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CURRICULUM VITAE

DAVID L. EATON

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PERSONAL

Born August 15, 1952, Helena, Montana
Married: Janet
Children: Lucas (7), Kaley (4)

EDUCATION

1978	University of Kansas Medical Center, Kansas City, Kansas, **PhD,** (Pharmacology)
1974	Montana State University, Bozeman, Montana, **BSc,** (Pre-medicine)

PROFESSIONAL POSITIONS

1992-Present	**Professor,** Department of Environmental Health, School of Public Health and Community Medicine, and Institute for Environmental Studies, College of Arts and Sciences, University of Washington
1991-Present	**Associate Chairman,** Department of Environmental Health, School of Public Health and Community Medicine, University of Washington
1986-1991	**Director,** Toxicology Program, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington
1986-1992	**Associate Professor,** Department of Environmental Health, School of Public Health and Community Medicine, and Institute for Environmental Studies, College of Arts and Sciences, University of Washington
1979-1986	**Assistant Professor,** Department of Environmental Health, School of Public Health and Community Medicine, and Institute for Environmental Studies, College of Arts and Sciences, University of Washington

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Ex. 1 Date 12-15-92
Witness LEATIOTA
JOANNE LEATIOTA

1978-1979	**Postdoctoral Fellow** in Toxicology, Department of Pharmacology, University of Kansas Medical Center, Kansas City, Kansas
1974-1978	**Predoctoral Trainee,** US Public Health Service, Department of Pharmacology, University of Kansas Medical Center
1974	**Research Assistant,** Department of Chemistry, Montana State University, Bozeman, Montana

FELLOWSHIPS, HONORS, AWARDS

Rohm & Haas Distinguished Professor of Public Health Sciences, 1992-97
NIH Pharmacology Research Associate Program Postdoctoral Fellowship Award, 1979-1981 (declined)
Honorable Mention Overall, National Student Research Forum, Galveston, Texas, 1978
First Place Award, Pharmacology/Toxicology Division, National Student Research Forum, Galveston, Texas, 1978
First Place Award, University of Kansas Medical Center, Graduate Student Research Day, 1978
Who's Who in American Colleges and Universities, 1977
President, Graduate Student Council, University of Kansas Medical Center, 1977
First Place Award, Pharmacology/Toxicology Division, National Student Research Forum, Galveston, Texas, 1977
Second Place Overall, National Student Research Forum, Galveston, Texas, 1977
First Place Award, University of Kansas Medical Center, Graduate Student Research Day, 1977
Graduate with Distinction, Montana State University, 1974

CERTIFICATION

Diplomate of the American Board of Toxicology, 1981, recertified 1985, 1990

PROFESSIONAL ORGANIZATIONS

International Society for the Study of Xenobiotics (ISSX), 1992-present
American Association for Cancer Research, 1988 - present
Sigma Xi 1987-present
Pacific Northwest Association of Toxicologists (Northwest Chapter, SOT); founding member, 1984-present, Vice President, 1984-1985; President, 1987-88, Councilor, 1988-89
Society of Toxicology, 1984-present
Society for Environmental Toxicology and Chemistry, 1981-present, Regional Chapter Councilor, 1989-90
American Association for the Advancement of Science, 1977-present

PROFESSIONAL ACTIVITIES

NATIONAL AND INTERNATIONAL

Member, NIEHS Site Visit Team and Reviewer, Program Project Grant review, Oregon State University, 1992.
Editorial Board, *Environmental Carcinogenesis & Ecotoxicology Reviews,* 1992-present
Treasurer, American Board of Toxicology, Inc., 1991-94
Member, Science Advisory Board, The Institute for Wildlife and Environmental Toxicology, Clemson University, Clemson, S.C. 1990-94
Councilor, Mechanisms Specialty Section, Society of Toxicology, 1990-93
Chairman, Membership Committee, Society of Toxicology, 1992-93
Membership Committee, (elected committee), Society of Toxicology, 1990-93
Board of Directors, American Board of Toxicology, Inc., 1990-94
Board of Publications, *Society of Environmental Toxicology and Chemistry,* 1989-91
Editorial Board, *Toxicology and Applied Pharmacology,* 1989-present
Invited Participant, United States - Japan Cooperative Program on Development & Utilization of Natural Resources, Joint panel on toxic microorganisms symposium on "Cellular and Molecular Mode of Action of Selected Microbial Toxins in Foods and Feeds," National 4-H Center, Chevy Chase, MD, Oct. 31- Nov. 2, 1989
Member, Society of Toxicology *ad hoc* "Tox-90's Education Committee," 1988-present
Invited Participant, Advisory workshop on significance and utilization of SARA Title III data, sponsored by USEPA, Chemical Manufacturer's Association and the National Academy of Sciences, NAS Headquarters, Washington, D.C., Oct. 18-19, 1988
Participant, 1987 Gordon Research Conference on Mechanisms of Toxicity, Kimball Union Academy, Meriden, New Hampshire, July 27-31, 1987
Member, NIH *Ad Hoc* Scientific Review Panel for NIEHS contract on "Methods development for *in vitro* human metabolism of xenobiotics," February 19-21, 1985, Research Triangle Park, NC
Committee on Public Communications, Society of Toxicology, 1984-1985

Manuscript Referee, *Aquatic Toxicology; Archives of Environmental Contamination and Toxicology; Biochemical Pharmacology; Biochemistry; Chemico-Biological Interactions; Environmental Science and Technology; Cancer Research; Fundamental and Applied Toxicology; Journal of Pharmacology and Experimental Therapeutics; Molecular Pharmacology; Pharmacology & Toxicology; Toxicology and Applied Pharmacology; Transactions of the American Fisheries Society*

COMMUNITY AND STATE

Member, Northwest Consumer Food Safety Council, 1990-94
Chairman, Science Advisory Board (5 member board established by Initiative, pre-empting above SAB), Washington State Department of Ecology, 1989-1991; Member 1989-92.
Member, Science Advisory Board (14 member board established under legislative act), Washington State Department of Ecology, 1988-90
Member, Technical Work Group on Environmental/Regulatory Policy for the Puget Sound Water Quality Authority, 1987

Site Visitor, Western Washington University, Bellingham, WA; Requested to evaluate current occupational health and safety practices relating to toxic chemical storage and use at Western Washington University. Provided written report to Dean of the Graduate School, October 8, 1986

Member, Mayor's Advisory Task Force on PCB's, 1984-86

Member, Advisory Task Force to Assess Health Impacts of Sediment and Shellfish pollution at Eagle Harbor, WA. For Coalition of County and State Health Departments. October 1984-85

Member, Mayor's Health Advisory Panel on Gas Works Park, 1984

Member, Gypsy Moth Advisory Committee, Department of Agriculture, Washington State Medical Association, and Office of the Mayor, City of Seattle, 1983

Member, Emergency Insect Criteria Committee, State Department of Agriculture, 1982-86

Member, Washington State Pesticide Advisory Board (Gubernatorial Appointment), 1982-1985, reappointed 1986-1989

Member, State Department of Social and Health Services, *Ad Hoc* Committee to Review Health Implications of Totally Recycled Water Systems, 1981

UNIVERSITY

Co-Chairman for Exhibits, University of Washington Health Sciences Open House, 1991

Member, Search Committee for new Chairman of Department of Environmental Health, 1990-91

Member, Civil Engineering Program Review Committee, University Programmatic Review by the Graduate School, 1990

Member, Environmental Studies Review Committee, campus-wide review of Environmental Studies programs and future directions, for the Office of the Provost, 1990

Chairman, University of Washington Technical Oversight and Advisory Committee for Ruston/Vashon Arsenic contamination remedial investigation feasibility study; consultant to Black and Veatch and Washington Department of Ecology, 1987

Member, Search Committee for Occupational Medicine faculty position (Chair, Dr. Linda Rosenstock), 1987

Member, Appointments, Promotions and Tenure Committee, Department of Environmental Health, 1986-present

Member, Arsenic Pathways Review committee, to assist study group and local health departments interpret and present findings of CDC/UW. Arsenic Pathways Study, 1986

Member, Alcohol and Drug Abuse Institute Executive Council, 1986-present

Member, Small Grants Review Committee, Alcoholism and Drug Abuse Institute, University of Washington, 1984-86

Chair, Curriculum and Teaching Policy Committee, Institute for Environmental Studies, 1983-present

Member, Search Committee for Dean of School of Public Health and Community Medicine, University of Washington (Chairman, Dean William Richardson), 1982

Member, Curriculum and Teaching Policy committee, Department of Environmental Health, 1981-86; Chairman, 1985-86

Member, Search Committee for Director of Laboratories, Department of Environmental Health (Chairman, Dr. Ken Jackson), 1980

Core Faculty Member, Environmental Pathology Training Grant, Department of Pathology, University of Washington, 1980-present
Member, Graduate Faculty, University of Washington, 1979-present

PRESENTATIONS

NATIONAL AND INTERNATIONAL

Seminar Speaker, Molecular basis for species differences in aflatoxin carcinogenicity, Chemical Industry Institute for Toxicology, Research Triangle Park, NC, June 4, 1992.
Invited Speaker, "Risk Assessment in Managing Environmental Exposure Communications and Media Relations", Medical Group Management Association, Occupational Medicine Assembly Conference, Orlando, FL, February 18, 1992
Invited Speaker, Principles of Toxicology; Metals Toxicology; Carcinogenesis, mutagenesis and Teratogenesis lectures, as part of "*Risk Assessment, Management and the Communication of Drinking water Contamination*, Continuing Education program sponsored by USEPA and NEHA, Portland, OR, June 27, 1991
Invited Alumni Speaker, The biochemical and molecular basis for species differences in susceptibility to aflatoxin B_1 carcinogenesis, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS April 9, 1991
Invited Seminar Speaker, The biochemical basis for species differences in aflatoxin carcinogenicity" Department of Environmental Health Sciences, Johns Hopkins University School Hygiene and Public Health, Baltimore, MD, February 12, 1990
Invited Seminar Speaker, "The role of biotransformation in species susceptibility to aflatoxin B_1 carcinogenesis", Medical Research Council, Toxicology Division, Carlshalton, England, Sept. 5, 1989
Participant and Presenter, Third International Conference on Glutathione S-Transferases, "Conjugation of aflatoxin B_1-8,9-epoxide by human liver glutathione S-transferases", Edinburgh, Scotland, August 31 - September 3, 1989
Invited Guest, National Public Radio Broadcast, Discussion on Alar in Apples, June 21, 1989
Invited Speaker, Pesticides in our food and Water. Putting the Risks in Perspective",, public information seminar sponsored by the National Environmental Health Association Annual Meeting, Seattle, WA June 25, 1989
Invited Speaker, "Approaches to Risk Assessment and Risk Communication for Forest Use Herbicides", presented to Regional Forest Managers and other agency officials, Portland, Oregon, April 16, 1987
Invited Speaker, "Estimating Occupational and Public Health Risks of Pesticides", Northwest Aerial Applicators Association Convention, Pendleton, OR, November 12, 1986
Invited Speaker, "Toxicology of Wood Preservatives: Pentachlorophenol, Creosote and Arsenicals", given for Montana Department of Agriculture as part of wood preservative applicators' certification training program, Kalispell and Helena, MT, September 30 and October 1, 1986

Session Co-Chairman, "Human Health Implications of Contaminated Seafood", International Symposium on Toxic Chemicals and Aquatic Life: Research and Management; provided closing remarks and session summary, Seattle, WA, September 16-18, 1986

Seminar Speaker, "Effects of enzyme induction on hepatobiliary disposition and DNA binding of Aflatoxin B_1 in the rat"; Presented at Philadelphia College of Pharmacy and Science, Department of Pharmacology and Toxicology, October 12, 1984

COMMUNITY AND STATE

Invited Speaker, "Fundamentals of Toxicology," Part 1 & Part 2, 1992 Northwest Federal Safety and Health Conference, Olympia, WA, April 29, 1992

Invited Speaker, "Basic Principles of Risk Assessment", American Public Works Association, Washington State Chapter, Spring Conference, March 26, 1992

Invited Speaker, "Toxic Chemicals, Human Health and the Environment", Science Enhancement for Teachers Program, University of Washington, April 27, 1991

Invited Speaker, "Are rodents good models for human responses to cancer-causing chemicals? Aflatoxin as an example. WEST '91 Conference, Seattle, Convention Center, April 11, 1991

Invited Speaker, "America's Epidemic of Chemicals and Cancer - Myth or Fact?" Inland Empire Agricultural Chemical Association 18th annual convention, Spokane, WA Dec. 11, 1990

Invited Panel Participant, Land Based Marine Pollution in the Pacific Northwest, sponsored by the Ocean Studies Council, University of British Columbia, Vancouver, BC, Nov. 2, 1991

Program Organizer and Speaker, "Human Health Risk Assessment: Process and Limitations", principal lecture for a symposium on uncertainties in risk assessment and risk management, given to upper level managers in the Washington Department of Ecology, March 13, 1990

Invited Speaker, "Risk Assessment in Foods", and Panel participant, "Chemical Residues in Foods", presented at Food Safety in Northwest Supermarkets, sponsored by Washington State University Cooperative Extension, Culinary Botany Northwest, and Department of Environmental Health, UW. October 12, 1989

Invited Guest, "Pesticides in Our Food", The Jim Altoff Show, KING 1090 Radio, October 9, 1989 (1 hour)

Invited Speaker, "Pesticide residues in our food and water- a hazard to your health, or a negligible risk? Saturday Seminar Series, sponsored by the Office of University Relations and UW Extension, Sept. 30, 1989

Seminar Speaker, "Biochemical basis for Species Differences in Aflatoxin carcinogenesis", Washington State University Pharmacology/Toxicology Program Seminar, July 25, 1989

Invited Participant, "Town Meeting", local television talk show focusing on health controversies over pesticide residues in food, KOMO TV, Seattle, WA, April 13, 1989.

Invited Speaker, "Strengths and Weaknesses of Toxicological Evidence", presented at Continuing Education Program on "Legal Aspects of Occupational Health", University of Washington Education Resource Center, February 8, 1989, Seattle, WA.

Co-organizer and Speaker, "Pesticides in the Urban Environment: Health and Environmental Responsibility", program for 1988 annual convention of the International Pesticide Applicators Association, held in Bellevue, WA on Sept. 28-30, 1988

Invited Speaker, City Club (a downtown business leaders civic group). Discussion of the procedures and difficulties encountered in utilizing science in decision making regarding public health risks. Seattle, WA, February 23, 1988

Invited Speaker, "Utilization of Scientific Information in Risk Management Decisions", presented to Division Mangers and Budget Coordinators for METRO, Battelle Conference Center, Seattle, WA, October 14, 1987

Invited Speaker, "Problems in Risk assessment and Risk Communication", King County Health Department annual retreat, Seattle Center, Seattle, WA, March 18, 1987

Invited Speaker, "Taking Care of Yourself - Pesticide Use and Human Health", presented at 77th Annual Meeting of the Western Washington Horticultural Association, Olympia, WA, January 6, 1987

Invited Speaker, "Toxic Chemicals and Public Health: Putting the Risks in Perspective", presented to the Seattle Chapter of the American Society of Safety Engineers, October 27, 1986

Invited Speaker, "Principles of Toxicology", given at "Toxic Substances and Public Health Workshop", sponsored by the Indian Health Service, Seattle, WA, October 22, 1986

Invited Speaker, "Pesticides and Public Health: Risks and Uncertainties", presented at the International Pesticide Applicators Association Convention, Spokane, WA, October 2, 1986

Invited Speaker, "Basic Principles of Toxicology", Risk Assessment Workshop for State Legislators Olympia, WA. Sponsored by Washington Department of Social and Health Services, September 25, 1986

Invited Speaker, "Basic Principles of Toxicology and Risk Assessment", presented to Community Health nurses and doctors for the Seattle-King County Health Department, Seattle, WA, June 10, 1986

Invited Speaker, "Potential health effects of occupational Exposure to PCB's", presented to Snohomish County PUD utility workers, March 17, 1986

Invited Speaker, "Health Implications of Gaseous Emissions Associated with Midway Landfill", February 6, 1986, Kent, WA; sponsored by Washington State Department of Ecology

Course Co-Organizer and Instructor, "Basic Principles of Toxicology for Environmental/Health Practitioners"; two day course given in Tacoma, WA (July 16 & 17, 1985), and Spokane, WA (August 29 & 30, 1985)

Speaker, "Toxicology of PBC's, dioxins and dibenzofurans", given at a public meeting in Kitsap County at the request of Seattle City Light Environmental Affairs Division, Seattle, WA, June 17, 1985

Invited Speaker, "An evaluation of the health concerns over dioxins found in Eagle Harbor, WA", given at a community meeting at the request of Dr. Willa Fisher, Director of the Kitsap County Health Department, July 11, 1985

Invited Speaker, "Health Impacts of Environmental Pollutants", given for the Continuing Medical Education Series, Riverton Hospital, July 19, 1985

Invited Speaker, "Health Effects of PCB's", given to Seattle City Light employees on November 1, 1984, January 9, November 19, November 21, December 4, and December 11, 1985

Course Organizer and Speaker, "Toxic Chemicals: Communicating Risks to the Public", forum for Scientist-media exchange, sponsored by the Society of Toxicology and the Department of Environmental Health, Battelle-Seattle Conference Center, Seattle, WA, April 27, 1985

Invited Speaker, Public Perception of Health Impacts of Chronic Pesticide Exposure; International Pesticide Applicators Association Convention, Fife, WA, September 27, 1984

Course Organizer and Instructor, Toxicology Review Course for EPA Personnel; Seattle, WA, September 5, 1984-January 15, 1985 (attended by 45 EPA staff)

Invited Speaker, Pesticides use for Gypsy Moth control; presented at Controversies in Poisoning Management and Occupational/Environmental Exposures, Children's Hospital and Medical Center, Seattle, WA, April 28-29, 1983

Lecturer, Recent Developments in biological Monitoring; Continuing Medical Education Course, Seattle, WA, March 18-19, 1983

Lecturer, Toxicology; short course presented to Hazards Assessment Program employees of NOAA, 3 lectures; NOAA Sand Point Facilities, Seattle, WA, March 10, 1983

Speaker, Health implications of Gypsy Moth control programs; Public Information Seminar, sponsored by Department of Environmental Health and Institute for Environmental Studies, University of Washington, March 10, 1983

Invited Speaker, Health effects of chronic pesticide exposure: a training program of health personnel: prevention, recognition and treatment of pesticide-related illness; sponsored by Yakima Valley Farm Workers Clinic and the Migrant Health Clinics in Washington, Oregon and Idaho, Pasco, WA, January 21-22, 1983

Invited Speaker, Clinical toxicology of pesticides; Northwest Poison Control Center Conference, Spokane, WA, April 22-23, 1982

Invited Speaker, A survey of toxic torts; Washington State Trial Lawyers Association Annual Meeting, Vancouver, BC, Canada, July 10, 1981

Invited Speaker, Carcinogens in the environment; Washington State Office of Environmental Education in Health Education, Everett, WA, March 10, 1981

Invited Speaker, Phenoxy acid herbicides--fact and fiction; Huxley College of Environmental Studies, Western Washington University. Bellingham, WA, January 27, 1981

Invited Speaker, Central nervous system effects of industrial chemicals and physical agents; 1980 Occupational Health and Medical Conference, Spokane, WA, October 22-24, 1980

Invited Speaker, PCB's and human health. Washington State Office of Environmental Education and Health Education. Seattle, WA, February 27, 1980

UNIVERSITY

Seminar Speaker, "Delaney Amendment of Carcinogenic Substances". The 1992 Symposium on Science, Technology, and Society, The Influence of Scientific Evidence on National Policy, April 28, 1992

Seminar Speaker, "Glutathione S-transferases: Species and individual susceptibility to chemical carcinogens." Department of Environmental Health, UW, February 5, 1988

Course Co-Organizer and Speaker, "Working With Pesticides: Health and Safety Issues", Northwest Center for Occupational Safety and Health, University of Washington, Seattle, WA, January 16, 1987

Seminar Speaker, "Role of Glutathione-S-Transferase in Detoxification of Aflatoxin B_1", Department of Environmental Health, University of Washington, Seattle, WA, December 4, 1986

Invited Speaker, "Basic Principles of Toxicology", given at "Worker Health and Safety at Hazardous Waste Sites", continuing Education Program, Department of Environmental Health, University of Washington, Seattle, WA, December 4, 1986

Panel Member and Discussant, "Scientist and the Media: Making Headlines on Your Own Terms", February 12, 1986; sponsored by University of Washington Medical Sciences Information Center

Speaker and Program Moderator, Professional exchange on hazardous/toxic substances in and around the home - use and disposal; sponsored by Institute for Environmental Studies, University of Washington, Seattle, WA, June 18, 1984

Invited Speaker, Chemicals and cancer: Putting the risks in perspective; Continuing Education Course, Biologists Look at Life and Human Affairs, Biology Program, University of Washington, Seattle, WA, April 16, 1984

Course Organizer and Instructor, Legal aspects of toxicology; Continuing Education Course, Educational Resource Center, Department of Environmental Health, University of Washington, SeaTac Hotel, Seattle, WA, December 2-3, 1983

Course Organizer and Instructor, Principles and practice of toxicology in environmental health; developed for Continuing Competency Education for Environmental Health Practitioners Program, University of Washington, Seattle, WA, January 25-March 15, 1982

Speaker, Central nervous system effects of chemicals and physical agents; Continuing Education Course, Educational Resource Center, University of Washington, Seattle, WA, April 20, 1981

Invited Speaker, Heavy metals in the environment; School of Public Health and Community Medicine's 10th Anniversary Celebration, University of Washington, Seattle, WA, September 25, 1980

Invited Speaker, Occupational toxicology: principles of toxicology; Department of Environmental Health Continuing Education Program, University of Washington, Seattle, WA, September 19, 1980

Seminar Speaker, Carrier-mediated transport systems in isolated hepatocytes; Department of Environmental Pharmacology, University of Washington, Seattle, WA, November 5, 1980

Seminar Speaker, Effects of trace metals on metallothionein, glutathione, cytochrome P-450 and heme oxygenase activity in rats. Department of Pathology, University of Washington. Seattle, WA, April 11, 1980

CURRENTLY FUNDED INDIVIDUAL AND COLLABORATIVE RESEARCH

Program Director, NIH/NIEHS ES-04696 (Superfund program project), "Effects-Related Monitors of Toxic Exposure"; Annual Direct Costs, $1,682,598; 6/01/90 - 3/01/95; (Sheldon D. Murphy was Program Director from 9/29/87 until his death in May, 1990).

Co-Principal Investigator, NIH/NIEHS ES-04696 Superfund research project, "Glutathione perturbations with toxic exposures"; Annual Direct Costs, $96,263; 09/29/87 - 03/31/95

Principal Investigator, NIH/NIEHS ES-04696, Superfund Training Core; Annual Direct Costs, $94,303; 04/01/92 - 03/31/95.

Principal Investigator, NIH/NIEHS, ES-05780, "Species differences in glutathione S-transferases", $129,527 Annual Direct Cost, 05/01/88 - 04/31/94.

Principal Investigator, NIH/NIEHS/NCI, ES-03933, "Effect of enzyme inducers on liver preneoplastic lesions"; $127,580 Annual Direct Cost, 05/01/91 - 04/30/95

Principal Investigator, Washington Department of Health/UW Cooperative agreement for research projects in Toxicology and Public Health, 7/1/89-6/30/94, $243,000 TDC

BIBLIOGRAPHY

(*Indicates students, post-doctoral fellows or research technologists under the direction of Dr. Eaton).

RESEARCH PAPERS IN REFEREED JOURNALS

1. **Eaton DL,** Klaassen CD. Effects of acute administration of taurocholic and taurochenedeoxychlic acid on biliary lipid excretion. *Proc Soc Exp Biol Med* 151:198-202, 1975.
2. **Eaton DL,** Poisner AM. Plasma pseudorenin in rats after alteration in the renin-angiotensin system. *Proc Soc Exp Biol Med* 154:6-8, 1977.
3. **Eaton DL,** Klaassen CD. Carrier-mediated transport of ouabain in isolated hepatocytes. *J Pharmacol Exp Ther* 285:480-488, 1978.
4. Iwamoto I, **Eaton DL,** Klaassen CD. Uptake of morphine and nalorphine by isolated rat hepatocytes. *J Pharmacol Exp Ther* 206:181-190, 1978.
5. **Eaton DL,** Klaassen CD. Carrier-mediated transport of the organic cation procaineamide ethobromide by isolated rat liver parenchymal cells. *J Pharmacol Exp Ther* 206:595-606, 1978.
6. Iga T, **Eaton DL,** Klaassen CD. Uptake of unconjugated bilirubin by isolated rat hepatocytes. *Am J Physiol* 236:C9-C14, 1979.
7. **Eaton DL,** Klaassen CD. Effects of microsomal enzyme inducers on carrier-mediated transport systems in isolated rat hepatocytes. *J Pharmacol Exp Ther* 208:381-385, 1979.
8. **Eaton DL,** Klaassen CD. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin, kepone and polybrominated biphenyls on transport systems in isolated rat hepatocytes. *Toxicol Appl Pharmacol* 51:137-144, 1979.
9. **Eaton DL,** Stacey NH, Wong KL, Klaassen CD. Dose-response effects of various metal ions on rat liver metallothionein, glutathione, heme oxygenase and cytochrome P-450. *Toxicol Appl Pharmacol* 55:393-402, 1980.
10. **Eaton DL.** Biliary excretion of 2,4,5-trichlorophenoxyacetic acid in the rat. *Toxicol Lett* 14:175-181, 1982.
11. **Eaton DL,** *Toal BF. Evaluation of the Cd/hemoglobin affinity assay for the rapid determination of metallothionein in biological tissues. *Toxicol Appl Pharmacol* 66:134-142, 1982.
12. **Eaton DL,** *Toal BF. A simplified method of quantitating metallothionein in biological tissues. *J Sci Total Environ* 28:375-384, 1983.
13. *Carpenter LA, **Eaton DL.** The disposition of 2,4-dichlorophenoxyacetic acid (2,4-D) in the rainbow trout, *Salmo gairdneri. Arch Environ Contam Tox* 12:162-173, 1983.
14. *Stinson MD, **Eaton DL.** Concentrations of lead, cadmium, mercury and copper in the crayfish (*Pacificasticus lenisculus*) obtained from a lake receiving urban runoff. *Arch Environ Contam Toxicol* 12:693-700, 1983.
15. Woods JS, Fowler BA, **Eaton DL.** Studies on the mechanisms of thallium-mediated inhibition of hepatic mixed function oxidase activity: Correlation with inhibition of NADPH Cytochrome c (P-450) reductase. *Biochem Pharmacol* 33:571-576, 1984.
16. Woods JS, **Eaton DL,** *Lukens C. Studies on porphyrin metabolism in the kidney: Effects of trace metals and glutathione on renal uroporphyrinogen decarboxylase. *Mol Pharmacol* 26:336-341, 1984.

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17. Kalman D, **Eaton DL,** Schumacher RS, Covert D. Biological availability of lead in a paint aerosol. I. Physical and chemical characterization of a lead paint aerosol. *Toxicol Lett* 22:301-306, 1984.
18. **Eaton DL,** Kalman DA, *Garvey D, Morgan M, Omenn GS. Biological availability of lead in a paint aerosol. 2. Absorption, distribution and excretion of intratracheally instilled lead paint particles in the rat. *Toxicol Lett* 22:307-313, 1984.
19. **Eaton DL.** *Short Communications:* Effects of various trace metals on the binding of cadmium to rat hepatic metallothionein determined by the Cd/hemoglobin affinity assay. *Toxicol Appl Pharmacol* 78:158-162, 1985.
20. *Monroe DH, *Holeski CJ, **Eaton DL.** Effects of single dose and repeated dose pretreatment with 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) on the hepatobiliary disposition and covalent binding to DNA of Aflatoxin B_1 in the rat. *Food Chem Toxicol* 24:1273-1281, 1986.
21. **Eaton DL,** *Richards JA. Kinetic evaluation of carrier-mediated transport of ouabain and taurocholic acid in isolated rat hepatocytes. Evidence for independent transport systems. *Biochem Pharmacol* 35:2721-2725, 1986.
22. *Holeski CJ, **Eaton DL,** *Monroe DH, *Bellamy GM. Effects of phenobarbital on the biliary excretion of Aflatoxin P_1-glucuronide and AflatoxinB_1-*S*-glutathione in the rat. *Xenobiotica* 17:139-153, 1987.
23. *Monroe DH, **Eaton DL.** Comparative effects of butylated hydroxyanisole (BHA) on the *in vivo* and *in vitro* biotransformation of Aflatoxin B_1 (AFB) in rat and mouse. *Toxicol Appl Pharmacol* 90:401-409, 1987.
24. Geraci JP, Dunston SG, Jackson KL, Mariano MS, *Holeski C, **Eaton DL.** Bile loss in the acute intestinal radiation syndrome in rats. *Rad Res* 109:47-57, 1987.
25. **Eaton DL,** *Monroe DH, Kalman DA and *Bellamy G. Identification of a novel dihydroxy-metabolite of aflatoxin B_1 formed both *in vivo* and *in vitro* in rats and mice. *Chem Res Toxicol* 1:108-114, 1988.
26. Franzblau A, Rosenstock L and **Eaton DL.** Use of inductively coupled plasma-atomic absorption spectroscopy (ICP-AES) in screening for trace metal exposures in an industrial population. *Environ Res* 46:15-24, 1988.
27. *Monroe DH and **Eaton DL.** Effects of modulation of hepatic glutathione on biotransformation and covalent binding of aflatoxin B_1 to DNA in the mouse. *Toxicol Appl Pharmacol* 94:118-127, 1988.
28. *Ramsdell HS and **Eaton DL.** Modification of aflatoxin B_1 biotransformation *in vitro* and DNA binding *in vivo* by dietary broccoli in rats. *J Toxicol Environ Health* 25:269-275, 1988.
29. **Eaton DL,** *Stapleton PL. Simultaneous determination of cytosolic glutathione *S*-transferase and microsomal epoxide hydrolase activity toward benzo[*a*]pyrene-4,5-oxide by high performance liquid chromatography. *Analytl Biochem* 178:153-158, 1989.
30. *Ramsdell HS, **Eaton DL.** Species susceptibility to aflatoxin B_1 carcinogenesis: Comparative kinetics of biotransformation. *Cancer Res* 50:615-620, 1990.
31. *Ramsdell HS, **Eaton DL.** Mouse liver glutathione S-transferase isoenzyme activity toward aflatoxin B_1-8,9-epoxide and benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide. *Toxicol Appl Pharmacol* 105:216-225, 1990.
32. Kavanagh TJ, Grossmann A, Jaecks EP, Jinneman JC, **Eaton DL,** Martin GM, Rabinovitch PS. Proliferative capacity of human peripheral blood lymphocytes sorted on the basis of glutathione content *J Cell Physiol,* 145:472-480, 1990.

33. *Ramsdell HS, Parkinson A, Eddy C, **Eaton DL.** Bioactivation of aflatoxin B_1 by human liver microsomes. Role of cytochrome P450IIIA isoenzymes. *Toxicol Appl Pharmacol* 108:436-447, 1991.
34. *Trenga CA, Kunkel DD, **Eaton DL,** Costa LG. Effect of styrene oxide on rat brain glutathione. *Neurotoxicology* 12:165-178, 1991.
35. *Chen ZY, **Eaton DL.** Differential regulation of cytochrome P450 $IIB_1/2$ by phenobarbital in hepatocellular hyperplastic nodules induced by aflatoxin B_1 or diethynitrosamine plus 2-acetylaminofluorine in male F344 rats. *Toxicol Appl Pharmacol* 111:132-144, 1991.
36. *Borroz KI, *Ramsdell HS, **Eaton DL.** Mouse strain differences in glutathione S-transferase activity and aflatoxin B_1 biotransformation. *Tox Lett* 58:97-105, 1991.
37. Rosenstock L, Keefer M, Daniell WE, McConnell R, Claypoole K, Barnhart S, Costa LG, Demers P, **Eaton DL,** Morris S, Murphy SD. Chronic central nervous system effects of acute organophosphate pesticide intoxication. *The Lancet* 338:223-227, 1991.
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39. Heckbert SH, Weiss NS, Hornung SK, **Eaton DL,** Motulsky AG. Glutathione *S*-transferase and epoxide hydrolase activity in human leukocytes in relation to the risk of lung and other smoking-related cancers. *J Nat'l Cancer Inst* 84:414-422, 1992.
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2. Nazar-Stewart V, Motulsky AG, **Eaton DL,** *White, EJ, Hornung, SK, Leng, Z-T, Stapleton, P and Weiss, NS. The Glutathione S-Transferase mu gene as a marker for susceptibility to lung carcinoma. *New Eng J Med.*
3. Kavanagh TJ, Grossman A, Jinneman JC, Kanner SB, *White CC, **Eaton DL,** Ledbetter JA, Rabinovitch PS. Glutathione depletion by 1-chloro-2,4-dinitrobenzene inhibits T-cell receptor-stimulated transmembrane signal transduction in purified subsets of human peripheral blood lymphocytes. *Toxicol Appl Pharmacol.*

4. *Chen ZY, **Eaton DL.** Association between centrilobular origin and advanced differentiation phenotypic characteristics of hepatic hyperplastic nodules induced by aflatoxin B_1 in male F344 rats. *Carcinogenesis*.
5. *Buetler, TM, *Slone, D and **Eaton, DL.** Comparison of the aflatoxin B1-8,9-epoxide conjugating activities of two bacterially expressed alpha class glutathione S-transferase isozymes from mouse and rat, *Biochem Biophys Res Commun.*
6. Buetler, TM and **Eaton, DL.** Amino acid sequence comparison, classification, and phylogenetic relationship between 65 glutathione S-transferases. *Environ Carcin Ecotox Rev.*
7. *Chen, ZY, *White, CC and **Eaton, DL.** Decreased expression of cytochrome P450 mRNA and related steroid hydroxylation activities in hepatic hyperplastic nodules in male F344 rats. *Toxicol Appl Pharmacol.*
8. Nazar-Stewart, V., **Eaton, DL,** White, E, *Stapleton, P and Weiss, NS. Markers for glutathione S-transferase and epoxide hydrolase enzymes in lung tissue and risk for lung carcinoma among smokers.

BOOKS

Eaton DL, Groopman JD, eds. *The Toxicology of Aflatoxins: Human Health, Veterinary and Agricultural Significance.* Academic Press, New York, in press, 1993.

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9/14/92

UW TEACHING RESPONSIBILITIES :

Courses with principal responsibility:

ENVH/ENVS 515, Environmental and Occupational Toxicology, typical enrollment of 30-40 students, taught annually from 1980-1991 (as ENVH/ENVS 415 from 80-82).
ENVH/ENVS 305/405, Toxic Chemicals in the Environment, typical enrollment of 50-65 students, taught annually from 1982 - 1984, even numbered years from 86-present. Annually from 1992 on.
ENVH 567 Environmental and Occupational Carcinogenesis, new course developed and taught on odd-numbered years from 1993 - on.

Annual Guest Lectures:
ENVS 101, Introduction to Environmental Studies, Fall and Spring quarters, 2-4 lectures.
HUBIO 555, Medicine, Health and Society, Winter quarter, 2 lectures.
ENVH 411, Introduction to Environmental Health, Spring quarter, 1 lecture.
ENVH 590c, Ecogenetics, Winter quarter, 1 lecture.
HUBIO 567, Medical Pharmacology, Spring quarter, 2 lectures.
C MED 520/530, Biology and Disease of Laboratory Animals, Fall quarter, 1 lecture.
ENVH 567, Industrial Carcinogens, 1-2 lectures.

Students/Fellows Precepted:

Post-Doctoral Fellows:
Dr. Howard Ramsdell, 1987-90.
Dr. Zhi-Ying Chen, 1988-present
Dr. Martin Buetler, 1990-present
Dr. Evan Gallagher, 1991-present

Doctoral Students:
Carolyn Holeski (with Dr. N. Karle Mottet, Pathology), 1986
David H. Monroe (Ind. Ph.D.), 1987
Kirk Van Ness, in progress

Master's Students (year graduated):
Brian Toal, 1981
Leslie Carpenter, 1981
Stephan Dobratz, 1983
Jeffry Defenbach, 1984
David Monroe, 1984
Priscilla Anderson, 1986
Julia Richards, 1988
Karyn Michelson, 1989
Denise Bender, 1990
Carol Trenga (with Dr. Lucio Costa), 1990
Steve Channel (with Dr. Terry Kavanagh), 1990
Dana Stahl, in progress
Margaret Stinson, 1981
Don Garvey, 1982
Jeff Bailet, 1984
Carl Lukens, 1984
Kim Lowry, 1985
Heidi Hagelstein, 1987
Denise Hamel, 1989
Lynn Berlad, 1989
Ingrid Borroz, 1991
John Heinonen, 1992

EXPIRED GRANTS

Principal Investigator, NIH/NIEHS, Carrier mediated transport of xenobiotics in isolated hepatocytes. 6/1/83-5/31/86, $245,000 DC

Principal Investigator, NIH/NIEHS/NCI, ES-03933, "Modification of aflatoxin disposition by dietary enzyme inducers"; $324,964 TDC , 05/01/88 - 04/30/91, (renewed as "Effects of enzyme inducers on liver preneoplastic lesions, NIEHS)

Co-Investigator, Dana Foundation Grant in Genetics and Environmental Health, (PI, Dr. Arno Motulsky); 09/01/85-06/30/92; sub-project, "Genetic Differences in Glutathione S-transferase activity".

Co-Investigator, US Forest Service (USDA); "Health Effects Implications of Pacific Northwest Region Vegetation Management", 10/01/86-9/30-88;

Co-Investigator, Milbank Memorial Fund, "Illness and Injuries Among Washington State Agricultural Workers", PI-Dr. Linda Rosenstock, 11/01/86-04/30/88;

Co-Investigator, NIH/NIOHS; "Peripheral markers of styrene toxicity", PI, Dr. Harvey Checkoway, 12/01/88-11/30/90;

Co-Investigator, NIH/NHLBI; "Metabolism of propranolol in uremic rat liver", PI, Dr. Danny Shen, Dept. Pharmaceutics; 04/01/88-03/31/92

Numerous small institutional grants: SPHCM BRSG (2), Graduate School Young Investigator Award, American Cancer Society Institutional Grants (2), Departmental Industrial Hygiene Research Grant.

PROFESSIONAL CONSULTING ACTIVITIES

CONSULTING FIRMS

Battelle Human Affairs Center, Seattle, WA. (Dr. James Woods) Advisory board member for Battelle contract to evaluate scientific review process utilized by EPA for pesticide evaluation for registration.

Environmental Toxicology International, Seattle, WA. (Dr. Kathryn Kelly) Prepared evaluation of potential human health risks associated with DDT soil contamination at a utility sub-station.

Harding-Lawson Associates, Novato, CA. (Dr. Michael Zander) Reviewed human health risk assessment portion of feasibility study of Milltown Reservoir CERCLA site containing arsenic residues.

URS Engineers, Seattle, WA. (Mr. Bob Dexter) Expert consultant on health effects of industrial pollutants.

CH2M Hill, Seattle, WA. (Mr. John Anthony) Expert consultant to evaluate potential human health hazards associated with commercial development of an abandon creosote plant site into condominium complex. (In association with Woodward-Clyde Associates, San Francisco, CA.)

K.S. Crump & Company, Ruston, LA. (Dr. Kenny Crump) Assisted in the development and evaluation of "Worst-Case Analysis" for forest herbicide use on public lands in the State of Washington.

Tetra Tech, Inc. (Dr. Robert Pastorak) Served as toxicology and risk assessment consultant on various projects associated with evaluating risks of consumption of contaminated seafood from Puget Sound.

Tetra Tech, Inc. (Dr. Gary Pascoe) Assisted in the development of a survey of pesticide use in the greater Puget Sound area, including evaluation of potential environmental and human health significance.

Parametrix, Inc. (Dr. Ramon Beluche) Reviewed human health components of various consultant documents on hazardous and solid waste site evaluation and clean-up plans.

Woodward-Clyde Consultants, (Dr. William Zbitnoff) Peer-reviewed human health risk assessment on potential airborne exposures and ground water contamination from municipal landfill.

PUBLIC AGENCIES

Department of Agriculture, State of Washington. (Mr. Art Losey) Provided expert evaluation of human health implications and risk assessment of chemical options, especially carbaryl, for control of Gypsy Moth in urban areas.

Seattle/King County Health Department, Seattle, WA. (Mr. Chuck Kleeberg) Provided numerous expert consultations on local environmental health problems, including: Gas Works Park - evaluation of human health risks associated with a City Park located on a former coal gasification plant site which was heavily contaminated with polyaromatic hydrocarbons; assisted City/County in evaluating health risks from several solid waste landfill sites; assisted in evaluating human health risks from consumption of contaminated seafood in Puget Sound area.

Bureau of Land Management, Washington, D.C. Evaluated worst-case analysis of herbicides for federal forest lands.

Tacoma/Pierce County Health Department, Washington. (Mr. Doug Pierce) Assisted in evaluating human health risks associated with arsenic residues in the community from ASARCO smelter emissions.

Kitsap County Health Department, Washington. (Dr. Willa Fisher) Assisted in evaluating human health risks associated with consumption of shellfish contaminated with polyaromatic hydrocarbons and other industrial waste products in Eagle Harbor, WA.

Seattle City Light, Environmental Affairs Division (Ms. Clair Dyckman) Reviewed toxicology and environmental effects of pesticides used by SCL, and prepared written evaluation of pesticide inventory and use practices.

Northwest Poison Control Center, Children's Hospital and Medical Center. (Dr. William Robertson) Provide occasional telephone consultations in pesticide and unusual toxic chemical poisoning.

U.S. Forest Service, Pacific Northwest Region. (Mr. Gary Larsen, Group Leader, Vegetation Management) Reviewed toxicologic data on forest use of herbicides, and assisted U.S. Forest Service in preparation, evaluation and interpretation of risk assessments (worst case analyses) as part of EIS.

City of Seattle, Solid Waste Division (Ms. Lin Robinson). Provided written evaluation of a draft Endangerment Assessment for the Midway Landfill.

LITIGATION EXPERIENCE

Aaby, Knies and Robinson, Bellingham, WA. (Mr. Robert Knies, plaintiff's attorney) Expert witness on occupational accident case involving high level 2,4-D exposure.

Reed, McClure, Moceri & Thonn, P.S., Seattle, WA. (Mr. Dennis Smith, defendant's attorney) Provided expert consultation and review on occupational exposure to ethylene oxide.

King County Prosecutor's Office, Seattle, WA. (Mr. Michael Linnabury, representing county as defendant.) Provided extensive evaluation, review and expert testimony regarding scientific information relevant to purported claims of adverse human health impacts resulting from living near a municipal landfill site. County was a defendant.

Richter, Wimberley & Ericson, P.S., Spokane, WA. (Mr. Gary Bloom, Plaintiff's attorney, class-action suit) Provided expert consultation and opinions regarding potential human health effects of chronic exposure to cyanide in drinking water (Spokane River aquifer).

Malagone & Velure, Eugene, OR. (Mr. Peter McSwain, plaintiffs' attorney) Reviewed documents and provide opinion (unfavorable) on occupational injury claim form solvent (toluene) exposure and possible relatedness to cardiovascular accident.

Ferguson & Burdell, Seattle, WA. (Mr. Thomas Wolfendale, defendant's attorney) Expert evaluation of personal injury claim related to exposure to carbonless copy paper.

Winer, Bennet, Harman & Tauman, P.C., Portland, OR. (Mr. Charles Tauman, plaintiffs' attorney) Provided expert evaluation and opinion regarding relationship between neurological deficits and chronic, very high level exposure to organic solvents in a work place.

U.S. Department of Justice, District of New Mexico. (Mr. Raymond Hamilton, Assistant U.S. Attorney, on behalf of U.S. Forest Service as defendant) Provided expert opinion (in testimony) on extent or significance of public health effects that complainants alleged would occur if application of carbaryl to national forest lands was allowed.

G. Lee Raaen, Attorney-at-Law, Seattle, WA. (Lee Raaen, plaintiff's attorney) Provided consultation and expert testimony on relationship between arsenic exposure and diagnosed peripheral neuropathy.

Agency Legal Services Bureau, Attorney General's Office, State of Montana. (Opal Winebrennar, Assistant Attorney General) Provided consultation and expert opinion on behalf of defendant (State of Montana) in workers compensation case claiming that leukemia resulted from herbicide (2,4-D) exposure.

Bogle and Gates, Seattle, WA. (Mr. David Bateman, defendant's attorney, for Olympic Pipeline Company.) Assisted Department of Engineering Program health risk assessment and public communications of risk surrounding pipeline leak of gasoline products in residential neighborhood.

Seattle City Attorney's Office, Seattle, WA. (Mr. Dennis McClairen and others) Provided consultation and expert opinion on likelihood of health risks occurring to residents near a municipal landfill.

Reed, McClure, Moceri, Thonn & Moriarty, Seattle, WA (Mr. Dennis Smith, Defendant's attorney) Provide consultation as to state of knowledge in 1960's of toxicologic hazards of various chemicals identified at a hazardous waste site.

Williams, Kastner & Gibbs, Seattle, WA (Mr. Roy Umlauf, defendant's attorney) Provide consultation as to effects of specific chemicals identified at a hazardous waste site.

U.S. Department of Justice, Washington, D. C. (Mr. Kevin Murphy, Trial Attorney, Lands Division) Expert Testimony in criminal prosecution case for illegal disposal of pesticides, Spokane, WA.

Betts, Patterson & Mines, Seattle, WA (Mr. John Allison, defendant's attorney) Provide research, consultation and expert testimony on potential health impacts of consumption of groundwater contaminated with small amounts of EDB.

Shidler, McBroom & Gates, Seattle, WA (Mr. Joel Summer, plaintiff's attorney) Provide, research, consultation and expert opinion on herbicide (paraquat)-related death.

Preston, Thorgrimson, Ellis & Holman, Seattle, WA (Mr. Ross Macfarlane, attorney for pulp and paper processor) Provide consultation and guidance on proper disposal of "hogged" fuel ash to ensure protection of human health and environment.

Underwood, Campbell, Brock & Cerutti, P.S., Spokane, WA (Mr. Jay Leipham, defendants attorney). Provide research, consultation and expert opinion regarding health impacts of consumption of groundwater contaminated with nitrates.

Moulton, Bellingham, Longo & Mather, P.S., Billings, MT (Ms. Tamara A. Scully, defendants attorney); Provide research, consultation and expert opinion regarding health impacts of atrazine herbicide.

Lukins & Annis, P.S., Spokane, WA. (Mr Ted Stiles, defendants attorney). Provide research, consultation and expert opinion regarding health impacts of malathion exposure.

Perkins Coie, Seattle, WA (Mr. Mark Schneider, defendants attorney). Provide research, consultation and expert opinion regarding health and environmental impacts of trichlorethane and trichloroethylene in groundwater.

Heller, Ehrman, White & McAuliffe, Seattle, WA (Mr. Steve Anderson, defendants attorney). Provide research, consultation and expert opinion regarding health impacts of variety of pollutants associated with Superfund hazardous waste site.

Helsell, Fetterman, Martin, Todd & Hokanson, Seattle, WA (Mr. Mark Rising, defendants attorney); Provide research, consultation and expert opinion regarding health and environmental impacts of waste products from steel production process.

Carney, Stephenson, Badley, Smith, Mueller & Spellman, P.S. (Mr. Dave Bratz, defendant's attorney) Provide research, consultation and expert opinion regarding toxicologic effects of methyl ethyl ketone peroxide.

Rivkin, Radler, Dunne & Bayh, Uniondale, NY (Mr. Edward T. Finneran, III, defendant's attorney); Provide research, consultation and expert opinion regarding health and environmental impacts of dioxane in groundwater.

Williams, Kastner & Gibbs, Seattle, WA (Mr. Mark Myers, defendants attorney). Provide research, consultation and expert opinion on health impacts of occupational exposure to wood products containing copper arsenate preservative.

Huey, Guilday, Kuersteiner & Tucker, P.S., Tallahassee, FL (Mr. Ralph DeMeo, defendant's attorney). Provide research, consultation and expert opinion on health risks of EDB in groundwater

Snohomish County, Solid Waste Division (Ms. Karen Nahkajuri) Provide testimony regarding extent, or lack, of potential public health risks from chemicals at planned municipal landfill.

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DEFENDANT'S EXHIBIT 3

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A meta-analysis of exposure to phenoxy acid herbicides and chlorophenols in relation to risk of soft tissue sarcoma

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Summary. This analysis combines the results of the epidemiologic studies which appeared in the literature from 1979–1987 concerning the associations between phenoxy acid herbicides (PH) and chlorophenols (CP) and the occurrence of soft tissue sarcoma. Cohort studies and case-control studies are considered separately. After specifying criteria for inclusion, the total number of deaths due to cancer occurring in selected cohorts of people exposed to PH or CP was determined. The proportion of soft tissue sarcoma cases observed versus expected in the cohort studies, calculated using United States SEER registry data, resulted in a proportional mortality ratio of 3.5 (95% confidence interval of 0,7–10.3). Excluding the initial reports, the selected case-control studies had homogeneous risk estimates with a summary odds ratio of 1.1 (95% confidence interval of 0.9–1.4). Other attempts to assess the combined data did not yield results indicating an association. These meta-analyses do not provide strong support for an association between the specified herbicides and soft tissue sarcoma.

Key words: Epidemiology – Soft-tissue neoplasms – Dioxins – Pesticides

Introduction

In 1979, case-series and case-control epidemiologic studies from Sweden indicted phenoxy acid pesticides (PH) and chlorophenols (CP) as human carcinogens. Of particular concern was exposure to the dioxin contaminant (2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD) of the phenoxy acid herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and chlorophenols. These reports were followed by a series of epidemiologic studies from all over the world of the risk for cancer in general [1, 2, 18, 24, 26, 28, 31, 38, 39], and for soft tissue sarcomas and lymphomas in particular, associated with exposures to PH and CP. This report presents meta-analyses of data pertaining to soft tissue sarcoma (STS), and draws conclusions by assessing summary measures and the consistency of results.

Methods

To our knowledge, we obtained and reviewed all of the papers published from 1979–1987 dealing specifically with epidemiologic studies of PH and CP and STS risk. A collection of papers and reports were provided by Dynamac Corporation to the authors. In addition, the authors conducted a comprehensive literature search using MEDLINE for all years through 1986, and by review of papers cited in the collected material. The 1986 and 1987 issues of environmental and occupational health journals were reviewed to obtain recent articles that might not have otherwise been included. All articles with mention of PH, CP, or dioxin and cancer were evaluated. The analyses presented in this paper include only cohort and case-control studies; case series reports were excluded because of the lack of comparison groups, and proportional mortality studies were excluded because the data provided in these studies could not be accommodated in our analytical approach.

Cohort studies. Cohort studies of the association between PH, CP and human cancer came from two different sources; 1) industrial exposures involved in the manufacture of these products and 2) exposures involving the application of these substances. The studies from manufacturing industries included cohorts who routinely came into contact with these substances as a part of their work as well as several groups of workers who experienced high intensity exposures of short duration due to accidents. Table 1 presents a listing of the cohort studies of exposures associated with PH and CP and STS mortality or incidence [2, 16, 24, 26, 28, 31, 36, 38, 39]. A number of commentaries presented additional data on overlapping groups of subjects from these cohorts [7, 12, 19, 22, 25]. However, the subject groups selected for this analysis were mutually exclusive.

The studies varied in exposure criteria, although all persons were considered exposed in some degree to PH or CP. Those studies that were more general with regard to exposure (e.g., all pesticides) or with only a short follow-up period were excluded. Some studies had disease incidence endpoints; others measured mortality. As this analysis used cancer-specific and total cancer mortality, studies with only incidence data were excluded. All the studies were limited to white males with the exception that 3.2% of the Michigan study subjects were American Indian, Black or Hispanic males [26]. No age-specific person-years of follow-up were available from the reports, prohibiting a direct re-analysis. Few of the studies presented the total number of person-years of follow-up.

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Table 1. Summary of cohort studies and risk estimates for soft tissue sarcoma[a]

Location [Ref.]	Exposure	No. of subjects of risk	O/E	Estimate	95% Confidence interval	Comments
Sweden [2] Axelsson et al. 1974	PH	207	0/0.03[b]	–	–	Railroad workers exposed > 45 days from 1957–72; followed 1957–1978; 10 years latency incorporated
Finland [28] Riihimaki 1982	PH	1926	0/0.1	–	–	Herbicide applicators exposed ≥ 14 days from 1955–71; followed 1972–1980; 10 years latency incorporated
Denmark [24][c] Lynge 1985	2,4-D; cresol	3390 940[d]	4/1.09 1/0.16	3.7 6.3	1.0–9.4 0.2–34.8	2 herbicide manufacturing plants; all employees from 1947–1982; followed 1947–1982; 10 years latency incorporated; 4 STS (2 leiomyosarcomas, one dermatofibrosarcoma of the back, one sarcoma of the retroperitoneum)
Michigan [26] Ott et al. 1980	2,4,5-T	2187	1/0.4	2.5	0.1–13.9	Chemical workers employed 1940–1980; followed 1940–1982; no latency incorporated; includes 61 subjects involved in a 1964 accident
Sweden [18] Hogstedt & Westerlund 1980	PH	161	0/0.1	–	–	Forest workers employed > 5 days from 1954–1967; followed 1954–1978
West Virginia [38] Zack & Suskind 1980	2,4,5-T	121	1/0.05[b]	22.2	0.6–123.8	Chemical workers accidentally exposed and diagnosed with chloracne in 1949; followed 1949–1978; no latency incorporated; number expected for STS grouped with "other cancer sites"
Federal Republic of Germany [31] Theiss et al. 1982	2,4,5-T	74	0/0.02	–	–	Chemical workers accidentally exposed in 1953; followed to 1979; no latency incorporated
West Virginia [39] Zack & Gaffey 1983	2,4,5-T	884	1/0.18[b]	5.7	0.1–31.8	Plant chemical workers with potential exposure; > 1 year employment from 1955–1977; followed 1955–1977; no latency incorporated; number expected for STS was grouped with "other cancer sites"
Sweden [36][a] Wiklund & Holm 1986	PH	354620 7215[d]	nc nc	0.9 0.8	0.8–1.0 0.3–1.9	Swedish agricultural and forestry workers in 1960 compared to other Swedish employed men; followed 1961–1979; high exposure category included those in silviculture

[a] CI = confidence interval; O/E = observed/expected; nc = not calculated as necessary data not presented; "–" indicates that zero (0) cases were observed so a risk estimate was not calculated; PH = phenoxy acid herbicides; 2,4-D = 2,4-dichlorophenoxyacetic acid; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; STS = soft tissue sarcoma. Reference 2 superseded an initial study [1]. Reference 26 superseded three earlier reports [4, 8, 27]

[b] Estimated expected number based on the information that 0.5% of the total cancer cases ascertained in the study could be expected to have STS, as calculated from SEER registry data [37]

[c] These risk estimates related to cancer incidence rather than mortality

[d] Higher exposed subgroup

Table 2. Summary of case control studies and risk estimates for soft tissue sarcoma

Location [Ref.]	Exposure[a]	No. of cases	No. of controls	Risk estimate	95% Confidence interval	Comments[b]
Sweden [16] Hardell & Sandstrom 1979	CP & PH	52	206	5.7[c]	2.7–11.8	Hospital cases admitted from 1970–1977, general population controls, extensive matching. Positive exposure if > 1 day and 5 years preceding diagnosis
	PH	46	201	5.3[c]	2.4–11.5	
	CP	40	193	6.6[c]	2.1–20.9	
Sweden [10] Eriksson et al. 1981	CP & PH	110	219	4.7[c]	2.3–9.5	Incident cases in 5 counties from 1974–1978; positive exposure if > 1 day at least 5 years preceding diagnosis
	PH	92	207	17.0[c]	2.1–140.0	
	CP	96	214	3.3[c]	1.3–8.6	
New Zealand [30] Smith et al.	PH	82	92	1.2	0.6–2.6	Incident cases 1967–1980, registry cancer controls matched on year of diagnosis and age. Unmatched in analysis. Positive exposure if > 1 day and 5 years preceding diagnosis
	CP	82	92	1.5	0.5–4.7	
New York State [13] Greenwald et al. 1984	Vietnam service	281	281	0.5	0.2–1.2	Incident cases from 1962–1980, 18–29 years old; controls from motor vehicle license listing and mortality files, partial pathology review
	Agent orange	252	252	0.7	0.3–1.8	
England [3] Balarajan & Acheson 1984	Agricultural and forestry occupations	1961	1961	1.1	0.8–1.5	Incident cases > 15 years old from 1968–1976. Cancer controls. Occupation at diagnosis from registry. Matching factors not stated but matched analysis
	Farming[d]	1961	1961	1.7[c]	1.0–2.9	
Italy [33] Vineis et al. 1986	Agricultural occupations in high herbicide use area: males & females	68	158	1.8	1.0–3.2	Incident cases ≥ 20 years old from 1981–1983. Controls selected from voting list and mortality files
	Surviving women with high herbicide exposure	20	56	2.7	0.6–11.1	
Washington [35][d] Woods et al. 1987	Occupations with exposure to PH	128	694	0.8	0.5–1.2	Incident registry cases 1981–1984, 20–79 years; population control group matched on age and vital status; interview data
	Occupations with exposure to CP	128	694	1.0	0.7–1.5	
	Farmers	128	694	1.3	0.8–1.9	
	Hx chloracne	128	694	3.3	0.8–14.0	
Kansas [17] Hoar et al. 1986	Farmers	133	948	1.1	0.7–1.6	Incident cases from 1976–1982, ≥ 21 years old. Controls from general population; random digit dialing, Medicare lists and mortality files; telephone interviews; pathology review
	Herbicide use (mostly 2,4-D)	133	948	0.8	0.5–1.3	

[a] CP = chlorophenols, PH = phenoxy acid herbicides
[b] Unless otherwise noted, studies are limited to white males
[c] $P < 0.05$
[d] Could not recalculate odds ratios and confidence limits, reported as stated in manuscript

For each study, relative risk estimates and confidence intervals (based on the Poisson distribution) were calculated directly from the data where available (Table 1). Many of the risk estimates were elevated, but the confidence intervals were wide and no risk estimate was statistically significant at the 0.05 level. In all these studies, exposure was relatively well documented through personnel records. However, the number of exposed subjects in each study was small, and STS's are very uncommon, with an overall age-adjusted incidence rate of 2.4 per 100,000 [37], indicating that the studies had a low power to detect elevated risk estimates. Thus, conclusive results cannot be determined from these individual reports.

The combined analysis for the cohort studies involved the calculation of a crude proportional mortality ratio (PMR) and 95%

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confidence interval across all studies assessing the percentage of all cancer deaths due to STS. The percentage of cancer deaths in the combined cohorts due to STS was compared to the same percentage calculated for white males ≥ 20 years as derived from the combined 1973–1981 SEER registries [37]. Younger males were excluded from the comparison population as all of the studies were of employed or retired males. Many studies reported data or results with and without a latency period incorporated in the analysis; if so, the data taking latency into account were selected.

Case-control studies. There were eight case-control studies (Table 2) which varied with respect to selection of living or deceased cases, the source of cases, and the selection of controls [3, 10, 13, 16, 17, 30, 33, 35]. For the case-control studies, odds ratios and confidence limits were recalculated (with some exceptions as noted) using the normal approximation to the logarithm of the odds ratio as suggested by Woolf [34]. Most of the studies had moderately increased risk estimates (Table 2). The classification of a subject as exposed varied both by the source of data (i.e., personal interview or available documents) and the criteria for exposure (i.e., job titles versus specific contact with a substance).

Studies in which the appropriate data could be abstracted were used to calculate a summary odds ratio and 95% confidence interval. The data from one 2 × 2 contingency table per study were included. Subjects with unknown exposures were excluded from analysis. One study in which the raw data could not be abstracted was excluded [35]. For each study where different pesticides were analyzed together and separately, the data representing combined pesticide exposure were selected. If data using both occupational titles and specific chemical exposure were available, the specific chemical exposure data were used. If only occupational titles were used as surrogate measures for exposure, but the data were stratified by exposure potential (e.g., residence in a high PH use county), the category with criteria defining the highest potential exposure was selected for the summary analysis.

In some instances, cases and controls were matched in the reported analyses. Sometimes the authors noted that dissolution of the matching did not affect the results and presented unmatched analyses; for others this procedure could be accomplished from data included in the manuscripts. Dissolving the matching never resulted in a substantial change in the results. Therefore, where applicable, the matching was dissolved for the studies utilized in the summary odds ratio analyses.

The odds ratios from the case-control studies were plotted against the number of cases in each study. This plot was done to determine if the odds ratios tended to cluster around a central point, with larger samples more closely clustered around a single true population value (if such exists).

Dose-response evaluation. For an assessment of dose-response in both the cohort and case-control studies, risk estimates are presented where results were derived for presumably low or general exposure categories as well as high exposure groups within a population. Categories of higher exposure were defined by methods indicating heavy exposure (spraying or mixing the material, or exposure during an accident), clinical evidence of high exposure (a history of chloracne), a longer duration of exposure, or other measures as constructed and presented by a paper's authors. A second analysis of dose-response compared risk estimates within the time period covered by a study, presuming that exposures have declined as technology has improved.

Results

The PMR for the combined cohort studies was 3.5 (95% confidence interval 0.7–10.3) (Table 3). The PMR using only cases confirmed by pathological review was 1.2 (95% confidence interval 0.03–6.55).

Table 3. Crude proportional mortality ratio for STS based on 7 combined cohorts of white males exposed to phenoxy acids or chlorophenols compared to all white male deaths, 10 SEER registries combined, 1973–1977[a]

[Ref.]	Number in cohort	Causes of death			
		Number deaths	Cancer deaths	STS deaths	STS deaths post review
[2]	207	20	6	0	0
[28]	1926	144	26	0	0
[26]	2187	370	81	1	0
[18]	161	29	5	0	0
[38]	121	32	9	1	1[b]
[31]	74	21	7	0	0
[39]	884	163	35	1	0
Observed			169	3	1
Expected[c]				0.85	0.85
PMR				3.53	1.18
Lower limit of 95% CI				0.73	0.03
Upper limit of 95% CI				10.31	6.55

[a] STS = soft tissue sarcoma; CI = confidence interval, calculated based on Poisson distribution
[b] The specific diagnosis was malignant fibrous histiocytoma, confirmed by expert pathology review. The duration between first exposure and death was 30 years
[c] Expected numbers calculated using percentage of all cancer deaths, NCI's SEER program, 1973–1977, white male ≥ 20 years, which is 0.5% for STS [37]

Table 4. Data used for summary odds ratio analyses[a]

Exposure[c] [Ref.]	Exposed STS cases	Exposed controls	Non-exposed STS cases	Non-exposed controls	OR & 95% CI[b]
PH & CP [16]	19	19	33	187	5.7 (2.7–11.8)
PH & CP [10]	25	13	85	206	4.7 (2.3– 9.5)
PH [30]	18	17	64	75	1.2 (0.6– 2.6)
PH (Agent orange) [13]	7	10	245	242	0.7 (0.3– 1.8)
PH (mostly 2,4-D) [17]	22	192	111	756	0.8 (0.5– 1.3)
Agricultural & forestry occupations [3]	93	82	1868	1879	1.1 (0.8– 1.5)
Agricultural occupations in high PH use area [33]	29	47	39	111	1.8 (1.0– 3.2)

[a] Studies could only be included if numbers of cases and controls by exposure status could be determined from report
[b] Individual confidence intervals are calculated using the normal approximation to the logarithm of the odds ratio as suggested by Woolf [34]; OR = odds ratio, CI = confidence interval
[c] STS = soft tissue sarcoma, PH = phenoxy acid herbicides, CP = chlorophenols

The Mantel-Haenszel odds ratio combining data across all case-control studies (Table 4) was 1.4 (95% confidence limits 1.1, 1.7, $P < 0.001$). However, the Breslow-Day test for homogeneity revealed that the individual esti-

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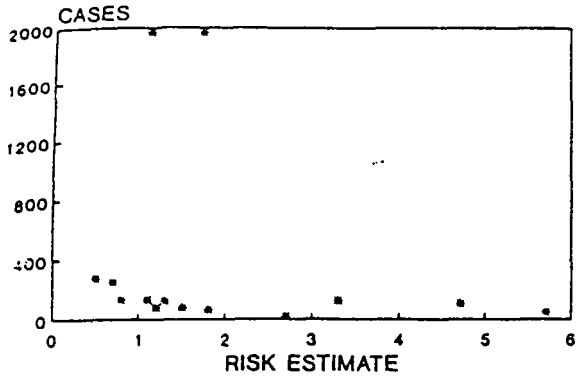


Fig. 1. Distribution of odds ratios from the case-control studies by the number of cases in each study

mates were disparate ($P < 0.001$). The Swedish studies that initially reported these associations had the highest estimates and have also received the most criticism [5–7, 14, 15]. Without the Swedish studies, the Breslow-Day test was insignificant ($P < 0.25$), and the summary odds ratio was 1.1 (95% confidence interval 0.9–1.4). No association was evident ($\chi^2_{MH} = 0.7$, $P < 0.42$).

Figure 1 shows odds ratios from the case-control studies (both statistically significant and not) compared to the number of cases in each study. There is no indication that the odds ratios from studies with more cases cluster more tightly around a single population value compared to odds ratios from smaller studies. Thus there is no implication that the studies are all samples from a single exposure population with a single true odds ratio.

Three of the seven study locations in Table 5 [24, 33, 38, 39] displayed an increased risk with increased exposure levels. In addition, the category in the Washington State study [35] implying definite and intense exposure, as indicated by a history of chloracne, had a relatively higher estimate of 3.3. In the Michigan cohort [26], the one case which occurred was in the high exposure category. The exposure categories were relatively less reliable in the remainder of the studies [13, 36].

A second approach to evaluate dose-response was based on the assumption that manufacturing processes and environments have improved over time and have been accompanied by a decrease in contaminants and lower exposure levels for workers. Figure 2 displays risk estimates against the midyear of eligibility of the cases, specifically, the midyear of the time period when a case

Table 5. Summary of study results by degree of exposure[a]

Location [Ref.]	Method	Degree of exposure[b]	STS risk estimate	95% Confidence interval
Sweden [36]	Cohort	Low	0.9	0.8–1.0
		High	0.8	0.3–1.9
Denmark [24]	Cohort	General	3.7	1.0–9.4
		High	6.3	0.2–34.8
Michigan [26]	Cohort	Low	–	–
		Moderate	–	–
		High	(1 obs/0 exp)	–
West Virginia [38, 39][c]	Cohort	General	5.7	0.1–31.8
		High (accident)	22.2	0.6–123.8
New York State [13]	Case-control	General	0.5	0.2–1.2
		High	0.7	0.3–1.8
Italy [33]	Case-control	General	1.8	1.0–3.2
		High	2.7	0.6–11.1
Washington [35]	Case-control	None (PH)	1.0	–
		Low (PH)	0.6	0.3–1.1
		Moderate (PH)	1.0	0.6–1.7
		High (PH)	0.9	0.4–1.9
		None (CP)	1.0	–
		Low (CP)	0.9	0.5–1.6
		Moderate (CP)	0.9	0.6–1.5
		High (CP)	0.9	0.5–1.8
		High (chloracne)	3.3	0.8–14.0

[a] "–" indicates that zero (0) cases were observed, so a risk estimate was not calculated. The number of subjects in the exposure groups can be determined from Tables 1 and 3 and Reference 35

[b] STS = soft tissue sarcoma, PH = phenoxyacid herbicides, CP = chlorophenols

[c] Crude risk estimates were calculated. Since expected numbers were not available in the paper, the SEER registry data [37] showing that 0.5% of the total cancer cases ascertained in the study could be expected to be soft tissue sarcomas were used to generate an expected number. In this paper, the soft tissue sarcoma case was misclassified in the "skin cancer" category

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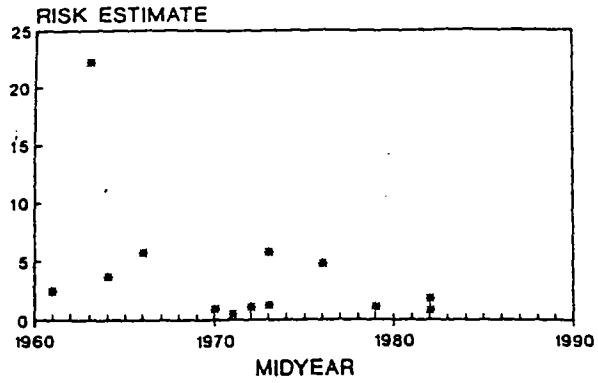


Fig. 2. Distribution of risk estimates by midyear of eligibility for case ascertainment

could be ascertained either through a diagnosis or death due to STS. All studies, including both manufacturing and application exposures, are included. Since survival for the tumors under study is relatively poor, the graph does not distinguish between cases identified at death versus diagnosis. There is no definite trend of decreasing risk estimates associated with a later case ascertainment.

Discussion

A major concern when combining the results of studies is the possibility that negative or inconclusive results remain unpublished and, therefore, unknown. The selection bias that can result may invalidate the conclusions of a collective evaluation of all published studies on a given topic. Because of the widespread use of CP and PH in agricultural and lumbering occupations and in military endeavors, such as Vietnam, studies of these agents have been conducted in a relatively politicized atmosphere. This has resulted in the publication of a number of negative or inconclusive reports. Thus, publication bias may not be a major problem here.

The biological plausibility of dioxin contaminants behaving as tumorigens has been demonstrated in several animals studies identifying this substance as a general chemical carcinogen [9, 23, 29, 32]. Recent reviews conclude, however, that the evidence for carcinogenicity of PH irrespective of their dioxin contaminant is inadequate [11, 20, 21].

In some studies in this series, it was evident that exposure periods overlapped with the time periods where cases could be ascertained. Most studies addressed this problem by imposing a latency period – disease had to occur after a designated time from first exposure. Even so, most studies used 5 or 10 years, which may be too short. The durations between first exposure at work and death for two pathologically confirmed STS cases were 32 and 30 years [7, 38]. It is impossible to recalculate estimates using different latency periods without the raw data.

It is likely that there was misclassification for exposure, particularly when occupational titles or interview data from subjects or their next of kin were the only indicators of exposure. If misclassification occurred unselectively among the exposed versus the non-exposed, risk estimates are biased toward one.

Studies in which pathological material was not reviewed probably resulted in the misclassification of some cases as STS is difficult to categorize. The pathology material from four cases occurring in three chemical worker cohorts [26, 38, 39] along with three other cases was reviewed at the Armed Forces Institute of Pathology and by another independent pathologist [12]. Two of the first four cases were found to be incorrectly classified as STS. The two expert reviewers agreed only 71% of the time as to histological subtype and in only two of seven cases did these reviewers and the original pathologist agree. In the case-control studies reporting results from pathological reviews, a substantial number of subjects originally classified with STS were reclassified to non-STS diagnoses, e.g. 6.3% [30], 9.3% [13], 19% [17] and 23.2% [33]. Thus, any expected numbers for STS generated from general population cancer registries can be assumed to include a large number of misclassified cases. It would be misleading, therefore, to compare study cohorts in which diagnoses have been corrected by pathology review to general population statistics. Therefore, the unreviewed PMR in Table 3 is the more valid PMR.

The cohort studies give inconclusive results even when combined (Table 3). The proportion of deaths is higher than expected; however, the confidence intervals include 1.0. One other case of STS (a confirmed malignant fibrous histiocytoma) occurred among one of the chemical worker cohorts [26] but was not included in the reported study or on Table 3. This subject died in 1983, and the last day of follow-up for this SMR study was December 31, 1982. Including this case yields 4 observed (prior to pathologic review) versus 0.85 expected for a PMR of 4.71 (95% confidence limits of 1.28 and 12.05). Other cancer deaths occurring in 1983 in the study cohort are not included, but based on the total number of cancer deaths between 1940–1982 ($n = 81$) adding one year's worth of deaths would have little effect on the percent of STS cases expected, and consequently the PMR.

The case-control study summary results are more informative. When the initial studies were excluded – as initial cases or clusters that stimulate a hypothesis are not counted in an individual study – the summary odds ratio for STS was 1.1 with a tight confidence interval (0.9–1.4). No conclusions indicating a high risk or no risk can be drawn from Fig. 1, which was based on the case-control studies.

Individual study results by degree of exposure presented in Table 5 and Fig. 2, in which year is a proxy for dose, are not strongly suggestive of a dose-response effect. Some risk estimates for the high exposure groups are elevated, but the confidence limits are wide and include 1.0, indicating low power and small sample sizes in these subcategories. One difficulty with the approach in Figure 2 is that the case ascertainment period for many of the studies covered a wide range of years.

The cohort results are equivocal. The case-control studies, with the exception of the earliest reports, do not support the proposed associations. The two most recent

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studies in Washington and Kansas appear to be well-conducted studies that were designed to avoid the problems encountered in earlier studies [17, 35]. The Kansas study did not find associations with herbicide use and STS. The results of the Washington study were conclusively negative, although there was an elevated but nonstatistically significant association of STS and a history of chloracne, indicating exposure to TCDD. In summary, this assessment does not provide strong evidence that exposure to PH and CP is associated with the occurrence of STS.

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Appendix

Appendix Table 1 (data for Fig. 1)

[Ref.]	Odds ratio	95% confidence interval	No. cases
[35]	1.3	0.8– 1.9	128
[35]	3.3	0.8–14.0	128
[30]	1.5	0.5– 4.7	82
[30]	1.2	0.6– 2.6	82
[13]	0.7	0.3– 1.8	252
[13]	0.5	0.2– 1.2	281
[17]	0.8	0.5– 1.3	133
[17]	1.1	0.7– 1.6	133
[33]	1.8	1.0– 3.2	68
[33]	2.7	0.6–11.1	20
[16]	5.7*	2.7–11.8	52
[10]	4.7*	2.3– 9.5	110
[3]	1.1	0.8– 1.5	1961
[3]	1.7*	1.0– 2.9	1961

* $P < 0.05$

Appendix Table 2 (data for Fig. 2)

Eligibility years [Ref.]	Risk estimate	95% confidence interval
1972–1980 [28]	–	–
1961–1979 [36]	0.9	0.8– 1.0
1947–1982 [24]	3.7	1.0– 9.4
1940–1982 [26]	2.5	0.1– 13.9
1954–1978 [18]	–	–
1949–1978 [38]**	22.2	0.6–123.8
1955–1977 [39]**	5.7	0.1– 31.8
1970–1977 [16]	5.7*	2.7– 11.8
1974–1978 [10]	4.7*	2.3– 9.5
1967–1980 [30]	1.2	0.6– 2.6
1962–1980 [13]	0.5	0.2– 1.2
1968–1976 [3]	1.1	0.8– 1.5
1981–1983 [33]	1.8	1.0– 3.2
1981–1984 [35]	0.8	0.5– 1.2
1976–1982 [17]	1.1	0.7– 1.6

* $P < 0.05$

** Crude estimates calculated based on the estimate that 0.5% of the total cases could be expected to have STS as calculated from SEER registry [10]

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Human Exposure to 2,3,7,8-TCDD and Risk of Cancer

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ABSTRACT

Most of the evidence for the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in humans has centered around whether it causes malignant lymphomas (ML) and soft-tissue sarcomas (STS). A critical review of the literature indicates that the evidence does not support a causal role for TCDD in the etiology of ML. For STS, the evidence does not specifically incriminate TCDD either, although there is room for doubt. Cancers of other sites, particularly of the respiratory system and thyroid which were found to be statistically significantly in excess in either of the two largest studies of combined cohorts of occupationally exposed workers, were identified as candidate tumors for which a possible etiological role of TCDD might need investigation in future studies.

Key Words: 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD, phenoxyacetic acids, chlorophenols, soft-tissue sarcoma, malignant lymphoma, cancer.

I. INTRODUCTION

There have been suggestions from case reports that exposure to phenoxyacetic acid herbicides, chlorophenols and their contaminant dioxins, especially 2,3,7,8-TCDD, may be associated with the development of malignant lymphomas (ML) and soft-tissue sarcomas (STS).[1–10] A large number of analytic epidemiological studies have investigated this putative association. Many studies examined the risk of ML among agricultural workers or among persons exposed to different groups of pesticides in general, but did not examine specific pesticides.[11–34] A majority of these studies have shown an association between non-Hodgkin's lymphoma (NHL) or Hodgkin's disease (HD) and agricultural practice, or groups of pesticides such as insecticides and herbicides.[11–23] Since these studies did not investigate specifically the role of phenoxy herbicides/chlorophenols/dioxins, they are not considered in this review. However, they leave open the question of which of the numerous exposures prevalent in agricultural occupational populations (including specific insecticides, herbicides, fungicides, fumigants, rodenticides, fertilizers, solvents, other chemicals, and oncogenic viruses of cattle and chickens) is responsible individually or jointly for this association. It is to be noted that phenoxy herbicides and chlorophenols are just 2 of the 600 to 1100 pesticides or active pesticide ingredients agricultural workers could be exposed to occupationally, and that 18 out of 29 pesticides evaluated by the International Agency for Research on Cancer (IARC) were judged to be animal carcinogens.[35] This review does not consider risk of cancer among ground troops in the Vietnam war since it has not been shown that this group had levels of TCDD exposure above background. However, persons in the military who sprayed herbicides in Vietnam are considered since their exposure to TCDD has been well documented.

II. CASE-CONTROL STUDIES OF MALIGNANT LYMPHOMAS

Ten distinct case-control studies have examined the risk of ML from exposure to phenoxys/chlorophenols,[36–50] Table 1. Four of the ten studies observed a statistically significant association in the study population as a whole,[37–39,46] and three more studies reported a statistically significant association only in a subset of the study population.[42,43,48] Thus, 70% of case-control studies which have investigated phenoxy acids and chlorophenols have reported some form of a statistical association between these compounds and ML.

Two of the ten studies reported a statistically significant association with NHL in general,[46] or skin NHL in particular,[43] but did not indicate whether the phenoxy acids that subjects were exposed to included 2,4,5-T. Eight studies provided information on possible TCDD exposure either specifically or by implication.[36,38,39,42,44,45,48–50] Only one out of these eight studies reported a statistically significant association with phenoxys containing TCDD.[38] Thus, at least 70% of the total of ten case-control studies did not show a statistically significant association between ML and exposure to 2,3,7,8-TCDD.

III. CASE-CONTROL STUDIES OF SOFT-TISSUE SARCOMAS

There have been 15 distinct case-control studies of STS,[39–40,48,51–68] Table 2. One study did not find an association with Agent Orange (2,4,5-T and 2,4-D in a 50:50 ratio) exposure in the military in Vietnam among Vietnam veterans, or with exposure to herbicides outside of the military.[60] Another reported a statistically significant 1.7-fold risk associated with farming, but no increased risk was observed among other agricultural workers with possible exposure to phenoxy acids/chlorophenols.[51] These two studies are not considered further since they provide no information specifically on phenoxy/chlorophenol exposure. Of the remaining 13 studies which did,

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Table 1
Case-Control Studies of ML and Exposure to Phenoxy Acids or Chlorophenols

Disease	Exposure	Odds ratio	Ref.
HL	2,4,5-T	1.8	36, 37
	PCP	1.86[a]	
AL	Phenoxys ex D & M only[e]	3.5[a]	38
	Phenoxys	4.8[a]	
	Chlorophenols	4.3[a]	
	2,4-D & MCPA	33.7[a,b]	
HL	2,4,5-T	1.3[b]	39, 40
	2,4-D	2.6[a]	
ID	Phenoxys	3.8	46
HL	Phenoxys	4.9[a]	
HL (skin)	Phenoxy acid	10.0[a]	43
HL (all)	Phenoxy acid	1.3	
	Chlorophenols	1.2	
HL	Phenoxys	0.9	48
	Chlorophenols	1.0	
	2,4-D	0.7	
	2,4,5-T	1.0	
	Phenoxys (E & L >15 years)[f]	1.7[a]	
	Farmer	1.3[a]	
	Sprayed forests with herbicides	4.8[a]	
NHL	2,4,5-T	1.6	41, 42, 47
	2,4-D	1.5	
	2,4-D[c]	1.1	
	2,4-D[b,d]	1.8[a]	
NHL	Phenoxys	1.4	44
	Chlorophenols	1.3	
	2,4,5-T (gorse)	1.4	
NHL	Phenoxys	1.0	45
	Chlorophenols	1.4	
	2,4,5-T (gorse)	0.9	
NHL	Phenoxys	0.9[b]	49, 50
	Chlorophenols	1.1[b]	
	Agent Orange	1.1	
HD	Phenoxys	0.9[b]	
	Chlorophenols	1.1[b]	
	Agent Orange	0.8	

[a] Statistically significant.
[b] Calculated from publication.
[c] Adjusted for organophosphate exposure.
[d] Adjusted for fungicide exposure.
[e] Phenoxys excluding 2,4-D and MCPA only.
[f] Exposure and latency > 15 years.

nly 4 showed a statistically significant association with exosure to these compounds in the study group as a whole.[52,55,57,68] hree more studies showed a statistically significant association n a subset of the study population.[48,53,66] Thus, a little over alf of the case-control studies investigating exposure to phenxy/chlorophenols reported a statistically significant associaon in one form or another between this exposure and STS.

ven studies provided some specific information on exosure to 2,3,7,8-TCDD or TCDD-containing phenoxys/chlo-

Table 2
Case-Control Studies of STS and Exposure to Phenoxy Acids or Chlorophenols

Exposure	Odds ratio	Ref.
Phenoxy acids	6.8[a]	52
Chlorophenols	3.3[a]	
Non-2,4,5-T Phenoxys	4.2	
2,4,5-T and other phenoxys	17.0[a]	
Phenoxys	1.3	53
Non-2,4,5-T Phenoxys	0.6	
2,4,5-T	1.8	
Phenoxys during 1950s	2.4[a]	
2,4,5-T during 1950s	2.9[a]	
PCP (high grade)	3.9[a]	
Non-TCP chlorophenols (high grade)	5.3[a]	
Phenoxys	5.3[a]	55, 56
Chlorophenols	6.6[a]	
2,4-D or MCPA	5.7[b]	
Phenoxys	3.3[a] (Pop.)	57
Phenoxys	2.2 (Cancer)	
2,3,7,8-TCDD	3.5[a] (Pop.)	
2,3,7,8-TCDD	3.1[a] (Cancer)	
Chlorophenol (low grade)	1.0[b] (Pop.)	
Chlorophenol (low grade)	1.0[b] (Cancer)	
Chlorophenol (high grade)	0.3[b] (Pop.)	
Chlorophenol (high grade)	0.5[b] (Cancer)	
Chlorophenols	1.4[a]	68
Phenoxys	0.7	
Agent Orange	0.7	
Phenoxys (live men)	0.9	66
Phenoxys (live women)	2.7	
Phenoxys (dead men)	0.2[b]	
Phenoxys (dead women)	1.1	
Phenoxys (live women, age <75 years, exp. 1950–1955)	15.5[a]	
Phenoxys	0.8	48
Chlorophenols	1.0	
High level phenoxys (with Scandinavian names)	2.8	
High and low chlorophenol (with Scandinavian names)	7.2[a]	
Lumber grader	2.7[a]	
Agent Orange, dioxin, or 2,4,5-T	0.7	54
Phenoxys	No association	39, 40, 58, 59
2,4,5-T	No association	
Phenoxys	1.4	61–63
Chlorophenols	1.3	
Phenoxys	0.7	64
Chlorinated dioxins (tetra- and higher)	No association	65
Vietnam veterans	0.8	60
Agent Orange	No association	
Herbicides	No association	
Pesticides	No association	
Insecticides	No association	
Chemical work or phenoxys/chlorophenols	1.8 (Cancer)	67

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Table 2 (continued)
Case-Control Studies of STS and Exposure to Phenoxy Acids or Chlorophenols

Exposure	Odds ratio	Ref.
Chemical work or phenoxys/ chlorophenols	1.6 (Pop.)	
Gardeners	Inf. (Cancer)	
Gardeners	4.1[a] (Pop.)	
Phenoxys/chlorophenols	4.2 (Pop.)	
Gardeners, groundsmen	0.7	51
Foresters, woodmen	1.0	
Acricultural workers	1.0	
Agricultural & forestry	1.2	
Farmers, farm managers, market gardeners	1.7[a]	

Note: Pop. = population controls; Cancer = cancer controls.

[a] Statistically significant.
[b] Calculated from publication.

rophenols, or stated that exposure to phenoxys may include 2,4,5-T.[39,48,52–55,57,61–66] Of these 11 studies, only 3 observed a statistically significant association with these compounds in the study group as a whole.[52,55,57] A further two studies showed such an association in a subset of the study population.[53,66] Thus, less than half of the case-control studies investigating TCDD exposure have reported a statistically significant association of any sort between STS and TCDD exposure.

IV. COHORT STUDIES OF WORKERS IN MANUFACTURING/FORMULATING PLANTS

Eight independent cohorts of persons with possible exposure to TCDD during the manufacture/formulation of 2,4,5-T or 2,4,5-TCP have been published to date,[69–87] Table 3. Five were small and obviously of inadequate size to evaluate risk of STS or ML.[69–73,83,84,86] Three of these did not record a single case of STS or ML;[69–73] one of them recorded one case each of STS and HD;[83] and one observed a nonstatistically significant twofold risk of NHL, but recorded no case of STS or HD.[86] Of the remaining three larger studies,[79,80,85,87] none reported a statistically significant excess of STS or ML in the study group as a whole, although one study[79,80] reported a statistically significant excess of STS in the subset of workers with chloracne based on two cases, one of which on histological review was found not to be a case of STS. Another study[87] reported a statistically significant excess of STS (based on four cases) in workers in the shipping department of the plant where exposure to several other chemicals occurred, as well as phenoxy acids, but not among workers involved in phenoxy production. Furthermore, MCPA was the major phenoxy acid produced in this plant, although limited amount of exposure to 2,4,5-T and 2,4,5-trichlorophenol also occurred. In this same cohort, a statistically significant risk of ML was observed not among workers engaged in the production of phenoxy acids, but rather in workers manufacturing other chemicals.

Isolated reports of a statistically significant risk of cancer of the bladder,[84] hemopoietic/lymphatic systems,[78] prostate,[80] rectum, uterine cervix, lung[87] have been made on one occasion only, and these reports have not been confirmed by the other studies. Two studies reported a significant excess of stomach cancer;[71,80] however, in one of them,[80] none of the stomach cancers occurred among the subset of workers with chloracne, although 0.4 were expected.

V. COHORT STUDIES OF WORKERS SPRAYING/APPLYING PHENOXY HERBICIDES

Cancer mortality (Table 4) and incidence (Table 5) have been studied in 15 distinct cohorts of sprayers/applicators.[85,88–107] None of these studies reported a statistically significant increased risk of STS, and only one reported a statistically significant risk of 1.4 for ML.[105] Of the 15 cohorts, only 10 specifically reported possible exposure to 2,4,5-T with potential exposure to TCDD.[88–102] Eight of these were too small to evaluate risk of STS or ML adequately, and none reported a case of either tumor.[88–94,96,97,102] Of the remaining two larger cohorts,[95,101] none reported a statistically significant risk of STS or ML.

There were also isolated reports of statistically significant risks of cancer of the testicles,[101,102] skin,[93,102] and lung[94] among workers in cohorts with the potential for exposure to TCDD. In cohorts not specifically identified as having a potential for TCDD exposure, isolated cancers reported to be significantly in excess include cancer of the nose,[85] bladder,[103] skin,[105] and lung.[104,106,107]

VI. POOLED COHORT STUDIES IN REGISTRIES

The results of two registries from the International Agency for Research on Cancer (IARC) and the U.S. National Institute of Environmental Health Sciences (NIEHS) and the National Institute of Occupational Safety and Health (NIOSH) U.S.A., which have pooled together a large number of occupational cohorts including many of the published ones, have recently been reported,[108,109] Table 3. The NIOSH registry[109,110] studied workers with possible exposure to TCDD during the manufacture/formulation of 2,4,5-T, 2,4,5-TCP, and hexachlorophene, whereas the IARC/NIEHS registry[108] includes workers exposed to all types of phenoxy acids and chlorophenols both during their manufacture and use, and thus consisted of both TCDD-exposed and -unexposed subjects.

A statistically significant excess of NHL or HD was not

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Table 3
Cohort Studies of Workers in Manufacturing/Formulating Plants — Observed Numbers of Deaths/Cases, and Relative Risks (RR)

Study	Compound	Total cancer	RR	STS cases	RR	NHL cases	RR	HD cases	RR	Ref.
	2,4,5-T	8	1.2	0		0		0		69
	2,4,5-T	2	—	0		0		0		70
	2,4,5-TCP	7	1.7	0		0		0		71
		17	1.7	0		0		0		72
Zober	Update of									73
Cohort 1	References	9	1.3	0		0		0		
Cohort 2	71 and 72	11	1.7	0		0		0		
Cohort 3		3	0.5	0		0		0		
Chloracne, etc.		16	1.4	0		0		0		
	2,4,5-T 2,4-D MCPA	1	—	0		0		0		74
	TCDD	3	1.9	1		0		0		75
	2,4,5-T 2,4,5-TCP	61	1.0	1		5	2.4	1		76
	Update of Reference 76	81	1.0	1		5	1.9	1		77
	2,4-D 2,4,5-T MCPA	26	1.2	0		2	1.8	1		78
	Update of References 76 and 77	95	1.0	2	5.0	6	2.1	1		80
	2,4,5-T 2,4-D Chlorophenols (chloracne)	6	0.7	1		0		0		81
	Update of Reference 81	5	0.6	2[a]	20.0	0		0		79
	2,4,5-TCP	9	1.0	1		0		1		83
	2,4,5-T 2,4,5-TCP	9	0.8	1		0		0		84
	MCPA, 2,4,5-T	297	1.0	1	1.1	2	0.4	1		85
	2,4,5-T 2,4-D/B/P MCPA/B/P, 2,4,6-TCP	37	1.0	0		2	2.3	0		86
Lynge										
Males	MCPA, 2,4-D, 2,4,5-T	159	1.0	5 4	2.7 3.7[a]	7[b]	1.3	[b]		87
Females		49	0.9	0		1[b]		[b]		
NIOSH Combined Study										
Fingerhut	2,4,5-T 2,4,5-TCP, PCP, hexachlorophene	265	1.2	4	3.4	10	1.4	3	1.2	109,110
IARC/NIEHS Combined Study										
Kogevinas	2,4,5-T/TP, 2,4,-D/B/P, MCPA/B/P, PCP, 2,4,5-TCP, 2,4,6-TCP, 2,3,4,6-TCP	505	>1	4	2.1	11	?	<1		108

[a] Statistically significant.
[b] Non-Hodgkins lymphoma and Hodgkin's disease combined.

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Table 4
Cohort Mortality Studies of Users (Sprayers and Applicators) — Observed Numbers of Deaths and Relative Risks

Compound	Total cancer cases	RR	STS	RR	NHL	RR	HD	Ref.
2,4-D	26	0.7	0		0		0	88
2,4,5-T								
2,4-D, 2,4,5-T	5	0.8	0		0		0	89
2,4-D/P, 2,4,5-T/P	18	1.1	0		0		0	90
2,4,-D, 2,4,5-T	2	0.7	0		0		0	91
Update	6	1.1	0		0		1	92
2,4-D, 2,4,5-T	13	0.9	0		0		0	93
2,4,-D, 2,4,5-T	84	1.1	—		—		—	94
—	47	0.8	—		—		—	103
—	232	1.1	—		—		—	104
MCPA, 2,4-D	297	1.0	1		—		—	85
2,4-D, 2,4,5-T	169	—	1	1.4	3*	0.9	*	95
2,4-D, 2,4,5-T	12	0.8	1		0		0	96
2,4-D, 2,4,5-T	6	0.9	0		0		0	97

* Non-Hodgkin's lymphoma and Hodgkin's disease combined.

observed in either of these two studies. In fact, in the NIOSH study, the risk of NHL in workers exposed to TCDD for 1 or more years was 0.93, while in the IARC/NIEHS registry, the risk for exposure to phenoxys/chlorophenols was 0.97. Both registries reported a nonstatistically significant two- to threefold risk of STS among persons with the potential for exposure to TCDD, based on *four* cases each.

In the NIOSH registry, STS risk was statistically significant in workers exposed to TCDD for 1 or more years when a latency period of 20 or more years was allowed for. (These workers had an estimated mean serum TCDD level at the time of last exposure of 3600 ppt.) However, the workers had spent much longer periods of employment exposed to chemicals other than 2,4,5-T, 2,4,5-trichlorophenol, or TCDD. Moreover, only 2 of 12 plants reported the total of 4 cases of STS (2 in each plant). Two of the four cases (one from each plant) were subsequently found not to be STS on histology review. Moreover, in one of the plants, there was evidence that the risk given for STS may have been overestimated for the following reasons: (1) a proportion of the person-years of observation for persons *known* not to have died from STS was excluded from the analysis; (2) 139 workers with chloracne, and thus possible high TCDD exposure representing at least 24% of the cohort, were not included in the analysis, and it was *known* that no death from STS as the underlying cause of death had occurred among them; (3) 45% of the cohort who were alive were excluded from the dose-response analysis. Unfortunately, it is not known by how much the STS risk given in the paper was therefore overestimated because of these problems. It is estimated though that substantial risk would still remain. No cases of STS occurred among workers exposed to TCDD for less than 1 year, even though for those with a 20-year latency it was estimated that their mean serum level of TCDD at the time of last exposure was possibly around 640 ppt. However, interpretation is limited because of insufficient power in this subgroup. Therefore, although the NIOSH study was a well-designed and carefully executed study, and of significant importance since it involved the largest number of workers with the highest TCDD exposure ever studied, the results with regard to STS are tentative because of the paucity of histologically confirmed STS (total = 2), insufficient statistical power, and problems mentioned.

In the IARC/NIEHS registry, there was no difference in risk of STS among phenoxy/chlorophenol-exposed persons with a potential for TCDD exposure and those without (twofold risk), and the increased risk was seen in the sprayer cohorts but not

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Table 5
Cohort Cancer Incidence Studies of Users (Sprayers, Applicators) — Observed Numbers of Cases and Relative Risks

Compound	Total cancer cases	RR	STS	RR	NHL	RR	HD	Ref.
2,4-D, 2,4,5-T	26	0.7	0		0		0	98
2,4-D, MCPA	30	—	0		0		0	106
2,4-D, 2,4,5-T	8	0.8	0		0		0	89
2,4-D, 2,4,5-T	8	1.6	—		—		—	91
2,4-D, 2,4,5-T	35	—	0		1		0	93
2,4-D, MCPA	169	—	1		2		2	107
2,4-D, 2,4,5-T	558	0.9	6	0.9	21	1.0	11	99–101
—	631	0.7	—		45[a]	1.4[b]		105
2,4-D, 2,4,5-T	21	1.2	1		1		0	102
2,4-D, 2,4,5-T	10	—	0		0		2	97

[a] Non-Hodgkin's lymphoma and Hodgkin's disease combined.
[b] Statistically significant.

n the production cohorts in which TCDD exposure would be xpected to be highest. The observation of similar increased isk of STS in both TCDD-exposed and TCDD-unexposed ersons raises the possibility that the increased risk of STS een in the NIOSH study may be due not to TCDD but to other oncomitant exposure(s). Thus, caution should be exercised in nterpreting these findings at this time.

Thyroid cancer mortality was statistically significantly increased for workers exposed to phenoxys/chlorophenols in the ARC/NIEHS registry with four observed deaths, and a four-old increased mortality, both for sprayers and production vorkers. Risk was highest in workers with probable TCDD xposure. Unfortunately, the NIOSH study did not provide nformation on this site.

A statistically significant increased risk of all cancers combined was reported in the NIOSH registry, and the risk increased with duration of exposure and latency. Also, a statistically significant excess of respiratory cancer was observed at igher exposures. Slightly elevated risks of all cancers, and ancer of the lung were also reported in the IARC/NIEHS egistry, however, they were not statistically significant, and o dose-response relationship was evident.

A mortality study of the Seveso population in Italy exposed y environmental contamination as a result of an industrial cc·'·ent has been published.[111] Unfortunately, follow-up was for the first 10 years since the incident, hence a sufficient latency period has not been accrued. Cancer of the corpus uteri was statistically significantly increased in women residing in the area where dioxin contamination was lowest. SMRs for certain cancers were highly elevated; these were soft-tissue sarcoma, HD, gallbladder and biliary duct, peritoneum, melanoma, pleura, other endocrine and thyroid, but they were based on one to three deaths only, and there were no consistent patterns that might help relate their occurrence specifically to TCDD at this time. It is pertinent to note that this cohort is not plagued as much as occupational cohorts by the issue of other concomitant exposures and thus might be of importance in the future in helping toward determining whether any observed risk of cancer is due to TCDD or other exposures.

VII. ASSESSMENT OF EVIDENCE

A. Prelude

Any evaluation of the evidence for a causal association between 2,3,7,8-TCDD and cancer in humans must take into account the following:

1. Occupational exposure to TCDD occurs either during the manufacture of phenoxy acids, chlorophenols, and hexachlorophene, or during the use of these compounds

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(spraying or application). Occupational exposure to TCDD is on average considerably much higher in manufacturing workers than in sprayers and applicators, e.g., in the NIOSH study, the mean serum TCDD level in a sample of manufacturing workers at the time exposure ceased was estimated to be 2000 ppt with a range of up to 32,000 ppt;[110] the corresponding highest serum TCDD level ever recorded in a sprayer was <1600 ppt.[112]

2. In virtually all the case-control studies, for practical purposes, risks given were for exposure during the *use* of phenoxy acids and chlorophenols, rather than during the manufacture of these substances. Hence, risks reported in case-control studies and cohort studies of sprayers/applicators should be expected to be lower than those given for exposure in manufacturing plants.
3. Workers exposed to phenoxy acids/chlorophenols are concomitantly exposed to a wide variety of different pesticides. For example, in the NIOSH study, it was stated that TCDD-exposed workers were also potentially exposed to some of the thousands of chemicals handled in the factories concerned, and in fact had spent five times as long exposed to these other chemicals than to TCDD. Similarly, every single individual who has sprayed phenoxy acids has also sprayed other chemicals. Moreover, some of these other chemicals are well-known causes of STS or ML in humans or animals. For example, the insecticide ethylene dibromide, which is used as a fumigant for various grains (corn, wheat, rice, etc.), causes hemangiosarcomas in animals;[113] similarly, arsenicals widely used as insecticides, herbicides, and wood preservatives cause angiosarcoma of the liver in humans and possibly lymphomas in humans and animals;[113] vinyl chloride, which causes angiosarcoma of the liver and other sites and lymphomas in humans and animals, has been used as a pesticide aerosol propellant.[113,114] Manufacturing plants producing phenoxys and chlorophenols not only produce arsenicals and ethylene dibromide, but also make p-aminobiphenyl, a known cause of angiosarcomas in animals.[84,113]

This means that an excess occurrence of STS or ML in sprayers or workers in pesticide manufacturing plants will not be unexpected. The issue would be to identify which of these chemicals is (are) responsible. In case-control studies in particular, the demonstration of an excess risk in sprayers or agricultural workers who had used phenoxys/chlorophenols is by itself not enough to conclude that these compounds are the cause of the excess. To determine this, further analyses must be done which attempt to obtain a risk estimate for exposure to phenoxys/chlorophenols/dioxins while controlling for other candidate chemicals shown to be, or likely to be, associated with the excess. *It is a fact that every single case-control study of ML in civilian populations included in this review has reported a statistically significant association with one or more concomitant exposures;* these include DDT (RR = 1.5), chloramben (RR = 2.2);[36] methyl bromide (RR = 2.8), insecticides (RR = 1.9);[37] asbestos (RR = 2.0), organic solvents (RR = 2.4);[38] fungicides (RR = 2.1), triazines (RR = 2.5), insecticides (RR = 2.8), amides (RR = 2.9), trifluralin (RR = 12.5);[39] organophosphates (RR = 1.9), diazinon (RR = 2.0), chlordane (RR = 2.1), malathion (RR = 2.2), dyfonate (RR = 2.4);[42] solvents (RR = 1.9), creosote (RR = 9.4 and 10.7);[46] solvents (RR = 1.4 and 3.4);[43] solvents (RR = 1.4), lead arsenate (RR = 1.6), DDT (RR = 1.8);[48] animal oncogenic viruses — meat works (RR = 1.8).[44,45] It is noted that this list is made up predominantly of pesticides. In this situation, it is reasonable to say that an observed excess risk cannot be attributed to exposure to phenoxys/chlorophenols/dioxins unless reasonable attempts have been made to control for these other exposures. It is pertinent to note that investigators have not explored the role of these other exposures to any significant degree in case-control studies of STS as was done for ML, and in the few instances this was attempted, there was a suggestion also that the pattern was the same, although mostly, statistically significance was not achieved because of the smaller sample size of STS studies.[40,48,52,53,55,57,63,65,67]

B. Malignant Lymphoma

At least 70% of all case-control studies of ML and phenoxyacetic acid/chlorophenol exposure do not show a statistically significant association for exposure to 2,3,7,8-TCDD. Only one case-control study of ML, conducted in Sweden, reported a statistically significant association with exposure to TCDD-containing phenoxy acid.[38] Paradoxically, in that study, although based on small numbers, the crude odds ratio for exposure to 2,4-D and MCPA only was calculated to be 33.7 (p <0.0001) as compared with 4.8 for exposure to all phenoxy acids combined including the TCDD-containing 2,4,5-T. In fact when subjects exposed to 2,4-D and MCPA *only*, and chlorophenols were excluded, the odds ratio for mixed exposure to phenoxy acids (TCDD and non-TCDD phenoxys) was only 3.5 (p <0.0001). This paradox was not limited to this study only — in the study by Hoar et al.,[39] the odds ratio for 2,4-D exposure was 2.6 (p <0.05) and only a nonsignificant 1.3 for 2,4,5-T; in another study by Hoar Zahm et al.,[42] the odds ratio for 2,4-D, controlling for fungicide exposure, was 1.8 (p <0.5) and for 2,4,5-T, a nonsignificant 1.6; in the studies by Cantor et al.[36] and Everett et al.,[37] the odds ratio for pentachlorophenol (PCP) exposure was 1.9 (p <0.05) and for 2,4,5-T, a nonsignificant 1.8. In the remaining four studies which provided information for 2,4,5-T and non-2,4,5-T phenoxys, neither was associated with an increased risk of ML.[44,45,48-50] These data therefore do not support a causal role for 2,3,7,8-TCDD in the etiology of ML.

For these data to be consistent with a causal role for TCDD,

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one would have to entertain the possibility that dioxins/furans other than 2,3,7,8-TCDD, which occur in both 2,4,5-T and non-2,4,5-T phenoxys do so at toxic equivalent concentrations greatly in excess of the concentration at which TCDD exposure occurs in 2,4,5-T. However, a study of dioxin and furan concentrations in older preparations of 2,4-D and 2,4,5-T in Sweden at a detection limit of 0.01 to 0.05 ppm does not support this possibility since the highest concentration of any isomer was that of TCDD (1.1 ppm) which was found only in 2,4,5-T preparations. No other dioxin isomers were detected, and only tetra- and penta-dibenzofurans were detected at a concentration of 0.1 ppm or less.[115] Similar results were obtained from analyses of European samples of 2,4,5-T.[116]

It is important to note that the single investigator reporting a statistically significant excess of ML due to exposure to TCDD-containing phenoxys also tested fat samples from phenoxy-exposed patients with ML for the presence of TCDD. Rather unexpectedly, no elevated levels of TCDD were found in the patients.[117] This again suggests that the excess risk of ML in that study was not due to TCDD exposure.

None of the 18 cohort studies of workers potentially exposed to TCDD have reported a statistically significant excess of ML. This does not seem to be due to inadequate sample size of many of these studies since the two largest studies of workers in manufacturing plants where TCDD exposure is *highest* (NIOSH and IARC/NIEHS), which have combined many such reports together, did not find an increased risk of ML. Moreover, in the only cohort study of workers in manufacturing/formulating plants in which it was possible to separate out to a large extent workers engaged in phenoxy acid production from workers engaged in the production of other chemicals (pigments), a statistically significant excess of ML observed in the factory as a whole was found to be confined to workers engaged in making pigments, and not among workers handling phenoxys.[87] It is concluded therefore that the overwhelming evidence does not support a causal association between ML and exposure to 2,3,7,8-TCDD. This conclusion is consistent with the animal data. No association between TCDD exposure and ML in male animals has been reported, except for the development of thymic lymphomas in mice given high toxic doses of TCDD during infancy.[118]

C. Soft-Tissue Sarcoma

Only five case-control studies have reported a statistically significant association between STS and phenoxys/chlorophenols containing TCDD.[52,53,55,57,66] Four of these studies were conducted by the same investigators in Sweden,[52,53,55,57] and the fifth was done in Italy.[66] The Italian study did not find an association between STS and phenoxy acid exposure in the study as a whole, neither in male STS cases whether living or dead, nor in female dead cases. A nonstatistically significant twofold risk for phenoxy exposure was observed in living female STS cases, based on only five exposed cases, and a statistically significant relative risk of 15.5 was reported for living women less than 75 years of age exposed to phenoxys between 1950 and 1955. However, there were no official records documenting whether or not 2,4,5-T was used during 1950 to 1955. Also, two of the five cases were chondrosarcomas of the lower limb and thorax, hence not true STS, and in the third case, the histology was uncertain.

The Swedish studies were unique in that they were not only conducted by the same investigators, but also were done in the same country using the same questionnaire instrument and study design. Thus, a high degree of replicability in findings is expected. Unfortunately, this has not been the case (Table 6). The inconsistency evidently makes interpretation of the Swedish studies difficult, especially in reference to TCDD-containing phenoxys and chlorophenols. Furthermore, there is evidence of bias in these studies. Although the main tool in these studies was a mailed questionnaire, all respondents who had reported work in agriculture and forestry or whose response to questions on exposure to phenoxys/chlorophenols was unclear were contacted again and reinterviewed by telephone on exposure to these compounds. In both the first two studies,[52,55]

Table 6
Lack of Replicability of Swedish Soft-Tissue Sarcoma Studies

Compound	First study	Second study
Eriksson		
2,4,5-T & other phenoxys	17.0[a]	1.8
Phenoxys with no TCDD	4.2[a]	0.6
Chlorophenols	3.3[a]	—
Chlorophenols with no TCDD	—	5.3[a]
Phenoxy acids exposure in 1950s	—	2.4[a]
2,4,5-T exposure in 1950s	—	2.9[a]
Phenoxy acids exposure in 1960s	—	0.7
Phenoxy acids (highest exposed group)	—	2.1
Hardell		
Chlorophenols	6.6[a]	0.7 (Pop.) 0.8 (Cancer)
Chlorophenols (high grade)	—	0.3 (Pop.) 0.5 (Cancer)
Phenoxys	5.3[a]	3.3[a] (all phenoxys) 3.1[a], 3.5[a] (TCDD Ph)

Note: Pop. = population controls; Cancer = cancer controls; Ph = phenoxys.

[a] Statistically significant.

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there was evidence of interviewer bias,[56] in spite of the fact that interviewers were blind to the status of cases and controls (Table 7). In the first study by Eriksson et al.,[52] the relative risk of STS for exposure to phenoxy acids using information from both questionnaire and telephone interview was 6.8 as compared with 4.1 using the questionnaire information only; similarly, in the first study by Hardell and Sandstrom,[55] the relative risks were 5.3 and 2.6, respectively (a twofold difference). The telephone interviews resulted in exposed controls being disproportionately reclassified as unexposed. Similarly, there was evidence of possible recall bias in these studies as shown in the second study by Hardell and Eriksson[57] — a statistically significant risk of 4.1 was obtained for exposure to phenoxy acids using population controls, and a borderline statistically significant risk of 2.4 was obtained using cancer controls (Table 7).

Table 7
Swedish Studies

Interviewer Bias
(Phenoxy Acids)

Odds ratios		
Questionnaire	Interview & questionnaire	Ref.
2.6[a]	5.3[a]	55
4.1[a]	6.8[a]	52

[a] Statistically significant.

Recall Bias

Odds ratios	
Using population controls	Using cancer controls
4.1[a]	2.4

[a] Statistically significant.

Apart from these difficulties, these studies do not provide evidence to incriminate TCDD specifically because the initial finding of a 17-fold risk associated with TCDD exposure[52] was not subsequently confirmed. As seen in Table 6 for both repeat studies, risks for exposure to phenoxys/chlorophenols which do not contain TCDD were either greater than or equal to those for exposure to TCDD-containing compounds. A similar paradox is that the risk for STS obtained in the Swedish studies for exposure to phenoxys/chlorophenols during occupational *use* is much higher than those reported in the NIOSH study which included workers in manufacturing plants with much higher TCDD exposure. Furthermore, whereas in the Swedish studies the potential exposure to TCDD was brief, e.g., in one of these studies,[55] of the 11 cases exposed to 2,4,5-T, only 2 were exposed for more than a year (all 10 controls were exposed for less than a year), the NIOSH study has not shown as yet any increased risk of STS in workers who were exposed to TCDD for less than a year and who had mean TCDD levels much higher than those reported among Swedish STS cases. As with ML, it is pertinent to note that adipose tissue collected by Hardell from STS patients who had sprayed phenoxy herbicides did not show increased levels of TCDD.[117] Also, the absence of dioxin isomers other than 2,3,7,8-TCDD in the different phenoxy herbicide preparations at concentrations significant enough to explain the occurrence of excess STS[115,116] does not support a causal role for dioxins in general in the etiology of STS, as claimed by Swedish investigators.[53] This is consistent with the fact that a majority of case-control studies of STS have not shown an association with TCDD. It is to be noted also that TCDD is not known to cause STS in male animals. However, since the two largest cohort studies (NIOSH and IARC/NIEHS) reported an excess of STS, in spite of the paucity of confirmed cases of STS (only two) in the NIOSH study, and in spite of the absence of risk in manufacturing workers in the IARC/NIEHS study, a remotely possible role of TCDD in the etiology of STS cannot be completely ruled out in the future. The following is now necessary: (1) further follow-up of the NIOSH, IARC/NIEHS, and Seveso cohorts, and accrual of more STS cases to determine if there is a real excess risk in TCDD-exposed cohorts; and (2) nested case-control studies within the NIOSH and IARC/NIEHS cohorts should be conducted to investigate specifically whether any excess risk observed is due to TCDD exposure or other concomitant chemical exposure(s). This requirement is obligatory considering that (1) no case-control study has adequately controlled for concomitant exposures; (2) in the NIOSH study, workers spent five times as long exposed to other chemicals than to TCDD; and (3) in the IARC/NIEHS study, the non-statistically significant twofold risk of STS was the same in both TCDD-exposed and unexposed subjects. The identification of plants within the two registeries in which workers were engaged in the production of phenoxys/chlorophenols almost exclusively will also be a great asset to the study of possible carcinogenic effects of TCDD, phenoxys, and chlorophenols.

D. Other Cancer Sites

As is evident from the review, there has been no consistent demonstration of excess risk of cancers of other sites either in the cohort studies. A significant excess of cancer of the lung, stomach, skin, bladder, and testicles only has been reported in more than one individual cohort study. However, such reports have been fragmentary and not corroborated in the majority of studies. Further investigations of these cancer sites are needed. The demonstration of a statistically significant excess risk of respiratory cancer in the NIOSH study and of thyroid cancer in the IARC/NIEHS study in TCDD-exposed workers makes these sites of particular interest for a possible causal role of TCDD in their etiology since TCDD exposure at very high doses has been shown to cause lung cancer in male and female animals,[119,120] while the thyroid is the site

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n to be most sensitive to the carcinogenic action of TCDD imals.[119] These sites therefore warrant further detailed vestigations in the future.

III. CONCLUSION

It is concluded that whereas the evidence from this review oes not support a causal role for TCDD specifically in the ccurrence of ML and possibly also STS, TCDD-exposed occupational male populations have shown an excess of certain ncers, particularly lung and thyroid cancers, which are known be caused by TCDD in male animals. Therefore, it is possible at TCDD may be carcinogenic in humans. However, the idence indicates that previous epidemiologic studies may ve focused unduly on sites which may not be the targets, or e most sensitive targets, for its carcinogenic action (ML, and ossibly STS). There is a need therefore for serious consideration to be given in the future to evaluating the role of TCDD the etiology of cancers other than ML and STS.

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REGULATORY TOXICOLOGY AND PHARMACOLOGY 13, 150–169 (1991)

Use of Epidemiology Data to Assess the Cancer Risk of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

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Most countries have assessed the human cancer risk of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) by extrapolating from animal data. The 1600-fold variation for acceptable intakes estimated by the Americans, Canadians, and Europeans, however, indicates a large scientific uncertainty about TCDD toxicity. Regulators are attempting to use human epidemiology data to eliminate some of the limitations inherent in using animal data to quantitatively assess cancer risk in humans, particularly now that techniques for measuring TCDD levels in adipose tissue and serum are available. This paper presents an overview of the major epidemiologic studies that associate cancer with TCDD exposure. The actual exposures experienced by the study populations are emphasized. The TCDD serum levels of the Seveso, Italy, residents and of the NIOSH Dioxin Registry participants indicate that these populations hold the best promise for future assessment of the human carcinogenic risk of dioxin. Based on the animal data, however, the calculated risk from these exposures is still moderate compared with high background cancer incidence present in most industrialized countries. © 1991 Academic Press, Inc.

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a by-product of the manufacture of polychlorinated phenols, particularly the herbicide 2,4,5-trichlorophenol (2,4,5-TCP) and the germicide hexachlorophene (Ayres *et al.*, 1985). TCDD seems to be the most toxic of the dioxin isomers that were evaluated in animal toxicology studies (IARC, 1978). Human evidence of adverse health effects associated with TCDD is derived from occupational, environmental, and military exposure. Routine occupational use of compounds contaminated with TCDD and industrial accidents caused the heaviest exposure.

Most countries have extrapolated the human risk of TCDD from animal data. These values are shown in Table 1. U.S. regulatory agencies have estimated risk-specific doses based on linear-at-low-dose extrapolation from the Kociba bioassay data (Kociba *et al.*, 1978, 1979). These doses represent the level in pg/kg body wt/day that may result in at most a lifetime risk of 10^{-6} for cancer. European and Canadian regulators generally do not believe that estimation of dioxin risk by the traditional

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TABLE 1

RISK-SPECIFIC (10^{-6}) DOSES AND ACCEPTABLE DOSES OF TCDD PUBLISHED BY VARIOUS COUNTRIES AND U.S. AGENCIES

Country	Dose (pg/kg/day)	End point	Model for RA[a]
U.S. EPA	0.006	Cancer	LMS[b]
U.S. CDC	0.03	Cancer	LMS
U.S. FDA	0.06	Cancer	LMS
Federal Republic of Germany	1.0	Cancer + reproductive	SF[c] (1000)
The Netherlands	4.0	Cancer + reproductive	SF (250)
Canada (Health and Welfare)	10.0	Cancer + reproductive	SF (100)

[a] RA, Risk assessment.
[b] LMS, Linearized multistage model.
[c] SF, Safety factor.

linear-at-low-dose extrapolation method is appropriate because of the evidence that TCDD is not mutagenic. Canada, Germany, the Netherlands, and Switzerland base a level of 1–10 pg/kg/day as a maximum daily intake on the application of a 100–1000 safety factor to the "no observed effect level" identified in the animal bioassay (Kociba *et al.*, 1978, 1979). No specific risk is calculated when this safety factor approach is used. As shown in Table 1, these estimates of acceptable intakes, which vary over 1600-fold, indicate the large scientific uncertainty surrounding TCDD potency estimates.

Using animal bioassay data to quantitatively assess cancer risk in humans has been criticized because of the inherent uncertainties in extrapolating from laboratory animals to humans. Predictions are frequently made even though the mechanism of carcinogenesis is unknown, pharmacokinetic data are unavailable, the metabolic processes vary widely among species and between animals and humans, or extremely large doses are required to produce cancer in chronic bioassays.

To eliminate some of these uncertainties, investigators are attempting to use human epidemiology data to quantitatively assess cancer risk in humans. Use of epidemiology data has many advantages because humans are evaluated and relevant exposure levels and routes of exposure can be analyzed. The majority of the available epidemiologic studies on the association of cancer to TCDD exposure provide little evidence that dioxin is a potent carcinogen in humans. However, the converse is also true—currently available evidence is inadequate to rule out TCDD as a human carcinogen. The epidemiologic studies are limited by small numbers of exposed persons and hence low statistical power for identifying an effect, insufficient follow-up considering the latency required for a chronic disease such as cancer, and imprecise exposure assessments.

The conclusions that can be drawn from the available human studies of TCDD exposure are particularly limited because of the uncertain accuracy of the exposure assessment. Techniques for measuring TCDD levels in either adipose tissue or serum are now available. Unfortunately, fat biopsies are invasive, and approximately 10 g of adipose tissue is needed to determine 1–10 parts per trillion (ppt) of TCDD (Stanley *et al.*, 1985). A significant breakthrough occurred when Patterson *et al.* (1988) found a high correlation between adipose tissue and serum levels of TCDD when expressed

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on a lipid-weight basis. The mean of the partitioning ratio of adipose tissue to serum TCDD was 1.09 with a 95% confidence interval (CI) of 0.97–1.21. The ability to measure TCDD in serum years after exposure provides an opportunity to link observed health effects in human populations exposed to TCDD with actual measurements of exposure.

The following is an overview of the major epidemiologic studies associating cancer with TCDD exposure. The actual exposures experienced by the study populations and the availability of data on TCDD levels in adipose tissue or serum are emphasized. The information that the epidemiologic studies provide about the human carcinogenic risk from dioxin is assessed from available exposure data.

MAJOR EPIDEMIOLOGIC STUDIES OF THE ASSOCIATION OF CANCER WITH TCDD EXPOSURE

BASF, Ludwigshafen, Federal Republic of Germany—1953

On November 17, 1953, an accidental reaction occurred during the hydrolysis of 1,2,4,5-tetrachlorobenzol to 2,4,5-trichlorphenoxyacetic acid (2,4,5-T) in the BASF Ludwigshafen plant in the Federal Republic of Germany (FRG), and TCDD was formed in this reaction. In 1982, Thiess *et al.* published a mortality study on the 70 persons who were exposed in 1953 and on the 4 additional persons who were exposed for short periods during cleaning and testing procedures in 1954 and 1955. Of the 74 persons, 66 suffered chloracne. All 74 persons were traced and the vital status of each was assessed through June 30, 1980, and their mortality experience was compared with both internal and external control groups. External comparison groups consisted of the total population of the local town, the local district, and the FRG. Internal comparison groups consisted of two control groups matched by age and date of first employment and gathered from cohorts in other studies for which follow-up had been completed.

Among those exposed to dioxin, 21 persons had died compared with 18 or 19 predicted by each external control group. Seven cancer deaths occurred among the exposed group compared with 4.1 expected from the external controls. The only significant site-specific finding was 3 stomach cancer deaths compared with 0.024–0.034 expected. When compared with the 2 matched internal control groups, excesses of stomach and lung cancer were found in the dioxin-exposed group (3 stomach vs 0 or 1 and 3 lung vs 0 or 1).

Recently, Zober *et al.* (1990) published the results of a mortality study of BASF employees who had been exposed to TCDD during or after the 1953 accident and followed through 1987. Approximately 250 employees were divided into three cohorts based on exposure status, and their mortality experience was compared with FRG rates. The composition of cohort C1 in this study was comparable to that of the cohort studied by Thiess *et al.* (1982); i.e., 66 individuals were identical. Three deaths were from stomach cancer compared with one expected in the C1 cohort (not significant). For those individuals with chloracne ($N = 114$), a statistically significant increase in risk for all malignant neoplasms existed for those individuals ≥ 20 years after first exposure. The standard mortality ratio (SMR) was 139. The study of Zober *et al.* (1990) was sufficiently powerful to identify a 2.5- to 3-fold increase in risk for all

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malignant neoplasms combined. However, the study has essentially no power to identify increased risks of even threefold for specific cancer sites.

In 1985, 32 years after the industrial accident, adipose tissue was taken from six workers who were exposed to TCDD at BASF (Schecter and Ryan, 1988). Each of the six workers had experienced chloracne as well as other acute effects from the exposure. The average concentration of TCDD on a lipid-weight basis was 49 ppt with a range of 11–141 ppt. Assuming that the human body is a one-compartment model for dioxin distribution and excretion and assuming a dioxin half-life of approximately 7 years (Pirkle *et al.*, 1989), the average 49 ppt level 32 years after exposure corresponds to an adipose tissue level at the time of the accident of approximately 1000 ppt on a lipid-weight basis. For a 70-kg man, assuming 15% lipid content, the mean body burden at the time of the accident would have been approximately 10.5 μg, which corresponds to a dose of 150 ng/kg body wt. Furthermore, if we assume that these workers had no further TCDD exposure and that their average age at the time of exposure was 35 years, their lifetime (70 years) average daily dose (LADD) proportionality could be estimated from

$$(150\ \text{ng/kg})(1/35\ \text{years})(1\ \text{year}/365\ \text{days}) = 12\ \text{pg/kg/day}.$$

The lowest concentration of TCDD in adipose tissue measured 32 years after the accident was 11 ppt on a lipid-weight basis, which corresponds to approximately 280 ppt at the time of the accident. Based on the same assumptions and calculations, the LADD for 280 ppt is approximately equivalent to 3 pg/kg/day. This calculation, which is only an approximation, was based on limited adipose tissue data.

If variable dose rate is an important factor in risk assessment or if doses received at certain life stages result in a disproportionate risk, then the use of LADD is questionable. Such information does not exist for dioxin. However, dioxin does have a long half-life in humans. Thus, the use of LADD to assess carcinogenic risk is probably an acceptable assumption.

The LADD calculated by the method shown here is actually the average daily dose received over the worker's remaining lifetime, which is approximately 35 years. An alternative approach, which would average the dose received over the entire 70-year life, results in LADDs of 6 pg/kg body wt/day for the average TCDD exposure and 1.5 pg/kg body wt/day for the lowest exposure. Arguments can be made to support either approach; for our purposes, the discrepancy is probably not large, considering the other approximations used in these calculations.

The TCDD levels measured in adipose tissue or serum are dependent upon the worker's nutritional status, which can be affected by disease or life-style changes. For example, the malnutrition associated with many chronic diseases could cause a decrease in adipose tissue, resulting in an increase in lipid and serum TCDD levels. Conversely, a substantial gain of adipose tissue would result in a dilution or lowering of lipid and serum TCDD levels. Although these potential fluctuations of individual lipid and serum TCDD levels over time introduce another area of uncertainty in the LADD calculations, the average or median values of the worker population are protected from this instability to some extent.

Zober *et al.* (1990) reported serum TCDD levels for 28 BASF workers, who were tested in 1986. Cohort C1 had the highest median value of 24.5 ppt on a lipid-weight basis; the other two cohorts had serum TCDD levels that were essentially the same as background (9.5 and 8.4 ppt). These serum TCDD levels are similar to what would

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have been measured in adipose tissue, given the high correlation between adipose tissue and serum TCDD when expressed on a lipid-weight basis (Patterson *et al.*, 1988). In 1989, two workers from cohort C1, who had been exposed in the 1953 accident, had serum TCDD levels of 255 and 553 ppt (Table 8 in Zober *et al.*, 1990). Currently, one (255 ppt) has acute myeloid leucosis and the other (553 ppt) has liver carcinoma without cirrhosis (Zober *et al.*, 1990). By using a 7-year half-life, these serum levels can be extrapolated to approximately 10,000 and 20,000 ppt at the time of exposure. However, these are isolated examples of high exposure; the foregoing calculations reveal that as a result of the accident, the majority of the BASF workers have experienced a lifetime TCDD exposure that is not excessively higher than known background TCDD exposure levels. The available data collected from studies measuring human adipose tissue, blood, and milk levels of TCDD support the existence of a background level of TCDD in the general population, at least in industrialized countries. Ryan *et al.* (1985) measured TCDD in human fat obtained from 1979 to 1981 in Ontario. The tissues were taken from the abdominal region of 21 deceased hospital patients of both sexes, primarily 60 years of age and older. The average level of TCDD was 10.7 ± 5.4 ppt; the highest level was 21.8 ppt.

Graham *et al.* (1986) reported a mean level of 7 ppt TCDD in adipose tissue from 35 autopsy specimens from St. Louis, and Patterson *et al.* (1986) reported a mean level of 7 ppt TCDD in 35 adipose tissues from autopsy specimens from Georgia and Utah. Both these studies found that levels of TCDD increased with age, as expected. Ono *et al.* (1986) reported a mean level of 9 ppt TCDD from 13 adipose specimens in Japan. The U.S. Environmental Protection Agency found a mean level of 5 ppt TCDD is an analysis of 46 composite human adipose tissues prepared from over 900 specimens representing three age groups and all regions of the United States (Stanley *et al.*, 1986).

Commoner *et al.* (1986), who used a half-life estimate of 5 years and simplified models of pharmacokinetics to calculate body burdens of dioxin, showed that a continuous daily dose of approximately 1 pg TCDD/kg body wt must occur in order to maintain the background adipose tissue levels at 10 ppt in industrialized countries.

Monsanto Co., Nitro, West Virginia—1949

One other significant accident during the trichlorophenol (TCP) process resulted in TCDD exposure for which the workers have been followed approximately 30 years, a sufficient period to allow for chronic diseases such as cancer to be clinically diagnosed. The accident occurred on March 8, 1949, at the Monsanto Co. plant in Nitro, West Virginia. The factory produced 2,4,5-T from 2,4,5-TCP.

In 1980, Zack and Suskind reported on the results of a standardized mortality analysis conducted on all male workers who developed chloracne attributable to the 1949 accident. The vital status of the 121 members of this cohort was ascertained as of December 31, 1978, almost 30 years after the accident. All 121 members of the cohort were traced.

The mortality of the cohort members was compared with that of the U.S. population. The SMR for deaths from all causes was 69; 32 deaths were observed and 46.4 were expected. Excess cancer deaths were found in only three sites: 5 deaths from lung cancer (2.85 expected), 3 deaths from neoplasms of lymphatic and hematopoietic

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tissue (0.88 expected), and 1 fibrous histiocytoma (0.15 expected). None of these was statistically significant.

The Zack and Suskind study lacks the power necessary to determine a statistically significant increase in cancers of most sites. The cohort was very small, and no internal control group was used for comparison. All members of the cohort had chloracne, which signifies TCDD exposure. However, exposure to TCDD need not result in chloracne in order for the potential chronic effects of TCDD to occur. Therefore, finding an appropriate internal control group that would not be misclassified by an inaccurate exposure assessment would be difficult at this plant.

Zack and Gaffey (1983) reported on the mortality experience of Nitro plant male hourly employees who were working at the plant a few years after the accident. The study cohort consisted of employees working for at least 1 year between January 1, 1955, and December 31, 1977. Workers were considered exposed if they were assigned to an area of 2,4,5-T production. The data might be confounded because other chemicals used at this plant included *p*-aminobiphenyl (PAB), a known human bladder carcinogen. 2,4,5-T exposure was assessed for decedents only and was determined by the worker's assignment to a 2,4,5-T operation. Employees with plantwide responsibilities were considered not to have been exposed to 2,4,5-T. Vital status of each cohort member was ascertained as of December 31, 1977.

Mortality rates of the total plant study cohort were compared with those of the age- and race-standardized U.S. population. All 884 men in the study cohort were traced; 163 deaths were identified. The all-cause SMR was 103 and the SMR for all malignant neoplasms was 113. Significantly elevated SMRs occurred for bladder cancer and for arteriosclerotic heart disease: The SMR for bladder cancer was 989 (9 deaths observed and 0.91 expected), and the SMR for arteriosclerotic heart disease was 133. Zack and Gaffey (1983) attributed the excess bladder cancer to PAB exposure.

The cohort of this mortality analysis was larger than the cohort of employees with chloracne studied by Zack and Suskind (1980). However, whether all members of this cohort were actually exposed to TCDD or what the level of exposure was is not clear. The criteria used to determine exposure to dioxin are questionable and could reduce the strength of any association due to random misclassification bias, as with the chloracne study. Although there is no quantitative exposure information on the Monsanto workers, their TCDD levels are assumed to be similar to those of the BASF workers. Therefore, a significant excess of cancers is unlikely to occur among these workers.

NIOSH Dioxin Registry

The National Institute for Occupational Safety and Health (NIOSH) maintains the NIOSH Dioxin Registry, which consists of approximately 6000 workers in 14 U.S. facilities who were identified as being employed in the production of dioxin-contaminated chemicals such as 2,4,5-T and 2,4,5-TCP (Fingerhut *et al.*, 1984). These sites were assessed for adequacy of personnel and medical records and exposure data before being included in the registry (Halperin *et al.*, 1982). Recently, NIOSH investigators reported results from a cross-sectional epidemiologic study of 400 living workers who were employed at 2 of the 14 plants (Fingerhut *et al.*, 1989; Sweeney *et al.*, 1989). Serum from 135 workers and from 54 unexposed referents was analyzed for TCDD on a lipid-weight basis. The mean TCDD level among the exposed workers was 266

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ppt (range, 2–3389 ppt) compared with 8 ppt (range, 1.7–19.7 ppt) among the unexposed referents (Sweeney *et al.*, 1989). Using a half-life of 7 years, the investigators calculated blood levels on a lipid-weight basis of up to 30,000 ppt TCDD at the approximate time of exposure. The mean extrapolated blood TCDD level present in workers at the time of termination of employment was 2658 ppt (Sweeney *et al.*, 1989).

We would like to compare these workers' lifetime TCDD exposure to background levels, as we did for the BASF workers. However, the NIOSH workers did not experience an accidental one-time exposure but instead were exposed continuously over their working lifetimes. Estimating LADD under these conditions is much more difficult because we need to know not only cumulative exposure but also how long the workers were exposed. The mean body burden at termination of employment can be calculated by using the mean extrapolated blood TCDD level of 2658 ppt in a 70-kg man, assuming 15% lipid content, from

$$(2658 \text{ ng/kg})(70 \text{ kg})(0.15)(1 \ \mu\text{g}/1000 \text{ ng}) = 28 \ \mu\text{g}.$$

A body burden of 28 μg corresponds to a dose of approximately 400 ng/kg body wt.

Assuming that the workers' average age at termination of employment was 55 years and their average working life was 25 years and that the workers had no further TCDD exposure after termination of employment, the mean dose of 400 ng/kg body wt at termination of employment can be averaged over their working life plus their remaining life (70 − 55 years) to calculate LADD proportionality:

$$(400 \text{ ng/kg})(1/(25 + 15) \text{ years})(1 \text{ year}/365 \text{ days}) = 27 \text{ pg/kg/day}.$$

These calculations only estimate an approximate LADD to compare with background levels in industrialized populations and are not meant to indicate absolute levels. A LADD for TCDD of approximately 27 pg/kg/day, which is over 25 times the estimated background dose, is a substantial exposure.

Results from a NIOSH mortality study of workers from the 14 sites are expected to be available in late 1990. Currently, no direct measurements of exposure exist for these workers; instead, duration of exposure is used as a surrogate of dose. An exposure matrix is being constructed from process and task descriptions, safety records, and analytical data reporting TCDD levels in substances produced at the companies (Fingerhut *et al.*, 1989). It is probably reasonable to assume that the workers enrolled in the mortality study have approximately the same exposure as that measured for the workers in the morbidity study at 2 of the 14 plants. To calculate an approximate lifetime cancer risk that this dose represents, the calculated LADD of 27 pg/kg/day can be compared with the Food and Drug Administration's (FDA) risk-specific dose based on the rat studies of Kociba *et al.* (1978, 1979). FDA's dose of 0.06 pg TCDD/kg body wt/day would result in a lifetime risk of at most 10^{-6} for cancer; therefore, 27 pg/kg/day would correspond to a risk of $(27/0.06) \times 10^{-6} = 4.5 \times 10^{-4}$. Thus, using the worst-case estimate, at most 4 to 5 of 10,000 workers with this level of TCDD exposure would be expected to die of cancer. The NIOSH Mortality Study may not be powerful enough to determine this excess risk, given the large background cancer rate in the United States.

Herbicide Exposure in Sweden—1970s

In 1979, Hardell and Sandstrom reported on a matched case–control study of soft-tissue sarcoma cases (STS) performed in northern Sweden where forestry is a major

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occupation. The cases were identified from admissions records of an oncology unit from 1970 to 1977; tumor histology was reviewed by a pathologist, who identified 52 cases of STS and 208 controls. Four controls matched by age, sex, year of death, and residence were selected for each case. Deaths from malignant tumors or suicide were excluded from the control selection. Exposure to chemicals, especially phenoxyacetic acids or chlorophenols, was assessed via a questionnaire mailed to the living cases and controls or to next-of-kin surrogates for the dead cases and controls. Supplementary information was gathered through a telephone survey by an interviewer blinded to the case/control status. In addition, a questionnaire was also sent to the employers of persons who stated work in the forest, pulp industries, or sawmills as an occupation, to verify use of phenoxy herbicides.

Exposure to phenoxyacetic acids or chlorophenols was reported in 36.5% of the cases and in 9.2% of the controls. This finding results in an odds ratio for STS of 5.7 (95% CI = 2.9–11.3). To validate the information gathered from surrogates of the dead cases and controls, the living and dead groups were analyzed separately. The odds ratio for STS was 9.9 for the 21 living patients and their controls and 3.8 for the 31 dead patients and their controls. Thus, the surrogates may have underestimated the risk. Analyses were also performed separately for those exposed only to phenoxyacetic acids (mixed with 2,4,5-T) and for those exposed only to chlorophenols; the odds ratios remained high and statistically significant for each (5.3 for phenoxyacetic acids and 6.6 for chlorophenols).

A larger case–control study by these same investigators was published in 1981 (Eriksson *et al.*, 1981). The study included all patients with STS, living or dead, in southern Sweden, where herbicides are used for agriculture, who had been reported to the national cancer registry from 1974 to 1978. Their histology slides were examined by two pathologists. For every living case, two controls were selected who were matched by age and residence; for every dead case, the controls were matched by age, residence, and year of death. Cancer cases and suicides were excluded. After the pathology review, 11 cases were omitted because of a previous wrong or uncertain diagnosis; 110 cases and 220 controls remained in the study. Exposure was assessed by postal questionnaire, as in the previous study, and in addition, an attempt was made to determine exposure to phenoxy acids free from polychlorinated dibenzodioxins and dibenzofurans. Cases with exposure to phenoxy acids and chlorophenols within 5 years of diagnosis of STS were excluded.

The results of the study in southern Sweden were similar to those for northern Sweden: The odds ratio for STS associated with exposure to phenoxy acids or chlorophenols was 5.1 (95% CI = 2.5–10.4) and to phenoxy acids alone was 6.8 (95% CI = 2.6–17.3). The investigators also subdivided the cases and controls into two categories based on the expected presence or absence of TCDD in the phenoxy acids. For example, 2,4,5-T is known to contain TCDD, whereas 4-chloro-2-methylphenoxyacetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), monochlorophenoxyacetic acid, and others are believed not to contain TCDD. The odds ratio associated with 2,4,5-T was 17; with non-TCDD herbicides it was 4.2. Thus the authors concluded that the risk of STS was also associated with uncontaminated phenoxy acids. However, 2,4-D may have been contaminated with TCDD because the facilities for 2,4,5-T production were also used to produce 2,4-D (Lilienfeld and Gallo, 1989).

These Swedish case–control studies on STS (Eriksson *et al.*, 1981; Hardell and Sandstrom, 1979) have been interpreted as showing strong evidence for an association

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of STS with TCDD. The major limitations of the studies concern the amount of exposure to TCDD. Exposure history was assessed retrospectively by questionnaire at a time when herbicide use was receiving media attention (Coggon and Acheson, 1982). This publicity could have influenced the STS cases to overestimate their exposure (information bias) or have contributed to recall bias (2,4,5-T was banned in Sweden in 1977). Furthermore, medical surveillance of herbicide workers could have been increased, possibly leading to bias. On the other hand, the odds ratios identified in the Swedish studies are quite high—too large to be explained by information, recall, or detection biases. The latency periods in the studies were sufficient (mean latency = 20 years) for carcinogenic effects to be initiated, the investigators matched on known confounders of risk for STS, and all tumor diagnoses were reviewed by pathologists—a crucial control technique when dealing with a tumor as difficult to diagnose as STS.

The publicized deficiencies of the Swedish studies generally represent limitations inherent in case–control studies. Unfortunately, the only realistic approach to study rare tumors such as STS is by case–control; cohort analyses of STS are inappropriate because many person-years and 15–20 years of follow-up are required to obtain tumors of this type.

Recently, Hardell and Eriksson (1988) conducted a case–control study of STS in Sweden to test the reproducibility of their previous findings. Only patients with the diagnosis of STS based upon histopathologic examination were included. Two control groups were used to evaluate the effect of recall bias: a population-based referent group and an additional group of patients with another type of malignant disease. Exposure was assessed by questionnaire, and follow-up telephone interviews were performed on those subjects who had worked in forestry, agriculture, horticulture, carpentry, or in sawmills for some period since 1948, when phenoxyacetic acids and chlorophenols were first used in Sweden. All questionnaires and interviews were analyzed in a blinded manner.

Cases exposed to phenoxyacetic acids produced an odds ratio of 3.3 for STS when compared with the population referents and an odds ratio of 2.2 when compared with the cancer referents. Because the risk ratio decreased when the control group with malignant disease other than STS was used, some recall bias cannot be excluded; yet we cannot rule out that phenoxyacetic acids may be associated with a general carcinogenic effect. No differences in risk were obtained for exposure to chlorophenols, in contrast to previous studies; however, the number of cases (4) exposed to chlorophenols in this study was low. Hardell and Eriksson (1988), who also assessed the risk of STS associated with exposure to TCDD as a contaminant in phenoxyacetic acids, found an odds ratio of 3.5 compared with the population-based control group and an odds ratio of 3.1 compared with the cancer control group.

Another Swedish case–control study of STS failed to find an association with phenoxy herbicides. In 1986, Wiklund and Holm reported on the risk of STS associated with exposure to phenoxy herbicides in 354,620 Swedish men employed for at least 1 week in 1960 in agriculture or forestry. The control group consisted of 1,725,845 Swedish men employed in other industries during the same time period. The cohort was subdivided into six groups based on occupation. Cases of malignant tumors were identified via a nationwide cancer registry for the years 1960–1979. The relative risk for STS was 0.9, and subanalyses did not reveal any group with an elevated risk of STS.

Limitations of this study include use of occupational classification from a national census as a surrogate for TCDD exposure and possible underascertainment of STS

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cases through use of a national cancer registry. Agricultural and forestry workers may not have access to high-quality medical care, and therefore cases of STS may be underdiagnosed. On the other hand, the study is large and the follow-up period long enough to reveal any excess STS cases.

Herbicide Exposure in New Zealand and the United States

Phenoxy herbicides, particularly 2,4,5-T, were widely used in New Zealand since the late 1940s. Researchers there undertook a case–control study of STS after the Swedish results were published (Smith *et al.*, 1984). All male cases of STS reported to New Zealand's national cancer registry between 1976 and 1980 were included in the study. All histology reports were reviewed by pathologists. Controls were selected for each case from patients in the registry with other types of cancer. Cases and controls were contacted by a single telephone interviewer who was blinded to the case/control status.

Exposure was ascertained by questions regarding employment and use of specific chemicals. Interviews were completed for 82 cases and 92 controls. The results, however, were not consistent with the Swedish studies. For cases having phenoxyherbicide exposure that lasted more than 1 day and occurred more than 5 years preceding cancer registration, the estimate of relative risk (the odds ratio) was 1.3 (90% CI = 0.5–4.5). Using only cancer patients as controls may have introduced misclassification bias in that dioxin exposure could have possibly contributed to the cancers.

The investigators undertook a further study of 51 more recently diagnosed STS cases (Smith and Pearce, 1986). Cases from 1976 through 1982 were selected from the same cancer registry as before. Histology reports were reviewed for each case, as in the earlier study. The 315 controls were selected from controls being used concurrently for another study of lymphoma and multiple myeloma. Only six cases were exposed, resulting in an odds ratio estimate of 0.7 (90% CI = 0.3–1.5).

The National Cancer Institute conducted a case–control study of STS, Hodgkin's disease, and non-Hodgkin's lymphoma in Kansas and found farm herbicide use to be associated with non-Hodgkin's lymphoma (Hoar *et al.*, 1986). All cases of STS, Hodgkin's, and non-Hodgkin's lymphoma among white male Kansas residents from 1976 to 1982 were identified through a University of Kansas population-based cancer registry. Pathology specimens for 87% of the cases were reviewed by a panel of three pathologists. Controls were white males from the Kansas general population, matched to each case by age and vital status. Three controls were used for each case. The patients and controls, or their next of kin, were interviewed by telephone. Questions on farming practices included which herbicides and insecticides were used, how many days per year the men were exposed, and what protective equipment was used.

For the 22 cases of STS that reported having used herbicides, the odds ratio was 0.9 when compared with 192 controls. For the 40 cases of non-Hodgkin's lymphoma involving herbicides, the odds ratio was 1.6. Men exposed to herbicides more than 20 days per year had an odds ratio of 6.0 for increased risk of non-Hodgkin's lymphoma (95% CI = 1.9–19.5). Also, a significantly increased odds ratio was associated with the non-Hodgkin's lymphoma cases who had used phenoxyacetic acids. The study results, which are based on questionnaire data, may be subject to recall bias. Only three patients had ever used 2,4,5-T in this study; the herbicide most frequently used was

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2,4-D. Therefore, the study results suggest that the herbicide 2,4-D is not related to the development of STS. However, the results do not provide adequate evidence to refute the Swedish study that found an association between 2,4,5-T and STS.

No quantitative data on TCDD exposure exist for any of the studies on the association of herbicide exposure to STS or other cancers. Although useful from a qualitative perspective, the data cannot be used to calculate the human carcinogenic risk of dioxin.

South Vietnam—1962–1971

In January 1962, a program to use herbicides for defoliation and crop destruction began in Vietnam under the code name "Ranch Hand." Six herbicides were used (Agents Orange, Purple, Pink, Green, White, and Blue). During the 9 years the program was operational, approximately 19 million gallons of these herbicides were sprayed over South Vietnam, of which approximately 12 million gallons were Agent Orange (Wolfe *et al.*, 1985).

Other than Agents White and Blue, most of the herbicides used in Vietnam were mixtures of 2,4-D and 2,4,5-T (Halperin *et al.*, 1982). Agents Pink, Green, and Purple each contained up to 66,000 ppb of TCDD; Agent Orange was used exclusively after July 1, 1965, and contained 2000 ppb of TCDD (Ayres *et al.*, 1985). In early 1978, the Veterans Administration established the Agent Orange Registry to identify all Vietnam veterans exposed to Agent Orange, to provide a mechanism for follow-up, and to obtain information on their current health status (Young *et al.*, 1985). The usefulness of the data in this registry is limited because TCDD exposure and health problems are self-reported and the participants are self-selected.

In 1979, the U.S. Air Force began a prospective epidemiologic study of chronic health effects among Air Force personnel who conducted the aerial spraying of Agent Orange ("Ranch Handers"). Wolfe *et al.* (1985) reported on the analysis of health information data for 1174 Ranch Handers and controls who were sent questionnaires and for 1045 Ranch Handers and controls who underwent physical examination.

Five controls, who were matched by race, age, and job category, were selected for each Ranch Hander. The controls were assigned to Air Force units that flew cargo aircraft in Southeast Asia. Each living Ranch Hander and the first of his living controls were selected for the questionnaire and physical examination phases. An exposure index for each job category was developed from mission records and tour data that included the amount and type of herbicide sprayed each month as well as the number of Ranch Handers in each job category who were involved in the spraying. The investigators were also able to determine the months each individual was assigned to the Ranch Hand operation. Exposure was determined as the effective number of gallons of herbicides to which each individual had been potentially exposed. Both the interviewer and the examining physician were blinded as to the exposure status of the subject.

The few deaths which had occurred among the cohort at the time of this study did not indicate increased mortality compared with the controls. This finding is not unexpected because of the young age of the cohort. Results were essentially negative. After adjustment for education, however, significantly more Ranch Handers than controls demonstrated hypochondria, depression, hysteria, and schizophrenia.

Kang *et al.* (1986) reported on a study of Vietnam veterans in which 234 veterans with STS who had served in the U.S. military between 1964 and 1975 were compared

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with 13,496 patients without STS selected from a Vietnam-era patient population. No association was found between service in Vietnam and STS. However, in this study Vietnam service was used as a measure of exposure to TCDD and not enough time had elapsed for the diagnosis of STS.

Kang *et al.* (1987) did another case–control study in which 217 STS patients who were selected from the Armed Forces Institute of Pathology were matched to 599 controls for service in Vietnam, exposure to chemicals, medical history, and life-style. The investigators found that the risk of STS was nonsignificantly increased for veterans with combat experience when compared with the risk for those without (odds ratio = 2.6), and also nonsignificantly increased for the combat veterans assigned to the region that was sprayed the most (odds ratio = 8.6). Again, the classification of exposure is questionable, and the study has little power to determine an effect.

Thomas and Kang (1989) have recently reported the results of a study of Army Chemical Corps Vietnam veterans, i.e., men who were responsible for the mixing and application of herbicides. The study cohort consists of approximately 1000 veterans who served between 1965 and 1971 and whose vital status was followed through December 31, 1987. The only potentially important finding was 3 diagnoses of Hodgkin's disease compared with 0.002, which was expected based on prevalence rates among all male veterans in the Army's Agent Orange Registry.

In an early study of Vietnam veterans in Australia (Fett *et al.*, 1984), the only excess neoplasm found was 2 cases of STS vs 0.64 expected, whereas in the control group no STS was found and 0.84 was expected. The study used service in Vietnam as a surrogate for exposure.

Fett *et al.* (1987a,b) published two reports on a retrospective cohort study that examined the number and causes of deaths among Australian Army veterans who served during the Vietnam era. Of those studied, 19,205 served in Vietnam and 25,677 served only in Australia. All men were traced from the end of their service, between 1966 and 1973, until January 1, 1982. Thus, the maximum follow-up time was 16 years. The first paper (Fett *et al.*, 1987a) reported that the mortality of Vietnam veterans was well below that expected based on the Australian male death rates (SMR = 73) but statistically significantly higher than that of non-Vietnam veterans (relative risk = 1.3; 95% CI = 1.1–1.5). The low mortality rate of the cohort compared with the Australian general male population could be explained by missed deaths, the enlistment requirement for good health, or the continual health screening practices of the Army. When stratified by assigned corps, the engineers' death rate for Vietnam veterans was 2.5 times that of non-Vietnam veterans ($P = 0.001$). Battle injuries were not important in contributing to the relative mortality of Vietnam and non-Vietnam veterans.

The second paper (Fett *et al.*, 1987b) reported the analysis of specific causes of death. Over 98% of the deaths among veterans were included in five classes of causes of death. Approximately three-fourths of the deaths were due to accidents, poisonings, and violence (external causes). The other four classes were neoplasms (14%), diseases of the circulatory system (7%), diseases of the digestive system (2%), and mental disorders (2%). In the last two classes, alcohol abuse was a factor in most deaths among Vietnam veterans. The mortality rates for the Vietnam veterans were significantly increased only for external causes (relative risk = 1.3) and diseases of the digestive system (relative risk = 9.0). However, the numbers of deaths from specific cancers are quite small and the follow-up period is too brief to ascertain carcinogenic effects. As

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in other studies of Vietnam veterans, service in Vietnam was the surrogate for exposure to any potentially putative agent.

Levels of TCDD in adipose tissue taken from Vietnam-era veterans have no correlation with known exposure to TCDD and service in Vietnam (Gross *et al.*, 1984; Lee and Hobson, 1985). Investigators from the Veterans Administration reported on the results of adipose tissue analyses from 20 veterans who believed they were exposed to Agent Orange in Vietnam, 10 veterans who had not served in Vietnam and who had no known exposure to TCDD, and 3 Air Force officers who had handled Agent Orange in field tests or in disposal operations (Lee and Hobson, 1985). Of the 10 unexposed veterans, 9 had 3–14 ppt TCDD in their adipose tissue. All 3 officers had repeated contact with TCDD and had been exposed within the previous 2 years; yet their adipose tissue contained 4, 5, and 6 ppt TCDD. The veterans who believed they were exposed to Agent Orange had tissue levels ranging from 0 to 99 ppt; the two high levels were 35 and 99 ppt and none of the others were above 13 ppt. The veterans were divided into three groups as to their likely past exposure to Agent Orange, based on military records. The most heavily exposed group consisted of the 2 veterans with levels of 35 and 99 ppt TCDD in their adipose tissue and 1 veteran with no measurable TCDD. The other two groups had similar levels of adipose tissue TCDD; none were above 13 ppt TCDD. The adipose tissue TCDD levels also did not correlate with perceived or real health status.

Kang *et al.* (1989) recently reported similar results. Mean TCDD levels in adipose tissue from 40 Vietnam veterans (13.4 ppt) did not differ appreciably from mean levels from either 80 non-Vietnam veterans (12.5 ppt) or 80 civilians (15.8 ppt). Also, the results showed no association between adipose tissue TCDD levels and any estimate of Agent Orange exposure opportunity based on military records.

With the exception of military personnel who actually handled or sprayed Agent Orange in Vietnam, most Vietnam veterans have current TCDD body burdens no different from the average background level. From the Veterans Administration data, the highest adipose tissue TCDD level was 99 ppt on a lipid-weight basis approximately 20 years, or 3 half-lives, after exposure. This finding corresponds to 800 ppt at the time of exposure, a body burden of 8.4 μg, or a dose of 120 ng/kg. The LADD is calculated by

$$(120 \text{ ng/kg})(1/50 \text{ years})(1 \text{ year}/365 \text{ days}) = 6.5 \text{ pg/kg/day}.$$

This calculation assumes that the veteran was 20 years old at the time of exposure. The adipose tissue TCDD level of 13 ppt corresponds to 104 ppt at the time of exposure, a body burden of 1 μg, a dose of 16 ng/kg, and a LADD of 0.9 pg/kg/day.

ICMESA, Seveso, Italy—July 10, 1976

On July 10, 1976, an accident occurred at the ICMESA factory during production of 2,4,5-TCP, which is used as an intermediate for hexachlorophene. The factory was located near Seveso, about 20 miles north of Milan. A plugged exhaust pipe caused an exothermic reaction that released high concentrations of TCDD into the atmosphere of an urban area southeast of the factory, which had a population of several thousand. Two to three days after the accident, small animals such as birds, rabbits, and chickens were dying, and a few children and adults were complaining of skin lesions and nausea

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(Fanelli *et al.*, 1980). The company analyzed the soil, dust, and vegetation in and outside the factory in an attempt to assess the amount and location of TCDD. Because concentrations as high as 15,000 ppb were found in grass, but much less was found in soil, all vegetation was cleared (Reggiani, 1978).

Approximately 2 weeks after the accident, the contaminated area was divided into three zones based on the soil analyses. Zone A with >10 μg TCDD/kg soil was evacuated. Zone B with 0.1–10 μg TCDD/kg soil was not evacuated, but recommendations were issued to reduce the possibility of exposure. Zone R had <0.1 μg TCDD/kg soil, and safety measures were restricted to prohibition of food production and consumption (Reggiani, 1978).

The Seveso Cancer Registry was set up in 1981 to record cases of cancer occurring among the population in the entire Lombardia region (Puntoni *et al.*, 1986). Cancer cases were identified through admission and discharge hospital forms. In 1986, the 1975–1981 site-specific cancer incidence rates were available. At that time, the incidence of STS was higher in the exposed group (517 per 100,000) than in the unexposed group (3.3 per 100,000) (Merlo and Puntoni, 1986; Puntoni *et al.*, 1986). The numbers were very small, however, and comparable STS incidence rates did not exist for the area before 1975. Worldwide STS incidence rates are usually 2 per 100,000 (Waterhouse *et al.*, 1982).

In 1989, Bertazzi *et al.* published the results of a 10-year mortality study of the population involved in the Seveso incident (Bertazzi *et al.*, 1989). Exposure was categorized by residence: At the date of the accident or their first entry into the area, subjects were classified as living in Zone A, Zone B, or Zone R. Persons living outside the contamination boundaries were considered to be unexposed. Both national and local mortality rates were used as reference standards. The cohort of people who had been living in the uncontaminated study area was determined to be the most suitable control population because they shared dietary habits and social and educational background with the exposed population. This population was followed according to the same criteria and methods and for the same amount of time.

Mortality of all persons residing in the area between the date of the accident and December 31, 1984, was examined for the period July 10, 1976, to December 31, 1986. More than 99% of the subjects were traced. The all-cause mortality rates were similar among the zones and to the reference rate. In the analysis, cause-specific mortality results for Zone A indicated an elevated mortality from circulatory diseases in both sexes. One death from cancer of the gallbladder and biliary tract among females resulted in a significantly high relative risk of 21.9. The results for Zone B were nonsignificant among males. Among females, however, the two deaths from cancer of the gallbladder and biliary tract gave a nonsignificant relative risk of 3.5, and one death from STS was statistically significant when compared with the control population. In Zone R, two deaths from STS among males resulted in a nonsignificant but high relative risk. Females had a significantly elevated relative risk for uterine cancer. The vital status of all the chloracne cases was ascertained ($N = 193$); no deaths have occurred.

The authors suggest that the excess deaths from cardiovascular problems may have been due to stress in the aftermath of the accident. The increased relative risks of specific cancer sites, even when statistically significant, were based on very few cases, and therefore the study results cannot be considered a reliable indication of a TCDD effect. Other limitations of the study include an insufficient latency period (10 years) for cancer and possible misclassification of exposure status. The residents of the un-

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contaminated area could have been exposed to dioxin, because the only exposure indicator used was soil levels of TCDD. Residents of Zone A were evacuated after a few weeks, whereas residents of Zone B remained in an area considered to be very contaminated. Their compliance with issued regulations regarding consumption of products from the area has not been estimated. Also, data suggest that some parts of Zone R were more contaminated than initially thought (Caramaschi *et al.*, 1981). Nevertheless, >10 years of follow-up is needed before the chronic effects of the Seveso accident can be fully evaluated.

Centers for Disease Control (CDC) has approximately 30,000 frozen blood specimens from people exposed to TCDD at Seveso. A few of the specimens have been analyzed and the serum TCDD levels have been estimated on a lipid-weight basis at the time of the accident. Analyses of serum from 19 individuals who lived in Zone A indicate a range of TCDD levels from 828 to 56,000 ppt; the latter measurement was made in a child who was 4 years old at the time of the accident (Needham *et al.*, 1989). The most recently reported half-life for TCDD of 7.1 years was used for this analysis (Pirkle *et al.*, 1989). The presence of chloracne was not correlated with a threshold level of dioxin; significant overlap of TCDD levels existed between those with and without chloracne (Patterson *et al.*, 1989).

A serum level of 56,000 ppt on a lipid-weight basis in a 4-year-old child with a 30% body lipid level corresponds to a body burden of approximately 340 μg,

$$(56{,}000 \text{ ng/kg})(20 \text{ kg})(0.30)(1\ \mu\text{g}/1000 \text{ ng}) = 340\ \mu\text{g},$$

which is equivalent to a dose of approximately 17,000 ng/kg. If we assume that the child had no further exposure, his LADD (74 years) proportionality could be estimated by

$$(17{,}000 \text{ ng/kg})(1/70 \text{ years})(1 \text{ year}/365 \text{ days}) = 660 \text{ pg/kg/day}.$$

The lowest serum level of TCDD among the persons with chloracne, 828 ppt, was measured in a 16-year-old girl and corresponds to the following body burden, dose, and LADD:

$$(828 \text{ ng/kg})(50 \text{ kg})(0.20)(1\ \mu\text{g}/1000 \text{ ng}) = 8.3\ \mu\text{g body burden}$$

$$(8.3\ \mu\text{g}/50 \text{ kg})(1000 \text{ ng}/1\ \mu\text{g}) = 166 \text{ ng/kg dose}$$

$$(166 \text{ ng/kg})(1/55 \text{ years})(1 \text{ year}/365 \text{ days}) = 8.3 \text{ pg/kg/day LADD}.$$

These approximate calculations reveal that the Seveso residents clearly have some of the highest TCDD exposures known to date; thus, they have significant potential to document whether dioxin exposure results in any chronic effects. The 4-year-old child with the highest exposure has a calculated LADD of 660 pg TCDD/kg body wt/day, which is quite high compared with the other documented human exposures to TCDD. However, when this approximate LADD is related to FDA's risk-specific dose based on the rat study (Kociba *et al.*, 1978, 1979), we find that the child's risk of death from cancer would result in a 10^{-2} risk at most. Because FDA's dose of 0.06 pg TCDD/kg body wt/day will result in at most a lifetime risk of 10^{-6} for cancer, 660 pg/kg/day would correspond to a risk of $660/0.06 \times 10^{-6} = 1.1 \times 10^{-2}$.

DISCUSSION

The evidence for an association of TCDD with human cancer is equivocal. The workers known to be exposed to TCDD at the Monsanto plant in Nitro, West Virginia,

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in 1949 have shown a nonsignificant excess of lymphatic and hematopoietic cancers, and the workers exposed at the BASF plant in Germany in 1953 have shown an excess of stomach cancer. The risk ratio for each type of cancer was small. Moreover, the positive findings of the BASF and Monsanto studies have not been duplicated in other epidemiologic studies because the cohorts have frequently been too small to have sufficient power to identify a small increase in cancer risk; were too recently exposed to develop cancer, which has a long latency period; or were exposed to other chemicals concurrently. The small number of workers involved in these accidents and the modest levels of TCDD exposure experienced by the workers make it unlikely that an excess of cancer will be determined in epidemiologic studies of these cohorts compared with the high background incidence rate of cancer in the United States.

An assessment of the available data for an association between TCDD exposure and STS reveals inconsistent findings. Case–control studies are the only practical method to study risk factors for very rare diseases such as STS, but then assessing exposure retrospectively introduces a number of inherent problems. Also, selection of appropriate controls who must have low or zero probability of exposure to TCDD is very difficult. Very high relative risks of STS were found in the Swedish studies, but the risks were associated with exposure to "TCDD-contaminated herbicides" and therefore included exposure to a myriad of chemicals. Although the most recent study of Hardell and Eriksson (1988) did associate STS with TCDD, the results are not useful in risk assessment because quantitative exposure information was not available. In addition, corroborative evidence has not been found from studies in other countries.

Serum specimens from the Ranch Handers are being analyzed by CDC, but the Vietnam experience will not likely reveal a human carcinogenic risk from dioxin. Although the Ranch Handers have shown no increase in the incidence of cancer, the cohort is composed of people who are still young; furthermore, the TCDD exposure from Agent Orange experienced by the Ranch Handers was, for the most part, not much different from the TCDD exposure experienced by a person living in an industrialized country. Such exposures imply excess lifetime cancer risks too minute to isolate in industrialized populations with high annual rates of cancer incidence. Moreover, although the results of additional epidemiologic studies on the Ranch Handers, which will relate serum levels of dioxin to an assessment of mortality, are anticipated, the follow-up periods covered by the studies will still be inadequate to result in many cancers.

Serum from the Seveso residents indicates that this population was significantly exposed to TCDD; CDC has estimated that several hundred of the participants probably had exposures resulting in serum concentrations of TCDD from 800 to over 50,000 ppt on a lipid-weight basis at the time of the accident (Needham *et al.*, 1989). Although the incidences of some cancers among those in the Seveso cohort are slightly increased over background, the numbers are too small to place much confidence in the findings. Also, the Seveso accident, which is relatively recent, has been studied for only 10–12 years to date. An additional important limitation of the Seveso data concerns the young age of the highly exposed cohort members, the majority of whom were children or young adults at the time of the accident in 1976. Therefore, the problem of a short latency period is compounded unless dioxin has the potential to increase the incidence of childhood cancers, and no evidence in human or animal studies supports such speculation.

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The results from the NIOSH Mortality and Morbidity Studies, which use data from NIOSH's Dioxin Registry, are expected in late 1990. This cohort also has documented high exposures to TCDD, although the percentage of the cohort that actually experienced the higher levels is not clear. The period of follow-up is long (up to 30 years), the serum levels of 135 workers at termination of employment average 2658 ppt, and because the exposure is occupational in nature, age is not a limiting factor (Sweeney *et al.*, 1989). Yet, the calculated *maximum* lifetime cancer risk predicted from the TCDD exposures experienced by the NIOSH cohort is only 4.5×10^{-4}, and it is unlikely that this level of excess cancer risk can be determined by an epidemiology study.

The findings of the epidemiology studies, which have quantitative exposure data, are summarized in Table 2. The studies are severely limited by low statistical power, except for the NIOSH cohort, whose power will depend upon the percentage of cohort members having high exposure histories and long follow-up periods. The maximum carcinogenic risks from dioxin implied by the studies (Table 2) were calculated by relating the approximate LADD of each of the exposed populations to FDA's risk-specific (10^{-6}) dose based on the Kociba rat study (Kociba *et al.*, 1978, 1979). The true risk may range from zero up to the maximum shown in Table 2. The risk to the Monsanto workers is unknown, but is probably $<2 \times 10^{-4}$, the calculated risk to the BASF workers. With the documented high exposure levels, the Seveso and NIOSH data hold the best promise for future use in the assessment of the human carcinogenic risk of dioxin, with the qualification that the maximum calculated risk of the Seveso and NIOSH cohorts based on the animal data is still moderate compared with high background cancer incidence rates for most industrialized countries. Therefore, we cannot be certain that the Seveso and NIOSH epidemiology studies will be sensitive enough to identify a significant excess of cancer.

TABLE 2

MAXIMUM CARCINOGENIC RISK TO POPULATIONS AND WORKERS EXPOSED TO TCDD

Population exposed	Excess cancer	Power considerations		Maximum[a] risk
		Positive	Negative	
BASF—FRG	Stomach	Long latency	Small cohort Low exposure	2×10^{-4}
Monsanto— West Virginia	? Lung ? Lymphatic ? Hematopoietic	Long latency	Small cohort Low exposure	? $<2 \times 10^{-4}$
NIOSH Dioxin Registry	Results expected late 1990	High exposure Long latency	Highly exposed subcohort may be small	4.5×10^{-4}
Vietnam veterans	None	None	Short latency Young cohort Low exposure	1×10^{-4}
Seveso	? None	High exposure	Short latency High exposures in young	1×10^{-2}

[a] From FDA's risk-specific (10^{-6}) dose based on the Kociba rat study (Kociba *et al.*, 1978, 1979).

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BIOLOGICALLY MEASURED HUMAN EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND HUMAN CANCER

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ABSTRACT

The development of methods to biologically measure human exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) has become an important breakthrough for epidemiological research. Because of the long half-life of 2,3,7,8-TCDD in humans, exceeding 5 years, biological measurements give an indication of human exposure occurring over many years. In this paper, we review cancer epidemiology studies of 2,3,7,8-TCDD in the light of biological measurements of exposure and conclude that no human cancer has so far been established to be caused by exposure to 2,3,7,8-TCDD. However increased cancer risks in two cohort studies involving significant exposure and sufficient time of follow-up raise the possibility that 2,3,7,8-TCDD is a human carcinogen.

INTRODUCTION

The development of methods to measure 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) in human adipose tissue, and subsequently in the blood, have become important breakthroughs for epidemiological research. Because of the long half-life of 2,3,7,8-TCDD in humans, exceeding 5 years, biological measurements give an indication of human exposure occurring over many years and reflect cumulative dose received from occupational exposure. Such information is valuable for causal inference since it allows identification of those studies involving significant exposure to 2,3,7,8-TCDD, and comparison of the results from different studies taking into account differences in exposure. Below, we review cancer epidemiology studies of 2,3,7,8-TCDD in the light of biological measurements of exposure.

METHODS

Cancer epidemiology studies were identified which could be linked to biological measurements of exposure to 2,3,7,8-TCDD. Most of these studies involved biological measurements on a subset of the cohorts being studied. On the basis of these measurements, inference could be made about the likely extent of exposure of the whole cohort. In some cases, such as herbicide spraying, there have been no cohort studies involving workers with biologically measured exposure to 2,3,7,8-TCDD. However the extent of exposure associated with spraying 2,4,5-T contaminated with 2,3,7,8-TCDD can be assessed on the basis of a study of serum 2,3,7,8-TCDD levels of a group of professional sprayers (1). Causal inference was based on the findings of the collected studies taking into account the extent of exposure to 2,3,7,8-TCDD and the adequacy of follow-up in terms of latency.

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RESULTS

Studies of 2,3,7,8-TCDD-exposed populations that have used biological measurements to assess exposure, and which could be linked to cancer studies, are presented in Table 1. The levels presented may be compared to levels in the general population which average around 5-10 pg/g lipid (2). The cohort with the highest average levels of 2,3,7,8-TCDD among those subjects measured involves Seveso residents who were exposed following an explosion in a chemical manufacturing plant (3). However the measurements involved only 19 subjects. The cohort with the second highest average level involves U.S. chemical plant workers with an average of 233 pg/g based on 253 measurements (4). The average level for 16 employees in the BASF chemical plant in Germany was estimated to be 74 pg/g (5). This is an approximate estimate derived from data presented in a bar graph. Ranch Hand Veterans were exposed to 2,3,7,8-TCDD contaminated Agent Orange in Vietnam with the highest exposure occurring among the enlisted non-flying personnel (6). A median of 24 pg/g was reported for this group but the range went up to 618 pg/g indicating that some of the men had high exposures.

Table 1: Exposed populations that have biological measurements of 2,3,7,8-TCDD and can be linked with cancer studies

Population	Number of Measurements	Serum TCDD Level[1] Average (Range)	Years Since Last Exposure	Reference
U.S. Chemical Workers	253	233 (2 - 3,400)	15 - 37	(4)
BASF Employees	16	74 (0 - 553)	18 - 34	(5)
Ranch Hand Enlisted Personnel	407	24[2] (0 - 618)	15 - 25	(6)
Seveso Residents[3]	19	12,558 (828 - 56,000)	<1	(3)
New Zealand 2,4,5-T Sprayers	9	53 (3 - 131)	16[4]	(1)

[1] pg per gram of lipid
[2] Median value reported
[3] Residents of Zone A
[4] Years since last significant exposure (Reference 7)

Findings reported for the four cancer epidemiology studies that have used biomarkers of exposure and indicate significant exposure to 2,3,7,8-TCDD are presented in Table 2. The highest exposed group identified in Table 1 involves Seveso residents. However duration of follow-up of this cohort is insufficient for causal inference. After 10 years of follow-up, no significant findings have emerged in the highest exposure group (Zone A) (8).

Table 2: Cancer findings of studies with biological markers of exposure

Population	Number of Subjects	Years Since First Exposure	Study Findings *SMR (95% C.I.)*	Reference
U.S. Chemical Workers	1,520[1]	≥20	All Cancers 1.5 (1.2-1.8) Respiratory Cancers 1.4 (1.0-1.9) Soft Tissue Sarcoma 9.2 (1.9-2.7)	(4)
BASF Employees	114[2]	≥20	All Cancers 2.0 (1.1-3.4) Lung Cancer 2.5 (0.8-5.9)	(5)
Ranch Hand Veterans	1,261	15-25	All Cancers 0.7 (0.3-1.1)	(9)
Seveso Residents of Zone A	556	10	All Cancers[3] 0.9 (0.3-2.9) Lung Cancer[3] 2.0 (0.5-8.1)	(8)

[1] Subcohort of workers with ≥ 1 yr of exposure and ≥ 20 yrs latency.
[2] Subcohort of workers that developed chloracne and with ≥ 20 yrs since first exposure.
[3] Males only.

The most important findings are those reported for the U.S. chemical plant workers since the cohort was large, involved significant exposure to 2,3,7,8-TCDD and included a sub-group with sufficient duration of follow-up (4). The main findings for those with at least 1 year of exposure and at least 20 years of follow-up are presented in Table 2. Noteworthy findings also appear in the study of BASF employees (5). Results are presented in Table 2 for a subcohort of workers that developed chloracne and had at least 20 years of follow-up.

Duration of follow-up of Ranch Hand Veterans is not yet sufficient to make the study useful for causal inference (Table 2). In addition, the median exposure of 24 pg/g (Table 1) even for the most heavily exposed group is not as great as those reported for the above cohorts.

DISCUSSION

Studies that use biological measures of human exposure to 2,3,7,8-TCDD help to place results reported for other studies in perspective in terms of relevant levels of exposure. Results from the study of professional pesticide applicators in New Zealand show that significant exposure beyond background levels can be found in ground-level applicators who have sprayed 2,4,5-T for many years. Evidence that exposure to 2,3,7,8-TCDD causes human cancer is largely based on studies of subjects who recall periods of exposure less than 90 days. On the basis of the findings reported for the New Zealand study, it seems unlikely that exposures of such short duration would significantly increase serum levels of 2,3,7,8-TCDD beyond background levels. Thus, if exposure to phenoxyherbicides contaminated with 2,3,7,8-TCDD causes cancer in humans, the epidemiologic evidence would likely come from studies of individuals with exposure occurring over many years. There are no such studies and therefore pesticide user studies are not relevent to causal inference concerning 2,3,7,8-TCDD effects.

Based on the study findings reported in Table 2, the most informative human study concerning 2,3,7,8-TCDD published to date is the cohort of U.S. chemical plant workers (4). Although this study did not reveal an increased risk of cancers of the lymphatic or hematopoietic tissue which have been postulated to be related to 2,3,7,8-TCDD exposure, it supports new hypotheses concerning some other cancer sites. It provides the only human evidence that soft tissue sarcoma might be related to 2,3,7,8-TCDD exposure. In addition, it raises the possibility that there might be a generalized increased risk of cancer resulting from high levels of exposure to 2,3,7,8-TCDD since there was an increase in mortality from all cancers combined, largely attributable to cancers of the respiratory and gastrointestinal tracts. However on the basis of current publications of findings in this cohort, it is not possible to exclude effects from other occupational exposures such as asbestos which must be the presumed cause of the two mesotheliomas reported.

It is noteworthy that the BASF cohort found increased risks of all cancers combined, also largely attributable to respiratory and gastrointestinal tract cancers. However a case of pleural mesothelioma was reported for this small cohort. Thus, although two cohorts provide evidence that 2,3,7,8-TCDD may increase risks of a variety of cancers and there are no cohorts involving significant exposure which provide evidence against this hypothesis; asbestos, which has been found to increase the risks of both respiratory and gastrointestinal tract cancers, probably accounts for at least part of the observed excess cancers in both the BASF cohort and the U.S. chemical plant workers cohort.

In conclusion, when the results of cancer epidemiology studies are reviewed in light of biological measurements of exposure, no human cancer site has yet been established to be caused by exposure to 2,3,7,8-TCDD. However there is evidence for an increase in the overall risk of cancer based on two studies, with no evidence to the contrary. It is expected that follow-up over the next ten years of these and other cohorts experiencing significant exposure will provide valuable information for causal inference concerning 2,3,7,8-TCDD and human cancer.

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EXPOSURE OF FLORIDA AIRBOAT AQUATIC WEED APPLICATORS TO 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

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ABSTRACT

Florida airboat handgun applicators were monitored for exposure to 2,4-dichlorophenoxyacetic acid (2,4-D). Four applicators were monitored with air samplers, α-cellulose patches, and 24 hr urine samples on 10 separate days. Estimated total body exposure averaged 15 ± 2 mg/hr, of which 74% was to the legs and feet with an additional 18% to the hands and arms. Estimated respiratory exposure was about 0.03% of the total body exposure. Twenty-four hr urinary 2,4-D ranged from 0.190-0.645 mg. The use of disposable coveralls and effective hand protection would markedly reduce this exposure.

INTRODUCTION

The exposure of agricultural workers to pesticides has received increasing attention from federal regulatory agencies. Workers associated with the application of chemicals appear to be the most highly exposed group within the 'farmworker' designation. Exposure studies of 2,4-D and 2,4,5-Trichlorophenoxyacetic acid may also be useful in estimating Vietnam veteran exposure to dioxin in Agent Orange (1). Chlorophenoxyacetic acid herbicide exposure and related studies have been reviewed (2).

The purpose of this study was to determine the 2,4-D exposure of Florida airboat handgun herbicide applicators.

EXPERIMENTAL

Materials and Methods Two, two-man airboat crews from Polk County Environmental Services were chosen for this study. Polk County Environmental Services is the county agency responsible for mosquito and aquatic weed control. Airboat crews are routinely used for water hyacinth control in lakes, rivers, streams, and canals. Vital statistics for the participating subjects appear in Table 1.

Table 1. Vital statistics of Florida airboat spray crews applying 2,4-D for aquatic weed control, Polk County, Florida.

Subject	Age	Height	Weight lb (kg)	Length of employment
JD	27	6' 0"	155 (70.4)	1 yr 6 mo
CH	54	5' 8"	165 (74.9)	6 yr
KP	26	5' 9"	150 (68.1)	1 yr
SS	43	5' 9-1/2"	165 (74.9)	2 yr

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These crews apply the dimethylamine salt of 2,4-D at a rate of 2 qt of 47.4% 2,4-D amine/50 gal water. The formulation of 2,4-D amine was 3.8 lbs 2,4-D acid equivalent per gallon (Southern Mill Creek Prod., Inc., Tampa, FL 33601) or a concentration in the finished spray of 0.038 lb AI/gal (4.6 gm AI/l). Eight ounces of Nalco-Trol® drift additive (Nalco Chemical Co., Chicago, IL 60638) was added to each 50 gal to control drift. While one crewman drove the airboat, the other applied 3-4 tanks over a 1 hr period. The men then changed places, so that each subject applied 2,4-D and operated the airboat for approximately 1 hr while exposure was monitored. 2,4-D was applied with a Bean® adjustable handgun with trigger grip. During the exposure period each man wore α-cellulose pads and a personal air sampler from which exposure estimates were later drawn. These methods have been described in detail (3). The α-cellulose pads were preextracted by tumbling 10 pads in 1 ℓ of methylene chloride for 10 min. Foam plugs were preextracted with 40 ml of methylene chloride followed by 40 ml of methanol as described below.

After a period of exposure, α-cellulose pads were placed in 1 pt Mason® jars. Personal air sampler exposure periods were timed with a sampler clock, and the foam plug retainers were then covered with aluminum foil. All samples were returned to the laboratory on ice and stored at -20°C for periods ranging from 0-48 hr prior to extraction.

The α-cellulose pads were sized to 2.5 in. x 2.5 in. with a paper cutter and then sectioned into quarters with scissors. Cutting surfaces were rinsed with methanol between samples. The quarters were placed in 8 oz jars and shaken 2X in 40 ml of methanol at 350 rpm for 10 min. The combined methanol extractions were taken almost to dryness at 40°C on a rotary evaporator and transferred into 10 ml of 1:1 acetonitrile:water. One ml of this extraction was reduced to dryness in a 10 ml centrifuge tube on a N_2 evaporator at 40°C. The remaining residue was transferred in 3 rinses of methanol to a 250 ml boiling flask for methylation.

Foam air sampler plugs were removed from their glass retainers with forceps and shaken 2X in 80 ml of methanol for 10 min at 350 rpm. The plug was squeezed with a pestle after each extraction and the methanol was decanted into a 250 ml boiling flask. The combined methanol extracts were taken almost to dryness at 40°C on a rotary evaporator. Ten ml of methanol was added for methylation.

An adaptation of the Sell and Maitlen (4) methylation method was used for GLC analyses. A glass boiling bead and 1 ml of BF_3 in methanol (Pierce Chemical Co., Rockford, IL 61105) was added to each flask. This solution was boiled gently on a hot plate until almost dry (solution darkens and white fumes appear). Ten ml of deionized water was added to the flask and it was allowed to cool to room temperature. Ten ml of isooctane was added, the flask was shaken for 30 sec, and the isooctane brought into the neck of the flask with deionized water. The isooctane was transferred to a brown glass storage bottle over anhydrous sodium sulfate. All samples were stored at -20°C. Recoveries (mean ± S.E.M.) of standard 2,4-D were: foam plug 90 ± 2% (10 μg level); α-cellulose pads 95 ± 2% (1 μg level). No interfering materials were detected in preextracted pads or foam plugs through the above procedure.

Twenty-four hr urine samples were taken from noon of each monitoring day until noon of the following day. One week after spraying stopped three consecutive 24-hr urine samples were collected. Urine samples were returned to the laboratory and stored at -20°C prior to extraction and derivatization. Urine samples were thawed, total volumes measured, and 15 ml aliquots placed into a 250 ml boiling flask. A glass bead, 15 ml of water, and 2 ml of 37% potassium hydroxide were added. This mixture was boiled on a hot plate for 30 min, allowed to cool, and transferred quantitatively to a 250 ml separatory funnel with 100 ml of 1:1 methanol:water. This mixture was extracted once with 25 ml of methylene chloride and the methylene chloride discarded. The solution was acidified with 2 ml of concentrated phosphoric acid and extracted three times with 25 ml of methylene chloride. The combined methylene chloride extracts were taken to dryness on a rotary evaporator at 40°C and the residue derivatized for gas chromatography as described above.

Blank and fortified urines were extracted and derivatized with each set of field urines. Also, 100 ng of standard 2,4,5-T (2,4,5- Trichlorophenoxyacetic acid, methyl ester) was added to each urine except for control "blanks." Internal 2,4,5-T was used to denote inefficient extractions and/or reactions. Fortified urines contained 1.5 µg of standard 2,4-D. Blank urines were blank throughout. Recovery of standard 2,4-D from fortified urine samples averaged 103 ± 3% (mean ± S.E.M.). Calculation of urinary 2,4-D included the correction for the ratio of M. W. 2,4-D methylester/2,4-D acid, i.e., 1.17.

Standard 2,4-D acid was obtained from Dow Chemical Co., Midland, MI 48640, with a listed purity of 99.9%. Standard 2,4-D methylester and 2,4,5-T acid were prepared by Nanogen, Inc., Watsonville, CA 95077.

Tracor 550 gas chromatographic conditions for the methylated product were: 1.8 m, 2 mm I.D. silanized glass column, 5% SP2100/1% SP2401 on 100-120 mesh Supelcoport®, 210°C injection, 165°C column, Ni^{63} electron capture 300°C, N_2 40 ml/min.

Methods for estimating specific body areas from body height and weight have been previously described (3).

RESULTS AND DISCUSSION

Table 2 contains the mean flux (µg/cm² hr) onto the exposure pads themselves, by pad location. Comparing these means through an F-test yielded no statistically significant differences among workers for any of the seven pad locations. Comparing the overall means by pad location yielded F=20.6, df=6/266, significant at the 99% level. Five exposure groupings, differing at the 99% level, emerge: shin (highest); thigh; wrist; forearm and hand; chest and shoulder; and back (lowest). For these groupings, hand accumulation rate, presented in Table 2 in mg/hr, was converted into flux by dividing by 900 cm², a representative value for the surface area of two hands. None of the three accumulation rates presented in Table 2 showed any significant difference among workers. Mean overall estimated total body exposure was 15 ± 2 mg/hr. The respiratory exposure estimate was 0.002 ± 0.001 mg/hr.

Table 2. Flux of 2,4-D on body pads and accumulation rates on body regions of Florida aquatic weed spray applicators.

	Subject				Mean	
	JD	CH	KP	SS	± SEM	n
Flux (µg/cm² hr) on various pads:						
Back	.07[a] ± .02[b]	.05 ± .03	.01 ± .01	.08 ± .04	.05[c] ± .01[b]	39[d]
Chest	.20 ± .06	.13 ± .03	.19 ± .04	.41 ± .11	.23 ± .04	39
Forearm	.62 ± .19	.16 ± .04	.71 ± .27	.52 ± .20	.50 ± .10	39
Shin	2.42 ± .81	1.53 ± .29	2.78 ± 1.09	2.83 ± 1.08	2.38 ± .42	39
Shoulder	.19 ± .07	.17 ± .05	.20 ± .11	.23 ± .09	.20 ± .04	39
Thigh	1.07 ± .21	.56 ± .12	1.12 ± .32	.78 ± .11	.88 ± .11	39
Wrist	.79 ± .26	.28 ± .06	.69 ± .17	1.08 ± .42	.61 ± .10	39
Accumulation rate (mg/hr) on body regions:						
Hands	.24 ± .04	.33 ± .07	.61 ± .11	.70 ± .30	.46 ± .08	39
Respiratory	.001 ± .001	.001 ± .000	.002 ± .001	.003 ± .002	.002 ± .001	38
Est. total body	16 ± 4	10 ± 2	17 ± 4	18 ± 5	15 ± 2	39

[a]Mean over sampling period, per subject.
[b]Standard error of the mean.
[c]Mean over sampling period, all subjects combined.
[d]Number of replications, all subjects combined.

F-test comparisons of % exposure to body areas showed no significant difference among workers, and these data were combined in Table 3. Approximately 74% of the estimated total body exposure was to the legs and feet. Arms (~10%) and hands (~8%) were next, followed by chest, head and neck, and back. Respiratory exposure, as a percentage of the estimated total body exposure, was low.

Table 3. Percent of estimated total body exposure of Florida 2,4-D aquatic weed spray applicators to their various body areas.

Body area	Mean % ± SEM	n
Head + neck	1.4[a] ± 0.1[b]	40[c]
Chest	5.8 ± 0.6	40
Back	1.2 ± 0.3	40
Arms	9.8 ± 1.0	40
Hands	8.4 ± 1.2	40
Legs + feet	73.6 ± 1.9	40
Resp.	.028 ± .009	38

[a]Mean percent of estimated total body exposure, all subjects combined.

[b]Standard error of the mean.

[c]Number of replications, all subjects combined.

Correlation of urinary 2,4-D with estimated total body exposure proved unsuccessful (Table 4). This difficulty is illustrated by subject CH. CH had the highest mean urinary excretion concentration and rate, but the lowest mean estimated total body exposure. The difficulty of correlating estimated 2,4-D exposure with urinary metabolites has been noted by Al-Jabery (5). Lavy et al (6) also had difficulty in correlating estimated exposure to urinary metabolites in 2,4,5-T applicators.

2,4-D is rapidly excreted by man. Sauerhoff et al. (7) recovered 87.6-106.3% of the ingested dose of 2,4-D in human urine over 144 hr. The elimination half-life ranged from 10.2 ± 0.4-28.4 ± 2.4 hr for a single 5 mg/kg oral dose. Kohli et al. (8) recovered 75% of a 5 mg/kg oral dose of 2,4-D in human urine over 96 hr after administration, with an elimination half-life of 33.0 ± 3.1 hr. Feldman and Maibach (9) recovered from human urine 100% of an i.v. dose of 2,4-D over a 120 hr period, the elimination half-life being 13 hr; however, only 5.8 ± 2.4% of a topical dose to the forearm was recovered in 120 hr. According to these authors, the variation observed in dermal penetration resulted from physiological variation among the subjects. This variation, combined with low dermal penetration, overlapping doses, and variable exposure estimates probably precludes any correlation of 2,4-D dermal dose and urinary 2,4-D in our study.

In the Lavy et al. (6) study, 2,4,5-T backpack sprayers were dermally exposed to 0.20-2.54 mg/kg and excreted 0.023-0.074 mg/kg 2,4,5-T. Dermal exposure for Florida 2,4-D handgun applicators ranged from 0.134-0.250 mg/kg and they excreted 0.0027-0.0086 mg/kg. Potential exposure for Florida applicators was about five times less, and excretion about ten times less.

Table 4. Mean urinary excretion of 2,4-D by subject, and correlation between daily urinary excretion and daily estimated total body exposure (ETBE).

	JU	CH	KP	SS
Mean ETBE (mg/hr) from Table 2	16[a] ± 4[b] (10)[c]	10 ± 2 (10)	17 ± 4 (10)	18 ± 5 (9)
Mean urinary 2,4-D (ppb)	116 ± 17 (10)	671 ± 198 (10)	404 ± 67 (8)	464 ± 161 (9)
Mean urinary 2,4-D (µg/24 hr)	190 ± 27 (10)	645 ± 160 (10)	315 ± 53 (8)	402 ± 120 (9)
Daily ppb 2,4-D vs. same day ETBE	+.44[d]	-.00	+.34	-.34
Daily µg/24 hr 2,4-D vs. same day ETBE	-.03	-.16	+.34	-.32
Daily ppb 2,4-D vs. previous day ETBE	-.08	+.76	-.96	-.36
Daily µg/24 hr 2,4-D vs. previous day ETBE	+.22	+.69	+.23	-.30

[a]Mean over sampling period.

[b]Standard error of the mean.

[c]Number of replications.

[d]Correlation coefficient.

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than for forest workers. The spray concentration of 2,4,5-T was 0.16 lb AI/gal compared to 0.038 lb AI/gal 2,4-D in this study. Under similar application conditions, the pesticide concentration apparently determines exposure level, a point also made by other authors (10, 11).

CONCLUSIONS

From the pattern of exposure observed here, a practical reduction would be afforded by the use of disposable coveralls and hand protection. Exposures reported here are low and would not appear to represent either acute or chronic hazard to Florida handgun 2,4-D applicators.

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Occupation and soft-tissue sarcoma in northeastern Italy

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The influence of occupation and exposure to different agents on the risk of developing soft-tissue sarcoma (STS) was assessed in a case-control study based on 93 cases of STS (53 men and 40 women) and 721 controls (371 men and 350 women), conducted in northeastern Italy. No risk elevation was found in subjects employed in agriculture (odds ratio [OR] for > 10 years = 0.8, 95 percent confidence interval [CI] = 0.4-1.5), nor in those who reported exposure to pesticides or herbicides (OR = 0.4, CI = 0.1-1.2). Similarly, neither occupation in the furniture, upholstery, and mechanics industries, nor exposure to livestock or meat processing, wood dust, metal dust, and dyes or paints were associated with STS risk. Workers who reported exposure to chemical agents or to benzene or other solvents for more than 10 years had, respectively, a 1.8-fold (CI = 0.7-4.4) and a 2.2-fold (CI = 0.9-5.5) higher risk of developing STS. Although the small number of STS cases limits the interpretation of the study results, these findings weigh against the hyphothesis that pesticides, herbicides, or other exposures related to agriculture, play an important role in the etiology of STS. The direct associations with exposure to chemical agents and benzene or other solvents, albeit not statistically significant, may provide a useful hint for future investigations.

Key words: Agriculture, benzene, case-control study, chemicals, Italy, occupation, soft-tissue sarcoma.

Introduction

Soft-tissue sarcomas (STS) are a group of rare and heterogeneous malignant neoplasms[1-3] whose etiology is still largely unknown.[4] Most of the epidemiologic studies have focused on STS risk associated with occupation in agriculture and related exposures, but inconsistent results have been reported.

Two Swedish case-control studies, conducted in the late 1970s and early 1980s, showed a nearly sixfold excess risk in individuals exposed to phenoxyacetic acid herbicides and chlorophenols.[5,6] An excess of STS also was noted among industrial workers exposed to herbicides[7] and among farmers in Washington State (United States).[8] Other studies, including a record linkage between occupational histories and the Swedish Cancer Environmental Registry[9] and a study on a cohort of licensed pesticide applicators,[10] did not confirm such association. Little information is available on the influence of occupations other than agriculture and related exposures on the risk of developing STS.[4,11,12]

In the present study, the role of agricultural and non-agricultural occupations and exposures in STS risk was assessed, using data from a case-control study conducted in northeastern Italy.

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Materials and methods

Since June, 1985, we have been conducting a hospital-based case-control study on risk factors for STS in the Friuli-Venezia Giulia region, northeastern Italy. Methods and results concerning the association between STS and nonoccupational risk factors (based on 88 cases and 610 controls) have been described elsewhere.[12]

Cases

The cases were histologically confirmed STS, diagnosed within two years before interview, who had been admitted as inpatients or referred for follow-up to the outpatient clinics of the Aviano Cancer Center or to the general hospitals in the study area. The present analysis is based on 93 cases (53 men and 40 women), aged 16 to 79 years (median age: 52 years), interviewed before June 1991. According to the classification of Enzinger and Weiss,[13] the most frequent cell type was sarcoma of the fibrous tissue (43 percent), followed by myomatus sarcomas (17 percent), liposarcomas (13 percent), and various other cell types (27 percent). Kaposi's sarcoma linked to human immunodeficiency virus infection and visceral sarcomas were excluded. All the histologic specimens were reviewed by one pathologist (A.C.).

Controls

The control group consisted of 721 patients (371 men and 350 women), aged 17 to 79 years (median age: 54 years), admitted to the study hospitals for a wide spectrum of diseases. Specifically excluded from the comparison group were all patients whose diagnoses at hospital admission included malignant disorders, conditions related to alcohol and tobacco consumption, or any disease which might have resulted in diet modification, *e.g.* disorders of respiratory and digestive tracts, cardiovascular diseases, diabetes, etc. Hematologic, allergic, or autoimmune diseases also were excluded. Twenty-eight percent of the controls were admitted for traumatic conditions (mainly fractures and sprains), 32 percent for musculoskeletal diseases (mainly low-back pain and disk disorders), 17 percent for minor conditions requiring surgical operations, and 23 percent for other illnesses such as disorders of the ear, nose, throat, skin, or teeth.

Questionnaire

Information was elicited on socioeconomic and anthropometric indicators including: education and social class (based on the head of the household's occupation);[14] smoking and consumption of alcohol, and of 14 selected food items; and medical history. The questionnaire also included information on the age at starting and stopping employment in 17 industries or occupations, on the subject's role in the industry or occupation in terms of direct involvement in production aspects, and on exposure to 15 occupational agents or groups of agents. Exposure histories were truncated, for both cases and controls, at the time of diagnosis.

Data analysis

For each occupation or exposure with at least five 'exposed' cases, odds ratios (OR), adjusted for age and sex by the Mantel-Haenszel procedure,[15] and their 95 percent confidence intervals (CI),[16] were computed. Sex- and age-adjusted tests for trend in risk were based on the Mantel chi-square test.[17]

Results

STS cases and controls were comparable regarding sex, age, education and social class (Table 1). The overall percentage of industrial manual workers among STS cases (45 percent) and controls (38 percent) were also similar (not shown in Tables).

Table 1. Distribution of cases of soft-tissue sarcoma and controls by sex, age, education, and social class. Pordenone, Italy, 1985-91

	Cases ($n=93$)		Controls ($n=721$)	
	No.	%	No.	%
Sex				
Male	53	57	371	52
Female	40	43	350	49
χ^2_1	0.80, $P=0.37$			
Age (years)				
≤ 39	21	23	179	25
40-49	17	18	118	16
50-59	23	25	165	23
60-69	19	20	168	23
≥ 70	13	14	91	13
χ^2_4	0.89, $P=0.93$			
Education (years)				
≤ 5	54	58	425	59
6-8	14	15	143	20
≥ 9	25	27	153	21
χ^2_1 for trend	0.53, $P=0.47$			
Social class				
I-II (highest)	7	8	34	5
III	27	29	211	29
IV-V	55	59	446	62
χ^2_1 for trend	0.73, $P=0.39$			
Other	4	4	30	4

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Table 2. Distribution of cases of soft-tissue sarcoma and controls by duration of various occupations or exposures, Pordenone, Italy, 1985-91

	Cases ($n=93$)				Controls ($n=721$)			
	1-10 Years		> 10 Years		1-10 Years		> 10 Years	
	No.	%	No.	%	No.	%	No.	%
Occupation[a]								
Agriculture	2	2.2	17	18.3	34	4.7	149	20.7
Chemical	1	1.1	1	1.1	2	0.3	1	0.2
Rubber	1	1.1	0	0.0	1	0.1	1	0.1
Dyestuff	0	0.0	1	1.1	4	0.6	1	0.1
Painting	0	0.0	0	0.0	6	0.8	6	0.8
Printing	0	0.0	0	0.0	2	0.3	1	0.1
Petroleum	0	0.0	0	0.0	2	0.3	0	0.0
Pharmaceutical	0	0.0	0	0.0	0	0.0	2	0.3
Furniture/upholstery	1	1.1	7	7.5	36	5.0	45	6.2
Asbestos	0	0.0	1	1.1	0	0.0	0	0.0
Coal/gas	2	2.2	0	0.0	5	0.7	2	0.3
Food processing	1	1.1	2	2.2	8	1.1	8	1.1
Mechanics	2	2.2	5	5.4	21	2.9	40	5.5
Leather tanning	0	0.0	0	0.0	2	0.3	0	0.0
Nuclear	0	0.0	0	0.0	0	0.0	0	0.0
Exposure								
Livestock/meat processing	2	2.2	10	10.8	12	1.7	58	8.0
Chemical agents	2	2.2	8	8.6	16	2.2	35	4.9
Gas	1	1.1	3	3.2	8	1.1	17	2.4
Metal dust	6	6.5	5	5.4	26	3.6	38	5.3
Plastic resins/glues	1	1.1	3	3.2	18	2.5	21	2.9
Oil	0	0.0	4	4.3	12	1.7	14	1.9
Radiation	0	0.0	0	0.0	1	0.1	2	0.3
Dyes/paints	4	4.3	4	4.3	32	4.4	32	4.4
Wood dust	5	5.4	6	6.5	47	6.5	46	6.4
Benzene/solvents	2	2.2	8	8.6	29	4.0	27	3.7
Asbestos	0	0.0	1	1.1	4	0.6	4	0.6
Electricity/radar	0	0.0	1	1.1	0	0.0	3	0.4
Coal tar	0	0.0	1	1.1	6	0.8	5	0.7
Herbicides/pesticides	3	3.2	3	3.2	29	4.0	56	7.8
Aluminum	2	2.2	0	0.0	13	1.8	14	1.4

[a] Only individuals employed in production aspects are included.

The distribution of STS cases and controls according to the duration of employment in major industrial occupations and reported exposure to various agents, or groups of agents, is provided in Table 2. Only occupations in agriculture, and the furniture or upholstery and mechanics industries, as well as exposures to livestock or meat processing, chemical agents, metal dust, dyes or paints, wood dust, solvents or benzene, and herbicides or pesticides were reported by at least five STS cases. The related ORs are given in Table 3.

No significant association of STS risk with occupation in agriculture (OR for >10 years = 0.8, CI = 0.4-1.5), or exposure to herbicides or pesticides (OR = 0.4, CI = 0.1-1.2) emerged (Table 3). Conversely, an approximately twofold increase in STS risk was noted among subjects who reported more than a 10-year exposure to chemical agents (different from pesticides or herbicides) (OR = 1.8, CI = 0.7-4.4) or to benzene or other solvents (OR = 2.2, CI = 0.9-5.5) (Table 3).

Analyses also were performed for men and women separately. Although no significant differences between sexes emerged, it should be noted that STS risk was somewhat higher in women exposed to benzene or other solvents for more than 10 years (OR = 3.7, CI = 0.7-17.0) than in men (OR = 1.8, CI = 0.6-5.6) (not shown).

Discussion

The present results provide further evidence against the possibility that employment in agriculture and, in

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Table 3. Odds ratios (OR)[a] and 95% confidence intervals (CI) for soft-tissue sarcoma for selected occupations and exposures,[b] according to duration. Pordenone, Italy 1985-91

Occupation or exposure	Duration of occupation or exposure				χ^2_1 (trend)
	<=10 years		>10 years		
	OR	CI	OR	CI	
Agriculture	0.4	0.1-1.7	0.8	0.4-1.5	0.79, $P=0.37$
Furniture/ upholstery	0.2	0.0-1.5	1.1	0.4-2.7	0.11, $P=0.74$
Mechanics	0.7	0.1-3.3	0.9	0.3-2.4	0.19, $P=0.66$
Livestock/meat processing	1.4	0.2-7.0	1.4	0.6-3.0	0.96, $P=0.33$
Chemical agents	1.1	0.2-5.6	1.8	0.7-4.4	2.13, $P=0.15$
Metal dust	1.8	0.6-5.0	0.9	0.3-2.6	0.13, $P=0.72$
Dyes/paints	0.9	0.3-2.9	0.9	0.2-2.7	0.07, $P=0.79$
Wood dust	0.8	0.3-2.3	0.9	0.3-2.4	0.09, $P=0.77$
Benzene/ solvents	0.5	0.1-2.2	2.2	0.9-5.5	1.90, $P=0.17$
Herbicides/ pesticides	0.7	0.2-2.5	0.4	0.1-1.2	3.14, $P=0.08$

[a] Nonexposed subjects as reference category; adjusted for age in quinquennia, and sex, using the Mantel-Haenszel procedure.
[b] Only occupations and exposures with at least five exposed cases.

particular, exposure to pesticides or herbicides play an important role in the etiology of STS. Because the study was based on only 93 cases of STS, there was insufficient power to evaluate all the subgroups—exposed to specific agricultural factors—that might be at risk. However, the study was able to exclude, at the level of statistical significance, an increased STS risk of greater than 20 percent from more than 10-years' exposure to herbicides or pesticides. This may be a useful contribution, considering the rarity of the disease and the recent debate on this topic.

In fact, the results of some epidemiologic studies seem to indicate that farmers have elevated risks for a few nonepithelial cancers, including STS.[18,19] However, the evidence remains inconclusive. A risk of 0.9 was found in a cohort of Swedish agricultural and forestry workers potentially exposed to phenoxy acid herbicides,[9] and a case-control study conducted in New Zealand was also negative.[22] STS risks lower than unity were found among agricultural and forestry workers and gardeners in a study based on 15 cancer registries in England and Wales.[21] Moreover, no evidence of increased STS risk emerged among professional pesticide applicators in the US[22] or in Germany.[23] A population-based case-control study of STS and malignant lymphoma from Kansas (US) showed a nearly sixfold higher risk of developing non-Hodgkin's lymphoma among herbicide users, but no increased risk for STS.[24]

In Italy, the association between STS risk and agricultural exposure to phenoxy herbicides has been investigated in one population-based case-referent study from an area where rice growing is a predominant agricultural activity.[25] The study, which included 68 cases (37 men and 31 women) and 158 controls, found a relative risk of 0.9 in men and a nearly threefold, nonsignificant, excess STS risk among women who potentially were exposed to phenoxy herbicides.[25]

Among nonagricultural occupations, an increased, albeit inconsistent, risk of STS among woodworkers has been reported.[11,26] One possible explanation for the reported excess STS risk among woodworkers is the frequency of exposure to phenoxy acid herbicides or chlorophenols in that occupation.[3,27] The present investigation, in agreement with other studies on woodworking and STS risk which showed negative results,[28,29] found no association either with occupation in the furniture or upholstery industry or with reported exposure to wood dust. The slightly increased risk of STS associated with livestock handling or meat processing, although not statistically significant, seems to be consistent with an excess STS risk among abattoir workers reported by Pearce *et al.*[30]

Eight STS cases (seven men and one woman) in this study reported an exposure to chemical agents for more than 10 years, with a nearly twofold, albeit nonsignificant, increased risk of STS. Three men reported exposure to sodium bicarbonate and two to ferrocyanide. Exposure to other chemicals (*e.g.* formaldehyde) found to be important in some studies on STS[31,32] was not documented in the present investigation. Similarly, STS cases exposed for more than 10 years to benzene or other solvents had a nearly twofold, although nonsignificant, increased risk of STS. Although benzene exposure was associated consistently with an elevated risk of acute leukemia,[33-35] the few studies which have investigated the association of benzene exposure with STS showed very modest, nonsignificant risk elevations.[36,37]

Both positive and negative results should be viewed with caution, considering the limits of the present study. Interview data were not validated with information from other sources, and questions were restricted to a selected series of general items. Importantly, not all potentially relevant occupations (*e.g.* leather industry[38] were well-represented in the study area and the overall number of exposed cases in most industrial occupations precluded detailed analy-

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ses of time-risk relationships and more precise estimations of ORs. It is unlikely, however, that these findings are affected seriously by bias, since there is little scope for supposing a differential recall between cases and controls for these broad categories of occupations and exposures. As yet, no hypothesis on STS etiology has gained sufficient attention in Italy to represent a major distorting factor. Moreover, a companion study of lymphoid neoplasms, based on the same design and data collection method, actually did find elevated risks among individuals employed in agriculture.[39,40]

In conclusion, the present study, which took place in an area where approximately one-quarter of the study population had a history of employment in agriculture, weighs against the possibility that herbicides and pesticides, or other exposures related to agriculture, play an important role in the etiology of STS. The ORs above unity for exposure to benzene or other solvents and chemicals agents, albeit statistically nonsignificant, may provide useful leads for future investigations.

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PESTICIDES AND CANCER RISKS IN AGRICULTURE

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The work environment in agriculture is complex, with many potentially hazardous exposures, but the overall mortality from cancer and other causes is rather low among farmers. However, several studies have consistently indicated an excess of certain cancer forms. Lymphomas, leukemias, multiple myeloma and also malignancies of connective tissue attract especial interest, as being possibly associated with the use of pesticides. Phenoxy acid herbicides may play an etiological role, especially for non-Hodgkin's lymphoma, whereas the findings are more ambiguous for Hodgkin's disease and soft-tissue sarcoma, perhaps indicating an interaction with co-factors. The issue has been controversial for many years, however, and one of its aspects involves the use of phenoxy acids in the Vietnam war. Furthermore, DDT has been associated with lung cancer in mixed exposure situations, and with chronic lymphatic leukemia. Arsenical pesticides may have caused skin cancer in vine-growers. Further studies, especially of specific user groups and producers, may avoid the complex exposure situation in agriculture.

Key words: **DDT, Phenoxy acids, Leukemia, Lymphoma, Sarcoma.**

INTRODUCTION

Agriculture means a complex work environment with exposures to dusts of organic and inorganic materials, micro-organisms including zoonoic viruses, pesticides, fertilizers and fumigants. Other pertinent exposures may relate to repair and maintenance of machinery and buildings, often implying heavy but short time exposures to agents like gasoline, oils, lubricants, paints and wood preservatives, mineral fibres, etc. Engine exhausts are inherent in modern agriculture, and other exposure to combustion products may derive from the burning of waste material, e.g. plastic sacks used for transportation of fertilizers or animal food. Usually, inhalation would be the most important route of exposure to these various agents, but skin absorption can also be considerable for some chemicals.

In spite of the many potentially adverse occupational exposures, that might be identified in the agricultural environment, the overall mortality of farmers tends to be low in comparison to that found for other occupational groups.[1,2,3,4] Tobacco and alcohol-related disorders especially, including cancer, seem to occur less frequently among farmers, as reported by several authors.[5,6,7] Many of the potentially adverse exposures that nowadays can be found in agriculture appeared only recently, and may therefore not yet have caused any clear effects on health and survival. The exposure to pesticides in agriculture nevertheless necessitates particular concern, since these agents exert strong biological activities and therefore have a potential also for adverse health effects. These involve cancer risks as subject to consideration in this review, especially from the epidemiological point of view and with emphasis on the controversial phenoxy acid herbicides.

PESTICIDES AND PESTICIDE EXPOSURE

In contrast to other chemicals (except drugs), pesticides are subject to special registration in many countries, after some sort of evaluation of the toxic properties. This procedure, together with recommendations for protection, might be thought of as hindering untoward effects on humans. At the time of registration, there has often been incomplete toxicologic information, however, especially on long-term effects. This point can be well illustrated by the fact that a working group at the International Agency for Research on Cancer (IARC), as late as in March 1987, had to conclude that the information on carcinogenicity from animal testing was inadequate for such widely used pesticides as the

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phenoxy acid herbicides 2,4-D and 2,4,5-T; for MCPA there were no data.[8]

Many years after their introduction on the market, several pesticides have become increasingly suspected of carcinogenic activity. Still, relatively few of these compounds have been labelled as carcinogens or as probably or possibly carcinogenic to humans, e.g. referred to as IARC groups 2A and 2B, respectively.[8] Some examples in this respect are shown in Table 1; none has been classified as carcinogenic without reservations (IARC group 1). Also, those compounds considered as possible or (especially) probable carcinogens may be thought of as being most likely to actually cause cancer in humans.

The uptake of pesticides by exposed workers has been studied in some instances and seems to be best assessed for phenoxy acids. Hence, urinary concentrations of workers exposed to phenoxy acids have been reported from many countries as summarized by IARC.[9] For small groups of spraymen, mean concentrations of up to 8 mg l^{-1} have been found as in forestry workers (for 2,4-D in tractor spraying;[10]). Spraying in farming was found to result in considerably lower levels, e.g. 0.2–0.3 mg l^{-1}. Professional spraymen may sometimes reach three to six times higher concentrations, however.[11]

Not surprisingly, aerial spraying is more favourable than tractor and other ground-spraying, with an approximately ten-fold reduction in exposure as reflected in the urinary herbicide concentrations. Still, aerial spraying tends to be less well accepted by the public, mainly for ecological reasons and especially with regard to spraying of forest land.

The contamination of clothes by pesticides has been common in the past, especially over the front of the thighs and legs. This is noteworthy, since the skin absorption seems to be particularly good from the thighs. A habit of combining suntanning with the spraying of pesticides of low acute toxicity seems to have been common in some countries, e.g. in Sweden, resulting in a wetting of the naked skin by phenoxy acids or other pesticides and probably leading to efficient absorption through the skin.

In clinical contacts with patients with alleged symptoms from pesticide exposure, one may also be told that clogged nozzles of the spraying equipment were sometimes cleaned by blowing with the mouth. This procedure apparently means another important route of exposure in some instances. Absorption of pesticides through the skin, and even by oral contact, is therefore sometimes likely to play a greater role than exposure by inhalation. As a consequence, the dose obtained by an individual may to a great extent depend on behavioural factors and vary considerably, even among individuals performing the same type of spraying. Only limited protection should therefore be expected through the standards set for air concentrations of pesticides in many countries.

Table 1. Some pesticides and pesticide exposures* considered by IARC, as grouped with regard to carcinogenicity to humans

Compound or exposure	Degree of evidence Humans	Animals	Group†
Amitrol‖	I	S	2B
Chlordecone (Kepone)§	ND	S	2B
Chlorophenols*‡	L	—	2B
Chlorophenoxy herbicides*‖	L	—	2B
Creosotes‡	L	S	2A
DDT§	I	S	2B
Dichlorobromopropane§	ND	S	2B
Hexachlorobenzene‡	ND	S	2B
Mirex§	ND	S	2B
Toxaphene§	I	S	2B

†Carcinogenic to humans (group 1), probably (group 2 A) or possibly (group 2 B) carcinogenic to humans. S = sufficient, L = limited and I = inadequate evidence of carcinogenicity. ND = no data. Information obtained from IARC monographs up to vol. 41, 1987 and supplement 7.[8]
‡Fungicide; wood preservative.
§Insecticide.
‖Herbicide.

OBSERVATIONS ON CANCER AND PESTICIDE EXPOSURE

The first reports more clearly associating a cancer risk with exposure to pesticides appeared in the 1970s. For example, Swedish railroad workers with a mixed exposure to amitrol, diurone, phenoxy acids, potassium chlorate, monurone and other agents were found to have suffered from significantly more cancers than expected, especially those who had a combined exposure to amitrol and phenoxy acids.[12,13] An excess of lung cancer was also seen in pesticide applicators in the DDR, again exposed to a mixture of preparations, both insecticides and herbicides.[14,15] A case series of soft-tissue sarcoma patients was reported by Hardell in 1977, suggesting exposure to phenoxy acids as playing an etiological role.[16] This clinical observation prompted epidemiologic studies in Sweden of soft-tissue sarcomas, carrying over also to a study on lymphomas.[17,18,19] and followed by investigations in other countries, as discussed below.

There has also been a number of cancer studies among farmers and other agricultural groups since the late 1970s, but with less specific information with

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regard to pesticide exposure. A review of most of these studies by Blair *et al.*[20] suggested that in spite of a generally low cancer morbidity among farmers, some cancer forms tend to quite consistently appear in excess, namely of the lip, stomach, skin (non-melanotic forms), prostate, brain and connective tissue, as well as Hodgkin's disease, multiple myeloma and leukemia. A decreased risk seems to be present for colorectal, bladder and lung cancer. Rather recently, additional studies have essentially confirmed this cancer pattern,[21,22,23] and some interest has also been directed to testicular cancer in some other studies, three out of four being positive in this respect regarding agricultural workers.[24,25,26,27]

Etiologically, solar radiation seems to explain the excess of lip and skin cancer, whereas physical activity may account for the low morbidity from colorectal cancer,[28,29] and the well-known lower smoking rate in rural populations may determine the decreased rate of bladder and lung cancer. For the latter, one may speculate that exposure to endotoxins in dust from hay and grain[30] may also play a preventive role.[31]

Stomach cancer may be thought of as relating to nitrates in food and water as a precursor of *N*-nitrosoamines. Interestingly, a large number of pesticides may react with nitrite to form *N*-nitroso compounds, e.g. atrazin, carbaryl, ferbam, glyphosate, MCPA (as an amine salt), simazine, thiram, ziram and several others.[32] However, in view of the latency required for cancer to develop, factors likely to have caused stomach cancer in rural areas must have appeared at an earlier point in time than the various pesticides used in agriculture. It is also of some interest in this context, that a rather recent report somewhat surprisingly shows an inverse association between nitrates and nitrites in saliva and stomach cancer rates.[33]

Some of the other increased cancer rates among farmers like those of the prostate and brain have no good explanations, although Blair *et al.*[20] in their review noted some relation of prostate cancer to poultry breeding and horticulture. Some studies seem to suggest pesticide use in agriculture as possibly playing a role in the etiology of brain tumours.[34,35,36]

A number of studies have considered leukemias and agricultural activities. As summarized by Blair *et al.*,[20] there is no consistent etiological explanation although poultry production seemed associated in some studies but not in others. Furthermore, in studies from the U.S. various leukemia forms have shown associations with farming, as involving insecticide use, but also dairy production, as well as the growing of crops. A tendency to a higher risk was seen for those born after 1900, a phenomenon suggesting factors in modern agriculture to be responsible. A simiar age-pattern has been found in some of the studies of multiple myeloma, and again, an increased risk appeared as possibly associated with pesticide use.[20]

A recent series of Swedish studies on acute myeloid leukemia, chronic lymphatic leukemia and multiple myeloma may help shed some further light on etiological factors for these disorders, however, especially as the exposure information is relatively detailed.[37,38,39] Hence, with regard to acute myeloid leukemia, there appeared a risk ratio of 2.3 for exposure to poultry but other determinants were also identified (see Table 2). For chronic lymphatic leukemia, a risk ratio of 6.0 was obtained for DDT exposure. Horse contact also appeared as a risk factor, and so did exposure to engine exhausts, all of these exposures being present in agriculture.

DDT has also appeared as a risk factor, although rather weak, in the aforementioned case–control studies on soft-tissue sarcoma and lymphoma in Sweden, as well as among a small group of forestry foreman,[40] and also in the U.S.[41] Some indications of an increased risk of lung cancer in relation to DDT exposure have also been obtained in a few studies with mixed pesticide exposures, which leads to a confounding of the situation, however.[15,42,43] Alveolar cell carcinoma has been reported in individuals with granulomatous disease of the lungs after inhalation of DDT powder.[44]

For multiple myeloma,[38] engine exhausts appeared as a determinant, in addition to creosote and contact with fresh wood, the latter exposure also being related to chronic lymphatic leukemia but only with borderline significance.[39] It is noteworthy that farming as an occupation did not remain as a risk factor, when these other determinants as occurring in farming were accounted for, i.e. engine exhausts, fresh wood and creosote. A puzzling observation that multiple myeloma seemed to correlate significantly negatively with background radiation, i.e. mainly tantamount to living in wooden houses, whether causally or spuriously, remains unsolved (whereas the chronic lymphatic and acute myeloid leukemias had no association and a positive relation, respectively). It also remains to be seen, however, whether other studies with as detailed exposure information will confirm or refute these various associations.

Some reports suggest arsenical pesticides as causing skin cancer in vineyard workers,[45,46] and an excess of lung cancer has been seen in pesticide producers with arsenic exposure[47] and users, although with mixed exposure.[15] Also, cases of liver angi-

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Table 2. Some significantly increased risk ratios, RR, by Mantel–Haenszel analyses* or logistic regression† regarding farming activities, acute myeloid leukemia (AML), chronic lymphatic leukemia (CLL) and multiple myeloma (MM)[37,38,39]

Disease	Risk factor	RR	95% CI	RR, other factors‡
AML	Poultry	2.3†	1.0–5.1	Electrical work: 3.8; psychotherapeutic drugs: 3.6; styrene: 18.9, three cases
CLL	DDT	6.0*	1.5–23	Engine exhausts: 2.2
	Horse	2.5*	1.2–5.1	(Fresh wood: 2.4; solvents 1.7)
MM	Creosote	4.7*	1.2–18	Engine exhausts: 2.1
	Fresh wood	2.6*	1.1–5.7	

‡RRs in parentheses with 95% CI including unity; for AML a dose–response pattern was also seen with regard to background radiation from building materials. MM with reversed association in this respect.

sarcoma have been reported as associated with arsenical pesticides.[48,49] The overall importance of arsenical pesticides in cancer causation in agriculture seems quite limited, however, since the use of such pesticides is restricted.

SOFT-TISSUE SARCOMAS, LYMPHOMAS AND PHENOXY HERBICIDES

As mentioned, studies with non-specific exposure information have indicated an excess risk of malignancies of the connective tissue, Hodgkin's disease and non-Hodgkin's lymphoma among farmers.[20] In this regard, the studies from Sweden[17,18] may be taken as a confirmation of the risk and as an elucidation the specific factors involved as showing a risk ratio of about 5–6, for soft-tissue sarcoma and phenoxy acid exposure alone, and a risk of 3–6 for chlorophenol exposure alone; taking phenoxy acids and chlorophenols either alone or together, the risk ratios were about 5. In one of the studies,[18] an attempt was made to distinguish the effect from phenoxy acids without tetrachloro-dibenzo-dioxin (TCDD) contamination, but effects were seen regardless of the possibility of this impurity's presence. However, the risk appeared to be higher when TCDD might have been involved.

Similarly, as for soft-tissue sarcomas, malignant lymphomas of the Hodgkin and non-Hodgkin type taken together were also found to be associated with exposure to phenoxy acids and chlorophenols, with risk ratios of about 5 and 4, respectively. In addition, there appeared to be an about three-fold risk as related to solvent exposure, especially involving tetra- and trichloroethylene, benzene and styrene.[19]

These Swedish studies have prompted a number of cohort and case–control studies to confirm or refute the possible associations of these malignancies and exposure to phenoxy acids and chlorophenols. Some results of the more important of these various studies are given in Tables 3–5, but a further comment may be justified in certain respects for some of the studies, especially regarding validity and power. Existing reviews may also be consulted for additional information and other aspects as also involving TCDD exposure.[66,67,68]

First, regarding the cohort studies, only the largest of these [52,55] would have some power to detect a risk of soft-tissue sarcoma as also appearing in one of them (Lynge, 1985).[52] The situation with regard to power is somewhat better for lymphoma although not found in any clear excess. The observation of nasal cancer in one of them[55] is of some interest, since nasal cancer has been associated especially with chlorophenol exposure, e.g. in sawmill work, wood dust being controlled.[69] An increased risk was also seen for phenoxy acid exposure, although not reaching significance. It may also be remarked that the latency periods elapsed so far since exposure in many of the studies, e.g. with regard to exposure in Vietnam, is still quite short. In the Swedish studies referred to, the average latency periods were 15–20 yr for the two disorders studied.[70]

The case–control studies listed in Table 4 may all be characterized as overall non-positive (except for the study on ovarial mesothelioma), but showing

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Table 3. Results in some cohort studies on phenoxy acid exposure, soft-tissue sarcoma (STS) and other cancer

Author and year	Character of population	Agents involved	Type of cancer(s)	Obs/expected or risk ratio; remarks
Hogstedt and Westerlund (1980)[40]	244 forestry workers	2,4-D; 2,4,5-T	All cancer	5/1.4 (foremen) 3/8.4 (workers)
Barthel (1981)[15]	1658 males in agriculture	2,4-D; MCPA and other pesticides	Lung cancer	50/27.5
Riihimäki *et al.* (1983)[50]	1926 herbicide sprayers	2,4-D; 2,4,5-T	All cancer	26/36.5 (no type in excess)
Lynge (1984, 1985)[51,52]	4563 chemical workers	(2,4,5-T) 2,4-D; MCPA and other	All ca STS Lung cancer	156/160.6 5/1.8 11/5.3
Ott *et al.* (1980)[53]	204 chemical workers	2,4,5-T	All cancer	1/1.3
Lawrence *et al.* (1985)[54]	1496 Vietnam veterans	2,4,5-T 2,4-D	STS	RR = 1.09 (95% CI, 0.18–6.7 vs non-Vietnam veterans)
Coggon *et al.* (1986)[55]	5784 users and producers	MCPA	All ca Nasal ca	297/314.0 3/0.61

Table 4. A ranking of risk ratios, RR, obtained in case–control studies of soft-tissue sarcoma and ovarial mesothelioma in relation to specific information of phenoxy herbicide exposure

Author	RR	Remarks
Donna *et al.* (1984)[56]	4.4	Ovarial mesothelioma
Woods *et al.* (1987)[57]	2.8	Scandinavians only
Vineis *et al.* (1986)[58]	2.7	Women with early exposure
Olsson (1987)[59]	2.4	Finland and Sweden
Smith *et al.* (1984)[60]	1.6	Probably or definitely exposed
Hoar *et al.* (1986)[61]	0.9	Use of 2,4-D on crops
Kang *et al.* (1986)[62]	0.8	Vietnam veterans
Greenvald *et al.* (1984)[63]	0.7	Vietnam veterans
Smith and Pearce (1986)[64]	0.7	RR = 1.1 by including earlier data from New Zealand

increased risk ratios for certain subgroups, e.g. for women with early and relevant phenoxy acid exposure.[58] A quite remarkable observation is reported by Woods *et al.*,[57] who in a study in Washington State found an increased risk among a subset of cases and controls with Scandinavian names. This finding together with the observations from Sweden[17,18] and Denmark[51,52] could indicate the operation of some genetic or cultural co-factor. It may be mentioned, however, that a record-linkage study using census data on occupation and cancer registry information in Sweden[71] failed to confirm the findings from the case–control studies. On the other hand, a British study based on data from cancer registration has shown a slightly but significantly increased risk of soft-tissue sarcomas among

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Table 5. A ranking of risk ratios, RR, obtained in case–control studies of Hodgkin's (H) and non-Hodgkin's (n-H) lymphoma in relation to specific information on phenoxy herbicide exposure

Author	H/n-H	RR	Remarks
Woods *et al.* (1987)[57]	n-H	4.8	Forestry herbicide applicators; 1.3 for farmers. No excess in Scandinavians
Hoar *et al.* (1986)[61]	H n-H	0.9 1.6	Exposure for more than 20 days/yr with RR = 6.0 for n-H
Pearce *et al.* (1986)[65]	n-H	1.3	Cancer controls; RR = 1.0 with population controls

farmers, farm managers and market gardeners but not in other subgroups in forestry and farming.[72] Based on occupational titles, the elevated risks seen in the Swedish case–control studies of soft-tissue sarcoma and lymphoma were reduced to 1.4 or less as indicated in Table 6.[70]

Regarding lymphomas, the aforementioned study from Washington State[57] indicate an increased risk for individuals in farming and forestry with exposure to phenoxy herbicides. A study from Kansas[61] strongly supports the findings from Sweden regarding non-Hodgkin's lymphoma with a clear and strong dose–response relationship, but, quite remarkably, no risk was seen either for Hodgkin's disease or soft-tissue sarcoma. The overall exposure situation may have been different to that in Sweden, however, with a resulting differential effect on the cancer pattern. Again, the observations with regard to ethnicity attract interest in view of the report by Woods *et al.*[57]

The studies from New Zealand have provided no clear support for any effect of phenoxy acid exposure with regard to soft-tissue sarcoma or non-Hodgkin's lymphoma, even if some slightly increased risk ratios appeared in the analyses of certain subgroups. Instead, an indication was obtained that meat work could be a risk factor both for soft-tissue sarcoma and non-Hodgkin's lymphoma.[60,65] Fencing appeared as another risk factor for non-Hodgkin's lymphoma.

CONTROVERSIES AND DISCUSSIONS

For regulatory purposes, evaluations have been made from time to time regarding the possible can-

Table 6. Exposure to phenoxy acids (Ph) and chlorophenols (Ch) of soft-tissue sarcoma (Eriksson *et al.*[18]) and lymphoma (Hardell *et al.*[19]) cases in comparison to referents

Type of cases; referents	Farming and forestry work			Other occupations		
	Ph	Ch	None	Ph	Ch	None
Soft-tissue sarcomas	13	1	17	1	10	68
Referents	5	3	39	0	5	167
Risk ratios	6.4	—	1.1	—	4.9	(1.0)
Lymphomas	35	5	37	6	15	71
Referents	23	1	114	1	7	189
Risk ratios	4.1	—	0.9	—	5.7	(1.0)

The risk ratios with regard to occupation (farming and forestry work) can be derived from the table and are 1.4 for soft-tissue sarcomas and 1.2 for lymphomas.

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cer effects of pesticides, and the possible carcinogenic effects of the phenoxy acids have become especially controversial. Most of the evaluations of this kind have not been published in the open literature and can hardly be considered as scientific in character, but rather as complying to economic interests. The resulting, quite problematic, situation has hardly been improved by the fact that the use of phenoxy acids in the Vietnam war has also led to alleged disorders in the Vietnamese population[67] and to compensation claims from veterans suffering from cancer and other illnesses. The issue became even more intricate as a result of falsifications of figures and facts from the published studies by a Royal Commission on the Use and Effects of Chemical Agents on Australian Personnel in Vietnam.[73,74,75] Hence, instead of making a serious and independent evaluation, this Australian commission simply copied several hundred pages of a submission, conclusions included, as compiled by lawyers representing a chemical company, namely Monsanto Australia.[76]

However, in contrast to the Australian approach, there are also some remarks on a scientific basis to the Swedish studies on soft-tissue sarcoma and lymphoma associated with phenoxy acids and chlorophenols. A couple of these comments might be considered here,[77,78] particularly with regard to the question of a possible misclassification of exposure as subject to concern, i.e. with a suspicion of either memory bias among the subjects regarding their exposure or a tendency by the investigators to ascribe exposure to the cases rather than to the controls. Now, in this critique, little attention seems to have been paid to the fact that measures were undertaken to avoid such a bias, i.e. the application of a special epidemiologic technique in two of the studies, that might have revealed a bias in this respect.[79,80] The principle can be seen in Table 6, where exposed and non-exposed subjects within the domain of farming and forestry work are compared to a reference outside this domain. Had there been an observation bias with regard to exposure, this would have meant an overstreaming of unexposed cases to the exposed category and/or of exposed controls or referents to the unexposed subjects, resulting in a decreased risk for non-exposed individuals within farming and forestry in comparison to the outside reference group. No such phenomenon appears, however, but the possibility remains that there is for some reason an increased risk among farmers and forestry workers, and that this increased risk in combination with an observation bias happens to be split up just the way it is, i.e. so that the non-exposed farmers and forestry workers get just the normal risk. It would be remarkable, however, with such a precise splitting up of a generally operating risk, not only in one but in two consecutive studies on this particular issue and for two different disorders. It is also difficult to explain the basis for any operation of a general risk as detached from herbicide exposure in these occupations. Furthermore, for chlorophenol exposure, there was a good agreement between the answers obtained in the questionnaires and the information given by the employers. Unfortunately, however, no employer data on exposure were available for phenoxy acids.

In view of the points just referred to, it is remarkable to see how the debate[78] on the possible cancer risks from phenoxy acids tends to accumulate various perceptions of bias, stemming from unidentified sources, and as witnessing that the original reports can hardly have been read with sufficient care. It should not be forgotten either, that if a significant recall bias had existed, studies on colon cancer and liver cancer undertaken in close connection with the soft-tissue sarcoma and lymphoma studies would also have reported an effect due to phenoxy acid and chlorophenol exposure.[81,82] The existence of specific information on the validity has also been acknowledged,[9] and further views on the scientific debate around the phenoxy acids can be found elsewhere.[66]

In view of the additional studies that have recently appeared on the scene, and as briefly reviewed above, the overall picture as to the effects of phenoxy acids remains somewhat confused. The results of the study by Hoar *et al.*[61] have now focused the interest on non-Hodgkin's lymphoma as a possible effect of phenoxy acids, but on the other hand the well-conducted studies from New Zealand do not provide much support for any effects at all. However, non-positive studies do usually not attract much criticism, and there is perhaps a reason in this context to make some remarks relating also to the validity of the studies from New Zealand.

First, it should be recalled that random misclassification (as for that matter systematical misclassification) of exposure might cause a bias towards a risk ratio of unity, and especially so if another risk factor is also operating. Therefore, it would have been desirable for exposure to both phenoxy acids and chlorophenols to have been more clearly displayed in the data. There is also an unexplained and remarkably increased risk for railway workers of 3.2 with regard to soft-tissue sarcoma,[60] at the same time as the percentage of railway workers among the controls is as high as 7.6% (7/92). A similarly high figure for railway workers is found in the study of non-Hodgkin's lymphoma,[65] i.e. 12 and 11% for

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cancer and population controls, respectively. Since the composition of a study population is supposed to be reflected by the controls in a case–control study,[83] the surprisingly high proportion of railroad workers raises the question whether there could have been some sort of selection bias with regard to the controls in these studies. However, there is no obvious reason why a bias in this respect should occur.

EPILOGUE

In an attempt to summarize the possible cancer effects from pesticides, it might be suggested that arsenic compounds are rather likely to be carcinogenic, as in good agreement with other observations on arsenic and cancer, although the studies on arsenic pesticides alone would be less convincing. DDT has also become more clearly, if not convincingly, associated with human cancer and there is sufficient evidence for carcinogenicity in animals. Therefore, even if the direct evidences for a cancer risk are rather limited in humans, the overall information seems rather convincing. The quantitative role of DDT for those cancer forms appearing in excess among farmers is not at all clear, however.

A definite evaluation of the carcinogenic effect of phenoxy acids seems to remain rather remote, since the epidemiologic studies appear as inconsistent but still without any clearly identifiable flaws. The conclusion at present might therefore be that these compounds are unlikely to be complete carcinogens and might even require particular circumstances to exert activity. Animal data do not provide much help for judgements on carcinogenicity as they are missing for MCPA and inadequate for 2,4,5-T and 2,4-D, even if some animal studies do exist that are indicative of a cancer risk.[9] A study from New Zealand, showing a significant relation between small-intestinal adenocarcinoma in slaughtered sheep and exposure to phenoxy acid or picolinic acid or both, through sprayed feedstuff, is quite remarkable.[8]

No particular guidance regarding the carcinogenic effects of phenoxy acids is obtained from studies of genotoxic effects such as chromosome aberrations, sister chromatid exchanges and micronuclei, since again, these studies are equivocal both in humans and in experimental systems.[8,85] It could be of some interest in this context, however, that both 2,4,5-T and 2,4-D are able to inhibit intercellular communication.[8] Furthermore, 2,4-D and MCPA can induce peroxisome proliferation in the liver of Chinese hamsters and decrease serum lipid levels,[86] i.e. similar to the drug clofibrate. Hepatic peroxisome proliferators are known to be carcinogenic, presumably through producing hydrogen peroxide and reactive oxygen radicals.[87,88] Judging from the existing knowledge based on experimental research as well as epidemiologic observations, and thereby taking the inconsistencies noted into consideration, it seems as if further investigations into phenoxy acids might very well help also the general understanding of carcinogenic mechanisms, namely if the somewhat puzzling pattern seen at present would turn out as a model for various interfering phenomena and co-factors in the process of carcinogenesis.

With regard to epidemiology, it seems rather clear, however, that cancer risks are associated at least with the use of mixtures of pesticides in agriculture, whereas the effects of isolated compounds are more difficult to assess. It may not necessarily be further studies on farmers, but rather on special groups of other users and producers, that will help assess more definitely the health hazards from pesticides, since the farmers may have a too complex exposure situation. It should also be a concern for the future, that even more potent herbicides seem to be under introduction at present, i.e. compounds of which only a few grams are needed per hectare of land, obviously making the hygienic control and the epidemiologic risk evaluations even more difficult than today.

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Phenoxy herbicides and soft-tissue sarcomas in female rice weeders

A population-based case-referent study

by Paolo Vineis, MD,[1] Benedetto Terracini, MD,[1] Giovannino Ciccone, MD,[1] Alessandro Cignetti,[2] Eva Colombo,[1] Adalberto Donna, MD,[3] Lidio Maffi,[1] Roberto Pisa, MD,[4] Paolo Ricci, MD,[5] Ermanno Zanini,[3] Pietro Comba, DSc,[6]

VINEIS P, TERRACINI B, CICCONE G, CIGNETTI A, COLOMBO E, DONNA A, MAFFI L, PISA R, RICCI P, ZANINI E, COMBA P. Phenoxy herbicides and soft-tissue sarcomas in female rice weeders: A population-based case-referent study. *Scand J Work Environ Health* 13 (1987) 9–17. A population-based case-referent study was conducted in an area of northern Italy where rice growing is the predominant agricultural activity and phenoxy herbicides have been used since 1950. Manual rice weeding was formerly performed by a seasonal female working population; in the early 1950s these women were concurrently exposed to chemical herbicides. Sixty-eight persons representing incident and histologically revised cases (31 women) and 158 population referents (73 women) were interviewed. The cases were histologically confirmed independently by two blinded pathologists, and exposure to phenoxy herbicides was assessed by two blinded pesticide researchers. An age-adjusted odds ratio of 0.91 was found for the living men (with suspect exposures; no man diagnosed as a case had been exposed with certainty to phenoxy herbicides). Among the living women the relative risk was 2.7 (90 % confidence interval 0.59–12.37), and it further increased when attention was restricted to women exposed in the whole 1950–1955 period and to younger age groups.

Key terms: case-control study, occupational exposure, rice growing.

Chlorinated phenoxy acids, namely, 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), 2,4-dichlorophenoxy acetic acid (2,4-D) and 2-methylchlorophenoxyacetic acid (MCPA), have been manufactured since the early 1940s and extensively used as herbicides in agriculture and forestry since the 1950s. A mixture of 2,4,5-T and 2,4-D (Agent Orange) was used as a defoliant during the Vietnam war.

An association between occupational exposure to phenoxy herbicides, both in their manufacture and use, and the subsequent occurrence of soft-tissue sarcomas has been suspected in some reports (2, 8, 9, 14, 18), but not confirmed by others (12, 27). A few studies have not been sufficiently informative (10, 21, 24, 26). (See also the reviews in references 1 and 6). The International Agency for Research on Cancer, chiefly on the basis of reports by Eriksson et al (8) and Hardell & Sandström (14), evaluated the evidence on the carcinogenicity of phenoxy acid herbicides to humans as limited (15).

Most of the aforementioned authors emphasized the need for further knowledge. Therefore we decided to undertake an investigation in the provinces of Vercelli, Novara, and Alessandria (northern Italy), where rice growing is the predominant agricultural activity. The total population in this area is approximately 1.5 million. Phenoxy herbicides were introduced in large amounts in the early 1950s and have been extensively used up to now. According to official statistics (16, 17), more than 4.8 t of 2,4-D and 21.7 t of MCPA were used in the three provinces in 1959 (data not available for 2,4,5-T). The corresponding figures for 1969 were 24.5 t for 2,4-D, 29.4 t for MCPA and 125.1 t for 2,4,5-T. 2,4,5-T was officially banned in 1970. Before the introduction of chemical herbicides, a female working population was seasonally engaged in rice weeding. For a period around 1950—1955 rice weeding was still done manually although chemical weeding was being experimented with. It is therefore reasonable to assume that the rice weeders working during this period were exposed to phenoxy herbicides, mainly through skin contact.

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Subjects and methods

Case identification and ascertainment

New cases of soft-tissue sarcoma with a proved or suspected histological diagnosis in 1981 – 1983 (age of person ≥ 20 years) were identified through all of the pathology departments of the three provinces (hospitals of Vercelli, Novara, Alessandria, Borgomanero, Biella, Casale Monferrato). The search was extended to six pathology departments in the city of Torino and to the National Cancer Institute in Milan, where residents in the three provinces could be referred for treatment. Overall, 135 potential cases were identified (116 in hospitals located in the three provinces and 19 in Torino or Milan). Visceral sarcomas were excluded.

Histological specimens (with the exception of the cases diagnosed in Torino and Milan and four other cases) were independently reviewed by two pathologists (AD, RP), blindly with respect to the exposure status and to previous diagnosis. The classification of the World Health Organization (WHO) was adopted for the soft-tissue sarcomas (7).

Of the 135 identified cases, 18 were excluded because the person's town of residence was unknown or the person was not traced after five attempts (table 1). After the histological review, 26 cases were discarded. For five others the two pathologists agreed on the diagnosis of soft-tissue sarcoma but did not agree on the histological type. After a check of the clinical records, four cases turned out to be visceral sarcomas and were excluded. For nine histologically confirmed cases, we met with refusal to participate, and for two in which the subject was deceased no relative could be traced. Of the remaining 75 cases, 68 were incident (ie, newly diagnosed in 1981 – 1983), and seven turned out to be prevalent (ie, diagnosed prior to 1981) during a check of the clinical records. The seven prevalent cases were not included in the analyses. Of the 68 subjects remaining, 44 were living and 24 were deceased.

The age-standardized annual incidence rate (including interviewed and noninterviewed subjects and excluding prevalent and visceral cases) was 2.8/100 000 [95 % confidence limits (CL) 1.3—4.2] for the men and 2.0 (95 % CL 0.8 – 3.1) for the women (age ≥ 20 years). These rates do not take into account the histological revision in order to be comparable to the rates reported in 1976 – 1977 by the Cancer Registry of the Province of Varese (an area close to the study area). The latter rates (per 100 000, age ≥ 20 years) were 2.3 (95 % CL 0.4 – 4.1) for the men and 1.5 (95 % CL 0.06 – 2.9) for the women (23). (All the rates have been age-standardized on the basis of the world population.)

Table 1. Distribution of cases and referents by categories of inclusion/exclusion.

	Cases	Referents
Originally enrolled	135	208
Excluded or lost		
Residence unknown	10	•
Untraced individual	8	20
Refusal[a]	9	26
Relative not traced[b]	2	–
Change of residence[c]	1	4
Histologic diagnosis not confirmed	26	•
Visceral sarcoma[d]	4	•
Prevalent case[d]	7	•
Histologically confirmed and interviewed nonvisceral cases	68	•
Interviewed referents	•	158

[a] Including individuals not available for interview due to illness, deafness or mental disorders.
[b] Relatives of deceased individuals were not available.
[c] Moved to other provinces; in strict sense not eligible.
[d] The four visceral and seven prevalent sarcomas were identified as such after a check of the clinical records after the cases had been enrolled. (See the text.)

Living referents

A random sample of eligible referents (30 living men and 30 living women aged ≥ 20 years from each province) with a distribution, by municipality, representative of the population of each province was drawn from electoral rosters. The overall number of referents was approximately twice the expected number of eligible cases. In fact, a total of 168 individuals from 65 municipalities were identified versus 180 expected. (Four electoral offices did not respond at all, and a few offices provided a lower than required number of individuals, for a total of 12 losses.)

Deceased referents

Thirty-seven of the 135 persons originally enrolled as cases were deceased at the time of the interview. Therefore, for each of these 37 persons, two deceased individuals, to be matched to the deceased subject by age (± 4 years), sex, and year of death, were identified as referents from the same municipality as the deceased subject through the demographic offices of the municipalities. There was no selection of the cause of death, with the exception of the exclusion of one suicide (for ethical reasons).

Seventeen deceased subjects with soft-tissue sarcoma had two matched referents. Only one referent met the matching criteria for six cases. No individual with the corresponding requirements was found for five cases. The municipalities did not respond for eight referents. And, finally, no referent was sought for five persons with soft-tissue sarcoma because they were enrolled in the National Cancer Institute in Milan after the scheduled end of the study. A total of 40 deceased referents were therefore known for interview purposes, and in fact the relatives of 36 were interviewed.

Interviews

Each enrolled subject was given a code number unrelated to the nature of their case-referent status. All subsequent procedures until the final stage of the analysis were blind. Each living subject was mailed an invi-

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ation to be interviewed, with no mention of the precise purpose of the study. Whenever possible, the invitation was followed by telephone calls in an attempt to schedule an interview date. Otherwise, additional letters inviting the individual to suggest a possible date for the interview were sent with a prepaid envelope inside. For each individual, at least five attempts (either by telephone or by a home visit by the interviewer) were made before the individual was considered untraceable. For deceased persons the next-of-kin was identified, and the same procedure was followed.

A simplified postal questionnaire was sent to subjects who, for any reason, refused a direct interview but agreed to provide information by mail. Overall, information on 16 cases and 37 referents was obtained in this way. The postal questionnaire was suitable for an exposure assessment identical to that provided by the direct interviews.

All the interviews were carried out by a trained interviewer (LM). The questionnaires collected (i) demographic information, (ii) a life-long smoking history, (iii) a life-long occupational history (including job titles, addresses of the plants or farms, and types of products or crops), (iv) a history of radiological therapeutic treatment, and (v) a history of residence changes, including a specific question on periods spent in rice-growing areas. In addition the questionnaire included a list of job titles potentially involving exposure to phenoxy herbicides or chlorophenols (including jobs in sawmills, the paper and leather industries, highway and railway maintenance, and flower and market gardening). Two additional sheets were filled out for those who had worked in agriculture, one for rice growing and one for other crops. In these sheets information was collected on the involvement in jobs entailing the handling, transportation, and distribution of herbicides; herbicide spraying procedures; other specific jobs involving (or not) herbicide exposure (ie, weeding and water regulation in rice growing); knowledge of exposure to herbicides (and more specifically to phenoxy acids or chlorophenols) or insecticides. This part of the questionnaire was prepared in cooperation with pesticide researchers.

Demographic information (including current occupation and educational level) for nonrespondents was collected from demographic offices of the municipalities of residence.

Assessment of exposure

Two experts with experience in chemical aspects of agriculture (EZ, AC) were asked to assess the exposure to phenoxy acids for each job recorded in the interviews. This assessment was done independently by the two and in a blind manner. The following criteria were used for reading and interpreting the questionnaires: category 1 = not exposed, category 2 = exposure could not be ruled out, and category 3 = certainly exposed. Attempts to distinguish between skin contact and other paths of exposure and between low and high levels of exposure proved not to be feasible. Rice weeders were considered to be exposed to phenoxy herbicides when they worked after 1950 and did not work exclusively in a small rice allotment of their own. Category 2 was used particularly for people engaged after 1950 in corn, wheat and pasture growing. As a first step, a separate exposure assessment was performed by each of the two experts. Subsequently, a joint assessment was made, in which they reached a common evaluation for each recorded job. The latter assessment was used in the analyses.

Herbicide spraying in the area has been chiefly done by tractor.

Coding of occupational histories

In addition to the aforementioned exposure assessment, all occupational histories were coded according to the classification for job titles of the International Labour Organization (4) and the United Nations classification for economic activities of plants or farms (22). All coding work was done by a single person (GC) in a blind manner.

Analyses

The analyses were performed with an HP-61 calculator using programs for epidemiology (25). Age-adjusted relative risks were computed according to Mantel-Haenszel's estimator (19); 90 % confidence intervals were obtained with Miettinen's approximate procedure (20) (the lower limit of each interval corresponding to a 95 % one -tailed confidence limit). In addition to age (in four groups), therapeutic X rays and smoking were included as potential confounders. The X-ray exposure represents a known risk factor for soft-tissue sarcoma (3), but there is no evidence of association with herbicide exposure. Smoking is not known to be a risk factor for this kind of tumor, but it was conservatively included as a potential confounder.

Results

Response and characteristics of the subjects

Table 2 reports the educational levels represented by the persons with soft-tissue sarcoma broken down into respondents and nonrespondents. The presented educational levels are those of the persons scheduled for interview (ie, the next-of-kin of deceased subjects). Responding men showed an unbalanced distribution for the cases and referents in the different educational categories (< 6 years: 26 cases and 37 referents, ≥ 6 years: 10 cases and 43 referents; unknown: 1 case and 5 referents); the distribution of the women was more balanced (< 6 years: 17 cases and 42 referents; ≥ 6 years: 12 cases and 30 referents; unknown: 2 cases and 1 referent).

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Table 2. Educational levels (years of schooling) for the responding and nonresponding living subjects or the next-of-kin of deceased subjects. Nonresponding individuals include refusals, untraced individuals, individuals whose relatives were not identified, and individuals who had changed residence.

Years of schooling	Living subjects				Next-of-kin of deceased subjects			
	Nonrespondents		Respondents		Nonrespondents		Respondents	
	Cases	Referents	Cases	Referents	Cases	Referents	Cases	Referents
<6	10	18	28	56	4	3	15	23
≥6	1	11	14	65	–	1	8	8
Unknown	5	13	2	1	–	4	1	5

Table 3. Distribution of the living subjects by age group and exposure level (1 = not exposed, 2 = exposure could not be ruled out, 3 = exposed). Exposure refers to any calendar period. The number of subjects for whom information came from postal questionnaires is presented in parentheses.

Exposure level	Age group (years)							
	<55		55–64		65–74		≥75	
	Men	Women	Men	Women	Men	Women	Men	Women
Category 1								
Cases	7 (3)	5 (2)	6[a] (1)	1	1	6 (3)	7 (1)	4 (2)
Referents	36 (8)	36 (10)	12 (2)	6 (2)	5	9 (5)	1 (1)	2 (1)
Category 2								
Cases	1 (1)	–	1	–	–	–	–	1 (1)
Referents	4	1	2	1	–	–	–	–
Category 3								
Cases	–	–	–	1	–	3 (1)	–	–
Referents	2	2	–	2	–	1	–	–

Table 4. Distribution of the living female subjects by age group and exposure level (1 = not exposed, 2 = exposure could not be ruled out, 3 = exposed). Only women exposed continuously during 1950–1955 have been included.

Exposure level	Age group (years)			
	<55	55–64	65–74	≥75
Category 1				
Cases	5	1	6	4
Referents	36	6	9	2
Category 2				
Cases	–	–	–	–
Referents	–	–	–	–
Category 3				
Cases	–	1	2	–
Referents	–	1	–	–

Among the men 26 (30.5 %) of 85 referents and 15 (40.5 %) of 37 subjects with soft-tissue sarcoma had worked in agriculture during at least some period of their worklife. The women with such occupations were 21 (28.5 %) of 73 referents and 14 (45.5 %) of 31 women with soft-tissue sarcoma. Two male referents and no men with soft-tissue sarcoma were assigned a job title code of rice grower. The respective numbers for the women were three and three (crude odds ratio 2.5). This code was the only job title code mentioning rice growing, and it was assigned when rice growing was the only activity performed in a given work period.

Living subjects

Table 3 reports the absolute numbers of living subjects with soft-tissue sarcoma and their referents by exposure levels and age groups for the two sexes. No men with soft-tissue sarcoma were definitely exposed, and exposure could not be ruled out for only two, the age-adjusted odds ratio being 0.91 (90 % CL 0.21–3.91).

Among the alive women, four subjects with soft-tissue sarcoma and five of their referents were certainly exposed, and one and two, respectively, belonged to exposure category 2. This distribution corresponds to an odds ratio of 2.42 (90 % CL 0.56–10.34) when exposure categories 2 and 3 are combined and an odds ratio of 2.70 (90 % CL 0.59–12.37) for category 3 alone. The odds ratios were age-adjusted in the four age groups.

Table 4 shows the distribution of the living female subjects who were engaged in relevant jobs in the whole 1950–1955 period. Three with soft-tissue sarcoma and one referent met these criteria, all from exposure category 3. Unexposed were 16 women with soft-tissue sarcoma and 53 female referents, as shown in table 3.

Deceased subjects

Table 5 reports the age and exposure distribution for the deceased subjects. There were 24 next-of-kin of persons with soft-tissue sarcoma interviewed and 36 next-of-kin of referents. Three women and no men with soft-tissue sarcoma belonged to exposure category 3, and one woman and one man with soft-tissue sarcoma were classified into category 2. The odds ratio (age-adjusted in two age groups) for the women in exposure categories 2 and 3 was 1.05 (90 % CL 0.21–5.1). When matched pairs or triplets were considered, there

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iving subjects or the next-of-kin of
luals whose relatives were not iden-

of-kin of deceased subjects		
idents	Respondents	
eferents	Cases	Referents
3	15	23
1	8	8
4	1	5

posed, 2 = exposure could not be
s for whom information came from

-74		≥75			
Women		Men		Women	
6	(3)	7	(1)	4	(2)
9	(5)	1	(1)	2	(1)
–		–		1	(1)
–		–		–	
3	(1)	–		–	
		–		–	

oups for the two sexes. No men
ma were definitely exposed, and
: ruled out for only two, the age-
eing 0.91 (90 % CL 0.21 – 3.91).
vomen, four subjects with soft-
'e of their referents were certainly
d two, respectively, belonged to
This distribution corresponds to
(90 % CL 0.56 – 10.34) when ex-
nd 3 are combined and an odds
CL 0.59 – 12.37) for category 3
os were age-adjusted in the four

distribution of the living female
gaged in relevant jobs in the whole
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to category 2. The odds ratio (age-
groups) for the women in expo-
3 was 1.05 (90 % CL 0.21 – 5.1).
s or triplets were considered, there

Table 5. Distribution of deceased subjects by age group and exposure status. The number of subjects for whom information came from postal questionnaires is presented in parentheses.

Exposure status	Age group (years)							
	<55		55–64		65–74		≥75	
	Men	Women	Men	Women	Men	Women	Men	Women
Unexposed								
Cases	5	1	5	1	2 (1)	1	1	3
Referents	5 (4)	–	6	2	5 (2)	1	1	4
Exposed[a]								
Cases	–	–	–	–	–	1	1	3
Referents	1	–	2	–	–	–	3 (1)	6 (1)

[a] Categories 2 (exposure could not be ruled out) and 3 (exposed) have been combined under this heading.

were only two: One was a triplet in which the subject with soft-tissue sarcoma and the two referents had been definitely exposed to phenoxy herbicides (category 3), and the second was a triplet in which one of the two referents (but not the sarcoma subject) belonged to category 3. Therefore a matched analysis of deceased female subjects was uninformative.

When the towns of residence of the deceased referents were compared with those of the living referents, important differences appeared. Six of the 23 deceased male referents lived in four towns of rice-growing areas versus 3 of 62 living referents. Among the women, 6 of 13 deceased referents lived in two towns with the same characteristics, versus 2 of 60 living referents. This comparison strongly suggests that overmatching of deceased subjects was introduced into the study by the inclusion of residence as a matching factor.

Confounding

Therapeutic X rays and smoking were considered as potential confounders. Overall, only two men and one woman with soft-tissue sarcoma, and one male and no female referents had been exposed to therapeutic radiation before 1981. Confounding by this variable, therefore, can be ruled out.

As for smoking, 24 (65 %) of 37 men and 3 (11 %) of 28 women with soft-tissue sarcoma and 54 (65 %) of 83 male referents and 21 (40 %) of 52 female referents were smokers or exsmokers. (For two male referents information on smoking was not available.) These findings indicate a clear deficit of smokers among the women with soft-tissue sarcoma. Such a pattern can be interpreted as a consequence of the greater proportion of agricultural workers among the persons with soft-tissue sarcoma.

Histology and site of the sarcomas

Table 6 reports the distribution of the cases by histological type according to the WHO classification. Table 7 gives the histological type and site of the tumors for the exposed cases. Among the exposed women with soft-tissue sarcoma (categories 2 and 3 of exposure), there were two chondrosarcomas, two Kaposi sarcomas, two malignant fibrous histiocytomas, one chordoma, and one leiomyosarcoma. (One case was uncertain with respect to histological type.) No gross differences were noticed between the exposed and unexposed women with respect to the sites of the tumors. Their locations were as follows: head and neck (0 exposed/3 unexposed), retroperitoneal (1 exposed/2 unexposed), upper limbs (1 exposed/4 unexposed), lower limbs (3 exposed/7 unexposed), thorax (1 exposed/3 unexposed), abdomen (1 exposed/1 unexposed), pelvis (2 exposed/1 unexposed), site not specified (0 exposed/1 unexposed).

Table 6. Distribution of the interviewed incident histologically revised cases by histological type, according to the classification of the World Health Organization.

Histological type	Men	Women
Dermatofibrosarcoma protuberans	1	–
Liposarcoma	2	1
Myxoid liposarcoma	3	3
Leiomyosarcoma	4	2
Rhabdomyosarcoma	1	1
Angiosarcoma	1	–
Malignant hemangio pericytoma	1	–
Other vascular malignant tumors	–	1
Synovial sarcoma	1	1
Malignant schwannoma	3	1
Other malignant tumors of the peripheral nerves	2	–
Malignant tumors of nonchromaffin tissue	–	1
Chordoma	–	1
Chondrosarcoma of soft parts	2	3
Malignant giant cell tumor of soft parts	1	–
Malignant fibroxanthoma (malignant histiocytoma)	3	5
Kaposi sarcoma	7	6
Uncertain	1	4
Total[a]	33	30

[a] The original diagnosis of the five interviewed incident cases which were not reviewed were one dermatofibrosarcoma protuberans, one angiosarcoma, one malignant histiocytoma, and one Kaposi sarcoma among the men and one malignant schwannoma among the women. These cases were included in the analyses because of the confidence of the high level of the histological diagnosis. One of the men belonged to exposure category 2, while the others were unexposed.

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Table 7. Sex, age, exposure category, job title, years of exposure, and histology and site of tumor (if a case) for the exposed subjects with soft-tissue sarcoma (cases) and the exposed referents.

Case/referent status	Sex	Age (years)	Exposure category[a]	Job title	Years of exposure	Histology and site of tumor
Living case	Female	84	2	Rice weeder	Unknown	Uncertain histology, lower limb
Living case	Female	71	3	Rice weeder	1950 – 1963	Kaposi, upper limb
Deceased referent	Female	77	3	Rice weeder	1950 – 1958	·
Living case	Male	55	2	Rice weeder	1950 – 1984	Dermatofibrosarcoma protuberans, site unknown
Deceased referent	Male	62	2	Farm laborer in rice growing	1950 – 1952	·
Deceased referent	Female	82	3	Rice weeder	1950 – 1960	·
Deceased referent	Female	84	3	Rice weeder	1958 – 1971	·
Deceased referent	Male	86	2	Wheat, corn and pasture grower	1950 – 1974	·
Deceased referent	Male	43	3	Water regulator in rice fields	1954 – 1966	·
Living case	Female	74	3	Rice weeder	1950 – 1967	Chondrosarcoma, lower limb
Deceased referent	Male	79	2	Wheat and corn grower	1950 – 1971	·
Deceased referent	Female	77	3	Rice weeder	1950 – 1960	·
Deceased referent	Female	79	3	Rice weeder	1950 – 1970	·
Deceased referent	Female	81	2	Corn and wheat grower	1950 – 1967	·
Deceased referent	Male	62	2	Wheat, pasture and corn grower	1950 – 1970	·
Living referent	Male	54	2	Flower gardener	1950 – 1984	·
Living referent	Female	56	2	Corn and pasture grower	1952 – 1984	·
Deceased case	Female	79	2	Wheat and corn grower	1950 – 1980	Chordoma, pelvis
Living referent	Male	47	3	Rice grower	1960 – 1984	·
Living referent	Female	52	3	Rice weeder	1963 – 1968	·
Living referent	Female	64	3	Rice weeder	1960 – 1971	·
Living referent	Male	44	3	Rice weeder	1955 – 1968	·
Deceased case	Male	88	2	Wheat and corn grower	1950 – 1980	Leiomyosarcoma, upper limb
Living referent	Female	69	3	Rice weeder	1955 – 1970	·
Deceased case	Female	77	3	Rice weeder	1950 – 1966	Malignant fibrous histiocytoma, pelvis
Living case	Female	73	3	Rice weeder	1961 – 1963	Chondrosarcoma, thorax
Deceased case	Female	84	3	Rice weeder	1950 – 1960	Kaposi, lower limb
Living case	Male	64	2	Wheat and corn grower	1960 – 1970	Liposarcoma, head-neck
Living referent	Female	62	3	Rice weeder	1950 – 1978	·
Deceased case	Female	75	3	Rice weeder	1950 – 1970	Malignant histiocytoma, retroperitoneal
Living case	Female	60	3	Rice weeder	1950 – 1963	Leiomyosarcoma, abdomen
Living referent	Female	47	2	Market gardener	1974 – 1984	·
Living referent	Female	50	3	Wheat, corn and pasture grower	1970 – 1984	·
Living referent	Male	65	2	Wheat, corn and pasture grower	1975 – 1983	·
Living referent	Male	47	2	Wheat, corn and pasture grower	1978 – 1983	·

(continued)

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te of tumor (if a case) for the exposed

ars of posure	Histology and site of tumor
known	Uncertain histology, lower limb
50 – 1963	Kaposi, upper limb
50 – 1958	·
50 – 1984	Dermatofibro-sarcoma protube-rans, site unknown
50 – 1952	·
50 – 1960	·
58 – 1971	·
50 – 1974	·
54 – 1966	·
950 – 1967	Chondrosarcoma, lower limb
950 – 1971	·
950 – 1960	·
950 – 1970	·
– 1967	·
950 – 1970	·
1950 – 1984	·
1952 – 1984	·
1950 – 1980	Chordoma, pelvis
1960 – 1984	·
1963 – 1968	·
1960 – 1971	·
1955 – 1968	·
1950 – 1980	Leiomyosarcoma, upper limb
1955 – 1970	·
1950 – 1966	Malignant fibrous histiocytoma, pelvis
1961 – 1963	Chondrosarcoma, thorax
1950 – 1960	Kaposi, lower limb
1960 – 1970	Liposarcoma, head-neck
1950 – 1978	·
1950 – 1970	Malignant histiocytoma, retroperitoneal
1950 – 1963	Leiomyosarcoma, abdomen
1974 – 1984	·
1970 – 1984	·
1975 – 1983	·
78 – 1983	·

(continued)

Table 7. Continued.

Case/referent status	Sex	Age (years)	Exposure category[a]	Job title	Years of exposure	Histology and site of tumor
Living referent	Male	30	2	Wheat, corn and pasture grower	1967 – 1984	·
Living referent	Male	47	2	Corn and pasture grower	1953 – 1957 1970 – 1982	·
Living referent	Male	63	2	Wheat and corn grower[b]	1959 – 1984	·
Deceased referent	Male	77	3	Farm laborer in rice growing	1950 – 1968	·

= exposure could not be ruled out; 3 = exposed.
Also engaged in railway maintenance (1953 – 1978).

Table 7 reports the ages at the time of the interview, vital status, job titles, years of start and cessation of exposure, and the level of exposure (as assessed by experts) for the exposed subjects, in addition to the histological types and tumor sites of those with soft-tissue sarcoma.

Exposure of persons with prevalent cases of soft-tissue sarcoma and those with visceral sarcomas

Of the five women interviewed who were considered to have prevalent cases of soft-tissue sarcoma, four were unexposed and one was definitely exposed, and they belonged to the 55- to 64-year age group. Two persons with visceral sarcoma were interviewed; they were both unexposed.

Discussion

Testing the hypothesis of an association between exposure to phenoxy acids in agricultural practice and soft-tissue sarcomas required a study to be carried out (i) on a population basis and (ii) in an area where phenoxy acids have been widely used. The present study meets both these requirements.

The most important exposures took place in the early 1950s, due to the concurrent presence of manual weeding and chemical spraying. Indeed, an increased risk of soft-tissue sarcoma was shown among the female rice weeders who had been employed in the early 1950s. Exposure among the men was negligible.

Although the present study is limited by a low power, on qualitative grounds our findings are consistent with both those obtained by Hardell & Sandström (14) and Eriksson et al (8) in similar population-based studies and with those of reports on small series of cases among workers exposed to chlorophenols and/or their derivatives in the chemical industry (9, 18).

Studies failing to suggest an association either (i) selected referents from cancer patient files (2) or adopted an approach involving proportional mortality ratios, which implied some loss in sensitivity (10, 21), or (ii) used a crude categorization of exposures, in terms of occupation at the time of diagnosis (2) or at the time of death (21). A misclassification of exposure may lead to an underestimation of the relative risk, if not a systematic bias (28).

Intensity and duration of exposure should also be kept in mind when different studies are compared, although no clear picture of the levels of exposure in different jobs and different countries is available.

The statistical power of the present study is low since we had planned a sample size suitable for increases in risk of the same order of magnitude of those reported by Swedish authors (8, 14). This outcome is reflected by the large confidence intervals around the relative risks. The one-tailed lower confidence limit was, in most instances, lower than one. Indeed, the target population (female rice weeders affected by soft-tissue sarcomas) was inherently limited in size.

Major biases were prevented.

The similarity of the incidence rates of soft-tissue sarcoma in the study area and in the near province of Varese suggests that case ascertainment was likely to be complete. The analysis was restricted to histologically revised incident cases. Population referents were selected from electoral rosters. Respondence was reasonable. (Nonrespondents were 9 of 80 for the confirmed incident cases and 26 of 208 referents.) Selection bias is therefore a remote explanation of the findings. As table 8 suggests, almost all of the exposed living females were in fact in the lowest educational level (relative risk in the category <6 = 3.86, 90 % CL 0.87 – 17.15). The interviewer was thoroughly trained, all questionnaires were coded blindly with respect to caseness, and exposures were blindly assessed by expert pesticide researchers.

However, the relative risks may have been underestimated because of the following three types of problems:

1. A median latency time of 15 years has been estimated for soft-tissue sarcomas after exposure to chemical agents (13); Fingerhut et al (9) reported figures in the range 9 – 29 years. Thus a proportion of the cases induced in rice weeders by exposures in the

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Table 8. Distribution of living female subjects by age group, exposure category, and educational level.[a]

Length of schooling	Age group (years)							
	<55		55–64		65–74		≥75	
	Unexposed	Exposed[b]	Unexposed	Exposed[b]	Unexposed	Exposed[b]	Unexposed	Exposed[b]
<6 years[c]								
Cases	1	–	1	1	3	3	1	1
Referents	12	2	5	3	7	1	2	–
≥6 years								
Cases	4	–	–	–	3	–	1	–
Referents	24	1	1	–	2	–	–	–

[a] There are two values missing.
[b] Categories 2 (exposure could not be ruled out) and 3 (exposed) are combined under this heading.
[c] Age-adjusted odds ratio: 3.86 (90 % confidence limits 0.87–17.15).

early 1950s may have been diagnosed before 1981, when our study started.

2. Elderly women (≧75 years) were less likely to be engaged in highly-exposing jobs in the 1950s, since they were already 45 years of age or older in that period. Their inclusion in the study may have introduced an underestimation of the risks.

3. The procedure of matching deceased referents to the deceased persons with soft-tissue sarcoma with respect to town of residence may have artifactually raised the proportion of exposed referents. The proportion of definitely exposed women was similar among the deceased (3 of 10) and living (4 of 21) women with soft-tissue sarcoma but quite different among the deceased (5 of 13) and living (5 of 60) referents. The choice of a group of living referents, extracted from the general population, for comparison with the deceased subjects inducted as cases is considered to be the best option in case-referent studies; the only flaw is the quality of the interview, obtained from the next-of-kin of deceased subjects (5, 11). If we compare all of the female subjects with soft-tissue sarcoma (living + deceased) with the living referents only, the age-adjusted odds ratio is 2.71 (90 % CL 0.51–14.17). We intend, in any case, to select another reference group of deceased individuals, unmatched to the subjects with soft-tissue sarcoma in respect to town of residence.

In conclusion, our study showed an odds ratio of 2.7 for living women who were exposed to phenoxy herbicides in any period of their life. When we restricted our attention to living women less than 75 years of age and exposed in the 1950–1955 period, an age-adjusted odds ratio of 15.5 (90 % CL 1.3–180.3) was determined (table 4). When exposure to phenoxy herbicides among living females who had a regular job in agriculture (ie, excluding unexposed nonagricultural workers) was analyzed, the age-adjusted odds ratio (Mantel-Haenszel) for exposure category 3 was 3.0.

The number of definitely exposed subjects with soft-tissue sarcoma and referents was negligible among the men. There is no specialized job such as pesticide applicator in rice growing in Piedmont, and annual herbicide spraying is performed, among numerous other jobs, by unspecialized rice growers or farm laborers.

No dose-response relationship could be considered because we were not able to distinguish between high and low levels of exposure in the exposure assessment (with the exception of the a priori hypothesis concerning the 1950–1955 period). Most of the exposed subjects (both those with soft-tissue sarcoma and the referents) had been employed in rice weeding for more than 10 years (table 7).

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ional level.[a]

	≥75	
posed[b]	Unexposed	Exposed[b]
3	1	1
1	2	–
–	1	–
–	–	–

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Epidemiologic Studies of Cancer in Agricultural Workers

Neil Pearce, PhD, and John S. Reif, DVM, MSc

The incidence of cancer in agricultural workers is generally low, in part due to the low prevalence of cigarette smoking in this group. However, agricultural workers have elevated risks for several specific cancer types including leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, and cancers of the lip, stomach, prostate, brain, and connective tissue. Two major groups of risk factors have been proposed as causes of hematologic malignancies in agricultural workers. The first group includes various agricultural chemicals. In particular, several studies have found increased risks of malignant lymphoma and soft tissue sarcoma in persons exposed to phenoxy herbicides. However, the evidence is inconsistent and there is a wide variation in relative risk estimates. The second group of risk factors includes various animal viruses. There is currently little evidence concerning the zoonotic nature or human carcinogenicity of these viruses. However, leads have been suggested by recent evidence of increased risks of hematologic malignancies in abattoir workers, veterinarians, and meat inspectors. A third hypothesis, for which little evidence is currently available, is that agricultural work may involve prolonged antigenic stimulus leading to lymphoproliferation. The factors responsible for the increased risks for cancers other than hematologic malignancies are not well understood but may also involve exposure to chemicals or viruses.

Key words: agricultural chemicals, animal viruses, antigenic stimulation, occupational cancer

INTRODUCTION

Agricultural work involves a wide variety of tasks, each involving potentially carcinogenic exposures. Farmers and farm laborers may come into contact with animal viruses, bacteria and fungi, pesticides, solvents, fuels and oils, and dusts [Blair et al., 1985; Lawhorne, 1976]. Other agricultural workers experience some of the same exposures. These include fencing workers, abattoir workers, meat inspectors, and veterinarians. In this paper, available data on cancer risks in agricultural workers are reviewed. We begin by reviewing studies of overall cancer mortality and site-specific cancers in farmers. The major hypotheses concerning cancer risks in agricultural workers are then discussed, and the epidemiologic evidence for each is

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TABLE I. Studies of Overall Cancer Mortality in Agricultural Workers

Reference	Country and time period	Study type	Study population	Total cancers in agricultural workers	Relative risk	95% confidence interval
Howe and Lindsay (1983)	Canada 1965–1969	Mortality	10% sample of all males	75	0.87	0.68–1.09
Registrar-General's (1978)	England and Wales 1970–1972	Mortality	Males aged 15–64	—	0.92	—
Pearce and Howard (1978)[a]	New Zealand 1974–1978	Mortality	Males aged 15–64	659	0.99	0.92–1.07
Wiklund (1986a)	Sweden 1961–1979	Incidence	All agricultural workers	24,763	0.82	0.81–0.83
Walrath et al. (1985)[b]	United States 1970–1984	Mortality	Veterans with life insurance aged 31–84	824	0.90	0.84–0.96

[a]Previously unpublished data from this study.
[b]Adapted from Blair et al. (unpublished).

reviewed. Finally, we discuss some of the difficulties of investigating cancer risks in agricultural workers and suggest some priorities for further research.

STUDIES OF CANCER MORTALITY IN AGRICULTURAL WORKERS

A recent publication reviewed the epidemiologic studies of the cancer experience of agricultural workers in a number of countries and regions [Blair et al., 1985]. The present article is not intended to review all the available studies. Instead, one major study has been selected from each country. In each instance, a cancer mortality or incidence study was selected (proportional mortality and case-control studies were not considered) on the basis of the study size, availability of site specific data, and availability of appropriate data for the calculation of confidence intervals. The characteristics of the selected studies [Howe and Lindsay, 1983; Office of Population Census and Surveys, 1978; Pearce and Howard, 1986; Wiklund and Holm, 1986a; Walrath et al., 1985] are presented in Table I.

Cancers for Which Agricultural Workers Have Decreased Risks

Despite the presence of potentially carcinogenic exposures, overall cancer incidence and mortality in farmers is generally lower than for the general population (Table I). In particular, farmers have lower than average risks for cancers of the lung, bladder, nose, colon, rectum, and liver (Table II). These patterns are clearest in the Swedish study since this involved the largest numbers of cases. However, similar patterns occur in Canada, England and Wales, New Zealand, and the United States (Tables I and II). The decreased risks for cancer of the lung and bladder are presumably due to the low prevalence of cigarette smoking in agricultural workers [Blair et

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TABLE II. Studies of Cancer Types That Show a Decreased Risk for Agricultural Workers

Country	Lung		Bladder		Nasal	
	Relative risk	95% confidence interval	Relative risk	95% confidence interval	Relative risk	95% confidence interval
Canada	0.69	0.42–1.08	—	—	—	—
England and Wales	0.84	—	0.70	—	—	—
New Zealand	0.86	0.74–1.00	0.91	0.47–1.59	—	—
Sweden	0.36	0.34–0.38	0.61	0.57–0.65	0.92	0.73–1.15
United States	0.83	0.70–0.98	0.50	0.31–0.77	—	—
	Colon		Rectum		Liver	
Country	Relative risk	95% confidence interval	Relative risk	95% confidence interval	Relative risk	95% confidence interval
Canada	0.53	0.14–1.36	1.16	0.31–2.97	—	—
England and Wales	1.20	—	1.00	—	—	—
New Zealand	1.17	0.92–1.48	0.80	0.54–1.15	0.59	0.24–1.22
Sweden	0.72	0.69–0.76	0.89	0.84–0.94	0.44	0.37–0.52
United States	0.71	0.57–0.88	0.79	0.54–1.12	0.83	0.50–1.30

al., 1985], a pattern that has been observed in New Zealand [Department of Statistics, 1983], Sweden [Central Bureau of Statistics, 1965], and the United States [Sterling and Weinkam, 1976]. The decreased risks of colon cancer in agricultural workers may be due to the protective effect of high physical activity [Blair et al., 1985; Garabrant et al., 1984]. The reasons for the low risks of cancer of the rectum, liver, and nose are currently unknown [Blair et al., 1985].

Cancers For Which Agricultural Workers Have Increased Risks

Agricultural workers have also been found to have elevated risks for several types of cancer. Increased risks have been most firmly established for hematologic malignancies, although there is little evidence for an increased risk of Hodgkin's disease in the countries considered here (Table III). Farmers also appear to have increased risks for cancers of the lip, stomach, prostate, and brain (Table IV). Some proportionate mortality studies have also found excess risks for connective tissue [Blair et al., 1985], but this was not observed in the Swedish study considered here (Table IV).

The excess risks presented in Tables III and IV are small, but this is to be expected given the crude occupational information used in these studies. Furthermore, it is likely that the elevated risks for specific cancers are due to exposures confined to particular subgroups of agricultural workers. The excess risks in these subgroups would thus be greater than those presented in Tables III and IV. These findings are also of interest when considered in conjunction with time trends in mortality for these cancer sites. Mortality from multiple myeloma, non-Hodgkin's lymphoma, leukemia, and cancers of the brain and connective tissue has increased in the United States during the period 1950–1980, particularly in farming states [Pickle et al., 1987]. Similar patterns have been observed for mortality and incidence in New Zealand [Pearce et al., 1986; Smith et al., 1984; Pearce et al., 1985], but there has been little increase in incidence in Sweden [Wiklund and Holm, 1986a].

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TABLE III. Studies of Cancer Types That Show an Increased Risk for Agricultural Workers: Hematologic Malignancies

Country	Hodgkin's disease		Non-Hodgkin's lymphoma		Multiple myeloma	
	Relative risk	95% confidence interval	Relative risk	95% confidence interval	Relative risk	95% confidence interval
Canada	—	—	0.62	0.13–1.81	—	—
England and Wales	1.03	—	1.12	—	—	—
New Zealand	0.79	0.16–2.29	1.41	0.91–2.08	1.55	0.82–2.65
Sweden	1.06	0.93–1.20	0.98	0.91–1.06	1.20	1.09–1.33
United States	—	—	—	—	—	—

Country	Leukemia		Total hematologic malignancies	
	Relative risk	95% confidence interval	Relative risk	95% confidence interval
Canada	0.33	0.00–1.84	0.55	0.22–1.14
England and Wales	—	—	—	—
New Zealand	1.18	0.78–1.72	1.29	1.00–1.63
Sweden	0.95	0.89–1.02	1.04	1.00–1.08
United States	1.13	0.85–1.47	—	—

TABLE IV. Studies of Cancer Types That Show an Increased Risk for Agricultural Workers: Cancers Other Than Hematologic Malignancies

Country	Stomach		Lip		Brain	
	Relative risk	95% confidence interval	Relative risk	95% confidence interval	Relative risk	95% confidence interval
Canada	—	—	—	—	1.77	0.48–4.53
England and Wales	0.97	—	—	—	0.99	—
New Zealand	0.85	0.56–1.24	—	—	1.10	0.75–1.55
Sweden	1.05	1.01–1.09	1.92	1.74–2.11	1.01	0.94–1.08
United States	1.07	0.82–1.38	—	—	1.04	0.60–1.51

Country	Prostate		Connective tissue	
	Relative risk	95% confidence interval	Relative risk	95% confidence interval
Canada	1.16	0.42–2.53	—	—
England and Wales	1.22	—	—	—
New Zealand	1.61	1.12–2.25	—	—
Sweden	0.96	0.94–0.99	0.98	0.85–1.13
United States	1.06	0.88–1.27	—	—

GENERAL HYPOTHESES FOR THE INCREASED CANCER RISKS IN FARMERS

The factors responsible for the increased risks of certain cancers in agricultural workers are not well understood. Hematologic malignancies have been most extensively considered, and most studies have focused on two general groups of risk

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factors: agricultural chemicals and farm animal viruses. A third hypothesis, which has not been studied in depth, is a possible role of chronic antigenic stimulation. These general hypotheses will be discussed with particular reference to hematologic malignancies. The factors responsible for the increased risks for cancer of sites other than the lymphatic and hematopoietic system are even less well understood but may also involve exposure to chemicals or farm animal viruses [Reif et al., in press].

CHEMICALS

Exposure to agricultural chemicals has been the primary focus of most epidemiologic studies of agricultural workers. Initial studies attempted to relate risks for leukemia [Blair and Thomas, 1979; Blair and White, 1981] and other hematologic malignancies [Burmeister et al., 1983; Cantor, 1982; Cantor and Blair, 1984; Saftlas et al., 1987] with specific agricultural practices based on county of residence. In general, these studies have not provided a clear pattern of exposure and risk, although associations between herbicide use, fertilizers, insecticides, and various crops such as corn and soybeans have been found by this ecologic approach. In the last decade, more studies have collected information on individual chemical exposures, particularly to insecticides and herbicides.

Insecticides

Arsenic is the insecticide that has been most clearly established as a human carcinogen. It is an established lung carcinogen and may also cause hematologic malignancies [Axelson, 1980]. Arsenic compounds have been used as pesticides for more than a century, particularly in sheep-dips and vineyards, although their use has declined in recent decades [Pershagen, 1983]. They are still used for the preservation of farm fencing materials and a recent New Zealand study found a small excess risk of non-Hodgkin's lymphoma in fencing workers [Pearce et al., 1986, 1987]. However, arsenic compounds have not been widely used in other branches of agriculture and would appear to be of limited importance for cancer causation in agricultural workers [Axelson, 1987].

Studies of other insecticides have included manufacturers of organochlorine compounds, and termite and agricultural applicators. Individual exposures have not been documented but most workers appear to have been exposed to various organochlorines including chlordane, heptachlor, DDT, dieldrin, and aldrin. These studies have recently been reviewed by Blair in an unpublished manuscript who concluded that they show small but consistent excesses of lung cancer. Although the specific insecticides that caused the lung cancer excess could not be identified, the organochlorine pesticides were the most likely candidates since they were the predominant insecticides during the relevant time periods.

These findings are of considerable interest, but they do not explain the cancer mortality patterns in other agricultural workers who generally have low lung cancer risks (Table II). Of more relevance are the case-control studies of hematologic malignancies and soft tissue sarcoma that have collected information on insecticide exposure. In an unpublished manuscript by Blair et al., it was reported that weak but consistent excesses exist for some commonly used organochlorines including DDT and lindane. One unpublished study by Blair and two case-control studies [Hoar et al., 1986; Hoar et al., 1988] have also reported associations between hematologic

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TABLE V. Relative Risk Estimates From Case-Control Studies of Soft Tissue Sarcoma, Non-Hodgkin's Lymphoma and Hodgkin's Disease and Exposure to Phenoxy Herbicides*

Study	Exposed cases	Relative risk	95% confidence interval
Soft tissue sarcoma			
Hardell and Sandstrom (1979)	13	5.3	2.4–11.5
Eriksson et al. (1981)	14	6.8	2.6–17.3
Hardell and Eriksson (1988)	9	3.3	1.4–8.1
Smith et al. (1984)	21	1.3	0.6–2.9
Smith and Pearce (1986)	6	0.7	0.3–1.9
Hoar et al. (1986)[a]	15	1.4	0.8–2.3
Woods et al. (1987)	[b]	0.9	0.4–1.9
Vineis et al. (1987)	5	2.4	0.5–12.4
Non-Hodgkin's lymphoma			
Hardell et al. (1981)	27	4.7	2.6–8.3
Hoar et al. (1986)	24	2.2	1.2–4.1
Pearce et al. (1987)	44	1.0	0.6–1.7
Woods et al. (1987)	[b]	1.2	0.8–1.9
Blair et al. (unpublished)	8	2.0	0.7–5.2
Hoar Zahm et al. (1988)	[b]	1.5	0.9–2.4
Persson et al. (1989)	12	1.6	0.7–3.5
Hodgkin's disease			
Hardell et al. (1981)	14	5.0	2.4–10.2
Hoar et al. (1986)[a]	13	1.0	0.5–1.7

*Includes 32 cases of Hodgkin's disease and 84 cases of non-Hodgkin's lymphoma.
[a]Adapted from Blair et al. [unpublished].
[b]Not reported.

malignancies and several organophosphate insecticides, which have largely replaced the organochlorines.

Herbicides

The carcinogenic effects of herbicides have been more intensively studied than those of insecticides. Attention has been focused on the phenoxy herbicides, a group which includes 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D); 2,4,5-T has been of particular interest because early formulations were contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). 2,4-D and other commonly used phenoxy herbicides are not contaminated by TCDD but are contaminated with other dioxins and dibenzofurans.

The current interest in phenoxy herbicides was initiated by a series of case-control studies in Sweden that found increased risks of soft tissue sarcoma [Hardell and Sandstrom, 1979; Eriksson et al., 1981] and malignant lymphoma [Hardell et al., 1981] in agricultural workers who had been exposed to phenoxy herbicides (Table V). These findings have been further investigated in case-control studies in Sweden [Hardell and Eriksson, 1988; Persson et al., 1989], New Zealand [Smith et al., 1984; Pearce et al., 1987; Smith and Pearce, 1986], the United States [Hoar et al., 1986, 1988; Woods et al., 1987], and Italy [Vineis et al., 1987].

The findings of these studies are summarized in Table V. The principal feature of the table is the considerable variation in relative risk estimates. For soft tissue sarcoma, the original two Swedish studies [Hardell and Sandstrom, 1979; Eriksson et

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al., 1981] found relative risks of 5 or 6, and a more recent study [Hardell and Eriksson, 1988] found a relative risk of 3.3. The estimates in the two New Zealand [Smith et al., 1984; Smith and Pearce, 1986] and two United States [Hoar et al., 1986; Woods et al., 1987] studies were much closer to 1.0. Vineis [Vineis et al., 1987] found a two-fold risk of soft tissue sarcoma in female rice weeders in Italy who had contact with 2,4,5-T.

Several case-control studies have found elevated risks for non-Hodgkin's lymphoma in persons exposed to phenoxy herbicides. Hardell's study [Hardell et al., 1981] found a six-fold risk, whereas a more recent Swedish study by Persson [Persson et al., 1989] found a relative risk of 1.6 for malignant lymphoma (primarily non-Hodgkin's lymphoma). Hoar [1986] found a two-fold risk in Kansas, but the risk was more than seven-fold in persons who reported using herbicides for more than 20 days per year. In the initial findings of a similar study in Nebraska [Hoar et al., 1988], a relative risk of 1.5 has been reported both for 2,4,5-T and 2,4-D. In Iowa and Minnesota, Blair reported in an unpublished manuscript that a two-fold risk occurring 20 years after exposure to 2,4,5-T was found, but little evidence was found for an increased risk for 2,4-D. On the other hand, Pearce [Pearce et al., 1987] in New Zealand, and Woods [Woods et al., 1987] in Washington State, found little evidence of elevated risks for non-Hodgkin's lymphoma in persons exposed to phenoxy herbicides. The two case-control studies of Hodgkin's disease also show a marked disparity in their findings (Table V).

A series of cohort studies in Sweden has also yielded findings at variance with those of the Swedish case-control studies. A study of 354,620 Swedish men who were classified as farmers or forestry workers in the 1960 census found a relative risk of 0.9 for soft tissue sarcoma (95% confidence interval 0.8–1.0), whereas a relative risk of approximately 1.5 would have been expected in this group on the basis of the case-control study findings [Wiklund and Holm, 1986b]. A further analysis of the same cohort found no evidence of an increased risk of non-Hodgkin's lymphoma or Hodgkin's disease [Wiklund et al., 1988]. A similar study of pesticide applicators found relative risks of 1.0 for non-Hodgkin's lymphoma (95% confidence interval 0.6–1.5) and 1.2 for Hodgkin's disease (95% confidence interval 0.6–2.2), whereas relative risks of approximately 4.5 were expected on the basis of the case-control study findings [Wiklund et al., 1987].

The wide range of relative risk estimates is puzzling, and a number of possible explanations have been offered.

The Swedish studies have been criticized with regard to the possibility of recall bias or interviewer bias when comparing the exposure history of cancer cases with those of healthy controls drawn from the general population. Such tendencies could have been fostered "by suggestion during previous interviews in hospital and by the effects of newspaper publicity on patients and their relatives" [Coggon and Acheson, 1982]. However, a simple check, based on a principle suggested by Axelson [Axelson, 1980], was carried out by examining the findings for occupations in which some, but not all, workers had been exposed to phenoxy herbicides. It was concluded that serious recall bias was unlikely to have occurred. Swedish case-control studies of other cancer sites have used the same study design and have not found six-fold risks, although the relative risk estimates were slightly elevated [Hardell, 1981; Hardell et al., 1982, 1984]. Hoar's Kansas study [Hoar et al., 1986] used a general population control group and found little evidence for an increased risk for soft tissue sarcoma or Hodgkin's disease

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(Table V). The New Zealand non-Hodgkin's lymphoma study used both general population controls and other cancer controls at a time when there was considerable media publicity concerning possible health effects of 2,4,5-T and found similar reported exposure patterns in the two groups [Pearce et al., 1986]. The recent Swedish soft tissue sarcoma study [Hardell and Eriksson, 1988] also used both general population controls and cancer controls and also found similar reported exposure patterns in the two control groups. Thus, although the information bias hypothesis cannot be excluded, there is currently little evidence to support it [Axelson, 1985].

The validity of the control group should also be considered for those studies that have not found an increased risk. The New Zealand studies used other cancer patients as controls in order to minimize the possibility of information bias. Selection bias could occur using this study design if other cancers were also caused by phenoxy herbicide exposure. However, any such bias is likely to be small [Smith et al., 1988] since the overall cancer mortality in New Zealand farmers is virtually identical to national rates (Table I). Furthermore, as noted above, one New Zealand study [Pearce et al., 1986] and one Swedish study [Hardell and Eriksson, 1988] used both general population and other cancer controls, and in both studies, the two control groups gave similar findings.

Differences in case ascertainment could also be responsible for the differing study findings. The New Zealand case-control studies of soft tissue sarcoma were confined to cases registered under International Classification of Diseases (ICD) [World Health Organization, 1977] code 171, whereas only one half of the Swedish cases were registered under ICD code 171. However, the effect estimates in this subgroup were similar to those in the cases registered under other ICD codes (L Hardell, personal communication).

Thus, although all the published case-control studies have strengths and weaknesses, it is difficult to identify methodological problems that could cause five-fold differences in the relative risk estimates. Accordingly, the characteristics of the populations and exposures studied should also be considered.

If the TCDD contaminant of 2,4,5-T is involved, then it is possible that the levels of contaminants have been different in Sweden, New Zealand, the United States, and Italy. However, available evidence does not suggest major differences between Sweden and New Zealand [Smith et al., 1984]. Furthermore, the Kansas study predominantly involved exposure to 2,4-D, and the Swedish studies also do not necessarily implicate TCDD since, in at least one study [Eriksson et al., 1981], an elevated risk for non-Hodgkin's lymphoma was also observed in persons exposed to 2,4-D and other phenoxy herbicides that do not contain TCDD [Axelson, 1987].

Differences in spraying practices could also be important. Owing to the climate, spraying in Sweden is usually carried out intensively during a 2–3-month period, whereas spraying in New Zealand and Washington State occurs intermittently over a longer period [Woods et al., 1987]. These differences could result in Swedish herbicide sprayers receiving a relatively high absorbed dose. This conjecture is consistent with the finding of Hoar [Hoar et al., 1986] of markedly increased risks of non-Hodgkin's lymphoma (but not soft tissue sarcoma or Hodgkin's disease) in persons spraying herbicides for more than 20 days per year. However, a re-analysis of the New Zealand non-Hodgkin's lymphoma data found a relative risk of 1.1 (95% confidence interval 0.3–4.1) in persons spraying 20 days or more per year (Pearce, unpublished data).

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A further possibility is that phenoxy herbicide exposure could be carcinogenic only when occurring jointly with other exposures present in Sweden and Kansas, but not New Zealand [Pearce et al., 1986, 1987]. Some support for this conjecture has been provided by the study of Woods [Woods et al., 1987]. Overall there was little evidence of elevated risks, but when the analysis was restricted to subjects with Scandinavian surnames, the odds ratio estimates for soft tissue sarcoma (but not non-Hodgkin's lymphoma) were elevated for phenoxy herbicide exposure (odds ratio = 2.8) and chlorophenol exposure (odds ratio = 7.2). Furthermore, the distribution of histologic types was similar to that in the Swedish studies. Woods et al. [1987] have suggested that factors specific to Scandinavian descent may contribute to increased risks of soft tissue sarcoma when exposed to certain chemicals. The relevant factors could be genetic, a hypothesis that is consistent with at least some studies of the binding affinity of the TCDD contaminant in tissues [Woods et al., 1987]. Alternatively, the relevant factors could be environmental, including the use of other agricultural chemicals in combination with phenoxy herbicides and chlorophenols. For example, in the recent Swedish malignant lymphoma study [Persson et al., 1989] the relative risk associated with exposure to phenoxy herbicides in combination with exposure to solvents and fresh wood was 2.7 (90% confidence interval 0.7–9.4), whereas exposure to these factors alone was associated with relatively modest increases in risk. Consideration of such joint effects can be expected to play an increasingly important role in occupational epidemiology [Axelson, 1988].

The hypothesis that phenoxy herbicides may only be carcinogenic in combination with other factors is of particular interest in light of recent developments in the understanding of mechanisms of carcinogenesis. The phenoxy herbicides are not classic mutagens, and there is currently little evidence that they are direct animal carcinogens. However, it is plausible that they could be tumor promoters. Promotion may involve a variety of mechanisms including interference with DNA repair mechanisms, cytotoxity, inhibition of cellular differentiation, immunosuppression, mitogenesis, and inhibition of intercellular communication [Williams, 1984]. These effects do not appear to involve genetic change and would require concomitant exposures to other carcinogens. Similar mechanisms have been suggested for arsenic, which is an established human carcinogen [Pershagen, 1983] despite the lack of evidence of animal carcinogenicity [Landrigan, 1981].

Viruses

Although epidemiologic evidence for the role of oncogenic animal viruses in human cancer is currently weak [Blair et al., 1985], a number of potentially zoonotic viruses exist in the agricultural environment. These include the herpes virus that causes Marek's disease in poultry [Witter, 1972], the avian leukosis virus [Burmeister and Purchase, 1970], papilloma viruses in cattle [Jarrett et al., 1978; Jarrett, 1980; Smith and Campo, 1985], and perhaps other currently undetected viruses. The rapid proliferation of information concerning retroviruses suggests that other members of this family will be found in domestic animals as well as humans.

Most interest has centered on the bovine leukemia virus (BLV), an exogenous C-type retrovirus that has been established as the etiologic agent of the adult form of bovine lymphosarcoma (BLS) [Miller et al., 1969]. The virus is related closely to HTLV-1, the cause of adult T-cell leukemia in humans [Jakobs, 1983]. BLV has been found in herds in most countries, including the United States and New Zealand

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[Parrish et al., 1982]. The virus has also been found in meat and unpasteurized milk [Ferrer et al., 1981]. Although BLV is killed by pasteurization, consumption of unpasteurized milk is extremely common among families and rural residents [Donham et al., 1977].

BLV can also infect sheep and goats, and can cause cancer in sheep [Jakobs, 1983]. It is capable of infecting human cells and inducing syncytia in tissue culture [Diglio and Ferrer, 1976]. Preliminary human studies have not shown evidence of infection [Donham et al., 1977], although this may be because current laboratory techniques or knowledge of the mechanism of infection are not sufficiently developed. In vivo, BLV infected cells or tumor cells do not express BLV [Kettman et al., 1978]. Therefore the failure to find antibodies to BLV in human seroepidemiologic studies should not be taken as definite evidence against infection.

Numerous reports of human leukemia clusters have been published that have attempted to show a relationship between cattle or the dairy industry and the occurrence of various hematologic malignancies [Blair and Thomas., 1979; Bartsch et al., 1975; Heath, 1970; Henricson and Ringertz, 1968]. Most studies have shown little evidence of an association, although a geographic correlation has been demonstrated in Sweden and the Soviet Union [Khoklova and Rakhamin, 1970] between areas with a high frequency of BLS and high human leukemia rates. In Iowa, Donham [Donham et al., 1980] found a high positive correlation between the incidence of acute lymphatic leukemia (ALL) in males and cattle density, which was most striking for dairy cattle. A Swedish case-control study found that leukemia patients had BLS in their cattle herds more frequently than did neighborhood controls [Kvarnfors et al., 1975]. However, a recent United States case-control study [Donham et al., 1987] found that the prevalence of antibodies to BLV in dairy herds to which cases of acute lymphatic leukemia (ALL) had been exposed was actually lower than that found in herds with which the controls had had contact.

Abattoir Workers

Thus, most studies of farm animal viruses have not found an association with human cancer, although this may be due to the difficulties of assessing exposure to these viruses in farmers. Possible leads have been suggested by recent studies of abattoir workers, a group that may have high exposure to animal viruses [Pearce et al., 1988].

Table VI presents the findings of five New Zealand case-control studies that have found increased risks of soft tissue sarcoma (STS), non-Hodgkin's lymphoma (NHL), and acute myeloid leukemia (AML) in abattoir workers [Pearce et al., 1986; Smith et al., 1984; Pearce et al., 1986,1987; Smith and Pearce, 1986]. The table also shows the cohort study by Johnson [Johnson et al., 1986a] of mortality in 13,844 white male members of a meat-cutters union in Baltimore, MD, of whom 3,025 had worked in abattoirs. The findings are difficult to interpret due to the small numbers of deaths, but Johnson's data show some consistency with the New Zealand findings. In particular, there were elevated risks for Hodgkin's disease (ICD 201) and for the category of "cancer of other lymphatic tissue," which included non-Hodgkin's lymphoma cases registered under ICD 202 and multiple myeloma (ICD 203). No increased risk was observed for non-Hodgkin's lymphoma cases registered under ICD 200. There was also no increased risk for all leukemias combined, but specific data for acute myeloid leukemia were not presented.

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TABLE VI. Relative Risk Estimates for Studies Showing Excess Risks of Soft Tissue Sarcoma, Non-Hodgkin's Lymphoma or Acute Myeloid Leukemia in Abattoir Workers*

Study	Site	ICD Code	Exposed cases	Relative risk	95% confidence interval
Smith et al. (1984)	STS	171	19	2.8	1.1–7.3
Smith and Pearce (1986)	STS	171	11	1.6	0.8–3.5
Pearce et al. (1987)	NHL	200	24	1.8	0.9–3.5
Pearce et al. (1987)	NHL	202	19	1.7	0.9–3.4
Johnson et al. (1986a)	NHL	200	1	0.4	0.0–2.2
	NHL, MM	202,203 208	5	2.2	0.7–5.1
Johnson et al. (1986b)	HD	201	4	2.4	0.8–7.5
Pearce et al. (1986)	AML	205.0	9	2.5	1.1–5.7

*STS, soft tissue sarcoma; NHL, non-Hodgkin's lymphoma; MM, multiple myeloma; HD, Hodgkin's disease; AML, acute myeloid leukemia.

Abattoir work may involve significant chemical exposures; 2,4,6-TCP, which contains TCDD as a contaminant, has been used in the treatment of pelts [Smith et al., 1984; Glover et al., 1975]. However, the New Zealand studies found that pelt department workers have similar risks to those of other abattoir workers [Pearce et al., 1988]. Various plastics have been used to wrap meat, and the thermal decomposition products may include benzene, phthalic anhydride, and phthalates. Johnson et al. [1986b] found a three-fold risk of mortality from myeloid leukemia and non-Hodgkin's lymphoma among female workers in the meat department of retail food stores. Elevated lung cancer rates in butchers and meatcutters have also been observed in several countries [Reif et al., 1989] and have also been attributed to exposures in the wrapping process [Johnson et al., 1986a,b].

However, a recent Swedish study [Gustavsson et al., 1987] did not find that lung cancer risks were associated with wrapping, and it appears that in most abattoirs only a few workers are directly exposed to fumes from the wrapping process. Johnson et al. [1986a] have also noted that the greatest risk for Hodgkin's disease in their study of male abattoir workers was associated with slaughtering, particularly of cattle, pigs, and sheep. This finding is consistent with the suggestion of a viral etiology [Pearce et al., 1986; Johnson et al., 1986a], and this is currently the leading hypothesis to explain the findings for abattoir workers [Pearce et al., 1988].

Other Relevant Populations

Veterinarians are another occupational group with exposure to farm animals. There is inconsistent evidence that veterinarians may be at increased risk for cancer of hematologic malignancies. Matanowski and Lilienfeld [1976] reported an SMR of 1.48 for Hodgkin's disease in a mortality study of 20,000 veterinarians. Blair and Hayes [1982] studied 5,016 deaths in white male veterinarians and found elevated risks for Hodgkin's disease (PMR = 1.87, 95% confidence interval 1.11–2.96) and cancer of other lymphatic tissue (PMR = 1.92, 95% confidence interval 1.26–2.82). Interestingly, the PMR for all hematologic malignancies was elevated in meat inspectors (PMR = 3.36, 95% confidence interval 1.44–6.57), a finding consistent with the increased risks in abattoir workers.

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Chronic Antigenic Stimulation

A third group of possible etiologic factors is suggested by the hypothesis that multiple myeloma and other hematologic malignancies may occur after prolonged antigenic stimulation [Greene, 1982; Blattner, 1982]. Gallagher et al. [1983] found elevated risks of multiple myeloma in farmers and in persons reporting a history of allergy. However, the role of allergies did not explain the excess risk for farmers. Cunningham [1976] has suggested that ingestion of animal proteins, particularly bovine protein, results in chronic stimulation of lymphoid tissue which may act in combination with other factors such as oncogenic viruses to induce malignant change. There are other sources of chronic antigenic stimulation that may also be more prevalent in agricultural communities. Chronic exposure to mitogenic substances from a grain elevator has been postulated to be a factor in a cluster of Hodgkin's disease cases [Schwartz, 1978], and a proportionate mortality study of workers in the grain industry [Alvanja et al., 1987] found elevated risks for hematologic malignancies. It has also been suggested that multiple myeloma may develop following chronic antigenic stimulation from repeated courses of allergen vaccines for diseases such as hay fever [Woodroffe, 1972]. Thus, the chronic antigenic stimulation hypothesis warrants further investigation, particularly with regard to hematologic malignancies in abattoir workers who have continual exposure to animal protein.

DISCUSSION

Given the wide variety of exposures involved, it is perhaps not surprising that the reasons for the increased risks for certain cancers in agricultural workers remain unclear. The problem of multiple exposures is a limitation that applies to all epidemiologic studies and has not prevented the discovery of other environmental carcinogens such as alcohol and cigarette smoking. In principle, the carcinogenic effects of specific herbicides and insecticides can be validly measured, providing appropriate information is collected on all other potentially confounding agricultural exposures. Nevertheless, an identification problem occurs when agricultural workers are exposed, perhaps simultaneously, to several chemicals. This problem is of particular concern when assessing the contributions of the so-called inert ingredients. For example, if a particular herbicide is always used in combination with particular types of solvents and emulsifiers in a particular country, then it may be very difficult to identify the agents responsible for an increased cancer risk associated with use of the herbicide. These considerations imply that the most efficient means of ascertaining the health effects of pesticide exposure in agricultural workers may be to study workers who produce these pesticides, rather than the agricultural workers who use them. However, exposures in pesticide producers may be much lower than exposures in users. Furthermore, if particular pesticides are only carcinogenic in combination with other exposures in the agricultural environment, then these effects may not be identified in studies of production workers. Thus, it is also important to study professional pesticide applicators who use a variety of agricultural chemicals, provided that it is possible for the effects of specific chemicals or combinations of chemicals to be isolated. Studies of farmers are unlikely to clarify the situation because of the difficulty of identifying the effects of specific risk factors [Axelson, 1987]. In this regard, the international collaborative cohort studies being conducted by the Inter-

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national Agency for Research on Cancer [1990] and the United States National Institute for Occupational Safety and Health [Fingerhut et al., 1984] will be of particular value in establishing whether phenoxy herbicides cause cancer in agricultural workers. These cohort studies of production workers and professional pesticide applicators will avoid many of the methodological problems, real or imaginary, of the previously conducted case-control studies. Studying the potential human carcinogenicity of animal viruses presents even more problems, since there are difficulties with documenting exposures to animal viruses in agricultural workers, or even in determining whether exposure occurs at all. In this respect, there may be considerable advantages to studying abattoir workers. They form a large enumerable group suitable for cohort studies [Johnson et al., 1986a,b]. They do not experience the wide variety of exposures experienced by farmers but have been demonstrated to have similarly increased risks for hematologic malignancies. Furthermore, exposure to animal viruses and other biological agents is likely to be high in slaughtering but relatively low in other abattoir workers. Thus, further studies of abattoir workers, rather than studies of farmers, may be the most productive avenue for investigating the causes of the increased risks for those cancer sites associated with contact with farm animals.

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Soft Tissue Sarcoma Risk in Swedish Licensed Pesticide Applicators

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Two Swedish studies have suggested that exposure to phenoxy acid herbicides may cause soft tissue sarcoma. This association has been subsequently investigated in a number of epidemiologic studies. Results are inconsistent.

In this study the risk of soft tissue sarcoma in a cohort of 20,245 Swedish pesticide applicators, of whom 72% were estimated to have been exposed to phenoxy acid herbicides, was analyzed. The cohort was observed by means of the Swedish Cancer Register from date of license until Dec 31, 1984, or until death if it occurred prior to that date. The mean follow-up time was 13.9 years. Seven patients with soft tissue sarcoma were observed compared with 7.7 expected on the basis of the incidence in Sweden. The relative risk was found to be 0.9 (95% confidence interval, 0.4 to 1.9).

This finding is consistent with the hypothesis that exposure of phenoxy acid herbicides does not increase risk of soft tissue sarcoma.

Two Swedish studies have reported a sixfold increase in the risk of soft tissue sarcoma following exposure to phenoxy acid herbicides.[1,2]

This relationship has been investigated in a great number of subsequent studies.[3–34] Reviews of these are somewhat contradictory.[35–36] One review of delayed health hazards of pesticide exposure concluded that neither phenoxy herbicides not dioxins can be said unequivocally to cause cancer in humans.[35] A review of health effects of phenoxy herbicides suggested that exposure may be associated with an increased incidence of cancer.[36]

Phenoxy acid herbicides were introduced in Sweden in the late 1940s. Use of these herbicides increased greatly until the most recent years, when a slight decrease has been observed.[37]

The compound most widely used in Swedish agriculture is MCPA (4-chloro-2-methylphenoxyacetic acid; CAS: 94-76-6). In the mid-1960s two other phenoxy acid herbicides were introduced: mecoprop ((±)-2-(4-chloro-*o*-tolyloxy) propionic acid; CAS: 7085-19-0) and dichlorprop ((±)-2-(2,4-dichlorophenoxy) propionic acid; CAS: 120-36-5). These two compounds constituted approximately 25% of the total use of phenoxy acid herbicides in 1970 and about 40% in 1985. The compound 2,4-D (2,4-dichlorophenoxyacetic acid; CAS: 94-75-7) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid; CAS: 93-76-5) have also been used, although to a lesser extent. Phenoxy acid herbicides contain toxic contaminants such as dioxins and dibenzofurans. MCPA, 2,4-D, mecoprop, and dichlorprop are not supposed to contain 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-para-dioxin; CAS: 1746-01-6), which is the most toxic dioxin. However, 2,4,5-T was prohibited in Sweden in 1977 because of contamination with 2,3,7,8-TCDD.

Most of the phenoxy acid herbicides sold in Sweden are used in agriculture or forestry. Since 1965 a license has been mandatory for using the most acutely toxic pesticides in agriculture.[38–40] Licensed pesticide applicators handle phenoxy acid herbicides to a greater extent than do agricultural and forestry workers in general. In two surveys we have found that 72% of licensed pesticide applicators compared with 16% of agricultural and forestry workers used phenoxy acid herbicides one day or more during the 1950s, 1960s, or 1970s. In the remainder of the Swedish population, the proportion of persons exposed to phenoxy acid herbicides can be estimated to be about 1%.[41] Of those, very few have been exposed occupationally. Exposure from private use of such herbicides is very low.

Swedish licensed pesticide applicators are thus an appropriate group of subjects for a study of the effects of phenoxy acid herbicides exposure. The object of the present study was to analyze the risk of soft tissue sarcoma.

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Methods

Cohort

Since 1965, completion of a four-day course on pesticide application has been a prerequisite for obtaining a license for using the most acutely toxic pesticides in agriculture. As of 1976, a total of 20,296 licenses had been issued. Thirty-three duplicates and 18 persons with incomplete identification were excluded, resulting in a cohort of 20,245 persons. Distribution of the cohort by year of birth is shown in Table 1. Almost 50% of the cohort were born in 1935 or later. Approximately 99% were men and 1% women. One third lived in the southernmost part of Sweden, where the use of herbicides is highest in the country. Half of the licenses were issued in 1965 or 1966.

Exposure

A questionnaire was mailed to a random sample of 273 persons in the cohort to study the use of pesticides and protective clothing, tobacco use habits, and occupations during the 1950s, 1960s, and 1970s. The response rate was 83%. The sample size was chosen so that the range of the 95% confidence interval for the percentage of phenoxy acid herbicide users would be ± 5%, if the sample showed that 75% of respondents had been users. The proportion of persons exposed to phenoxy acid herbicides one day or more was estimated to be 72% (66% to 78%). The proportions in the five year-of-birth groups are shown in Table 2. Herbicide use during the 1950s, 1960s, and 1970s was 19%, 49% and 67%, respectively.

Forty-two percent of those who used phenoxy acid herbicides during the 1950s reported that they never or seldom wore any protective clothing, compared with 23% in the 1960s and 9% in the 1970s (Table 3). Gloves were the most commonly used protective clothing. The survey also showed that most cohort members were employed in agriculture and/or forestry (70%) and horticulture (10%). Only a few worked full-time as pesticide applicators. The remaining 20% held a variety of occupations closely related to agriculture, but some worked in occupations such as building and construction.

TABLE 1
Distribution by Year of Birth of the Cohort of Licensed Pesticide Applicators in Agriculture

Year of Birth	Percent (N = 20,245)
–1904	2.3
1905–1914	10.0
1915–1924	19.6
1925–1934	19.9
1935–1944	23.8
1945–1954	22.7
1955–	1.8
Total	**100**

TABLE 2
Estimated Exposure to Phenoxy Acid Herbicides Among the Cohort of Licensed Pesticide Applicators in Agriculture, by Year of Birth

Year of Birth	Percent Exposed
1905–1914	32
1915–1924	70
1925–1934	65
1935–1944	81
1945–	79

TABLE 3
Use of Protective Clothing Among Licensed Pesticide Applicators who Applied Phenoxy Acid Herbicides in the 1950s, 1960s and 1970s

Use of Protective Clothing	1950s (n = 53)	1960s (n = 120)	1970s (n = 157)
Never or seldom used any protective clothing	42	23	9
Used protective mask	19	44	58
Used protective glasses	26	31	51
Used protective gloves (as only protection)	49	75	89
Used protective dress	19	19	16
	23	33	41

Follow-up

The cohort was observed by means of the Swedish Cancer Register from date of license until Dec 31, 1984, or until death if it occurred prior to that date. The Cancer Register was established in 1958. Reporting of all malignant and some benign tumors has been compulsory for almost all physicians and pathologists from the beginning. A modified version of the 7th revision of the Classification of Diseases (ICD 7), has been used throughout to code anatomic sites.[42]

This study included all registered cases of malignant tumors in connective tissue or muscle (ICD 7 = 197). The histologic diagnoses included were: angiosarcoma, leiomyosarcoma, rhabdomyosarcoma, myogenic sarcoma, fibrosarcoma, malignant fibrous histiocytoma, myxosarcoma, liposarcoma, extraskeletal chondrosarcoma, malignant synovialoma, undifferentiated and sarcomatous tumor, spool-shaped sarcoma, Enzinger tumors, giant cell sarcoma, and embryonal rhabdomyosarcoma. The cases were reviewed by a pathologist and all were confirmed.

Statistical Analysis

The expected number of cases (E) was calculated from the age-specific incidence in 5-year classes for respective years in the entire Swedish population. The ratio of cases observed (O) to the number expected forms the standardized incidence ratio (SIR). Observed and expected numbers of cases were also calculated 5 and 10 years, respectively, after date of license to allow for "latency." The 95% confidence intervals were calculated by means of a Poisson distribution table.

Results

The number of person-years of follow-up in the cohort was 281,550. When no "latency" time was allowed, the

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TABLE 4
Cases of Soft Tissue Sarcoma, SIR, and 95% Confidence Interval in the Cohort of Licensed Pesticide Applicators for Latency Periods of 0, 5, and 10 Years

Latency Time, yr	Cases		SIR	95% Confidence Interval
	Observed	Expected		
0	7	7.7	0.9	0.4–1.9
5	6	6.0	1.0	0.4–2.2
10	4	3.9	1.0	0.3–2.7

TABLE 5
The 7 Cases of Soft Tissue Sarcoma

Age at Diagnosis	Year of Diagnosis	Year of License	ICD (7th)	Histologic Diagnosis
63	1978	1966	197.0	Fibrosarcoma
61	1975	1965	197.3	Rhabdomyosarcoma
60	1983	1965	197.3	Malignant fibrous histiocytoma
52	1973	1965	197.3	Undifferentiated
41	1967	1965	197.1	Leiomyosarcoma
38	1979	1965	197.0	Rhabdomyosarcoma
28	1978	1969	197.3	Fibrosarcoma

observed number of soft tissue sarcomas was 7, compared with 7.7 expected (Table 4). The corresponding SIR was 0.9 (0.4 to 1.9). The SIR for 5 years' latency was 1.0 (0.4 to 2.2) and for 10 years', 1.0 (0.3 to 2.7). The observed numbers of cases were 6 and 4, respectively.

Among those to whom a license was issued in 1965 or 1966, 6 cases were observed and 5.0 expected; the SIR was calculated to be 1.2 (0.4 to 2.6). The mean observation time was 16.1 person-years.

Some data for the seven patients with soft tissue sarcoma are shown in Table 5. For all patients, diagnosis was based on histologic examination of biopsy material, and the diagnoses were confirmed in a review by a pathologist. Age at diagnosis ranged from 28 to 63 years.

Discussion

In this cohort of licensed pesticide applicators in Sweden no excess risk of soft tissue sarcoma was found, although 72% (confidence interval, 66% to 78%) of the persons in the cohort were estimated to have been exposed to phenoxy acid herbicides one day or more. If the relative risk for exposed versus unexposed persons is truly 6,[1,2] the SIR in this study should have been 4.5.

Latency periods have been estimated to be 15 and 20 years, respectively, in the two Swedish case-control studies.[41] The mean follow-up time in this study was 13.9 years. However, time since first exposure is longer, because 19% were exposed one day or more during the 1950s. Regardless, the cohort may have been observed for too short a time to reveal any excess risk and it is therefore important to continue the follow-up.

Estimation of the use of protective clothing is of importance since dermal absorption of pesticides is higher than uptake from the oral cavity or the lungs.[43] The absorption of phenoxy acid herbicides can be 30 to 40 times higher without protective clothing.[44] However, in some cases wearing contaminated clothing may increase the exposure.[45]

The use of protective clothing among pesticide applicators was not frequent, despite special education. One explanation could be that it is more uncomfortable than the acute reaction to exposure.

Pesticide applicators are recruited primarily from farmers, who have a decreased risk of cancer in general.[46] Farmers are healthier and thus utilize health services less than other occupational groups do.[47] One can therefore suspect that some tumors have not been detected. However, in a study of a sample of cases not reported in the Cancer Register, the registration deficit was found to be 4%.[48] In a follow-up study, agricultural workers were found not to have any increased registration deficit for all cancers. We have no data regarding a possible deficit for soft tissue sarcoma. Since the relative risk for soft tissue sarcoma in Swedish farmers was found to be 0.98 (0.84–1.15), one can assume no deficit or a very small one for this type of tumor.

The initial Swedish case-control studies have been criticized for basing exposure data on recall of the patient or of relatives if the patient was deceased.[49] The studies were made during the 1970s, when phenoxy acid herbicides and their possible health effects attracted much attention in Sweden, which could have influenced the data on exposure.[37,49]

One advantage of the present study is that the exposure status ("having a license for pesticide application") was established before the disease was detected, and therefore has not been influenced by media attention. Another advantage is the high proportion of exposed subjects in the cohort in comparison with the general population.

A major disadvantage of the present cohort study is the lack of individual exposure data. We have information for only a sample of the cohort and thus the estimates of pesticide use and use of protective clothing refer to the whole cohort and not to individuals.

In summary, the results of this study are consistent with the hypothesis that exposure to phenoxy acid herbicides does not increase risk of soft tissue sarcoma.

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Soft Tissue Sarcoma and Occupational Exposures

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The associations between soft tissue sarcoma (STS) and occupational exposures were studied in a case–referent study in the southeast of Sweden. Exposure information was obtained through mailed questionnaires to 96 cases, 450 randomly selected population referents, and 200 cancer referents. Odds ratios (OR), were calculated for various occupational groups, and particularly, for occupations with potential exposure to chlorinated phenoxy herbicides and chlorophenols. In the analyses based on population referents, increased risks for soft tissue sarcoma were seen for especially gardeners (OR = 4.1), but also railroad workers (OR = 3.1); construction workers with exposure to impregnating agents (OR = 2.3), asbestos (OR = 1.8), or pressure impregnating agents (OR = 1.7); and unspecified chemical workers with potential exposure to phenoxy herbicides and/or chlorophenols (OR = 1.6). A similar pattern appeared when cancer referents were used although the numerical values of the odds ratios became different. A grouping of jobs resulted in Mantel–Haensel OR from 1.5 to 1.9 for farmers and forestry workers, dependent on referents used and even more increased OR for railroad workers and unspecified chemical workers with potential exposure to phenoxy herbicides and chlorophenols. The results of the study confirm rather than refute that phenoxy herbicides and chlorophenols could be of etiologic importance for STS; the high risk for gardeners, although based on a small number of individuals, was unexpected and remains unclear. Also, since other cancers were used as referents, no definite problems of recall bias should obtain in this material. None of the exposed groups had a higher proportion of smokers than the unexposed group. *Cancer* **66:806–811, 1990.**

SOMEWHAT CONFLICTING RESULTS about a possible association of cancer and occupational exposure to pesticides have been reported in a number of studies from different countries. An early observation of increased cancer risks in connection with herbicide exposure appeared in the early 1970s in a study of Swedish railroad workers engaged in brush and weed killing.[1,2] Two Swedish case–referent studies on soft tissue sarcoma (STS) in the late 1970s and early 1980s[3,4] showed odds ratios (OR) of 5.3 and 6.8 for exposure to chlorinated phenoxy herbicides and OR of 6.6 and 3.3 for exposure to chlorophenols, respectively.

A recent Swedish case–referent study has again suggested an association of STS with exposure to phenoxy herbicides with a roughly three-fold increase in risk but the earlier seen association with exposure to chlorophenols was not reproduced.[5] It is worth noting, however, that record linkage in the so-called Cancer–Environmental Registry has failed to find any association between STS and agricultural or forestry work.[6] Another Swedish study on licensed pesticide applicators has also failed to find any association.[7]

In a study from Kansas, no association between agricultural herbicides and STS was found[8] whereas an association appeared in the same material for STS and insecticides used in farming.[9] The current case–referent study was undertaken to obtain further elucidation of the

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possible associations between STS and different occupational exposures, especially those likely to involve use of phenoxy herbicides or chlorophenols.

Materials and Methods

From the Regional Cancer Register at the University Hospital in Linköping, Sweden, 96 cases of STS with ICD 7 codes 174, 196 (except osteosarcomas), and 197 were identified during the period 1975 through 1982. All were men, 25 to 80 years old at time of diagnosis, from the region delivering cases to the hospital, *i.e.*, the counties of Östergötland, Jönköping, and Kalmar. Sixty-four are alive and 32 are dead. Two different sets of referents were drawn, one consisting of 200 alive and 250 dead individuals, randomly selected in 1982 from the population in these counties and in the same ages as the cases. The second referent group consisted of 200 individuals from the same three counties who had a cancer diagnosis other than sarcoma during the study period. Of these, 111 were alive and 89 were dead, all with ages 25 to 80 years at time of diagnosis.

Information regarding exposure was collected through a contracted organization working with epidemiologic materials in various respects. Each person when alive, or the next-of-kin of dead individuals, received an introductory letter and a questionnaire with 16 main questions and subspecifications about occupations in general and some specific jobs in particular. Questions were asked about occupational exposure to pesticides, wood preservatives, solvents, and other chemicals, as well as various leisure time exposures and smoking habits.

If the questionnaire had not been properly completed or when a person indicated work as a farmer or forestry worker, additional telephone interviews were made (blindly with regard to case–referent status) by the contracted organization. After receiving the returned questionnaires, the first page with information about case–referent status, name, address, and personal identification number was removed and not attached again until the final processing of the material for the analyses.

Detailed questions about pesticide exposure were asked, but very few subjects were able to provide specific information about the names of the pesticides to which they had been exposed. The analyses were, therefore, limited to evaluating job associated risks only; analogous to using job-exposure matrices, certain work tasks were considered to have involved exposure in the various respects accounted for in the "Results" section.

In addition to calculations of crude OR, the statistical analyses of the data were based on the Mantel–Haensel procedure for the calculation of adjusted OR (MH-OR).[10] In some of the analyses, adjustments were made for age and smoking. Ninety percent confidence intervals (CI) were calculated according to Cornfield's method as described by Breslow and Day.[11] A calculated OR was considered statistically significant if the lower limit of its associated CI exceeded unity. The statistical analyses were performed with the Epilog statistical package for personal computers (Epicenter Software, Epilog, version 3, Pasadena, CA, 1986).

Results

After losses due to refusals, failure to identify a next-of-kin, questionnaires which were not returned, and ethical restrictions regarding recently dead individuals, the final study material consisted of 550 individuals, Table 1. Odds ratio analyses were performed for all occupational groups that could be defined from the questionnaires. Significantly increased risks were seen for gardeners and construction workers exposed to asbestos (Tables 2 and 3). Somewhat elevated risks also appeared for other occupational categories, although none of them significant (Tables 2 and 3).

Further analyses were conducted on specific jobs (called "risk jobs" in the tables) where exposure to phenoxy herbicides and/or chlorophenols were likely to have occurred, namely farmers, forestry workers, gardeners, railroad workers, road workers, unspecified chemical workers (with or without stated exposure to phenoxy herbicides and/or chlorophenols (PHC)/non-PHC), sawmill workers, construction workers, and carpenters. Farmers and forestry workers were grouped together since almost all forestry workers in southern Sweden also have been working with farming.

Another grouping of all potentially chlorophenol exposed workers, *i.e.*, sawmill workers, construction workers and carpenters, was also made. In these further analyses, eight cases of lymphomas (ICD 7 codes 200–202) were removed from the cancer referent group because of the many reported findings of an association between lymphomas and exposure to phenoxy herbicides and chlorophenols.[12] In total, 331 individuals were exposed to one or more of these particular jobs. The unexposed category

TABLE 1. Distribution of Alive and Dead Individuals Among Cases and Referents

	Alive	Deceased	Total
Cases	32	39	71
Cancer referents	58	106	164
Population referents	159	156	315
			550

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TABLE 2. Elevated Risks for Some Specific Jobs and Smoking With Odds Ratio > 1.2 and Two or More Cases Exposed*

	OR	90% CI	No. of cases exposed	No. of referents exposed
Gardeners	Infinite	2.8	6	0
Railroad work	1.9	0.3–9.1	4	5
Construction work, asbestos exposure	5.9	1.3–36	7	3
Unspecified chemical work, potential exposure to phenoxy herbicides or chlorophenols	1.8	0.8–4.0	14	20
Farmer work, exposure to fertilizers	1.3	0.6–2.7	16	30
Construction work, other insulation exposure	3.3	0.9–12	8	6
Construction work, exposure to impregnating agents	4.8	0.7–53	2	4
Construction work, exposure to pressure impregnating agents	3.5	0.4–43	3	2
Sawmill work, sawing	1.5	0.4–5.6	5	8
Smokers	1.3	0.3–4.5	5	9

CI: confidence interval; OR: odds ratio.

* Other cancer cases were taken as referents.

was taken as individuals in jobs, where exposures to phenoxy herbicides or chlorophenols were unlikely.

In Table 4, the comparisons involved cancer referents and in Table 5 population referents. In these two tables, the exposed individuals could have had a combination of jobs with potential exposure to phenoxy herbicides and/or chlorophenols. In Tables 6 and 7 the same analyses are presented but here the individuals were exposed only to one such job, *i.e.*, no combinations of jobs were accepted, which reduced the number of individuals eligible in this context.

Age and smoking were controlled for in the analyses. A latency time requirement of 5 years was applied in these analyses, *i.e.*, an occupational exposure less than 5 years before diagnosis was not taken into account. Separations of alive and dead individuals did not change the results in any specific direction, and these analyses are therefore omitted in this report.

Discussion

The results from this study, although based on small numbers, corroborate some of the findings from other epidemiologic studies regarding an increased risk of STS among farmers and forestry workers. However, other occupations such as gardeners, railroad workers, unspecified chemical workers, and workers in contact with wood, had an increased risk of STS. These occupations may imply exposure to either phenoxy herbicides or chlorophenols, or both, but obviously other agents also.

It is somewhat surprising, however, that the occupational group with the highest risk for STS was gardeners.

TABLE 3. Elevated Risks for Some Specific Jobs and Smoking With Odds Ratio > 1.2 and Two or More Cases Exposed*

	OR	90% CI	No. of cases exposed	No. of referents exposed
Gardeners	4.1	1.0–14	6	7
Railroad work	3.1	0.6–14	4	6
Construction work, asbestos exposure	1.8	0.6–4.8	7	18
Unspecified chemical work, potential exposure to phenoxy herbicides or chlorophenols	1.6	0.8–3.3	14	41
Farmer work, exposure to fertilizers	1.3	0.6–2.6	16	57
Construction work, other insulation exposure	1.3	0.5–3.2	8	28
Construction work, exposure to impregnating agents	2.3	0.5–8.9	4	8
Construction work, exposure to pressure impregnating agents	1.7	0.3–7.3	3	8
Sawmill work, sawing	1.5	0.4–4.5	5	15
Smokers	1.5	0.4–4.6	5	15

OR: odds ratio; CI: confidence interval.

* A population sample was taken as referents.

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TABLE 4. Exposure to Some Risk Jobs Associated With the Use of Phenoxyacetic Acids and Chlorophenols*

Age (yr)			Unexposed	Farmers, forestry workers	Gardeners	Railroad workers	Road workers	PHC	Unspecified chemical workers (non-PHC)	Sawmill workers, construction workers, and carpenters
≥54	Nonsmokers	Cases	5	3	0	0	0	1	2	1
		Cancer Ref	4	3	0	0	0	0	0	0
	Smokers	Cases	7	1	0	1	1	0	2	1
		Cancer Ref	16	4	0	2	1	0	4	3
55–69	Nonsmokers	Cases	1	4	2	0	0	0	1	3
		Cancer Ref	5	7	0	0	0	0	1	3
	Smokers	Cases	6	11	2	3	0	1	2	8
		Cancer Ref	22	13	0	1	0	0	6	8
70–80	Nonsmokers	Cases	2	2	2	0	0	0	0	2
		Cancer Ref	4	11	0	1	1	0	1	8
	Smokers	Cases	1	7	0	0	0	0	3	6
		Cancer Ref	15	14	0	0	3	0	6	16
		Cases	22	28	6	4	1	2	10	21
		Cancer Ref	66	52	0	4	5	0	18	38
	OR		1.0	1.6	—	3.0	0.6	—	1.7	1.7
	M-H OR		1.0	1.9	—	2.6	0.9	—	2.1	2.6
	90% CI		—	(0.9–4.3)	—	(0.5–16)	(0.0–12)	—	(0.7–6.1)	(1.0–6.6)

OR: odds ratio; CI: confidence interval; M-H: Mantel-Haensel; PHC: unspecified chemical workers exposed to phenoxy herbicides and/or chlorophenols; Ref: referent.

* Unexposed are individuals never exposed to any of the risk jobs. Combined jobs, *i.e.*, exposure to more than one of the risk jobs can occur. Cancer cases other than sarcoma and lymphoma are taken as referents.

This association has, to our knowledge, not been reported in any other study. The chemical exposure situation among gardeners is unclear. Only two of a total of 13 gardeners stated that they had worked with pesticide spraying, and only one of these (a case) could tell what kind of pesticide he had used (Toxidol, a bromophos preparation).

Higher occupational risks appeared, especially for those individuals with exposure to more than one of the jobs studied. The number of cases and referents were very much reduced when only one specific and nonoverlapping job was studied, indicating that seasonal work or movement between the studied jobs was common.

The use of a cancer referent group should have mini-

TABLE 5. Exposure to Some Risk Jobs Associated With the Use of Phenoxyacetic Acids and Chlorophenols*

Age (yr)			Unexposed	Farmers, forestry workers	Gardeners	Railroad workers	Road workers	PHC	Unspecified chemical workers (non-PHC)	Sawmill workers, construction workers, and carpenters
≤54	Nonsmokers	Cases	5	3	0	0	0	1	2	1
		Population Ref	20	8	0	0	0	0	6	7
	Smokers	Cases	7	1	0	1	1	0	2	1
		Population Ref	41	16	2	2	2	0	13	18
55–69	Nonsmokers	Cases	1	4	2	0	0	0	1	3
		Population Ref	5	17	0	0	2	0	2	13
	Smokers	Cases	6	11	2	3	0	1	2	8
		Population Ref	27	25	2	1	2	1	5	20
70–80	Nonsmokers	Cases	2	2	2	0	0	0	0	2
		Population Ref	10	18	2	1	1	1	2	7
	Smokers	Cases	1	7	0	0	0	0	3	6
		Population Ref	24	29	1	1	4	1	7	17
		Cases	22	28	6	4	1	2	10	21
		Population Ref	127	113	7	5	11	3	35	82
	OR		1.0	1.4	4.9	4.6	0.5	3.8	1.6	1.5
	M-H OR		1.0	1.5	4.4	4.8	0.7	4.2	1.7	1.4
	90% CI		—	(0.7–3.0)	(1.0–18)	(0.8–24)	(0.0–6.2)	(0.4–41)	(0.7–4.3)	(0.7–3.0)

OR: odds ratio; CI: confidence interval; M-H: Mantel-Haensel; PHC: unspecified chemical workers exposed to phenoxy herbicides and/or chlorophenols; Ref: referent.

* Unexposed are individuals never exposed to any of the risk jobs. Combined jobs, *i.e.*, exposure to more than one of the risk jobs can occur. A population based sample is taken as referents.

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TABLE 6. Exposure to Some Risk Jobs Associated With the Use of Phenoxyacetic Acids and Chlorophenols*

Age (yr)			Unexposed	Farmers, forestry workers	Gardeners	Railroad workers	Road workers	PHC	Unspecified chemical workers (non-PHC)	Sawmill workers, construction workers, and carpenters
≤54	Nonsmokers	Cases	5	1	0	0	0	0	2	0
		Cancer Ref	4	3	0	0	0	0	0	0
	Smokers	Cases	7	1	0	1	0	0	2	0
		Cancer Ref	16	2	0	0	0	0	3	1
55–69	Nonsmokers	Cases	1	2	1	0	0	0	0	0
		Cancer Ref	5	5	0	0	0	0	1	1
	Smokers	Cases	6	5	1	0	0	1	1	2
		Cancer Ref	22	10	0	0	0	0	6	6
70–80	Nonsmokers	Cases	2	1	1	0	0	0	0	0
		Cancer Ref	4	5	0	0	1	0	1	2
	Smokers	Cases	1	4	0	0	0	0	2	2
		Cancer Ref	15	3	0	0	1	0	4	6
		Cases	22	14	3	1	0	1	7	4
		Cancer Ref	66	28	0	0	2	0	15	16
	OR		1.0	1.5	—	—	—	—	1.4	0.7
	M-H OR		1.0	1.6	—	—	—	—	1.6	1.1
	90% CI		—	(0.6–4.4)	—	—	—	—	(0.4–5.4)	(0.2–4.7)

OR: odds ratio; CI: confidence interval; M-H: Mantel-Haensel; PHC: unspecified chemical workers exposed to phenoxy herbicides and/or chlorophenols; Ref: referent.

* Unexposed are individuals never exposed to any of the risk jobs. No combined jobs are accepted, as reducing the material. Cancer diagnoses other than sarcoma and lymphoma are taken as referents.

mized the recall bias problem that often has been discussed when case-referent studies focus on malignant diseases. Hence, a recall bias, if existing, is likely to be similar for the cases and for the cancer referents. Furthermore, an association between the exposures and some other cancers than sarcoma (and lymphoma) would result in a decreased risk. Instead, the OR in this study increased when using cancer referents. This may indicate that the population working in the particular jobs with potential exposure, *i.e.*, mostly outdoor and physically active work, is healthy with regard to other cancers. The STS risk for railroad workers was higher when population referents were in-

TABLE 7. Exposure to Some Risk Jobs Associated With the Use of Phenoxyacetic Acids and Chlorophenols*

Age (yr)			Unexposed	Farmers, forestry workers	Gardeners	Railroad workers	Road workers	PHC	Unspecified chemical workers	Sawmill workers, construction workers, and carpenters
≤54	Nonsmokers	Cases	5	1	0	0	0	0	2	0
		Population Ref	20	5	0	0	0	0	6	4
	Smokers	Cases	7	1	0	1	0	0	2	0
		Population Ref	41	7	0	2	0	0	7	10
55–69	Nonsmokers	Cases	1	2	1	0	0	0	0	0
		Population Ref	5	11	0	0	0	0	2	5
	Smokers	Cases	6	5	1	0	0	1	1	2
		Population Ref	27	12	0	1	0	1	2	10
70–80	Nonsmokers	Cases	2	1	1	0	0	0	0	0
		Population Ref	10	12	0	0	0	1	0	2
	Smokers	Cases	1	4	0	0	0	0	2	2
		Population Ref	24	16	0	0	0	1	3	4
		Cases	22	14	3	1	0	1	7	4
		Population Ref	127	63	0	3	0	3	20	35
	OR		1.0	1.3	—	1.9	—	1.9	2.0	0.6
	M-H OR		1.0	1.4	—	1.8	—	2.1	2.0	0.7
	90% CI		—	(0.6–3.4)	—	(0.1–23)	—	(0.1–33)	(0.7–6.7)	(0.1–2.3)

OR: odds ratio; CI: confidence interval; M-H: Mantel-Haensel; PHC: unspecified chemical workers exposed to phenoxy herbicides and/or chlorophenols; Ref: referent.

* Unexposed are individuals never exposed to any of the risk jobs. No combined jobs are accepted, as reducing the material. A population sample is taken as referents.

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volved rather than cancer referents, indicating the possibility of a hidden association between railroad work and some cancer forms represented among the cancer referents.

Also, the fact that no systematic differences were found between answers from alive and the next-of-kin of dead individuals indicates that there should be no important recall bias in this material. It may be noted in this context that in a recent validation study from US, no difference was found between cases and referents with regard to recall of previous work areas, that is, the risk of recall bias is unlikely to be of any importance in case–referent studies based on occupational titles.[13] The proportion of smokers did not differ much between the unexposed and the exposed groups except for farmers, forestry workers, and gardeners, who had a somewhat lower proportion of smokers. Hence, with regard to these occupations, there might have been some uncontrolled confounding from smoking affecting the referent side[14,15] when cancer referents were used.

Even if the size of this study is rather small as a result of the relative rareness of STS, and despite the lack of specific exposure information, the overall results seem to confirm than refute earlier observations suggesting a relation of STS and, especially, exposure to phenoxy herbicides. However, this material shows a lower degree of risk in the combined group of occupations with potential exposure for chlorophenols, whereas construction workers with specified exposure for impregnating and insulating agents had relatively higher risks for STS. The strong effect seen for gardeners is difficult to explain and has to be considered further in new studies.

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The Evaluation of Trends in Soft Tissue Sarcoma According to Diagnostic Criteria and Consumption of Phenoxy Herbicides

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The possible association between exposure to phenoxy herbicides and development of soft tissue sarcomas has been studied in both case–control and cohort studies. In these studies soft tissue sarcoma cases have been identified from either deaths with malignant neoplasms of the connective tissue (ICD-8 171) as the underlying cause of death, from incident cancer cases with the same topography code, or from incident cases of extraskeletal sarcomas, excluding lympho and reticulosarcomas. The current study shows that the choice of source material has a considerable influence on the registered occurrence of soft tissue sarcomas in a well defined population. In 1978 to 1982 an annual number of 33 deaths from ICD-8 171 was registered in Denmark, whereas the annual number of diagnosed extraskeletal sarcomas was 231. The distribution over histologic types differed between tumours coded to the connective tissue and extraskeletal sarcomas coded to specific organs. Furthermore, during the period 1943 to 1982 the mortality from ICD-8 171 increased slightly, whereas the incidence from all extraskeletal sarcomas decreased slightly, except for males since 1970. Time trends based on any of the three source materials should, however, be interpreted with caution.

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SOFT TISSUE SARCOMAS (STS) defined as extraskeletal sarcomas other than lymphosarcomas and reticulosarcomas constitute only 1% of incident cancer cases in developed countries.[1,2] Nevertheless, they have attracted substantial attention since 1977, when clinical observations in Sweden indicated an association with exposure to phenoxy herbicides.[3] Two subsequent case–control studies, one in northern[4] and one in southern Sweden[5] showed associations with herbicide spraying with relative risks of 5.3 and 6.8, respectively. The three main types of phenoxy herbicides, 2,4,5-T, 2,4-D and MCPA, have been widely used as weed killers in agriculture and forestry, and 2,4,5-T and 2,4-D were used extensively for defoliation in the Vietnam War.

In 1981, four cases of STS were reported from small cohorts of US workers who had been exposed accidentally to the toxic dioxin 2,3,7,8-TCDD in manufacture of 2,4,5-T.[6,7] These observations prompted several further studies, the results of which have been inconsistent. No cases of STS were thus found in a cohort of Finnish men, who had sprayed 2,4,5-T and 2,4-D[8,9]; a weak, statistically nonsignificant association between STS and 2,4,5-T and 2,4-D exposure was found in a New Zealand case–control study[10]; an excess risk at the border of statistical significance was found in a cohort of workers in manufacture of MCPA in Denmark[11]; and no consistent pattern of excess risk of STS was seen with either duration or frequency of herbicide use in a recent case–control study from Kansas.[12]

In evaluating these inconsistent results, attention has been given to methologic weaknesses inherent in case–control studies, such as the possible recall bias in self-reported exposure data, and to the accuracy of the exposure data based on employment records. Little attention has been paid, however, to differences in source materials used for identification of STS cases. Table 1 shows that STS cases in the reported studies have been identified from either death certificate data, from cancer registry data with information on topography only, or from cancer registry data with information on both topography and morphology. These studies therefore cover either deaths from connective tissue tumours (ICD-8 171 or equivalent codes in other ICD versions), incident cancer cases with the same site-specific ICD code, or incident cancer cases with morphology codes for sarcomas in combination with topography codes for soft tissues. These three sources for case identification are all available in Denmark. The current study illustrates how

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TABLE 1. Epidemiologic Studies of the Relationship Between Soft Tissue Sarcoma and Exposure to Phenoxy Herbicides Classified by Study Design and Disease Indicator

Studies reference	Disease indicator	Inclusion of STS cases in organs	No. of STS cases	RR	95% confidence interval
Case–control studies					
Sweden, north/ph exposure (Hardell and Sandström[4])	Incidence	+	46	5.3	2.4–11.5
Sweden, south/ph exposure (Eriksson[5])	Incidence	+	99	6.8	2.6–17.3
New Zealand/ph exposure (Smith *et al.*[10])	Incidence	–	82	1.6	0.7–3.3*
England & Wales/farmers, *etc.* (Balarajan and Acheson[21])	Incidence	–	1961	1.15†	0.83–1.59
New York State, US/Vietnam service (Greenwald *et al.*[22])	Incidence	+	281	0.70	0.17–2.92
[illegible] patients, US/Vietnam service (Kang *et al.*[23])	Incidence	–	234	0.83	0.63–1.09
Veterans, Mass, US/Vietnam service (Kogan and Clapp[24])	Mortality	–	ND	5.16‡	2.39–11.14
Kansas, US/ph exposure (Hoar *et al.*[12])§	Incidence	+	133	1.0	0.7–1.6
Italy/ph exposure (Vineis *et al.*[25])‖	Incidence	–	17	2.70	0.6–12.4*
Cohort studies					
Railway workers, Sweden (Axelson *et al.*[26])	Mortality	–	0	—	—
Forest workers, Sweden (Hogsted and Westerlund[27])	Mortality	–	0	—	—
Sprayers, Finland (Riihimaeki *et al.*[9])	Incidence¶	–	0	—	—
Sprayers, Victoria, Australia (Royal Commission[28])	Mortality	–	1	1.43	0.04–7.96
Census-based cohorts, Sweden (Wiklund and Holm[29])	Incidence	+	258#	0.9	0.8–1.1
Ranch Handers, US (US Air Force[30])	Mortality	–	0	—	—
Vietnam veterans, Australia (Australian Veterans Health Studies[31])	Mortality	–	2	3.1	0.4–11.3
2, 4, 5-T manufacture, Dow Chemical, US (Ott *et al.*[32])	Mortality	–	0**	—	—
2, 4, 5-T manufacture, Monsanto, US (Zack and Suskind[33])	Mortality	–	1**	ND	ND
TCDD-exposed, Dow Chemical, US (Cook *et al.*[34])	Mortality	–	1**	ND	ND
TCDD-exposed, Monsanto, US (Zack and Gaffey[35])	Mortality	–	1**	ND	ND
TCDD-exposed, BASF, GFR (Thiess *et al.*[36])	Mortality	–	0	—	—
MCPA-manufacture, Denmark (Lynge[11])	Incidence	+	5	2.72	0.88–6.34
MCPA-manufacture, UK (Coggon *et al.*[37])	Mortality	–	1	1.06	0.03–5.93

* 90% confidence interval.
† Subcohort of farmers, farm managers and market gardeners, N = 42, RR = 1.7, CI95 = 1.00–2.88.
‡ Compared to non-Vietnam veterans from same era.
§ Worked or lived on farmland.
‖ Only living women included.
¶ Also analyzed for mortality.[8]
Only subcohorts "land and/or animal husbandry" and "silviculture" included.
** In total three observed cases equivalent to 2.9% of deaths in the four cohorts, where approximately 0.07% was expected,[6] subsequent pathology review showed only 2 of these cases to be STS.[38]
VA: Veterans Administration; Mass: Massachusetts; ND: no data; STS: soft tissue sarcoma; RR: relative risk.

the choice of source material influences the registered occurrence of, and trends in, STS in a well-defined population.

Material and Methods

Mortality Data

All deaths with malignant neoplasm of the connective tissue as the underlying cause of death during the period 1951 to 1982 were selected for analysis from the annual death certificate registrations. For the years 1951 to 1957 these were coded as ICD-6 197, for 1958 to 1968 as ICD-7 197, and for 1969 to 1982 as ICD-8 171; for abbreviation the notation "deaths ICD-8 171" is used. Mortality data for this rubric are not available before 1951.

Incidence Data

Population-based cancer registration was commenced in Denmark in 1942. From 1943 to 1977, notified cases were coded according to a modified version of ICD-7,[13] and from 1978 according to ICD for Oncology (ICD-O).[14] A computer program has been developed whereby ICD-O codes are translated to the modified ICD-7 codes, thus allowing consistent tabulations over time. Malignant neoplasms of the connective tissues have been coded as ICD-7 197; for abbreviation the notation "incident cases ICD-7 197" is used. Soft tissue sarcoma cases located to specified organs have been classified by means of a special Danish code,[13] excluding lymphosarcomas and reticulosarcomas and excluding cases with bone tumors (ICD-7 196) and connective tissue tumors

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TABLE 2. Average, Annual Number of Soft Tissue Sarcoma Cases and Age-Standardized Rate per 100,000 in Denmark 1978–1982 Based on Different Indicators for Occurrence of the Disease

Disease indicator	Men		Women	
	No. of cases	Age-standardized rate*	No. of cases	Age-standardized rate*
Deaths ICD-8 171	18	0.55	15	0.37
Incident cases ICD-7 197	39	1.21	28	0.67
Incident cases other STS	75	2.23	89	2.25
All incident STS cases	114	3.44	117	2.93

* World Standard Population.
STS: soft tissue sarcoma.

(ICD-7 197); for abbreviation the notation "incident cases other STS" is used.

Tabulations

Both mortality and incidence data have been tabulated by sex, 5-year age groups, and 5-year calendar periods. Age-standardized rates based on the World Standard Population are used for comparison over time.

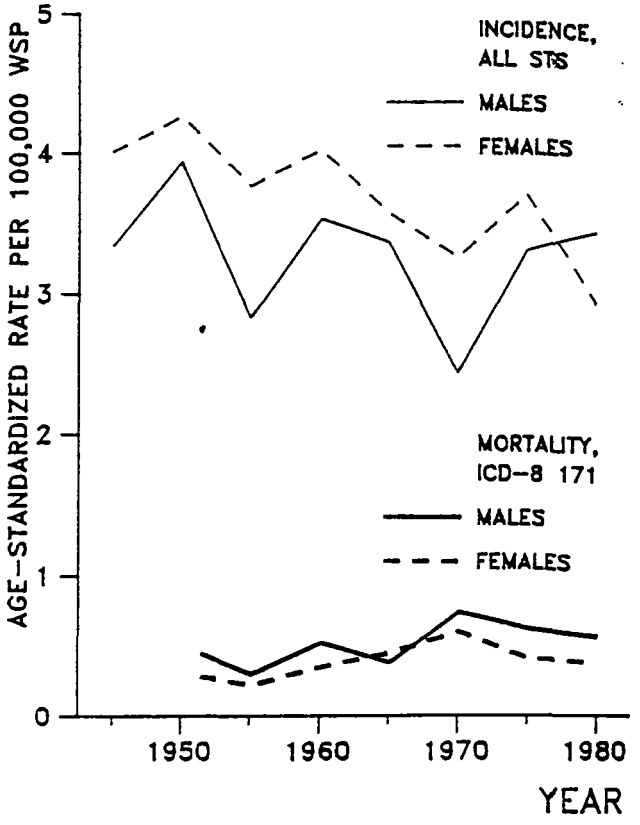


FIG. 1. Age-standardized (World Standard Population) mortality from malignant neoplasms of the connective tissue (ICD-8 171) and age-standardized incidence of all soft tissue sarcomas in Denmark, 1943 through 1982. Notice: Incidence figures for 1943 through 1977 include neuroblastomas.

TABLE 3. Incident Cases of ICD-7 197 and Incident Cases of Other STS Among Males and Females in Denmark 1978–1982 by Histologic Subtype

ICD-O morphology code Behavior 3–9	Men (%)		Women (%)	
	ICD-7 197 (N = 195)	Other STS (N = 376)	ICD-7 197 (N = 141)	Other STS (N = 443)
8800-8804 Sarcoma NOS	13	20	18	19
8810-8814 Fibrosarcoma	11	8	17	6
8830 Malignant fibrous histiocytoma	23	8	20	4
8850-8860 Liposarcoma	23	9	21	7
8890 Leiomyosarcoma	6	18	9	31
8900-8920 Rhabdomyosarcoma	10	7	5	1
9540-9560 Neurogenic sarcoma	—	5	—	5
Other specified sarcomas*	14	26	11	27
Total	100	100	100	100

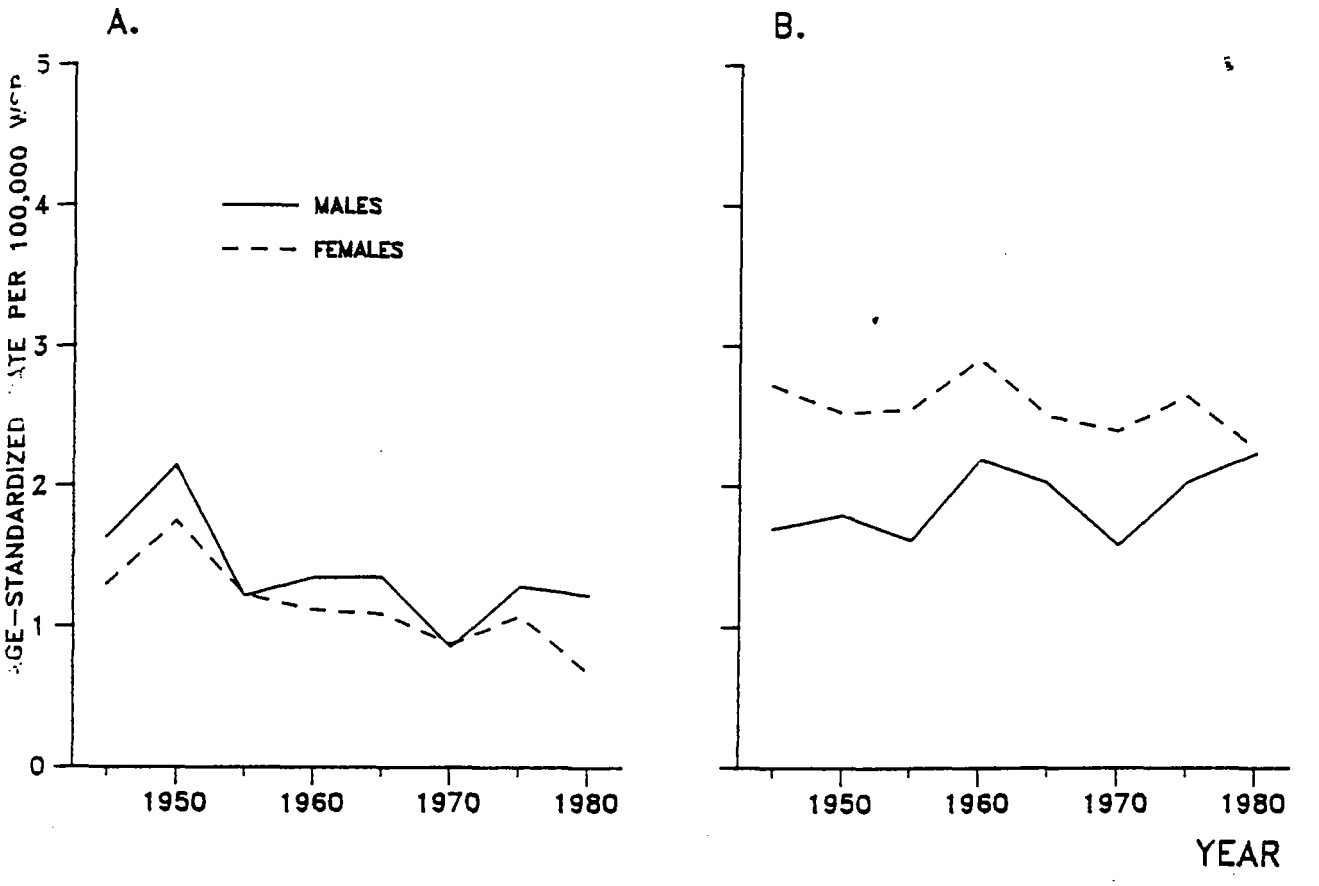
* Other specified sarcomas: 8710, 8831-8840, 8891-8895, 8930, 8980-8991, 9020-9044, 9120-9140, 9170, 9220-9240, 9270-9290, 9330, 9471, 9480-9481, 9530-9539, 9581.
STS: soft tissue sarcomas.

Results

Table 2 shows the average annual number in 1978 to 1982 of deaths from ICD-8 171, of incident cases coded to ICD-7 197, and of incident cases of other STS. The mortality figures give an annual number of STS cases in Denmark of 33 and age-standardized rates per 100,000 of 0.55 and 0.37 for men and women, respectively. A total annual number of 231 incident STS cases is observed when the two groups of "incident cases ICD-7 197" and "incident cases other STS" are combined. The age-standardized rates per 100,000 for all STS cases are 3.44 and 2.93 for males and females, respectively.

Table 3 shows the distribution by histologic types of incident cases of ICD-7 197 and of incident cases of other STS among men in 1978 to 1982. Malignant fibrous histiocytoma and liposarcoma are the predominant cell types among incident ICD-7 197 cases, whereas sarcoma not otherwise specified (NOS) and

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FIGS. 2A AND 2B. Age-standardized (World Standard Population) incidence of (A) malignant neoplasms of the connective tissue (ICD-7 197) and of (B) other soft tissue sarcomas in Denmark, 1943 through 1982. Notice: Incidence figures for other soft tissue sarcomas for 1943 through 1977 include neuroblastomas.

leiomyosarcoma are more frequent among the incident cases of other sarcomas than among the incident cases of ICD-7 197.

Figure 1 shows the time trends of STS in Denmark based on age-standardized mortality figures and age-standardized incidence figures for all STS, respectively. A slight increase is seen in the mortality from deaths ICD-8 171. The trend in incidence of all STS has been decreasing for both male and female patients until 1970, and a slight increase has been registered in the incidence for men after this point in time. Age-standardized rates for incident cases of ICD-7 197 and for incident cases of other STS are presented in Figures 2A and 2B. The incidence of ICD-7 197 has been decreasing for both sexes, whereas a slight increase is seen in the incidence of other STS in male patients and the incidence of other STS has been virtually stable over time for women.

Discussion

Soft tissue sarcomas form a morphologically heterogeneous group of tumors and risk factors are known for a few of these, *e.g.*, thorotrast[15] and vinyl chloride[16] for angiosarcoma of the liver, and von Recklinghausen neurofibromatosis for neurosarcoma.[17] There are indications of ionizing radiation being a risk factor for other types of STS as well, and of childhood STS tending to aggregate in siblings and to be associated with other cancers in family members.[2] Phenoxy herbicide may be an additional risk factor common for various types of STS, as suggested by the observation in Sweden and subsequent studies.

In the Swedish case–control studies, of STS and exposure to phenoxy herbicides, selection of cases was based on pathology registration systems, where incident cases of both sarcomas in the connective tissue and sarcomas of other anatomical sites could be identified. In subsequent studies, STS cases have been identified from death certificates, from cancer registers with topography codes only or from cancer registers similar to those used in Sweden. The current study shows that the mortality figures reflect only a fraction of the disease, and that both the occurrence of STS and the time trends in STS vary

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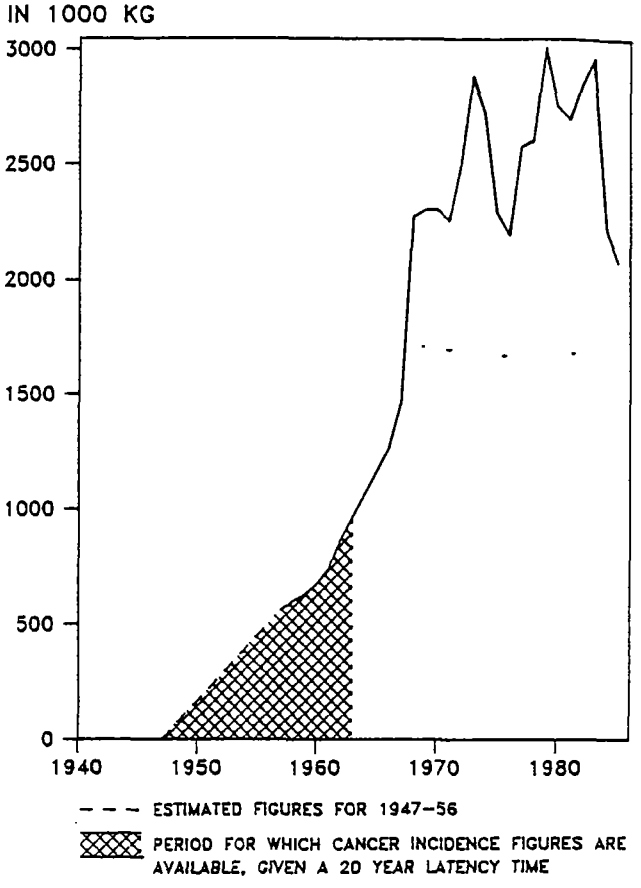


FIG. 3. Annual consumption of phenoxy herbicides in Denmark, 1947 through 1985.

considerably in a well-defined population, depending on which of the three data sources is used.

These differences do not necessarily imply, that the estimated relative risks, for development of STS after exposure to phenoxy herbicide, will vary between studies where STS cases are identified in different ways. The data source used for identification of STS cases has a major influence, however, on the statistical power of cohort studies. An illustration of this is given in the Danish cohort study of workers employed in manufacture of MCPA where five STS cases were found in the Danish Cancer Registry when information on both topography and morphology was used for identification of cases. Only one of these cases had the topography code ICD-7 197 on the cancer register record, and only this patient had the ICD-8 171 code on the death certificate.[11]

It has previously been pointed out that the frequency distribution of histologic types of STS ascertained in the New Zealand case–control study differed from those in the Swedish studies. Variation in pathologic classification or differences in etiologic factors have been put forward as possible explanations.[18] In this study differences were found between the distribution of histologic subtypes of incident cases of ICD-7 and incident cases of other sarcomas. Our data thus indicate, that a possible explanation for the difference found between New Zealand and Sweden is that the New Zealand study included only incident cases of ICD-7, whereas the Swedish studies included incident cases of all STS.

New Zealand data show both the incidence (ICD-9 171) and the mortality of STS to be higher for men than for women, and show trends over the period 1955 to 1979 to be increasing for both sexes.[10] The age-standardized incidence (ICD-7 197) and mortality figures for Denmark for 1978 to 1982 are lower, than the equivalent figures for New Zealand for 1975 to 1979. There is little difference between the figures for the two sexes in the incidence of all STS in Denmark; and the trends in incidence of all STS show a slight decrease over time in Denmark except for men since 1970. These differences between the figures for New Zealand and for Denmark are noticable, because New Zealand is known to have a large consumption of 2,4,5-T,[18] whereas very limited quantities of 2,4,5-T have been used in Denmark.[19]

However, as for other rare cancers time trends in STS should be interpreted with caution. The proportion of histologically verified cancer cases notified to the Danish Cancer Registry has increased from 68% in 1953 to 1957 to 90% in 1978 to 1982,[20] thus increasing the proportion of cases potentially coded by morphology as sarcomas. In the Kansas case–control study only 81% of the STS cases identified from cancer registry data could be confirmed in a pathology review.[12] Therefore, a possible effect of an increase in the proportion of histologically verified cases in a cancer registry might also be that some "STS cases" are eliminated.

Time trends may also be influenced by changes in classification. Thus, neuroblastomas could not be separated from neurofibrosarcomas for the period 1943 through 1977 where the modified version of the ICD-7 code[13] was used; and although the modified ICD-7 code allows site-specific coding of all extraskeletal sarcomas, fibrosarcomas and chondrosarcomas may have been coded to the bone. This is unlikely to happen with the ICD-O code,[14] where topographic and morphologic data are coded separately.

An additional reason, for reservation in interpretation of time trends in STS in relation to time trends in phenoxy herbicides consumption, is the latency time. In Denmark, 1982 was the last year for which cancer incidence figures are available. If we assume that there is a 20-year latency period between exposure to phenoxy herbicide and a possible excess risk for development of STS, the exposure for which we are now able to measure a possible effect, is the phenoxy herbicide consumption

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before 1962. An illustration of this is given in Figure 3. As the consumption of phenoxy herbicides before 1962 was limited, negative results based on time trends should at present be interpreted with caution, but surveillance of the trend in the near future is warranted.

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A cohort mortality study of forestry workers exposed to phenoxy acid herbicides

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Abstract

A cohort mortality study was undertaken of forestry workers at a public electrical utility who had worked for six months or more during 1950–82 and who were routinely exposed to herbicides including phenoxy acids. A total of 1222 men with 25 274 years at risk experienced 80 deaths. Ascertainment of vital state at the end of follow up was 95·5%. The male population of the province (Ontario) was used as the reference group. Overall, no excess mortality was found in this cohort relative to the reference population. A statistically significant increase in deaths occurred, however, due to suicide (SMR = 210, 95% confidence interval, 95% CI 105–376) for the cohort as a whole. No deaths were seen due to cancers such as soft tissue sarcoma and non-Hodgkin's lymphoma that have been cited by other authors as being associated with exposure to phenoxy acid herbicides. Although the cohort is not large, the absence of deaths due to these cancers is consistent with findings from other studies with sufficient numbers to allow examination of specific risks. The cohort is still young, however, and at the end of follow up most had not reached an age when increased incidence of cancer would normally be expected.

Phenoxy acid herbicides comprise a large part of vegetation management programmes in Canada. Several epidemiological studies have linked exposure to these herbicides with specific cancers such as soft tissue sarcomas and non-Hodgkin's lymphomas.[1–4]

A cohort mortality study was undertaken at a public utility (Ontario Hydro) that is responsible for the generation, transmission, and distribution of electricity to the province. The group under study belonged to the forestry trade. The workers use herbicides including phenoxy acids for brush control and line clearing along rights of way of power transmission and distribution lines. Concern regarding the possible associations with cancer and exposure to phenoxy acid herbicides prompted the investigation.

Control of vegetation is an integral part of forestry activities at this utility with herbicide application accounting for most of the control programme since 1950. Brush control and line clearing are performed throughout the year and although frequency varies seasonally, most forestry workers are exposed to herbicides on a weekly basis throughout the year. In the summer foliar application is province wide whereas in the colder months, basal sprays and stump treatments are routine. Exposure to herbicide occurs by breathing the aerosols or by direct contact either from touching recently sprayed vegetation or spray equipment; key factors affecting exposure include weather conditions, the height of brush and terrain, and the type of spraying operation.

Although measurements of personal exposure were not made until 1980, it can be assumed that failure to wear protective equipment and poor work practices in the 1950s and 1960s resulted in exposures to herbicides that were high and that were certainly greater than those likely to be experienced by most members of the general population. The work activities of this occupational group also indicate that exposure to herbicides may be regarded as proportional to duration of employment in the forestry trade at the company.

Table 1 gives a list of herbicides that were used during the period under study. Not all herbicides were used over the entire study period; 2,4-dichlorophenoxyacetic acid (2,4-D) and until March 1979, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were the most commonly used. Work with phenoxy acid herbicides increased steadily after 1950 because of the need to control greater land area under new lines, and because this means of vegetation management was the most labour and cost efficient. In a 10 year period, the amount of active ingredient of phenoxy acid herbicide used increased by almost 50%.

The exact 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD) content of the 2,4,5-T used within the company was not known, but in 1974 the permissible concentration of TCDD in 2,4,5-T registered in Canada was less than 0·5 ppm. This was reduced to

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Table 1 Herbicide use within Ontario Hydro

Chemical families of herbicides (generic/trade name)	*Approximate period of use**
Phenoxy acids:	
2,4-D (weed spray)	1950–82
2,4-D + 2,4-DP (Desormone; Silvaprop; Diphenoprop)	1979–82
2,4,5-T	1950–79
2,4-D + 2,4,5-T (Brushkill)	1950–79
2,4,5-TP (Silvex, Kuron)	1952–79
Atrazine + 2,4-D	1973–82
Picloram/2,4-D (Tordon 101)	1965–82
S-Triazines:	
Atrazine	1970–82
Simazine (Simmaprim)	1970–82
Substituted urea:	
Dioxin (Karmex)	1955–82
Monuron (Ureabor; Telvar "W")	1950–73
Tebuthiuron (Spike)	1978–82
Other:	
Aminotrizole (Amitrol)	1968–82
Ammonium sulphamate (Amate)	1950–71
Arsenic trioxide	1952–4
Bromacil (Hyvar)	1958–82
Dicamba (Banvel; Dycleer)	1973–82
Fosamine ammonium (Krenite)	1981–2
Glyphosate (Roundup)	1975–82
Maleic hydrazide (MH 30)	1970–5
Paraquat (Gramoxone)	1963–82
Picloram (Tordon 10K)	1965–82
Sodium TCA	1950–82
Stump spray	1950–82

*Relative to study period.

0·1 ppm in 1975 and was required to be undetectable with analytical methodology that was sensitive to concentrations of 0·1 ppm TCDD. Before 1974, TCDD content of 2,4,5-T may have been 60–120 ppm. In Canada currently registered 2,4-D must have a total dioxin content of no more than 30 parts per billion (ppb) and no more than 10 ppb of any dioxin isomer.[5] Because of a different formulation process, TCDD is not expected to occur in 2,4-D, but the legislative requirement is that it must be undetectable at a detection limit of 1 ppb.

By the nature of the routine duties of the forestry workers at this utility, employment during the period of study may be considered synonymous with exposure to phenoxy acid herbicides. The object was to determine whether exposure to phenoxy acids in this group of workers was associated with a mortality experience different from that of the total male population of Ontario.

Subjects and methods

A cohort of 1222 workers was identified who had worked six months or more in the forestry trade at a public utility (Ontario Hydro) at some time during the period 1 January 1950 to 31 December 1982. The cohort was identified from records within the company. The follow up period coincided with the period of ascertainment—namely, 1950–82 and was accomplished using internal company records, drivers' licence records (Ministry of Transportation and Communications) and the Canadian Mortality Data Base (Statistics Canada). Vital state was verified only from official sources. For all deaths but one, the underlying cause of death was obtained from the official death certificate. Cause of death and the International Classification of Diseases (ICD) code were recorded as stated on the death certificate to maintain comparability with the statistics used for the reference population. For one person, only the fact of death could be confirmed from official records and the cause of death was obtained from other contacts.

Person-years at risk were calculated with a program based on the standard person-years approach.[6,7] Expected numbers of deaths were derived from Ontario provincial death rates for males using 15 five year age groups and seven calendar periods centred on the official five year censuses. Standardised mortality ratios (SMRs) were calculated for main ICD cause of death categories and for suicides, lung cancer, and stomach cancer. Tests of statistical significance were applied assuming that the observed deaths had a Poisson distribution, the mean and variance of which were equal to those for the expected number. The 95% confidence intervals (95% CI) were calculated according to Byar's formula.[8]

Results

Ascertainment of vital state was complete for 95·5% of the cohort. Fifty five members were lost to follow up after a mean duration in the forestry trade of 4·2 years.

Of the 1222 cohort members, age specific person-years could not be calculated for two because of the unavailability of birth dates. Therefore, the person-years calculation for the cohort includes 1220 members who contributed 25 274 person-years during the 33 year follow up. The employment state of the cohort as of the end of follow up is indicative of the trade's stability, most being still employed as foresters (table 2).

Sixty per cent of the cohort has been followed up for 20 years or more with the mean length of follow

Table 2 Employment state by duration in forestry trade

Employment state as of 31 December 1982	*Years in forestry trade*		
	<5	*5–14*	*≥15*
Active employment in forestry trade	99	182	260
Active employment elsewhere in corporation	34	100	69
Retired	1	3	39
Left service	156	117	27
Deceased	21	18	41
Vital state unknown	39	16	—
Total	350	436	436

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up equal to 21 years (table 3); the main contributors are those cohort members with a long duration in the forestry trade. For the 361 cohort members who worked 20 years or more, the average duration in the trade was 28·6 years with a range of 20–46 years. About 30% of the person-years at risk are allocated where time since first entry to the forestry trade is 15 years or longer and years employed in the trade exceeds 10 (table 4).

The 80 deaths identified for the period 1950–82 contributed to an SMR of 94 for mortality from all causes indicating that this cohort experienced an overall mortality lower than that for men of corresponding age in the general population of Ontario, but not significantly so. This was also the case for mortality from circulatory diseases, diseases of the nervous system, respiratory system, genitourinary system, and digestive system (table 5).

Seventeen deaths occurred due to malignant neoplasms (table 6). One neoplasm, acoustic neuroma, was benign and death was due to a complication of therapy. Inspection of the death certificate for one other cohort member showed that the cause of death, bladder cancer, was incorrectly coded. The ICD code applied by the vital statistics official to the underlying cause reflected a complication of the treatment of the disease (abdominal wall fistula) as probably the underlying cause of death. Due to the small number of deaths for each type of cancer, expected numbers were calculated only for lung and stomach cancer. No significant excesses in cancer mortality were seen: there were five observed deaths from lung cancer *v* 4·57 expected and two observed deaths from stomach cancer *v* 1·05 expected. The SMR of 210 for suicide with 11 observed deaths *v* 5·25 expected was the only statistically significant finding ($p = 0·04$).

Table 3 Duration of follow up and employment state

Employment state as of 31 December 1982	Years of follow up			
	<10	10–19	⩾20	Total
Active employment in forestry trade	187	116	238	541
Active employment elsewhere in corporation	8	23	172	203
Retired	—	—	43	43
Left service	16	53	231	300
Deceased	18	14	48	80
Vital state unknown	44	8	3	55
Total	273	214	735	1222

Table 4 Distribution of person-years by duration of employment in the forestry trade and time since first exposure

Duration in forestry trade (y)	Time since first exposure				
	<10	10–14	15–19	⩾20	Total
<5	6289·0	860·6	719·5	1055·1	8924·2
5–9	3926·9	636·9	528·3	676·0	5768·1
10–14	—	2809·4	579·2	839·8	4228·4
⩾15	—	—	1957·8	4395·8	6353·6
Total	10215·9	4306·9	3784·8	6966·7	25274·3

*Table 5 Observed (Obs) and expected (Exp) deaths, SMRs, and 95% CIs for major causes of death**

Cause of death	Obs	Exp	SMR (95% CI)
All causes	80	85·02	94 (75–117)
Diseases of circulatory system	27	29·39	92 (61–134)
External causes (accidents)	25	23·04	109 (70–160)
Neoplasms (malignant and benign)	18	16·48	109 (65–173)
Diseases of nervous system and sense organs	2	2·06	97 (11–350)
Diseases of respiratory system	3	3·3	91 (19–265)
Diseases of digestive system	4	4·54	88 (24–225)
Diseases of genitourinary system	1	1·00	100 (1–556)

*Male Ontario population rates as a standard.

Table 6 Numbers of deaths due to malignant neoplasms

Type of cancer	No (1950–82)
Lung	5
Stomach	2
Pancreas	2
Colon	2
Bladder*	1
Thyroid	1
Leukaemia (acute myelogenous)	1
Prostate	1
Tongue	1
Kidney	1
Total	17

*One other death was coded on the official death certificate to "diseases of the digestive system" but bladder cancer should be regarded as the underlying cause of death.

Discussion

As expected, this cohort shows evidence of the healthy worker effect. The low SMRs for mortality due to all causes and diseases of the circulatory system reflect the selection and survival of healthy and fit people into the physically demanding forestry occupation.[9–12]

A slight but non-significant excess of deaths occurred for all causes of mortality for those employed less than five years, which is a finding characteristic of most occupational cohorts.[13]

No clear explanation exists for the significant twofold excess in suicides or the fact that suicide risk decreases with years spent in the forestry trade. With one exception, there was no personal medical information which might help to explain this excess. One suicide death might have been accounted for by the cohort member's medical history that included a progressively disabling neurological disease.

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Hogstedt and Westerlund,[14] in their cohort study of forestry workers in Sweden, reported an increase in violent deaths. Six of the 12 "violent deaths" were suicides. The standardised proportionate mortality ratios and standardised mortality odds ratios calculated by Kogan and Clapp[15] in their study of Massachusetts Vietnam veterans showed statistically significant excesses for suicides. Other studies relating either to exposure to phenoxy acid herbicide or to forestry work have not shown such excesses but few were cohort investigations in which a complete picture of the range of outcomes was obtained. In my study, the suicides appeared to be clustered in the period 1957–67 and in view of this, consideration might be given to the theory of imitative behaviour.[16] Alternatively, a different explanation is worthy of speculation. Although it may seem that increased risk of suicide would be unrelated to forestry work or more specifically to exposure to phenoxy acid herbicide, some published reports describe neurological toxicity associated with exposure to phenoxy acid herbicides. A farming student committed suicide by ingestion of the dimethylamine salt of 2,4-D; the necropsy examination showed degenerative ganglion cell changes in the brain, although the substance did not appear to concentrate in the brain. The authors,[17] with the provision that the cerebral changes were not due to anoxia, suggested that the nervous tissue must have been extremely sensitive to this substance. Oliver[18] described personality changes and neurological disturbances in two laboratory workers who were exposed to 2,3,7,8-tetrachlorodibenzo-1,4-dioxin. In a health survey of workers in a factory which made 2,4,5-T, a correlation was found between the severity of chloracne, known to be associated with dioxin exposure, and a high score on the manic scale of the Minnesota multiphasic personality inventory.[19] It is therefore reasonable to consider whether neurological toxicity associated with exposure to phenoxy acid herbicide might have psychiatric manifestations such as suicide.[20] Whether the increased risk of suicide is a chance finding resulting from testing for multiple hypotheses or whether it is real merits further study.

The knowledge that this study was being conducted prompted an inquiry from one cohort member who had recently experienced a basilar artery thrombosis with infarction of the occipital lobe at age 53 years. Both he and his physician raised the question of a causal relation with exposure to herbicides. The documented exposure for this cohort member while employed with the company was 2,4,5-T, arsenic trioxide, and monuron. The death of one member of the cohort, at 44 years of age was also due to basilar artery thrombosis. Such a diagnosis is uncommon at those ages and although the study does not permit a full determination of incidence of specific health outcomes, the question of basilar artery thrombosis as a late complication of exposure to phenoxy acid herbicides is worthy of consideration. At least one report exists citing changes in the central nervous system due to an unusual type of severe atherosclerosis of the cerebral arteries in a 57 year old subject working in production of sodium 2,4,5-trichlorophenoxyacetate and butyl ester of 2,4,5-trichlorophenoxyacetate acid, and with documented chloracne linked to chronic TCDD intoxication.[21]

Although this investigation was prompted by concern regarding findings in Sweden of soft tissue sarcomas and exposure to phenoxy acid herbicides,[1] no deaths from cancer of this type were found. Nor did the study show any deaths due to non-Hodgkin's lymphoma, which other authors have shown to be associated with exposure to this type of herbicide.[2 3] Because of the small size of the cohort, the statistical power to detect real increases in risk of non-Hodgkin's lymphoma or soft-tissue sarcoma, however, is low. This study only had sufficient statistical power (84%) to detect a sevenfold increase in non-Hodgkin's lymphoma. For soft tissue sarcoma, the power was 80% to detect a 27-fold increase, with two observed deaths. Despite this, the absence of deaths due to soft tissue sarcoma or non-Hodgkin's lymphoma is consistent with findings in other studies[22–24] that, as described by Johnson,[25] had sufficient numbers to examine the risks for these cancers.

The cohort is characterised by a group of young men who entered the forestry trade in the 1950s and who have mostly remained in this trade group throughout their career. Many of the cohort members are only now approaching the age when mortality would be expected. At the end of follow up, 96% of the person-years experience was contributed by subjects less than 55 years of age with an average age at the end of follow up of 43 years. It would therefore still be possible for an excess of cancer deaths to be observed in further follow up. Although the follow up period of 33 years might appear adequate, the results can only be interpreted with caution.

Although not directly relevant to the purpose of this investigation, it is of interest that only one death was due to acute myelogenous leukaemia. An increased risk of this leukaemia subtype has been found in occupational groups thought to be exposed to electromagnetic fields.[26–28] The primary function of the forestry trade is to perform line clearing adjacent to Ontario Hydro lines and associated apparatus and linemen at this utility work close to both transmission and distribution lines. Movement between the forestry and line trade is not uncommon. Leukaemia is a cancer with a relatively short latent period, and if a leukaemogenic risk associated with electromagnetic fields surrounding power lines exists, some evidence might be expected even in a small cohort.

In summary, the results agree with findings from

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other studies in which no association was found between phenoxy acid herbicide exposure and certain cancers. The cohort is small, however, and extended follow up is required. The excess of suicides in the cohort was an unexpected finding and prompts speculation regarding the biological plausibility of the result in relation to exposure to herbicide. Evidence from the epidemiological studies completed thus far cannot discount the carcinogenic potential of phenoxy acid herbicides. It would seem from this study, however, that if such a risk exists, it is low and requires a long latent period.

Consultation on the analysis was provided by Mr Jim Julian, a statistician with McMaster University, Hamilton, Ontario.

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Mortality and incidence of cancer at four factories making phenoxy herbicides

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Abstract

To assess the possible carcinogenicity of phenoxy herbicides and related chlorophenols and dioxins, the International Agency for Research on Cancer is coordinating an international collaborative study of workers exposed to these compounds in their production or use. Four British cohorts of chemical manufacturers which have been recruited to the survey are described. They comprise a total of 2239 men employed during 1963–85. These subjects were traced to 31 December 1987 through the National Health Service Central Register and the National Insurance Index, and their mortality compared with that in the national population. Two deaths were from non-Hodgkin's lymphoma with 0·87 expected. Both deaths occurred more than 10 years after first exposure to phenoxy compounds. One further non-Hodgkin's lymphoma was registered in a living subject with probable exposure to phenoxy compounds. No cases of soft tissue sarcoma or Hodgkin's disease were recorded. A non-significant excess of lung cancer (19 deaths observed, 14·2 expected) is probably attributable to chance or a confounding effect of smoking. In one cohort only there was increased mortality from circulatory disease (34 deaths observed, 20·4 expected). A nested case-control study did not point to any occupational cause for this excess, but further evaluation will be needed during continued follow up.

The regulation of pesticides requires that the utility of products be balanced against any risks to the environment and to the health of manufacturers, users, and those eating contaminated foods. Judgements about the toxicity of new compounds in man are usually based on extrapolation from laboratory experiments in vitro and in animals. Many pesticides have now been in use long enough, however, that it is possible to assess even their chronic effects more directly. Such assessment is important because laboratory investigation may not always reliably predict toxicity in man.

One group of compounds that have attracted particular attention are the phenoxy herbicides (for example, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4-dichlorophenoxyacetic acid (2,4-D), 2 methyl-4 chlorophenoxyacetic acid (MCPA)). Discovered in the 1940s, these chemicals have found extensive worldwide use in agriculture and forestry, as well as being sold on a smaller scale for domestic application in gardens. Initial case-control studies in Sweden suggested that exposure to phenoxy compounds and structurally related chlorophenols might carry an increased risk of soft tissue sarcoma[1,2] and lymphoma.[3] This idea was supported by the findings of four cohort studies of chemical manufacturers in the United States.[4–7] Subsequent investigations, however, have been less conclusive.[8–22] Several have shown associations between the manufacture or use of phenoxy compounds and soft tissue sarcoma or lymphoma, but risk estimates have been smaller than in the earlier studies and usually not statistically significant.

The statistical uncertainty surrounding small increases in risk for rare diseases presents a problem in evaluation. To help resolve this issue, the International Agency for Research on Cancer (IARC) has established an international collaborative survey of workers exposed to phenoxy acids and related compounds.[23] The study includes cohorts from six British companies. Data on two have already been published.[14,24] Here we describe the other four British workforces in the IARC survey, and report their mortality and cancer incidence. The findings are relevant not only to the assessment of phenoxy compounds but also to other chemicals that have been manufactured and formulated at the same factories.

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Table 1 Definition of cohort

Factory	*Phenoxy herbicides and chlorophenols produced and formulated (unless otherwise stated, dates are for both production and formulation)*	*Other main products*	†*Definition of cohort*	*Number of men analysed*	*Number of men traced*
A	2,4,5-T 1968–78 2,4-D and 2,4-DP 1954– 2,4-DB 1965– MCPA and MCPP 1954– MCPB 1970– PCPA and PAA (produced only) 1969–	Picric acid Dinitro-o-butyl phenol Dinitro-o-cresol Simazine Aminotriazole Oxynils	All manual employees during April 1975–October 1985	1104	1082
B	2,4,5-T (formulated only) 1970–74 2,4-D, 2,4-DP and 2,4-DB 1969– MCPA and MCPP 1969– MCPB formulated 1969– produced 1975–	Dithiocarbamates Organophosphorus compounds Carbamates Dinocap Toluidine compounds Urea herbicides Imidazole compounds Phthalimide compounds	All weekly paid employees during March 1969–November 1985	271	261
C	2,4,5-T (formulated only) 1959–81 2,4,-D (formulated only) 1959–81 MCPB 1956– PBA (produced only) 1958–75	Aminophylline Metronidazole Sulphonamides Oxynils Dinocap Asulam Sodamide Diflufenican	(1) All process workers on the phenoxy plant during January 1963–December 1984 (2) All formulators and packers during January 1982–December 1984 (3) All maintenance workers during July 1967–December 1984	345	343
D	2,4,5-T (formulated only) 1960–79 2,4-D 1949– 2,4-DP 1955– MCPA 1951– MCPP produced 1955–formulated 1965– 2,4,6-TCP (produced only) 1983–	Metallic soaps Plasticiser esters Naphthenic acid Alkyl phenols Phthalate esters	All weekly paid employees during April 1969–December 1985	519	503

*2,4,5-T = 2,4,5-Trichlorophenoxyacetic acid, 2,4-D = 2,4-dichlorophenoxyacetic acid, 2,4-DP = 2,4-dichlorophenoxypropionic acid, 2,4-DB = 2,4-dichlorophenoxybutyric acid, MCPA = 2 methyl-4 chlorophenoxyacetic acid, MCPP = 2 methyl-4 chlorophenoxpropionic acid, MCPB = 2 methyl-4 chlorophenoxybutyric acid, PCPA = parachlorophenoxyacetic acid, PAA = phenoxyacetic acid, PBA = phenoxybutyric acid, 2,4,6-TCP = 2,4,6-trichlorophenol.
†As only one woman met these criteria, the study was restricted to men.

Method

Subjects worked at four factories each of which manufactured and formulated a range of chemicals including phenoxy herbicides (table 1). The compound 2,4,5-T was produced at factory A during 1968–78, but at the other factories it was only formulated. Manufacturing processes were similar at all four factories. The relevant substituted phenol was reacted with monochloroacetic acid to produce phenoxyacetic compounds, with α-chloropropionic acid to make phenoxypropionic compounds, and with γ-butyrolactone to produce phenoxybutyric compounds. The 2,4,6-trichlorophenol was synthesised at factory D by chlorination of dichlorophenol.

Table 1 sets out the criteria for entry to the cohort. Scope for recruitment was limited by the availability of complete records from which subjects could be identified. At factories A, B, and D information was obtained from personnel or wages files. At factory C process and maintenance workers were identified from bound registers kept by shift foremen, and formulators and packers from personnel records. For each member of the cohort we abstracted (as far as they were available) the name, date of birth, address, national insurance number, and job history.

Job histories were used to classify subjects according to their potential exposure to phenoxy compounds and chlorophenols. At factories A and D records were sufficiently detailed to distinguish jobs that entailed work with these chemicals. The few subjects who worked only in non-phenoxy plants were considered to have only "background" exposure. At factory C process operators were identified from the shift register on the phenoxy plant, and thus are known to have worked with phenoxy compounds. Maintenance workers and formulators and packers were also considered to be exposed, although a few may not have come into contact with the compounds if they only worked in the job for a short period. The employment records at Factory B

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Table 2 Observed and expected mortality by cause: all subjects

Cause of death	Deaths observed	Analysis based on national rates			Expected numbers locally adjusted		
		Deaths expected	SMR	95% CI	Deaths expected	SMR	95% CI
All causes	152	136·22	112	94–131	134·73	113	96–132
Circulatory disease	74	63·76	116	91–146	63·94	116	91–145
Respiratory disease	8	11·78	68	29–134	11·74	68	29–134
Injury and poisoning	19	12·27	155	93–242	11·06	172	103–268
All cancer	37	36·84	100	71–138	36·66	101	71–139
Cancer of lung	19	14·15	134	81–210	14·21	134	80–209
Soft tissue sarcoma	0	0·18	0	0–2087	0·18	0	0–2058
Hodgkin's disease	0	0·43	0	0–855	0·38	0	0–976
Non-Hodgkin's lymphoma	2	0·87	229	28–827	0·73	272	33–983

Deaths were also observed from cancer of mouth (1), oesophagus (1), stomach (2), small intestine (1), large intestine (2), rectum (2), bowel not further specified (1), pancreas (2), larynx (1), bone (1), testis (1), and bladder (1).

did not distinguish between work on different processes, and individual exposures therefore could not be assigned with confidence. Management estimated that approximately 50% of employees would have worked with phenoxy compounds during campaign periods (January–May), but all staff were likely to walk through the production areas on occasions. No environmental or personal monitoring for phenoxy compounds, chlorophenols, or contaminant dioxins had been carried out at any of the factories.

Subjects were traced through the National Health Service Central Register and National Insurance Index up to 31 December 1987. For those who had died we obtained a copy of the death certificate with the underlying cause of death coded to the ninth revision of the International Classification of Diseases. We were also notified of any cancers registered among living members of the cohort.

Mortality ratios standardised for age (in five year bands), sex, and five year calendar period of death were calculated by the person-years method with rates for England and Wales as standard. In some analyses a second set of mortality ratios was derived with expected numbers of deaths for each factory adjusted in proportion to standardised mortality ratios (SMRs) during 1974–85 for the local authority area in which the factory was situated. Confidence intervals for mortality ratios were based on the Poisson distribution.

Results

A total of 2256 men satisfied the criteria for entry to the cohort, but 17 had to be excluded from the analysis because their date of birth was unavailable. Of the remaining 2239 men, 2189 (97·8%) were traced, including 152 who had died and 19 who had emigrated. These 19 were followed up to the date that they left the country, while untraced subjects were considered to be at risk up to their last known date of employment. Table 1 shows the numbers of men traced from each factory.

Table 2 shows mortality by cause for the full cohort. The total of deaths observed during the follow up period was a little higher than expected from national rates, and this was due largely to excesses of circulatory disease (SMR = 116, 95% CI 91–146) and of deaths from injury and poisoning (SMR = 155, 95% CI 93–242). Overall cancer mortality was close to expectation, but there was a small excess of deaths from lung tumours (19 observed *v* 14·15 expected). Two deaths from non-Hodgkin's lymphoma were recorded compared with

Table 3 Mortality by factory for selected causes

Cause of death	Factory A		Factory B		Factory C		Factory D	
	Deaths observed	Deaths expected	Deaths observed	Deaths expected	Deaths observed	Deaths expected	Deaths observed	Deaths expected
All causes	62	44·70	17	11·90	22	30·13	51	49·50
Circulatory disease	34	20·42	5	5·29	12	14·31	23	23·75
Respiratory disease	1	3·41	1	1·25	0	2·33	6	4·79
Injury and poisoning	7	4·77	3	1·43	3	2·59	6	3·48
All cancer	12	12·26	5	2·83	6	8·34	14	13·42
Cancer of lung	6	4·63	3	0·99	4	3·22	6	5·30
Non-Hodgkin's lymphoma	0	0·31	0	0·07	1	0·20	1	0·29

Expected numbers are based on national rates.

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Table 4 Mortality from selected causes in men at Factories A, C, and D with greater than background exposure to phenoxy compounds and/or chlorophenols

Cause of death	Subjects followed up from first exposure above background		Subjects followed up from 10 years after first exposure above background	
	Deaths observed	SMR	Deaths observed	SMR
Circulatory disease	59	117	31	110
All cancer	30	102	13	80
Cancer of lung	14	123	6	92
Non-Hodgkin's lymphoma	2	282	2	588

SMRs are based on national rates.

0·87 expected, but there were no deaths from Hodgkin's disease or soft tissue sarcoma. Adjustment for local differences in mortality had little effect on expected numbers.

Table 3 summarises mortality patterns by factory. The excesses of lung cancer and of injury and poisoning were spread across all factories, whereas the high rate of circulatory disease was confined to factory A (34 deaths observed *v* 20·42 expected). With adjustment for local mortality, the expected number of circulatory deaths at factory A rose to 24·49, and the excess fell just short of statistical significance at a 5% level. The two deaths from non-Hodgkin's lymphoma occurred at factories C and D.

Table 4 shows mortality ratios for subjects at factories A, C, and D with more than background exposure to phenoxy compounds or chlorophenols. Standardised mortality ratios for circulatory disease and all cancers were similar to those for the full cohort, whereas that for lung cancer was rather lower, especially after allowance for a latency of 10 years from first exposure. The two cases of non-Hodgkin's lymphoma both occurred more than 10 years after first exposure to levels above background—one man had been a packer for 21 years and the other a process operator in phenoxy acid synthesis for one month.

Only one further case of non-Hodgkin's lymphoma had been registered among living members of the cohort—a process worker at factory B. There were no registrations of Hodgkin's disease or soft tissue sarcoma.

Eight of the 19 deaths from injury and poisoning occurred while the men concerned were still working at the factories under study. Of these, two were due to accidents at work, two to accidents outside work, three to suicide or possible suicide, and one to homicide.

To investigate further the excess of circulatory disease at factory A, we carried out a nested case-control study. Each of the 34 cases at the factory was matched with four controls who were under follow up at the time that the case died, and whose date of birth was as close as possible to that of the case. Year of birth was matched to within one year for 82% of controls, and the mean year of birth of controls was one year later than that of cases. Associations with having ever worked in various departments were examined by conditional logistic regression for matched sets.[25] Table 5 summarises the findings. No individual department was clearly associated with a risk of circulatory disease. Risk was significantly increased among the aggregate of smaller departments (the 'other' category in table 5), but detailed examination did not suggest a common underlying factor.

Discussion

Although the workforces that we studied were exposed to a multiplicity of chemicals, this only presents a problem of interpretation in relation to positive findings. Any reassurance deriving from

Table 5 Risk of circulatory disease at Factory A according to department

Department	Subjects ever employed in department		Relative risk	95% CI
	Cases (n=34)	Controls (n=136)		
Oxynils	0	16	0	—
Nitration products	1	1	4·0	0·3–64·0
Triazines	0	4	0	—
Engineering	4	19	0·8	0·3–2·6
Effluent	2	8	1·0	0·2–4·7
Plant cleaning	2	5	1·6	0·3–8·2
Other	15	19	6·2	2·3–16·4

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risks which were not increased extends to all exposures experienced by the cohort. Unfortunately, levels of exposure to specific chemicals could not be precisely estimated. In particular, no hygiene data were available for phenoxy compounds, for the chlorophenols from which they were made, or for the dioxins with which they may have been contaminated. From descriptions of processes and methods of operation provided by longstanding employees, however, we estimate that exposures during the early part of the study period would generally have been higher than more recently. Thus, any risks from current working practices at the factories are likely to be lower than those in the workers studied.

No soft tissue sarcomas or Hodgkin's lymphomas were recorded in the study cohort during the period of follow up. There were, however, two deaths from non-Hodgkin's lymphoma compared with 0·87 expected, and one further case was registered in a subject who was still alive. Expected numbers of incident cancers could not be reliably estimated because the completeness of cancer registration in Britain has varied between regions and over time, but our findings accord with those of most previous studies in suggesting that any risk of soft tissue sarcoma or lymphoma from occupational contact with phenoxy compounds is small.[8–22] A more precise estimate of the risk of these rare tumours will be possible from analysis of the full IARC survey with its much larger study population.

The largest excess of cancer found was for lung tumours (19 deaths observed *v* 14·2 expected), and this derived from all four factories. Most of the deaths occurred, however, less than 10 years after first exposure to phenoxy compounds or in subjects with only background exposure. After allowance for a latency of 10 years from first exposure, the increased risk disappeared (table 4). This and the absence of a comparable association with lung cancer in most other cohort studies of phenoxy workers[5 7 8 14 19 20] suggests that phenoxy compounds are unlikely to be responsible for the excess. A more likely explanation is chance or a confounding effect of smoking. Data on the smoking habits of cohort members were not available.

The only diagnostic category for which we found a clear increase in risk was circulatory disease, and this was confined to factory A where 34 deaths were observed *v* 24·5 expected (after adjustment for local patterns of mortality). The finding is particularly remarkable since occupational cohorts usually exhibit a deficit of cardiovascular deaths because of the healthy worker effect. The limitation of the excess to one factory and the absence of similar associations in other cohorts of phenoxy workers[5 7 14 19 20] argue against a hazard from the phenoxy process, but it was possible that some other aspect of work at factory A might be responsible. We therefore carried out a nested case-control study to examine other possible occupational risk factors, but could find none that would explain the observation. The excess could perhaps be due to a combination of smoking and other non-occupational causes, but it will require further evaluation during continued follow up.

Despite the limitations imposed by small numbers and the potential difficulties in unravelling the effects of multiple exposures, it is important that studies such as this are carried out. They provide an essential audit of the extrapolation from laboratory data on which most decisions for the regulation of pesticides are based. We will continue to follow up these workforces and review their mortality and cancer incidence at intervals.

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Phenoxy herbicides and chlorophenols: a case control study on soft tissue sarcoma and malignant lymphoma

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Summary A case control study on patients with soft tissue sarcoma and malignant lymphoma was undertaken to test whether there was any association between these diseases and past exposure to chlorinated phenoxy acid herbicides or chlorophenols. It was carried out over the period 1982–1988 in Victoria, Australia. Thirty males with soft tissue sarcoma and 52 males with malignant lymphoma were matched by age, place of residence and sex with one population control and one cancer control each. Exposure was assessed by personal interviews conducted by an occupational hygienist. Exposures within 5 years prior to diagnosis of each matched case were ignored, both for the cases and their matched controls.

The estimated relative risks for definite or probable exposure to chlorinated phenoxy compounds or chlorophenols for at least 1 day were 1.0 (95% confidence interval (CI): 0.3–3.1) for soft tissue sarcoma and 1.5 (95% CI: 0.6–3.7) for malignant lymphoma. When the criterion for exposure was raised to more than 30 days, the estimated relative risks were 2.0 (95% CI: 0.5–8.0) for soft tissue sarcoma and 2.7 (95% CI: 0.7–9.6) for malignant lymphoma. Additional analyses were carried out for exposure of at least 1 day to phenoxy herbicides alone or chlorophenols alone. None of the estimated relative risks was significantly greater than unity.

A report of a case control study published in 1979 by Hardell and Sandström in Sweden was the first to claim an association between exposure to phenoxy herbicides and soft tissue sarcoma in humans (Hardell & Sandström, 1979). The authors also claimed to have found a link between exposure to chlorophenols and the same cancer and it was suggested that the carcinogenic agent in both types of chemicals could be a polychlorinated dibenzodioxin. This paper was soon followed by one reporting a similar study in a different area of Sweden (Eriksson *et al.*, 1981) and another one suggesting a link between exposure to these chemicals and malignant lymphoma (Hardell *et al.*, 1981). Prior to 1979 the claims of serious health effects from phenoxy herbicides had been confined to birth defects and other adverse pregnancy outcomes. The three Swedish studies were the first linking exposure of phenoxy herbicides with human cancer and they aroused considerable concern among public health authorities around the world.

In 1981 the Health Department of the State of Victoria, Australia, decided to support an investigation of the possible association between exposure to phenoxy herbicides or chlorophenols and the development of soft tissue sarcoma or malignant lymphoma, using a matched case-control study design. The design specified each case of soft tissue sarcoma or malignant lymphoma to be matched by age, sex and place of residence to one control without cancer drawn from the Victorian population and one control with cancer drawn from the same group of hospitals as the cases. Only living cases and controls were to be used and exposure was to be assessed by means of face-to-face interviews with the subjects themselves; no interviews with relatives were to be conducted. The results of this study are now reported.

The main chlorinated phenoxy herbicides used in Victoria have been 2,4-dichlorophenoxy acetic acid (2,4-D), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and 4-chloro-2-methylphenoxy acetic acid (MCPA) and their esters and amines. Other chlorinated phenoxy agricultural chemicals used to a much lesser extent are 4-chlorophenoxy acetic acid (CPA), 2-[4-chloro-2-methylphenoxy] propionic acid (mecoprop), (±)-2-[2,4,5-trichlorophenoxy] propionic acid (fenoprop), sodium 2-[2,4-dichlorophenoxy] ethyl sulphate (2,4-DES-sodium), 4-[4-chloro-2-methylphenoxy] butyric acid (MCPB), 4-[2,4-dichlorophenoxy] butyric acid (2,4-DB) and methyl (±)-2-[4-(2,4-dichlorophenoxy) phenoxy] propionate (diclofop-methyl). At least two factories in Victoria have been involved in the manufacture of phenoxy herbicides and chlorophenols. Phenoxy herbicides have been widely used in Victoria to control broad-leaved weeds in cereal crops (wheat, oats, barley) and to control blackberry and other noxious weeds. Small amounts of chlorinated phenoxy compounds are used to prevent pre-harvest dropping of fruit.

Clofibrate, a chlorinated phenoxy drug, has been used to treat coronary heart disease, diabetes and high cholesterol.

The main chlorophenol used in Victoria is pentachlorophenol and its sodium salt. Other chlorophenols or salts used have been 2,4,6-trichlorophenate, sodium 2,3,4,6-tetrachlorophenate, 2-benzyl-4-chlorophenol, 3-methyl-4-chlorophenol, 3,5-dimethyl-4-chlorophenol, 3,5-dimethyl-2,4-dichlorophenol and bis(5-chloro-2-hydroxyphenyl) methane. Chlorophenols have been used mainly as wood preservatives, and also in leather tanning, as paint preservatives and anti mould treatments for walls, as disinfectants, as preservatives in adhesives, for slime control in paper pulp, for preserving size used on textiles, for mothproofing wool, as herbicides, and for various other fungicidal and bactericidal purposes.

Subjects and methods

The population from which the cases and cancer controls were selected consisted of male cancer patients registered by the Victorian Cancer Registry after 1 January 1982 who were aged 30 years or more at registration, who were patients at any of six major Melbourne hospitals and who were still alive at the time of selection for the study. Since 1 January 1982 all cases of cancer diagnosed in Victoria, with the exception of non-melanoma skin cancer, have been required to be registered with the Cancer Registry. The study was restricted to six hospitals because the Cancer Registry was not permitted to contact patients before written informed consent of the patient had been obtained by the hospital concerned. The lower age limit of 30 years was specified in order to exclude young men whose cancers were unlikely to

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be caused by occupational exposures, taking into account a reasonable latency period. Only living patients were included as it was considered that occupational data from relatives was likely to be incomplete and possibly inaccurate.

Cases were men with soft tissue sarcoma either coded as ICD 171 (World Health Organisation, 1977) or coded to other sites, and men with malignant lymphoma coded as ICD 200, ICD 201 or ICD 202. The study continued until interviews had been obtained from 30 patients with soft tissue sarcoma and 52 with malignant lymphoma. The histological diagnoses for the 82 cases are shown in Table I, together with their ICD codes. Histological types and ICD codes recorded on the Cancer Registry were confirmed from individual hospital records. No review of pathology specimens was undertaken. The cases were first diagnosed between 1976 and 1987 with 89% first diagnosed after 1 January 1982. The median age at diagnosis was 59 years (range 37 to 87) for soft tissue sarcoma cases, 39.5 years (range 28 to 57) for Hodgkin's disease cases and 59.5 years (range 30 to 87) for non-Hodgkin's lymphoma cases.

For each case, one control with another type of cancer was randomly selected by Cancer Registry staff, matching for sex, age within 3 years and Statistical Division of current residence. There are 12 Statistical Divisions in Victoria, with the city of Melbourne comprising one. Patients with leukaemia, multiple myeloma or sarcoma of bone were not eligible as cancer controls because the aetiology of these diseases may be similar to that of lymphoma or soft tissue sarcoma. The cancer controls had 23 different ICD codes from ICD 140 to ICD 194 with the largest groups being ICD 162 — bronchus and lung (17 controls), ICD 185 — prostate (nine controls), ICD 153 — colon (six controls) and ICD 154 — rectum (six controls).

Population controls were selected at random from the Electoral Register by Cancer Registry staff. It is compulsory for all Australian citizens over the age of 18 years to be on the Electoral Register. One population control was matched to each case by sex, age within 3 years and Statistical Division of current residence. Population controls who had had cancer (apart from non-melanoma skin cancer) were not eligible.

All selection and matching of cases and controls was done by Cancer Registry staff, independently of the principal investigators (the authors). Five controls differed by age from their matched cases by more than 3 years (3.1 to 5.4 years). One case was resident in New South Wales, although he attended a Melbourne hospital. He was matched with two controls resident in the nearest Statistical Division in Victoria. All other controls satisfied the matching criteria.

Written consent for a personal interview was sought from each case and cancer control. In the letters the purpose of the study was described as an investigation of possible associations between occupations and cancer. Herbicides or other chemicals were not mentioned. Initially, written consent was also required from population controls but the response rate was so low that the interviews with the eight population controls who consented were considered to be possibly biased and were discarded. From then on population controls were contacted in writing and given the opportunity to refuse to participate. If there was no written refusal, the interviewer then contacted the potential control by telephone or in person and, if verbal consent was obtained, the man was interviewed. This procedure meant that the interviewer could not be blind with regard to the population controls. However in a face-to-face interview involving a person's life history, blindness with respect to cancer status is virtually impossible.

Response rates in cases and controls were calculated after excluding 101 patients who were sent letters but who did not satisfy the eligibility criteria, 55 who were found to be dead by the time the letter would have been received, 14 who were reported to be no longer at the address on the Cancer Registry and 18 who were sent an incorrect letter which stated that either the patient or his relative could be interviewed (administrative error by one hospital). Two of those sent the incorrect letter were interviewed and included in the study as cases but were not included in the calculation of the response rates. Of the 301 remaining cancer patients who were sent letters, 187 agreed to be interviewed, 25 refused and 89 did not reply. Assuming that those who did not reply were in fact refusals, the response rates were 70% for cases and 56% for cancer controls. This was the most pessimistic view as some of those who did not reply might have never

Table I Histological types and ICD-9 codes of cases

	No. patients	*ICD code*
Histology of soft tissue sarcoma cases (30)		
Malignant fibrous histiocytoma	9	all 171
Leiomyosarcoma	5	152, 158, three 171
Sarcoma, not otherwise specified (NOS)	2	both 171
Liposarcoma, well differentiated	2	both 171
Liposarcoma, NOS	1	171
Myxoid liposarcoma	1	158
Spindle cell sarcoma	1	171
Fibrosarcoma, NOS	1	158
Dermatofibrosarcoma, NOS	1	173
Epitheloid leiomyosarcoma	1	171
Synovial sarcoma, NOS	1	171
Clear cell sarcoma of tendons and aponeuroses	1	171
Kaposi's sarcoma	1	173
Malignant haemangiopericytoma	1	171
Chondrosarcoma NOS (extraosseous)	1	171
Malignant neurilemmoma	1	171
Histology of malignant lymphoma cases (52)		
Hodgkin's disease – nodular sclerosis NOS	6	all 201
Hodgkin's disease – mixed cellularity	3	all 201
Hodgkin's disease – lymphocytic predominance	1	201
Reticulosarcoma	15	all 200
Lymphocytic, poorly differentiated NOS	3	all 200
Immunoblastic type	2	both 200
Mixed lymphocytic-histiocytic NOS	2	both 200
Lymphocytic, poorly differentiated, nodular	8	all 202
Mixed lymphocytic-histiocytic, nodular	4	all 202
Lymphoma, NOS	2	both 202
Undifferentiated cell type NOS	2	both 202
Nodular NOS	2	both 202
Convoluted cell type NOS	1	202
Hairy cell leukaemia	1	202

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received their letters due to death or change of address. It is likely that some of the cases and cancer controls refused, or did not reply to, the request for interview because they were too ill. Eleven were later found to be within 3 months of death and another six gave other health-related reasons for refusing. Sixteen of those who agreed to interview could not be interviewed because of illness or administrative problems.

Of 160 population controls selected, 30 were not able to be contacted and six were ineligible because they had had cancer prior to the introduction of compulsory cancer registration. Of the remaining 124 contacted, 37 refused and 87 were interviewed, giving a response rate of 70%.

Nine eligible cancer controls and five eligible population controls who were interviewed were later excluded by Cancer Registry staff (without knowledge of the interview results). They were found not to match any cases or were superfluous to the requirement of one population control and one cancer control for each case (the control with the closest match was retained if two were available).

Of those who consented, 30 men with soft tissue sarcoma and 52 men with malignant lymphoma, as well as 164 matched controls (82 cancer controls and 82 population controls), were interviewed and included in the study. Unfortunately the distribution of interviews throughout the study was not uniform because of early administrative problems and the change to the method of obtaining population controls when it was realised that written consent was not feasible for these controls. Cases were interviewed between 1982 and 1988 with 80% interviewed prior to 1986, cancer controls between 1982 and 1988 with 18% interviewed prior to 1986 and population controls were all interviewed between 1986 and 1988. However all interviews were conducted by the same person (J.G.S.) who was blind as to the case/control status of the cases and the cancer controls and who was unaware of the distribution of cases and controls at the time. The extra controls and ineligible subjects who were interviewed, but later excluded helped to ensure the interviewer's blindness. The percentage of subjects reporting exposure did not vary significantly with the year of interview over the 6 years of the study ($P = 0.63$, chi square test, 5 degrees of freedom) and there was no apparent trend over the years ($P = 0.69$, chi square test for trend). The lengthy duration of the study was due to the rarity of soft tissue sarcoma cases and some administrative problems.

The interviewer was an occupational hygienist with experience in pesticide exposures. In the interview, which usually lasted about 45 min, a comprehensive occupational history was obtained, plus details on education, leisure activities, and alcohol and tobacco consumption. Details on the nature and duration of exposure were sought if the subject reported any occupation or activity likely to involve the chemicals of interest. The fact that clofibrate could be a source of exposure to chlorinated phenoxy compounds was not realised until after the first ten subjects had been interviewed. From then on all subjects (except for one accidental omission) were asked about medications for diabetes, high blood pressure or high cholesterol to determine exposure to clofibrate. In addition to the occupational history, four specific questions were asked concerning work in the country or living on a farm, work with asbestos, use of pesticides, herbicides or wood preservatives, and work with lead. Positive answers were probed for further details. The questions on asbestos and lead were for camouflage purposes only. The subject was not told of the main purpose of the study, namely exposure to phenoxy herbicides or chlorophenols.

Exposures to phenoxy herbicides or chlorophenols or clofibrate were coded as none, possible or definite/probable (Table II). This was done by the interviewer while still blind as to each subject's status and matched triad. In some instances this required extensive consultation with industry experts because subjects quoted superseded trade names or had worked in factories which were no longer in operation. For the analyses, exposures within 5 years prior to the year of diagnosis of a case were ignored, both for the case and his matched controls. A subject whose total lifetime exposure was less than 8 h (i.e. 1 day) was counted as not exposed.

The main analyses were carried out by comparing matched triads for exposures, i.e. case *vs* both matched controls. Soft tissue sarcomas and lymphomas were analysed separately. The method of conditional logistic regression described by Breslow and Day (1980, Chapter 7) was used to estimate relative risks and their approximate confidence intervals and test the null hypotheses. In the regression model for exposure to chlorinated phenoxy compounds or chlorophenols there were two indicator variables, one for possible exposure and one for definite exposure. The estimated relative risks reported are for definite exposure, adjusted for the estimated risk of possible exposure, i.e. with the possible exposure

Table II Exposures to chlorinated phenoxy compounds or chlorophenols (Numbers exposed for more than 30 days shown in parentheses)

	Soft tissue sarcoma			*Malignant lymphoma*		
Exposure	*Case*	*Pop. control*	*Cancer control*	*Case*	*Pop. control*	*Cancer control*
Definite/probable						
Spraying phenoxy herbicides	5[a](4)	4[b](1)	2(1)	7[d](3)	9[b,d](1)	4[c](2)
Other phenoxy acids		2(0)				
PCP wood preservatives[e]			1(1)	1(0)	1(0)	1(0)
Sodium PCP as house painter				2(2)		1(1)
PCP in carpet glues as carpet layer				1(1)		
Chlorophenol disinfectant as cleaner					1(1)	
Clofibrate medication		1(1)				
Total definite/probable	5(4)	7(2)	3(2)	11(6)	11(2)	6(3)
Possible						
Unknown disinfectants	2(2)			1(1)		1(1)
Unknown wood preservatives or possibly handling treated wood	1(0)		2(2)			
Possibly exposed to PCP laurate at woollen mill		1(1)				
Unknown herbicides			1(0)	3(0)	2(0)	
Unknown chemicals in tanneries					3(3)	
Total possible	3(2)	1(1)	3(2)	4(1)	5(3)	1(1)

[a]One subject also made phenoxy acid derivatives in a laboratory (≤ 30 days); [b]One subject also used pentachlorophenol wood preservatives (≤ 30 days); [c]One subject also exposed to sodium pentachlorophenate in manufacture of animal glue (> 30 days, already counted as exposed > 30 days to phenoxy herbicides); [d]One subject also possibly exposed to sodium pentachlorophenate in latex glues as shoemaker (possible exposure > 30 days, definite exposure to phenoxy herbicides ≤ 30 days); [e]PCP = pentachlorophenol or pentachlorophenate.

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variable in the model. The score statistic was used to test each null hypothesis. Because there were three levels of exposure to the chemicals of interest — none, possible, definite—the test statistic was compared with the chi square distribution with two degrees of freedom.

The *a priori* hypotheses of the study were whether exposure of at least 1 day to chlorinated phenoxy herbicides or chlorophenols was associated with the development of soft tissue sarcoma or malignant lymphoma. Subsequent analyses were also carried out to investigate whether there was any risk associated with exposure of at least 1 day to chlorinated phenoxy herbicides alone or chlorophenols alone and whether there was any risk associated with exposure to either group of chemicals for more than 30 days.

In addition, analyses of the matched pairs, cases *vs* population controls and cases *vs* cancer controls, were carried out. For the matched pairs analyses, the classical methods described by Breslow and Day (1980, p. 182) were used to estimate relative risks and test the null hypotheses. The score test statistic was calculated and compared to the chi square distribution with two degrees of freedom. Approximate confidence intervals were calculated using conditional logistic regression (program STRAT, Breslow & Day, 1980 or software package GLIM, Adena & Wilson, 1982). The confidence intervals reported for the matched pairs are of limited validity, because no adjustment has been made for the fact that the two comparisons made to test each hypothesis are not independent, i.e. the same cases are used in each matched pair comparison.

Matched pair analyses of cases *vs* population controls only were carried out to test for possible effects of smoking tobacco (non-smoker, past smoker or current smoker of at least one cigarette per day for as long as 6 months) and drinking alcohol (non-drinker, past drinker or current drinker of more than 100 grams of alcohol per year). A 5 year latency period prior to the year of diagnosis of the matched cases was applied as in previous analyses. Comparisons of cases with cancer controls were not made for smoking and drinking because several of the cancers among the cancer controls are known to be strongly associated with tobacco and alcohol, namely lung, larynx, kidney and bladder. In each of the two regression models for smoking and drinking there were two indicator variables, one for past smoking/drinking and one for current smoking/drinking. For these analyses the estimated relative risk for current smokers after adjustment for the risk for past smokers is reported and the estimated relative risk for past smokers after adjustment for the risk for current smokers is reported. Similarly the estimated relative risks are reported for current and past drinkers.

As a hypothesis generating exercise, all occupations of at least 5 years' duration prior to the date of interview were coded according to the Australian Standard Classification of Occupations (Castles, 1986). Cases and controls were compared to see if there were any significant clusters of soft tissue sarcoma or malignant lymphoma cases in each occupation. A latency period of 5 years prior to the year of diagnosis of the matched cases was applied. Any occupation which had a cluster of five or more cases was then analysed using the classical method for matched triads and dichotomous exposures described by Breslow & Day (1980, p. 169).

Results

Forty-three men (16 cases, 18 population controls, nine cancer controls) were definitely or probably exposed and 17 men (seven cases, six population controls, four cancer controls) were possibly exposed to chlorinated phenoxy compounds or chlorophenols for at least 1 day prior to 5 years before the year of diagnosis of the case in each matched triad. The exposures are listed in Table II. The total amount of exposure for those involved in spraying phenoxy herbicides ranged from 8 h to 122 weeks, the latter being the length of exposure of a population control. The total amount of exposure for those using pentachlorophenol as a wood preservative ranged from 14 h to 190 days, the latter being the length of exposure of a cancer control. There were no significant differences between population controls and cancer controls with respect to definite exposure (soft tissue sarcoma controls 7/30 *vs* 3/30, $P = 0.30$; malignant lymphoma controls 11/52 *vs* 6/52, $P = 0.29$, Fisher exact test two tailed). Hence the cancer controls and population controls were combined and matched triad analyses were done.

The data were tabulated according to the exposure of each subject in the triad. The matched triad data for testing the two main hypotheses of the study are shown in Table III. The estimated relative risks with approximate 95% confidence intervals are shown in the first line of Table IV. Neither of the relative risks is significantly different from unity ($P > 0.$ for both).

Analyses were also carried out for phenoxy herbicides and chlorophenols separately, using the same exposure criteria. In the phenoxy herbicide analysis, exposure to chlorophenols or clofibrate was counted as no exposure, and in the chlorophenol analysis, exposure to phenoxy herbicides or clofibrate was counted as no exposure. The estimated relative risks are shown in the second and third lines of Table IV. None is statistically significant ($P > 0.4$ for all comparisons).

An analysis was also carried out for exposure of more than 30 days to either chlorinated phenoxy compounds or chlorophenols prior to 5 years before the year of diagnosis of the matched cases (a subject exposed for 30 days or less was counted as not exposed). The estimated relative risks are shown in the fourth line of Table IV. They are higher than the risks for one day's minimum exposure but they are still not statistically significant ($P > 0.2$ for both comparisons). Because of the small numbers exposed for more than 30 days, the confidence intervals are relatively wide.

The results of the matched pair analyses are shown in Table V. The confidence intervals are wider than those obtained for the matched triad analyses because only half the number of controls are used in each comparison and because pairs in which both the case and his matched control are

Table III Exposure of matched triads to chlorinated phenoxy compounds or chlorophenols for at least one day, 5 years' latency period

	Exposure of controls						
Exposure of case	*Both none*	*1 none, 1 possible*	*Both possible*	*1 none, 1 definite*	*1 possible, 1 definite*	*Both definite*	*Total*
A. Soft tissue sarcoma							
None	10	4	0	8	0	0	22
Possible	2	0	0	1	0	0	3
Definite	4	0	0	1	0	0	5
Total	16	4	0	10	0	0	30
B. Malignant lymphoma							
None	25	3	0	7	0	2	37
Possible	2	0	0	1	0	1	4
Definite	6	2	0	2	1	0	11
Total	33	5	0	10	1	3	52

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equally exposed are ignored in the analysis. (There were no triads in which the case and both his controls were equally exposed so the matched triad analysis was able to make full use of the data.) None of the estimated relative risks for the matched pairs was significantly different from unity ($P > 0.1$ for all comparisons).

The estimated relative risks for smoking tobacco or drinking alcohol are shown in Table VI. No statistically significant associations were found ($P > 0.1$ for all comparisons).

When all occupations were examined for significant clusters of cases it was found that the 30 soft tissue sarcoma cases had 67 occupations of at least 5 years' duration prior to the date of interview, among which there were 52 different occupation codes. The 52 malignant lymphoma cases had 111 occupations of at least 5 years' duration with 80 different occupation codes. After applying a 5 years' latency period and analysing the data as matched triads, no statistically significant clusters were found for any occupation when coded to a six digit level of specialization ($P > 0.1$). When similar occupations were combined to the extent that the first four digits of the occupation codes were the same, there was still no significant clusters ($P > 0.1$). However the study was designed to test two specific hypotheses rather than generate new ones, and the number of cases was too small to be likely to find statistically significant clusters unless a very strong association were present.

Thirteen cases (16%) and 25 cancer controls (30%) volunteered opinions as to the causes of their cancers although this was not asked by the interviewer. No cases, who gave an opinion, suspected phenoxy herbicides or chlorophenols and only one cancer control did so. Thus recall bias on the part of the subjects was not apparent.

Discussion

The results of this study do not support the hypotheses that exposure to chlorinated phenoxy herbicides or chlorophenols causes soft tissue sarcoma or malignant lymphoma. In the main analysis the relative risks of developing soft tissue sarcoma or malignant lymphoma following exposure of at least 1 day to these chemicals were estimated to be 1.0 (95% CI: 0.3–3.1) and 1.5 (95% CI: 0.6–3.7) respectively.

When the relative risks were estimated from the matched pairs, the risks calculated from cases *vs* cancer controls were generally higher than the risks estimated from cases *vs* population controls, although none of the relative risks was statistically significant at the 0.1 level. If a higher relative risk had been found when comparing cases with population controls than when comparing cases with cancer controls, it could have been explained in terms of recall bias. As it is, however, there is no obvious explanation for the variation between estimates of relative risk apart from chance.

The number of subjects exposed was too small to be able to estimate with reasonable power the relative risks for exposure of more than 30 days to phenoxy herbicides or chlorophenols or for exposure of at least 1 day to chlorophenols alone.

In the three early Swedish studies the estimated (matched) relative risks for exposure of at least 1 day to chlorinated phenoxy herbicides or chlorophenols were 6.2 and 5.1 for soft tissue sarcoma (Hardell & Sandström, 1979; Eriksson *et al.*, 1981) and 6.0 for malignant lymphoma (Hardell *et al.*, 1981). In recent years the Swedish researchers have carried out two more case-control studies on soft tissue sarcoma in which the estimated relative risks were much lower. In a study reported in 1988 the estimated relative risk (unmatched, stratified) for phenoxy acetic acids alone was 3.3 and for chlorophenols alone was less than unity (actual estimate not reported; Hardell & Eriksson, 1988). Conversely, in a study published in 1990, no significant risk was found for phenoxy herbicides alone (RR 1.3, 95% CI: 0.7–2.6) but the estimated relative risk for chlorophenols alone was significant (RR 5.2, 95% CI: 1.7–16.3; Eriksson *et al.*, 1990). The estimated relative risk (matched) for exposure to either phenoxy herbicides or chlorophenols was 1.8 (95% CI: 1.1–3.0) in the 1990 study.

Many other cohort and case-control studies have been carried out to investigate the possible association between phenoxy herbicides or chlorophenols and soft tissue sarcoma or lymphoma. Of at least 18 studies of cohorts or workers known to be exposed to phenoxy herbicides or chlorophenols, only one has found a statistically significant association with either of these cancers. This is a study by Lynge (1985) who found a significant excess of soft tissue sarcomas in workers employed in manufacturing phenoxy herbicides in Denmark. A recent nationwide cohort study of 12 USA plants manufacturing chemicals contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin found an increased incidence of soft tissue sarcoma but the statistical significance of the results are in doubt because of misclassification of soft tissue sarcoma on death certificates (Fingerhut *et al.*, 1991).

Case control studies investigating actual exposures to the chemicals of interest, rather than presumptive exposures associated with occupations or military service in Vietnam, have been carried out in New Zealand (Smith *et al.*, 1983,

Table IV Estimated relative risks for exposure to chlorinated phenoxy compounds or chlorophenols, 5 years' latency period, matched triads, (95% confidence intervals in parentheses)

Exposure	*Soft tissue sarcoma*	*Malignant lymphoma*
Phenoxy herbicides or chlorophenols ⩾ 1 day	1.0 (0.3–3.1)	1.5 (0.6–3.7)
Phenoxy herbicides ⩾ 1 day	1.3 (0.4–4.1)	1.1 (0.4–3.0)
Chlorophenols ⩾ 1 day	0 (–)[a]	1.4 (0.3–6.1)
Phenoxy herbicides or chlorophenols > 30 days	2.0 (0.5–8.0)	2.7 (0.7–9.6)

[a]No soft tissue sarcoma cases were exposed to chlorophenols so confidence interval could not be calculated.

Table V Estimated relative risks for exposure to chlorinated phenoxy compounds or chlorophenols, 5 years' latency period, matched pairs, (95% confidence intervals in parentheses)[a]

Exposure	*STS vs pop. cont.*	*STS vs cancer cont.*	*ML vs pop. cont*	*ML vs cancer cont.*
PH or CP ⩾ 1 day	0.7 (0.2– 2.6)	1.7 (0.4– 7.0)	0.9 (0.4– 2.4)	2.6 (0.8– 8.8)
PH ⩾ 1 day	0.8 (0.2– 3.7)	2.5 (0.5–12.9)	0.8 (0.3– 2.2)	1.8 (0.5– 6.0)
CP ⩾ 1 day	0 –	0 –	1.2 (0.3– 5.4)	3.0 (0.2–44.9)
PH or CP > 30 days	2.0 (0.4–10.9)	2.0 (0.4–10.9)	3.0 (0.6–14.9)	3.0 (0.5–17.0)

[a]Abbreviations: STS = soft tissue sarcoma, ML = malignant lymphoma, pop. = population, cont. = controls, PH = phenoxy herbicides, CP = chlorophenols.

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Table VI Estimated relative risks for smoking tobacco or drinking alcohol, 5 years' latency, cases *vs* population controls, (95% confidence intervals in parentheses)[a]

	Soft tissue sarcoma vs *population controls*	*Malignant lymphoma* vs *population controls*
Current smoker	2.8 (0.9– 9.0)	2.2 (0.7 –6.7)
Past smoker	1.1 (0.3– 3.8)	2.2 (0.7 –7.1)
Current drinker	2.3 (0.6– 8.9)	0.6 (0.2 –2.0)
Past drinker	2.9 (0.4–25.0)	0.4 (0.04–3.3)

[a]Smoker = smoking as much as one cigarette per day for as long as 6 months, drinker = consuming more than 100 grams of alcohol per year.

1984; Smith & Pearce, 1986; Pearce *et al.*, 1986, 1987), Kansas, USA (Hoar *et al.*, 1986), Western Washington State, USA (Woods *et al.*, 1987) and Northern Italy (Vineis *et al.*, 1986). None of the main results of these studies showed a statistically significant association except for the study by Hoar *et al.* (1986) which reported a significant association between non-Hodgkin's lymphoma and phenoxy herbicides (odds ratio 2.2, 95% CI: 1.2–4.1). Hoar *et al.* found no increased risk of soft tissue sarcoma or Hodgkin's disease. The largest case control study, by Woods *et al.* (1987), reported an odds ratio of 0.9 (95% CI: 0.5–1.5) for non-Hodgkin's lymphoma and phenoxy herbicides and no increased risk of soft tissue sarcoma (actual odds ratio not reported).

In the present study considerable efforts were made to obtain the most accurate exposure data possible by the use of face-to-face interviews and by obtaining data from the subjects themselves, rather than next-of-kin of deceased subjects. The matching of cases and controls was maintained throughout the analysis to maximise the power to detect increased relative risks if they existed. The opinions expressed by subjects as to the causes of their cancer indicated a lack of any recall bias.

Two weaknesses in the study are its small size and the relatively low response rates. Larger sample sizes would have made the study impractical. The rarity of soft tissue sarcoma meant that it took over 5 years to accrue 30 living soft tissue sarcoma patients who would consent to be interviewed. It was considered undesirable to obtain larger numbers of cases by interviewing relatives of dead patients as the data would be considerably less reliable. The sample sizes were sufficient to detect a relative risk of 5 at the 0.05 level of significance with power of 90% for soft tissue sarcoma and 99% for malignant lymphoma.

The response rates were low compared to the Swedish studies by Hardell and his colleagues but not very much lower than in the New Zealand case-control studies (79% to 88%) (Smith *et al.*, 1983, 1984; Pearce *et al.*, 1986, 1987). It is understandable that response rates will decrease with the amount of effort required. Face-to-face interviews are more demanding than postal questionnaires which were used in Sweden or telephone interviews which were used in New Zealand. Age group (<65 *vs* ≥ 65 years) and place of residence (city *vs* country) were not significantly associated with response rate. Response rates for cancer patients varied with different hospitals and gradually decreased over the period of the study, probably because of waning enthusiasm by hospital staff involved in sending out letters and following up non-responders. This is unlikely to have introduced a bias with respect to the exposures of interest however.

It proved to be too difficult to arrange for pathological review of soft tissue sarcoma specimens. In a recent study a pathological review of soft tissue sarcoma diagnoses (Alvegård & Berg, 1989), 5% of the soft tissue sarcomas reviewed were re-diagnosed as non-sarcomatous tumours. This paper referred to two other studies in which 7% of 'soft tissue sarcomas' and 6% of 'bone and soft tissue sarcomas' were non-sarcomatous tumours respectively. It therefore seems possible that, in our 30 cases diagnosed as soft tissue sarcoma, one or two cases may have been wrongly diagnosed. It is unlikely that this would make a significant difference to the main conclusion.

One possible source of bias in the comparison between cases and population controls was the fact that the cases were drawn from a population of public (non fee paying) patients at six Melbourne hospitals, whereas the population controls were drawn from all registered voters in the State of Victoria (all social classes). Most patients with cancer in Victoria would attend a Melbourne hospital at some stage in their disease. Matching for statistical division of residence helped to eliminate a possible selection bias introduced by the hospital locations and catchment areas. However there remained a potential selection bias due to possible social class differences between public (non-fee paying) patients and the population controls.

One measure of social class in Australia is educational level. Cases had slightly more education than their matched cancer controls, although the difference was not statistically significant ($P = 0.08$ for age of leaving school, $P = 0.4$ for highest qualification ever obtained, Wilcoxon signed rank test). Cases had significantly less education than their matched population controls ($P = 0.006$ for age of leaving school, $P = 0.02$ for highest qualification ever obtained). However there was no association found between definite exposure to chlorinated phenoxy compounds or chlorophenols (categorised as none, 1–30 days, >30 days) and age of leaving school (dichotomised as ≤ 15 years and >15 years) ($P = 0.92$, chi square test for trend) or between definite exposure and highest qualification ever obtained (dichomotised as primary or secondary school only *vs* trade certificate, diploma or degree) ($P = 0.62$, chi square test for trend). If it can be assumed that educational level is a good measure of social class, then it can be concluded that the social class differences between cases and controls did not affect the relative risks concerning exposure to chlorinated phenoxy compounds or chlorophenols.

This study has found no statistically significant association between exposure to phenoxy herbicides or chlorophenols and the development of soft tissue sarcoma and lymphoma. However it is a relatively small study and the findings should be viewed as one piece of evidence in the large and growing literature concerning this question.

Note: Details of this study are included in a thesis by one of us (J.G.S.) submitted to the University of Sydney.

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BRIEF COMMUNICATION

Incidence Rates of Lymphomas and Soft-Tissue Sarcomas and Environmental Measurements of Phenoxy Herbicides

Paolo Vineis, Fabrizio Faggiano, Martine Tedeschi, Giovannino Ciccone*

Several studies (*1-6*) have suggested an association between phenoxy-herbicide exposure and the occurrence of soft-tissue sarcomas and non-Hodgkin's lymphomas, but other studies (*7,8*) have not confirmed such an association. Data are particularly conflicting in the case of soft-tissue sarcomas, whereas a recent editorial (*9*) has suggested that the evidence for an association between phenoxy-herbicide exposure and the occurrence of non-Hodgkin's lymphomas is stronger.

We report here the results of an investigation into the occurrence of malignant lymphomas and soft-tissue sarcomas in an Italian community where phenoxy herbicides—ie, 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and their propionic derivatives (2,4-DP and 2,4,5-TP, respectively) and 4-chloro-2-methylphenoxyacetic acid—have been extensively used.

Methods

The study area is represented by the provinces of Novara and Vercelli (northern Italy; total population, 900 000). In these provinces, rice growing is the main agricultural activity, and phenoxy herbicides have been used in rice weeding since 1950.

Table 1. Annual age-adjusted incidence rates for non-Hodgkin's lymphomas*in the entire study area and in two subareas defined on the basis of measurements of levels of 2,4-D and 2,4,5-TP: 1985-1988

Area†	Incidence rates (95% CI) Men	Women
Entire study area (N = 253)	8.8 (7.4-10.4)	5.8 (4.7-7.0)
Category A‡ (N = 24)	11.7 (6.5-19.3)	6.2 (2.8-11.8)
Category B§ (N = 30)	18.2 (11.1-28.0)	7.4 (3.5-13.6)
Other municipalities not included in category B	8.1 (6.7-9.7)	5.7 (4.7-6.9)

*Per 100 000, ages 15 through 74 years.
†N = No. of cases.
‡Thirty-two municipalities where phenoxy herbicides have been measured in the soil or water.
§Thirteen municipalities along a canal with higher levels of phenoxy herbicides.

We have identified all cases of Hodgkin's disease [International Classification of Diseases (ICD) code 201], non-Hodgkin's lymphoma (ICD codes 200 and 202), and soft-tissue sarcoma (ICD code 171) newly diagnosed in 1985 through 1988 in men and women aged 15 through 74 years living in the two provinces. Cases were identified through a survey conducted in the four hospitals of the two provinces and in extraprovincial institutions to which residents of the two provinces could be referred. After prevalent cases were discarded, 43 cases of Hodgkin's disease, 141 cases of non-Hodgkin's lymphoma, and 30 cases of soft-tissue sarcoma were identified in men, and 20 cases of Hodgkin's disease, 112 cases of non-Hodgkin's lymphoma, and 19 cases of soft-tissue sarcoma were identified in women.

Gas chromatographic analyses of soil and water contaminated by 2,4-D and 2,4,5-TP were performed in 1974 through 1975 in several areas of the two provinces. The results of these analyses have been published (*10*). The same analyses have been used in the present study for assessment of exposure. Water was sampled every 1 or 2 weeks during the 2-year period, whereas the soil was sampled two to five times per year. Areas where exposure occurred are separated into two categories according to two different criteria. Category A includes areas where 2,4-D or 2,4,5-TP has been detected in water and/or soil in at least one of the measurements (32 municipalities); category B includes areas where the highest levels of water contamination (2,4-D, 0.07 to 0.46 ppm; 2,4,5-TP, 0.10 to 0.19 ppm) were found (13 municipalities located along a canal, Roggia Mora). Categories A and B partially overlap.

Age-adjusted incidence rates have been computed by means of the direct standardization method (*11*), with the world population used as a reference (*12*). Ninety-five percent confidence intervals (CIs) of the rates have been computed according to the Poisson distribution (*13*). The incidence rate ratio (RR) was computed as a measure of association; the corresponding 95% CIs were based on the formula $\exp[\ln RR \pm 1.96\ SD(\ln RR)]$ (*11*).

Results

In Table 1 the age-adjusted incidence rates of non-Hodgkin's lymphomas are reported for the entire study area and in the two subareas previously defined in

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categories A and B. The incidence rate has clearly increased among males in the areas in category A (phenoxy herbicides measured at any level) and more markedly in the areas that meet the criterion for category B (municipalities along the canal with higher levels of contamination). The rate ratio comparing the latter area with the remaining municipalities was 2.2 (95% CI, 1.4 to 3.5) among males. A slight increase among females was also observed.

The annual incidence rates (per 100 000) for Hodgkin's disease in the entire study area were 3.3 (95% CI, 2.4 to 4.4) for males and 1.5 (95% CI, 0.9 to 2.3) for females. For soft-tissue sarcomas, these rates were 1.8 (95% CI, 1.2 to 2.6) for males and 0.9 (95% CI, 0.5 to 1.4) for females. Absolute numbers were too small to allow meaningful interarea comparisons.

Discussion

We found a higher incidence rate of non-Hodgkin's lymphomas in males living in a rice-growing area where 2,4-D and 2,4,5-TP have been identified in the soil and the water. The rate in the most polluted municipalities was two times higher than that in the rest of the territory. This observation is consistent with previous reports (*3,4,14*). No association between exposure to phenoxy herbicides and development of Hodgkin's disease or soft-tissue sarcomas has been detected, but the absolute numbers of cases, particularly soft-tissue sarcomas, were small.

All of the hospitals that serve the two provinces have been surveyed, so that case ascertainment is likely to be complete. Prevalent cases have been discarded. The incidence rates that we have estimated are comparable to those reported from cancer registries in Western Europe (*12*). Analytic measurements in soil and water were available for the years 1974 through 1975, ie, 10 to 11 years before cancer incidence was measured; this allows for a reasonable latent period. Definition of exposure categories was done a priori on the basis of the analytic measurements.

Despite the weakness of the approach (ecologic analysis), the reported association between the development of non-Hodgkin's lymphomas and phenoxy-herbicide contamination is of interest because of the availability of objective measurements of the relevant chemicals in the soil and water. It is more likely that the excess number of non-Hodgkin's lymphomas in the area of higher pollution, if it is real, is attributable to occupational rather than to environmental exposure. A case-control study on lymphomas and occupational exposures is currently planned in this area.

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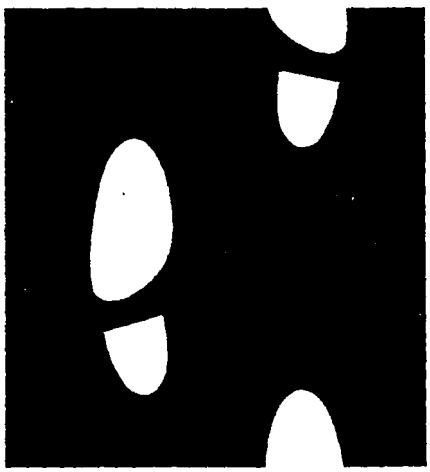
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Original article

Risk factors for adult soft tissue sarcoma in northern Italy

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Summary. The role of several potential risk factors in the etiology of soft-tissue sarcoma (STS) was examined in a hospital-based case-control study, conducted in the Friuli-Venezia Giulia region, northeast Italy, between 1985 and 1991. A total of 93 STS cases (53 males and 40 females, median age: 52 years) and of 721 controls (371 males and 350 females, median age: 54 years) were interviewed. Significant increased risks were associated with a history of herpes zoster infection (odds ratio (OR): 2.3, 95% confidence interval (CI): 1.1–4.9), chicken-pox (OR: 2.1, 95% CI: 1.2–4.1) and mumps (OR: 2.0, 95% CI: 1.1–3.8). None of the other medical conditions investigated – socio-economic and anthropometric indicators, tobacco smoking, consumption of alcoholic beverages, coffee and tea – seemed to affect STS risk. No risk elevation was found in subjects employed in agriculture (OR – for >10 years employment = 0.8, 95% CI: 0.4–1.5), nor in those who reported exposure to pesticides or herbicides (OR = 0.4, 95% CI: 0.1–1.2). Workers who reported exposure to chemical agents or to benzene or other solvents for more than 10 years had, respectively, a 1.8-fold (95% CI: 0.7–4.4) and a 2.2-fold (95% CI: 0.9–5.5) higher risk of developing STS.

Key words: case-control study, risk factors, soft-tissue sarcoma

Introduction

Soft-tissue sarcomas (STS) are a group of rare malignancies, derived from mesenchymal tissues other than bones and cartilage, which account for less than 2% of the overall cancer mortality in western countries, in luding Italy [1–2]. Due to the rarity of STS and to the heterogeneous cell types, few epidemiologic studies have investigated the etiology of STS [3].

Most of the epidemiologic research has focused on occupational risks, namely occupation in agriculture and exposure to phenoxyherbicides and chlorinated phenols [5–7], but inconsistent results have been reported. A small fraction of STS seems to be attributable to genetic susceptibility [3, 8–10], heavy external ionizing radiation [3, 11, 12] and immunosuppression [13–15]. The role of other potential risk factors is not known, although some etiologic hypotheses have been made, in particular on the role of infectious agents [3].

In this paper, the results of a case-control study [4, 5] on the role of several occupational and non-occupational factors in the etiology of STS are reported.

Materials and methods

Since June, 1985, we have been conducting a hospital-based case-control study on risk factors for STS in the Friuli-Venezia Giulia region, Northeast Italy.

Cases

The cases were histologically confirmed STS, diagnosed within two years before interview, who had been admitted as in-patients or referred for follow-up to the out-patient clinics of the Aviano Cancer Center or to the general hospitals in the study area. The present analysis is based on 93 cases (53 men and 40 women), aged 16 to 79 years (median age: 52 years), interviewed before June 1991. According to the classification of Enzinger and Weiss [16], the most frequent cell type was sarcoma of the fibrous tissue (43%), followed by myomatus sarcomas (17%), liposarcomas (13%) and various other cell types (27%). Kaposi's sarcoma linked to human immunodeficiency virus infection and visceral sarcomas were excluded.

Controls

The control group consisted of 721 patients (371 men and 350 women), aged 17 to 79 years (median age: 54 years), admitted to the aforementioned hospitals for a wide spectrum of diseases. Specifically excluded from the comparison group were patients whose cause of hospital admission was of malignant disorders, conditions related to alcohol and tobacco consumption, as well as any disease which might have resulted in diet modification, e.g. disorders of respiratory and digestive tracts, cardiovascular diseases, diabetes, etc. Haematologic, allergic or autoimmune diseases were also excluded. Twenty-eight percent of the controls were admitted for traumatic conditions (mainly fractures and sprains), 32% for musculoskeletal diseases (mainly low-back pain and disk disorders), 17% for minor conditions requiring surgical operations and 23% for other illnesses such as disorders of ear, nose, throat, skin or teeth.

Questionnaire

The questionnaire included several socio-economic indicators like education, occupation, social class (based on the head of the household's occupation [17], sibship size, birth order) and life style habits, such as smoking, frequency of consumption of alcoholic and methylxanthine-containing beverages and frequency of weekly consumption of selected food indicators.

Information was obtained about acquired disorders potentially related to the immune system – chronic infectious diseases, auto-

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immune diseases, allergic conditions, common childhood infections, immunization practices, tonsillectomy and radiation exposure for diagnostic and therapeutic purposes. Age at starting and stopping employment in 17 industries or occupations, the subject's role in the industry or occupation in terms of direct involvement in production aspects, and history of exposure to 15 occupational agents or groups of agents were also investigated.

Data analysis

The effect of several potential risk factors on STS risk was estimated by means of odds ratios (ORs) adjusted for age and sex [18], and their 95% confidence intervals (CIs) [19].

Results

None of the socio-economic indicators investigated in the present study seemed to affect substantially the risk of STS, since cases and controls were similarly distributed as regards education, social class, sibship size and ponderal index (Table 1). Cases and controls also showed similar habits of tobacco use and alcohol consumption (not shown).

Table 2 illustrates the distribution of STS cases and controls according to medical history. A similar proportion of cases and controls reported a history of chronic infectious diseases, while a positive association emerged for chicken-pox (OR = 2.1, 95% CI: 1.2–4.1) and mumps (OR = 2.0, 95% CI: 1.1–3.8) and a history of herpes zoster infection (OR = 2.3, 95% CI: 1.1–4.9). None of the other medical conditions investigated was associated with the probability of developing STS, including exposure to therapeutical radiations and tonsillectomy (Table 2).

Table 1. Distribution of 93 cases of soft-tissue sarcomas and 721 controls, according to socio-economic and anthropometric indicators. Pordenone, Italy, 1985–1991.

	Cases (n = 93) # (%)	Controls (n = 721) # (%)	MH-OR (95% CI)[a]
Education (years)			
≤5	54 (58)	425 (59)	1
6–8	14 (15)	143 (20)	0.73 (0.32–1.52)
≥9	25 (27)	153 (21)	1.30 (0.96–4.02)
X^2_1 for trend			1.04, p = 0.31
Social class			
V–IV (lowest)	55 (59)	446 (62)	1
III	27 (29)	211 (29)	1.08 (0.64–1.84)
II–I	7 (8)	34 (5)	1.72 (0.65–4.46)
X^2_1 for trend			0.86, p = 0.35
Other	4 (4)	30 (4)	–
Sibship size[b]			
1–2	19 (20)	152 (21)	1
3–4	35 (38)	240 (33)	1.14 (0.60–2.20)
≥5	39 (42)	327 (46)	0.79 (0.39–1.61)
X^2_1 for trend			0.27, p = 0.60
Body mass index[b]			
≤23	30 (33)	265 (37)	1
24–26	35 (38)	240 (33)	1.07 (0.60–1.95)
≥27	27 (29)	215 (30)	0.91 (0.49–1.68)
X^2_1 for trend			0.01, p = 0.99

[a] Odds ratio (OR) and 95% confidence interval (CI), adjusted for age and sex according to the Mantel-Haenszel (MH) procedure.
[b] For some items, the sum does not add up to the total because of missing values.

Table 2. Distribution of 93 cases of soft-tissue sarcoma and 721 controls, according to history of selected diseases and medical procedures. Pordenone, Italy, 1985–1991.

Condition[a]	Cases (n = 93) # (%)	Controls (n = 721) # (%)	MH-OR (95% CI)[b]
Chicken-pox			
No	19 (32)	233 (47)	1
Yes	40 (68)	263 (53)	2.11 (1.15–4.11)
Mumps			
No	16 (25)	211 (39)	1
Yes	49 (75)	327 (61)	1.98 (1.06–3.76)
Measles			
No	11 (17)	121 (24)	1
Yes	54 (83)	384 (76)	1.68 (0.82–3.58)
Chronic infectious diseases			
No	84 (91)	644 (90)	1
Yes	8 (9)	73 (10)	0.79 (0.33–1.81)
Herpes-zoster			
No	82 (88)	680 (94)	1
Yes	11 (12)	40 (6)	2.26 (1.06–4.94)
Allergic conditions			
No	72 (77)	565 (79)	1
Yes	21 (23)	153 (21)	1.13 (0.65–1.97)
Past exposure to therapeutical radiations			
No	91 (98)	697 (97)	1
Yes	2 (2)	23 (3)	0.73 (0.12–3.30)
Tonsillectomy			
No	69 (75)	565 (79)	1
Yes	23 (25)	151 (21)	1.23 (0.70–2.16)

[a] For some items, the sum does not add up to the total because of missing values.
[b] Odds ratio (OR) and 95% confidence interval (CI), adjusted for age and sex according to the Mantel-Haenszel (MH) procedure.

As concerns occupational risk factors, no significant association of STS risk with employment in agriculture (OR for >10 years = 0.8, 95% CI: 0.4–1.5), or exposure to herbicides and/or pesticides (OR = 0.4, 95% CI: 0.1–1.2) emerged (Table 3). Conversely, an approximately two-fold increase in STS risk was noted among subjects who reported more than 10-year exposure to chemical agents (other than pesticides or herbicides) (OR = 1.8, 95% CI: 0.7–4.4) or to benzene or other solvents (OR = 2.2, 95%: 0.9–5.5) (Table 3).

Analysis according to strata of STS histologic subgroup did not suggest any discrepancy with the overall risk estimates (not shown).

Discussion

The findings of the present study suggest that past viral infections may increase the risk of STS. Episodes of herpes-zoster infection were more common among STS cases than among controls, particularly in the three years preceding STS diagnosis. A similar relationship was reported in other studies on malignant lymphomas, with the greatest risk near the time of lymphoma diag-

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Table 3. Odds ratio of soft-tissue sarcoma for selected occupations and exposures.[a] Pordenone, Italy 1985–91.

Occupation or exposure	Odds ratio[b,c] (95% CI) by duration of occupation or exposure		
	≤10 years	>10 years	X^2_1 (trend)
Agriculture	0.4 (0.1–1.7)	0.8 (0.4–1.5)	0.79; p = 0.37
Furniture/ upholstery	0.2 (0.0–1.5)	1.1 (0.4–2.7)	0.11; p = 0.74
Mechanics	0.7 (0.1–3.3)	0.9 (0.3–2.4)	0.19; p = 0.66
Livestock/meat processing	1.4 (0.2–7.0)	1.4 (0.6–3.0)	0.96; p = 0.33
Chemical agents	1.1 (0.2–5.6)	1.8 (0.7–4.4)	2.13; p = 0.15
Metal dust	1.8 (0.6–5.0)	0.9 (0.3–2.6)	0.13; p = 0.72
Dyes/paints	0.9 (0.3–2.9)	0.9 (0.2–2.7)	0.07; p = 0.79
Wood dust	0.8 (0.3–2.3)	0.9 (0.3–2.4)	0.09; p = 0.77
Benzene/solvents	0.5 (0.1–2.2)	2.2 (0.9–5.5)	1.90; p = 0.17
Herbicides/ pesticides	0.7 (0.2–2.5)	0.4 (0.1–1.2)	3.14; p = 0.08

[a] Only occupations and exposures with at least 5 exposed cases.
[b] Non-exposed subjects as reference category.
[c] Adjusted for age in quinquennia and sex by the procedure of Mantel-Haenszel.

nosis [20, 21], indicating that first occurrence of herpes zoster infection may be favored by the early phases of STS, possibly on account of an early impairment of the immune system.

Common childhood infections were more frequently reported by cases than controls, in particular a history of chicken-pox and mumps. Although none of such viruses have been directly linked with STS or with other malignancies, viruses have long been suspected to play some role in the origin of human sarcomas [22–25]. Attention has been recently focused on the association between cytomegalovirus and Kaposi's sarcoma, a type of STS which is overrepresented among the immunosuppressed patients [26].

The association between past infectious diseases and STS, if it exists, may have important implications for understanding STS etiology, and it should be further investigated in studies in which the medical histories can be confirmed through hospital records, and, possibly, by means of serologic markers.

In agreement with other work showing no association between STS risk and exposure to radiations, neither diagnostic nor therapeutic exposure was associated with STS risk in the present study [27, 28]. Similarly, none of the socio-economic and anthropometric indicators was found to affect STS risk, nor were cigarette smoking, alcohol, coffee and tea consumption. These findings are consistent with a few previous investigations [27–29] which were also negative as concerns these factors, with the exception of a case-control study in which the risk of STS was directly associated with adult body weight [29].

As regards the association between occupation and STS risk, the results of the present study provide further evidence against the possibility that employment in agriculture and, in particular, exposure to pesticides or herbicides play an important role in the etiology of STS. Although the study was based only on 93 cases of STS, and found no significant association, it was able to exclude, at the usual level of statistical significance, an increased STS risk from more than 10-year exposure to herbicides or pesticides greater than 20%. This may, hence, be an useful contribution, particularly in consideration of the rarity of the disease and of the recent debate on the topic. The results of some epidemiologic studies seem to indicate that farmers have elevated risks for a few non-epithelial cancers, including STS [for a review, see 30 and 31]. However, the evidence remains inconclusive. A risk of 0.9 was found in a cohort of Swedish agricultural and forestry workers potentially exposed to phenoxy acid herbicides [32], and a case-control study conducted in New Zealand was also negative [33]. STS risks lower than unity were found among agricultural, gardeners and forestry workers in a study based on 15 cancer registries in England and Wales [34].

Conversely, in this study, workers who reported to have been exposed for more than 10 years to unspecified chemical agents or to benzene or other solvents had a nearly two-fold, albeit nonsignificant, increased risk of STS. Although benzene exposure was consistently associated with an elevated risk of acute leukemia [35], the few studies which have investigated the potential role of exposure to benzene on the risk of developing STS showed very modest, nonsignificant risk elevations [27, 36].

Weaknesses and strengths of hospital-based case-control studies have long been discussed [18]. The geographic area herein examined is not covered by a cancer registry, thus STS patients included in the study group may not be representative of all STS cases occurring in the population. The collaborating hospitals, however, included the majority of diagnostic and therapeutical facilities for STS available in the area and, therefore, most STS cases should have been referred there. Furthermore, a good comparability between cases and controls should have been achieved by interviewing controls coming from the same catchment area of STS cases and admitted to hospitals for a wide spectrum of acute conditions.

A more important limitation of these data comes from the power of the study, which was low for many of the variables examined, thus hampering the interpretation of the findings. Most of all, as regards the purported role of past infectious diseases which emerges from the present study, the potential role of recall bias is of concern, particularly since only interview-data were available and no validation on past medical records or serology has been possible. The performance of the interviews in a hospital setting should have assured, however, a more complete ascertainment of medical history and a closer comparability between cancer cases and controls than those obtainable in a community setting [37].

In conclusion, also bearing in mind such limitations and the difficulties of studying epidemiologically the etiology of rare and heterogenous diseases like STS, the

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present study investigated a few risk factors that were not previously considered. Some of the reported associations, like the increased risk related to history of past infectious diseases and exposure to chemical agents and benzene, may be useful in providing leads for further investigation.

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IN THE CIRCUIT COURT OF THE NINTH JUDICIAL CIRCUIT OF FLORIDA, IN AND FOR ORANGE COUNTY.

CASE NO: CI 89-8657
DIVISION: 32 THOMPSON

KIMBERLY MOYER, as Personal Representative of the Estate of ROBERT W. MOYER, II, deceased.

Plaintiff,

vs.

DOW CHEMICAL COMPANY, et al.,

Defendants.
_____/

NOTICE OF TAKING DEPOSITION
*DUCES TECUM

TO: TO ALL ATTORNEYS ON ATTACHED MAILING LIST

PLEASE TAKE NOTICE that the undersigned attorneys will take the deposition of:

NAME AND ADDRESS:	David Eaton, Ph.D. c/o Alan Wagner, Esq. Carlton, Fields, Ward, et al., First Florida Bank Building, P. O. Drawer 190 Tallahassee, FL 32302
DATE AND TIME:	December 15, 1992 at 9:30 a.m.
PLACE OF TAKING:	Larsen & Smith, Inc., Court Reporters, 1325 4th Ave., Suite 1740, Pudget Sound Plaza, Seattle, WA. 98101 (206/623-6717)

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DUCES TECUM

See Subpoena marked as Exhibit "A" and attached hereto.

I HEREBY CERTIFY that a true copy of the foregoing has been furnished to the attorneys listed on the attached mailing list by U. S. Mail, this the 19th day of October, 1992.

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HUMAN HEALTH EFFECTS OF INCREASED HERBICIDE USE: PERCEIVED v. ACTUAL RISKS

Synthetic chemicals used to control unwanted plant growth have become an indispensable part of commercial agriculture, forest management and home gardening. The use of synthetic organic chemicals as herbicides is a relatively recent phenomenon; prior to the early 1940's, most herbicide application was in the form of inorganic salts such as lead arsenate. Since the development and marketing of the phenoxy acid herbicides in 1944, herbicide use in the U.S. has grown more rapidly than any other class of chemical pesticide. In 1963, annual sales of herbicides were on the order of 100 million pounds; by 1975 sales increased to 645 million pounds, surpassing the total usage of insecticides in the United States. By 1964, nearly twice as much of the crop area in the United States was treated with herbicides as with insecticides or fungicides. Although the ecological and health risks associated with widespread insecticide use became a prime issue of the environmental movement in the 1960's, herbicides as a chemical class were generally viewed by both scientists and the general public as rather innocuous compounds. Indeed, classical measures of toxicity such as acute lethality (LD_{50}) are universally much lower for herbicides than for many widely used insecticides.

The widescale use of the now notorious herbicide mixture, "Agent Orange," in the jungles of Viet Nam brought to focus potential adverse health effects that might result from exposure to the phenoxy acid herbicides 2,4-D and 2,4,5-T. Veterans returning from Viet Nam began to attribute a wide variety of ill effects to their exposures to Agent Orange years earlier. Their suspicions of toxicity from Agent Orange were fueled by the discovery that many batches of this herbicide mixture were contaminated with the highly toxic chemical, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD is considered the most toxic man-made chemical known, and is capable of producing cancer and birth defects in experimental animals at very low doses compared to many other known carcinogens and teratogens. Moreover, in contrast to the active herbicide components which are readily eliminated from the body and are rapidly broken down in the environment, TCDD is highly persistent and tends to accumulate both in the environment and the human body.

Citizen complaints of ill health effects from herbicide spraying began to appear in he early 1970's, particularly in the forest lands of the Pacific Northwest, where aerial application of phenoxy acid herbicides is widespread. Of particular concern were reports of increased occurences of miscarriages and birth defects among rural residents of these areas. Based on a preliminary finding of increased miscarriage rates in the Alsea, Oregon area which appeared to correlate wth 2,4,5-T application,

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the EPA ordered an emergency suspension of most uses of 2,4,5-T. However, 2,4-D use was not affected by this ruling. this regulatory action, hotly contested by the manufacturers of 2,4,5-T, has transformed what was perceived to be a scientific question to a highly emotional debate between industry and citizens, with scientists mostly caught in the middle.

The dilemma facing many scientists, particularly those trained in toxicology, is the inability of traditional scientific methods to discern between actual versus perceived risks with the degree of certainty that the scientific community demands before recognizing such risks as "fact". Traditional regulatory approaches governing the safety of chemicals have tended to rely upon the addage of "innocent until proven guilty", accepting as proof only those data which meet the predefined criteria of "statistical significance". To the layperson, however, an n of 1 is generally adequate proof that x caused y, and there is little appreciation for the "scientific method". How then is the toxicologist to deal with the public's questions regarding the health effects of chemicals, when, in the eyes of the layperson, circumstantial evidence abounds that x caused y, yet scientifically there is no statistical proof that such a relationship exists. The case of herbicides is an excellent example with which to study this dilemma.

What are the perceived risks of herbicides? Obviously this depends upon the individual's experiences and understanding of chemical hazards. There is probably no other class of chemicals for which a greater range in perceived risks exist than the phenoxy acid herbicides. In a recent book entitled "A Bitter Fog", author Carol Van Strum graphically depicts her perception of the damage that herbicides have on human health and the environment. Speaking of an incident in which her children were apparently directly sprayed by a roadside crew with a combination of 2,4-D and 2,4,5-T she states "The children did no more fishing that spring day in 1975. They were too upset, and their eyes, noses and mouths burned. I brought them to the house and washed them, changed their clothes, gave them soda to rinse their mouths, but the bitter taste and burning wouldn't go away. By evening, all were sick with nausea, vomiting, cramps, headaches, diarrhea. One of the boys had an uncontrollable bloody nose...". She continued, "The day after the spray truck came, we went back to the river and saw that it had changed. The leaves of alder and willow on both sides of the river wilted, their new growth in unnatural spirals. The water had an oily scum on it. Floating or beached on the shore were dead crayfish and trout, and the sodden bodies of two merganser ducklings. Caddis larvae and snails that usually traced patterns in the silt on the river bottom were dead. Songbird nests along the shore were empty, except for one, in which a hermit thrush still sat, dead." Later, she describes a second episode: "Within 24 hours we were sick again, with the same symptoms as before. This time, there was a further complication, uterine bleeding in both myself and my daughter, who had not yet begun to menstruate at that

time..... In the next weeks we had an epidemic of deformities among the chicks, ducklings, and goslings that hatched on the farm: crossed beaks, stunted or missing wings, toes or whole feet on backward, and stunted growth." Numerous magazine and newspaper articles have also described the perceived impacts of herbicide use on human health and the environment in a similarly graphic manner. Thus, there are numerous anecdotal reports describing the ability of herbicides to produce a wide range of adverse health effects, including neurological effects, birth defects, reproductive disturbances, cancer and a variety of 'subjective' effects such as nausea, headache and malaise. If, in fact, these reports are true, how could such highly toxic, dangerous chemicals be allowed for such widespread use for nearly 40 years? This, of course, is the question many citizens have been asking of the EPA, the chemical industry and the scientific community after hearing of such reports. Many in the scientific community, upon hearing of such reports, have responded with a great deal of skepticism, citing the bulk of the scientific literature on phenoxy acid herbicides that suggests that such widespread, severe toxicologic responses could not occur at normal application rates of phenoxy acid herbicides. However, extrapolation of laboratory data to predict what might happen in the "field" is difficult, at best, and scientists should recognize the limitations of classical laboratory studies for predicting actual effects.

What is known about the actual health risks associated with long-term, low level exposure to dioxins and/or phenoxy acid herbicides? The answer to even this question is exceedingly complex. There are some data in laboratory animals which indicate that 2,4,5-T can cause certain birth defects at relatively high doses - that is, at doses which are sufficiently high enough to produce evidence of toxicity to the mother (generally greater than 50 mg/kg/day). Whether the birth defects which result are a result of the chemical per se, or are an indirect effect of maternal toxicity, is unclear. TCDD, on the other hand, is an extremely potent teratogen, capable of inducing cleft lip and palate in fetuses at doses in the order of several nanograms/kg/day, or one one-millionth the effective dose for 2,4,5-T listed above. However, extrapolation of such results to humans is extremely difficult because of large differences in species susceptability to TCDD. There is a five thousand fold difference in the acute lethality of TCDD between guinea pigs and hamsters. We have little idea where humans fit on this scale, although monkeys appear to be relatively sensitive to both the acute toxic and teratogenic effects of TCDD. A second confounding factor in predicting human effects from exposure to phenoxy acid herbicides is the wide range in levels of TCDD contamination of different herbicide formulations. Certainly the single most important factor in assessing risk from a chemical is to establish the dose, or amount, of exposure. In virtually every human instance of herbicide exposure there has been no information as to the level of dioxin contamination, or an accurate indication as to the extent of exposure to the herbicide

itself. Factors such as the amount of body surface area exposed, the percent of the dose deposited on the skin that is actually absorbed, the concentration of herbicide in the spray, the concentration of herbicide in the air, the duration of breathing such vapors, and the frequency and duration of exposure over time are paramount to an accurate estimation of exposure.

Several epidemiologic studies on workers exposed occupationally to phenoxy acid herbicides have identified an increased risk of developing certain types of cancer (soft tissue sarcoma, stomach cancer and lymphomas). These studies have been challenged by other scientists because of mis-identification of pathologic tumor type and other procedural problems. There have been several other epidemiologic studies which have not found any correlation between herbicide exposure and an increased risk for cancer. Thus, there appears to be inadequate and conflicting data in the scientific literature regarding the risk of developing cancer from phenoxy acid herbicide exposure.

Recent studies on residents of Seveso, Italy, where a chemical manufacturing plant explosion resulted in extraodinarily high levels of contamination of the environment with TCDD, revealed an apparent increase in abnormal birth outcomes in women residing in the area, as well as a dramatic decline in the birth rate in the year following the accident. Whether the decline in birth rate was a result of an increase in contraceptive use (unlikely), an increase in elected abortions because of the incident, an actual toxicological effect from the dioxin (increase in miscarriages/spontaneous abortions) or some other factor is not known. Because the exposure to dioxin (TCDD) in this episode was several orders of magnitude above that which would be encountered in other environmental exposures, such as from the application of herbicides in forest management, or from living in the dioxin contaminated environment of Times Beach, Missouri, it is difficult to extrapolate the human exposure data from Seveso to these comparatively low level situations.

We continue to find new areas that are contaminated with dioxin, and this has generated a substantial amount of concern among the general public, the scientific community and regulatory agencies. The levels of dioxin reported at most of these sites are generally in the order of a few parts per billion (ppb). One ppb is roughly equivalent to a single drop of pure chemical diluted in 13 thousand gallons of water! Never-the-less, such low levels of TCDD are capable of producing biologic effects in some experimental animals if present in the <u>diet</u> for a prolonged period of time. The perceived risks of dioxin in the environment are very great. Whether the actual risks approach the perceived risks is an unanswered and much debated question.

The concern over phenoxy acid herbicides and dioxin has grown to include all herbicides in the eyes of many people. Application of any herbicide is often greated with resistence by nearby residents, regardless of the nature of the chemical being

used. While the magnitude of the concern is often out of proportion with the risk, it is important that scientists maintain an open mind and consider all possibilities of detrimental effects of herbicide use, recognizing the limitations of current scientific methodologies to accurately predict such effects. The lessons learned from asbestos, ionizing radiation, DDT, thalidomide and numerous other substances, where traditional scientific methods failed to adequately predict the risks to human health and/or the environment before damage was done, should be kept in mind by the scientist who readily dismisses perceived risks as impossible because they are not consistent with the available scientific data. However, it is equally important that scientific data not be used out of context to project irrational fears of potential adverse health effects from chemical application. Many of the perceived risks of herbicides are the result of unwarranted extrapolation of a single, unconfirmed laboratory report as pertinent to any potential human exposure situation. The harm generated by such predictions may be more damaging than the chemical itself. The mental anguish and physical stress placed on a pregnant woman who is told that herbicides will be sprayed in her neighborhood is probably more harmful to the developing child, if the woman perceives that the chemical is likely to harm her baby, than the chemical itself.

Scientists must make an effort to put risks in perspective, utilizing all available scientific information but recognizing the limitations and deficiencies in each study. More emphasis should be placed on establishing relative risk rather than absolute risk. The general public often does not understand the significance of, for example, an increased risk of cancer of one in ten thousand if chemical x is present as a contaminant in the diet at 1 ppm over a lifetime of exposure. They are more likely to understand the risk if the same risk were expressed in relative terms; for example, as being roughly equivalent to the increased risk of developing cancer from eating x ounces of charcoal broiled steak over a lifetime, or from sitting in the sun for x many hours. Although such comparisons are crude estimations at best, the magnitude of the error is not likely to be greater than the error of determining absolute risk.

The risks to human health that might result from increased herbicide use in "low-till" farming are probably minimal when put in perspective with other risks encountered daily. However, such an opinion must be qualified for each circumstance: the nature of the chemical; who is at risk for exposure (the applicator, the farmers family, nearby neighbors, etc), and at what level; what other sources of exposure they might encounter; what is known about the toxicologic effects of the chemical, etc. There will undoubtedly be controversy over the use of herbicides for many years to come. Hopefully, the scientific community will respond with reasoned concern, utilizing science to its fullest, yet recognizing its limitations, in an attempt to put the risks of herbicide use in perspective.

Brief Review of the history of the "dioxin" Controversy

1944 - First use of phenoxy acid herbicides

1950 - Dow chemical finds highly toxic contaminant

1957 - Chick edema disease - chemical poison in feed

1961 - TCDD identified as contaminant responsible for occupational Chloracne

1962 - Operation Ranchhand begins

1967 - Chick edema factor identified as HCDD

1969 - Report by Orians + Pfeiffer on ecological effects in Viet Nam

1969 - Bionetics Research Lab reports teratogenicity of 2,4,5-T

1969 - Study of birth records in Viet Nam - inconclusive

1969 - Globe, Arizona - National Forest - citizen complaints receive national media coverage

1969 - Presidents' CEQ recommends cancellation of 2,4,5-T on food crops + home use

1970-74 many congressional and administrative hearings

1976 - Seveso, Italy accident

1978 - CBS movie "Agent Orange: Viet Nam's Deadly Fog"

1978 - EPA issues RPAR for 2,4,5-T

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1979 - EPA investigates miscarriages in Alsea, OR

1979 - EPA issues emergency suspension of 2,4,5-T

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LYNDA L. BROTHERS
SPECIAL COUNSEL

November 10, 1989

Risk Assessment and Communication
Conference Work Group

Dear Members:

Thank you for attending the meeting in our offices at Heller, Ehrman on November 2, 1989. As promised, enclosed please find a copy of the articles contributed by Chuck Kleeberg.

In addition, this letter confirms our next meeting for January 19, 1990 at 5:00 p.m. at the Heller, Ehrman offices. I have contacted Dave Eaton, who has enthusiastically agreed to attend and to talk with us on risk and risk assessment.

I look forward to seeing you all on the 19th. If anybody has any information they would like to distribute to the group, please feel free to send it to me at the above address. Also, bring ideas for our next meeting at which we will focus on risk communication. I have also enclosed a copy of the work group mailing list, subject to changes and additions as we go forward.

Very truly yours,

Lynda Brothers

Lynda L. Brothers

cc: David Eaton, Ph.D.
Enclosures

Ex. 33 Date 12-15-92
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from Chuck Clusky

****
* COMBINED PUBLIC BULLETIN BOARDS *
****
This area combines GENERAL, EMERGENCY, and LEGISLATIVE bulletins...

<S>can <R>ead <E>nter <D>elete <C>hoose
<->MAIN
Commands:

HAZARD VS. OUTRAGE:

HOW THE PUBLIC SEES HEALTH RISK

Presentation by Peter M. Sandman, Ph.D., Rutgers University
To the ASTHO Annual Meeting, April 6, 1989. Vail, Colorado.

Notes by: A. Lamont Carstens, Supervisor, Community Liaison Section, Dept. of Health, Mail Stop: ET-36, Olympia, Wa. 98504

Community messages of risk to the public involves two questions:

1.1. How does one get the public to take real risks seriously and react when truly threatened? How can one convince apathetic or disempowered people to be motivated to take self-protective measures?

2. How does one calm people down? How can one help people to deal with anxieties provoked by inadequate information and avoid hysteria when the risk is not serious?

Anxiety-producing situations may not be hazardous; hazardous situations may not produce anxiety.

Risk often is viewed as the product of two factors:

magnitude - how bad what happens is
X
probability - how often it happens

However, this formula misses an essential ingredient in public response. That ingredient is outrage. To understand public response, it would be clearer to call the product of magnitude x probability, "hazard." Risk then becomes the sum of hazard plus outrage.

The public and scientists differ in their definition. Scientists use the word "risk" as a hazard. The public responds to a perceived risk as an outrage. The scientific mind may say something is not dangerous but the public mind, if it finds something disgusting, responds with a sense of alarm and outrage. The sense of outrage is real and must be dealt with.

In the literature of public risk assessment, twenty-six variables systematically turn up. Eleven of these precursors or measures of public health outrage reaction are discussed here. Distinctions people use in assessing risk, from low-risk to high-risk, include:

1. The distinction between risks that are voluntary and those which are coerced. This is as true for communities as for individuals. Coercion is seen as an assault which creates risk to those receiving the force.

2. A natural vs. a man-created risk. Radon is viewed as an act of God. A tanker oil spill is seen as an act of man and triggers greater outrage.

3. The familiar vs. the exotic or unfamiliar. Radon collects in the basement, a familiar location. Watching on TV an EPA cleanup by men in spacesuits creates a greater level of anxiety.

4. Not memorable vs. memorable. Memorable relates to how easy it is to conjure up thoughts of how bad things could be or how things could go wrong. Non-memorable relates to those situations we have lived in without a sense of apprehension. Memorable reactions can be stimulated by imagination or associations as well as real events. Talk of building waste incinerators conjures up in many minds thoughts of belching industrial smokestacks.

5. Not dreaded vs. a sense of dread. AIDS and cancer cause dread. A number of AIDS or cancer deaths will cause more alarm in a community than an equal number of deaths from other diseases. A 55-gallon drum marked "materials" will cause less anxiety than the same drum and content marked "waste."

6. Knowable vs. not knowable. "Knowability" relates to a number of factors such as scientific knowledge, familiarity, and detectability. Beliefs also may be treated as fact.

   Debate as to what is knowable may lead to conflict. Fear and outrage may increase as debate develops regarding what a risk level really is. The scientific capacity to detect minute amounts of substances increases this debate. Rates in discussing risk assessment takes a different meaning from rates in standard settings.

   An agency arguing that views of risk are exaggerations may be viewed by the public as disinterested and thereby increase fear and outrage.

7. Controlled by the individual (self) vs. controlled by others. Control is determined by who implements. Visualize the difference between sharpening the butcher knife and cutting the rib roast vs. holding the roast while someone else cuts. In many conflicts between government agencies and communities, the communities view themselves as holding the meat while the agency wields the knife.

   An agency sends mixed, contradictory messages when it tries to control or impose and at the same time try to say "don't worry." An agency which has a history of adverse impact on communities and people must acknowledge that history if it is to reduce dread and other anxiety-producing feelings.

Being knowledgeable is a part of the sense of control and voluntary participation. For an agency to share control means that it must allow the community to implement key controlling factors such as doing the study to determine need, desirability, et al.

8. Fair vs. unfair. One element of public perception of fairness is the perception as to distribution of risks and benefits. The belief is that benefits should benefit those sharing the risk and not skip the risk-bearers. For instance, outrage may increase over a garbage incinerator proposal which includes bringing garbage from outside the area. Public perception of fairness may also have a "my risk vs. your risk" element.

9. Morally irrelevant vs. morally relevant. Developing public values that some factor, for example, environmental contamination, is wrong--EVIL--gives moral relevance to the issue. However, some morally relevant issues have less tradeoffs. The more incensed the public becomes, the higher its sense of risk. When the public becomes very angry, it may demand a zero-risk solution. If the public agency shows no signs of getting to zero risk, the public becomes angry again.

10. Trusted vs. not trusted. Health agencies are trusted by the public when they deliver a warning, when they say that something is dangerous. ("They are the experts.") The health agency may not be trusted when it says something is safe. (Others are saying it is not safe.) Distrust may be one of the community's chief measures of the size of risk.

11. Good process vs. bad process. The processes of interaction between the governmental agency and the community may contribute to the community's sense of risk.

    A. Openness vs. secrecy. Openness reduces a public sense of risk. Agencies should tell what they will do and what happened. The public is more interested in being told than in what they are told.

    B. Acknowledge prior misdeeds vs. denial and stonewalling. The American public is tolerant of repentant sinners but distrusts those who legalistically deny that sin has occurred. "We goofed," is a necessary defense for public agencies.

    C. Caring communication vs. jargon and argument. What the speaker termed "technobabble" is distancing language.

Five conclusions were presented:

1. Risk means outrage, not hazard, to people. The public view of risk relates to their sense of outrage, not to health impact.

2. No one learns much about hazard while experiencing a high level of outrage. Substantive issues are not the person's issues when feelings are running strong. Substantive data, such as data on hazard, becomes ammunition by looking at data as something to distort in order to prove the conclusions of outrage. The only valid, relevant data is that which supports the outrage.

3. Outrage simultaneously is a:

   A. Distraction from hazard.

   B. Protection of the values we really care about. Outrage factors are factors people value--really care about. They reflect things society wants taken seriously.

4. Where hazard is high, the job is to make outrage higher. For example, while 80-90% of the hazard of smoking is to the smokers and only 10-20% to non-smokers, the outrage of the passive smoker is responsible for most control measures. MADD is another example of translating a serious hazard into serious outrage and getting progress on the problem.

5. Where the hazard is low, the job of risk communication is not to explain that the risk is low. Rather, the job of communication is to keep the level of outrage low. The job is to find ways to keep the issues at the low-risk, non-outrage stage of the eleven factors listed above.

The communication must speak to the variables of outrage, not just to the hazards.

Dr. Denman Scott, ASTHO president-elect, observed that the process works. When syringes washed up on the Rhode Island shoreline, his first words were "it's disgusting." He then could say further, "It's disgusting but, incidentally, you will not get AIDS if you step on one of these needles on the beach."

Your Seattle City Light

Memorandum

DATE: June 20, 1989

TO: Distribution List

FROM: May Gerstle via Julia Greenlee

SUBJECT: Electric Magnetic Fields Communications Conference

The attached report summarizes highlights from the Electric Magnetic Fields (EMF) Communications Conference sponsored by the Western Systems Coordinating Council held June 7-9. Of particular interest are the comments on risk perception and the importance of addressing the political and societal issues in planning for utility projects. The pivotal role of public information and involvement was stressed throughout the conference.

I hope that this report will be useful to you in the planning and decision-making process. Julia Greenlee would be glad to answer any questions or to discuss this report further if you are interested. In light of the articles in the New Yorker magazine the EMF issue may be in for increased attention and scrutiny.

JG:ph

Attachment

Distribution List

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Saven
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Walters
EMF Workgroup

cc: Gerstle
Bailey
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Forslund
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19232

531-L(1-83)

Questions regarding:

- o ODC hormone promotion
- o Cell communication effect
- o Melatonin and breast cancer
- o Tumor cell growth
- o Decreased cancer-killing ability

Other Effect Issues

- o Reproduction
  - Electric blanket use
  - Chick embryos (pulse magnetic fields)
- o Melatonin
  - Mood, behavior
  - Seasonal breeding
- o Cellular effects
  - Calcium (primarily electric fields)
  - Communication between cells
- o Behavior, learning

EPA's Position

Potential for cancer and behavior changes. Utilities should exercise caution before allowing any increase in exposure.

New York Studies

Several areas of potential concern.

Conclusions

- o Some nonhazardous field effects and potential for effects on human health or performance.
- o Several epidemiological studies suggest 60-hertz fields may be weak cancer promoters.
- o Limited evidence for effects on reproduction.
- o More long-term research needed.
- o Trend developing to recommend limiting chronic field exposures.

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Morgan's research involved the following schedule of activities:

- o Performing scoping studies
- o Developing and testing questionnaires
- o Developing and testing common materials
- o Performing public perception studies

The studies included face-to-face interviews, perception focus groups, and decision focus groups.

According to the early results of the research:

- o Public perception of the funding source is critical to credibility.
- o Relative risk is not often realised by public or an especially compelling argument.
- o Attention to an issue implies risk.
- o Need doubted. This was highlighted as a significant public perception that was also emphasized by Herbert Thier. Thier encouraged utilities to thoroughly test need within their own organizations and not to approach the public until they can convincingly demonstrate need.

What people need to know, according to Morgan, are basic facts, basic principles, and one or two advanced facts.

Granger Morgan is in the process of developing the next step in the research that will focus on strategies and outcomes based on the results of the research on public perception.

Summary of Presentation by Dr. Herbert Thier

Dr. Herbert Thier, from the University of California, Berkeley, gave an excellent presentation on Science Education as a precursor to risk communications.

Useful EMF risk communication aids should:

- o Aim toward clear objectives
- o Define overall risk communication strategy
- o Establish content
- o Develop and integrate detailed risk messages and evaluations

Correlation is not a synonym for causation; it is a somewhat hard distinction for the public to make.

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Quantitative risk assessment - risk hazards prioritize regulatory action.

The acceptance of risk is a political question that requires political action; perception is reality.

In dealing with public perception of EMF, it is important to define what is the message and who conveys it.

Frequently you communicate risk; the public hears certainty.

Public perception determines acceptance of risk.

Factors that influence perception are:

Less Risky	More Risky
Voluntary	Involuntary
Controlled	Uncontrollable
Beneficial	Not beneficial
Familiar	Unfamiliar
Controlled by self	Controlled by others
Fair	Unfair
Not dreaded	Dreaded
Not memorable	Memorable
Chronic	Acute
Diffused in time	Focused in time and space
Immediate	Delayed
Not fatal	Fatal

You need to establish ongoing interaction between you and the community.

The utilities' goal is to inform the public; the decision making is the prerogative of the public.

According to Thier, the only thing that works with the public is the argument of actions taken versus words or comparisons. Have you as a utility minimized the hazards and made improvements? Put another way, the question to ask is "What have you done to make things safer?"

Risk management decisions involve:

- Risk identification
- Risk analysis
- Risk comparison
- Resource allocation

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When you send the solution instead of alternatives to the public, the solution becomes the definition of the problem. You set up a power situation that results in only two reactions--either submit or oppose. You rule out possible alternatives and force the public into an adversarial relationship. The attention needs to be focused on fundamental interests.

- o Take time to build an understanding in the community about what the problem is and go out to the community early.

- o Don't trivialize risk.

- o Moral categories mean more than risk data.

- o Policy decisions are seen in terms of being either risky or safe.

- o Equity and control issues underly most risk controversies.

- o Risk decisions are better when the public shares the power.

- o Risk communications are easier when emotions are seen as legitimate.

- o Public involvement must be thoroughly integrated into decision-making process.

There must be a result from participating. The public impacts decision making and the Utility learns something.

Different forums must be provided for different public sectors.

Public hearings are the least effective way to resolve issues. Public involvement must be done before public hearings. Giant forums are not effective consensus-building devices.

- o The role of public involvement is to build and maintain relationships with the public. It is the accumulative effect of relating to the public consistently over time.

Media Relations

- o Environmental risk is not a big story

- o Politics are more newsworthy than science

- o Reports cover viewpoints, not truth

- o The risk story is simplified to a dichotomy

- o Reporters try to personalize the risk story

- o Claims of risk are usually more newsworthy than claims of safety

- o Reporters do their job with limited expertise on subject and time

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ENVH 567

Environmental Carcinogenesis

Winter quarter, 1993

Instructor: David L. Eaton, Ph.D.
Environmental Health and Environmental Studies
685-3785

I. **Basic Concepts of Chemical Carcinogenesis**
   - A. Epidemiology and the Etiology of Cancer - What role do chemicals play? (1)*
   - B. The multi-stage nature of carcinogenesis (1)

II. **Biochemical and molecular events in chemical carcinogenesis**
   - A. Biotransformation of chemical carcinogens (1)
     discussion focus: aromatic amines; PAHs
   - B. genotoxicity (1)
     discussion focus: N-nitroso compounds; aflatoxin
   - C. protooncogenes and signal transduction (1)
     discussion focus: G-proteins, protein kinase C, and phorbol esters
   - D. tumor suppresor genes (1)
     discussion focus: P53
   - E. hormones, hormone receptors & growth regulation (1)
     discussion focus: estrogens; TCDD
   - F. apoptosis in carcinogenesis (1)
     discussion focus: experimental methods to assess apoptosis
   - G. role of cell proliferation in carcinogenesis (1)
     discussion focus: Saccharin; nitriloacetic acid
   - H. chemo- and dietary interventions and anticarcinogenesis (1)
     discussion focus: phenolic antioxidants as chemoprotectants

III. **Experimental models in chemical carcinogenesis**
   - A. initiation-promotion regimens (1)
     discussion focus: Mouse skin papillomas
   - B. preneoplastic lesions/hyperplastic foci (1)
     discussion focus: Solt-Farber protocol
   - C. anomolies in chemical carcinogenesis
     1. peroxisomal proliferation; male rat specific nephropathy (1)
        discussion focus: Trichloroacetic acid; 2,3,5-Trimethylpentane
     2. B6C3F1 mouse hepatomas; transgenic animals (1)
        discussion focus: chlorinated hydrocarbons (DDT, dieldrin)
     3. solid state (e.g. fiber) carcinogens (1)
        discussion focus: asbestos

IV. Assessment of chemical carcinogens and cancer risk
   - A. mathematical modeling- linearized multistage , PBPK and MVK models (1)
   - B. NTP/NCI Bioassay program - experimental design & limitations (1)

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*Number of lectures shown in parentheses (Tentative schedule).

FORMAT - Each 90 minute session will be divided approximately equally into two parts - a "didactic" lecture by the instructor or guest lecturer on the scheduled topic, and a group discussion on the assigned reading, lead by one of the students in the class. The discussion should go beyond the material presented by the instructor, and should be focused on the assigned reading (review article or book chapter), plus one additional recent "original research" article on the specific discussion topic(s), selected and distributed at least one week in advance by the student responsible for leading the discussion. Generally, the discussion focus will be on a specific chemical which produces or acts via the general processes discussed in the lecture. The discussion leader should familiarize herself/himself with other literature on the topic, and be able to demonstrate a relatively high level of understanding of the topic. A bibliography of recent articles (dated 1991 or later) and a 2-3 page summary of the topic of discussion must be turned in within one week following the discussion. A student will be responsible for only one discussion session during the quarter.

GRADING: Grades will be based on two written exams and the performance of the student as a discussion leader. The mid-term will be a take-home exam, whereas the final will be an in class, closed book exam. Each exam and the discussion leader responsibilities will be worth 33% of the grade.

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Readings List for ENVH 567 - Environmental and Occupational Carcinogenesis
(* indicates required reading)

I. Basic Concepts of Chemical Carcinogenesis

*Fraumeni, J. F., Jr. Epidemiology of Cancer. IN: *Origins of Human Cancer: A Comprehensive Review*, J. Brugge, T. Curran, E. Harlow and F. McCormick, Editors, Cold Springs Harbor Laboratory Press, 1991; pp. 171-181.

*Willett, W.C. Diet and human cancer. IN: *Origins of Human Cancer: A Comprehensive Review*, J. Brugge, T. Curran, E. Harlow and F. McCormick, Editors, Cold Springs Harbor Laboratory Press, 1991; pp. 191-198.

Perera, F. P., Santella, R. , Brandt-Rauf, P., Kahn, S., Jiang, W. and Mayer, J. Molecular epidemiology of lung cancer. *Origins of Human Cancer: A Comprehensive Review*, J. Brugge, T. Curran, E. Harlow and F. McCormick, Editors, Cold Springs Harbor Laboratory Press, 1991; pp. 219-236.

*Barrett, J. C. Relationship between mutagenesis and carcinogenesis. *Origins of Human Cancer: A Comprehensive Review*, J. Brugge, T. Curran, E. Harlow and F. McCormick, Editors, Cold Springs Harbor Laboratory Press, 1991; pp. 101-112.

*Knudson, A. Genetic events in human carcinogenesis. *Origins of Human Cancer: A Comprehensive Review*, J. Brugge, T. Curran, E. Harlow and F. McCormick, Editors, Cold Springs Harbor Laboratory Press, 1991; pp. 17-25.

*Weinstein, I. B., Borner, C. M., Krauss, R. S. et al. Pleiotropic effects of protein kinase C and the concept of carcinogenesis as a progressive disorder in signal transduction. IN:*Origins of Human Cancer: A Comprehensive Review*, J. Brugge, T. Curran, E. Harlow and F. McCormick, Editors, Cold Springs Harbor Laboratory Press, 1991; pp. 113-124.

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*Harris, C.C. Chemical and physical carcinogenesis: advances and perspectives for the 1990s. Cancer Res. 1991 Sep 15. 51(18 Suppl). P 5023s-5044s.

Trush, M.A. Kensler, T.W. An overview of the relationship between oxidative stress and chemical carcinogenesis. Free Radic. Biol. Med. 1991. 10(3-4). P 201-9.

Pitot, H.C. Dragan, Y.P. Facts and theories concerning the mechanisms of carcinogenesis. FASEB J. 1991 Jun. 5(9). P 2280-6.

Biotransformation and Carcinogenesis

*Guengerich, F.P. Metabolic activation of carcinogens. Pharmacol. Ther. 1992. 54(1). P 17-61.

Tsuchida, S. and Sato, K. Glutathione transferases and cancer. Crit. Rev. Biochem. Mol. Biol. 1992. 27(4-5). P 337-84.

Stiborov'a, M. Frei, E. Schmeiser, H.H. and Anzenbacher, P. The role of peroxidases in the activation of chemical carcinogens. Drug.Metabol. Drug. Interact. 1991. 9(3-4). P 177-90.

Kawajiri, K. Fujii-Kuriyama, Y. P450 and human cancer. Jpn. J. Cancer. Res. 1991; 82(12). P 1325-35.

*Miles, J.S. and Wolf, C.R. Developments and perspectives on the role of cytochrome P450s in chemical carcinogenesis. Carcinogenesis.

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"America's Epidemic of Chemicals and Cancer" - Myth or Fact?[1]

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There is no debate that cancer is a devastating and deadly disease. One in three people living in the U.S. today will contract some form of cancer in his or her lifetime, and one in four will die from it, if current rates continue. However, the contention that we are in the throes of an unprecedented cancer epidemic, and that this "epidemic" is caused in large part by cancer-causing pesticides and industrial chemicals is simply not supported by the scientific data available on cancer incidence and etiology (causes).

Are cancer rates increasing in epidemic proportions? It is certainly true that both the total number of people and the fraction of all deaths attributable to cancer have increased dramatically in the past 50 years. However, cancer is largely a disease of old age, and thus it is necessary to adjust such statistics for changes in the age distribution of a population over the years. A 1988 report from the National Cancer Institute states that "the age adjusted mortality rates for all [types of] cancers combined, except lung cancer, has been declining since 1950 for all individual age groups except 85 and above". Statistics from the American Cancer Society yield the same conclusion.

The reason for excluding lung cancer in this evaluation is to examine cancer trends without the strong influence that the dramatic increase in lung cancer incidence (and deaths) have on *total* cancer rates. The available scientific evidence would support the supposition that we *are* in an "epidemic" of lung cancer. For most of the first half of this century, lung cancer mortality was not even in the "top five" types of cancer-related deaths. Lung cancer is now the leading cause of cancer-related deaths in both men and women. There is abundant scientific evidence to demonstrate that 85 - 90% of all lung cancers in men, and perhaps 70% in women, is directly attributable to smoking, a habit which increased steadily in popularity from the early 1900's through the 1950's. *Per capita* consumption of cigarettes increased 5-fold in men from 1900 to 1960, and with it a concomitant increase in lung cancer. The risks of several other types of common cancers are also increased by smoking (e.g. cancers of the bladder and esophagus). Approximately one-third of *all* cancer deaths could be eliminated by the elimination of smoking from our society. The supposition that many of these smoking-related cancers may be related to carcinogenic pesticides and chemicals in tobacco is grossly misleading- while the statement in its absolute sense is likely to be true, the *source* of these carcinogenic pesticides and chemicals is *mother nature*, not the chemical and agricultural industry.

What proportion of cancers can be related to environmental pollution from synthetic pesticides and industrial chemicals? The often-cited statistic that "70 to 90% of all cancers are caused by environmental influences and are hence theoretically preventable" is frequently incorrectly interpreted as meaning that *chemical pollution* is responsible for 70 - 90% of cancers. The original source of this statement stems from studies on human cancer incidence reported in the late 1960's. These studies suggested that most cancers could not be directly traced to genetic or hereditary factors, and therefore it was concluded that the majority of cancers must have an "environmental" etiology. In this context, the term "environmental" includes not only chemicals in the environment,

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[1]This article has been prepared for the fourth edition of an undergraduate textbook entitled "Environmental Science: A Framework for Decision Making", by Daniel D. Chiras; Benjamin Cummings Publishing Company, Menlo Park, California, 1990.

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but lifestyle factors such as smoking and alcohol, dietary factors such as the proportion of fat and fiber in the diet, "natural" carcinogens that occur in nearly all foods, cancer-causing viruses such as hepatitis B, and occupational exposures to substances such as asbestos.

Of the variety of environmental factors other than smoking, dietary factors are now generally thought to represent the largest source of cancer risk, perhaps related to 30-40% of all cancers. Although synthetic chemicals such as industrial pollutants and pesticides present in trace amounts in our food supply may contribute to dietary risk, recent studies have suggested that this contribution is trivial relative to other "non-pollutant" factors. For example, the risk of breast cancer in women, second only to lung cancer in incidence and mortality, is significantly increased by high fat diets, and the amount of fiber in the diet substantially influences the risk of colon cancer, a major site of cancer in both men and women.

Recently scientists have found that by far the largest source of exposure to cancer-causing chemicals comes not from industrial pollution, but from chemicals that occur naturally in our diet. All plants produce toxic chemicals as a means of protection against insects, fungi and animal predators. It has been estimated that we ingest in our diet about 10,000 times more of "nature's pesticides" than man-made chemical residues. Many of these chemicals are potent mutagens and carcinogens, and are frequently present at levels thousands of times higher than the trace levels of synthetic pesticide residues and industrial chemicals sometimes found in food crops. For example, natural carcinogenic chemicals are found in mushrooms, parsley, basil, fennel, pepper, celery, figs, and mustard, to name a few. The vast majority of natural chemicals present in foods (tens of thousands) have never been tested to determine if they might be carcinogenic. There is also substantial scientific evidence that cooking of foods, especially meats, forms highly mutagenic and perhaps carcinogenic chemicals from natural precursors such as certain protein components. Taken together, the dietary risk factors from natural sources, often present in relatively high amounts, are far more important than the pesticide residues and industrial chemicals that can often be detected at exceedingly small concentrations in our diets. Unfortunately, because of the relatively high exposure to carcinogens from natural sources, the complete elimination of synthetic industrial chemicals from our diet, if it were possible, would not likely have any significant beneficial effect on cancer incidence and mortality.

There is little question that extensive exposure to some industrial chemicals can increase the risk of certain types of cancer. Occupational exposures to potentially carcinogenic chemicals can be a significant risk factor for cancer, and every effort should be made to identify and reduce workplace exposure to these chemicals. However, the reliance upon a 1978 report of the Department of Health Education and Welfare which stated that "20 to 38% of all cancers could be attributed to occupational exposure to just 6 industrial chemicals" is no longer valid, as even the authors of this report acknowledge that this was a substantial overestimation of the contribution of occupational exposures to total cancer incidence. Most authoritative sources now estimate that occupational exposures account for no more than 5 to 10% of all cancers, and many of these cancers are a result of extensive exposures to asbestos and a few other industrial carcinogens that were commonplace in the 50's and 60's, but have since been greatly reduced (although certainly not eliminated). Of course, 5% is too high, and every effort should be made to reduce this contribution further.

Finally, recent advances in the understanding of the biology of cancer suggest that "spontaneous" or "background" alterations in DNA may explain much of the etiology of cancer. The use of modern techniques in molecular biology have revealed that DNA is inherently unstable, can be altered by normal errors in DNA replication, and is subject to extensive damage from processes associated with normal cellular metabolism. Considering that within our life span, our cells undergo about 10 million billion (10^{16}) cell divisions, and that spontaneous errors in this process which lead to mutations and cancer accumulate with age, it is not surprising that cancer

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WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

LONG-TERM HAZARDS OF POLYCHLORINATED DIBENZODIOXINS and POLYCHLORINATED DIBENZOFURANS

JOINT NIEHS / IARC WORKING GROUP REPORT

IARC, LYON

JUNE, 1978

IARC INTERNAL

TECHNICAL REPORT

No. 78/001

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IARC MONOGRAPHS ON THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS

In 1971, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals.

The role of the monograph programme is to collect all available relevant experimental and epidemiological data about groups of chemicals to which humans are known to be exposed, to evaluate these data in terms of human risk with the help of international working groups of acknowledged experts in chemical carcinogenesis and related fields, and to publish and disseminate the conclusions of those working groups as a series of IARC Monographs.

Some Inorganic Substances, Chlorinated Hydrocarbons, Aromatic Amines, *N*-Nitroso Compounds, and Natural Products	Volume 1, 1972; 184 pages US$ 4.20; Sw. fr. 12.—
Some Inorganic and Organometallic Compounds	Volume 2, 1973; 181 pages US$ 3.60; Sw. fr. 12.—
Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds	Volume 3, 1973; 271 pages US$ 5.40; Sw. fr. 18.—
Some Aromatic Amines, Hydrazine and Related Substances, *N*-Nitroso Compounds and Miscellaneous Alkylating Agents	Volume 4, 1974; 286 pages US$ 7.20; Sw. fr. 18.—
Some Organochlorine Pesticides	Volume 5, 1974; 241 pages US$ 7.20; Sw. fr. 18.—
Sex Hormones	Volume 6, 1974; 243 pages US$ 7.20; Sw. fr. 18.—
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Some Aromatic Azo Compounds	Volume 8, 1975; 357 pages US$ 14.40; Sw. fr. 36.—
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Some Aromatic Amines and Related Nitro Compounds—Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals	Volume 16, 1978; 400 pages US$ 20.00; Sw. fr. 50.—
Some *N*-Nitroso Compounds	Volume 17, 1978; 365 pages US$ 25.00; Sw. fr. 50.—

Available from WHO, Distribution and Sales Service, 1211 Geneva 27, Switzerland; or from the United Nations Bookshop, New York, NY 10017, USA (retail only); or from the Franklin Institute Press, Benjamin Franklin Parkway, Philadelphia, PA 19103, USA.

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LONG-TERM HAZARDS OF POLYCHLORINATED DIBENZODIOXINS and POLYCHLORINATED DIBENZOFURANS

JOINT NIEHS / IARC WORKING GROUP REPORT

This report represents the views and opinions of an US National Institute of Environmental Health Sciences/International Agency for Research on Cancer ad hoc Working Group which met during 10-11 January 1978 in Lyon, France to review the history of human exposure to these chemicals, to collate current information, and to plan and forecast needed new directions.

IARC, LYON

JUNE, 1978

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1	General formulae for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans	10

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Participants in the NIEHS/IARC Working Group on the
Long-Term Hazards of
Polychlorinated Dibenzodioxins / Polychlorinated Dibenzofurans

Lyon, France, 10-11 January 1978

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Joint NIEHS/IARC Working Group Report on the
Long-Term Hazards of
Polychlorinated Dibenzodioxins / Polychlorinated Dibenzofurans

I. BACKGROUND

In February 1977, an IARC *ad hoc* Working Group met to evaluate the carcinogenic risk to humans of certain fumigants, the herbicides 2,4-D and 2,4,5-T, chlorinated dibenzo-*p*-dioxins, and miscellaneous industrial chemicals (IARC, 1977). What became clear both during the meeting and subsequently, while preparing IARC Monograph Volume 15 for publication, was the need not only for better coordination of ongoing and projected epidemiological efforts concerning the chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans, but also for a worldwide action plan which would serve to harmonize the various activities already in progress or in preparatory stages.

Purpose of the NIEHS/IARC Meeting

During 10-11 January 1978, in Lyon, France, a joint US National Institute of Environmental Health Sciences/International Agency for Research on Cancer *ad hoc* Working Group considered and discussed the feasibility of coordinating epidemiological studies on the long-term hazards associated with the chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans (PCDDs and PCDFs).

Eighteen invited scientists, from eight countries, presented introductory working papers summarizing the most up-to-date and relevant information available from their individual programmes (References - Part II).

Report Contents

This report represents the collective views and scientific opinions of the Working Group. The greater part of this document comprises epidemiological studies related to episodes of human exposure. The review begins, however, with a brief section concerning possible routes of human exposure, an overview of the pertinent chemical characteristics, and the salient toxicological properties of the structurally similar PCDDs/PCDFs. The Working Group report ends with recommendations for future activities.

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Introduction to the Problem

Human exposure in the workplace can occur when chlorinated dibenzo-*p*-dioxins (PCDDs) are formed during the production of certain compounds such as the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), the fungicide pentachlorophenol, and the germicide hexachlorophene (IARC, 1977). The dioxins, impurities/contaminants associated with these end-products, result most often from treatment of chlorinated benzenes at elevated temperature and pressure under alkaline conditions. *Via* the widespread use of these commercial products (Gosselin *et al.*, 1976; IARC, 1977) the general population may also become exposed.

In recent years, out-of-control chemical reactions during the production of 2,4,5-trichlorophenol have proceeded to the explosive stage thereby exposing persons to toxic levels of PCDDs. From certain of these accidents, moreover, not only did those occupationally involved receive dangerous exposure but also those inhabiting the surrounding areas received uncommon risk.

The structurally-related chlorinated dibenzofurans (PCDFs) are contaminants found in some polychlorinated biphenyl compounds - Aroclor, Clophen, Phenoclor (IARC, 1978a).

These unwanted - and until recently undetected and unrecognized - impurities are more toxic and represent a potentially greater environmental hazard than the compounds they contaminate (Blair, 1973; Huff & Wassom, 1973, 1974a,b; IARC, 1977, 1978a; NIEHS, 1973; Ramel, 1978). For instance, 2,3,7,8-tetra-CDD has been recently found in beef fat from cattle grazed on 2,4,5-T-treated rangeland and in breast milk from women living in areas where 2,4,5-T is used on rangeland or in forest areas (Meselson *et al.*, 1978). Various investigators (Buser & Bosshardt, 1978; Buser *et al.*, 1978b,d; Olie *et al.*, 1977) have also discovered PCDDs and PCDFs in fly ash and flue gas from municipal incinerators, and in dust from fungicide-treated wood (Levin *et al.*, 1977).

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II. CHEMICAL ASPECTS

The chlorinated dibenzo-*p*-dioxins (PCDDs) and chlorinated dibenzofurans (PCDFs) are two series of tricyclic aromatic compounds which exhibit similar chemical and physical properties (IARC, 1977, 1978a; NIEHS, 1973). The basic two-dimensional structures as shown in Figure 1 have eight possible points of chemical addition. From the monochloro to the octachloro derivatives, a variety of positional isomers are possible - 75 PCDDs and 135 PCDFs (Table 1).

The extreme toxic potency of some of these compounds, as well as the large number of potential isomers, warrant analytical methods exhibiting high sensitivity and specificity to monitor the environment. A desirable detection limit of one part per trillion (ppt or a picogram/gram sample) is being reached with current methodology. Essential requirements included efficient clean-up, good separation and selectivity, ultra-sensitive quantification, and validation (Buser, 1977; Buser & Rappe, 1978).

III. TOXICOLOGICAL ASPECTS

Animals

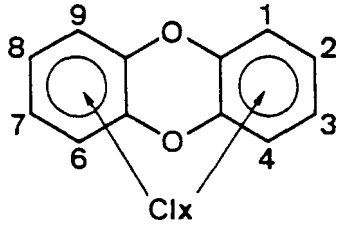
The prototype and most extensively studied isomer of the PCDDs and PCDFs is the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-tetra-CDD), perhaps the most potent man-made toxin presently known. The comparative oral lethal dose values for various PCDDs in mice and guinea-pigs (Table 2) show clearly that the 2,3,7,8-tetra-CDD and the 1,2,3,7,8-penta-CDD isomers are the most toxic (McConnell *et al.*, 1978a).

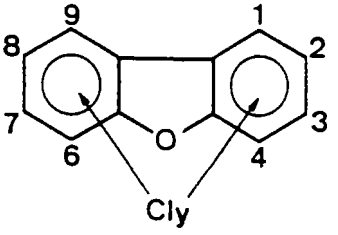
Toxic effects induced by PCDDs and PCDFs vary quantitatively and qualitatively among different species; however, within a single species the untoward consequences are markedly similar for all PCDDs and PCDFs that have been studied. For example, the toxic effects induced by 2,3,7,8-tetra-CDD which are most often observed in mice, guinea-pigs, and monkeys, are illustrated in Table 3. In a brief report, daily oral intake (12-61 days) of <1 µg/kg bw 2,3,7,8-tetra-CDD was stated as being lethal to young male rhesus monkeys (McNulty, 1977), whereas McConnell *et al.* (1978b) reported an LD_{50} in female rhesus monkeys as <70 µg/kg bw.

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Figure 1

General formulae for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans





	CHLORINATED DIBENZO-*p*-DIOXINS	CHLORINATED DIBENZOFURANS
Empirical formulae:	$C_{12}H_{7-0}Cl_{1-8}O_2$	$C_{12}H_{7-0}Cl_{1-8}O$
Molecular weight ranges:	218-460	202-444

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TABLE 1

Possible number of isomers for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans

Chlorine atoms	PCDD isomers	PCDF isomers
1	2	4
2	10	16
3	14	28
4	22	38
5	14	28
6	10	16
7	2	4
8	1	1
TOTAL	75	135

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TABLE 2[a]

Estimated single oral lethal dose$_{50-30}$ values of certain polychlorinated dibenzo-*p*-dioxin isomers

Chlorine isomer	Guinea-pigs		Mice	
	µg/kg bw	µ mole/kg bw	µg/kg bw	µ mole/kg bw
2,8	>300,000	>1,180	-	-
2,3,7	29,444	120.41	>3,000	>10
2,3,7,8	2	0.006	283.7	0.88
1,2,3,7,8	3.1	0.009	337.5	0.94
1,2,4,7,8	1,125	3.15	>5,000	>14
1,2,3,4,7,8	72.5	0.185	825	2.11
1,2,3,6,7,8	70-100[c]	0.178-0.255	1,250	3.19
1,2,3,7,8,9	60-100[c]	0.153-0.255	>1,440	>3.67
1,2,3,4,6,7,8	>600	>1.400	-	-
1-NO_2-3,7,8	>30,000	>90	-	-
1-NH_2-3,7,8	>30,000	>99	-	-
1-NO_2-2,3,7,8	47.5	0.129	>2,000	>5.4
1-NH_2-2,3,7,8	194.2	0.576	>4,800	>14.2

[a] From McConnell *et al.* (1978a)
[b] The LD_{50-30} was calculated by the Spearman-Karber method (Finney, 1964).
[c] Estimated range due to variability in replicates.

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TABLE 3[a]

Summary of the toxic effects of
2,3,7,8-tetrachlorodibenzo-*p*-dioxin

	Mice	Guinea-pigs	Monkeys (female)
Thymus involution	+++	+++	+++
Spleen reduction (white pulp)	+	+	+
Bone marrow hypoplasia	±	++	+
Liver megalocytosis/degeneration	+++	-	-
Bile duct hyperplasia	±	±	+++
Testicular degeneration	++	+++	NA[b]
Renal pelvis hyperplasia	-	++	+
Urinary bladder hyperplasia	-	++	-
Adrenal cortical atrophy (*Zona Glomerulosa*)	-	++	-
Haemorrhage intestinal	+	+	-
adrenal	-	++	-
Ascites	++	-	+
Cutaneous lesions	-	-	+++

[a] From Moore (1978)
[b] NA = not applicable

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A preliminary report on 2,3,7,8-tetra-CDF concerning the toxicity of PCDFs (Moore *et al.*, 1976), and a report on PCDFs (Oishi *et al.*, 1978) have been published.

The toxic syndrome produced by PCDDs and PCDFs may be divided into seven categories:

Chloracne - 2,3,7,8-Tetra-CDD and the tri-PCDF and tetra-PCDF were found to be active skin irritants and to induce acneform lesions in the skin of rabbit ears (Kimmig & Schulz, 1957a).

Classical chloracne is a hallmark of PCDDs exposure in humans and an analagous hyperkeratosis and the modulation of sebaceous structures to keratin cysts has been observed in monkeys, rabbits, and hairless mice.

Chloracne or acneform dermatitis is a common occupational dermatitis characterized by comedones, keratin cysts, pustules, papules and abscesses. In 1957, Kimmig & Schulz (1957b) found that 2,3,7,8-tetra-CDD was the agent responsible for causing occupational chloracne in employees of chlorophenol-producing factories. Further, in 1971 PCDDs were implicated as causing chloracne in male workers in a plant producing 2,4-D and 2,4,5-T (Bleiberg *et al.*, 1964; Poland *et al.*, 1971). Chloracne may appear weeks or months after the initial exposure to PCDDs and PCDFs.

Hepatoxicity - The degree of hepatic involvement appears to be dose-dependent, and the severity of the changes produced varies between species (Gupta *et al.*, 1973). Hepatic necrosis produced by 2,3,7,8-tetra-CDD is probably a contributing cause of death in rats and rabbits, while hepatic necrosis and liver insufficiency are less extensive in mice and are minimal in comparison in guinea-pigs and monkeys (Buu-Hoï *et al.*, 1972a; Harris *et al.*, 1973; Jones, 1975; Jones & Butler, 1974; McConnell *et al.*, 1978b; Schulz, 1968; Schwetz *et al.*, 1973; Vos *et al.*, 1973, 1974). Hepatic porphyrin accumulation has been observed in mice, rats, and chickens.

Hypoplasia of the lymphoid tissues - Particularly involved are the cortical cells of the thymus and this hypoplasia has been observed in mice, rats, guinea-pigs, and monkeys. The most significant findings in both mice and guinea-pigs treated with sublethal doses of 2,3,7,8-tetra-CDD

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were in the lymphoid system, resulting in suppression of cell-mediated immunity, particularly in young animals (Vos, 1977; Vos *et al.*, 1973). Low levels of 2,3,7,8-tetra-CDD that did not produce overt clinical or pathological changes still reduced host defences: 1 µg/kg bw given orally once weekly for 4 weeks to mice before infection with *Salmonella* increased mortality and decreased the time from infection to death (Thigpen *et al.*, 1975). The increased mortality may be caused by the endotoxin content of these gram negative bacteria, since 2,3,7,8-tetra-CDD markedly increases the susceptibility of mice to endotoxin (lipopolysaccharide of *Escherichia coli*) (Vos *et al.*, 1978). Treatment of female mice and rats with 2,3,7,8-tetra-CDD during the latter half of gestation and in the postnatal period resulted in a severe depletion of lymphocytes in the thymic cortex of the offspring (Vos & Moore, 1974). Cellular immunity was impaired.

Haematological changes in mice, rats and guinea-pigs treated with 2,3,7,8-tetra-CDD included lymphopenia and thrombocytopenia (Weissberg & Zinkl, 1973; Zinkl *et al.*, 1973); manifest also is an increased susceptibility to infection concomitant with the suppression of cell-mediated immunity. For 9 months, female rhesus monkeys received a diet containing 500 parts per trillion 2,3,7,8-tetra-CDD; within 6 months, the monkeys became anaemic and after 9 months pancytopenic (Allen *et al.*, 1977). The marked thrombocytopenia was associated with widespread haemorrhage. Death occurred in five of the eight monkeys between months 7 and 12 of the experiment at total exposure levels of 2-3 µg 2,3,7,8-tetra-CDD/kg bw. At autopsy, in addition to extensive haemorrhage, there was a distinct hypocellularity of the bone marrow and lymph nodes. Hypertrophy, hyperplasia and metaplasia of the epithelium in the bronchial tree, bile ducts, pancreatic ducts, salivary-gland ducts and palpebral conjunctivae were observed. Squamous metaplasia and keratinization of the sebaceous glands and hair follicles were present in the skin. Death was attributed to complications from the severe pancytopenia (Allen *et al.*, 1977).

General debilitation and wasting - Animals that receive a toxic or lethal dose of PCDDs or PCDFs exhibit a chronic and progressive weight loss with parallel mobilization of peripheral fat, increased serum triglyceride

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levels, and development of a fatty liver. Death due to PCDDs or PCDFs intoxication is delayed, as exemplified by an elapsed time period of 6 to 8 weeks following administration of a lethal dose and eventual death (Allen *et al.*, 1977; McConnell *et al.*, 1978a,b).

Embryotoxicity and teratogenicity of 2,3,7,8-tetra-CDD - In repeated or single doses of 2,3,7,8-tetra-CDD to mice, as little as 1-10 µg/kg bw cause increased frequencies of cleft palate and kidney abnormalities (see Table 4) (Courtney & Moore, 1971; Neubert & Dillmann, 1972; Neubert *et al.*, 1973; Smith *et al.*, 1976). In rats, embryo-lethal effects occur under experimental conditions (Sparschu *et al.*, 1970, 1971), and kidney anomalies (Courtney & Moore, 1971), intestinal haemorrhages and general oedema can be produced in the foetuses (Khera & Ruddick, 1973). Few follow-up studies of the effects of prenatal exposure on postnatal functions have been published. In mice, foetal kidney abnormalities caused by 2,3,7,8-tetra-CDD may progress into a hydronephrosis during the postnatal period (Moore *et al.*, 1973).

Chick oedema disease - Hydropericardium, ascites, subcutaneous oedema, liver necrosis and death were described in 1957 following the accidental administration of toxic fats in the feed of broiler chickens (ascites have been observed also in mice) (IARC, 1977). The toxic material was later identified in commercial oleic and stearic acids produced from inedible tallow recovered from animal hides; trichlorophenols and pentachlorophenols had been used in the curing of the hides. The oedema causative was termed toxic fat and more specifically chick oedema factor and was characterized by X-ray crystallography as 1,2,3,7,8,9-hexa-CDD (Cantrell *et al.*, 1969). 2,3,7,8-Tetra-CDD, hexa-CDD, and octa-CDD have been also identified in several commercial fatty acids (Firestone, 1973). Daily doses of 10 or 100 µg hexa-CDD/kg bw, or of 1 or 10 µg 2,3,7,8-tetra-CDD/kg bw, produced a positive response in the chick oedema bioassay; 0.5% octa-CDD in the diet had no effect (Schwetz *et al.*, 1973). Similar oedematous effects were observed in rats, pigs, dogs, and monkeys, but not in guinea-pigs (Vos, 1978). Decreased serium albumin may be associated with the oedema; oedema has been also shown to occur in chicks dosed orally with 2,3,7,8-tetra-CDF (McKinney *et al.*, 1976).

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TABLE 4[a]

Evaluation of reported embryotoxic and teratogenic effects induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxins in rats and mice

Species	Strain	Embryotoxic/ teratogenic effect	Dose (µg/kg bw)		Period of dosing (days)	Route	Reference
			lowest tested[b]	~ED_{50}[c]			
Rat		Intestinal haemorrhage	0.125	0.5?		oral	Sparschu *et al.*, 1971
	CD	Kidney abnormality	0.5	>1	6-15	s.c.	Courtney & Moore, 1971
Mouse	CD-1	Cleft palate	1? 3	>3	6-15	s.c.	" " "
		Kidney abnormality	1	1-3	6-15	s.c	" " "
	DBA/2J	Cleft palate	3	>3	6-15	s.c.	" " "
		Kidney abnormality	3	>3	6-15	s.c.	" " "
	C57BL/6J	Cleft palate	3	>3	6-15	s.c.	" " "
		Kidney abnormality	3	<3	6-15	s.c.	" " "
	NMRI	Cleft palate	3	6.5	6-15	oral	Neubert & Dillmann, 1972
			9	<9	9-13	oral	" " "
			15	40	13	oral	Neubert *et al.*, 1973
			5	15	11	oral	" " "

[a] From Neubert *et al.* (1973)

[b] The lowest dose with which an embryotoxic or teratogenic effect detectable at birth has been produced is indicated. Since sometimes only one dose level was tested, this does not necessarily represent the lowest dose from which an effect could result.

[c] ED_{50} - dose required to produce an embryotoxic effect in 50% of animals

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Other effects - In one or more species of laboratory animal, bone marrow hypoplasia, testicular degeneration, renal pelvis and urinary bladder hyperplasia and haemorrhage in the intestines and adrenal have been observed.

Enzyme Induction

2,3,7,8-Tetra-CDD and other halogenated dibenzo-*p*-dioxins and dibenzofurans stimulate a number of enzyme activities, most notably in the liver (IARC, 1977). 2,3,7,8-Tetra-CDD is a potent inducer of hepatic and renal microsomal drug metabolizing enzymes (Beatty & Neal, 1976; Buu-Hoï *et al.*, 1971, 1972b; Fowler *et al.*, 1975; Greig, 1972; Greig & DeMatteis, 1973; Hook *et al.*, 1975a,b; Lucier *et al.*, 1973, 1975a,b; Woods, 1973). Intoxication with 2,3,7,8-tetra-CDD results in a marked increase in the cellular smooth endoplasmic reticulum content of hepatic and renal cells (Fowler *et al.*, 1973, 1975). 2,3,7,8-Tetra-CDD can simultaneously activate and suppress certain microsome-associated foreign-compound and steroid-hormone-metabolising enzyme systems (Hook *et al.*, 1975a) as well as increase the activity of both renal and hepatic glutathione-S transferase (Kirsch *et al.*, 1975).

2,3,7,8-Tetra-CDD is the most active of the PCDDs in inducing hepatic δ-aminolevulinic acid (ALA) synthetase and aryl hydrocarbon hydroxylase (AHH) in chick embryo liver preparations (Poland & Glover, 1973a,b).

The PCDDs that induce ALA synthetase in chick embryo have two common properties: (1) halogen atoms occupy at least 3 of the 4 ring positions (2,3,7,8), and (2) at least one free, non-halogenated carbon atom is unoccupied (Poland & Glover, 1973a). The available toxicological data (Schwetz *et al.*, 1973) indicate that those PCDDs that are lethal at low doses, teratogenic or produce acne also induce ALA synthetase; those PCDDs that are not toxic generally do not induce ALA synthetase. The structure-activity relation of PCDDs to induce AHH in chick embryos was identical to that in inducing ALA synthetase (Poland & Glover, 1973a).

Mixed function oxidase enzyme systems of mouse strains 'non-responsive' to other aromatic hydrocarbons were induced by single doses of 2,3,7,8-tetra-CDD, as evidenced by increases in hepatic monooxygenase activities

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and in concentrations of cytochrome P-448 (Chhabra *et al.*, 1974; Kouri & Nebert, 1977; Nebert *et al.*, 1975; Poland *et al.*, 1974). Genetic resistance to induction of AHH by 3-methylcholanthrene in DBA/2J mice was overcome by treatment with 2,3,7,8-tetra-CDD. This result conflicts with the hypothesis that induction of AHH activity is a consequence of the formation of cytochrome P-448 (Chhabra *et al.*, 1976). A component that has a high binding affinity for 2,3,7,8-tetra-CDD was found in mouse liver cytosol (Poland *et al.*, 1976a).

2,3,7,8-Tetra-CDD induces AHH even in 'poorly responsive' strains of mice (Niwa *et al.*, 1975), not only in liver but also in lung, kidney and colon. The DBA/2N strain, which responds only weakly to the sarcomatogenic action of 3-methylcholanthrene, becomes highly susceptible after treatment with 2,3,7,8-tetra-CDD (Kouri, 1976; Kouri & Nebert, 1977).

2,3,7,8-Tetra-CDD is approximately 30,000 times more potent than 3-methylcholanthrene in inducing AHH activity in rat liver (Poland & Glover, 1974).

McConnell *et al.* (1978a) reported that the comparative toxicity of 13 PCDDs in mice and guinea-pigs (see Table 2) supports the idea that the relative potency (or rank order) of a congener to produce one toxic response is a good indicator of its relative potency (or rank order) to produce other toxic manifestations.

Hepatic Cytosol Binding Protein

A macromolecular binding species has been characterized in the hepatic cytosol fraction of mouse and rat liver which has the *in vitro* binding properties predicted for the receptor for the induction of AHH activity based on the *in vivo* biology (Poland *et al.*, 1976a). Namely: (1) ^{3}H-2,3,7,8-tetra-CDD binds to this cytosol protein reversibly with a high affinity (Kd = 0.27 nM) comparable to the ED_{50} for hepatic AHH induction (ED_{50} in mice = 1 nmole/kg bw); (2) the binding affinity of halogenated dibenzo-*p*-dioxins and dibenzofurans for this protein *in vitro* corresponds to their potency to induce hepatic AHH activity in the chicken embryo; (3) other compounds, such as the polycyclic aromatic hydrocarbons, which induce AHH activity and cytochrome P_1-450 also compete for this cytosolic

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binding protein, but compounds which induce other types of microsomal monooxygenase activities (e.g., phenobarbital) and steroids fail to bind. Thus, this cytosolic binding protein may be the receptor for the induction of AHH activity.

Structure-Activity Relationships

The pathologic effects produced by the toxic PCDD and PCDF isomers are similar to those of 2,3,7,8-tetra-CDD for a given species, differing only in the intensity of the toxic effect produced by a given isomer. The toxic PCDDs have chlorine atoms in at least three of the four lateral ring positions (2,3,7 and 8) with at least one unsubstituted ring position (the octa-CDD is comparatively inactive). To the extent that the toxicity has been determined, a similar structure-toxicity relationship has been observed for the PCDFs.

The structure-activity relation established for PCDDs and PCDFs for the induction of hepatic aryl hydrocarbon hydroxylase (AHH) activity and for binding to the hepatic cytosol binding species has been extended to other classes of chlorinated aromatic compounds. 3,4,3'4'-Tetrachloroazoxybenzene (TCAOB) and 3,4,3',4'-tetrachloroazobenzene (TCAB) are potent acnegens formed as trace contaminants in the synthesis of 3,4-dichloroaniline or herbicides based on this compound (Poland *et al.*, 1976b). At high doses in animals, TCAB is reported to produce thymic involution and liver damage similar to 2,3,7,8-tetra-CDD (Du Pont Inc., 1978). Both TCAOB and TCAB are potent inducers of hepatic AHH activity and bind to the hepatic cytosol binding protein with a high affinity. Congeners such as 3,5,3'5'-tetrachloroazoxybenzene and -azobenzene fail to induce AHH activity, fail to bind to the hepatic cytosol species, and fail to produce chloracne.

Of 16 halogenated biphenyl compounds tested, only 3,4,3'4'-tetrachloro-, 3,4,5,3'4'5'-hexachloro- and 3,4,5,3'4'5'-hexabromo-biphenyls induced hepatic AHH activity and bound to the hepatic cytosol binding species (Poland & Glover, 1977). The 3,4,3'4'-tetrachlorobiphenyl has been reported to produce chloracne. McKinney *et al.* (1976) found that of five hexachlorobiphenyls tested in chickens, 3,4,5,3'4'5'-hexachlorobiphenyl was by far the most toxic, and the only one that induced significant chick oedema and involution of the thymus.

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Pharmacokinetics

In the rat, following acute or chronic administration, 2,3,7,8-tetra-CDD is accumulated primarily in the liver and to a lesser extent in the fat, and largely eliminated unmetabolized in the faeces with a whole body-halflife of about 3 weeks. Some pharmacokinetic experiments suggest the formation of a polar metabolite appearing in the urine, but direct attempts to demonstrate metabolism with hepatic microsomes *in vitro* have been negative (IARC, 1977).

Mutagenicity

Only four dioxin isomers have been evaluated for mutagenicity: the 2,7-di-, 2,3,7,8-tetra, and octa-CDDs as well as the unsubstituted dibenzo-*p*-dioxin (Wassom *et al.*, 1978).

2,3,7,8-Tetra-CDD increased the reversion frequency to streptomycin independence in *Escherichia coli* Sd-4. In *Salmonella typhimurium*, frameshift mutations in strain TA1532, but not base substitutions in strain TA1530, were induced by toxic concentrations of 2,3,7,8-tetra-CDD (Hussain *et al.*, 1972). In plate assays, the response was positive with *S. typhimurium* TA1532, doubtful with TA1531 and TA1534, and negative with G46 and TA1530 (Seiler, 1973). McCann (1978) tested 2,3,7,8-tetra-CDD in *S. typhimurium*, both with and without metabolic activation, using a spot-test and the standard plate test with strains TA1532, TA1535, TA1537 and TA1538; all these tests were negative.

Octa-CDD was non-mutagenic in *S. typhimurium* strains G46, TA1530 and TA1531, and doubtful results were obtained with strains TA1532 and TA1534 (Seiler, 1973). Metabolic activation systems were not included in any of these microbiological assays.

Inhibition of mitosis and chromosomal abnormalities (dicentric bridges and chromatin fusion with formation of multinuclei or a single large nucleus) were observed in endosperm cells of the African blood lily (*Haemanthus Katherinae* Baker) treated with 2,3,7,8-tetra-CDD in the presence or absence of 2,4,5-T (Jackson, 1972).

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No chromosomal aberrations were observed in bone-marrow cells of male rats treated with 2,7-di-CDD, 2,3,7,8-tetra-CDD, or dibenzo-*p*-dioxin by oral intubation, with 2,3,7,8-tetra-CDD by intraperitoneal injection or orally (Green & Moreland, 1975). However, when Osborne Mendel rats of both sexes were treated twice weekly for 13 weeks with 2,3,7,8-tetra-CDD, a significant but weak increase in the number of chromosome aberrations in bone marrow cells was reported (Green *et al.*, 1977).

2,3,7,8-Tetra-CDD did not induce dominant lethal mutations in Wistar rats after oral administration to males for 7 days (Khera & Ruddick, 1973).

Carcinogenicity

Two reports indicate that chronic administration of low levels of 2,3,7,8-tetra-CDD to rats is associated with an increased incidence of neoplasia (IARC, 1977; Kociba *et al.*, 1978; Van Miller *et al.*, 1977).

Groups of 10 male Sprague-Dawley rats were fed a diet containing 2,3,7,8-tetra-CDD for 78 weeks in the following amounts (figures in parentheses are approximate weekly doses): 0, 1 part per trillion (0.0003 µg/kg bw), 5 ppt (0.001), 50 ppt (0.01), 500 ppt (0.1), 1 part per billion (0.4), 5 ppb (2.0), 50 ppb (24), 500 ppb (240), and 1000 ppb (500). The three highest dose levels (50, 500, and 1000 ppb) were toxic and killed all animals by the fourth week. Of the six remaining test groups, the overall incidence of neoplasms was 23/60 (38%); none occurred in the 1 ppt group. In the 5 ppt group, 5/10 animals had 6 neoplasms (ear-duct carcinoma, lymphocytic leukemia, adenocarcinoma, malignant histocytoma (with metastases), angiosarcoma, Leydig-cell adenoma); the following groups also showed neoplasms: 50 ppt, 3 observed in 3/10; 500 ppt, 4 observed in 4/10; 1 ppb, 5 observed in 4/10; 5 ppb, 10 observed in 7/10. Neoplasms were not observed in the controls (Van Miller *et al.*, 1977).

Groups of 100 Sprague-Dawley rats (50 males and 50 females) received for two years diets containing 0, 22, 210, and 22,000 parts per trillion (ppt), equivalent to 0.0, 0.001, 0.01, and 0.1 µg 2,3,7,8-tetra-CDD/kg bw/day. Continuous ingestion of 0.001 µg/kg bw/day did not cause any chemically-related changes in tumour incidence or toxicity; feeding with 0.01 µg/kg

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bw/day induced an increased incidence ($P<0.05$) of hepatocellular hyperplastic nodules (female: 18/50 *versus* 8/86), of focal alveolar hyperplasia in the lungs, and of urinary excretion of porphyrins (female). Dietary intake of 0.1 µg/kg bw/day caused an increased incidence ($P<0.05$) of hepatocellular carcinomas (female: 11/49 *versus* 1/86) and squamous-cell carcinomas of the lung (female: 7/49 *versus* 0/86), of the hard palate/nasal turbinates (male: 4/50 *versus* 0/85; female: 4/49 *versus* 0/86), and of the tongue (male: 3/50 *versus* 0/85). Further increased were adenoma of the adrenal cortex (male) and hepatocellular hyperplastic nodules (female). At this dose, certain age-related lesions were reduced (males: acinar adenoma of the pancreas; females: granulosal cell neoplasm of the ovary, benign and malignant tumours of the mammary gland, pituitary adenoma, and benign tumours of the uterus). Also chronic administration of 2,3,7,8-tetra-CDD caused multiple toxicologic effects, including increased mortality, decreased body weight gain, slight depression of certain hematologic parameters, increased urinary excretion of porphyrins and delta-aminolevulinic acid, increased serum levels of alkaline phosphatase, glutamyl transferase and serum glutamic pyruvic transaminase, and morphologic changes primarily of the hepatic, lymphoid, respiratory and vascular tissues of the body (Kociba *et al.*, 1978).

These two reports show that chronic administration of 2,3,7,8-tetra-CDD causes an increased incidence of neoplasms, but not whether 2,3,7,8-tetra-CDD acts as an initiator or promoter. This consideration is particularly important because unequivocal evidence is lacking that 2,3,7,8-tetra-CDD is a mutagen or is metabolized, and no evidence is available that 2,3,7,8-tetra-CDD and/or metabolite(s) bind covalently to macromolecules.

As summarized in Table 5, at least 24 long-term carcinogenicity studies using mice and rats are currently in progress (IARC, 1978b).

Humans

Toxic effects in humans - Toxicity due to 2,3,7,8-tetra-CDD has been reported after (i) occupational exposure during the industrial synthesis of 2,4,5-trichlorophenol (TCP) and 2,4,5-T, (ii) exposure in factories and in

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TABLE 5

Ongoing long-term carcinogenicity testing of chlorinated dibenzo-*p*-dioxins[1]

Compound	Number of studies	Route	Species
Dibenzo-*p*-dioxin	4	Oral (diet)	Mouse, rat
		Skin	Mouse
			Mouse (with DMBA[2])
2,7-di-CDD	4	Oral (diet)	Mouse, rat
		Skin	Mouse
			Mouse (with DMBA)
2,3,7-tri-CDD	3	Oral (gavage)	Mouse, rat
		Skin	Mouse
2,3,7,8-tetra-CDD	5	Oral (diet)	Rat
		Oral (gavage)	Mouse
		Oral (gavage)	Mouse, rat
		Skin	Mouse
1,2,3,6,7,8-hexa-CDD	3	Oral (gavage)	Mouse, rat
		Skin	Mouse
1,2,3,7,8,9-hexa-CDD	2	Oral (gavage)	Mouse
		Skin	Mouse (with 1,2,3, 6,7,8-hexa-CDD)
1,2,3,4,6,7,8,9-octa-CDD	3	Oral (diet)	Mouse, rat
		Skin	Mouse

[1]Data taken from Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity, No. 7 (IARC, 1978b). Details of these studies as well as the authors/institutes are available in this source reference document.

[2]DMBA = dimethylbenzanthracene

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the surrounding environment due to accidents occurring during the synthesis of TCP, and (iii) exposure to herbicides and other materials containing 2,3,7,8-tetra-CDD. Exposed subjects have been found to develop a wide variety of lesions and symptoms (Table 6). For instance, a typical exposure victim experiences a number and a variety of clinical signs and symptoms: e.g., early exposure symptoms may include a burning sensation of the eyes, nose, and throat followed by headache, dizziness, nausea, and vomiting. Some days later, severe itching, redness, swelling of the face, more marked over the eyelids, nose and lips, may develop. Within the initial weeks' post-exposure, inflamed nodules as well as pustules appear on the face, forearms, shoulders, neck, and trunk, leading then to comedones and cysts. After a month or two, acneform eruptions emerge and the skin becomes hyper-pigmented. At about the same time, aching muscles, mainly in the thighs and chest area, become manifest and aggravated on exertion. Insomnia, extreme irritability and loss of libido also occur during this stage.

Other than the consistently found clinical feature of acne, other findings in humans may include: neuromuscular symptoms (weakness and pain with nerve conduction abnormalities), *porphyria cutanea tarda*, hepatic dysfunctions, hyperlipidaemia, cutaneous hyperpigmentation and hirsutism, chronic eye irritation, emotional disorders, and neuropsychiatric syndromes.

Chloracne, one of the most constant and prominent features of 2,3,7,8-tetra-CDD exposure, has been described as a refractory acne characterized by inclusion cysts, comedones and pustules, with eventual scarring of the skin, more frequently originating on the face and sometimes spreading to other parts of the body. Many patients also have blepharoconjunctivitis and irritation of other mucous membranes. Sometimes the chloracne is preceded by erythematous and oedematous skin lesions. The latent period between exposure and the appearance of clear signs of chloracne ranges from a few weeks to several months (May, 1973).

An important and unique episode revolves around three scientists who were self-exposed to 2,3,7,8-tetra-CDD: 'A' heated trichlorophenol in an alkaline solution, 'B' heated potassium trichlorophenate, and 'C' worked in the same laboratory as 'B' and used a diluted solution of the synthesized dioxin (Oliver, 1975). 'A' and 'B' developed chloracne eight weeks after

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TABLE 6

Toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxins in humans

EFFECTS	REFERENCES
Dermatological	
chloracne	Bauer *et al.*, 1961; Bert *et al.*, 1976; Bleiberg *et al.*, 1964; Carter *et al.*, 1975; Dugois *et al.*, 1968; Firestone, 1978; Gianotti, 1977; Goldmann, 1972, 1973; Jensen & Walker, 1972; Jirásek *et al.*, 1973, 1974; Kimmig & Schulz, 1957a; May, 1973; Oliver, 1975; Poland *et al.*, 1971; Suskind, 1951
porphyria cutanea tarda	Bleiberg *et al.*, 1964; Jirásek *et al.*, 1973, 1974, 1976
hyperpigmentation and hirsutism	Bleiberg *et al.*, 1964; Oliver, 1975; Poland *et al.*, 1971
Internal	
liver damage[a]	Bauer *et al.*, 1961; Bleiberg *et al.*, 1964; Dugois *et al.*, 1968; Goldmann, 1972, 1973; Jirásek *et al.*, 1973, 1974; May, 1973; Ton That, 1977
raised serum hepatic enzyme levels	Bert *et al.*, 1976; Bleiberg *et al.*, 1964; Jirásek *et al.*, 1973, 1974; May, 1973; Poland *et al.*, 1971; Ton That, 1977
disorders of fat metabolism	Jirásek *et al.*, 1973; Oliver, 1975; Poland *et al.*, 1971
" " carbohydrate metabolism	Goldmann, 1972, 1973; Jirásek *et al.*, 1973, 1974; Poland *et al.*, 1971
cardiovascular disorders	Goldmann, 1972, 1973; Jirásek *et al.*, 1973, 1974
urinary tract "	Carter *et al.*, 1975; Goldmann, 1972, 1973
respiratory "	Bauer *et al.*, 1961; Goldmann, 1972, 1973
pancreatic "	Goldmann, 1972, 1973
Neurological	
polyneuropathies (peripheral neuritis)	Goldmann, 1972, 1973; Jirásek *et al.*, 1973, 1974
lower extremity weakness	Bauer *et al.*, 1961; Firestone, 1978; Goldmann, 1972, 1973; Jirásek *et al.*, 1973, 1974; Oliver, 1975; Poland *et al.*, 1971
sensorial impairments (sight, hearing, smell, taste)	Goldmann, 1972, 1973; Oliver, 1975; Poland *et al.*, 1971; Ton That, 1977
Psychiatric	
neurasthenic or depressive syndromes	Bauer *et al.*, 1961; Firestone, 1978; Goldmann, 1972, 1973; Jirásek *et al.*, 1973, 1974; Oliver, 1975; Poland *et al.*, 1971

[a]Mild fibrosis, fatty changes, haemofuscin deposition and parenchymal-cell degeneration were observed in a few cases.

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exposure, whereas 'C' showed no evidence of the characteristic acneform lesions. Delayed symptoms, probably due to 2,3,7,8-tetra-CDD, developed approximately two years later and 'B' and 'C' showed: personality changes (mainly loss of energy and drive); impairment of vision, taste and muscular coordination; sleep disturbances; gastrointestinal symptoms; and hirsutism. 'A' experienced none of these adverse effects. All three exhibited hypercholesterolaemia (>300 mg/100 ml).

Human exposure to PCDDs and PCDFs - Major sources of human exposure to PCDDs and PCDFs include:

- exposure in the workplace;
- exposure in factories and in the surrounding environment from industrial accidents;
- exposure to contaminated materials, wastes, or food in the general environment; and
- exposure in Vietnam and other intensive herbicide spraying operations. (Prior and subsequent to use as a defoliant, 2,4,5-T was used in weed-killing and forest-thinning operations in the United States of America and elsewhere).

Occupational exposure may occur:

- in manufacturing plants producing chlorinated phenols (tri-, tetra-, and pentachlorophenols), or phenoxy acid herbicides (2,4-D, 2,4,5-T), or PCBs;
- in factories utilising these chemicals for the production of other substances (hexachlorophene from 2,4,5-trichlorophenol);
- in factories manufacturing or repairing transformers and capacitors or having heat exchange or heat hydraulic systems containing PCBs; and
- in the use processes of these chemicals under various occupational conditions such as spraying of herbicides, using chlorinated phenols for a variety of applications (especially wood preservative), sawing or otherwise processing treated wood, and using hexachlorophene in sanitary occupations.

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The burning of materials impregnated with commercial 2,3,4,6-tetrachlorophenates yielded 150-1000 µg of mixed PCDDs/g chlorophenate. Although only found as a minor constituent, 2,3,7,8-tetra-CDD has been quantified at levels exceeding 10 µg/g chlorophenate (Rappe *et al.*, 1978b).

Pyrolysis of a technical grade PCBs-mixture yielded many PCDF isomers; the total yield could be as high as 3-25%. One of the main constituents is 2,3,7,8-tetra-CDF, the most toxic PCDF-isomer. Consequently, uncontrolled burning of PCBs can be an important environmental source of the hazardous PCDFs, and operations such as welding or soldering electrical equipment containing PCBs, or using casting waxes in foundries, may possibly entail a significant exposure (Buser *et al.*, 1978a). A PCB used in a heat exchange system for two years contained approximately 1.25 ppm of 2,3,7,8-tetra-CDF and a total of 15 ppm of PCDFs (Buser *et al.*, 1978c).

Apart from accidents such as the one which occurred in Seveso in 1976, general environmental exposure may originate from herbicide spraying and waste disposal.

An outbreak of PCDDs poisoning in humans, horses, and other animals occurred in Missouri in 1971 (Beale *et al.*, 1977; Carter *et al.*, 1975) following the spraying of contaminated oil for dust control in horse arenas.

Another possible source of exposure to PCDDs and PCDFs pollution are waste oils, and possibly other waste, when burned both in industrial and municipal incinerators. Under simulated environmental conditions, the combustion of a standard 2,4,5-T formulation led to formation of small amounts of PCDDs and PCDFs (Ahling *et al.*, 1977). Recently, Buser & Bosshardt (1978) quantified that the total amount of PCDDs and PCDFs in fly ash from an industrial incinerator heating facility was 0.2 ppm and 0.1 ppm, and in the fly ash from an industrial heating facility in Switzerland was 0.6 ppm and 0.3 ppm. More than 30 individual PCDDs can be identified in the fly ash, but the known highly toxic PCDD isomers are only minor constituents (Buser *et al.*, 1978b). The number of PCDF isomers was larger, but, in this case, the known highly toxic isomers are major constituents (Buser *et al.*, 1978d).

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An additional potential source of human exposure has been revealed recently. Beef fat taken from cattle grazed on 2,4,5-T-treated rangeland contained 2,3,7,8-tetra-CDD; of the 11/14 positive samples, the four with the highest levels had 12, 20, 24, and 70 ppt (Meselson *et al.*, 1978). In another study, 3/24 samples of beef fat contained 3-4 ppt 2,3,7,8-tetra-CDD (Kocher *et al.*, 1978). Moreover, in a preliminary report of an ongoing enlarged study of women living in areas where 2,4,5-T is used on rangeland, 4/18 breast milk samples contained approximately 1 ppt each (Meselson *et al.*, 1978).

Workers in wood processing industries are exposed to wood dust containing preservatives as well as accompanying impurities and degradation products. Wood dust from a saw-mill in which a 2,3,4,6-tetrachlorophenol formulation was used as a fungicide was found to contain 1-10 ppm PCDFs and <0.5 ppm PCDDs (Levin *et al.*, 1977).

In 1968, more than 1200 persons in South-west Japan were intoxicated by consuming a commercial rice oil contaminated with 1000 ppm PCBs. Nagayama *et al.* (1976) analyzed the rice oil (Yusho oil) and found 5 ppm PCDFs, the major constituents of which were tetra- and penta-CDFs. Buser *et al.* (1978c) have recently shown that 2,3,7,8-tetra-CDF was the main PCDF in the Yusho oil (0.45 ppm). The high level of PCDFs was caused by leakage from heat exchangers containing PCBs contaminated with PCDFs.

Workers manufacturing 2,4,5-trichlorophenol(TCP) or 2,4,5-T during normal production operations and/or following explosions taking place in these plants, may have been exposed to a variety of polychlorinated chemicals whose type and quantity depends on the particular chemical processes in use and on the phase of the reaction in which the accident took place. Apparently, for instance, PCDFs are produced in the earlier stages when mainly tetrachlorobenzene is present and PCDDs are produced nearer the end of the reaction when primarily trichlorophenol is extant. The concentration of chlorophenols and chlorobenzenes, for instance, was probably higher in the 1963 episode in The Netherlands, where the accident took place at the beginning of the reaction, than in the 1968 episode in the United Kingdom, or the 1976 accident in Italy, where the explosion took place at the end.

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A thorough description of the reported industrial accidents and other cases of intoxications observed in exposed workers is given in IARC Monographs' Volume 15 (IARC, 1977). In this present report, only those episodes specifically discussed at length by the NIEHS/IARC *ad hoc* Working Group will be considered. A sequential perspective of the known accidents is given here below (see also Table 7, pages 35-37).

In 1949, the first reported cases of industrial poisoning due to the formation of 2,3,7,8-tetra-CDD in uncontrolled exothermic reactions occurred during the manufacture of 2,4,5-trichlorophenol at a 2,4,5-T-producing factory in Nitro, West Virginia; 288 persons were affected (see Firestone, 1978; Suskind, 1978). In November 1953, an accident occurred in Ludwigshafen, Federal Republic of Germany, during the manufacture of TCP (Goldman, 1972, 1973; Hofmann, 1957; Thiess & Goldman, 1978); 53 workers were affected by chloracne.

Five cases of chloracne were reported following an industrial accident in an Italian TCP-producing factory (Hofmann & Meneghini, 1962). In 1963, an accident occurred at the 2,4,5-T-producing factory in The Netherlands; approximately 50 persons were affected by chloracne (Dalderup, 1974; Hay, 1976). In 1966, Dugois *et al.* (1968) observed 21 cases of chloracne after an accident in a French factory producing TCP in the Grenoble region. In 1968, an accident occurred at the TCP-producing factory at Bolsover, Derbyshire, United Kingdom (Jensen & Walker, 1972; May, 1973). Within the next 7 months, 79 workers developed chloracne. In 1971, 2,3,7,8-tetra-CDD contaminated waste oil caused an outbreak of poisoning in humans, horses and other animals (Carter *et al.*, 1975; Kimbrough *et al.*, 1977). In July 1976, an accident at the TCP-producing factory in Meda, Italy (Bert *et al.*, 1976) resulted in the contamination of a large, densely populated area, including the towns of Seveso, Meda, Cesano Maderno and Desio.

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Key information concerning the effects of human exposure to herbicides in Vietnam, especially to the so-called "Agent Orange"[1] (a 50:50 mixture of the *n*-butyl esters of 2,4-D and 2,4,5-T, containing up to 30 mg/kg or more 2,3,7,8-tetra-CDD), may be found in, among others, the following references: Committee on the Adverse Effects of Herbicides in Vietnam, 1974; IARC, 1977; Meselson *et al.*, 1972; Ton That, 1977; Westing, 1978.

IV. EXPOSURE EPISODES CONSIDERED IN DETAIL BY THE NIEHS/IARC *ad hoc* WORKING GROUP

The text below is a condensed version of the discussions which took place during the two-day NIEHS/IARC *ad hoc* Working Group Meeting:

Phenoxyacids exposure

Cohort studies on herbicide sprayers have been conducted or are being planned in Scandinavian countries. The more advanced of these concerns Swedish railroad workers with exposure to a variety of herbicides. These people exhibited a significantly increased tumour incidence (apparently dose-dependent) and tumour mortality (Axelson & Sundell, 1974). The excess of tumours was found particularly among workers with exposure to amitrole (aminotriazole) whereas those exposed to phenoxyacids (2,4-D and 2,4,5-T) showed only a slightly increased excess of cancers. The study was small-sized, comprising 2978 person-years at observation in the total cohort and with 18 deaths *versus* 20.54 expected. The original conclusion from this study was that amitrole exposure may have caused an excess of tumours, whereas there was probably no pertinent increase in tumour incidence associated with exposure to phenoxyacids. The study has been recently reanalysed using a case-control approach, and through stratification on amitrole when considering the effect from phenoxyacids, and *vice versa*. The results show a possible and previously masked tumour-inducing effect also from phenoxyacids.

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[1]More than 2,000,000 gallons of Agent Orange remaining from military defoliant operations were destroyed by incineration on board ship in the Pacific Ocean, 120 miles from Johnston Island. Temperatures not lower than 1250°C were used. The steel containers are to be melted (Walsh, 1977).

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Another retrospective cohort mortality study, conducted in Finland on workers of five companies involved in spraying 2,4-D and 2,4,5-T on brushwood, did not show any increase in mortality. During 1955-1971, in the younger group of workers (under 45 years of age), however, 4 cancer deaths were observed *versus* less than 2 expected (no statistically significant difference). A prospective follow-up study for the period 1972-1976 revealed fewer cancer-related deaths than expected in all age groups. Clinical and anamnestic investigations were also performed showing a picture of acute complaints during and following the spraying operations: headache, transient dizziness, fatigue, abdominal complaints, skin and mucous irritations and a few cases of persistant papulae.

It is interesting to note that some samples of 2,4,5-T used in Finland for spraying operations contained from 0.04 to 0.07 ppm 2,3,7,8-tetra-CDD. This may imply that the 2,4,5-T produced before 1965 contained lower levels of 2,3,7,8-tetra-CDD impurities than has been observed subsequently from 2,4,5-T used elsewhere. However, the analytical methods used in the determination of 2,3,7,8-tetra-CDD in the Finnish samples were antiquated in view of the rapid advancements made in analytical methodology and thus a confirmatory analysis of the samples using modern techniques was made by Rappe *et al.* (1978a). Five samples of 2,4,5-T ester dating from 1962 to 1967 were analysed for PCDDs and PCDFs; levels of 0.1-0.95 ppm 2,3,7,8-tetra-CDD and 0.1-0.15 ppm tetra-CDF were found (Rappe *et al.*, 1978a).

A second feasibility study was designed and conducted in Finland to determine whether one could obtain anamnestic, clinical, haematological, and immunological information on Finnish railway and forestry workers who had been exposed for several years to herbicides containing 2,3,7,8-tetra-CDD. The exposed group consisted of 30 men who were control-matched to persons of the same age coming from the same district with similar job backgrounds and living conditions. Of the tests made, including liver and immuno-function studies, no differences were observed between the exposed and control groups. Although the population study is too small to make any general conclusions as to the potential adverse health effects from long-term exposure to phenoxyacids, the investigation shows that follow-up is possible

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on the health status of persons exposed for several years to 2,3,7,8-tetra-CDD-containing phenoxyacids, and then to compare the exposed group results to carefully matched controls. The study is in progress.

Hexachlorophene exposure

An excess of malformations, some severe, has been reported among children whose mothers were employed as nurses in a hospital. The mothers were exposed to hexachlorophene soap during early pregnancy, and the hypothesis of a causal-relation between such an exposure and the occurrence of the malformations has been advanced (Halling, 1977a); five severe and six slight malformations were observed in 65 children in the exposed group, whereas only one slight malformation was observed in the 68 children of the unexposed group. This report has been followed by another study (to be published) of a similar group of children of exposed mothers in comparison to children of unexposed mothers. Again a high frequency of malformations among the offspring of exposed mothers was reported (Halling, 1977b) From these studies, however, it is not clear if exposure to other hazardous chemicals could be excluded. A retrospective-prospective study is being initiated among long-term workers of an United States' factory which used Seveso-produced 2,4,5-trichlorophenol in the manufacture of hexachlorophene. However, in the case of hexachlorophene, polychlorinated xanthenes have been also identified as contaminants at a higher level than 2,3,7,8-tetra-CDD (Baughman, 1974).

Phenoxyacids and chlorophenols exposure in forestry workers and in wood industry

At the Regional Hospital in Umea (Northern Sweden), Hardell (1977) observed that several patients suffering from mesenchymal tumours reported occupational or other exposures to phenoxyacids. More specifically, 87 mesenchymal tumours were diagnosed during the years 1970 through 1976. Of these cases, 55 were men (more than expected) and 43 of these 55 men were known by profession: 19 were either forestry workers, farmers and forestry workers, or workers in saw-mills and paper-mills where exposure to chlorophenols is common. Based on the official statistics of Sweden, one can calculate approximatively the expected fraction of tumours within these trades, resulting in an expectancy of approximately 11 cases *versus* the 19 observed.

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The Mount Sinai School of Medicine in New York is currently planning a field survey on long-term health effects on wood-preservative workers in Arkansas and Oklahoma in the United States of America.

2,3,7,8-Tetra-CDD exposure during 2,4,5-T or polychlorinated phenols production and following industrial accidents

The following cases, as well as others that have not been considered at the meeting, have been described in the IARC Monographs' Volume 15 (IARC, 1977) and only some of the previously available information will be given here. The ongoing epidemiological studies on these cohorts are summarized in Table 7.

As each individual cohort has a relatively small number of person-years, a study has been proposed in which all the cohorts would be pooled and the mortality compared to that expected from the national statistics.

The first reported cases of industrial poisoning due to the formation of 2,3,7,8-tetra-CDD in uncontrolled exothermic reactions occurring during the manufacture of TCP were seen in 1949 at a 2,4,5-T-producing factory in Nitro, West Virginia, USA; 228 persons were affected. These individuals were studied by Suskind & Ashe (1978) and followed-up for a period of four years. Symptoms included chloracne, nausea, vomiting, headaches, severe muscular aches and pain, fatigue, emotional instability and intolerance to cold. Laboratory findings showed raised total lipids and raised prothrombin time. Among those affected were not only workmen, but also laboratory personnel, medical personnel, and even the Safety Director who visited the areas of exposure. Several wives who had never visited the plant also developed acne, usually at the same time as their employee husbands.

It is important to note that information on the toxicity as well as on the stability (long persistence and slow degradability) of 2,3,7,8-tetra-CDD was not available at the time the first reports of acute toxicity were observed, and no measures were taken to decontaminate the factories and control the residue levels of 2,3,7,8-tetra-CDD. In fact, the presence of 2,3,7,8-tetra-CDD or other PCDDs and PCDFs was not even suspected. Workers, therefore, were probably exposed for a considerable time to 2,3,7,8-tetra-CDD following the first observations of acute toxic effects.

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TABLE 7

Epidemiological studies on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Human exposure episodes caused by accidental or occupational exposure occurring in factories producing 2,4,5-trichlorophenol and/or 2,4,5-trichlorophenoxyacetic acid

Town and Country	Year of accidental or occupational exposure	Number of exposed persons	Clinical follow-up and cohort studies	Observations todate	Proposed actions	Comments and recommendations of the NIEHS/IARC *ad hoc* Working Group
(a) Accidental exposures[1]						
Nitro, West Virginia, United States of America	1949	228	Only 36 individuals were followed, for a period of 4 years.	No significant long-term observations are yet available (some followed-up individuals had symptoms of severe intercostal neuritis).	A mortality cohort study is being planned.	Efforts should be made to trace the individuals who were exposed, possibly through the employment compensation files.
Ludwigshafen/Rhein, Federal Republic of Germany	1953	75	A mortality cohort study has been conducted 24 years after the accident; all exposed workers were traced for a total of 1525 person-years of observation.	6 Cancer deaths were observed : 3 of stomach cancer in the age group 60-69 (significantly higher than expected), 2 of oat-cell carcinoma of the lung and 1 of colonic adenocarcinoma	To continue the follow-up.	The results of the continuing follow-up study should periodically be made available.
Amsterdam, The Netherlands	1963	106	Preliminary observations on mortality are available; 93 exposed workers have been traced up to 1977.	Of 8 deaths, 5 or 6 were from cardiovascular diseases - 5 probably from myocardinal infarctions; the myocardial infarction death rate seems higher in the most heavily exposed workers affected by chloracne. One worker, who did not develop chloracne, died from pancreatic carcinoma 14 months after the accident; however, he complained of abdominal pain prior to the accident.	A full report is planned for 1978	Efforts should be made to trace all the workers who were exposed.

[1] More details about these accidents are given in the text, starting on page 34, and in IARC (1977).

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Table 7 - continued

Bolsover, Derbyshire, United Kingdom	1968	90	Of the workers belonging to the original cohort, 50% are still employed and routinely undergo clinical and laboratory investigations.	One workers is known to have died from coronary thrombosis.	An epidemiological cohort study will be planned when the results of the present clinical follow-up investigation become available.	Efforts should be made to trace all the workers who were exposed.
Meda, Brianza di Seveso, Italy	1976	?[2]	A clinical follow-up of the inhabitants from the more contaminated areas is being carried out. At the time of the accident, living in Zone A was a population of 730 people, with 200 of those being between 0-14 years of age. From these 730 persons, 623 (125 between 0-14 years of age) were followed-up. In Zone B lived a population of 4732 people, with 1293 between the ages 0-14 years. An accurate count of those followed in Zone B is not available, but 943 youngsters between 0-14 years of age are included in the follow-up.	The first clinical observations showed a high incidence of 2,3,7,8-tetra-CDD-dependent skin lesions among children living in the more contaminated area. Also, a number of persons residing in the zone surrounding the more contaminated areas had skin lesions, but with poorly defined clinical features; these lesions may be the result of 2,3,7,8-tetra-CDD exposure. Moreover, both clinical and laboratory findings suggest an increased incidence of hepatic sufference among adults living in the highly contaminated areas. A preliminary mortality study suggests that although the overall rates from 2 Seveso towns are not different from a nearby area, an increase of deaths from liver cirrhosis and leukemia was noted.	Among other ongoing investigations, a long-term morbidity study has been initiated.	An investigation should be made of the possible teratogenic and other adverse reproductive and developmental effects from 2,3,7,8-tetra-CDD exposure. A mortality register and population files, suitable for adequate descriptions of morbidity data, should be established. Further, the cancer registry that already covers a nearby province, should be extended to include the Seveso area.

[2]No reliable information was available to the Working Group about the actual number of exposed persons; the entire area has a population of approximately 200,000 people.

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Table 7 - continued

(b) Occupational exposure						
Czechoslovakia	1965-1969	80	A follow-up has been made regularly on 55 workers of the original cohort. The workers lost to follow-up are mainly foreigners who left the country.	Five patients died during the first 5 year-follow-up period: 2 died from bronchogenic carcinoma and 1 from a rapidly developing atypical atherosclerosis. One further death, probably from liver cirrhosis, has recently occurred. Many of the workers are now suffering from hypertension and show signs of hyperlipidaemia and hypercholesterolaemia as well as prediabetic symptoms and signs.	The follow-up will continue. A 10-year report will be prepared during 1978.	The results of the continuing follow-up study should periodically be made available.

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In November 1953, an accident occurred at a TCP factory in Ludwigshafen, Federal Republic of Germany; 75 workers were exposed to the reaction products during the accident and the subsequent cleaning work; most were affected by chloracne, 42 severely: 21 of the 42 suffered consequent damage to internal organs or disturbances of the nervous system. The most relevant features were polyneuritis, sensorial impairments and liver damage. The son of one of the workers developed chloracne following contact with his father's workclothes. An additional case of poisoning occurred 5 years later in a worker who was involved in repair work (welding one of the autoclaves) at the contaminated site; subsequently this worker died with necrotic pancreatitis.

All the 75 workers could be traced in a cohort study 25 years later; mortality was compared both with the regional and national mortality and with a control group of workers from the same factory. In the exposed group, 17 deaths were observed (11 to 25 expected depending on the control population); 6 from cancer (4 or less expected), 5 from cardiovascular diseases (as expected), 2 from suicides (0.2-0.6 expected), 1 from liver cirrhosis, 1 from a urogenital tract disease, and 2 from traffic accidents.

Of the 6 cancer deaths, 3 were from stomach cancer and occurred in the age group 60-69, which is significantly more than expected. This effect was more pronounced when a minimum observation period of 10 years was considered, and more so for stomach cancer after a 20-year observation period. (Use of a long observation period permits a better estimation of the relation between exposure and disease when long "latency" periods are involved, as for cancers).

A similar accident occurred at the 2,4,5-T-producing factory in The Netherlands in 1963; 106 men are likely to have been exposed to 2,3,7,8-tetra-CDD (and possibly PCDFs) as they were in the building where the accident occurred during the subsequent period, March-July 1963. These workers may be divided into three groups:

A: factory employees (exposed during all phases of the cleaning and reconstruction activities) - 44 men;

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B: clean-up crew (non-factory personnel, exposed during the months May-July) - 18 men; and

C: plumbing, painting and insulating personnel (non-factory workers, exposed during the months May-July) - 44 men

During the explosion in Amsterdam, 2 operators were present in the building. With the exception of these men, who were probably exposed primarily by the inhalation route, all other workers used safety masks and were probably exposed by dermal contact. Chloracne was the most common and prominent lesion observed following 2,3,7,8-tetra-CDD exposure (26/44 examined in the A, 10/16 in the B, and 8/16 in the C groups). Liver function tests (thymol turbidity and serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase) did not indicate liver damage. A few men complained of fatigue. The latent period between exposure and the appearance of chloracne, characterized by comedones, pustules and cysts on the face and sometimes on other parts of the body, was approximately 4-6 weeks. Sometimes, erythematous and oedematous skin lesions, possibly due to phenolic compounds, were noted one day after exposure. No skin lesions (chloracne) were reported in family members of the employees. Of the A group, 3/44 workers have died, 1 of pancreas carcinoma in 1964, 1 of myocardial infarction at the age of 69, and 1 of a traffic accident. Of the 17/18 traced individuals in the B group, 4 have died (3 from sudden death, probably of myocardial infarction, at ages 41, 53, and 65, and 1 of ill-defined pulmonary or cardiac cause at age 44). Only 32/44 of the C group were traced and 1 of them died of sudden death, probably myocardial infarction, at age 50.

Jirásek *et al.* (1973, 1974, 1976) studied 55 subjects of a cohort of 78 workers from a factory in Czechoslovakia producing 2,4,5-T and pentachlorophenol, who were affected by chloracne. The workers were probably exposed during the years 1965-1968 to unknown levels of 2,3,7,8-tetra-CDD. A significant symptom was the disturbance in porphyrins metabolism with signs of *porphyria cutanea tarda*. Many workers also suffered severe hepatic and neurological damage and had raised blood levels of cholesterol and total lipids. In the first years of follow-up, 5 deaths were observed, 2 from

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bronchogenic carcinoma at age 47 and 59 (only 0.12 lung cancer deaths were expected from national mortality statistics), 1 from atypical atherosclerosis at age 57, and 1 from the acute occupational intoxication. One further death, probably from liver cirrhosis, has recently been observed and 50 workers are still being followed. Many workers continue to show lipaemia and hypercholesterolaemia and have developed prediabetic changes and hypertension. The workers lost to follow-up are mostly foreigners who returned to their country of origin.

In July 1976, an accident in a TCP-producing factory in Meda, Italy resulted in the contamination of the towns of Seveso, Meda, Cesano Maderno, Desio, Nova Milanese and possibly others of a large, densely populated area called the Brianza di Seveso with a total population of about 220,000 inhabitants. A retrospective health survey conducted on the workers of the ICMESA factory revealed that two workers among those directly involved in the production of 2,4,5-trichlorophenol suffered from skin lesions previous to July 1976. These facts may be indicative of a previous 2,3,7,8-tetra-CDD exposure.

A large and intensive follow-up programme is in progress in the Seveso area; data were presented about the first dermatological, neurological, clinical and laboratory findings, as well as about foetal damage. Dermatological examinations, including the screening of 32,000 children, resulted in the finding of hundreds of children with skin lesions, 135 of which were eventually identified as chloracne; 59 cases were from the more contaminated area (A zone). However, precise description of diagnostic criteria and accurate anamnestic information, including, among others, data on places of residence, were not included. This information is needed before evaluating the meaning of the chloracne incidence data by territorial areas and efforts should be made obtain it. Chloracne cases were not systematically searched for in adults. Neurological examinations showed both signs of subclinical neurological damage and cases of clinically detectable polyneuropathy in adults (34/338 persons from A zone and 8/185 from the surrounding areas).

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In about 30% of 1654 adults from both A zone and nearby zones, hepatomegaly was reported to be present on clinical investigation. No information, however, is given on the criteria by which the hepatomegaly was evaluated. The same percentage of abnormalities was observed in one or more liver tests (mainly glutamyl transferase and serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase). Extensive monitoring of various haematological parameters from 25,000 persons is now in progress and the data are expected to become available during 1978. Results of this investigation should allow a better understanding as to whether or not these alterations are caused by exposure to 2,3,7,8-tetra-CDD and PCDDs.

So far, immunological investigations, cytogenetic research, and embryomorphology analysis on cases of therapeutical abortions have not given abnormal results. However, the study of the frequency of congenital malformations and spontaneous abortions seems to be very difficult to accomplish. The importance of a systematic study on this subject has been stressed by the Working Group, because the questions on the teratogenicity of 2,3,7,8-tetra-CDD in humans cannot be solved by any other study.

A preliminary mortality study has been possible in 2 of the 11 towns of the Seveso area. The 1975-1977 mortality rates from liver cirrhosis and leukemia in these two limited zones of the Seveso area were compared to that of a nearby area (province of Varese). Within an overall mortality which did not appear different, an increase of deaths from liver cirrhosis and from leukemia was noted. The validity and significance of these observations should be carefully evaluated.

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V. RECOMMENDATIONS

A chemical analytical programme should be encouraged to identify and standardize analytical methods used, to catalogue products contaminated with PCDDs and PCDFs, and to establish a register of known PCDD and PCDF standard samples.

The Working Group unanimously recommended that a system be developed for an international exchange of information and research coordination on the health effects of chlorinated dibenzo-*p*-dioxins/chlorinated dibenzofurans. Further, and in particular, the following three areas were indicated as deserving special attention:

1. Development of sensitive and reliable measurements of body burden; improved, more sensitive, and less expensive analytical technology should be generated. Standardized sampling (air, water, soil, tissues and body fluids, environmental), preparation, clean-up, and analysis should be promoted;
2. Development of common protocols for clinical examinations (including reproductive experience) and, in particular, design of a protocol for basic core information; and
3. Development of an international registry of exposed persons to serve as a basis for long-term follow-up: this was considered especially important because the relatively small size of populations involved in individual exposure episodes are an obstacle to risk assessment and thus pooling of data is almost a necessity.

The Working Group recommended that IARC explore the technical and financial feasibility, possibly in cooperation with other national and/or international organizations, of setting-up such a permanent mechanism which could include as a minimum starting programme:

a) a periodical, perhaps biennial, meeting to up-date the progress of ongoing studies, and to identify needed developments;

b) an exchange of relevant information accruing during the interval between meetings; and

c) a register of chloracne cases, containing simple, essential information in a standardized form. This PCDDs/PCDFs exposed-related sign may help to better define an exposed cohort population suitable for long-term follow-up.

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Part II - Working papers
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Arstila, A.U. & Sorvari, T.E., An epidemiological feasibility study on the effects of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) on Finnish forestry, road and railway workers: preliminary findings.

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TOXICOLOGY AND APPLIED PHARMACOLOGY 36, 491–501 (1976)

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The Dose-Dependent Pharmacokinetic Profile of 2,4,5-Trichlorophenoxy Acetic Acid Following Intravenous Administration to Rats

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The Dose-Dependent Pharmacokinetic Profile of 2,4,5-Trichlorophenoxy Acetic Acid Following Intravenous Administration to Rats. SAUERHOFF, M. W., BLAU, G. E., BRAUN, W. H., AND GEHRING, P. J. (1975). *Toxicol. Appl. Pharmacol.* 36, 491–501. The clearance of 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) from plasma and its elimination from the body of rats were determined after single intravenous doses of [^{14}C]2,4,5-T. Dose-dependent pharmacokinetics were found. Urinary excretion of unchanged 2,4,5-T accounted for approx. 95% of the ^{14}C activity excreted from the body. The results show that the distribution and elimination of 2,4,5-T are markedly altered when larger doses were administered. The pharmacokinetic data indicate that the statistical projection of results of experiments with large doses of 2,4,5-T, to predict the hazard of exposure to small amounts, is not justified because the capability of the body to handle the compound has been altered.

2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) is a plant growth regulator and herbicide. The absorption, metabolism, and excretion of 2,4,5-T have been studied in man and experimental animals. Five human male volunteers ingested a single oral dose of 5 mg/kg without presenting detectable clinical effects (Gehring *et al.*, 1973). The clearance of 2,4,5-T from the plasma as well as excretion from the body occurred by an apparent first-order rate process with a half-life of 23 hr. Essentially all of the 2,4,5-T was absorbed into the body and excreted unchanged in urine. Erne (1966) demonstrated that orally administered salts of both 2,4,5-T and 2,4-dichlorophenoxyacetic acid (2,4-D) were readily absorbed in rats and eliminated primarily by the kidneys. The half-life of clearance from plasma of both 100 mg/kg of 2,4,5-T and 50–200 mg/kg of 2,4-D was approx 3 hr. The elimination half-life of 2,4-D from tissues ranged from 5 to 10 hr. Grunow *et al.* (1971) reported that 7 days after oral administration of 50 mg/kg of 2,4-5-T to rats, only 69% of the dose was recovered, all in the urine. The main constituent in urine was unchanged 2,4,5-T. Approximately 15–30% of the dose recovered in urine was found as conjugates of 2,4,5-T.

Courtney (1970) found approx 90% of orally administered 2,4,5-T (50–100 mg/kg) unchanged in the urine of rats within 72 hr. Piper *et al.* (1973) studied the clearance of 2,4,5-T from plasma and its elimination from the body of rats after single oral doses of [^{14}C]2,4,5-T. The half-life values for the clearance of 2,4,5-T from plasma of rats given

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doses of 5, 50, 100, and 200 mg/kg were 4.7, 4.2, 19.4, and 25.2 hr, respectively. Half-life values for elimination from the body were 13.6, 13.1, 19.3, and 28.9 hr, respectively. These results establish dose-dependent pharmacokinetics. In interpreting the data of Piper *et al.* (1973) it is not possible to state whether dose-dependence is attributable to saturation of some excretory mechanism or to absorption.

To resolve questions on absorption and subsequent eliminaton of 2,4,5-T additional pharmacokinetic studies were conducted to determine the rate of 2,4,5-T clearance from plasma, the volume of distribution, metabolism, and routes and rates of 2,4,5-T elimination from rats following a single iv dose of either 5 or 100 mg/kg of [^{14}C]2,4,5-T.

METHODS

Animals. Sprague–Dawley (Spartan substrain) rats weighing 180–220 g were housed in metal metabolism cages designed for the separate collection of urine and feces. The rats were acclimated in their individual metabolism cages for 4 days prior to dosing. Food and water were available *ad libitum* to all rats throughout the acclimation and experimental periods. The cages were maintained in an air-conditioned room at a temperature of 24°C with a 12-hr light–dark cycle. Jugular cannulas were surgically implanted in all rats while anesthetized with methoxyflurane 72 hr prior to administration of the dose (Harms and Ojeda, 1974).

Dosage solution. [Ring-U-^{14}C]2,4,5-T (4.3 mCi/mmol, Mallinckrodt Chemical) was administered to rats as the sodium salt in an aqueous solution. A stoichiometric equivalent of NaOH was added and mixed with the 5- and 100-mg/kg dosing solutions, converting the free acid of 2,4,5-T to the soluble sodium salt. The two dosing solutions were prepared equimolar with respect to sodium by adding NaCl to the 5-mg/kg dosing solution.

The infrared spectrum of the sodium 2,4,5-trichlorophenoxyacetate (Na-2,4,5-T) obtained as a NUJOL mull was identical in all essential details to the spectrum of Dow Analytical Standard Na-2,4,5-T. An excess of HCl was added to an aliquot of the dosing solution and an additional infrared spectrum of the recovered free acid was obtained. This was compared to the spectrum of Dow Analytical Standard 2,4,5-T. The spectra of these materials were identical in all essential details. Thus, the addition of NaOH to 2,4,5-T did not cause chemical decomposition of the starting material.

Unlabeled Dow Analytical Standard 2,4,5-T (greater than 99% pure) and [^{14}C]2,4,5-T were prepared to contain 1 mg of 2,4,5-T/1.5 ml for the 5-mg/kg dosing solution and 20 mg of 2,4,5-T/1.5 ml for the 100-mg/kg dosing solution. Each solution contained approx. 5 μCi/1.5 ml of [^{14}C]2,4,5-T. Doses of 2,4,5-T were prepared to administer 5 mg/kg and 100 mg/kg of the free acid of 2,4,5-T. Dosing solutions were infused through a jugular cannula using a constant delivery syringe pump at the rate of 1 ml/min. After delivery of the dosing solution, the cannula was washed with physiological saline. The dose was determined gravimetrically by weighing the dosing syringes prior to and immediately following delivery of the dose. Five-microliter aliquots of the dose solutions were counted to quantitate the amount of ^{14}C activity administered to each rat.

The radiochemical purity of the dosing solution was determined using two thin-layer chromatography systems. Approximately 100,000 dpm were spotted on (1) alumina

(0.25-mm thickness, Brin
(50); and (2) silica gel (0.2
(90): methanol (5): glacial
The R_f[1] value of 2,4,5-T
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to be 99.0% radiochemic

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mg/kg dose.

Following the last coll
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washed with water and a
activity.

Blood plasma study. Fo
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or 100 mg/kg of [^{14}C]2,4,
cutting the tail vein with
tube. The tubes were imr
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Plasma samples were coll
dose, and at 0.5, 1, 2, 4, 8

Analysis of urine for 2,
were spotted on silica gel
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Liquid Scintillation Syste
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[^{14}C]-toluene as the inter
urine, plasma, and cage w
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Carbon dioxide formed d
methoxyethanol (10 ml) a

[1] R_f measured as the ratio c
tance from the origin to the so

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(0.25-mm thickness, Brinkman) and developed with glacial acetic acid (50): methanol (50); and (2) silica gel (0.25-mm thickness, Brinkman) and developed with chloroform (90): methanol (5): glacial acetic acid (5). Each plate was developed two dimensionally. The R_f[1] value of 2,4,5-T on both silica gel and alumina plates was 0.5. The diagonal area of each plate was scraped in 1-cm sections and the scrapings counted for ^{14}C activity in an Aquasol–water gel scintillation cocktail. The dosing solutions were shown to be 99.0% radiochemically pure.

Metabolism and balance study. Two rats of each sex received either 5 or 100 mg/kg of [^{14}C]2,4,5-T. Urine was collected every 12 hr throughout the experimental period at both dose levels. Urine traps were immersed in Dry Ice baths. Feces were collected at 12, 36, 60, and 84 hr at the 5-mg/kg dose, and every 24 hr through 168 hr at the 100-mg/kg dose.

Following the last collection interval, the rats were killed by decapitation and exsanguinated. The carcasses were skinned, and the liver, kidneys, a sample of perirenal fat, and skeletal muscle were removed from each animal. Each metabolism cage was washed with water and acetone. The cage wash was subsequently counted for ^{14}C activity.

Blood plasma study. Four rats of each sex were used to characterize the plasma concentrations of [^{14}C]2,4,5-T. Two male and two female rats were administered either 5 or 100 mg/kg of [^{14}C]2,4,5-T. Whole blood samples (approx. 70 μl) were obtained by cutting the tail vein with a scalpel and collecting the blood in a heparinized capillary tube. The tubes were immediately sealed at one end and centrifuged in a hematocrit centrifuge. The tubes were broken close to the plasma red cell interface and the plasma was drained into preweighed liquid scintillation vials for subsequent ^{14}C counting. Plasma samples were collected at 0.5, 1, 2, 4, 8, 12, 20, 28, and 36 hr after the 5-mg/kg dose, and at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 hr after the 100-mg/kg dose.

Analysis of urine for 2,4,5-T metabolites. One hundred-microliter aliquots of urine were spotted on silica gel and alumina thin-layer plates and developed with chloroform (90): ethanol (5): glacial acetic acid (5), and glacial acetic acid (50): methanol (50), respectively. Standards of [^{14}C]2,4,5-T were chromatographed with the urine samples. Plates were scanned on a Panax Thin Layer Scanner. The area on the plates containing [^{14}C]2,4,5-T was marked, scraped, and transferred to liquid scintillation vials for subsequent ^{14}C analysis. The remaining areas on the tlc plates were scraped and handled in the same manner. The percentage of total ^{14}C activity on each tlc plate associated with [^{14}C]2,4,5-T was calculated.

Radioactivity analysis. All samples were counted in a Nuclear Chicago Mark II Liquid Scintillation System utilizing external standard ratios to determine quench correction. The validity of the quench correction curve was checked periodically using [^{14}C]-toluene as the internal standard. Water and Aquasol were added to aliquots of urine, plasma, and cage wash, and analyzed for ^{14}C activity. Feces, kidney, carcass, and liver samples were prepared as 33% aqueous homogenates and oxidized in a Beckman Biological Material Oxidizer. Perirenal fat and skeletal muscle were oxidized directly. Carbon dioxide formed during the oxidation was trapped in 5 M 2-aminoethanol in 2-methoxyethanol (10 ml) and counted for ^{14}C activity following addition of ascintillation

[1] R_f measured as the ratio of the distance from the origin to the top of the spot divided by the distance from the origin to the solvent front.

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cocktail (10 ml). The cocktail contained 4 g of 2,5-diphenyloxazole (PPO) and 0.1 g of 1:4-di(2-(5-diphenyloxazolyl)benzene) (POPOP) per liter of 1:1 (v/v) toluene:2-methoxyethanol. Recovery of ^{14}C activity from spiked samples was 95 ± 5%.

Gas chromatography–mass spectrometry of 2,4,5-T. Urine samples (5 ml) were acidified with 1 ml of 1.0 N HCl and extracted twice with 5 ml of diethyl ether. The ether extracts were methylated with diazomethane, evaporated to dryness, and redissolved in 0.5 ml of hexane. A 2-μl aliquot of the hexane was injected into a LKB 9000 gas chromatograph–mass spectrometer (gc–ms). The gc column was glass 6 ft × 2.0 mm (i.d.), and packed with 10% OV-1 on Chromosorb W 80–100 mesh. The *m/e* 268, 270, and 272 peaks were monitored for sample quantitation. Recorded peaks were symmetrical; therefore peak heights and not peak areas were used for sample quantitation.

RESULTS

The concentration of 2,4,5-T in the plasma of rats as determined by ^{14}C activity is shown in Fig. 1 on a semilogarithmic plot of concentration versus time following iv doses of 5 and 100 mg/kg of [^{14}C]2,4,5-T. Clearance of ^{14}C activity at the 5-mg/kg dose indicates first-order clearance kinetics are operative. However, a nonlinear re-

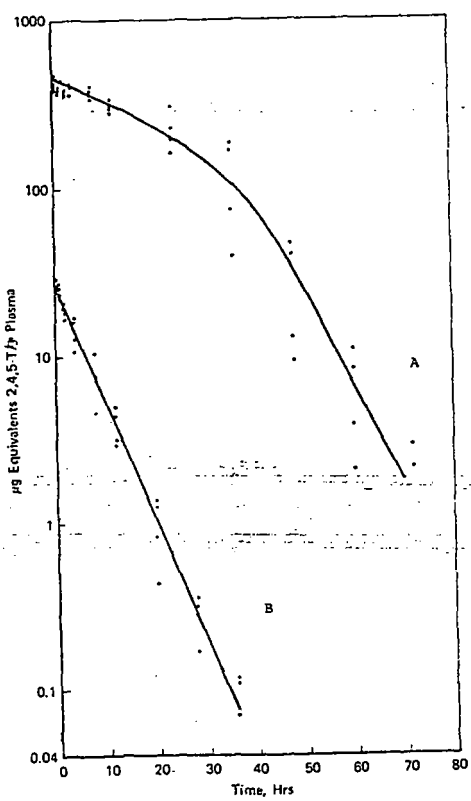


FIG. 1. Concentration of 2,4,5-T in the plasma of rats as a function of time following a single iv dose of [^{14}C]2,4,5-T at 5 mg/kg (B) and 100 mg/kg (A) as determined from the concentration of ^{14}C activity. Data for each dose level were obtained from four rats.

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linear parameter estimatio
(Wagner, 1973)

which is the Michaelis–Me
substrate concentration: V_m
where the enzyme is saturate
at $\frac{1}{2}V_m$.

The pharmacokinetic pa
from plasma at both dose le

RATE CONSTANTS (k)
[^{14}C]2,4,5-T FROM PLA
SINGLE

Dose (mg/kg)	
5	0.16 ±
100	0.030 ±
	0.13 ±

[a] Two male and two fer
[b] Calculated by linear r
[c] Maximum eliminatior
1973):

$c_1 - c_2$
$c_1 - c_3$
V_m/k_m =

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Projection of the blood p
allows calculation of the initi
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Table 2 shows the excreti
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in urine and feces during su
^{14}C activity from the body
corresponding half-life valu
lated by linear regression f
No sex differences were obs

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lationship is necessary to characterize the pattern for clearance with 100 mg/kg. Nonlinear parameter estimation can be used to fit the data of curve A to the equation (Wagner, 1973)

$$dc/dt = \frac{V_m \cdot c}{k_m + c}$$

which is the Michaelis–Menten equation for a one-enzyme reaction where c is the substrate concentration: $V_m = 16.6 \pm 1.82$ μg/hr/ml corresponds to the maximum rate where the enzyme is saturated and $k_m = 127.6 \pm 25.9$ μg/g is the substrate concentration at $\frac{1}{2}V_m$.

The pharmacokinetic parameters describing the rate of clearance of [^{14}C]2,4,5-T from plasma at both dose levels are presented in Table 1. The rate constant k describing

TABLE 1

RATE CONSTANTS (k) AND HALF-LIFE VALUES ($t_{\frac{1}{2}}$) FOR THE CLEARANCE OF [^{14}C]2,4,5-T FROM PLASMA AND VOLUMES OF DISTRIBUTION FOLLOWING A SINGLE INTRAVENOUS DOSE OF 5 AND 100 mg/kg[a]

Dose (mg/kg)	k (hr^{-1})	$t_{\frac{1}{2}}$ (hr)	V_d (ml/kg)
5	0.16 ± 0.01[b]	4.33 ± 0.27	190 ± 8
100	0.030 ± 0.008[b] (0–36 hr) 0.13 ± 0.03[c] (36–72 hr)	23.1 ± 4.9 (0–36 hr) 5.30 ± 1.23 (36–72 hr)	235 ± 10

[a] Two male and two female rats at each dose level.
[b] Calculated by linear regression analysis.
[c] Maximum elimination rate constant calculated by the following equations (Wagner, 1973):

$$c_1 - c_2 + K_m \ln(c_1/c_2) = V_m(t_2 - t_1)$$
$$c_1 - c_3 + K_m \ln(c_1/c_3) = V_m(t_3 - t_1)$$
$V_m/k_m = k$ (maximum elimination rate constant).

the linear portion of the plasma curve at the 100-mg/kg dose (36–72 hr) was not statistically different from the value when rats were given 5 mg/kg (0–36 hr). No sex differences were observed in the clearance of ^{14}C activity from plasma.

Projection of the blood plasma concentration curves back to the y intercept, $t = 0$, allows calculation of the initial concentration of 2,4,5-T in the plasma prior to excretion. Dividing the initial plasma concentration of [^{14}C]2,4,5-T in micrograms per milliliter into the dose in micrograms per kilogram gives the volume of distribution (V_d), milliliters per kilogram. V_d increased with dose from 190 ± 8 ml/kg to 235 ± 10 ml/kg for the 5- and 100-mg/kg dose levels, respectively.

Table 2 shows the excretion of ^{14}C activity by rats following single iv doses of 5 and 100 mg/kg of [^{14}C]2,4,5-T. Excretion is expressed as a percentage of the total dose found in urine and feces during successive time intervals. The rate constants for excretion of ^{14}C activity from the body of rats given a single iv dose of 5 and 100 mg/kg and the corresponding half-life values ($t_{\frac{1}{2}}$) are presented in Table 3. The constants were calculated by linear regression from data collected through the 84-hr collection interval. No sex differences were observed in the excretion of ^{14}C activity from the body.

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TABLE 2

PERCENTAGE OF A SINGLE INTRAVENOUS DOSE OF [^{14}C]2,4,5-T EXCRETED IN URINE AND FECES OF TWO MALE AND TWO FEMALE RATS DURING SUCCESSIVE TIME INTERVALS

Excretion interval (hr)	5 mg/kg		Excretion interval (hr)	100 mg/kg	
	Urine	Feces		Urine	Feces
0–12	52.4 ± 4.42[a]	2.43 ± 0.08	0–12	46.2 ± 2.91	—[b]
12–24	22.7 ± 1.89	—[b]	12–24	23.3 ± 2.45	3.97 ± 1.63
24–36	11.9 ± 2.79	0.16 ± 0.08	24–36	8.71 ± 1.41	—[b]
36–48	5.83 ± 1.35	—[b]	36–48	3.99 ± 0.48	3.06 ± 1.00
48–60	3.00 ± 0.65	0.14 ± 0.03	48–60	2.00 ± 0.28	—[b]
60–72	0.12 ± 0.05	—[b]	60–72	0.36 ± 0.30	0.31 ± 0.08
72–84	0.05 ± 0.03	0.05 ± 0.02	72–84	0.21 ± 0.11	—[b]
Total	96.0 ± 4.55	2.78 ± 0.82	84–96	0.34 ± 0.23	0.15 ± 0.18
			96–120	0.18 ± 0.10	0.05 ± 0.02
			120–144	0.33 ± 0.20	0.03 ± 0.02
			144–168	0.21 ± 0.17	0.03 ± 0.02
			Total	85.8 ± 3.50	7.59 ± 2.52

[a] Values represent mean ± SD.
[b] Feces collected over a 24-hr excretion interval.

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[a] Mean ±

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was 96.0 ± 1.0, 97.0 ± 1.4, a
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TABLE 3

RATE CONSTANTS (k) AND HALF-LIFE VALUES ($t_{\frac{1}{2}}$) FOR THE EXCRETION OF [^{14}C]2,4,5-T FROM THE BODY OF RATS FOLLOWING A SINGLE INTRAVENOUS DOSE OF 5 AND 100 mg/kg

Dose (mg/kg)	k[a] (hr^{-1})	$t_{\frac{1}{2}}$ (hr)
5	0.065 ± 0.020	10.7
100	0.063 ± 0.020 (0–84 hr)	11.0
	0.010 ± 0.005 (84–168 hr)	69.3

[a] Mean ± SD for four rats.

The elimination of ^{14}C activity from the body appears to be dose-dependent. The elimination rate constants and $t_{\frac{1}{2}}$ values for urinary elimination of ^{14}C activity over a 84-hr time interval at the two dose levels are not statistically different. However, there are differences in the amount of ^{14}C activity excreted in feces between each dosage group. Additionally, urinary elimination of ^{14}C activity at the high dose appears to be biphasic with a slower elimination of ^{14}C activity past the 84-hr collection interval. Eighty-four hours after administration, approx. 3.7% as compared with 0.6% of the initial dose remained in the body of rats at the 100- and 5-mg/kg dose groups, respectively.

To assess the overall recovery of ^{14}C activity, the carcasses of rats in the excretion studies were homogenized and the ^{14}C activity determined in aliquots of the homogenate. In addition, the cage wash was counted for ^{14}C activity. Total recovery of ^{14}C activity for the 5- and 100-mg/kg dose level was 99.5 ± 5.1% and 96.0 ± 3.0% (mean ± SD).

Tissue to plasma ratios were evaluated at the 5- and 100-mg/kg dose levels 8 hr after administration of the dose. The concentration of ^{14}C activity in plasma and selected tissues and the tissue/plasma ratios are presented in Table 4. Tissue to plasma ratios at the two dose levels were not statistically different with exception of the kidney. The results, however, indicate that the liver to plasma ratio is higher at the 100-mg/kg dose.

Kidney to plasma ratios were also evaluated at selected time intervals after the iv administration of 100 mg/kg of [^{14}C]2,4,5-T. The concentration of ^{14}C activity in plasma and kidney is presented in Table 5. Kidney to plasma ratios were less than 1 at the 4-hr interval. The kidney to plasma ratio increased with time following administration of the dose. At low plasma concentrations of ^{14}C activity, the ratio was 4.95 ± 0.59.

The percentage of ^{14}C activity in urine associated with [^{14}C]2,4,5-T in two thin-layer chromatography systems is presented in Table 6. Between 96.7 and 99.3% of ^{14}C activity in urine appeared as [^{14}C]2,4,5-T at the 5-mg/kg dose level while between 93.8 and 97.1% of ^{14}C activity appeared at the 100-mg/kg dose level. In order to confirm the identity of the material in urine with a similar R_f value to that of [^{14}C]2,4,5-T, urine samples were analyzed by gc–ms. The amount of 2,4,5-T in each urine sample was calculated based on the specific activity of the dose and percentage of dose excreted during each collection interval. The percentage of calculated 2,4,5-T at the high dose as determined by gc–ms was 96.0 ± 1.0, 97.0 ± 1.4, and 100.3 ± 7.5 for the 0–12, 12–24, and 24–36 hr collection intervals, respectively. Therefore, unchanged [^{14}C]2,4,5-T accounted for greater than

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TABLE 4

CONCENTRATION OF ^{14}C ACTIVITY IN SELECTED TISSUES AND PLASMA 8 HR AFTER THE ADMINISTRATION OF 5 AND 100 [^{14}C]2,4,5-T[a]

Dose (mg/kg)	Tissue	Concentration (μg/g)	Tissue/Plasma ratio
5	Liver	0.94 ± 0.12	0.24 ± 0.03
	Kidney	9.26 ± 2.23	2.40 ± 1.23
	Brain	—[b]	
	Fat	0.43 ± 0.07	0.11 ± 0.03
	Muscle	0.32 ± 0.02	0.08 ± 0.02
	Plasma	3.85 ± 0.85	
100	Liver	157 ± 9	0.38 ± 0.07
	Kidney	205 ± 15	0.49 ± 0.10
	Brain	6.6 ± 0.7	0.016 ± 0.040
	Fat	40.0 ± 8.8	0.10 ± 0.03
	Muscle	53.0 ± 9.4	0.12 ± 0.03
	Plasma	416 ± 36	

[a] Expressed as microgram equivalents of 2,4,5-T per gram; mean ± SD of four rats.
[b] Sample below detection limit.

TABLE 5

CONCENTRATION OF ^{14}C ACTIVITY IN KIDNEY AND PLASMA AT SELECTED TIME INTERVALS AFTER THE INTRAVENOUS ADMINISTRATION OF 100 mg/kg [^{14}C]2,4,5-T[a]

Time interval (hr)	Plasma concentration	Kidney concentration	Kidney/Plasma ratio
4	415 ± 19	297 ± 11	0.72 ± 0.02
8	359 ± 20	281 ± 17	0.78 ± 0.03
16	315 ± 21	297 ± 29	0.94 ± 0.04
32	142 ± 21	198 ± 18	1.40 ± 0.11
64	25 ± 9	122 ± 45	4.95 ± 0.59

[a] Expressed as microgram equivalents of 2,4,5-T per gram; mean ± SD of four rats.

TABLE 6

PERCENTAGE OF URINARY ^{14}C ACTIVITY AS [^{14}C]2,4,5-T[a]

System	Dose (mg/kg)	Time interval (hr) 0–12	12–24	24–36
Silica gel	5	97.0 ± 1.0	99.3 ± 1.2	99.0 ± 1.7
Al_2O_3	5	99.3 ± 0.6	98.7 ± 2.3	96.7 ± 3.5
Silica gel	100	93.8 ± 5.5	94.7 ± 3.3	95.0 ± 5.2
Al_2O_3	100	94.8 ± 2.5	97.1 ± 2.5	96.4 ± 3.9

[a] Mean ± SD of four rats from each excretion interval.

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93.8% of the ^{14}C activity in urine as determined by thin-layer chromatography and gc–ms at both dose levels.

DISCUSSION

Greater than 93.8% of the ^{14}C activity excreted in urine was unchanged [^{14}C]2,4,5-T. It can be inferred that this relationship also exists in plasma. Therefore, clearance of ^{14}C activity from plasma and urine represents clearance of 2,4,5-T to a good first approximation. The clearance of 2,4,5-T from plasma was clearly dose-dependent since there were marked differences in the shapes of the plasma clearance curves at the two dose levels.

It has been suggested that 2,4,5-T is cleared from the plasma by the kidney via the organic acid secretory pathway. Hook *et al.* (1974) have shown that kidney slices *in vitro* concentrate [^{14}C]2,4,5-T. The 2,4,5-T plasma-time profile indicates this active process may be saturated at 100 mg/kg because clearance from plasma occurs at a maximum rate during the first 36 hr and then decreases according to a first-order process. Clearance during the first 36 hr can also be described by a first-order process. The rate constant and half-life values are shown in Table 1. The rate constant characterizing the first-order process for 36–72 hr is the same value as the rate constant valid for the entire clearance curve at the low dose level. This pattern exhibited at the high dose with decreasing substrate concentrations is characteristic of a saturable active transport process.

Additional evidence in support of the concept of saturability of the excretory mechanism of 2,4,5-T comes from analysis of kidney tissue/plasma ratios. Eight hours after administration of 5 mg/kg the kidney/plasma ratio is 2.40 while at 100 mg/kg the ratio is 0.49. Kidney to plasma ratios at selected intervals following administration of 100 mg/kg demonstrate that as the plasma concentration of 2,4,5-T decreases, the ratio increases. The kidney cannot concentrate 2,4,5-T as effectively at the higher plasma concentrations associated with administration of 100 mg/kg. However, as plasma concentrations of 2,4,5-T decrease, the kidney can concentrate 2,4,5-T.

The plasma concentration data presented in Fig. 1 reveal additional dose-dependent effects. Projection of the curve back to the y-intercept (time = 0), allows calculation of the concentration of 2,4,5-T in plasma prior to excretion. This concentration is dependent on the distribution of 2,4,5-T to tissues and its subsequent entry into the cells and/or tissue spaces. The V_d of 2,4,5-T is significantly larger at the high dose. If first-order processes were operative, then the V_d would not differ at these two dose levels. A comparison of liver tissue/plasma ratios 8 hr after the iv administration of 5 and 100 mg/kg of [^{14}C]2,4,5-T reveals that the ratios are 0.24 and 0.38, respectively. Liver tissue, therefore, is exposed to a higher concentration of 2,4,5-T than would have been predicted assuming the first-order kinetics of a 5-mg/kg dose of 2,4,5-T. While these differences are not statistically different, a trend is indicated.

Examination of the excretion data (Table 2) reveals two significant features. At the 100-mg/kg dose level, a greater fraction of the dose as ^{14}C activity is excreted in the feces. This suggests that at high dose levels biliary excretion of 2,4,5-T and/or degradation products plays a more important role in the overall elimination of 2,4,5-T from the body.

At the 100-mg/kg dose level, there appears to be a second slow phase of ^{14}C activity excreted in urine between 84 and 168 hrs. In rats given 5 mg/kg, 0.6% of the dose remained in the body after 84 hr. In those given 100 mg/kg, 3.7% of the original dose remained and was gradually eliminated. This leads to two alternative conclusions: (1) at 100 mg/kg, a larger fraction of the dose has gained entry into compartments, tissues, and cells from which it is slowly released, and/or (2) a fraction of 2,4,5-T has been metabolically converted in the body to products which are less readily excreted. This latter hypothesis is supported by data presented in Table 6. Between 96.7 and 99% of ^{14}C activity in urine appeared as [^{14}C]2,4,5-T at the low dose while between 93.8 and 97% of ^{14}C activity appeared as [^{14}C]2,4,5-T at the high dose. While these are not significantly different, a trend is indicated.

There are direct toxicological implications for the dose-dependent elimination of 2,4,5-T from plasma as well as from the body. Systemic toxicity of a drug or foreign compound is often a function of the concentration and duration of that drug in plasma. If the drug or foreign compound is eliminated at a slower rate from the plasma, and/or different metabolites are formed at a high dose, then the ability of the drug and/or metabolite to induce toxicity at the high dose will be greater. Therefore, more severe toxicological manifestations than would have been predicted will often occur at the high dose level where nonlinear kinetics are operative. However, toxic effects have not been observed with a single iv dose of 100 mg/kg of 2,4,5-T.

There appear to be significant differences between the results of this study and that of Piper *et al.* (1973). Piper *et al.* administered 2,4,5-T orally, while in this study 2,4,5-T was administered by the iv route. Piper demonstrated dose-dependent elimination of 2,4,5-T from plasma. For any given oral dose, semilogarithmic plots of elimination versus time in plasma gave a linear relationship. At increasing doses, however, the elimination $t_{\frac{1}{2}}$ was longer which is a criterion of dose-dependent kinetics (Levy, 1968). Clearance curves did not show the classical Michaelis–Menten graphical representation of nonlinear kinetics as is shown in this study. One explanation for the data is that the study was not conducted over a sufficiently long time interval. An alternative is that oral absorption of 2,4,5-T is playing some undefined role in altering the clearance of 2,4,5-T from plasma.

The pharmacokinetic data presented in this report indicate that the statistical projection of results of experiments with large doses of 2,4,5-T to predict the hazard of exposure to small amounts is not justified because the capability of the body to handle the compound has been altered.

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REVIEW

Association between Soft Tissue Sarcomas, Malignant Lymphomas, and Phenoxy Herbicides/Chlorophenols: Evidence from Occupational Cohort Studies

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Association between Soft-Tissue Sarcomas, Malignant Lymphomas, and Phenoxys/Chlorophenols: Evidence from Occupational Cohort Studies. JOHNSON, E. S. (1990). *Fundam. Appl. Toxicol.* **14**, 219–234. Some case–control studies have reported a significant association between occupational use of phenoxy herbicides and chlorophenols and soft-tissue sarcomas and malignant lymphomas. However, persons who spray or apply these substances are concomitantly exposed to other potentially carcinogenic chemicals and oncogenic viruses, which have been found or suspected to play a role in the etiology of these tumors. No study has thoroughly controlled for these other exposures, some of which have been shown to be independently associated with these tumors even after controlling for exposure to phenoxy acids or chlorophenols. On the other hand, it has been found that an observed risk from exposure to phenoxy herbicides disappeared on controlling for some of these concomitant exposures in the rare instance this was attempted. Also, on several occasions, an association has been observed with occupations in which exposure to phenoxys and chlorophenols may occur, but not with the compounds themselves. Accordingly, a detailed review of the evidence from occupational cohort studies was conducted, to see if it corroborates that from case–control studies. It was found that the evidence does not unequivocally incriminate phenoxys and chlorophenols as a cause of these tumors. The results obtained with cohort studies of sprayers and applicators do not corroborate the association reported among this occupational group, in case–control studies. It is possible that the suspected association may well be due, partly or wholly, to one or more of the other concomitant exposures. However, in view of the fact that the majority of the cohorts need further follow-up to be informative, it is concluded that further studies of these cohorts are required before it can be determined whether or not these tumors are caused by exposure to phenoxy acids and chlorophenols. © 1990 Society of Toxicology

The phenoxy herbicides and chlorophenols have extensive use in agriculture, forestry, construction, and other industries. Commercial-grade preparations of these compounds are contaminated by the highly toxic dioxins and furans, some isomers of which are known to be potent carcinogens in laboratory animals (Kociba, 1984; National Toxicology Program, 1982; Pitot *et al.*, 1980). There are reports that in humans, exposure to phenoxy acid herbicides and chlorophenols increases risk for soft-tissue sarcomas (STS) and malignant lymphomas (ML). The potential for widespread human exposure to these substances and their contaminants is significant. Hence, what role if any, they play in the causation of these tumors, is of importance.

A large number of studies of different designs have previously reported on the relationship between malignant lymphomas (Hodgkin's disease, non-Hodgkin's lymphomas) and farming or agricultural work or exposure to pesticides (Binaschi *et al.*, 1985; Brace, 1986; Brownson and Reif, 1988;

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Buesching and Wollstadt, 1984; Burmeister, 1981; Burmeister *et al.*, 1983; Cantor, 1982; Delzell and Grufferman, 1985; Dubrow *et al.*, 1988; Fasal *et al.*, 1968; Gallagher *et al.*, 1984; Giles *et al.*, 1984; Milham, 1971; Pearce *et al.*, 1985; Priester and Mason, 1974; Saftlas *et al.*, 1987; Schumacher, 1985; Schumacher and Delzell, 1988; Stark *et al.*, 1983; Weisenburger *et al.*, 1986; Wiklund *et al.*, 1988; Williams *et al.*, 1977). Most have shown an increased risk (Binaschi *et al.*, 1985; Brace, 1986; Brownson and Reif, 1988; Buesching and Wollstadt, 1984; Burmeister, 1981; Burmeister *et al.*, 1983; Cantor, 1982; Dubrow *et al.*, 1988; Giles *et al.*, 1984; Pearce *et al.*, 1985; Saftlas *et al.*, 1987; Schumacher, 1985; Stark *et al.*, 1983; Weisenburger *et al.*, 1986; Wiklund *et al.*, 1988; Williams *et al.*, 1977). The excess risk was statistically significant in all but four studies (Brace, 1986; Dubrow *et al.*, 1988; Stark *et al.*, 1983; Williams *et al.*, 1977). Of these, the observed risk was associated with farming only, in the study group as a whole or a subgroup(s) of it (Binaschi *et al.*, 1985; Brownson and Reif, 1988; Buesching and Wollstadt, 1984; Burmeister, 1981; Dubrow *et al.*, 1988; Giles *et al.*, 1984; Pearce *et al.*, 1985; Schumacher, 1985; Stark *et al.*, 1983; Wiklund *et al.*, 1988), since pesticide/herbicide use was not investigated specifically. Seven studies investigated pesticide/herbicide use (Brace, 1986; Burmeister *et al.*, 1983; Cantor, 1982; Saftlas *et al.*, 1987; Schumacher and Delzell, 1988; Weisenburger *et al.*, 1986; Williams *et al.*, 1977). No association was reported in two of them (Brace, 1986; Schumacher and Delzell, 1988). One study observed a significant association with pesticides, but did not investigate farming as a risk factor (Weisenburger *et al.*, 1986). Three studies found statistically significant associations with both farming and general herbicide exposure, separately (Burmeister *et al.*, 1983; Cantor, 1982; Saftlas *et al.*, 1987). These findings are consistent with an elevated risk of lymphoma among farmers and agricultural workers. They leave open, however, the question of which specific exposure(s) (from among the various agricultural chemicals and animal oncogenic viruses) is responsible.

One specific exposure which has received considerable attention as a potential candidate is exposure to phenoxy acid herbicides or chlorophenols. There are case reports on the development of non-Hodgkin's lymphoma (NHL) following exposure specifically to phenoxy acid herbicides or chlorophenols (Bishop and Jones, 1981; Olsson and Brandt, 1981) and this possible association has been examined in no less than eight case-control studies of ML (Cantor *et al.*, 1985; Everett *et al.*, 1985; Hardell *et al.*, 1981; Hoar *et al.*, 1986; Hoar Zahm *et al.*, 1988a; Hoar Zahm *et al.*, 1988b; Pearce *et al.*, 1986, 1987; Woods *et al.*, 1987). Three of these (Everett *et al.*, 1985; Hardell *et al.*, 1981; Hoar *et al.*, 1986) reported a statistically significant association, with estimated relative risks of 1.9 for exposure to pentachlorophenol, 6.0 for exposure to phenoxy acids and chlorophenols, and 2.6 for exposure to 2,4-dichlorophenoxy acid, respectively. In two studies, the excess risk observed was not statistically significant (Cantor *et al.*, 1985; Hoar Zahm *et al.*, 1988b). In the remaining three studies, a statistically significant association was observed between ML and spraying of herbicides or occupations in which exposure to phenoxy acids/chlorophenols might occur, but no association was observed for exposure to the compounds themselves in the entire study population (Pearce *et al.*, 1986, 1987; Woods *et al.*, 1987).

There are also case reports of soft-tissue sarcomas following exposure to phenoxy acids or chlorophenols (Cook, 1981; Fingerhut *et al.*, 1984; Hardell, 1977; Honchar and Halperin, 1981; Hope *et al.*, 1984; Johnson *et al.*, 1981; Moses and Selikoff, 1981; Sarma and Jacobs, 1982) and an excess of STS has been seen in the Seveso region of Italy following an industrial release of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Puntoni *et al.*, 1986). Since Agent Orange, a mixture of the phenoxy acids 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), was used in the Vietnam

war, military personnel have been studied for the occurrence of STS and ML (Anderson *et al.*, 1985; Breslin *et al.*, 1988; Fett *et al.*, 1987a,b; Goun and Kuller, 1988; Holmes, 1986; Kang *et al.*, 1986; Kogan and Clapp, 1985, 1988; Lathrup *et al.*, 1983; Lathrop *et al.*, 1984; Lawrence *et al.*, 1985; Centers for Disease Control Vietnam Experience Study, 1987; Wolfe *et al.*, 1984; Wolfe and Michalek, 1985) with generally inconclusive results. However, the relevance of studies of military personnel is limited; the groups under study are too young, sufficient time since first exposure (latency) has not accrued, and exposure is inadequately characterized [except in the studies by Lathrop *et al.* (1984) and Wolfe and Michalek (1985)]. Accordingly, the weight of evidence for investigating the risk of STS from exposure to phenoxy acids or chlorophenols is derived mainly from a large number of more conventional case–control studies (Balarajan and Acheson, 1984; Eriksson *et al.*, 1981; Greenwald *et al.*, 1984; Hardell, 1981; Hardell and Eriksson, 1988; Hardell and Sandstrom, 1979; Hoar *et al.*, 1986; Hoar Zahm *et al.*, 1988a; Kang *et al.*, 1987; Smith *et al.*, 1982, 1983, 1984; Smith and Pearce, 1986; Sobel *et al.*, 1986; Vineis *et al.*, 1987; Woods *et al.*, 1987) and studies using proportional mortality ratio (PMR) analysis of routinely collected death certificates (Gallagher and Threlfall, 1984; Milham, 1982).

The case–control studies of STS comprise 12 distinct series, reported in 16 publications. One series (Sobel *et al.*, 1986) was anecdotal and of limited value. Of the 11 remaining series, all but one examined risk associated with exposure to phenoxy acids or chlorophenols per se. The single study which did not (Balarajan and Acheson, 1984) recorded a statistically significant risk for the occupational group of farmers, farm managers, and gardeners. Of the 10 studies which did, a statistically significant association between STS and these substances was observed in only 4 series (Eriksson *et al.*, 1981; Hardell and Eriksson, 1988; Hardell and Sandstrom, 1979; Vineis *et al.*, 1987), with estimated relative risks of 5.1, 3.3, 6.2, and 15.5 respectively, for exposure to phenoxy acids or chlorophenols; none of these three series reported a significant association with farming or agriculture. Two other series reported statistically significant associations with occupations in which exposure to phenoxys and chlorophenols might occur (railways, meat works, tannery, lumber grading) but no such relationship was observed for the compounds themselves (Smith *et al.*, 1984; Woods *et al.*, 1987). The significance of the finding by Woods *et al.* (1987) of a statistically significant increased risk of STS of 7.2, due to chlorophenol exposure among persons in the United States with Scandinavian names, is difficult to assess in view of the very many comparisons made in this study and the fact that this was observed neither for ML nor between STS and phenoxy acids.

With regard to the two PMR studies, the study by Gallagher and Threlfall (1984) did not show any significant risk of STS for any occupation, although significant associations were observed between sawmill and pulp workers and NHL. The other by Milham (1982) obtained a statistically significant risk of 1.52 for STS among farmers (not otherwise specified), but it was also noted that the 15 occupations with the highest PMRs for STS did not include those with exposure to phenoxy acids or chlorophenols. This study also observed a statistically significant risk of 1.94 for Hodgkin's disease among paper/pulp mill workers.

The evidence from the few case–control studies linking ML and STS with exposure to phenoxys and chlorophenols has met with controversy (Coggon and Acheson, 1982; Royal Commission, 1985). This controversy has centered around the quality and detail of exposure information and the possibility of bias in the ascertainment of exposure. Bias could be introduced from interviewers obtaining exposure history unequally in cases and controls (interviewer bias) or from unequal recall of exposure between case and control questionnaire respondents (recall bias). While the existence of these types of bias can be suspected (Coggon and Acheson, 1982) proof of their existence has been

TABLE I

COHORT STUDIES OF WORKERS IN MANUFACTURING/FORMULATING PLANTS: OBSERVED NUMBER OF DEATHS/NUMBER OF CASES

Study	Total deaths	Total cancers	STS	NHL	HD
1. Dalderup and Zellenrath	25	8	0	0	0
2. Pazderova *et al.*	6	2	0	0	0
3. Theiss and Frentzel-Beyme[a]	21	7	0	0	0
4. Lehnert and Szadowski[a]	43	17	0	0	0
5. Ott, Holder and Olson[b]	11	1	0	0	0
6. Cook *et al.*[b]	4	3	1	0	0
7. Cook *et al.*[b]	298	61	1	5	1
8. Ott *et al.*[b]	370	81	1	5	1
9. Zack and Suskind	32	9	1	0	1
10. Zack and Gaffey	58	9	1	0	0
11. Coggon *et al.*[c]	1039	297	0	2	1
12. Bond *et al.*					
2,4-D plant	68	17	0	2	1
Total cohort	111	26	0	2	1
13. Lynge[d,e]					
Phenoxy department		17	1	0	
Total cohort		208	5	(8)	

[a] These two studies were from the same plant.

[b] These four studies were from the same plant.

[c] This was a mixed cohort of sprayers and workers in manufacturing. All three cases of ML were from manufacturing.

[d] Figure in parentheses includes NHL and HD combined.

[e] Blanks indicate data not available.

difficult to establish. Similarly, PMR analyses suffer from certain types of bias (Johnson, 1986; Wong and Decoufle, 1982; Wong *et al.*, 1985) which makes it imprudent to interpret as causal the relatively small excess risk observed among farmers in the study by Milham (1982).

Cohort studies of adequate size are more definitive, and have the advantage over case–control studies that bias in the ascertainment of exposure is minimized, since exposure status is determined before the outcome of interest is known (Mausner and Bahn, 1974). Furthermore, in occupational settings, exposure status is more readily and reliably ascertained. Therefore, cohort studies of occupationally exposed groups should provide the best evidence with which to evaluate causation, since experimental studies cannot be done in humans.

Accordingly, it is of interest to review critically the evidence for the association between STS or ML and phenoxys and chlorophenols available from these types of studies. If the estimates of increased risk derived from cohort studies corroborate those from case–control studies, the case for causality is greatly strengthened, as these two types of studies are the prime analytic designs employed in epidemiology. There is added significance to this exercise, since the purported increased risk of these tumors due to phenoxy/chlorophenol exposure in case–control studies originates largely from exposure during the use of these compounds during spraying or application. The much higher exposure during the manufacture and formulation of these compounds (Patterson *et al.*, 1989) has featured very little in case–control studies, possibly because fewer persons are exposed this way than during their use. This review separates evaluation of cohorts involved in the use of these substances (which has more direct relevance to case–control studies) from that of cohorts

TABLE 2

COHORT STUDIES OF WORKERS IN MANUFACTURING/FORMULATING PLANTS FOR WHICH RELATIVE RISKS (RRs) OR CONFIDENCE INTERVALS WERE GIVEN

Study	STS		ML		HD	
	RR	Confidence interval	RR	Confidence interval	RR	Confidence interval
7. Cook *et al.*			2.4	0.8–5.6		0.0–5.6
8. Ott *et al.*			1.9	0.6–4.5	0.9	0.0–5.1
11. Coggon *et al.*			0.4	0.0–1.3	0.3	0.0–1.6
12. Bond *et al.* total cohort[a]			1.8		2.7	0.0–14.7
13. Lynge						
Total cohort[b]			(1.2)			
Phenoxy department	3.3					

[a] A relative risk of 3.1, which was statistically significant, was obtained for all hemopoietic/lymphatic cancers combined among workers in the 2,4-D plant.

[b] Figure in parentheses denotes ML unspecified.

involved in manufacture and formulation. A formal metanalysis will not be conducted as the data are too scanty, and the definition of exposure in most instances was not comparable from study to study.

COHORTS FROM MANUFACTURING AND FORMULATING PLANTS

Tables 1 and 2 summarize 13 cohort studies (representing nine distinct cohorts) of workers in manufacturing/formulating plants (Bond *et al.*, 1988; Coggon *et al.*, 1986; Cook *et al.*, 1980, 1986; Dalderup and Zellenrath, 1983; Lehnert and Lynge, 1985; Ott *et al.*, 1980, 1987; Pazderova-Vejlupkova *et al.*, 1981; Lehnert and Szadkowski, 1985; Thiess *et al.*, 1982; Zack and Gaffey, 1983; Zack and Suskind, 1980). All 13 studies except that by Lynge (1985) were mortality studies. Of the 12 mortality studies, 7 did not record any death from STS (Bond *et al.*, 1988; Coggon *et al.*, 1986; Dalderup and Zellenrath, 1983; Lehnert and Ott *et al.*, 1980; Pazderova-Vejlupkova *et al.*, 1981; Lehnert and Szadkowski, 1985; Thiess *et al.*, 1982) and the remaining 5 reported one death each (Cook *et al.*, 1980, 1986; Ott *et al.*, 1987; Zack and Gaffey, 1983; Zack and Suskind, 1980). In all 12 studies, the expected number of deaths from STS was less than one. Broadly speaking, about 180 cancer deaths should occur in a cohort in order to expect one death from soft-tissue sarcoma (Riggan *et al.*, 1983). As seen in Table 1, with one exception (Coggon *et al.*, 1986), none of the 12 mortality studies recorded total cancer deaths anywhere close to this figure. Even in the study by Coggon *et al.* (1986) the total number of cancer deaths among workers engaged in manufacturing/formulating was probably less than 180, since the 297 cancer deaths recorded in the cohort included sprayers as well.

No death from malignant lymphomas was recorded in 7 of the 12 mortality studies (Cook *et al.*, 1980; Dalderup and Zellenrath, 1983; Lehnert and Ott *et al.*, 1980; Pazderova-Vejlupkova *et al.*, 1981; Lehnert and Szadkowski, 1985; Thiess *et al.*, 1982; Zack and Gaffey, 1983). Based on the studies with available data (Bond *et al.*, 1988; Coggon *et al.*, 1986; Cook *et al.*, 1986; Ott *et al.*, 1987) about 100 deaths (total) should occur in an occupational cohort in order to expect one death from NHL. The highest number of total deaths recorded in these 7 studies was only 58 (Zack and Gaffey, 1983). In addition, about 30 and 60 deaths from cancers should occur in order to expect one death from non-Hodgkin's lymphoma (NHL) and Hodgkin's

disease (HD), respectively (Riggan *et al.*, 1983). Based on this criterion also, it is seen from Table 1 that the sizes of these 7 cohorts were clearly inadequate to investigate any but extremely large risks from ML, as the highest total number of deaths from cancers recorded in any of them was only 17 (Lehnert and Szadkowski, 1985). Of the remaining 5 studies with at least one ML death recorded (Bond *et al.*, 1988; Coggon *et al.*, 1986; Cook *et al.*, 1986; Ott *et al.*, 1987; Zack and Suskind, 1980), all except that by Coggon *et al.*, showed at least around a twofold nonsignificant increased risk of NHL or HD. [Note that the study by Ott *et al.* (1987) is an update of the study by Cook *et al.* (1986).] In one of these studies (Bond *et al.*, 1988) the increased risk of 3.1 (for a total of five deaths) of tumors of the hemopoietic/lymphatic systems among workers in a 2,4-D plant was statistically significant, but risks for lymphomas were not given separately. Two of the five deaths were due to NHL, one due to HD, and two due to leukemia.

The Danish cancer incidence study by Lynge (1985) reported an excess of STS among cohort members from two plants which manufactured phenoxy acids as well as a variety of other chemicals. Five cases of STS occurred in the cohort as a whole and the relative risk (RR) for STS was 2.72 [95% confidence interval (CI), 0.88–6.34]. When a 10-year latency was allowed for, the RR was 3.67 (95% CI, 1.0–9.39). The durations of employment for the five cases were 3, 3, 0.5, 30, and 90 months. Four of the five STS cases had potential for exposure, three of them having worked in the general shipping department, where presumably all other chemicals produced by the factory were handled as well, and the fourth in the phenoxy herbicides packaging department. This cohort was not complete for persons hired before the mid-1950s. The predominant phenoxy herbicides produced by this factory were 2-methyl-4-chlorophenoxyacetic acid or α2-methyl-4-chlorophenoxypropionic acid (MCPA/MCPP) and, to a lesser extent, 2,4-dichlorophenoxyacetic acid and 2,2,4-dichlorophenoxypropionic acid. Smaller amounts of 2,4,5-T (acid and esters), as well as hexachlorophene, were also produced. 2,4,5-Trichlorophenol (TCP) was produced for a very limited period (1951–1952), but only one of the STS cases had been employed during its production. The factory also produced large amounts of other chemicals such as aniline dyes, organic and inorganic dyes, and pigments, and formulated various insecticides. It is to be noted that of the four STS having possible exposure, four different histologic types were involved (liposarcoma, fibrosarcoma, dermatofibrosarcoma or hemangiopericytoma, and leiomyosarcoma). Only one of the four did not occur in an internal organ; based on Danish national data (Lynge *et al.*, 1987) one would have expected 1.4.

Eight cases of malignant lymphoma (one in a female) were observed in the entire Danish cohort, and the nonsignificant relative risks were 1.3 and 0.8 for males and females, respectively. No case of ML occurred among workers specifically engaged in the manufacture/formulation of phenoxy acids, even though 0.9 was expected. A statistically significant relative risk of 2.91 for ML was observed among workers engaged in the processing of products other than phenoxy acids.

COHORTS OF USERS (SPRAYERS, APPLICATORS, AGRICULTURE/FORESTRY WORKERS, ETC.)

A total of 16 occupational cohort studies of users from 12 distinct cohorts have been conducted to date (Axelson and Sundell, 1974; Axelson *et al.*, 1980; Barthel, 1976, 1981; Blair *et al.*, 1983; Coggon *et al.*, 1986; Green, 1986; Hogstedt and Westerlund, 1980; MacMahon *et al.*, 1988; Morgan *et al.*, 1980; Riihimaki *et al.*, 1982, 1983; Royal Commission, 1985; Wang and MacMahon, 1979; Wiklund *et al.*, 1987, 1988) (Tables 3 and 4). Two reports of the same study (MacMahon *et al.*, 1988; Wang and MacMahon, 1979) did not provide data on STS and ML, even though the update (MacMahon *et al.*,

TABLE 3

COHORT STUDIES OF USERS (SPRAYERS, APPLICATORS): OBSERVED NUMBER OF DEATHS/NUMBER OF CASES

Study	Total deaths	Total cancers	STS	NHL	HD
1. Riihimaki *et al.*[a]	144	26	0	0	0
2. (Incidence)					
Riihimaki *et al.*[a]		26	0	0	0
3. (Incidence)					
Barthel		30	0	0	0
4. (Incidence)		8	0	0	0
Hogstedt *et al.*	29	5	0	0	0
5. Green *et al.*	80	18	0	0	0
6. (Incidence)		8	0		
Axelson and Sundell[b]	10	2	0		
7. Axelson *et al.*[b]	20	6	0	0	1
8. (Incidence)		35	0	1	0
Morgan *et al.*	62	13	0	0	0
9. Wang and MacMahon[c]	311	47			
10. MacMahon *et al.*[c]	1082	232			
11. Blair *et al.*	378	84			
12. (Incidence) Barthel		169	1	2	2
13. (Incidence)					
Wiklund *et al.*[d]			6		
14. (Incidence)					
Wiklund *et al.*[d]				21	11
15. Coggon *et al.*[e]	1039	297	1	0	0
16. Royal Commission Report[f]	692	169	1	(3)	

[a–d] Same cohort.

[e] This was a mixed cohort of sprayers and manufacturing workers.

[f] Figure in parentheses denote ML unspecified.

1988) was large enough for cases of both diseases to have occurred; it was reported though that tumors of the hemopoietic/lymphatic systems were not in excess. The study by Barthel (1981) was subject to selection bias as only 70% of the study population was identified: one case of STS and two cases each of NHL and HD were observed, which were not significantly in excess. Of the other 13 studies, the results for one cohort for STS and ML were reported separately (Wiklund *et al.*, 1987, 1988). No occurrence of STS was re-

TABLE 4

COHORT STUDIES OF USERS (SPRAYERS, APPLICATORS) FOR WHICH RELATIVE RISKS OR CONFIDENCE INTERVALS WERE GIVEN

	STS		NHL		HD	
Study	RR	Confidence interval	RR	Confidence interval	RR	Confidence interval
13. Wiklund *et al.*	0.9	0.3–2.0				
14. Wiklund *et al.*			1.0	0.6–1.5	1.2	0.6–2.2
16. Royal Commission Report[a]	1.4		(0.9)			

[a] Figure in parentheses denotes ML unspecified.

ported in nine studies (Axelson and Sundell, 1974; Axelson *et al.*, 1980; Barthel, 1976; Blair *et al.*, 1983; Green, 1986; Hogstedt and Westerlund, 1980; Morgan *et al.*, 1980; Riihimaki *et al.*, 1982, 1983). In general, over 100 incident cases of cancer should occur in order to expect one case of STS; to expect one case each of NHL and HD, a total of at least 40 and 70 cases of cancer, respectively, should occur (Cutler and Young, 1975; Waterhouse *et al.*, 1982). Using these criteria, and those established above for mortality studies, it is seen from Table 3 that none of the nine studies without a case of STS was sufficiently large to examine risk of STS. In none of the remaining three studies with a recorded case(s) of STS was any statistically significant excess of the disease observed (Coggon *et al.*, 1986; Royal Commission, 1985; Wiklund *et al.*, 1988). Six cases of STS were recorded in the largest cohort (Wiklund *et al.*, 1988) and the relative risk was 0.89; 72% of the cohort had potential for exposure to phenoxy acids. However, the maximum follow-up period was only 18 years, and it is not known what proportion of the cohort had a time since first exposure longer than 18 years. Thus, it is difficult to assess whether the latency period was adequate.

Similarly, with respect to malignant lymphomas, only five studies reported any death from, or any case of lymphoma (Axelson *et al.*, 1980; Barthel, 1981; Morgan *et al.*, 1980; Royal Commission, 1985; Wiklund *et al.*, 1987). In none of these five studies was a significant excess of malignant lymphoma reported, although in at least two of them (Axelson *et al.*, 1980; Morgan *et al.*, 1980) a case occurred when none was expected. Six studies were obviously not large enough to observe a case of NHL or HD (Axelson and Sundell, 1974; Barthel, 1976; Green, 1986; Hogstedt and Westerlund, 1980; Riihimaki *et al.*, 1982, 1983). Two studies did not indicate the findings for these tumors (MacMahon *et al.*, 1988; Wang and MacMahon, 1979) and a further one (Blair *et al.*, 1983) mentioned only that there was no excess. No case of ML occurred among sprayers in the mixed cohort of sprayers and production workers by Coggon *et al.* (1986; personal communication, 1988).

ANIMAL DATA

A brief reference will be made to the animal data, to examine to what degree they complement those for humans.

2,4-Dichlorophenol and 2,4,5-trichlorophenol each acted as a skin tumor promoter when given after a single initiating dose of 0.3% dimethylbenz[*a*]anthracene (DMBA) in benzene (Boutwell and Bosch, 1959). In 2-year feeding studies in male and female rats and mice fed diets containing 2,4-dichlorophenol, there was no evidence of carcinogenic activity (National Toxicology Program, 1988a).

2,4,6-Trichlorophenol has been reported to cause an increased incidence of reticulum cell sarcoma and hepatoma in mice (Innes *et al.*, 1969). It was also found to be carcinogenic in male rats, inducing lymphomas or leukemias, and to be carcinogenic in male and female mice, inducing hepatocellular carcinomas or adenomas (National Cancer Institute Carcinogenesis Technical Report, 1979).

Pure pentachlorophenol (PCP) did not show evidence of carcinogenicity in rats even at the highest dose of 30 mg/kg, although there was suggestion that the animals could have tolerated a higher dose (Schwetz *et al.*, 1978). A neoplastic effect of PCP was not observed in mice observed for 18 months (Innes *et al.*, 1969). In a National Toxicology Program (NTP) study, it was concluded that there was evidence of carcinogenic activity for male mice fed diets containing technical-grade PCP, as shown by increased incidences of adrenal medullary and hepatocellular neoplasms. There was also an indication of carcinogenic activity for female mice exposed to technical-grade PCP, as shown by increased incidences of hemangiosarcomas and hepatocellular neoplasms. A carcinogenic activity was observed for male mice exposed to a

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purer grade of PCP, Dowicide EC-7, as shown by increased incidences of adrenal medullary and hepatocellular neoplasms, and for female mice also, as shown by increased incidence of adrenal medullary and hepatocellular neoplasms and hemangiosarcomas (National Toxicology Program, 1988b).

Male rats fed a diet containing a high dose of 2,4-dichlorophenoxyacetic acid showed a statistically significant increased incidence of brain tumors (Hazleton Laboratories, 1986). However, an expert panel reviewing this study concluded that for various reasons, there was insufficient evidence to conclude that these tumors were related to 2,4-D exposure, particularly as an increase of these tumors was not observed in female rats (Canadian Centre for Toxicology, 1987). Unfortunately, other studies of 2,4-D have been limited by insufficient sample size or inadequate reporting, and an unequivocal demonstration of carcinogenic activity has not been evident (Hazleton Laboratories, 1984; International Agency for Research on Cancer, 1977). Similarly, studies of the carcinogenicity of 2,4,5-T and MCPA in animals have generally been sparse and suffer from the same limitations as the 2,4-D studies (International Agency for Research on Cancer, 1977, 1983).

In summary, while there is significant evidence that chlorophenols have tumor-promoting and -initiating effects in animals, a similar effect has not been observed for any of the phenoxyacetic acid herbicides, partly because many of the studies of this group of compounds were limited, because of small numbers of animals used. The currently available data are inadequate to fully assess the carcinogenicity of these compounds in animals; thus it is not known whether or not phenoxys are carcinogenic in animals.

SUMMARY AND DISCUSSION

Soft-Tissue Sarcomas

None of the 12 mortality studies of manufacturing cohorts conducted to date have accumulated sufficient person-years of observation for adequate evaluation of risk of soft-tissue sarcomas, since the expected number of deaths from STS in each of them is less than one. It is estimated that if the expected number of deaths from STS in a study is one, an underlying risk of disease will have to be at least fivefold for it to be detected at the 95% level of significance and with a statistical power of 0.8 (Beaumont and Breslow, 1981). An increased risk of STS was recorded in the single cancer incidence study by Lynge (1985). However, in that study, three of the four cases of STS with potential for exposure to phenoxy acid herbicides worked in the general shipping department, where presumably they had potential for concomitant exposure to a large number of chemicals other than phenoxys or chlorophenols, which were also produced in the factory. It is to be noted among these studies that a case of STS occurred in three cohorts (reported in five studies) even though none was expected.

Similarly, 9 of the 13 usable studies of sprayers/applicators which provided information on STS specifically did not report a case of the disease, and are therefore not adequate at the present time to evaluate risk of STS. On the other hand, in none of the remaining four cohorts which were sufficiently large or which recorded a case(s) of STS, was any statistically significant excess of the disease observed. Similarly, in contrast to the situation with manufacturing cohorts, no case of STS occurred when none was expected in these cohorts of sprayers and applicators.

It is worth recollecting that only four of the ten series of case–control studies (and a possible fifth) which have specifically investigated the role of phenoxys and chlorophenols have detected any statistically significant association between STS and these compounds, in the study group as a whole or any subgroup of it.

Malignant Lymphomas

Eight of the twelve mortality studies of manufacturing cohorts have not accumu-

lated sufficient years of observation to adequately evaluate risk of non-Hodgkin's lymphoma and Hodgkin's disease. In none of the remaining four studies which were sufficiently large or which recorded a case(s) of ML in the cohort, was a statistically significant elevated risk of the disease observed. However, one case of Hodgkin's disease was recorded in one cohort when none was expected, and at least around a twofold excess of NHL or HD (which was not statistically significant) was observed in two cohorts.

Similarly, in the sprayer/applicator cohorts, of the six studies which were sufficiently large or which recorded a case(s) of ML, none showed a statistically significant increased risk of the disease. Eight studies were not sufficiently large to evaluate risk from ML. Nevertheless, a case of ML was recorded in two of them when none was expected.

As with soft tissue sarcomas, it is worth recollecting here also that only three of the eight case–control studies (and a possible fourth) which have specifically investigated the role of phenoxys and chlorophenols have detected any statistically significant association between ML and these compounds, in the study group as a whole or any subgroup of it.

One important issue that needs to be addressed additionally, for a complete and thorough review of this literature, is the problem of concomitant exposure to other chemical or biological agents which are potentially carcinogenic. Bearing in mind that the only evidence from case–control studies linking these compounds to cancer occurrence in humans is based on exposure during the use of these compounds, it is also pertinent to note that persons who occupationally use phenoxys *and chlorophenols (sprayers, applicators,* farmers) are invariably exposed to several other chemicals or oncogenic viruses concomitantly. Thus, unless these other potentially carcinogenic exposures were adequately controlled for in these studies, it could equally be argued that any increased risk observed in occupational groups which have used phenoxys/chlorophenols is due not to them, but to one or more of these other concomitant exposures.

The following are consistent with this hypothesis.

1. In relation to malignant lymphomas, out of a total of nine studies reviewed which have investigated both phenoxy acids/chlorophenols or herbicides, as well as some of the other concomitant exposures in any detail (Burmeister *et al.*, 1983; Cantor, 1982; Cantor *et al.*, 1985; Everett *et al.*, 1985; Hardell *et al.*, 1981; Hoar *et al.*, 1986; Hoar Zahm *et al.*, 1988a; Hoar Zahm *et al.*, 1988b; Saftlas *et al.*, 1987; Woods *et al.*, 1987), without exception, a statistically significant increased risk of ML was observed in all of these studies for one or more concomitant exposures other than phenoxy herbicides, such as nonphenoxy herbicides, insecticides, fungicides, solvents, fertilizers, etc. This consistency was not seen even with the candidate phenoxys/chlorophenols themselves. Furthermore, it appears that in nearly all of these instances, the highest statistically significant relative risk recorded was for one or more of these concomitant exposures rather than for exposure to phenoxy acids, chlorophenols, or herbicides.

Similarly with STS, few studies have investigated or provided data on the role of any of these other exposures individually (Eriksson *et al.*, 1981; Hardell and Eriksson, 1988; Hardell and Sandstrom, 1979; Hoar *et al.*, 1986; Hoar Zahm *et al.*, 1988a; Kang *et al.*, 1987; Woods *et al.*, 1987). Two of them (Hoar *et al.*, 1986; Hoar Zahm *et al.*, 1988a; Kang *et al.*, 1987) did not observe any statistically significant association with phenoxys or chlorophenols, but one recorded a statistically significant association with exposure to insecticides (Hoar Zahm *et al.*, 1988a).

2. No study has given the risk of ML or STS from exposure to phenoxys and chlorophenols after exhaustively controlling for these concomitant exposures, which may be extremely difficult to achieve, as it requires a priori knowledge of, and data on, all such possible exposures. It is noteworthy that in

the study by Woods *et al.* (1987) when other variables and some concomitant chemical exposures which were found to be significantly associated with ML in the study were entered in a logistic regression model, the risk for exposure to phenoxyacetic acids only was 0.85.

3. For both ML and STS, some case–control studies have reported a statistically significant association with occupations in which exposure to phenoxys/chlorophenols might occur, or even with spraying of herbicides, but no such association was observed with the compounds themselves. Furthermore, although an association was observed between ML and sawmill or paper/pulp mill workers in the two PMR studies, no such association was observed for either other occupation, or all occupations combined, in which exposure to phenoxys and chlorophenols might occur (Gallagher and Threlfall, 1984; Milham, 1982). Similarly, in the PMR study by Milham (1982) the 15 occupations with the highest risk of STS did not include occupations in which exposure to these compounds occurs, and no association between STS and all occupations (combined) in which exposure to phenoxys and chlorophenols might occur was observed.

4. In the Danish cancer incidence study of manufacturing cohorts by Lynge (1985) in which it was possible to separate phenoxy-exposed workers from those engaged in the production of other chemicals, the statistically significant excess risk of ML obtained for the factory was confined to workers engaged in the manufacture not of phenoxys, but of these other chemicals. In that same study it is noted also that the excess of STS occurred among workers in the shipping department of the factory where exposure to compounds other than phenoxys/chlorophenols occurred.

5. It is noteworthy that in none of the four cohort studies of users (i.e., sprayers and applicators) of reasonable size (Barthel, 1981; Coggon *et al.*, 1986; Royal Commission, 1985; Wiklund *et al.*, 1987, 1988), was any significant risk of STS or ML observed. This is not supportive of the case–control studies which have found significant risk in association with the use of phenoxy acids and chlorophenols.

It is important to note that since persons who use phenoxys and chlorophenols invariably are exposed to other candidate exposures simultaneously, it is obvious that studies in which risks of STS and ML have been given for exposure to phenoxys and chlorophenols mainly through their use (case–control studies, occupational cohort studies of users) may not be sufficient by themselves to evaluate risk associated with these compounds, unless these concomitant exposures have been adequately controlled for in the analysis, or the analysis is restricted to a study group or a subgroup within the data which used phenoxy herbicides or chlorophenols exclusively. Either of these two alternatives has been difficult to achieve, since the former requires a priori knowledge of and data on at least all the relevant concomitant occupational exposures, some of which have already been shown to have independent etiologic roles in the occurrence of ML and STS, while an adequate sample size may be a limiting factor for the latter even if such a group or subgroup can be identified. The value of existing case–control studies, and cohort studies of users, may therefore be restricted because of these factors. Parenthetically, there is need to investigate the role of these other concomitant exposures in the etiology of these tumors more thoroughly than has been done in the past. Future epidemiological studies should be directed toward further follow-up and/or pooling together of occupational cohort studies of workers exclusively or predominantly engaged in the manufacture of phenoxy acids and chlorophenols or of other suitable uniquely exposed groups.

CONCLUSION

It is concluded from this review that the weight of evidence does not unequivocally incriminate phenoxy acids or chlorophenols as a cause of malignant lymphomas and soft-tis-

sue sarcomas in humans, at least from exposure during the *use* of these compounds. This distinction between risk arising from exposure during use and risk arising during the manufacture of these compounds may be of importance in the future, as the intensity of exposure is considerably different in the two groups. Studies in which these compounds have been purported to be implicated have been plagued with the intractable problem of concomitant exposures, and it seems plausible that some of these other exposures, which were not all adequately controlled for in these studies, could well account for at least a significant part, if not all, of the apparent association. On the other hand, the vast majority of occupational cohort studies have not accumulated sufficient person-years of observation to date, yet even in the presence of this deficiency, cases of ML and STS have occurred when none is expected. Similarly, although there is at present no conclusive evidence that phenoxy acids cause cancer in animals, there is significant evidence that some of the chlorophenols do. It is therefore possible that the failure to demonstrate conclusive evidence of carcinogenicity in most of the animal and human studies may be the result of limiting factors in these studies rather than true lack of this effect. It is prudent therefore to wait for further animal and human data, particularly to wait until existing occupational cohorts have been combined and/or observed for longer periods, before a clear, decisive, and unambiguous evaluation can be made on the potential carcinogenicity of these very important compounds.

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FUNDAMENTAL AND APPLIED TOXICOLOGY 14, 235-242 (1990)

Assessing the Efficacy of Azaprophen and Physostigmine as a Pretreatment for Soman-Induced Incapacitation in Guinea Pigs by Response-Surface Modeling[1]

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Assessing the Efficacy of Azaprophen and Physostigmine as a Pretreatment for Soman-Induced Incapacitation in Guinea Pigs by Response-Surface Modeling. GENNINGS, C., CARTER, W. H., JR., HARRIS, L. W., CARCHMAN, R. A., CAMPBELL, E. D., BOYLE, R. M., TALBOT, B. G., AND SOLANA, R. P. (1990). *Fundam. Appl. Toxicol.* **14,** 235–242. Physostigmine (PHY) has the advantage over pyridostigmine of minimizing OP-induced incapacitation because it penetrates into the CNS. However, physostigmine is behaviorally toxic at relatively low concentrations. It is anticipated that this could be offset by a cholinolytic to prevent behavioral deficit due to the carbamate pretreatment alone. The therapeutic efficacy of physostigmine/azaprophen pretreatment therapy was evaluated in soman-challenged guinea pigs. Response surface methodology was employed to describe the relationship of the pretreatment combination with duration of incapacitation. The significance of the combination relative to PHY alone was evaluated in addition to dose combinations that yield optimal time to recovery. Analysis of the fitted response surface indicated that combination pretreatment with these compounds significantly reduces the time to recovery after soman challenge versus pretreatment with PHY alone. © 1990 Society of Toxicology

Physostigmine (PHY), an *N*-methyl carbamate and an inhibitor of acetylcholinesterase (AChE), in addition to being evaluated as a pretreatment against organophosphate exposure, has been used in the treatment of Parkinson's disease. Since PHY is behaviorally toxic at relatively low concentrations (Harris *et al.*, 1984b), it is anticipated this could be offset by a cholinolytic to prevent behavioral deficit due to the carbamate pretreatment alone (Mewaldt and Ghoneim, 1978; Harris *et al.*, 1984a).

Azaprophen (AZA; 6-methyl-6-azabicyclo-[3.2.1]octan-3-ol-2,2-diphenylproprionate) is a recently synthesized novel cholinolytic. It has structural features of both atropine and aprophen (Carroll *et al.*, 1987), both of which have been used successfully in conjunction with carbamate to antagonize intoxication by potent organophosphorus anticholinesterase agents (Fleisher and Harris, 1965; Leadbeater *et al.*, 1985).

A pretreatment dosage combination of PHY and cholinolytic which minimizes the dosage of PHY could provide a pretreatment which is without a behavioral decrementing effect. Because it penetrates into the CNS, PHY has the advantage over pyridostigmine of minimizing post-agent incapacitation

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Phenoxyacetic acid herbicides and chlorophenols and the etiology of lymphoma and soft-tissue neoplasms

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ABSTRACT

The phenoxyacetic acid herbicides and the chlorophenols are compounds of economic importance. The herbicide 2,4-D is widely used in agriculture, industry, and the home. Recently, concern has arisen over their safety as a result of studies linking these compounds with soft-tissue sarcomas and non-Hodgkin's lymphomas. We reviewed the available literature in order to advise a provincial government regulatory body and investigated methodologic issues by examining the pattern of reported cases in Alberta. We conclude that, taking into account the serious limitations on methodology in the available data, the evidence for a causal association is strongest for non-Hodgkin's lymphomas and probably reflects either a weak effect or, possibly, a confounding exposure associated with the use of 2,4-D. Given the worst-case assumptions, however, the potency of 2,4-D as a carcinogen is probably weak. Its intrinsic toxicity is less than that of alternative herbicides and the hazard posed by its use is probably much less than either the use of chemical alternatives or manual cleaning of vegetation, which carry a high risk of occupational injuries.

INTRODUCTION

Government and the agricultural community have become increasingly concerned about the safety and health aspects of the use of herbicides. This concern followed the demonstration that farmers were among those at elevated risk for certain types of cancers. This observation stands in contrast to the general observation that farmers have a decreased overall mortality experience. Subsequently, one group of herbicides, the phenoxyacetic acids, has generated particular concern as a result of recent research. The carcinogenicity of phenoxyacetic acid herbicides and chlorophenols remains an unresolved issue, however, due in part to difficulties in the classification of the cancers of concern, problems intrinsic to the study of a rare disease, and an uncertain toxicological data base. These chemicals are widely used in agriculture and industry. This report reviews the evidence for carcinogenicity of these compounds, as reflected in rates of soft-tissue sarcoma (STS) and non-Hodgkin's lymphoma (NHL) among occupationally-exposed users. Nosological problems with STS and

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limitations in study design are primary reasons why many studies have yielded inconclusive results. In this paper we review the studies on STS and NHL and offer recommendations for further study and public advisories. Table 1 lists the ICD-Oncology (1) classification numbers for the histologic types of greatest interest to this discussion.

We reviewed the world literature on this topic to provide an authoritative basis for regulation for Alberta Environment, the regulatory agency responsible for pesticide use and distribution in this western Canadian province where consumption of the herbicide is relatively heavy.

Table 1

Classification of histologic subtypes of soft-tissue sarcomas and lymphomas (1)

ICD No.	Description
Soft Tissue Sarcomas	
880	soft tissue tumors and sarcomas, NOS*
881–883	fibromatous neoplams
885–888	lipomatous neoplasms
889–892	myomatous neoplasms
904	synovial neoplasms
912–916	blood vessel tumors
918–920	osteomas, osteosarcomas
921–924	chondromatous neoplasms
925	giant cell tumors
926	miscellaneous bone tumors
938–948	gliomas
954–957	nerve sheath tumors
958	granular cell tumors and alveolar soft part sarcoma
Malignant Lymphomas	
959–63	lymphomas, NOS* or diffuse
964	reticulosarcomas
956–966	Hodgkin's disease
969	lymphomas, nodular or follicular
970	mycosis fungoides

* Not otherwise specified.

Phenoxyacetic acid herbicides and chlorophenols

Phenoxyacetic acids and the structurally-related chlorophenols are pesticides widely used for the control of weeds and fungi, and for wood preservation. (See Table 2 for names, abbreviations, and uses.) Commercial production of some of these pesticides may yield a number of contaminants, among them dioxins. Table 3 lists the known toxicity levels of these contaminants and the pesticide active ingredients, and are expressed by the LD_{50}. Toxicity in rats ranges over 6 orders of magnitude. Table 4 lists the pesticides in which dioxin contaminants are known to occur.

Contamination of herbicide preparations by dioxins greatly complicates toxicological assessments because the outcomes of concern are similar. 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD), one of the most studied dioxins, has received the most attention because of its toxicity in animal studies. In the early 1970s detection limits for the analysis of TCDD were in the parts-per-million range (2). Since the level of contamination in most herbicide products varied considerably, early reports quantifying dioxin levels must be interpreted cautiously. Even so, gross contamination of up to 47 ppm (3) and 100 ppm (4) has been reported. The carcinogen nitrosodimethylamine was also found to be a contaminant in MCPA at levels of from 200–600 micrograms to 2–18 mg/kg (3). Contamination levels and product formulations vary among contries and even among parts of the same country because the products are "designed" for specific regional conditions. Production quality control and analytical methods for the detection of contamination have improved dramatically in the last decade. Current allowable levels of contamination by TCDD are 0.1 mg/kg of the technical material, a standard instituted in Canada in 1975 and in the US in 1971 (2).

A factor further complicating the study of this group is that they have often been formulated with other herbicides, including other members of their chemical group. In 1953, 64% of the products available in Canada containing 2,4,5-T also contained 2,4-D (5). Recollection by study subjects of products used in the past is confused by a plethora of similar-sounding names and by changes in product concentration using the same or similar names by either the same manufacturer or a competitor. 2,4,5-T is no longer manufactured and is not sold in Canada or the US.

Table 2

Abbreviations, names, and uses of phenoxyacetic acid herbicides and chlorophenols

Abbre-viation	Commercial name	Chemical name	Uses
2,4-D	2,4-D	(2,4-dichlorophenoxy) acetic acid	herbicide
	dicamba	3,6-dichloro-2-methoxy benzoic acid	herbicide
DCP	dichlorophenol	2,4-dichlorophenol	2,4-D precursor
2,4-DP	dichlorprop	2-(2,4-dichlorophenoxy) propionic acid	herbicide
	fenoprop, Silvex	2-(2,4,5-trichlorophenoxy) propionic acid	herbicide, control of preharvest apple drop
HCB	hexachlorobenzene	2-2′-methylene bis (3,4,6-trichlorophenol)	disinfectant in toothpaste and mouthwash
	hexachlorophene		prescription drug
MCPA	MCPA	4-chloro, 2-methyl phenoxyacetic acid	herbicide
MCPP	mecoprop	2-[(4-chloro-o-tolyl)oxy]propionic acid	herbicide
PCP	pentachlorophenol		wood preservative, insecticide, disinfectant
2,4,5-T	2,4,5-T	(2,4,5-trichlorophenoxy) acetic acid	herbicide
TTCP	tetrachlorophenol	2,3,4,6-tetrachlorophenol	wood preservative
TCP	trichlorophenol		disinfectant, 2,4,5-T precursor
	triclosan	5-chloro-2-(2,4-dichlorophenoxyl) phenol	fungicie use in soaps, detergents, and medicated cosmetics

Table 3

Animal toxicity of the dioxin contaminants, phenoxyacetic acid herbicides, and chlorohenols (79,80)

Homologue name	Isomer	LD_{50} (mg/kg body weight)
di-dioxins	2,7	>2,000
	2,8	300
tri-dioxins	2,3,7	30
	tetra-dioxins2,3,7,8	0.002–5
	1,2,3,4	>1,000
penta-dioxins	1,2,3,7,8	0.003–0.338
	1,2,4,7,8	1.1–5
hexa-dioxins	1,2,3,4,7,8	0.07–.8
	1,2,3,6,7,8	0.07–1.3
	1,2,3,7,8,9	0.06–1.4
hepta-dioxins	1,2,3,4,6,7,8	>0.6
octa-dioxin		>1,000
2,4-D	acid	699
	sodium salt	997
	butyl ester	695
	dimethylamine salt	949
	iso octyl ester	896
	isobutyl ester	618
	butoxyethanol ester	850
dicamba		2,900
dichlorprop		800
fenoprop		1,000
MCPA		700–800
mecoprop		930
pentachlorophenol		50–140
2,4,5-T		500
tetrachlorophenol		470
trichlorophenol		1,000–3,000

Table 4

Dioxin contamination of some pesticides

Pesticide	Known contaminants
2,4-D	di-, tri-, and tetradioxins (but not 2,3,7,8-TCDD)
fenoprop	2,3,7,8-TCDD
hexachlorophene	2,3,7,8-TCDD
pentachlorophenol	hexa-, hepta-, octadioxins
2,4,5-T	2,3,7,8-TCDD
tetrachlorophenol	hexa-, hepta-, octadioxins
triclosan	di-, tri-, tetradioxins

Occupations in which exposure to the phenoxyacetic acid herbicides and chlorophenols are likely to occur are listed in Table 5. It is clear from the diversity of these occupations that the pesticides have wide and important use in the economy. A recent federally-sponsored study of the effect of a withdrawal of 2,4-D on the Canadian economy suggests that the cost would fall between $130 and $200 million per year (Canadian dollars), mostly incurred by the agricultural sector. Much of the cost would result from substituting other methods of brush and weed control, including manual removal and more expensive alternative herbicides (6). 2,4-D is no longer protected by patent and is much less expensive than commercial alternatives which are so protected (7). 2,4-D has also been more extensively studied than alternative herbicides, many of which are known to be more acutely toxic (8).

Biological effects of phenoxyacetic acid herbicides and chlorophenols

A recent field experiment conducted at the University of Guelph is useful in separating out "dislodgeable residues" from total recoverable 2,4-D in grass (9). Within hours of application less than 4.5% of the

2,4-D applied could be dislodged. This declined to less than 0.02% over 7 days even in dry conditions that would maximize retention. We estimate from the data that there is a *maximum* half-life of about 19 hours for the dislodgeable residues. Thus, in the absence of rain only about 1% of the application would be "dislodgeable" after 2 days. This estimate would be modified downward for different (more dilute) commercial formulations, rainfall, or mowing.

Pharmacokinetic data on the rate of absorption and the turnover of

Table 5

Occupations associated with possible pesticide exposure

agriculturalists	new pesticides under development
crop farmers and farm workers	herbicides, insecticides, fungicides insecticides, litter from treated wood chips
nursery and related workers	herbicides, insecticides, fungicides
farm machinery operators	herbicides, insecticides, fungicides
chemical processing operations	all pesticides
flour and grain milling	seed treatments, fungicides
sawmills	fungicides
wood treating occupations	fungicides
wood treating occupations	wood preservatives
laboring—wood	fungicides
pulp and paper	fungicides
textile and rope finishing and calendering	fungicides
leather manufacturing and processing	fungicides
rubber processing, fabricating & repair	fungicides, curing compounds
petroleum industry	slimicides
railway section and track workers	herbicides, wood preservatives
electrical power: erect, install, repair	herbicides
other construction trades	herbicides
air pilots	all pesticides
air transport operating occupations	all pesticides
grain handling	seed treatments, fungicides
polyvinyl adhesives manufacture application	preservative
photographic solutions	preservatives

specific herbicides and their solvent carriers are also important in interpreting possible health effects. 2,4-D is poorly absorbed through the skin. The metabolic fate of a subcutaneously injected ester of 2,4-D has been studied in the rat (10). 95% of the administered dose was excreted in the urine as free acid within 48 hours. Studies on farmers going through their work routine in Saskatchewan using dermal and clothing pads have provided data on absorption and excretion (11). The amount of 2,4-D metabolite in urine was significantly correlated with the amount of herbicide sprayed. The time required for excretion of the urinary metabolite to return to background concentration was related to the amount sprayed and the number of exposures.

Chromosomal aberrations, sister chromatid exchanges, and micronuclei are morphological changes in cells recognized to be early indications of genotoxicity. Mutagenic studies in microbial and yeast systems have yielded conflicting results (3). Davring and Hultgren (12) demonstrated that an ester of 2,4,5-T acts as a mitotic poison or clastogen in bone marrow cells from male mice. A risk assessment conducted in 1981 by the US Environmental Protection Agency (13) concluded that there was "substantial toxicological and epidemiological evidence that TCDD is a potent carcinogen". Shu et al. have concluded that the data overwhelmingly support a role for TCDD as a tumor promotor rather than as an initiator (14).

There are conflicting reports of chromosomal changes in humans exposed to phenoxyacetic acid herbicides. Yoder et al. described increased frequency of chromatid lesions in lymphocytes from agricultural workers during a season of work involving exposure to pesticides including 2,4-D and 2,4,5-T (15). Hogstedt et al., on the other hand, reported no such increase in a group of Swedish male workers exposed during the spraying of a number of pesticides, including 2,4-D, MCPA, and mecoprop (16). Crossen et al. observed a larger than normal number of sister chromatid exchanges in lymphocytes from workers exposed to 2,4-D and 2,4,5-T in New Zealand (17).

Other than studies evaluating chromosomal changes in lymphocytes, experimental testing of the effects of phenoxyacetic acid herbicides on the immune system is limited. In screening a number of pesticides, Vos et al. reported that 2,4,5-T had a histologically visible effect on the tissues of the immune system (18). Blakley administered sublethal levels of the *n*-butyl ester of 2,4-D dermally to female CD-1

mice (19). There was minimal clinical or pathological alteration and no effect on antibody production but the treatment did enhance B-lymphocyte proliferative responses. Subacute oral doses of the same pesticide failed to demonstrate immune effects (20). A recent report by Hoffman et al. demonstrated an immunosuppressive effect of TCDD in humans which was consistent with evidence from animal studies showing T4 lymphocyte depletion and a decreased T4/T8 ratio (21).

Thunberg (cited in Axelson, 3) has drawn attention to similarities between the effects of vitamin A deficiency and TCDD exposure, namely, epithelial lesions and infections, acne and keratosis, failure of normal growth, immunosuppression, reproductive abnormalities, teratogenesis, and lesions in the skeleton and nervous system.

Problems in study design

Epidemiological studies of pesticides share common methodological problems. Subjects are exposed to the substance at widely varying exposure levels and exposed intermittently and often concurrently with occupational exposures to other potentially toxic substances. Any risk assessment exercise must therefore interpret the available studies with caution. Six methodological problems are particularly significant in the study of phenoxyacetic acid herbicides and chlorophenols:

1) A fundamental requirement of these studies is to document the nature and duration of exposure. None of the available studies has determined exposure levels accurately. Except in the rare case of relatively well-documented exposure of production workers, most studies rely on either a detailed recall of distant events (sometimes from a deceased subject's relatives) or obtaining death certificate analyses with implicit assumptions regarding the extent of exposure based on occupational title or employment records. Recall is obviously subject to bias. Some retrospective studies have obtained information about exposure by asking interview questions about events that happened more than 20 years before the time of the interview. Pesticide formulations and application techniques have changed with time. Application work tends to be seasonal and information about exposure can be extremely inaccurate because detailed records are rarely kept. Some investiga-

tors have attempted to verify responses by checking supplier sales records. Even this approach cannot be regarded with confidence because, in general, only recent records can be confirmed.

2) The limitations of using death certificates as the sole source of data for cause of death or the decedent's occupation has been well documented (22, 23). The disease itself may be under- or over-reported on death certificates (23) and the occupation coded on the form will be the last occupation or that of longest duration. This occupation is not necessarily that of most interest toxicologically. Mortality rates may differ widely among occupational groups for reasons of selection that are not related to the chemical exposure and these differences often persist well past retirement (24). A chemical exposure profile can only be inferred from death certificates. Studies that rely on death certificates alone may seriously underestimate risk because of the lack of pathological confirmation of the malignancy and omission of incident cases in subjects dying of other causes. However, available population-based studies of mortality or disease frequency by occupation often omit essential data needed to derive refined risk estimates. They aggregate occupations so as to dilute the experience of exposed groups with that of unexposed groups (25).
3) There is considerable uncertainty over the latency period one should assume before the effects of interest appear. Different investigators have applied inconsistent criteria in selecting estimates of latency in the analysis of their data. Although 20 years is commonly cited as a reasonable latency period for exposure-induced cancer, it may be too long for hematopoietic and lymphatic malignancies and is likely to be too short a time to detect any peak in excessive incidence of carcinomas. The minimum appropriate latency period for STS and other types of sarcoma is unknown.
4) The power of a study depends upon the numbr of subjects and the magnitude of the risk involved (26). A rough estimate of the proportion of the population exposed to phenoxyacetic acid herbicides and chlorophenols on a regular basis can be made by examining the proportion of farmers in the general population. This varies from almost 0% in urban populations to 11% in Alberta and 20% in some rural areas of the US (27). If a power of 80% is considered to be the acceptable minimum, either large

numbers of study subjects or significant health effects are required. Figures 1 and 2 are power curves showing the relationship between number of cases and the assumptions regarding effects size. Figure 1 is based on the assumption that 10% of the population is regularly exposed; Fig. 2 is based on a corresponding figure of 20%. Groups of 50 or fewer subjects cannot reliably detect a risk ratio less than 3 by these criteria, even when there is a high ratio of controls to cases in a traditional case-control study model.

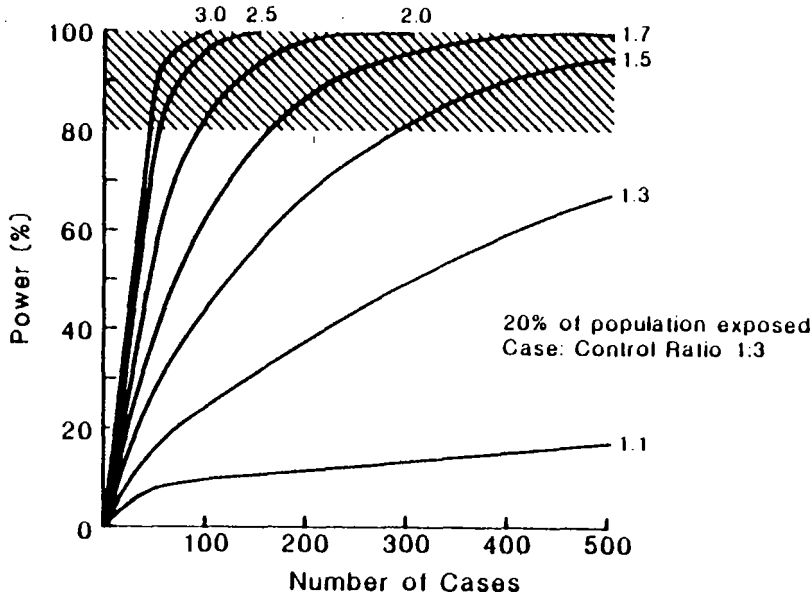


Fig. 1. Power functions: 20% of population exposed to the risk and a case-control ratio of 1:3.

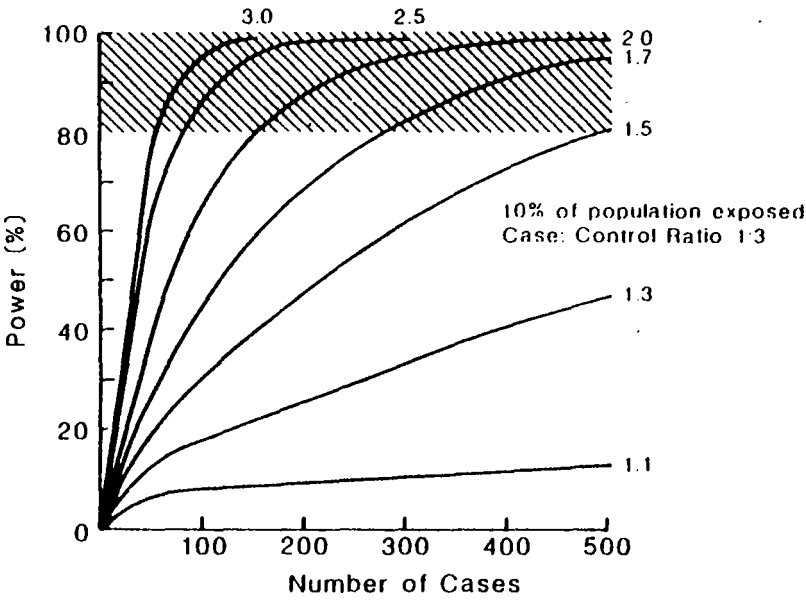


Fig. 2. Power functions: 10% of population exposed to the risk and a case-control ratio of 1:3.

5) In a cohort study the treatment of subjects lost to follow-up can alter the study's findings (28). The common practice of assuming that those lost to follow-up are in fact alive at the end of the study will underestimate mortality. Many investigators prefer this conservative approach as one less likely to yield a spurious association.
6) The age distribution of subjects chosen for the study can radically affect the outcome. Young people are generally omitted from case-control studies because their exposure is assumed to have been low. This may be a false premise because they may have been exposed prenatally or in their home environment. Laundry, for example, has proven to be an extremely efficient method for the redistribution of pesticide residues (29). The public's perception of pesticides has altered radically in recent years. As little as 10 years ago parents may not have considered pesticides to be as dangerous to their children as they do now.

Although these methodological problems are difficult to assess or to overcome, their individual and collective effects are likely to underestimate the risk associated with exposure to the phenoxyacetic acid herbicides. Thus, demonstration of an elevated risk in such studies should be considered as an improbable outcome suggestive if it is observed. The risk estimates themselves, in this view, serve not so much as measurements of the magnitude of risk but as indicators of an elevated risk of unknown but larger magnitude.

SOFT TISSUE SARCOMAS

The epidemiological study of soft-tissue sarcomas (STS) typifies may of the problems inherent in occupational epidemiology. The nature and duration of exposure are difficult to quantify, death certificate entries on occupation cannot give an adequate exposure profile, latency is unkown, and the treatment of subjects lost to follow-up can radically alter results. There are also problems specific to studies of STS such as the low overall incidence in the populations and the definition and identification of STS. Table 6 summarizes the results of the STS studies discussed.

The soft-tissue sarcomas present a difficult problem. Two disease classification systems are used for neoplasms. One is based on anatomic side (ICD-9) and the other on tissue type (ICD-O). Death certificates use ICD-9 only, but cancer registries use both. Because soft-tissues are scattered throughout the body they are difficult to identify in the anatomic (ICD-9) system. Data from the Alberta Cancer Board demonstrate that only 45% of the soft-tissue sarcoma cases can be identified as such. In other geographic areas as few as 30% of cases can be identified. The tumors themselves are rare and histologic differentiation of the neoplasms is difficult but improving with new technology as more specific cell markers are developed.

The study of soft-tissue sarcomas presents several unique problems:

1) The STS are classified awkwardly in the International Classification of Diseases (ICD) system. This system is organ-based. For example, soft-tissue sarcomas arising in the mesenchyme of the stomach would be classified under stomach rather than under ICD 171: Malignant Neoplasm of Soft and Other Connective Tissue. If ICD 171 is the only category used for patient identifi-

cation, 50–75% of cases may be missed (19). This can be confirmed by an examination of data from the province of Alberta. (We are indebted to Ms. S. Fincham and Dr. M. Koch for making these data available to us.) Figure 3 presents ten years of histologically selected cases from the Alberta Cancer Board as originally coded. Figure 3 shows all cases coded in ICD-9 171 against those coded otherwise. Only 45% of all histologically identified cases of soft-tissue sarcoma were coded as ICD-9 category 171. ICD-9 171 cannot, because of its restrictive criteria, be considered a representative sample of all cases.

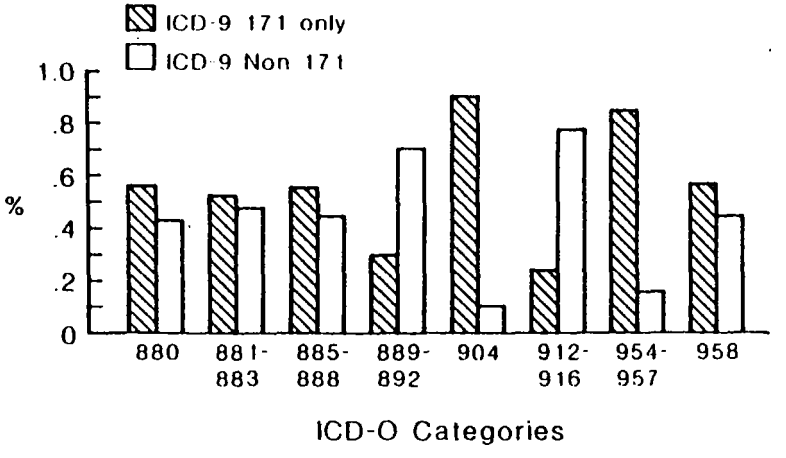


Fig. 3. Soft-tissue sarcoma ICD-O subclasses, showing the percentage of cases in each histological (ICD-O) classification that were classified by the turnover registry under ICD-9 category 171 compared to those classified in all other ICD-9 categories.

2) These tumors are rare. Again, referring to Alberta data, the combined annual incidence rate is only 3.89 per 100,000 population if determined from ICD-9 or 8.5 per 100,000 population if calculated from ICD-O categories (30). It is difficult to recruit sufficient patients for a case-control study and cohorts would have to be extremely large to identify even a five-fold increase in risk. There is also a bimodal distribution that includes a peak in

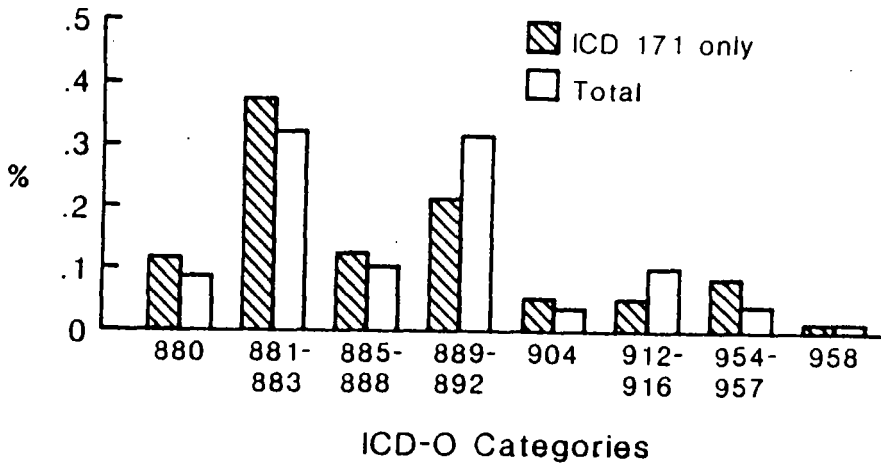


Fig. 4. The percentage of each ICD-O classification of soft-tissue sarcomas classified by the tumor registry under ICD-9 category 171, compared to those classified in all other ICD-9 soft-tissue sarcoma categories showing similar distributions.

early childhood and differing rates between males and females, females being underrepresented in exposed occupations. These features further reduce the number of cases available for the study of occupational associations of these tumors (31).

3) In spite of the common use of the term "soft-tissue sarcoma," there is considerable variation in its operational definition. Some pathologists include extra-skeletal osteomas in the group but others do not. Gliomas may or may not be included and some researchers exclude visceral sarcomas. Mesothelioma, a visceral sarcoma, is usually classified separately but there may be overlap in pathological definition.
4) Histological differentiation within the STS group is difficult and requires classification by a pathologist familiar with these subgroups (22,31,32). Woods et al. reviewed all STS cases in one study (32). He ultimately retained the original diagnosis in 45% of the cases, changed the histologic diagnosis within the general category of STS in 34%, and rejected 21% as not STS or of

indeterminate pathology. Such detailed typing is usually restricted to research institutions.

An association between sarcomas and pesticide exposure was first suggested in 1974 by Axelson et al. (33, 34). They found a significantly higher tumor incidence and mortality rate from malignancies in Swedish railroad workers exposed to the herbicide amitrole, a suspect animal carcinogen that is chemically unrelated to the phenoxyacetic acids. In the analysis, it was found that the affected workers had also been exposed to 2,4-D and 2,4,5-T and to diuron and monuron, two urea herbicides unrelated to the phenoxyacetic acids. In 1977 Hardell described a group of STS patients who gave histories of occupational exposure to phenoxyacetic herbicides (35). Axelson subsequently re-studied Hardell's cohort and suggested that the phenoxyacetic acid herbicide exposure might have been responsible for results in the earlier study but that a ten-year latency may be needed for the disease to appear (34). This conclusion generated a flurry of studies in Sweden and abroad (Table 6).

The initial findings by Hardell generated two case-control studies. In the first, Hardell mailed a questionnaire to STS patients and to controls drawn from the general population of northern Sweden (36). In the analysis he examined several possible confounders, among them exposure to DDT, diesel oil, and tobacco smoking. They found that

Table 6

Summary of studies on soft tissue sarcomas

Cohort type	Study outcome		
	Positive and stat. signif.	Positive, but not stat. signif.	Negative
manufacturers			42,43,44,45,46,47
forestry and agriculture		56,57	32,59*,62*,63
Vietnam veterans			51,52,54,55*
pesticide exposed	33-34*,35,36*,37*		60-61

* Histologically confirmed STS cases.

\- Indicates same study or population described in different reports.

exposure to phenoxyacetic acids or chlorophenols yielded a relative risk (RR) of 5.7 (95% confidence interval 2.9, 11.3). Exposure to phenoxyacids alone yielded an RR of 5.3 (2.4, 11.5) and to chlorophenols alone, an RR of 6.6 (95% confidence interval by test-based method: 1.4, 31.1). These are very high estimates of relative risk.

In the second study, Eriksson et al. (37) identified cases of STS from southern Sweden. They regarded persons in contact with cutting oils and in the shoe and leather industry as unexposed and used them in the control group. A relative risk of 6.8 (95% confidence interval 2.6, 17.3) was found for exposure to pehnoxyacids and 3.3 (1.3, 8.1) for exposure to chlorophenols. This estimate is remarkably consistent with the estimate in the second Hardell study (36).

Both the second Hardell (36) and the Eriksson et al. (37) studies examined all histologically diagnosed cases of STS (not just those under ICD 171) and excluded from analysis all who had sustained pesticide exposures within the previous 5 years only or for a duration of less than 1 day. Both investigators supplemented the questionnaire information with telephone interviews but only for pesticide exposure data. This raised the question of bias, which they attempted to control by using another group of cancer patients as a second set of controls, reasoning that recall bias from concern over health would generally be found among cancer patients. Use of this second control group did not uncover evidence of bias.

Manufacturing plant workers

Following the study of Eriksson et al. (37) a series of case reports appeared in the literature describing patients with STS who had worked in pesticide production plants (38-41). Subsequent review of the histology reclassified two cases as carcinomas, not sarcomas. One case report described the occurrence of STS in a father and son, both of whom worked in the same plant, and raised the possibility of a genetic predisposition to the effects of exposure. However, the histologic types were very different (Schwannoma and lipoma) and the two had very different exposure histories (40).

Several studies of US production plant cohorts (42-47) found no statistically significant excess of STS. Three of the studies were small (61, 121, and 204 persons) and had a very limited statistical power to detect increases. All had assumed a sufficient latency between

exposure and outcome of study. Two of the studies used chloracne as an indicator of exposure. Chloracne in man usually occurs as comedomes in a malar distribution and on the pre- and post-auricular portions of the face and head and is often accompanied by hirsutism, melanosis, or secondary infection (48, 49). The threshold level for development of chloracne varies considerably among individuals (49), making it a poor indicator of exposure.

Agent Orange

Forty million liters of a 50:50 mixture of 2,4-D and 2,4,5-T named Agent Orange was applied to the tropical jungle in Southeast Asia as a defoliant by the US military in the Vietnam War. The herbicide was applied to clear vegetation from around bases and camps and along communication lines, and to expose operational staging areas. Contamination by 2,3,7,8-TCDD at levels of 0.02-54 micrograms/g have been reported in the Agent Orange used at the time (46). Agent White and Agent Blue were two other defoliant sprays used by the US military. Agent White contained picloram, which has been demonstrated to be a carcinogen in rats and mice; Agent Blue contained salts of arsenic (50). Up to 2 million American military personnel and many more Vietnamese could have been exposed to one or more of these mixtures. In a study of 1,024 men who sprayed the herbicides in Vietnam ("Operation Ranchhand" personnel) there were no statistically significant excesses of cancer in a baseline mortality study (51). Despite the numbers, the power of the study was limited because of the rarity of STS and the low numbers of expected cases.

Kang et al. (52) gave the results of a hospital-based comparison group study of veterans who served between 1964 and 1975 and who were diagnosed in a Veteran's Administration (VA) Hospital as having STS (ICD 171). The controls were selected from the same hospital population. Military service information was abstracted from the US National Personnel Records Center as a surrogate for exposure data. No significant association between STS and previous military service in Vietnam was observed (OR = 0.83, 95% confidence interval 0.63, 1.09). This study must be interpreted with caution and has been highly controversial because personal exposure data were missing and outcome measurements were uncertain. In a follow-up study to examine potential bias in identifying STS, Kang et al. (54) stratified

the data on hospital type and found no statistically significant risks.

Many cases of STS may have been missed because only ICD 171 was used to identify the cases. During the Vietnam era there was poor, and sometimes biased, military record keeping (53), a problem aggravated by a fire in the central records depository that destroyed many records in the early 1980s. It is probably impossible to establish satisfactory estimates of individual exposure. Outcome measurements may be equally difficult to obtain. Patients in VA hospitals also may not contribute a representiative sample of the total veteran population. Unfortunately, case ascertainment was poor because many hospitals refused to particupate in Kang's study.

The only Vietnam veteran study to use histologically-identified STS was that by Greenwald et al. (55), who used interviews to collect data on 151 live and 130 dead cases, including military service, occupatioal history (emphasizing exposure to herbicides), specific chemical exposures, family history, medical history, smoking, and alcohol abuse. They found no significant association with any particular type of herbicide exposure. Some of their cases may not have had a sufficient latency period (15-20 years) for cancer to develop if the latency for STS is comparable to that for other cancer types.

Other studies

Three other studies of largely agricultural populations have reported detecting a significant excess of STS.

Balarajan (56) examined STS among farmers, agricultural workers, and related occupational groups in England and Wales. One of the four occupational subgroups in the study (including farmers, farm managers, and market gardeners) showed a relative risk of 1.7 (95% confidence interval, 1.00, 2.88). Unfortunately, only 70% of their cancer patient registrations had an occupation listed and it is not known whether there would have been a selective bias in recording occupation.

Vineis et al. (57) identified STS cases through hospital pathology departments in 3 provinces of Italy. Cases were verified histologically but visceral cases were excluded. Ascertainment was poor and introduced the possibility of bias by excluding cases with characteristics not shared with respondents (58). Vineis et al. found an increased risk of 2.7 (not significant at $p = 0.05$) among female rice weeders.

El Zayadi et al. (59) found that 10 out of 14 patients with hepatic

angiosarcoma in Egypt had a history of direct, chronic, recurrent exposure to agricultural pesticides. The types of pesticides were not described.

Most other studies of STS have found no association between phenoxyacid herbicide or chlorophenol use and soft-tissue sarcoma. Smith et al. (60, 61) did not find significant results in a case-control study of all cancers registered in the New Zealand Cancer Registry. Hoar et al. (62) used histologically verified cases and found no increase in risk for STS among white male residents of Kansas who gave a history of work with herbicides including phenoxyacid herbicides and chlorophenols. Wiklund and Holm (63) studied a cohort of Swedish men who had been employed in agriculture or forestry in 1960 and found no significantly increased risk either in the whole cohort or in any of several subgroups. In Washington state Woods et al. (32), reported that study subjects with any past occupational exposure to phenoxyacetic acid herbicides had an estimated RR of 0.80 (95% confidence interval 0.5, 1.2). They used histologically verified cases of STS.

In 1976 there was an industrial accident and explosion at a chemical plant in Seveso, Italy, exposing many local residents to trichlorophenols and dioxins. Local health authorities set up a health monitoring program and a cancer registry. By crudely comparing contaminated and uncontaminated areas, based on TCDD levels in soil, there appears to be a clear difference in the incidence of STS in 1980-81 (48). Unfortunately there are no comparably ascertained STS incidence rates from before the accident by which to establish any trend. Puntoni et al. suggest that it is highly unlikely that this increase could be due to the accident, however, as the latency period would then be only four years (64).

LYMPHOMAS

Lymphomas, like soft tissue sarcomas, arise from tissue of mesenchymal origin. They are divided into Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL) on histologic criteria. NHLs are commonly divided further in the ICD-9 into prolymphocytic lymphosarcoma, malignant lymphoma, lymphosarcoma, and reticulosarcoma (1). This classification scheme is considered obsolete by clinical

investigators, who have replaced this crude system with much more detailed nosologies based on morphology and immunological typing. Cancer registries use both systems. Studies on the association between lymphomas and the use of phenoxyacid herbicides or chlorophenols are summarized in Table 7.

In other settings, risk for NHL is associated with immunological disorders or immunosuppressive treatment. The possibility of an association with immunostimulation has not been fully documented but it is of interest that 2,4-D is a stimulus to B-cell proliferation, given that NHLs are predominantly of B-cell origin (20, 31).

Goldsmith and Guidotti (65) studied deaths due to lymphosarcoma in California males in 1971 and 1972. Based on 1970 census data they found a significant excess among farmers, farm managers, and farm laborers when compared to the expected number. In 1974, the American Cancer Society published a survey of malignancies in Iowa between 1969 and 1971. DeKraay (66) examined the distribution and suggested a link between pollution in drinking water on farms and lymphoma. In a death certificate study of Iowa farmers (67),

Table 7

Summary of studies on lymphoma

	Study outcome		
Cohort type	Positive and stat. signif.	Positive, but not stat. signif.	Negative
manufacturers			47
agriculture	45,62,65,66,67,68 71,72,77,78	32,82+	
forestry and wood	32,79*,80*	74	82+
pesticide exposure	69		32,75-76-77

* Hodgkin's disease only.

\+ includes fishing.

– indicates same study or population described in different reports.

Burmeister found a statistically significant SMR of 1.29 for lymphoma in spite of a significantly lower overall cancer rate. In a further case-control study (68), he found a statistically significant odds ratio of 1.26 for farming and NHL.

In 1979, Hardell (69) noted that several patients with lymphoma had reported exposure to phenoxyacids and chlorophenols. In a case-control study (70), he sent self-administered questionnaires to patients with NHL of the histiocytic type and to controls and obtained an OR of 6.0 (95% confidence interval 3.7, 9.7) for exposure to phenoxyacids or chlorophenols. He calculated an OR of 4.8 (2.9, 8.1) for exposure to phenoxyacids only.

Males in Wisconsin who had died of NHL, as recorded on their death certificates, were studied by Cantor (71), who found that farmers less than 65 years of age were more likely to have NHL than non-farmers (OR = 1.67,95% confidence interval 1.14, 2.5). Reticulum cell sarcoma was more common among farmers under 65 years of age, with an OR of 2.7 (1.54, 4.5).

Using computerized death certificates in Wisconsin, Saftlas et al. (72) examined causes of death in all persons whose "usual occupation" was described as farmer. Since farmers smoke less than non-farmers, smoking-related causes of death were removed from the analysis and a proportionate (corrected) mortality ratio (PCMR) was calculated on the remaining deaths. He found a statistically significant PCMR of 1.25 for the entire cohort of farmers. This could not be linked to specific types of farming when the degree of agricultural activity in each county was used as a surrogate for individual exposure.

As with soft-tissue sarcomas a series of case reports on lymphoma in pesticide production workers appeared in the literature (73, 74). Unfortunately, most manufacturing cohort studies have focused on STS and did not consider NHL. Only two studies specifically examined the risk for lymphoma. One did not uncover a statistically significant excess. One of the largest studies of manufacturing cohorts is that by Bond et al., who were primarily investigating an alleged association between 2,4-D exposure and brain tumors suggested by animal studies (47). Among 878 chemical workers potentially exposed to 2,4-D between 1945 and 1982, they found two deaths from NHL, for an SMR of 391 (95% confidence interval: 44, 1411). Although elevated, this finding is not easily interpretable because of the small number involed and resulting wide confidence interval. Although it

cannot be considered evidence for an association, the study is suggestive. In other studies (42-44) the sample sizes have been too small to attain convincing power and the latency periods too short.

Hoar et al. (62) studied white male residents of Kansas diagnosed with NHL. During a structured telephone interview participants were asked detailed questions about farming practices. Hoar et al. found no trend with years spent living or working on a farm but did find a significant trend between the risk of NHL and increasing years of herbicide use and number of days of herbicide exposure per year. With more than 20 days per year of exposure the OR was 6.0 (95% confidence interval: 1.9, 19.5). Subjects who reported usually mixing or applying the herbicide themselves showed an OR of 1.9 (95% confidence interval: 1.1, 3.3) compared to those who had someone else perform this function (OR = 1.1; confidence interval not available). Farmers who did not use protective equipment with herbicide use had a higher odds ratio (OR = 2.3; 95% confidence interval: 1.0, 4.2). Higher risks for herbicide use were also seen among farmers who used backpack or hand sprayers (OR = 2.3; 95% confidence interval: 1.0, 5.2). They also examined the known associations affecting risk for lymphoma, including conditions resulting in immunosuppression, drugs, family history, cigarette smoking, coffee consumption, and ionizing radiation. Other than herbicide use, only family history had a significant OR of 2.3 (95% confidence interval: 1.6, 3.2). Their sample of farmers had an unusually high percentage (77%) of people who "never used" herbicides. Farming practices in the "grain-belt" make this statistic highly improbable. However, the bias introduced by error in determining use of herbicides would tend to *underestimate* the risk calculated. This study has since been replicated in Nebraska.

Pearce et al. have conducted several studies in new Zealand (75-77). In a 1985 case-control study (75) of lymphoma and multiple myeloma, they found an OR of 1.25 for agriculture or forestry workers, using current or most recent occupation on death certificate. For ICD category 202, the OR was 1.76. They then inteviewed lymphoma cases registered under ICD 202 and found no significant results for exposure to phenoxyacetic acid herbicides or chlorophenols (76). Meat packing workers exposed to 2,4,6-trichlorophenol and zoonotic viruses demonstrated an OR=5.7 (90% confidence interval 2.3, 14.3; p=0.03). There was an elevated risk for fencing work but in New Zealand only 5% of the pesticides used in this type of work are chlorophenols. They

study was then expanded to include ICD category 200 (77). Neither the combined cohort nor the new classification showed a statistically significant excess associated with exposure to phenoxyacetic acid herbicides or chlorophenols. It is interesting to note that ICD category 200 had two results that were high but failed to achieve statistical significance for either railway workers who sprayed chemicals or for Ministry of Works employees who sprayed chemicals on public lands.

Schumacher (78) studied white male Utah residents with a diagnosis of NHL occurring after their 35th birthday and who died before their 75th birthday. Occupations as reported on death certificates were analyzed for cases and for a series of controls with adenocarcinoma of the colon. Stratifying the data by year of diagnosis revealed an OR for any type of agricultural occupation of 6.6 (90% confidence interval 1.2, 3.57) for those diagnosed between 1952 and 1966; 3.1 (95% confidence interval: 1.2, 8.1) for those between 1966 and 1971; and 1.0 by the period 1972-77. There may not have been a sufficiently long latent period between exposure and the latest time period studied.

Studies of Hodgkin's disease (HD) do not appear to demonstrate an association with herbicide exposure but do suggest an association with exposure to wood dust. Milham and Hesser (79) analyzed death certificate occupations for 1549 white males aged 25 years or more who had died of HD in New York state excluding New York City in 1940-53 and 1957-64. Their study revealed a preponderance of men whose work entailed exposure to wood. Grufferman et al. (80) also found an excess risk of HD among woodworkers in the Boston Standard Metropolitan Statistical Area. They studied histologically confirmed HD cases and compared them to the 1960 and 1970 census data for the same area. As will be discussed below, this observation may provide a lead for investigating the mechanism of carcinogenicity.

Mortality patterns for deceased members of the American Federation of Grain Millers Insurance Plan were studied by Alvanja et al. (81). Occupational information was taken largely from employment and union records. Questionnaires were sent to a sample. Alvanja et al. found a PMR=202 ($p<.005$) for disease of the lymphatic and hematopoietic system and a PMR=272 for other lymphatic neoplasms.

Woods et al. conducted a case-control study in Washington state (32) to specifically examine the risks of using the phenoxyacetic acid herbicides or chlorophenols. Among study subjects with past occupational exposure to phenoxyacetic acid herbicides the estimated

RR was 1.07 (95% confidence interval: 0.8, 1.4). For farmers the RR was 1.33 (95% confidence interval: 1.03, 1.7) and for forestry herbicide applicators it was 4.8 (95% confidence interval: 1.2, 19.4). When the analysis was restricted to anyone with exposure to phenoxyacetic acid herbicides in any occupation for 15 or more years during a period 15 years prior to cancer diagnosis the RR was 1.71 (95% confidence interval: 1.04, 2.8).

Occupation and risk of cancer in Denmark was studied by Olsen and Jensen (82) in a computerized death certificate study. They did not find a significant excess of NHL or HD in males or females employed in agriculture, forestry, or fishing. The inclusion of fishing, which has never been implicated as a suspect occupation, may have diluted the result.

Wiklund and Holm (83) considered that increasing pesticide use would have resulted in increased risks in recent decades but found no statistically significant change in the RR for NHL or HD over 1961-79 in agricultural occupations.

DISCUSSION

Taking into account the limitations inherent in the methodology of all available studies, we conclude that the evidence for carcinogenicity of the phenoxyacetic acid herbicides, specifically 2,4-D, is strongest for NHL and weak for STS. If the association between the herbicide and NHL is causal, the potency of the chemicals as a carcinogen must be weak.

There are only two studies of STS that show a convincing association with phenoxyacetic acid herbicides and chlorophenols, both from Sweden. Manufacturing plant cohorts which are assumed to be highly exposed did not show any association but, with two exceptions (45, 47), were too small in size to attain sufficient power. Under critical examination the association between STS and phenoxyacetic acid herbicide or chlorophenol exposure may be an irresoluble issue in the absence of epidemiologic evidence to support a conclusion. A study to be conducted in Canada has recently been announced. Canada has a climate and economic level similar to Sweden. This study will be coordinated by the University of Saskatchewan.

Studies of non-Hodgkin's lymphoma generally show a slightly increased risk. The effect of the phenoxyacetic acid herbicides would appear to be specific for NHL, since no effect has been demonstrated for Hodgkin's disease. Table 7 summarizes the data and reveals a pattern. Agricultural cohort studies all show a positive correlation, as do most studies in which pesticide exposure has been identified. In addition, studies that attempt to quantify pesticide exposure have tended to report higher risks (21, 62, 70).

There are many reasons why risk estimates may be inconsistent among cohorts. Death certificate studies and occupational health monitoring data may dilute results by the inclusion of subgroups at low risk, depending upon the composition of the occupational category that includes agriculture (25). The codes for the lymphomas and ICD 171 embrace a very wide variety of tumors. They are by no means equal in distribution or risk factors. The aggregation of diagnostic codes may be masking a highly specific effect limited to a rare tumor. Failure to distinguish among the many types of lymphoma may dilute risk estimates. Studies of non-Hodgkin's lymphomas present different but equally difficult classification problems than the soft-tissue sacromas, but show a clearer increase in risk. It cannot now be determined whether only one subtype of NHL or several show this increased risk. Cantor (71) found a higher risk for one subtype of NHL.

Present systems of categorizing the lymphomas, especially, for epidemiological purposes are probably almost useless in the interpretation of the carcinogenic behavior of chemical exposures because the immune system is so highly structured that it is likely that such effects are targeted in a very specific way, causing a particular type of lymphoma to the exclusion of others. We advocate a study in which cell surface antigen markers are used to specifically identify the lymphoma by a consistent typology, or the clinical system developed by the National Institutes of Health (84). We believe that terms such as "lymphosarcoma or reticulosarcoma" are no longer useful.

The phenoxyacetic acid herbicides may be carcinogens specific to the immune system by a mechanism unique to lymphatic tissue. TCDD has been shown to be an immunosuppressant and 2,4-D an immunostimulant. TCDD or the herbicide itself might cause neoplasms in the immune and lymphatic system, perhaps by altering the

surveillance function of lymphocytes. The data may suggest an interactive effect between phenoxyacetic acid herbicides or chlorophenols and something else in the agricultural or forestry environment. Hoar showed that farmers never exposed to herbicides had a risk close to that of non-farmers, suggeting that farming itself in the absence of exposure to herbicides confers no risk (62). Woods et al. showed that increased risks of NHL were observed among those with occupational exposure to organochlorine insecticides such as DDT and organic solvents, and to other chemicals typically encountered in agriculture, and the forestry and wood products industries (32). An important recent case-control study by Olsson et al. suggests that exposure to organic solvents may be a risk factor for NHL (85). If confirmed, this might shift attention to pesticide vehicles and diluents as well as their active ingredients. TCDD, a known contaminant in early 2,4,5-T formulations, is recognized to have an immunosuppressive effect (21) and appears to act as a promoter in carcinogenesis rather than as an initiator (14).

Confounding or interacting exosures may also play a role in the potential for cancer risk associated with herbicide use. A putative risk factor participating in an interaction does not have to be a man-made chemical. Lymphomas can be induced in animals with plant lectins, which are carbohydrate-binding proteins that stimulate mitosis in lymphocytes and act as hemagglutinins. They have been identified in legume seeds (peas and beans), solanacia (potatoes and tomatoes), triticeae (grains), and several animal species. As noted, a similar process could be responsible for the association observed between Hodgkin's lymphoma and wood dust. Other substances in the natural environment are known to be initiators in carcinogenesis including aflatoxins, and some phenylpropenes (86) such as safrole. Several types of viral cancer have been identified in farm animals and this may also occur in humans. Two studies (67, 71) of agricultural cohorts have identified increased risks of leukemia in groups exposed to grain, for NHL in groups exposed to milk production, and for both groups exposed to egg-laying chickens.

Further investigation of an association between phenoxyacetic acid herbicides or chlorophenols and non-Hodgkin's lymphoma seems justified. Because of the many reasons why risk estimates may be inconsistent among cohorts, we cannot endorse the approach of meta-analysis used by Bond et al. (87) of mapping probability densities from

published studies and drawing conclusions from pooled risk estimates. The quality, power, and consistency of method among the various available studies do not support such a sophisticated approach in our judgement. Targeted epidemiologic studies are needed that will quantify pesticide exposure and relate this to immunologic subtypes of non-Hodgkin's lymphoma. The potential for interactive effects between pesticides and other substances in the forestry or agricultural environment is a reasonable line of investigation. Future studies should address the possibility that phenoxyacetic acid herbicides, chlorophenols, or their contaminants act as promoters in carcinogenesis.

A recent review of this same topic by Lilienfeld and Gallo, (88) which placed more emphasis on 2,4,5-T, has come to the same general conclusions as our interpretation.

Recommendations for the phenoxyacetic acid herbicides

Government regulating agencies, school boards, parents, park superintendents, grounds maintenance personnel, and the agricultural community have become increasingly concerned about the safety and health aspects of herbicide use. This paper, for example, grew out of our own review of the toxicity of the phenoxyacetic acid herbicides prepared in part for the Pesticide Management Branch of Alberta Environment. It is our conclusion that the potential for carcinogenicity of 2,4-D is real but very limited and not potent. Because 2,4-D quickly binds to the surfaces of plants and degrades very quickly in the environment there is no compelling reason to restrict its use on the basis of it being a widespread environmental hazard. It may present an occupational hazard in pesticide application but not of great magnitude and is not likely to be a significant environmental hazard. The evidence associating 2,4-D and the uncommon class of malignancies known as the "non-Hodgkin's lymphomas" is increasingly convincing in spite of methodological limitations, some of which we have documented here. However, 2,4-D is remarkably free of acute toxic effects. Even given the potential for increasing the rate of these uncommon cancers, its use presents a limited risk that can be controlled by the use of personal protection and sensible precautions. With respect to health concerns 2,4-D presents advantages over alternative herbicides although it is not a perfect solution to the problem of safe vegetation management.

The study by Hoar in Kansas (62) showed a clear decrease in the risk for NHL associated with the use of protective clothing. Agricultural use of 2,4-D can continue with minimal risk if farmers and applicators use personal protection to limit the extent and duration of exposure. There is no evidence that existing guidelines and recommendations are inadequate if followed. These include protective outer clothing, avoiding contamination of clothing, avoiding prolonged skin contact, respiratory protection until the herbicide application is completed, and uncontaminated or decontaminated air supplies in farm vehicles.

Treatment of turf areas with 2,4-D for weed control can be performed safely in parks and playgrounds. Furthermore, most of the 2,4-D picked up on a child's clothing, for example, would not be directly applied to the skin. The efficiency of abrasion or rubbing as a means of picking up 2,4-D residue on skin or clothing would be minimal. The Guelph study used organic solvents and repeated abrasion (9). In practical terms the hazard would be vanishingly small after a day or two, even under the worst assumptions.

Our assessment of the evidence to date serves to confirm that current recommendations to parks, school boards, and citizens concenred with lawn care are, we suggest, safe and present no substantial risk to children. Turf areas treated with 2,4-D are safe to reenter within 48 hours under dry conditions and within 24 hours after watering, rainfall, or a heavy dew. Should a child inadvertently wander into a site or play on treated grass before these reentry periods have expired there is no cause for immediate concern or medical examination. 2,4-D is low in toxicity and is by now well-studied. Alternative pesticides now available are more hazardous, more persistent in the enviroment, and less well understood. Mechanical and manual removal of undesirable vegetation is very labor-intensive, expensive, and carries a high occupational health cost associated with ergonomic and safety hazards. Properly applied and with minimal precautions, 2,4-D is probably the safest alternative presently available for turf management in public areas, at least given the present level of documentation.

Herbicides have widely replaced intensive manual control of unwanted vegetation for reasons of economy and safety. Concern over the safety of available herbicides has forced a rethinking of vegetation management by those in industry as well as by those criticizing current

practices (89). The most intensive use of herbicides is to manage agricultural land and to control unwanted vegetation at industrial sites or corridors for transportation, energy, and communication facilities. Vegetation management in this setting is more than a matter of killing weeds safely. Its fundamental purpose is to provide cost-effective control of undesirable vegetation in a way that does not harm the environment and in a manner that protects the health and well-being of all affected. The parties primarily affected include applicators and field workers, community residents, farmers, and those whose safety is dependent on suppressing vegetation growth which may cause a fire hazard, a barrier to vision or visibility, destruction of facilities, impeded access to hazardous equipment, or entangled ground cover. Most approaches to managing vegetation without herbicides carry substantial safety and health implications: stoop labor to pull out weeds, widespread use of chainsaws, pulling stumps to remove trees, controlled burning, sending crews into inaccessible or dangerous terrain, and paving surfaces that would otherwise be left open. Changes in techniques of vegetation management would have an impact on operations in a number of industries besides agriculture and landscaping: power and telephone transmission lines, highway and road maintenance, park management, airport and other grounds management, railroad rights of way, and forestry, for example. A policy that encourages vegetation management by the means most appropriate to the application holds the most promise for protecting the health of the public.

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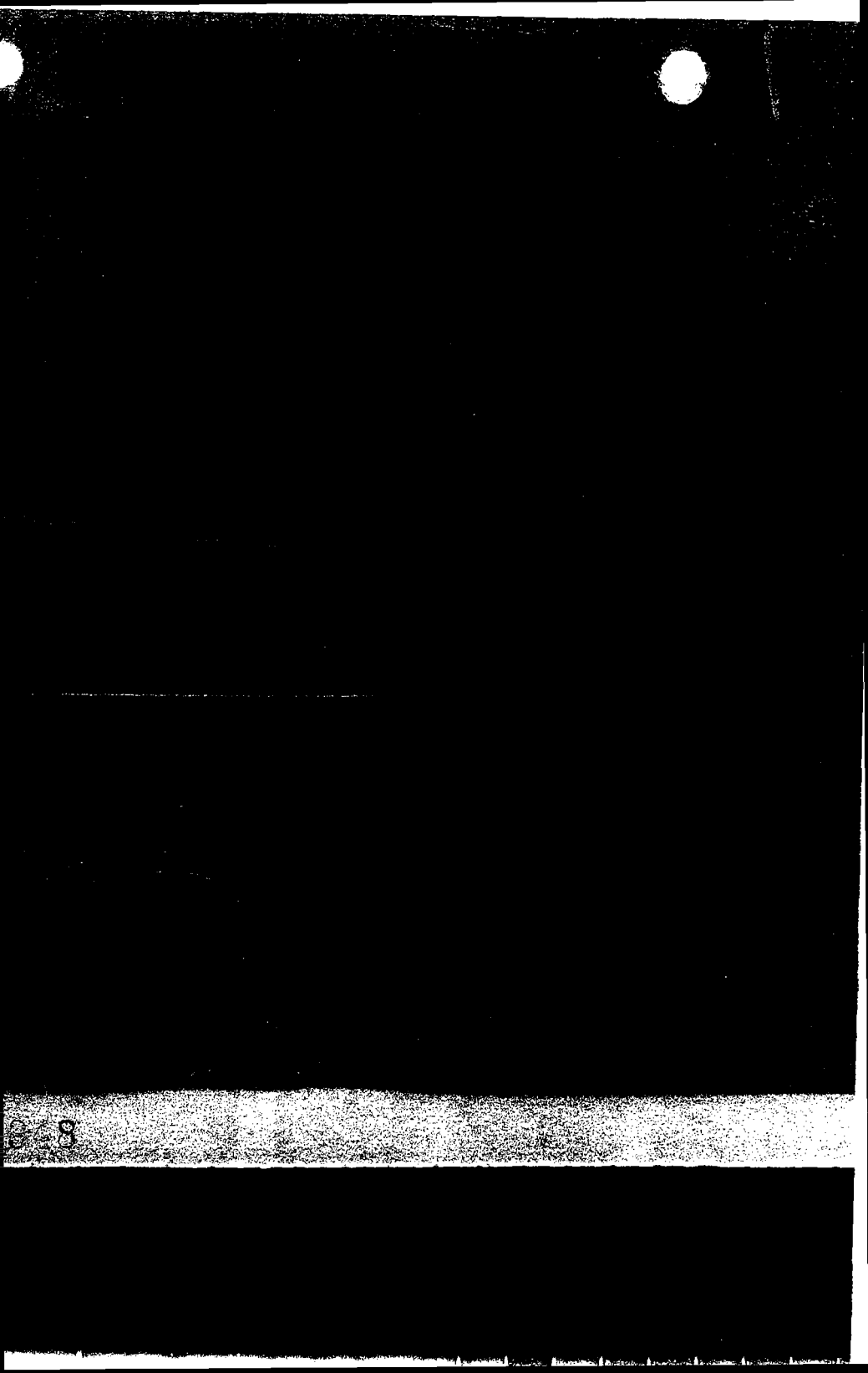
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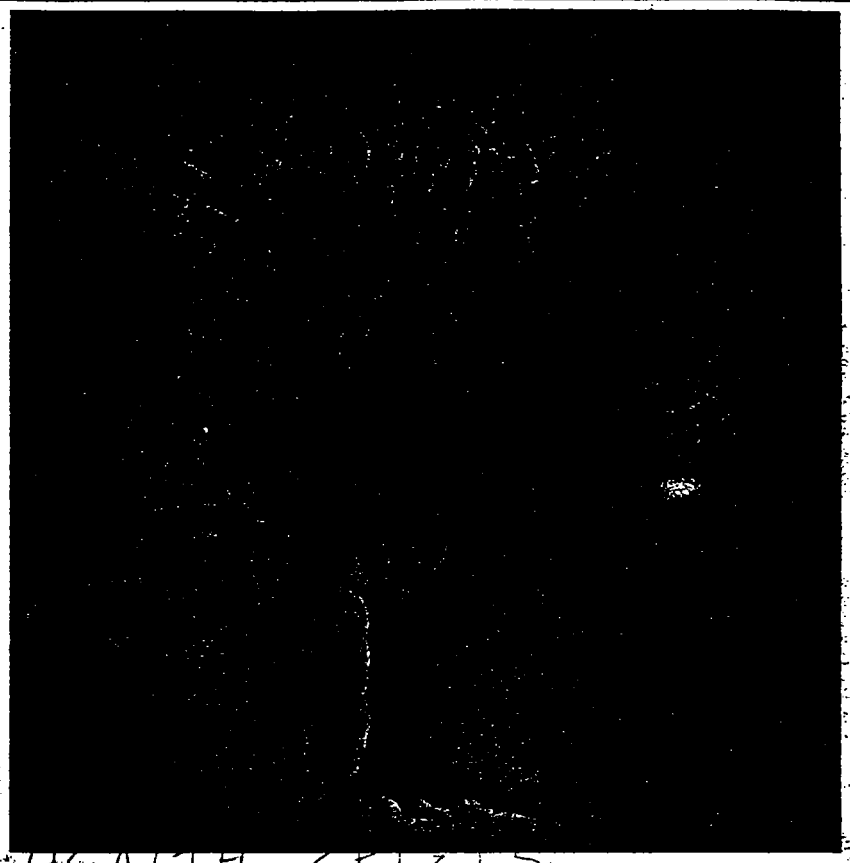
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HEALTH CRISIS

AGENT ORANGE DIOXIN

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THE HEALTH EFFECTS OF "AGENT ORANGE" AND POLYCHLORINATED DIOXIN CONTAMINANTS

Technical Report

Prepared by the Council on Scientific Affairs'

Advisory Panel on Toxic Substances

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Appendix

Table 1 — Occupational and Industrial Accidental Exposures to TCDD in Plants Producing 2,4,5-trichlorophenol and/or 2,4,5-T A1

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PREFACE

During the latter stages of the US' involvement in Vietnam, herbicidal mixtures of 2,4,-D and 2,4,5-T—otherwise identified by the military as Agent Orange—were sprayed over certain areas of the Vietnamese jungle for the express purpose of defoliating the jungle and destroying one of the enemy's means of concealment. Similar spray programs have been used on occasion in the US as a means of forestry management. For the past 30 years, mixtures of 2,4-D and 2,4,5-T have been used extensively and uneventfully by the homeowner and farmer for ridding lawns and agricultural acreage of unwanted broadleaf vegetation. In any event, large numbers of persons have been unintentionally exposed not only to varying amounts of 2,4,-D and/or 2,4,5-T, but also to a contaminant in the mixture—ie, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)—which may well be one of the most toxic substances known to man. Over the years, there have also been a number of industrial incidents wherein workers, as well as civilian populations, have been subjected to accidental exposure to these compounds. There are now pending a number of litigious actions and government regulatory responses that are based upon alleged adverse health effects from such exposures.

The purpose of this report then is to review the medical evidence regarding the toxicity and long-term health effects of TCDD.

In spite of the voluminous data on the biological effects of the phenoxy-type pesticides and their associated chlorinated dioxins, which oftentimes co-exist as contaminants of the pesticide formulations, there is still very little substantive evidence for many of the alleged claims that have been made against these compounds. The most serious of these allegations assert that Agent Orange, or compounds of a like nature, have caused malignant tumors, spontaneous abortions and birth defects. Although data from studies on experimental animals tend to support some of these claims, it is not certain that the animal data are extrapolatable to man. No laboratory animal can fully substitute for man; we must, therefore, depend on the results of ongoing epidemiologic studies on persons who are known to have been exposed.

Thus far, one of the most extensive "human experiments" is being conducted on the residents of Seveso, Italy. The whole story may not be known for another five years; but preliminarily, it appears that the dermatological effects were reversible; there has been no progression of neurological damage; differences in immune response between exposed and nonexposed persons have not been significant; and there has been no notable increase in infectious diseases. There may never be an entirely clear picture from Seveso of the effect of TCDD on reproduction or the fetus, because it is suspected that a number of women elected to have therapeutic abortions. No major pathology, however, was reported by the area physicians.

In the case of industrial exposures, the epidemiological studies on these populations may be more fruitful. First, the workplace generally will have an unexposed control group that can be closely matched to the exposed group. Secondly, in many cases there will be medical records on the individuals and measurements, or close approximations, of the exposure concentrations. By being able to identify and locate earlier workers—by means of retirement, union or other employment records—the task of medical surveillance over extended periods of time will facilitate the discovery of long-term health effects from the exposure.

There is a vast amount of literature available on the adverse health effects of 2,4-D, 2,4,5-T and TCDD; but one must view much of this information objectively, for

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data—especially from the early years—may be devoid of adequate controls.

Hopefully, the internationally coordinated studies of existing cohorts and the opportunity to share data from both past and future exposures, as proposed by the International Agency for Research on Cancer, will produce an irrefutable stand on the long-term consequences of exposure to TCDD.

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HEALTH EFFECTS OF "AGENT ORANGE" AND POLYCHLORINATED DIOXIN CONTAMINANTS

INTRODUCTORY

What is "Agent Orange"?

Agent Orange—or Herbicide Orange—was a label given by the US military forces to a 50:50 mixture of the n-butyl esters of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), together with a minor amount of the free acid 2,4,5-T (1% of the total mixture) and varying amounts (from 0.02 to 15 ppm, or a weighted mean of 1.98 ppm) of the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

2,4,5-T was an active ingredient in Agents Green, Pink, Purple and Orange, used by the military forces from 1962 thru 1970 to defoliate certain jungle areas of Vietnam. Approximately 276,000 gal of Green, Pink and Purple—containing 64 kg (143 lb) of TCDD—were sprayed over South Vietnam prior to 1965 before they were replaced by Agent Orange. Another formulation, Herbicide Orange II, similar to Agent Orange (except that the isooctyl ester of 2,4,5-T was substituted for the n-butyl ester of 2,4,5-T), was also sent to Vietnam; there is no available record to show how much of this formulation was actually used or later disposed of (Young et al, 1978).

According to Young et al (1978), about 25 million kilograms (55,940,150 lb) of 2,4-D and 20 million kilograms (44,232,600 lb) of 2,4,5-T—for a total of 166 kg (368 lb) of TCDD—were distributed over approximately 1.4 million hectares (3.5 million acres) of Vietnam from January 1962 to February 1971. It is significant to note that almost 40% of the entire amount of TCDD that was released in Vietnam—or 64 kg (143 lb)—was contained in Agents Green, Pink and Purple, which materials were used on about 36.4 thousand hectares (90 thousand acres) from 1962 thru 1964; this was during a time when very few American troops were committed to the field.

In addition to Agent Orange, other spray defoliants used in Vietnam included Herbicide White (about 5 million gallons) and Herbicide Blue (about 5,200 gallons). The active ingredient of Herbicide White was picloram (the triisopropanolamine salt of 4-amino-3,5,6-trichloropicolinic acid), which is very persistent in the environment and highly carcinogenic in rats and mice (Reuber, 1981). Herbicide Blue contained sodium cacodylate* (26%) and cacodylic acid (5%) (Young et al, 1978). Though the long-term effects in humans of either or both of the compounds is uncertain—on an acute basis, both are relatively non-toxic—picloram and cacodylic acid should be considered along with the rest of the chemical toxicants encountered in Vietnam.

What is TCDD?

TCDD—or 2,3,7,8-tetrachlorodibenzo-p-dioxin—is only one of 75 various chlorodioxins; there are 22 positional isomers of tetrachlorodibenzo-p-dioxin alone. The 2,3,7,8-tetrachloro isomer is considered to be the most toxic, while the 2,7-dichloro- and the octachloro- dioxins are the least toxic. It may form as a by-product of the synthesis of 2,4,5-trichlorophenol (TCP), a precursor of 2,4,5-T, when 1,2,4,5-tetrachlorobenzene is subjected to alkaline hydrolysis at elevated temperature and pressure. If the reaction

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* Sodium cacodylate is the sodium salt of cacodylic acid (hydroxydimethylarsine oxide).

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temperature is allowed to rise above 180°C, the sodium-2-hydroethoxide (a combination of the ethylene glycol solvent and caustic soda) decomposes exothermically and promotes the dimerization of sodium trichlorophenate to TCDD. The presence of TCDD as a contaminant of 2,4,5-trichlorophenol was disclosed by Kimmig et al (1957) and Schulz (1957) when workmen involved in the manufacture of TCP developed chloracne.

Halogenated dibenzodioxins are also apt to be created when certain chlorinated compounds in industrial and municipal wastes are incinerated (Anon, 1979; Bumb et al, 1980; Rappe et al, 1978; and Rawls, 1979), and they may be released by the burning of vegetation that has been treated with the chlorophenoxy herbicides and defoliants (Buu-Hoi et al, 1971). There is some argument whether such release will occur under field conditions, especially if burning takes place one or more months after spraying (Norris, 1978). According to a recent report from the American Society of Mechanical Engineers (1981), however, municipal waste incineration should pose no greater health risk from the polychlorinated dibenzo-p-dioxins than other forms of waste disposal, ie, landfill. The report estimates that the cancer risk will be increased by only one person in one million of the population.

TCDD as an impurity of 2,4,5-T was first revealed about 1959 by Tomita et al (1959). It, as well as other related dioxins, may also contaminate pentachlorophenol; hexachlorobenzene; 2,4,-dichlorophenyl-p-nitrophenyl ether; silvex; ronnel (fenchlorphos); erbon; trichloronate; hexachlorophene; 2,4,4'-trichloro-2'-hydroxydiphenyl ether and the polychlorinated biphenyls (PCB's) (Kimbrough, 1979).

About 20 years ago, commercially available 2,4,5-T contained anywhere from 1 to 70 ppm of TCDD. When the industry became aware of the contaminant's existence and toxicity, production operations were monitored and altered to reduce the level. Manufacturing operations are now able to control the amount of TCDD in commercial 2,4,5-T formulations to less than 0.01 ppm (with occasional batches as high as 0.05 ppm), a level believed to be non-hazardous to humans and other organisms. Data relating to an acceptable maximum level are presently under regulatory review by FIFRA (Federal Insecticide, Fungicide and Rodenticide Act); the recommended maximum concentration is now placed at 0.1 ppm (Council for Agricultural Science and Technology, 1978).

TCDD was first noted for its teratogenicity and fetotoxicity by Courtney et al (1970), who were testing the biological activity of 2,4,5-T in mice and rats--the sample they were using was later found to contain 30 ppm of 2,3,7,8-tetrachlorodibenzo-p-dioxin. The incidence of cleft palate was greater in both the C57BL/6 and AKR mouse strains, while the C57BL/6 mouse and the rat had a higher incidence of cystic kidney. All doses given the rat led to gastrointestinal hemorrhage in the fetuses; also, the increased ratio of fetal liver weight to body weight in the mouse suggested that TCDD was fetotoxic in this particular species.

An early major review of this subject was the "Perspective on Chlorinated Dibenzodioxins and Dibenzofurans" (Moore, 1973); unfortunately, little information was given on biological effects of TCDD in man. Evidence for long-term adverse health effects to man was still inconclusive in the extensive reviews by Young et al (1978) and Bovey et al (1980) and the International Agency for Research on Cancer (1977).

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WHAT ARE THE CURRENT SOCIAL AND MEDICAL CONCERNS?

Phenoxy herbicides came into commercial use as agricultural herbicides in the mid-40's with the introduction of 2,4-D, which was followed by 2,4,5-T in 1948. Both were used extensively and uneventfully in the control of woody and broadleaf vegetation in croplands, forests, rights-of-way and turf until 1969, when public concern mounted over the human health hazards of 2,4,5-T and/or TCDD. Consequently, in late 1970, the Secretary of Agriculture suspended those uses of the herbicide where humans might encounter greatest exposure to the TCDD. In 1971, the Environmental Protection Agency (EPA) initiated cancellation proceedings against certain other uses of 2,4,5-T. EPA then withdrew its cancellation notice in 1974 and initiated a plan for the development of data that would be needed for their decision-making process. In April 1978, EPA issued a notice of Rebuttable Presumption Against Registration (RPAR)*. In March of 1979, the RPAR process was partially interrupted by an emergency suspension to ban the use of 2,4,5-T in pastures, forests and rights-of-way home gardens, aquatic weeds, ditch banks and ornamental turf, excluding use on rangeland and rice; the suspension was prompted by reports of spontaneous abortions in humans in Alsea, Oregon, which abortions were allegedly caused by these herbicides (American Farm Bureau Federation, 1979 and US Environmental Protection Agency, 1979). Inasmuch as the EPA believed that silvex (or 2-2(2,4,5-trichlorophenoxy)propionic acid) was closely related to 2,4,5,-T and also apt to be contaminated with TCDD, the Agency suspended silvex as well.

Meanwhile, a number of Vietnam war veterans were reported to have had various medical complaints (for instance, weight loss, liver damage, recurrent skin rashes, deformed offspring, stillbirths, cancer, sterility, personality changes and "other illnesses"), which they ascribed to their exposure to Agent Orange some 8 to 18 years earlier. For example, a helicopter pilot who had served in Vietnam, and who had been discharged in apparent excellent health, began to experience symptoms of bowel irregularity, sleeplessness, nausea, headaches, night sweats and a general malaise. He was diagnosed as "psychotic", then found to have terminal cancer of the colon, which metastasized to the lymphatics. A class action suit was instituted in 1979 against five of the herbicide manufacturers—Dow Chemical, Monsanto, Hercules, Diamond Shamrock and Thompson-Hayward—on his behalf and extended to "all American servicemen whose health has been damaged because of contact with Agent Orange." The companies in turn filed a third-party action against the US government, passing the responsibility for alleged harm to the government for its negligent misuse of the chemicals.

A class action suit was likewise filed against the Veterans Administration and the US Department of Defense in January 1981.

At the last count, some 2.4 million Vietnam veterans, including 1,200 in Operation Ranch Hand helicopter crews, and 200 civilians who were involved in "dedrumming" and destroying about 40,000 55-gallon drums of surplus Agent Orange were presumably exposed. Other persons were also claimed to be within 1/3 mile of the spray areas.

The news media have stated that the potential number of "plaintiffs" will exceed 2.5 million people, from which about 40,000 veterans may possibly become sick or die as

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* RPAR is an intensive benefit/risk review of a currently registered pesticide to determine if new health effects data and risk criteria can justify its continued registration and unrestricted use.

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a result of Agent Orange. The reports go on to suggest that at least 2,000 children will suffer "catastrophic" birth defects. More than 1,250 lawyers from 150 law firms in the US are involved in the litigation to date (Elson, 1980).

Victor Yannacone, the New York attorney who filed the first suit on behalf of the above-mentioned helicopter pilot, has alleged that birth defects, in addition to those already listed, include: urinary and genital tract deformities, missing limbs, clubbed feet and mislinked blood vessels (Elson, 1980).

Agent Orange litigation has not been confined to the military, for a number of women were believed to be at risk of miscarriage after 13 spontaneous abortions occurred in Oregon's Alsea basin area. In this particular instance, silvex was employed by the US Forest Service in their spray programs for this area. See the section below, "critical reviews of...Alsea studies," for additional discussion of the incident.

Inasmuch as the phenoxy herbicides have been manufactured and used for the past 40 odd years, it is reasonable to expect that the above cases are not the only instances of frank exposure to these chemicals or their contaminants. The first of several accidental releases of TCDD, and other dioxins, attending the manufacture of 2,4,5-trichlorophenol (TCP) or 2,4,5-T occurred in 1949. A consequence of this industrial incident was a suit—asking $1.7 billion—that has been filed against the Monsanto Chemical Co. on behalf of 54 former Monsanto employees and representatives of eight deceased persons who worked in the Nitro, West Virginia plant. From 1948 to 1969, this plant manufactured 2,4,5-T; however, its major products were chemical compounds for the rubber industry (eg, p-aminobiphenyl and 4-nitrobiphenyl, both of which are now known to induce bladder cancers in humans). The men are said to have suffered from kidney diseases, leukemia and a variety of other ailments (Anon, 1981).

To date, there are at least 579 workers who are known to have been exposed to TCDD, including 156 employees in the ICMESA (Industrie Chimiche Meda Societa Anonima) plant near Seveso, Italy.

In July of 1976, TCDD was accidentally released from the ICMESA trichlorophenol synthesis plant when a safety disk in a steam-heated reaction vessel ruptured. The plume of reactor contents, including TCDD, rose into the air and fell in a cone-shaped pattern about a mile long and half-mile wide—an area of about 2.02 km^2 (about 500 acres or 0.8 sq mi). It has been estimated that from 650 to 1,700 grams of TCDD were dispersed into the environment. Some of the environmental samples of contaminated grass contained as much as 15,840 ug/m^2, or more than 15 ppm, of TCDD. On the basis of soil samples, the territory was divided into three zones according to their relative degree of contamination:

zone A—about 0.73 km^2 (180 acres or 0.3 sq mi) with a population of 736 persons—ranged from a low that was non-detectable to a high of 5,477 ug/m^2, or an average level of 235.5 ug/m^2;
zone B—2.25 km^2 (556 acres or 0.9 sq mi) and 4,699 people—had an average of 3 ug/m^2; while
zone R of 12 km^2 (2,954 acres or 4.6 sq mi) and 31,800 people was sporadically contaminated with an average of about 0.5 ug/m^2 (the low was undetectable and the high was less than 5 ug/m^2).

Serving as controls were some 180,000 persons who had been living under similar conditions outside the contaminated area (Homberger et al, 1979).

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Some of the persons in the area became ill immediately, while others experienced chemical burns of the skin, stomach pains and internal hemorrhage within a few days of the accident. With the death of the animals and vegetation, the inhabitants were evacuated and the area was sealed off by officials, who felt that it would be ten or more years before the contaminant would have decomposed to a level that would be sufficiently safe for resettlement (Rawls et al, 1976). Approximately 511 of the 736 evacuees from zone A were allowed to return to their living quarters by May 1977 (Homberger et al, 1979).

From Vietnam have come the observations of Dr. Ton-That Tung (Tung, 1971, 1973), that liver cancer incidence in Vietnam jumped from the eighth to the second most common form of cancer six years after the introduction of Agent Orange into that country in 1963. Abortions and congenital birth defects were also said to be more frequent. Ford et al (1978) offered one possible explanation for this alleged rise in cancer incidence: that aflatoxin-contaminated cereal grains may have become more prevalent during the war. Unfortunately, these statements of Tung and others cannot be scientifically verified, and without the cooperation of the Vietnamese government, there is no likelihood of a long-term follow-up.

One source of information about the consequences of TCDD on the Vietnamese people is the report of the National Academy of Sciences (1974).

TOXICOLOGICAL EVIDENCE OF A HEALTH HAZARD

Toxicity, Animal

—Laboratory Tests of Toxicity

One of the common failings of animal toxicity assessments is the use of test doses that are much higher than those that may be encountered by humans. Such massive doses are intended to shorten the time for a response or, in the case of a carcinogenicity test, to increase the probable incidence of tumors within a sample of animals. Then too, toxic response is determined by the pharmacokinetics, pharmacodynamics and adaptive considerations of the particular species of animal, and all of these factors differ—sometimes markedly—from one species to another. Animals with metabolic rates that are higher than man's (eg, rats and mice) often will be relatively more sensitive than man. Thus, extrapolation of the toxicologic data from one animal species or pure-bred strain to another, including man, can be fraught with error and be misleading. For example, as Purchase (1980) points out in his literature review of 250 compounds that had been tested for carcinogenicity: 43 of these chemicals varied in their effects on the rat and mouse—21 (8%) were carcinogenic only in the mouse, while 17 (7%) were carcinogenic only in the rat—and 5 (2%) varied among other species. Some 38% of the 250 compounds were non-carcinogenic in both rat and mouse and 44% were carcinogenic in both of these species.

—Dioxin (TCDD)

Two of the more pronounced biological effects of many of the chlorinated dioxins are their tendency to cause acne (especially, in the rabbit and monkey) and the accumulation of fluid (ascites) in the pericardium and peritoneal cavity of chicks. This so-called "chick edema" disease was noted in late 1957 (Sanger et al, 1958) when millions of broilers in the eastern and midwestern US died as a result of an apparent feed additive. The contaminant, or chick edema factor, was finally traced to tallows that had been recovered from hide stripping operations employing pentachlorophenol as a preservative

(Schmittle et al, 1958); the contaminant was identified as a mixture of 2,3,7-trichlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzo-p-dioxin (Tomita et al, 1959).

In general, chronic exposures lead to degeneration of the liver and thymus—one sensitive index of exposure is atrophy of the thymus—porphyria cutanea tarda, altered levels in serum enzymes and weight loss. Several animal species are also affected by its fetotoxicity (National Research Council, 1977; Neubert et al, 1972, 1973). Delayed effects from TCDD appear as pathological changes in the liver. Major organs to be affected are the liver, blood forming organs and reticuloendothelial system. Other than the usual progressive weight loss, the first clinical signs of toxicity, for the monkey at least, are marked by alopecia, facial edema and a dry, scaly dermatitis over the rest of the body.

TCDD by itself is extremely toxic; along with botulinus bacillus toxin and aflatoxin, it is one of the most lethal organic chemical compounds known to man. In terms of the acute oral dose, or LD_{50}, its toxicity ranges from 0.6 ug/kg of body wt for the guinea pig (the most sensitive of experimental animals) to 115 ug/kg of body wt for the New Zealand albino rabbit (Schwetz et al, 1973); single oral doses of 20 ug to 50 ug/kg/body wt have produced fatal liver necrosis in the rabbit (Schulz, 1957). Among primates, the LD_{50}(oral) for the Rhesus monkey is less than 70 ug/kg of body wt (McConnell et al, 1978).

As for the rat, its oral LD_{50} (male) is 22 ug/kg vs the female LD_{50} at 45 ug/kg (Schwetz et al, 1973), which is about 10,000 times less than the oral LD_{50} for 2,4,5-T. The fetotoxic dose, is about 1/400 of the above LD_{50} (or about 0.11 ug/kg) (Office of Science and Technology, Panel on Herbicides, 1971). Death is probably due to hepatic-cell necrosis.

Liver lesions caused by TCDD in the rat were subjected to a morphological study (Jones et al, 1974). Parenchymal cell necrosis in the centrilobular zone with mononuclear cell infiltration was noted after the first week of exposure. By the second week there was dilatation of the sinusoids in the centrilobular zone, more severe inflammation and increased necrosis in the parenchymal cells; loss was not made up by hyperplasia of the live cells. Inflammation regressed after 3 to 6 weeks, but focal areas of necrosis remained around central veins and mural fibrosis of the central vein developed.

No ill effects were discerned in young adult Sprague-Dawley rats that were fed either 0.001 or 0.01 ug of TCDD/kg 5 days/wk for 13 weeks (Kociba et al, 1976); the slight increase in relative liver weight at the 0.01 level was considered to be a physiological adaptation rather than a toxic response. There were a number of changes in tissue and organ function at higher levels. The 1.0 ug/kg/day dose, for example, caused some mortality (which was seemingly more marked in females), inactivity, decreased food consumption and body weight, icterus, elevated serum bilirubin and alkaline phosphatase, liver impairment, lymphoid depletion of the thymus and other lymphoid organs, increased excretion of urinary porphyrins and delta-aminolevulinic acid, together with minor hematopoietic changes (bone marrow production of red blood cells was not markedly inhibited). It was unclear whether the suppressed function of the reproductive organs would be a direct action of TCDD or the result of the debilitated condition of the animal. In general, the findings of this study supported prior studies of shorter duration.

A 2-year chronic toxicity and oncogenicity study of TCDD was conducted on male and female rats at three different dose levels: 0.1, 0.01 and 0.001 micrograms per kg per day (Kociba et al, 1978). At the 0.1 ug level, the fat and liver of the females contained 8,100 and 24,000 ppt of TCDD, respectively, at the end of two years. Some tumors (viz,

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hepatocellular carcinomas of the females and squamous cell carcinomas of the lung, hard palate/nasal turbinates or tongue) were induced, while the strain-related tumors (ie, of the pituitary, uterus, mammary glands, pancreas and adrenals) were suppressed. Fewer aged males had chronic renal disease, and it was less severe . Other toxic signs included increased mortality; decreased gain in body weight; depression of certain hematologic parameters; increased urinary excretion of porphyrins and delta-aminolevulinic acid (ALA); increased serum activities of alkaline phosphatase (AP), gamma-glutamyl transferase (GGT) and serum glutamic pyruvic transaminase (SGPT); as well as morphological changes in the hepatic, lymphoid, respiratory and vascular tissues. At the 0.01 ug level, there was slight poisoning: urinary excretion of porphyrins (in females) was increased, hepatocellular nodules were more numerous and there was an increased incidence of focal alveolar hyperplasia. No significant toxic effects were noted at 0.001 ug. No increase in tumors was evident at either of the lower levels. It should be noted that most of the above work used the Sprague-Dawley rat strain, a strain that characteristically has a high incidence of spontaneous tumors.

As little as 5 ppt to 5 ppb in the rat diet are said to induce various neoplasms (Van Miller et al, 1977); lung and/or liver tumors have developed at the 1 or 5 ppb level.

TCDD is a very potent inducer of aryl hydrocarbon hydroxylase (AHH), as well as the mixed-function oxidases and the cytochrome P_1-450 (P-448) enzymes of the liver, lung, placenta and kidney of the mouse and rat. In addition to altering normal enzyme activity, it may even potentiate the harmful action of other toxins or render an otherwise innocuous agent toxic. It is 30,000 times more powerful than 3-methylcholanthrene in inducing the activity of AHH in certain laboratory animals (Poland et al, 1974). Mouse liver fractions, which had been pretreated with TCDD and subjected to 3-methylcholanthrene, showed a significantly greater mutagenic effect than without the pretreatment. A similar experiment using benzo(a)pyrene as the polycyclic hydrocarbon was unremarkable (Felton et al, 1975); one is left to wonder whether other organic compounds are likewise modified mutagenically by TCDD. The tissue-specificity of TCDD's induction property appears as part of the work of Berry et al (1977), wherein samples of liver, lung and placental tissue from TCDD-treated female Sprague-Dawley rats were compared with tissue of untreated controls for the covalent binding capacity of benzo(a)pyrene to DNA.

Although TCDD appears to have no profound direct toxicity to the adult kidney, its property as an enzyme inducer conceivably may convert harmless substances to nephrotoxins (Fowler et al, 1977).

^{14}C-labeled TCDD was used to determine the tissue distribution and excretion of TCDD in the rat (Allen et al, 1975; Fries et al, 1975; Piper et al, 1973; and Rose et al, 1976). Excretion was largely via the feces (over 50%), with small amounts being eliminated in the urine and expired air. The compound accumulated, for the most part, in the fat and liver with lesser amounts (some 20% to 30% less), by decreasing order, in the spleen, bone, heart, lungs, testes, kidney, muscle, skin, pancreas, brain, stomach and adrenals. Accumulation reached a steady state within 13 weeks if administration was continued for several months. TCDD was metabolized only slightly by the rat. Most of it seemed to remain unmetabolized in the liver—esp, in the microsomal fraction—of the rat, mouse, and monkey (Matthiaschk, 1978) and guinea pig (Gasiewicz et al, 1979), which agrees with the findings from an intraperitoneal injection of male mice with an LD_{50} dose of ^{3}H-TCDD (Vinopal et al, 1973). Tulp et al (1978) have suggested that the metabolism of TCDD is unlikely to occur inasmuch as its molecular structure hinders formation of the intermediate 2,3-epoxide, which is the main route for other polychlorinated dibenzodioxins. More recent radiolabel studies by Ramsey et al (1979) have indicated

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that TCDD metabolized slowly in the rat liver to a variety of unidentified polar compounds, whose excretion rate in the bile was comparable to the authors' earlier disclosed rate of excretion in the feces. Poiger et al (1979) also have reported on the excretion of polar metabolites of TCDD in rat bile. The hamster's rate of TCDD metabolism was greater than that of the guinea pig's or rat's, which may help to explain the relative differences in toxicity between these species (Olson et al, 1980).

The acute LD_{50} of TCDD in the female Rhesus monkey, while not determined accurately by McConnell et al (1978), is still probably close to 70 ug/kg. This species thus appears to be intermediate in sensitivity between the guinea pig (0.6 ug/kg) and the rabbit (115 ug/kg) and more sensitive than the C57BL/6 mouse (284 ug/kg). The first toxic symptoms for the monkey were progressive weight loss, followed by blepharitis—a most sensitive marker for toxicity in this animal—then skin lesions, that are not unlike those experienced by humans, and the loss of fingernails and eyelashes. The authors also noted less reduction in spleen weight than for other species, and the thymus appeared to be completely involuted. Increase in liver weight also was less marked than in rodents, and there were fewer hepatic lesions. Perhaps this was due to less TCDD being retained in the monkey liver.

Adult female Rhesus monkeys were also fed 500 ppt of TCDD for up to nine months (Allen et al, 1977; 1978), during which time they ingested about 2-3 ug of TCDD/kg/day. For the first three months there was acne, alopecia and periorbital edema. Irregular menstrual cycles were accompanied by decreased 17-beta-estradiol and progesterone levels. Early abortions and the inability to conceive occurred at the six-month point. Death was mainly due to thrombocytopenia; in five out of eight animals, death was preceded by pancytopenia. Microscopic examinations revealed suppressed hematopoiesis of the bone marrow, hypocellularity of the lymphoid tissue, widespread hemorrhage, cardiac hypertrophy, edema and dilation, hyperplasia and metaplasia of all ductal tissue, hypertrophic gastritis with ulceration and dilation of the biliary system. Offspring were weak and died within a few weeks, possibly from the additional dioxin in the maternal milk.

One test animal that is particularly sensitive to CNS toxicants is the chicken. Sanderson et al (1981) of Australia treated both chicken eggs and hatchlings with "environmental levels" of 2,4,5-T contaminated with 0.03 ppm of TCDD. Although the general activity of the animal was unaffected, there was a dose-related increase in jumping and a retardation in visual learning.

—Picloram

A review from the National Cancer Institute on the carcinogenicity of this compound (Reuber, 1981) has focused on two NCI studies and two Dow Chemical Co. studies. The findings revealed that all neoplasms, including malignant ones, increased for both male and female Osborne-Mendel rats. The malignant neoplasms consisted of carcinomas—of the adrenal, thyroid and pituitary glands—and sarcomas. Mice of both sexes developed neoplasms of the spleen. Male rats also had chronic renal disease, parathyroid hyperplasia and polyarteritis; both rats and mice displayed testicular atrophy.

Toxicity, Human

—2,4-D

Normally, 2,4-D is not contaminated with TCDD. It often has been used and tested in combination with 2,4,5-T, which can contain TCDD; hence, there is merit to a limited discussion of how this compound is known to affect man.

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Ingested 2,4-D is absorbed almost entirely from the gastrointestinal tract and clears from the body by a first-order rate process; the half-life is about 17.7 hrs. It is excreted in the urine mainly as unmetabolized 2,4-D (82.3%) and the 2,4-D conjugate (about 12.8%). Accumulation, in the event of continued intake of the compound, is unlikely (Sauerhoff, 1977).

Three cases of polyneuritis have been attributed to rather massive percutaneous absorption of 2,4-D (Goldstein et al, 1959). In each instance, the symptoms were progressive, with severe pain, paresthesia and paralysis. All of the victims still suffered some neurological damage after about three years from date of onset. No details were given on the compositions of the formulations (ie, impurities and adjuvants).

The lethal, oral dose (based on a 23-yr-old male) is probably over 80 mg/kg; thus, an 80 kg man would require at least 6.4 grams of the compound. A fatal suicide attempt with an estimated 6.5 g gave signs of convulsions and nonspecific engorgement with blood of the lungs, liver and brain (the time from ingestion to death was 18 hrs) (Nielson et al, 1965).

In another incident (Berwick, 1970), a man accidentally ingested a 110 mg/kg dose of 2,4-D (isooctyl ester). Besides depression and irritation of the CNS, the victim suffered from myopathy, GI irritation, nephropathy and toxicity to the liver; however, he recovered fully with no signs of peripheral neuropathy.

Signs and symptoms of poisoning occur at an oral dose of around 3 to 4 grams (Hayes, 1963). There is one instance of a man who consumed 500 mg of purified 2,4-D each day for 21 days without ill effects. In another case, 18 iv doses—the last 12 of which were 800 milligrams or more—were delivered to a patient over the course of 33 days for the treatment of coccidioidomycosis; there were no side effects even after the 18th dose of 2,000 mg. The last injection of 3,600 mg produced acute illness—viz, unconsciousness, twitching of some of the muscles of the body, depression of the reflexes and involuntary urination—yet, by the end of 48 hours, the subject had completely recovered.

Myotonia soon after a heavy exposure of animals to 2,4-D is characteristic of a poisonous dose; this sign may also suggest intoxication in man, but it has not yet been reported (Hayes, 1963).

The porphyria that has been observed among 2,4-D and 2,4,5-T workers by Poland et al (1971) was most likely due to chlorobenzene impurities, or TCDD.

—2,4,5-T

Attempts to assess the health effects of 2,4,5-T from toxicologic data that neither indicate nor account for the presence of TCDD will be virtually without merit. If a report does not mention chloracne as being one of the clinical signs, then it is very unlikely that TCDD was present in such an amount as to cause organic damage.

As part of an experiment to determine the fate of ingested 2,4,5-T in man, five male volunteers (from 31 to 58 yrs of age) were given a single dose of 5 mg/kg of body wt of 2,4,5-T (which contained less that 0.05 ppm of TCDD). There were no discernible clinical signs save for a metallic taste, which persisted about 1-2 hours. Most of the 2,4,5-T (65%) went into the plasma where it was bound reversibly with protein. Its concentration in plasma reached a plateau after three days in those individuals who ingested the compound daily. Virtually all of the compound was excreted unmetabolized in the urine at a first-order rate (Gehring et al, 1973).

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2,4,5-T causes only a mild spastic condition and difficulty in swallowing (Hayes, 1963).

Based on the lack of known illnesses from occupational exposures, the American Conference of Governmental Industrial Hygienists (ACGIH) has recommended that the threshold limit value (TLV) for 2,4,5-T be set at 10 mg/m^3 (time-weighted average for an 8-hr working day) or 20 mg/m^3 for a short-term exposure (ie, up to 15 min for no more than 4 times per day).

—Silvex

The toxicology of this compound is mentioned here because it was involved in the Alsea dispute, and it too can be contaminated with TCDD.

Also known as fenoprop, 2-2(2,4,5-trichlorophenoxy)propionic acid is used on apples to intensify color and prevent fruit drop, to eradicate undesired plants in forests, to control aquatic weeds and to control a variety of weeds in turf and on industrial lands. Often used in combination with 2,4,5-T to enhance the herbicidal effect of the formulation on refractory woody plants, this compound was employed in US Forestry Service spray programs of the Alsea, Oregon area. Its use was suspended by the EPA after the announcement of a higher than usual number of spontaneous abortions in the region.

The oral LD_{50} for rats is 650 mg/kg; mice dosed with 15–46 mg/kg/day gave no signs of tumorigenicity (Hayes, 1975).

Seven men and one woman ingested 1 mg of silvex /kg of body weight: Biomedical tests showed that ingested silvex was rapidly absorbed; peak blood plasma concentration occurred within 2 to 4 hours. Elimination was rapid, primarily via the urine; within 24 hours after ingestion, 65% had been passed in the urine. The half-life for urinary clearance averages about 16 hours. No clinically demonstrable adverse effects were observed (Sauerhoff et al, 1977).

—TCDD

TCDD is recognized as a potent chloracnegen in the monkey, rabbit (where it appears only on the ear), nude mouse and man. Chloracne is typified by comedones—with or without cysts and pustules—in a malar distribution, along with melanosis and a secondary inflammation; the pre- and post-auricular forms of chloracne are sometimes accompanied by hirsutism in these areas. In more severe cases its location may become generalized.

This disorder was first described by Herxheimer in 1899. Still later, Bauer (in 1918) and Teleky (in 1927) noted that it was due to dermal contact with certain chlorinated hydrocarbons. Chloracne has now become one of the more common forms of occupational dermatitis. A review on the subject was presented by Schwartz et al (1957) and Crow (1970), and more recently by Taylor (1979). The role of polychlorinated dioxins in chloracne came to light when Schulz (1957) and Bauer et al (1961) were investigating the disease among a group of German workers who had been handling technical grade 2,4,5-trichlorophenol. Pure chlorophenol did not elicit in the rabbit the dermal response (ie, hyperkeratosis) of the impure compound (Kimmig et al, 1957); thus, they reasoned that an impurity, which was identified as a chlorinated dioxin, was responsible for the acne.

The acnegenic property of the chlorinated dioxins, as well as other other chlorinated compounds, is apparently related to the degree of chlorination of the parent compound together with the relative molecular positions of the chlorine atoms and their

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symmetry. Unchlorinated dioxin does not cause acne and it is non-toxic. Note too that 2,4,5-T per se does not cause chloracne; if the condition occurs upon exposure to a mixture of 2,4,5-T, it is most likely that TCDD is responsible. The potent chloracnegens are, in general, polychlorinated aromatics, such as polychlorobiphenyls (PCB's), polychloroterphenyls, polychlorodiphenyloxide and chloronaphthalenes (or halowaxes).

Chloracne may appear 2 to 3 weeks after the first exposure or after a delay of several months—and possibly even as long as two years (Oliver, 1975). Mild chloracne clears up within several months; severe chloracne is persistent and has been known to last for as long as 15 years following the cessation of exposure to the causal agent (May, 1973). Persons most responsive to chloracnegens are those who are prone to develop acne vulgaris. If there is no medical history of chloracne, then the likelihood of a significant exposure to, or adverse health effects from, TCDD is remote. Hence, chloracne is the clinical marker of TCDD exposure.

In addition to its acnegenic properties, TCDD is also a very active inducer of delta-aminolevulinic acid (d-ALA) synthetase and aryl hydrocarbon hydroxylase in both laboratory animals and man. The increase in GGT activity and abnormal lipid levels in eight men who contracted chloracne from occupational exposure to TCDD has been suggested as being due to the enzyme induction capability of TCDD (Walker et al, 1979). Quite likely, such induced d-ALA overstimulates the production of porphyrin, which may explain the cause of porphyria cutanea tarda (PCT) in certain experimental animals and man (Crow, 1978). PCT has also been described as an interference with the uroporphyrinogen decarboxylase, which prevents conversion of uroporphyrinogen (UP) into protoporphyrinogen. The UP is converted instead to uroporphyrin, which in turn accumulates in the liver until it is excreted in the urine. The disorder is enhanced clinically by alcohol, certain hormones and drugs, and a number of halogenated compounds (such as trichlorophenol) (Homberger et al, 1979). Seemingly, on the basis of tests with mice, TCCD-induced porphyria does not occur in the presence of an iron deficiency (Sweeney et al, 1979).

Other toxic effects in man have been suggested by Reggiani (1977) and include: hyperpigmentation, hirsutism, liver damage (eg, mild fibrosis fatty changes, hemofuscin deposition and parenchymal-cell degeneration), raised serum hepatic enzyme levels, fat and carbohydrate metabolism disorders, cardiovascular disorders, urinary and respiratory tract disorders, pancreatic disorders, polyneuropathies (eg, sensory impairments), weakness in lower extremities and neurasthenic or depressive syndromes.

The metabolism of TCDD in man is unknown, and for the present there is only limited information available on the metabolic pathways and metabolites that may occur in other mammals.

Mutagenicity: No clearly defined mutagenic effect has been observed in vitro with TCDD. The Ames test with Salmonella typhimurium (Gilbert et al, 1980) and one strain of Escherichia coli (Hussain et al, 1972) was positive; however, the test with another strain of S typhimurium was negative.

Hussain et al (1972), were presumably the first investigators to evaluate TCDD's mutagenic potential; they found that Escherichia coli (Sd-4), which measures the reversion to streptomycin independency, was positive (probably a base-pair substitution mutation). Salmonella typhimurium (TA1530 and TA1532 strains), which measures reversion to histidine protrophy, was positive (a frameshift mutation) for TA1532 only at higher concentrations of TCDD and lower survival frequency; the test was negative for TA1530. There could have been a weak prophage induction using E coli (K39) cells,

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though the effect was difficult to separate from that of the dimethyl sulfoxide solvent. By inference, TCDD could be mutagenic if it were to intercalate with DNA and lead to a frame shift mutation; the intercalating effect with DNA was confirmed by Kondorosi et al (1973) in their study of TCDD's transfection of Q(beta) RNA. Seiler (1973) likewise performed the Ames test and found it to be positive with the TA1532 strain of S typhimurium, yet his findings with other strains—in addition to the work of other researchers—casts doubt on the reliability of the test results.

More recently, Bronzetti et al (1980) experimented with the D7 strain of Saccharomyces cerevisiae to evaluate mitotic gene conversion and reverse mutation; both genetic effects were induced with the S_{10} fraction, but there was no induction without the fraction. Their in vivo test with male Swiss albino mice injected intravenously produced an effect within 20 days.

Other cytological changes from TCDD have been noted in the African blood lily (Jackson, 1972) and Drosophila (Davring et al, 1971).

Of more relevance to man are the in vitro studies on mammalian cells—ie, HeLa; Balb-3T3, normal mouse fibroblasts; SV101, virus (SV40)-transformed 3T3 mouse fibroblasts; human foreskin fibroblasts and normal human lymphocytes (Beatty et al, 1975). There was no significant growth inhibition in the cell cultures, nor were there discernible ultrastructural changes by electron microscopy. The authors did not dismiss the possibility that other test conditions and a higher level of organization than the single cell might reverse the reaction.

A review of the genotoxicity of 2,4,5-T on animals (Grant, 1979) points out that the effects could be due to 2,4,5-T, TCDD or a combination of the two. Moreover, the emulsifiers and solvents of 2,4,5-T could be responsible for the chromosomal aberrations.

Oncogenicity: DiGiovanni et al (1977) found little or no tumor-causing or tumor-promoting properties of TCDD, and Berry et al (1978, 1979) found a remarkable inhibitory effect on skin tumors in mice.

Toth et al (1978, 1979) found an increased incidence of liver tumors in male Swiss (H/Riop) mice that were fed both TCDD and TCDD admixed with 2,4,5-trichlorophenoxy-ethanol (a derivative of trichlorophenol), but they were not convinced that TCDD was by itself a carcinogen.

Even if TCDD were a weak tumor initiator, perhaps due to its relative chemical stability within the organism, there is other data of Berry et al (1977) to show that TCDD facilitates the biotransformation in rats of such tumor precursors as benzo(a)pyrene.

According to the National Toxicology Program (NTP), TCDD when administered by gavage was carcinogenic in the B6C3F1 mouse (NTP Rept 80-31; cited by Huff, 1980)—hepatocellular carcinomas were observed in both sexes of the mouse but only female mice and male rats developed follicular cell thyroid adenomas. Neoplastic nodules of the liver were noted in the female rat. Likewise, dermal application of TCDD was carcinogenic in Swiss-Webster mice (NTP Rept 80-32; cited by Huff, 1980); the females had integumentary fibrosarcomas, while results from the males were inadequate.

(See also the section on "toxicity, animal," which discusses the toxicity and oncogenicity studies of Kociba et al (1978).)

Teratogenicity: 2,4,5-T containing 30 ppm of TCDD in two strains of mice (C57BL/6 and

AKR) and one strain of rats, produced increased incidences of cleft palate in mice and cystic kidney in the rat (Courtney et al, 1970); when comparably dosed with a sample containing only 1 ppm of TCDD, teratogenesis was not evident (Emerson et al, 1971). A gavage dose in the rat of 0.125 ug/kg/day from the 6th-15th days of gestation showed only a slight effect that was much pronounced at the level of 0.5 ug/kg/day (Sparschu et al, 1971).

In utero exposure of mice to TCDD during the final half of the gestation period produced fetuses with poorly developed lymphatic systems and fatty infiltrates in the liver (Neubert et al, 1973).

Prenatally, TCDD causes hydronephrosis, which according to Gibson (1976), may be only a sign of delayed maturation and not permanent damage inasmuch as the test animals were taken by cesarean section. Gibson did find altered PAH transport from kidney slices of neonate animals even where there was no evidence of a lesion; the significance of these data on the adult form of the organism is unknown. TCDD also caused postnatal hydronephrosis in a suckling pup whose foster mother had been dosed beforehand with the compound (Moore et al, 1973).

Reproduction and Fertility, Animals: Lamb and co-workers (1980) fed male mice (C57BL/6) varying dosages of a simulated Agent Orange mixture (using the free phenoxy acids instead of the butyl esters) in order to determine the effect of TCDD on the male's reproductive function. During the 8-wk period of dosing, the animals exhibited a dose-related liver and thymus toxicity and a significant reduction in body weight gain. Both the liver and thymus showed either significant or complete recovery when the test diet was terminated; there was also no significant change in sperm concentration, motility or percent of abnormal sperm either during or after the exposure. At the end of eight weeks, the treated males were mated to untreated virgin females; no significant decrease in fertility or reproduction nor evidence of germ cell toxicity or mutagenicity was evident, which is said to correlate well with the multigeneration studies of Murray et al (1979) and the dominant lethal assays of Khera et al (1973). Also, the paternal exposure did not appear to affect the survival of the offspring nor the development of the neonates.

The three-generation reproduction study on the Sprague-Dawley rat led Murray et al (1977) to believe that fertility was only affected at the 0.01 and 0.1 ug/kg/day levels, not at 0.001 ug/kg/day; there were no other signs of organ damage. Seemingly, no embryotoxicity in this species occurs at or below 0.03 ug/kg/day according to Schwetz et al (1973).

Dominant lethal studies of TCDD on male Wistar rats were negative, though the numbers of pregnancies were reduced; oral doses of 4, 8 and 12 ug/kg/day were given to groups of 20 animals for seven days prior to mating with untreated virgin females, and follow-up continued through seven sequential matings. Testes of the surviving males appeared normal histologically, except the inflamed state of the epididymides suggested an autoimmune reaction (Khera et al, 1973).

Other adverse effects on the testes were shown in the 65-wk feeding studies on Sprague-Dawley rats by Van Miller et al (1977). TCDD levels of 0.05 to 1.0 ppm caused death within 4 weeks; the autopsied animals had a decided decrease in spermatogenesis. Male mice injected intraperitoneally with 0.4 mg/kg showed a 50% reduction in the rate of testicular DNA synthesis (Seiler, 1977). Other animals thus affected were the mouse and guinea pig (McConnell et al, 1976; 1978) and the chicken and monkey (Norback et al, 1973). These and other studies of the testes, wherein a weak dominant lethal effect was

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apparent, suggest that further research is needed, especially to determine if TCDD acts directly on the testes or by triggering a secondary response (Wassom et al, 1977/1978).

Adult female Rhesus monkeys were fed 500 ppt of TCDD for up to 9 months (Allen et al, 1977; 1978), during which time they ingested about 2-3 ug of TCDD/kg/day. For the first three months there was acne, alopecia and periorbital edema. Irregular menstrual cycles were accompanied by decreased 17-beta-estradiol and progesterone levels. Early abortions and the inability to conceive occurred at the 6-month point. Offspring were weak and died within a few weeks, possibly from the additional dioxin in the maternal milk.

Male Rhesus monkeys, fed for more than one year on a diet containing TCDD, displayed an absence of spermatids and mature spermatozoa (Allen et al, 1979).

Reproductive Effects in Humans: There are some 287,000 defective births in the US each year, about 9% of all live births; another 560,000 infant deaths, spontaneous abortions, stillbirths and miscarriages are due to defects in fetal development (March of Dimes Birth Defects Foundation, 1981). While about 20% of the defects are for genetic reasons and 3% to 5% are the result of chromosomal aberrations, there are less than 10% that can be attributed to environmental agents—ie, radiation, infections, maternal metabolic imbalance, and drugs and other chemicals. The reasons for the remaining 65% to 70% of birth anomalies is indeterminate; in all likelihood, there may be a combination of any or all of these three factors (Wilson, 1973).

A number of incidents over the past few years have focused on exposures of workers and the general public to lead, pesticides such as dibromochloropropane (DBCP) and chlordecone (Kepone) and the flame-retardant TRIS. Lead workers, for example, have shown evidence of deformed sperm (Lancranjan et al, 1975), while chemical and farm workers showed signs of oligospermia and azoospermia from their contact with DBCP and kepone (Whorton et al, 1977 and Cannon et al, 1978). The latest chemicals that appear to alter the quantity and quality of human sperm are toluenediamine and dinitrotoluene. Nine men who worked with these precursors of toluene diisocyanate (which is a polyurethane intermediate) experienced significantly reduced sperm counts and a reduced percentage of large forms of sperm (from CDC's Morbidity Mortality Wkly Rept **30**:199 (1981)). And should the findings from the Boston Drug Surveillance Program (Jick et al, 1981) be substantiated, some common defects—such as Down's syndrome, limb deformities, malignant brain tumors and hypospadias—and spontaneous abortions may be traced to certain vaginal spermicides that are used either pre- or post-conception.

Other agents that have profound effects on human health are tobacco smoke and alcohol. In fact, many of the disabilities that have been claimed for slight exposures to 2,4,5-T, TCDD and silvex are typical of those caused by these two agents. Thus, any analysis of human toxicology or epidemiologic data must consider the smoking and drinking habits of the subjects. The Alsea II results, in particular, are meaningless without such information. Solid, persuasive evidence relates smoking dose to the frequency of spontaneous abortions. The probability of miscarriage is almost double. Smoking also retards the rate of fetal growth and increases the risks of spontaneous abortion, fetal death and neonatal death in otherwise normal infants. Smoking during pregnancy may affect physical growth, mental development and behavioral characteristics of children at least up to the age of 11. Yet, evidence does not support a conclusion that maternal smoking increases the incidence of congenital malformations (US Surgeon General, 1979). An additional review on the subject is provided by Rylander et al (1981).

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A very limited analysis was made of 43 cigarette smokers (matched against 43 non-smokers) to see if there were any differences in sperm morphology between the two groups (Evans et al, 1981). The results confirm earlier work of a similar nature, indicating a higher frequency of morphologically abnormal sperm cells, which in turn may reflect genetic damage to the cells. Yet, a more detailed analysis of 344 males, extending over eight years, revealed no significant differences (at the 5% level) in sperm count, morphology or motility between non-smokers, light or moderate smokers and heavy smokers (Godfrey, 1981).

And now that marijuana smoking and other illicit drugs have become more prominent and socially acceptable, perhaps as an aftermath of the Vietnam war, we should be aware of how they alter the reproductive functions of both men and women. Hembry, an infertility specialist and endocrinologist at Columbia University, reports that an average of 16 joints/day for four weeks lowers the sperm count significantly and increases the percentage of abnormally shaped sperm, not to mention the possible genetic damage that may occur if such sperm were to successfully produce a conception (Brody, 1981).

The reproductive and other health hazards associated with alcohol consumption have been reviewed most recently by Eckardt et al (1981).

There is currently no direct evidence of any causal connection between 2,4,5-T or TCDD and neural tube damage in the human fetus (Field et al, 1979; Hanify et al, 1981). The latter group—in their survey of 37,751 babies born in Northland, New Zealand hospitals from 1960-77—did find a significant association between 2,4,5-T applications in the environment and occurrences of talipes, though TCDD exposure levels were unknown; the authors aver that the "causal relation remains to be established." Thomas (1980), in his examination of data from the Hungarian registry of birth defects, could detect no marked increase in the incidence rates of cleft palate and lip and cystic kidney disease during 1970-76, a time when there was a thirty-fold increase in the use of 2,4,5-T in Hungary. During this same period, the incidence rates of stillbirths, spina bifida and anencephalus were on the decline. Again, the extent of TCDD exposure was not clearcut, but it was assumed that the agricultural and forestry populations of Hungary—those most apt to have contact with the herbicide—were far more numerous than in Australia and the United Kingdom, and the amounts of TCDD in their 2,4,5-T formulations were no less than 0.1 ppm.

Information about in vivo chromosomal aberrations due to TCDD in humans, for that matter, cytological effects of TCDD on man in general, is not only meager but inconclusive.

HEALTH SURVEYS OF THE EXPOSED HUMAN POPULATIONS

Most toxicologists will agree that the results obtained from their animal experiments only presume a like effect in humans. In the final analysis, the most reliable test for the human animal is man himself. And while intentional human experimentation is becoming less acceptable in our society, we are occasionally afforded human experience with a particular toxicant as the result of some untimely accidental exposure to it. Such an opportunity may occur in connection with the workplace, or it may involve a very diverse fraction of the general population. In either event, there will frequently be a definable cohort for an epidemiologic study. Even so, one must be mindful that the most carefully designed study will often lack proper controls, have incomplete exposure data or will fail to account for one or more confounding variables.

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So it is with Agent Orange and the associated chlorodioxin. A number of exposures to TCDD among the industrial and general populations have occurred over a long period of time. A total of 14 accidents have been identified as occurring between 1949 and 1976, both here and abroad, in chemical plants that manufactured trichlorophenol. The data derived from the incidents are not entirely conclusive, yet many of the cases have been documented well enough to provide predictive evidence of the effects of TCDD on man. See Table 1 of the appendix for a summary.

Of Monsanto Employees

Of the 122 persons who were identified as having had chloracne following the TCP process accident at Nitro, West Virginia in 1949, 121 were selected for a cohort study (Zack et al, 1980). The results of the standardized mortality analysis revealed that the 32 actual deaths (from all causes) were less than the 46.41 expected deaths; this SMR of 0.69 was the only statistically significant value among the collected data. Nor was there any apparent excess of deaths from malignant neoplasms or circulatory diseases. Of the nine deaths from malignant neoplasms, five were due to lung cancers—four of them in cigarette smokers—three were from neoplasms of the lymphatic and hematopoietic tissue; one was blamed on a rare form of skin cancer, a fibrous histiocytoma.

Soon after the accident and on three occasions during 1949-53, Ashe et al (1949, 1950) and Suskind (1953) examined 12 of the workers who were severely affected with chloracne; an additional 26 persons with chloracne, who were not connected with the accident, were examined in 1953. The most frequent clinical symptoms were acneform lesions, followed by severe muscle pains in the upper and lower extremities, shoulders and thorax; fatigue; nervousness and irritability; decreased libido; dyspnea; vertigo and intolerance to cold. In addition to one case of sensory loss in the foot, there were four instances of hepatomegaly and other signs of liver impairment. Upon re-examination of several of the above workers in 1953, there was marked improvement in the skin lesions and a general subsidence of the non-cutaneous symptoms. Some persons had persistent but inexplicable complaints of pains in the back and lower extremities, nervousness, excess fatigue and dyspnea.

Another epidemiologic study of the 1949 accident will comprise a cohort of 437 persons; viz, the retirees and present and former Nitro employees, including those who were a part of the accident. The data are now being analyzed by Suskind and others, and their results will be published later.

Of Dow Chemical Employees

Ott et al (1980) examined the mortality experiences of a group of 204 workers who had been exposed to 2,4,5-T during its production from 1950-71. Many of the workers were also potentially exposed to a number of other chemicals during this period. Only 19 episodes of acute exposure were noted during 1954-70, which consisted largely of irritant exposures to the eyes and skin. No cases of chloracne or porphyria cutanea tarda were evident, and mortality of this limited sample was stated to be comparable to that of US white males as well as to the "background mortality experience" at this location.

A mortality study, undertaken on 61 Dow employees who had been engaged in the production of trichlorophenol and who had been accidentally exposed in 1964 to TCDD, did not disclose an elevated mortality rate; there were 4 actual deaths from all causes vs 7.8 expected. Overall cancer deaths were elevated (3 vs 1.5 expected) but no particular tumor type predominated. None of the findings was statistically significant (Cook et al, 1980). Of these workers, 49 developed chloracne. The three cancer deaths (identified as

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an adenocarcinoma, a fibrosarcoma and a glioma with metastases) were slightly above the expected number (3 vs 1.6), but they could not be ascribed to any predominant tumor type or particular organ/tissue site (Cook et al, 1980).

The latest Dow study from an interview questionnaire of 715 wives of men who worked in the Michigan division revealed no statistically significant differences in occurrences of miscarriages, stillbirths and fetal deaths or malformations. The two groups (ie, 370 wives of men with exposure to TCDD and 345 controls, wives of men without possible exposure) were matched for maternal age, complications during pregnancy and labor, existing medical conditions, smoking and mother's occupation. One weakness in the study, however, may be that the exposed group had 737 conceptions vs 2,031 for the controls (Anon, 1981).

Of Seveso Inhabitants

An earlier section of this report described an explosive release of TCDD from a trichlorophenol reactor in the vicinity of Seveso, Italy. This is the largest single population—over 37,000 persons—to have been potentially exposed to varying doses of this compound. Two years after the incident occurred, the acute and mid-term health effects were assessed; the mild chloracne, which occurred mainly in a small group of children, healed quickly. Subclinical peripheral nerve impairment was reported; there was also some liver involvement, but without apparent functional disorders. Immunoresponse was not altered nor was susceptibility to infectious diseases increased (Reggiani, 1979).

The long-term epidemiologic survey of the 220,000 residents of the Seveso area—conducted under the auspices of the NIEHS/IARC* working group—is expected to go on for at least five years. The most recent progress report of that group emphasizes the preliminary nature of all their findings and reiterates the above conclusions of Reggiani (Pocchiari et al, 1979, and Huff et al, 1980). Except for the skin, no organs or body functions were impaired. No derangement of gestation, no fetal lethality and loss, no gross malformations, no growth retardation at term and no cytogenetic abnormalities have yet occurred.

Meanwhile, analysis of the livers of 144 sheep which died in the Seveso area following the accident has disclosed that tympanism was the cause of death, not TCDD (Anon, 1980).

Of BASF Employees; Ludwigshafen, Germany

Seventy-five workers in a trichlorophenol plant were exposed to reactor products during an accident and its subsequent cleanup. Most of them suffered chloracne; 42 suffered severely, and 21 of these persons also had systemic damage to internal organs or CNS disturbances. Polyneuritis, impaired senses and liver damage were most prominent.
In 1978, a mortality study was conducted on the cohort: Of the 17 deaths (from 11 to 25 were expected, depending on the choice of control population), six were due to cancer (three from stomach cancer), five from cardiovascular diseases, one from cirrhosis of the liver and one from urogenital disease; all other deaths were either accidental or suicidal (Huff et al, 1980). No miscarriages or abortions were reported among the wives of exposed employees who were still at work in the plant (IARC, 1977).

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* NIEHS/IARC: National Institute of Environmental Health Sciences/International Agency for Research on Cancer

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Of NV Philips Employees; Netherlands

In this explosion of 1963 at a 2,4,5-T plant, 106 workers were probably exposed to TCDD (or related polychlorodibenzofurans). The two operators were exposed to TCDD largely by way of inhalation. Other workers, who were either part of the regular adjoining work force or were part of the clean-up and rebuilding crews, were outfitted in protective gear; they may have had only dermal contact. Chloracne occurred in 44 persons. Liver damage was not apparent, but there were a few complaints of fatigue. Of the eight deaths, one was from a pancreatic tumor and one was due to a myocardial infarction (at age 69); five other deaths also could have been from infarctions.

Of Swedish Railway Workers

The first study of this group, who had worked with both phenoxy acid (2,4-D and 2,4,5-T) and amitrole herbicides (Axelson et al, 1974), indicated that amitrole was probably solely responsible for the noted excess of tumors. A case-control analysis of the data has later revealed the possibility of a masked tumor-inducing effect of the phenoxy acids (Huff et al, 1980).

Of Other Swedish Workers

Follow-up on an earlier line of investigation led Hardell and co-workers to additional cases of soft-tissue sarcomas, which again seemed related to occupational exposures to phenoxy acids or chlorophenols. Malignant histiocytic lymphomas* were clustered among a group of hospital admissions in Sweden from January to September 1978; 14 out of 17 of the patients were at the time in occupations that utilized phenoxy acids or chlorophenols (eg, forestry, wood and paper mills, and farming). Inasmuch as both 6-fold tumor increases (Hardell, 1977 and 1979) were attributed to impurities, such as TCDD, a case-control (case-referrent) study by Eriksson et al (1981) was launched; their objective was to confirm the previous findings and to clarify whether or not the cause was due to the impurities. The new risk ratio (5.7) for soft-tissue sarcomas from exposure to phenoxy acids or chlorophenols was of the same order of magnitude as before; furthermore, a risk ratio of 4.2 was obtained for other phenoxy acids (such as MCPA, 2,4-D, mecoprop and dichlorprop) which are free of dioxins.

Of Finnish Railway and Forestry Workers

A similar group of 30 workers in Finland, who were exposed to TCDD-containing herbicides, will be compared to a matched control group to see if there are any long-term effects from their exposures. Thus far, no differences are apparent (Huff et al, 1980).

Of Dow/Monsanto Cohorts (Soft-Tissue Sarcomas)

Researchers at the National Institute for Occupational Safety and Health (NIOSH) (Honchar et al, 1981) have released the results of an examination of four cohorts of chemical workers from Dow and Monsanto who were exposed to 2,4,5-T or 2,4,5-trichlorophenol and contaminated with TCDD. From the 105 deaths among these cohorts

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* Tumor sites included: frontal sinuses, bladder, ileum, retroperitoneal, parotid and submandibular areas, femur and groin.

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were three from soft-tissue sarcoma (2.9% of the total); by comparison, the figure for similar deaths in 1975 among US males aged 20 to 84 yrs old was 0.07%. Casewise,

> one death (Monsanto) in 1978, at age 58, was from a malignant fibrous histiocytoma of soft-tissue origin, some 29 years after exposure to an accidental spill of TCP, which also resulted in chloracne;
>
> another Monsanto man, who had been involved in the synthesis of 2,4,5-T, was 49 at the time of death in 1972 from a generalized liposarcoma (he had no definite history of chloracne, yet his case cannot be discounted);
>
> the third case was a Dow TCP worker who died in 1975 at age 53 from fibrosarcoma—the subject had had facial dermatitis but no definite chloracne.

Since the publication of the above NIOSH report, other cases of soft tissue sarcomas have been announced. One of these, a Dow worker (age unknown) who also was stationed in the TCP-production area when the outbreak of chloracne appeared in late 1963-64, died of a malignant fibrous histiocytoma; he, presumably, had had frank chloracne (Anon, 1981; Cook, 1981). Cook cites one common factor besides TCDD exposure for the four victims: they were cigarette smokers. Hardell and Eriksson (1981), on the other hand, saw no difference in the smoking habits of their subjects, and they refuted Cook's hypothesis that smokers who contracted chloracne from TCDD were at increased risk of these sarcomas.

Their argument may be well founded when another disclosed case of soft-tissue sarcoma at Monsanto is considered (Moses et al, 1981). This man—a non-smoker—died at age 58 of a retroperitoneal neurogenic sarcoma (malignant schwannoma*). He had worked for the company for 32 years as a truck driver, hauler and maintenance man; though he had not engaged in the production of TCP or 2,4,5-T, he could have been potentially exposed to either or both of the chemicals (probably for the first time around 1948). He had no history of chloracne.

A father and son who were employed at the Sauget, Illinois chlorophenol plant of Monsanto were admitted almost simultaneously for diagnosis and treatment of soft-tissue sarcomas. The father, age 53, had a history of prolonged exposure prior to the tumor's development—a liposarcoma of the buttock and thigh. With resection and radiation therapy, the growth apparently was arrested. The 33-yr-old son, however, died shortly after the resection of an extensively metastasized fibrosarcomatous mesothelioma. Neither of the two men were known to have had chloracne (Johnson et al, 1981).

Of Other Industrial Workers

Porphyria cutanea tarda, of varying degrees of severity, and elevated urinary uroporphyrins were discovered among 11 out of 29 workers at Diamond Alkali's New Jersey plant. These workers, who were exposed to 2,4-D and 2,4,5-T in the manufacturing processes, were examined because of their chloracne symptoms (Bleiberg et al, 1964). The degree of hyperpigmentation was said to vary according to the severity of chloracne and was more prevalent in Negroes. The degree of hirsutism too was related to the severity of chloracne. The cause of porphyria cutanea tarda was assumed to be

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* According to the Task Force on Soft-Tissue Sarcoma, malignant schwannomas are said to comprise 4.9% of the soft-tissue sarcomas (Suit, 1978).

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chemically induced as a result of damage or insult to the liver and not of genetic origin. The skin manifestations of PCT, ie, epidermal fragility and vesiculobullous eruptions, occurred on those areas of the face, ears and hands that were exposed to sun or pressure.

A follow-up of Bleiberg et al was made in the same plant by Poland et al (1971) on 73 male workers. Moderate to severe chloracne was prevalent in only 13 out of 73 (18%) of the cases; again, it varied in degree and was accompanied by scarring, hyperpigmentation, hirsutism and eye irritation. There was no evidence of porphyria cutanea tarda and only one case of uroporphyrinuria. The authors have inferred that porphyria cutanea tarda and chloracne are independent syndromes though they may have the same etiologic agent. There was either no acne or only minimal lesions in 82% of the employees. Occurrence of chloracne, likewise, was not significantly greater in Negroes than whites, and was seemingly not related to job location or duration of employment (hence, level of exposure); cardiovascular findings were unremarkable except for three persons with a history of myocardial infarction.

Oliver (1975) has described three laboratory workers who were exposed to "pure" TCDD. Two of the three had typical chloracne. All had abnormal serum cholesterol levels but without porphyria. Two had gastrointestinal symptoms, one complained of blurred vision and impaired muscular coordination, and another complained of "neuralgia of the left thigh." Two years after the exposure, two of the men experienced personality changes, neurological disturbances and hirsutism.

Jirasek et al (1973, 1974) have reported from Czechoslovakia on what is probably the most thoroughly documented group of exposed workers. From 1965-69, 78 persons were affected by their contact with the sodium salt of 2,4,5-T, the butyl ester of 2,4,5-T and pentachlorophenol; 76 of them developed acne, hypertrichosis and hyperpigmentation. Complaints included fatigue, weakness and pain in the extremities, sweating and headache; many had polyneuropathy. Clinical findings revealed hepatomegaly, porphyria cutanea tarda, and increases in ALA and alkaline phosphatase (Suskind, 1980). The ten-year follow-up of 55 workers by Pazderova-Vejlupkova et al (1980, 1981) emphasized the non-uniformity of TCDD's effects on individual organs and the development of illness that affects virtually every important organ system. Complete recovery is rare, except for anxiety and depression, and persistence of an impaired metabolism of fats and carbohydrates can be a major cause of premature arteriosclerosis. Children who were born to exposed individuals were not adversely affected.

The Coalite episode, which occurred in Derbyshire, UK in April 1968, was first described by May (1973). This also involved an overheated reactor used to synthesize TCP; the explosive rupture of the kettle was accompanied by a detonation of the released glycol and chlorobenzene vapors. Seventy-nine cases of chloracne were initially recorded; many were severe, but they were resolved within four to six months. There was no evidence of depression, weight loss, malignancies or liver, kidney or cardiac impairment. The epidemiological survey of 1977-78, covered only 41 of the 90 exposed workers and revealed some abnormalities in blood chemistry and immune function. There were no chromosomal abnormalities in the circulating lymphocytes. The levels of serum cholesterol and triglycerides were elevated, while the gamma-glutamyl transpeptidase was abnormally high. Both IgD and IgM were lower than the controls. Twenty-nine of the original 41 workers were re-examined, but the immunoglobulins were not measured. The company is now reluctant to extend the investigation further, and it will no longer agree to medical examinations of the work force as long as the results are intended for publication (Hay, 1981).

A group of 190 workers (80 current and 110 previously employed) of the Vertek Chemical

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plant, Jacksonville, Arkansas, are being studied for possible adverse health effects from the plant's production of 2,4,5-T (Suskind, 1980). Results are presently unavailable.

Of Vietnam War Veterans

The Armed Forces Institute of Pathology is examining specimens of veterans' tissue to determine whether any of the illnesses in these men can be related back to their exposures to Agent Orange. By its very nature, the project can only reveal carcinogenesis and possibly the residual effects of acute toxicity.

From past experience, toxic substances tend to damage specific organs or organ sites; thus, if TCDD is typical, one should expect a cluster of cases according to clinical symptoms and/or organ type. The only clustering thus far in the 152 cases that have been assessed occurs with the skin and subcutaneous tissues; the dominant diseases are epidermal inclusion cysts and chronic, non-specific dermatitis. There were no consistent patterns for all other dermatologic diagnoses and malignancies. If any malignancies were to have been induced by TCDD, they should be appearing by now. Yet, no unusual morphological features nor clustering of tumors by diagnosis or site implicate Agent Orange (Irey, 1981).

Of "Dedrumming" Personnel

As mentioned earlier, about 200 civilians were assigned by the military to destroy the contents of about 40,000 55-gallon drums (2.22 million gallons) of surplus Agent Orange at the close of the Vietnam war.

Pre- and post-physical examinations, including X-rays, neurological examinations and extensive clinical chemical tests, were peformed on all workers along with environmental monitoring during this operation. Military personnel revealed no apparent physical effects (Young et al, 1978).

ENVIRONMENTAL FATE OF 2,4-D, 2,4,5-T AND TCDD

2,4-D and 2,4,5-T

Environmentally, 2,4-D is regarded as a non-persistent material. At least 12 different reactions may contribute to its breakdown, such that its half-life in soil is less than 0.5 months. Phenoxy herbicides, as a class, undergo rapid decomposition in the soil; both 2,4-D and 2,4,5-T are degraded to carbon dioxide, inorganic chlorides and water (Audus, 1960).

2,4,5-T also is of minor ecological concern, though compared with 2,4-D it is slightly persistent; its half-life in soil when applied at customary rates varies from 2 weeks to 4 months. And since it is soluble in water only to the extent of 0.2 ppb, and is firmly bound to soil particles, its migration thru the soil should be minimal (Crosby et al, 1971; 1973). There were even no significant differences in the persistence and disappearance of these two herbicides when they were tested with various soil types, including the tropical soils of Vietnam (Blackman, 1974).

TCDD

TCDD is distributed equally among the fat and liver of organisms and to a lesser extent in the kidneys and skin. It is eliminated, for the most part, via the feces. Currently, the

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highest environmental residues of TCDD as measured by EPA ranged from 20-60 ppt in a small sample of fat from beef cattle that had grazed on 2,4,5-T-treated rangeland (Young et al, 1978). This finding conflicts with Kocher et al (1978), who detected only 3-4 ppt in range-fed beef. Mahle et al (1977) found no TCDD in the milk of cows that had grazed on 2,4,5-T-treated pasture or rangeland. Likewise, TCDD was undetectable (not more than 10 ppt) in human milk that was sampled from an area near West Texas, where 2,4,5-T herbicides were used repeatedly over a period of 20 years (Shadoff et al, 1977). On the other hand, samples of human milk collected from Vietnam in 1970 and analyzed four years later contained 40-50 ppt of TCDD (Baughman, 1976).

An extensive examination of 30 species of flora and fauna at the Eglin AFB test site, Florida, which had been exposed to soil levels of TCDD between 10 and 1500 ppt (mean = 165 ppt), revealed no abnormal histopathology nor other deleterious effects (Young et al, 1978).

TCDD breaks down rapidly on leaves of plants, in water and on the surface of soil, through the action of sunlight and the presence of an organic hydrogen-donor (eg, a petroleum hydrocarbon), the reaction is a progressive dechlorination of the aromatic ring. Both Crosby et al (1977) and Nash et al (1978) confirm the extreme photosensitivity of TCDD in the presence of ultraviolet radiation and an organic hydrogen-donor, especially when the compound exists as a thin layer on plant material and the like. It does persist in the soil and in aquatic ecosystems, but—like 2,4,5-T—there is no evidence that leaching by water occurs.

From experimental work with Seveso soils, it appears that TCDD can be taken up by certain plants and translocated from the roots to the foliage (Cocucci et al, 1979), the compound may then be photochemically degraded, released into the atmosphere by transpiration and/or metabolized by the plant. This matter of translocation, however, is contrary to the earlier findings of Helling et al (1973) and Isensee et al (1971).

TCDD generally has a half-life in soil no longer than one year (Kearney et al (1972), or about 230 days. Under certain conditions it may persist longer than this if it was initially present with high amounts of 2,4,5-T in the soil (Young, 1976). Though microbial degradation is not a major means of elimination from the environment, there are at least five strains of bacteria that are known to assimilate it (Matsumura et al, 1973). There is also a possibility that mutants of existing microbial strains, or even artificially created Pseudomonas strains (Chatterjee et al, 1981), will become increasingly effective scavengers in the future.

When lake sediments containing TCDD were incubated in the laboratory, the compound was released from the sample mainly by evaporation. Its rate of metabolism was influenced by those conditions that also stimulated microbial growth in the sample (Ward et al, 1978).

Due to the paucity of environmental sampling, it is not yet possible to say that TCDD is bioaccumulating; although, there is the possibility that levels of 0.3 ppt might be found along rights-of-way where periodic applications of 2,4,5-T and silvex have been made (American Farm Bureau Federation, 1979). There is no evidence that tetrachlorodioxins are formed in the environment (Sundstrom et al, 1979).

The Sabine (Texas) River Authority recently removed 15 drums of chemical compounds, including 2,4,5-T and 2,4-D, from a waste disposal site that was located about 300 yards from the lake that furnishes about 20% of the drinking water to the city of Dallas and its suburbs. Buried in 1978, the waste does not appear to have left dangerous concentrations

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of TCDD in the soil. EPA is conducting further sampling to ensure that there is no serious contamination (US Environmental Protection Agency, 1980).

CRITICAL REVIEWS OF PRIOR STUDIES

Of EPA's Alsea Studies

In the Alsea basin of Oregon, 2,4,5-T and silvex were used on a seasonal basis to eradicate unwanted vegetation from the forest lands. In 1978, there was some concern that such spraying was responsible for a number of spontaneous abortions in the area. All told, 13 miscarriages were reported from nine women. As a result of much publicity and public concern, the EPA conducted a study—Alsea I—to assess the facts and to determine whether or not the spraying was indeed responsible. EPA concluded that there was no relationship between the spraying and the incidence of miscarriage; the rate was within the expected limits. The study was harshly criticised because the exposures were not documented and the statistical treatment was improper. Thus, Alsea II was begun in October of 1978, to study a much larger area and population than Alsea I and to compare miscarriages within the first 20 weeks of pregnancy with the spraying of 2,4,5-T. A high probability of an elevated miscarriage rate shortly after spraying was reported to the press, but this claim was proved to be without merit.

—By Oregon State University

A critical analysis of Alsea II was conducted by an interdisciplinary team of Oregon State University scientists (Wagner et al, 1979). It did not support any of EPA's conclusions in the Alsea II study. The team contended that:

there was no control population in the study;

data on miscarriages were unreliable and inaccurate;

medical practices were profoundly different in the three areas under consideration;

information on use of 2,4,5-T was inaccurate and incomplete;

variations in hospitalized miscarriages in all the areas were well within expectations;

differences existed in topography, climate and demography;

data collection was incomplete and failed to account for patterns of and differences in medical practice among areas.

The panel termed the EPA's hospitalized spontaneous abortion indices (HSAI) values to be unreliable because:

there was substantial variation in the magnitude of the HSAI's for any one month among the six years and

the peak HSAI in June was due to a single large excursion only in 1976, while the seasonal cyclic peaks were consistent with random variations.

Furthermore,

alleged increase in the use of 2,4,5-T was actually due to more complete collection of spray data,

there was no statistically significant cross-correlation except for the apparent increase in the June HSAI—deletion of the single June 1976 HSAI value removed the significant correlation—and

the HSAI increase in June 1976 was identified only with a location where human exposure was least likely to have occurred.

Hence, according to the OSU panel, there was:

no significant correlation between 2,4,5-T use and the HSAI, and

EPA's study was seriously flawed because of incomplete and inaccurate data.

—By FIFRA's Scientific Advisory Panel
This panel of the Federal Insecticide, Fungicide and Rodenticide Act reviewed the evidence and found neither immediate nor substantial threat to human health or the environment when silvex or 2,4,5-T was applied to rice, rangeland, orchards, sugarcane, and when used for certain non-crop purposes.

—By Others
Lamm (1979) also declared the Alsea II study to be epidemiologically unsound and the report useless. Both statistically and epidemiologically, the report does not support any relationship between herbicide spraying and miscarriages. Lamm (1980) has since claimed that only four out of the ten women who were cited by EPA in the Alsea II study underwent spontaneous abortions; the remainder were hospitalized for other reasons.

New Zealand's Allan H. Smith (1979) termed the Oregon study of highly doubtful validity.

Robinson (1979) of Austrialia emphasized that the EPA information in no way confirmed a hazard to human health from 2,4,5-T when it was used in a careful manner and according to label instructions on the container. A comprehensive review by the Australian Department of Health (1977) concluded that there was no evidence that 2,4,5-T caused human birth defects.

CURRENT AND PROPOSED STUDIES

International Agency for Research on Cancer (IARC)

An ad hoc Working Group, comprised of representatives of the National Institute of Environmental Health Sciences (NIEHS) and IARC, met in Lyon, France in January of 1978 to discuss the feasibility of a coordinated epidemiological study on the long-term hazards of the chlorinated dibenzo-p-dioxins and chlorinated dibenzofurans. A review of the meeting and an account of the long-term hazards are to be found in Huff et al (1980).

An outcome of the meeting was the unanimous agreement for an exchange of information and the coordination of research activities. Three items were believed worthy of special attention:

development of sensitive and reliable measurements of body burden, more sensitive and less expensive analytical methods and standardization of sampling procedures;

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development of standard protocols for clinical examinations as well as basic information; and

development of an international registry for long-term follow-up of exposed persons, including a registry of chloracne cases.

IARC has contracted with the National Institute of Environmental Health Sciences (NIEHS) for NIEHS to establish and maintain an international registry of persons outside the US who have been exposed to phenoxy herbicides and their contaminants (personal communication, Suskind, 1981).

US Air Force Epidemiological Study

—Project Ranch Hand Personnel
This project will arrange for the examination of the 1,200 men who were involved in the actual handling and spraying of Agent Orange. They, and the control population of 20,000, are to be followed over the next 20 years (Holden, 1981).

Armed Forces Institute of Pathology (AFIP)

Thru its Department of Environmental and Drug-Induced Pathology, AFIP is examining biopsy and autopsy tissue of all Vietnam veterans. To date, only 152 cases have been assessed; the findings have been presented earlier in this report under the heading "health surveys of exposed human populations."

National Cancer Institute (NCI)

The Epidemiology Branch of NCI is conducting a number of investigations where pesticide exposures are involved. In cooperation with the Federal Aviation Administration, a retrospective mortality study of 10,000 aerial pesticide applicators will be conducted; NCI will run a similar study on 4,500 pest control operators. The University of Minnesota, under contract to NCI, will conduct a case-control interview study of patients with diagnosed leukemia or non-Hodgkins lymphomas within the past year or over the next two years; some 300 cases of each disease and their matched controls will likely be included.

A soft-tissue sarcoma study also has been proposed; this will be conducted jointly with the Armed Forces Institute of Pathology (Cantor, 1981).

National Institute for Occupational Safety and Health (NIOSH)

NIOSH is establishing a registry of all US workers who may have had exposure to TCDD in the course of manufacturing 2,4,5-T. Results of the data analysis are not expected before about 1983 (Anon, 1980).

US Department of Agriculture

Cancer deaths among Forestry Service workers will be compared with a cohort of other employees who are not considered at risk.

Veterans Administration

An annotated review of the herbicide literature, including the phenoxy acids, their

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derivatives and associated chlorodioxins, will be completed around October 1981. The work was performed under a contract awarded December 1980 to JRB Associates.

A contract was awarded in May 1981 by the Veterans Administration to the University of California at Los Angeles for the design of an epidemiologic study of Vietnam veterans.

Approximately 45,000 Vietnam veterans who expressed concern about the hazards of Agent Orange have been examined; data on 25,000 of these men have been placed in a special Agent Orange Registry, which may serve later to identify them and to provide medical information, as well as indications of health trends over the long term.

The Chloracne Task Force was established to sift out those cases of dermatitis that either resemble or are truly chloracne. Cases of the former type will be re-examined by dermatologists who have an expert knowledge of the disease. Thus far, there have been only 700 cases of "skin conditions" out of the total of 3,500 claims that have been filed for damage from Agent Orange.

Others

Australia will fund a two-year study of its Vietnam veterans and their offspring to ascertain if there were any adverse effects of military exposure to Agent Orange. Some 60,000 veterans with an estimated 100,000 children will be involved (Anon, 1980).

Canadian officials recently have become alarmed after the Canadian Wildlife Service found TCDD in herring gull eggs, a common fish-eating bird, from Lake Ontario and a part of Lake Michigan. These preliminary results have led them to believe there may be US industrial contamination of these waters. A joint US-Canadian effort to study the situation further is a likelihood (Anon, 1980, 1981).

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SUMMARY

Agent Orange—as used in Vietnam—was a 50:50 mixture of the n-butyl esters of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), together with a minor amount of the free acid 2,4,5-T (1% of the total mixture) and varying amounts (from 0.02 to 15 ppm, or a weighted mean of 1.98 ppm) of the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

Commerical formulations of 2,4,5-T, as well as silvex (or 2-2(2,4,5-trichlorophenoxy)propionic acid), may contain TCDD as a contaminant. At one time the amount of TCDD was as much as 70 ppm; present methods routinely produce levels below 0.01 ppm, with occasional excursions to no more than 0.05 ppm. TCDD may form as a by-product in the synthesis of 2,4,5-trichlorophenol (a precursor of 2,4,5-T), particularly if the alkaline reaction mixture is allowed to rise uncontrollably beyond 180°C. It may also be produced by the pyrolysis of certain chlorinated compounds in industrial or municipal wastes and by the burning of vegetation that has been sprayed with the chlorinated phenoxy acid herbicides. Note that 2,4-D, a phenoxy herbicide closely related to 2,4,5-T, is not normally contaminated with TCDD.

In addition to the marked variations in the sensitivity and susceptibility of animal species to all toxic substances, there are significant differences between some of the toxic effects of TCDD in experimental animals and the human experience; thus, the animal data cannot be translated directly to man.

One of the more pronounced biological effects of TCDD, as well as a number of other chlorinated aromatic compounds, is a tendency to cause chloracne in certain animals and man. This particular skin disease is, in fact, regarded as the clinical marker for TCDD exposure. Systemic disorders in man from exposures to TCDD are unlikely to occur in the absence of chloracne. Such symptoms as impaired liver function, nephropathy, GI irritation, myopathy and neuropathy, including depression and irritation of the central nervous system, have been reported after exposure to large amounts of TCDD; however, these symptoms have not been progressive, and they have always cleared with time.

Other toxic effects of TCDD in test animals appear as pathological changes in the liver, peripheral nerves, blood forming organs and reticuloendothelial system.

TCDD is a very powerful enzyme inducer. In addition to altering normal enzyme activity, it may potentiate the harmful action of other toxins or even render an otherwise innocuous agent toxic.

TCDD can serve as a promoter of carcinogenesis or as an inducer of cancer in some strains of rats and mice. The carcinogenicity is always accompanied by considerable systemic toxicity in contrast to some other chemical carcinogens. By itself, 2,4,5-T is not a carcinogen, nor has it been shown to cause genetic changes in any animal species.

A consensus of studies using high doses of 2,4,5-T with 0.1 ppm or less of TCDD showed cleft palate in mice—but no other species—and embryotoxicity to the mouse, rat, hamster, sheep, monkey and rabbit.

TCDD does induce genetic changes by the Ames test with S typhimurium and E coli but dominant lethal and cytogenetic evaluations in rodents do not confirm that such changes will also occur in other animals, let alone man.

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While 2,4,5-T and 2,4-D pesticides have been used in agriculture, forest management, and commerical and residential landscaping for over 30 years, there is still no conclusive evidence that they and/or TCDD are mutagenic, carcinogenic or teratogenic in man, nor that they have caused reproductive difficulties in the human.

Both 2,4,-D and 2,4,5-T undergo rapid decomposition in the soil and are, therefore, of little environmental concern. TCDD does persist in soil longer than 2,4,5-T but, in general, its half-life in soil is no longer than one year; in the presence of ultraviolet light it breaks down rapidly when present as a thin film on plants, water and the surface of soil.

RECOMMENDATIONS

The Council on Scientific Affairs recommends that:

(1) The above studies on exposed, or allegedly exposed, persons continue to be supported and, if feasible, enlarged to include the cooperative engagement of all internationally known exposure data, as recommended by the International Agency for Research on Cancer (IARC).

(2) All physicians be alerted through AMA publications to the classical signs of chloracne and the possible signs and adverse effects of TCDD exposure. They should be encouraged to enlist in the present efforts to identify and treat those persons who have had serious exposures to TCDD, and to cooperate in the collection of vital information that is needed for the ongoing human epidemiologic studies.

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Table 1 — Occupational and Industrial Accidental Exposures to TCDD in Plants Producing 2,4,5-trichlorophenol and/or 2,4,5-T

Date	Site	Injured/exposed	Remarks
1949	Monsanto (Nitro, W Va)	122/250	Uncontrolled reaction. Some studies completed, others underway. 122 chloracne subjects used for cohort study: 32 deaths (all causes) vs 46.4 expd.; no excess deaths from malignant neoplasms or circulatory disease (Ashe et.al, 1949, 1950; Suskind, 1953; Zack et al, 1980).
1952-3	Boehringer (W Germany)	37/?	Occupational. Outcome unavailable (Bovey et al, 1980).
1953	BASF (Ludwigshafen, Ger)	55/75	Uncontrolled reaction. 17 deaths (11-25 expd.) (4 GI cancer — 3 stomach, 1 colon — and 2 oat-cell lung cancers). Continuing 25-yr follow-up. Most prominent were polyneuritis, impaired senses and liver damage (Huff et al, 1980; IARC, 1977).
1954	Boehringer (Hamburg, Ger)	?/31	Occupational. Outcome unavailable (IARC, 1977; Young et al, 1978).
1956	Rhone-Poulenc (France)	17/?	Uncontrolled reaction. 17 chloracne cases, also hyperlipidema and hypercholesterolemia (Bovey et al, 1980; IARC, 1977).
1956	Hooker (US)	?/?	Probably occupational. Outcome unavailable (Young et al, 1978).
1956	Diamond Alkali (N Jersey)	29/?	Probably occupational. 29 chloracne subjects studied, 11 had porphyria cutanea tarda (Bleiberg et al, 1964; Poland et al, 1971).
1963	Philips (Netherlands)	30/106	Uncontrolled reaction. 44 chloracne cases (42 severe, 21/42 had internal damage or CNS disturbances; viz, polyneuritis, impaired senses and liver damage); 8 deaths (6 possible myocardial infarctions); some fatigue; no liver damage. Full report planned (Huff et al, 1980).
1964	Spolana (Czechoslovakia)	?/78	Occupational. 78 chloracne, 5 deaths (2 bronchogenic cancer, 1 atypical atherosclerosis, 1(?) liver

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		cirrhosis, 1 industrial intoxication accident); many of remaining 50 studied over 10 yrs have hypertension, hypercholesterolemia, prediabetes and hyperlipidemia; significant amount of porphyria cutanea tarda and severe hepatic and neurologic damage. Study to continue (Huff et al, 1980; Pazderova-Velupkova et al, 1981).
1964	USSR 128/	Occupational. 128 cases of skin lesions, 69 of 83 examined had "acne"; 18 with neurasthenic syndrome; many with liver impairment (esp, among those with severe skin lesions) (Bovey et al, 1980; IARC, 1977).
1964	Dow Chemical (Midland) /204	Occupational. Mortality less than expected, comparable to US white males: from all causes, 11 vs 20.3; from malignant neoplasms, 1 vs 3.6; from cardiovascular diseases, 4 vs 9.1. No chloracne or PCT (Ott et al, 1980).
	/61	Deaths (all causes) not elevated (4 vs 7.8 expd.); deaths (all cancers) elevated (3 vs 1.5 expd.) but no predominant type or site (Cook et al, 1980).
1966	Rhone-Poulenc (France) ?/?	Uncontrolled reaction. 21 chloracne cases (Bovey et al, 1980).
1968	Coalite (United Kingdom) ?/90	Uncontrolled reaction. 79 chloracne cases; 1 death (coronary thrombosis). Cohort study planned. Company refuses to divulge any more information (Bovey et al, 1980; May, 1973, 1981).
1974	Bayer (W Germany) 5/?	Occupational. Outcome unavailable (Young et al, 1978).
1972-3	Linz Works (Austria) 50/?	Occupational. 5 chloracne cases; outcome unavailable (Young et al, 1978).
1976	ICMESA (Seveso, Italy) /156	Uncontrolled reaction. Over 500 residents treated for presumed toxic symptoms, 134 confirmed chloracne cases (Bovey et al, 1980). Long-term morbidity study underway — overall mortality rate normal so far. Cancer and mortality registries should be formed.

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Polychlorinated Biphenyls (PCBs), Dibenzo-p-Dioxins (PCDDs), Dibenzofurans (PCDFs), and Related Compounds: Environmental and Mechanistic Considerations Which Support the Development of Toxic Equivalency Factors (TEFs)

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ABSTRACT

Halogenated aromatic compounds, typified by the polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and diphenylethers (PCDEs), are industrial compounds or byproducts which have been widely identified in the environment and in chemical-waste dumpsites. Halogenated aromatics are invariably present in diverse analytes as highly complex mixtures of isomers and congeners and this complicates the hazard and risk assessment of these compounds. Several studies have confirmed the common receptor-mediated mechanism of action of toxic halogenated aromatics and this has resulted in the development of structure-activity relationships for this class of chemicals. The most toxic halogenated aromatic is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and based on *in vivo* and *in vitro* studies the relative toxicities of individual halogenated aromatics have been determined relative to TCDD (i.e., toxic equivalents). The derived toxic equivalents can be used for hazard and risk assessment of halogenated aromatic mixtures; moreover, for more complex mixtures containing congeners for which no standards are available (e.g., bromo/chloro mixtures), several *in vitro* or *in vivo* assays can be utilized for hazard or risk assessment.

Key Words: 2,3,7,8-TCDD, halogenated aromatics, toxic equivalency factors.

I. INTRODUCTION

Polyhalogenated aromatic compounds such as the polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), polychlorinated benzenes, polychlorinated phenols, polychlorinated terphenyls (PCTs), polybrominated biphenyls (PBBs), and chlorinated phenols, anilines, and benzenes are industrial chemicals which have been utilized for diverse commercial applications.[1-7] For example, PCBs were widely used as heat transfer fluids, organic diluents, plasticizers, lubricant inks, fire retardants, paint additives, sealing liquids, immersion oils, adhesives, dedusting agents, laminating agents, waxes, and as dielectric fluids for capacitors and transformers. PCNs and PCTs have enjoyed limited use as PCB replacement compounds and PBBs were primarily used as flame retardants. The monocyclic halogenated compounds are important intermediates in the synthesis of a wide array of commercial chemical products and the chlorinated phenols have been extensively used as fungicides. A number of other compounds, including the polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), alkylated PCDFs, polychlorinated diphenyl ethers (PCDEs), polychlorinated hydroxydiphenyl ethers; polychlorinated azo- and azoxybenzenes; polybrominated dibenzo-p-dioxins (PBDDs) and dibenzofurans (PBDFs), and mixed bromo/chloro dibenzofurans and dibenzo-p-dioxins have also been identified as (1) byproducts which form during the synthesis of the primary industrial halogenated aromatics (2) byproducts from other commercial processes, and (3) byproducts of combustion.

Most members of this family of chemicals exhibit several common properties. These compounds tend to be highly lipophilic and the degree of lipophilicity is increased with increasing ring chlorination. With few exceptions, the halogenated aromatics which contain more than one phenyl ring are also stable and resistant to breakdown by acids, bases, heat, and hydrolysis; these physicochemical characteristics are important factors which have contributed to the diverse industrial applications of these compounds. Not surprisingly, these properties also contribute to the widespread environmental problems associated with certain members of this class of chemicals, particularly the PCBs, PCDDs, and PCDFs which have been identified in almost every compartment of the global ecosystem.[5,6,8-33] Moreover, because of the liophilic nature of the PCBs, PCDDs, and PCDFs, these compounds bioaccumulate in the food chain, and residues have been detected in fish, wildlife, and human adipose tissue, milk, and serum.[34-62]

Public and regulatory concern over the potential adverse human health effects and environmental damage associated with exposure to halogenated aromatics has been heightened by several incidents in which potentially higher levels of human exposure have been reported. These include agricultural and occupational exposures to halogenated aromatics,[61,63-96] PCB fires,[97] and several accidental exposures which include the Yusho and Yu Cheng poisonings,[98-104] the explosion in Seveso,[105-110] and the exposures associated with Love Canal,[111] chlorinated phenoxy herbicides used in Vietnam (Agent Orange),[112-115] Times Beach, and the related exposures in Missouri,[117-121] and the PBB food chain contamination in Michigan.[122-127] These incidents, coupled with frequent press reports

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of PCB fires, numerous small spills and accidents, and continuing disposal problems, have generated considerable pressure to develop and validate methods for the hazard and risk assessment of toxic halogenated aromatics, with particular emphasis on the PCDDs, PCDFs, and PCBs. The problems associated with this process are numerous and are discussed in this review article.

II. FACTORS WHICH AFFECT THE HAZARD AND RISK ASSESSMENT OF TOXIC HALOGENATED AROMATICS

A. Sources of Halogenated Aromatics

The structures of the polycyclic halogenated aromatics (Figure 1) show that all of these compounds have multiple sites for halogen substitution in which there can be both differences in substitution patterns and the degree of halogenation. Table 1 summarizes the number of possible isomers and congeners for the major classes of polycyclic halogenated aromatics. Thus, there are 209 possible PCBs, PCDEs, and azo/azoxy benzenes, 135 PCDFs, and 75 PCDDs and PCNs. The number of possible polychlorinated o-, m-, and p-terphenyls are 3043, 3155, and 1951, respectively, and the number of possbile mixed bromo/chloro halogenated aromatics are exceedingly high (5020 possible compounds).[128]

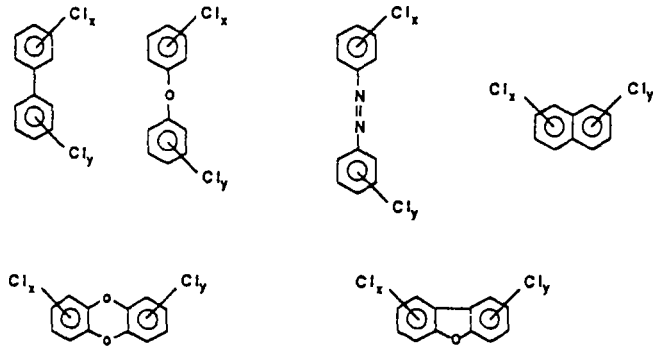


FIGURE 1. Structures of the polycyclic halogenated aromatics.

Table 1
Multiplicity of PCB, PCN, PCDE, PBB, PCDF, PBDF, PCDD, and PBDD Isomers and Congeners

No. of Cl atoms	1	2	3	4	5	6	7	8	9	10	Total
PCBs/PBB and PCDEs	3	12	24	42	46	42	24	12	3	1	209
PCDFs/ PBDFs	4	16	28	38	28	16	4	1	—	—	135
PCDDs, PBDDs and PCNs	2	10	14	22	14	10	2	1	—	—	75

Not surprisingly, the composition of the commerial PCB and PCN products are highly complex. A recent study[129] on the high resolution capillary gas chromatographic analysis of the commercial PCBs revealed that 132 individual congeners were present in these mixtures and the relative number of congeners and their concentrations in the commercial products is dependent on the degree of chlorination of the mixture. The commercial PCN mixtures are also complex,[130] however, the high resolution analysis and indentification of the individual congeners has not been reported.

The halogenated aromatics which are formed as byproducts in the synthesis of industrial chemicals or as byproducts of combustion are, with few exceptions, complex mixtures of isomers and congeners. Some of the important industrial sources of PCDDs and PCDFs are summarized in Table 2 and these include their formations as byproducts in the productions of PCBs,[3,5,6] chlorinated phenols and chlorinated phenol-derived chemicals,[131,132] hexachlorobenzene,[133] technical hexachlorocyclohexanes,[134] and chlorides of iron, aluminum, and copper.[135] PCDFs and PCDDs have also recently been identified in effluents, wastes, and pulp samples from the pulp and paper industry, and in finished paper products.[136-142]

In 1977, Hutzinger and co-workers first identified the presence of PCDDs and PCDFs as trace components in extracts of fly ash and in flue gas emissions from municipal waste incinerators in the Netherlands.[143] Analytical studies in several laboratories have since identified PCDDs and PCDFs emissions from municipal incinerators in all parts of the world.[25,144-147] The levels of these toxins which are emitted are highly variable and dependent on a number of factors including the incinerator design operating conditions and the nature of the waste products.[148-151] Several studies have also shown the formation of

Table 2
Identification of PCDDs and PCDFs as Byproducts in Industrial Chemicals and Industrial Processes

Industrial products containing PCDFs	Use	Ref.
Phenoxy alkanoic acid compounds	Herbicides	131, 132
Chlorinated phenols	Wood preservative	131, 132
Hexachlorobenzene	Fungicide and industrial waste	133
Trichlorobenzene and tetrachlorobenzene	Industrial use	303
Polychlorinated biphenyls	Industrial use	3, 5, 6
Chlorides of iron, aluminum and copper	Industrial use	135
Technical hexachlorocyclohexanes	Insecticide	134
Pulp and paper industry	Byproducts	136—142

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PCDDs and PCDFs in home heating systems which use both coal and wood as fuel sources.[152-155] Table 3 summarized the relative concentrations of PCDDs and PCDFs in soot from municipal waste incinerators and from home heating incinerators and higher levels have generally been observed in the municipal waste incinerators. The "Trace Chemistries of Fire Hypothesis"[145] suggested the PCDFs and PCDDs can arise from diverse combustion processes which include "natural" forest fires and volcanic eruptions. However, results from the analysis of dated aquatic sediment profiles have shown that there were time-dependent increases in the concentrations of PCDDs and PCDFs which correlated with the increased production and use of chlorinated industrial compounds.[29-33] These results confirm that the production of industrial halogenated organic chemicals and their subsequent incineration contribute more to the accumulation of PCDDs and PCDFs in sediments than do natural combustion processes.

The mechanisms associated with the heat-induced formation of PCDDs, PCDFs, and related compounds have been extensively investigated.[156-159] For example, in a flow reactor experiment which utilized methane gas, hydrochloric acid, and oxyen, over 300 compounds were formed and these included alkanes, alkenes, alkynes, aromatic hydrocarbons, and chlorinated aromatic hydrocarbons including PCDDs and PCDFs.[160] In addition, the combustion or pyrolysis of a wide spectrum of chemicals can yield PCDDs and PCDFs and these include PCBs,[161-163] chlorophenates[165,166] and chlorinated phenol-derived products,[132] polyvinyl chloride and chlorinated alkanes,[167,168] and chlorinated benzenes.[164] In addition, the pyrolysis of brominated diphenyl ethers and biphenyls also gave complex mixtures of PBDDs and PBDFs as byproducts.[169-171]

Table 3
Concentration of PCDD/PCDF in the Soot of Home Heating (ppb)[152-155]

Compound	Municipal waste incinerator fly ash	Home heating	
		Spot oil	Soot coal/wood
TetraCDDs	19.06	3.90	1.54
PentaCDDs	37.25	0.41	5.13
HexaCDDs	115.49	3.06	5.20
HeptaCDDs	275.88	1.37	2.76
OCDD	598.69	1.05	2.37
TetraCDF	79.45	28.91	50.80
PentaCDF	120.30	16.55	30.04
HexaCDF	116.34	6.24	11.67
HeptaCDF	108.24	1.78	3.23
OCDF	42.90	0.33	0.53
2,3,7,8-TCDD	0.60	0.10	0.21
2,3,7,8-TCDF	2.47	1.07	1.92

Halogenated aromatic compounds (including several brominated chemicals) have been identified in the emissions from automobiles using leaded gasolines.[172] Dibromoethane and dichloroethane are added to leaded gasolines and act as scavengers to prevent the deposition of lead compounds in engines. These compounds are not added to unleaded gasoline and not surprisingly no halogenated aromatics were emitted from automobiles using unleaded gasoline.[173] Mixed bromo/chloro dibenzofurans and dibenzo-p-dioxins have been identified as combustion byproducts from muncipal waste incinerators and this reflects sources of bromine in some waste streams. Rappe and co-workers have compared the GC-MS profiles of the PCDDs and PCDFs derived from several combusion sources.[24,25,144,174] Their data indicate that for extracts from several diverse combusion samples there was a remarkable qualitative similarity between their GC-MS fragmentograms for various isomer groups. These results suggest that there are common pathways for the formation of PCDDs and PCDFs during the combusion or pyrolysis of organic materials.

B. Environmental Impact of Halogenated Aromatics

It is apparent from the discussion noted earlier that PCDDs and PCDFs are formed from diverse processes which can result in the introduction of complex mixtures of these compounds into the environment. The relative contribution of these sources and other unidentified sources to the overall input of PCDDs and PCDFs into the environment is unknown. In contrast, environmental contamination by the commercial PCBs is largely the result of careless use and disposal practices. PCBs, PCDDs, and PCDFs have been indentified as contaminants in almost every compartment of the ecosystem and, without exception, the PCB levels are significantly greater than the PCDDs plus PCDFs. In addition, these halogenated aromatics are routinely identified as complex mixtures of isomers and congeners in extracts from most environmental samples.

PCBs, PCDDs, and PCDFs have been identified in ambient air samples from diverse locations.[14-19,21-27,175-178] For example, total PCDD and PCDF levels in air from rural locations in Germany or the U.S. are generally <0.1 to 1 pg/m^3, whereas these values can rise significantly depending on the degree of industrial and urban activities which generate combusion-derived PCDDs and PCDFs. It was also apparent from analysis of these atmospheric samples that they contained complex mixtures of isomers and congeners which varied in their relative concentrations. In one study, the atmospheric levels of several halogenated hydrocarbons were determined around Kobe, Japan[177] and the average concentration of PCBs was 2800 pg/m^3, whereas the PCDD and PCDF levels were 8.6 and 8.8 pg/m^3, respectively. It was stated that the PCB levels were probably due to emissions from sites where commercial PCBs were spilled or dumped; in contrast, the atmospheric PCDDs and PCDFs were combustion-derived since the distribution of congeners in air samples and fly ash samples were similar.

PCDDs and PCDFs have also been widely identified in sediment samples and the composition and dated profiles of these samples suggest that the contaminants are combusion-derived.[29-33]

The identification of PCBs in fish, wildlife, and human tissues has now been reported for over 2 decades[8-21,34-44] and the results of these analytical studies led to the ultimate ban on further use and production of these compounds. The precise congener compostion of PCB extracts from biota samples is variable and depends, in part, on which specific commercial PCB preparations were associated with a contaminated area.[13] High resolution analysis of the PCBs in a composite human milk sample from Michigan has been reported.[45] Not surprisingly, the PCBs found in the human milk sample were highly complex and the congener composition and their relative concentrations did not resemble any of the commercial PCB preparations. This fact raises obvious problems with regard to the hazard assessment of PCB mixtures and is discussed later in this review.

PCDDs and PCDFs have also been widely identified in environmental biota and in human milk, serum, and adipose tissue. Rappe and co-workers[46] identified PCDFs in fat samples from snapping turtle (Hudson River) and grey seals from the Gulf of Bothnia, and PCDDs and PCDFs have been identified in fish and wildlife samples throughtout the global aquatic and marine environments.[47-56] Congener-specific analysis has shown that the 2,3,7,8-substituted PCDDs and PCDFs were the major compounds present in most sample extracts, however, the relative concentration of the individual components were variable due to the different composition of the sources of the PCDD and PCDF pollutants.

In a recent study,[53] the analyses of PCDDs and PCDFs in fish samples from several of the Great Lakes has been reported. It was apparent from the results that the PCDD and PCDF levels were lowest in fish from Lake Superior and Lake Erie and highest in fish from Lake Ontario (Table 4). The results also showed that there were higher levels of total PCDFs than total PCDDs in all the fish extracts from Lake Erie, Lake Superior, Lake Huron, Lake Michigan, and Lake St. Clair. In addition, the predominant PCDF congeners were 2,3,7,8-TCDF, 1,2,3,7,8-pentaCDF, and 2,3,4,7,8-pentaCDF; the predominant PCDDs were 2,3,7,8-TCDD, 1,2,3,7,8-pentaCDD, and 1,2,3,6,7,8-hexaCDD. These compounds were also the major PCDD and PCDF congeners in the Lake Ontario fish extracts, however, in these extracts the total PCDD and PCDF levels were comparable. This was primarily due to the unusually high concentrations of 2,3,7,8-TCDD; these results suggested that Lake Ontario contains additional point sources of 2,3,7,8-TCDD which are probably associated with 2,4,5-trichlorophenol-derived chemical wastes which contain 2,3,7,8-TCDD as the major byproduct.

PCDF and PCDD residues have been extensively identified in human adipose tissue, blood serum, and milk in individuals in many different countries.[23,57-62] In most samples, the overall PCDD levels are higher than the concentration of PCDF, and OCDD is the dominant congener in most samples. Table 5 summarizes the PCDD and PCDF levels in human adipose tissue and milk samples from several countries. These data illustrate that only the toxic 2,3,7,8-substituted congeners are present in these samples and it is evident that the bioconcentration potential of individual PCDD and PCDF congeners are very different in humans compared to fish (i.e., see Tables 4 and 5).

Based on the distribution of PCDDs and PCDFs in human tissue, it is not possible to assign specific sources for these compounds, although it has been suggested that combustion processes are major contributors to the PCDDs and PCDFs which are bioavailable.[25] However, it should also be noted that PCDDs and PCDFs have been reported in a variety of foods including fish, domestic food animals, cow's milk, butter, chickens, and eggs[179] and these foods also contribute to the relative body burden of PCDDs and PCDFs in human tissues.

III. HALOGENATED AROMATIC HYDROCARBONS: COMMON BIOCHEMICAL AND TOXIC RESPONSES AND MECHANISM OF ACTION

The development of hazard assessment methodologies for a single toxin, a mixture of structurally related congeners, or a complete family of compounds such as the halogenated aromatic industrial compounds (Figure 1) requires an understanding of the toxic and biochemical responses elicited by these chemicals and their mechanism of action. There are at least four possible mechanistic scenarios for the halogenated aromatic family of chemicals (Figure 1) and these include (1) members of the same class of compounds (e.g., PCDDs) elicit different responses via different mechanisms; (2) some (or all) members of the same class of compounds elicit similar responses via a common mechanism; (3) some (or all) members of two or more different classes (e.g., PCDDs and PCDFS) within this family cause different responses via different mechanisms; (4) some members of all classes of the halogenated aromatic family of chemials elicit similar responses in animals and cells via the same mechanism of action. The successful development of methods for the hazard and risk assessment of the complex mixtures of halogenated aromatic compounds is directly dependent on the mechanisms of action of these compounds. It is evident that if each individual congener or class of compounds cause different responses and act via independent mechanisms, then the relative toxicities and carcinogenicities of every congener must be determined. This would be a formidable if not impossible task due to the highly complex mixtures of halogenated aromatics which are introduced into the environment and which have been detected in environ-

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Table 4
Levels of 2,3,7,8-Substituted PCDDs and PCDFs in Great Lakes Fish[53]

Congener	Range of values (ppt)				
	Lake Michigan	Lake Erie	Lake Superior	Lake Huron	Lake Ontario
2,3,7,8-TCDF	31—39	8.8—20	5.7—22	19—31	13—30
1,2,3,7,8-PentaCDF	3.9—8.0	0.9—2.5	0.6—2.4	3.3—9.2	3.2—5.9
2,3,4,7,8-PentaCDF	8.1—14	1.2—5.0	1.0—3.8	6.9—19	17—26
1,2,3,4,7,8-HexaCDF	0.9—2.4	0.1—0.3	0.1—1.3	0.6—3.0	5.5—21
1,2,3,6,7,8-HexaCDF	0.7—1.9	0.1—0.5	0.08—0.6	0.5—2.1	1.0—3.0
1,2,3,7,8,9-HexaCDF	<0.08	<0.2	<0.1	<0.1	<0.2
2,3,4,6,7,8-HexaCDF	0.3—4.0	0.2—1.2	0.06—0.8	0.8—2.8	0.6—2.3
1,2,3,4,6,7,8-HeptaCDF	0.5—1.7	0.1—2.4	0.08—1.1	0.1—1.0	0.4—1.5
OCDF	<6.7	<0.2	<1.7	<0.2	<1.8
2,3,7,8-TCDD	3.0—4.4	1.2—3.2	0.3—1.4	4.5—12	37—45
1,2,3,7,8-PentaCDD	7.0—12	1.8—5.6	0.7—4.2	5.3—15	7.4—10
1,2,3,4,7,8-HexaCDD	0.5—1.2	0.1—0.4	0.1—0.5	0.4—0.9	0.3—0.7
1,2,3,6,7,8-HexaCDD	4.1—10	1.0—4.0	0.4—2.0	2.6—5.2	3.4—6.3
1,2,3,7,8,9-HexaCDD	0.5—1.3	0.2—0.5	0.1—0.7	0.5—0.8	0.3—0.6
1,2,3,4,6,7,8-HeptaCDD	0.7—1.1	0.8—1.5	0.4—1.2	0.3—1.3	0.5—1.7
OCDD	0.8—1.2	1.1—5.1	0.4—1.2	0.6—1.1	0.9—1.8

mental and biological samples. However, there is strong evidence that several compounds within each class of the halogenated aromatic family elicit comparable responses in cells, laboratory animals, and humans. The following discussion briefly summarizes the evidence which supports a common mechanism of action for the toxic halogenated aryl hydrocarbons.

A. Toxic Responses

Several studies have reported that 2,3,7,8-TCDD and related toxic halogenated aromatics elicit a number of common toxic responses which include body weight loss, thymic atrophy, impairment of immune responses, hepatotoxicity and porphyria, chloracne and related dermal lesions, tissue-specific hypo- and hyperplastic responses, carcinogenesis, teratogenicity, and reproductive toxicity.[180-186] Figures 2 and 3 illustrate the dose-response toxic effects of selected representative halogenated aromatic hydrocarbons and show that selected congeners within each class elicit the same responses. The only major differences in these compounds are their relative toxic potencies. It should also be noted that the toxic effects of halogenated aromatics are dependent on the animal species or strain, their age, and sex. For example, the toxic halogenated aromatics such as 2,3,7,8-TCDD cause chloracne and related skin lesions in some strains of genetically inbred mice, rabbits, monkeys, and humans but not in other strains of mice, rats, guinea pigs, and hamsters. The interspecies differences in their response to halogenated aromatics is illustrated by the following LD_{50} values for 2,3,7,8-TCDD;[187,188] in the guinea pig (0.6 to 2.0 μg/kg), rat (22 to 45 μg/kg), chicken (25 to 50 μg/kg), monkey (70 μg/kg), rabbit (115 μg/kg), dog (100 to 200 μg/kg), mouse (114 to 284 μg/kg), bull frog (>1000 μg/kg), and hamster (1157 to 5000 μg/kg).

B. Biochemical Responses

One of the hallmarks of exposure to halogenated aromatics is their induction of both phase I and phase II drug-metabolizing enzymes in laboratory animals and mammalian cells in culture.[180-186] 2,3,7,8-TCDD and related toxic halogenated aromatics induce the cytochrome P-4501A1 and cytochrome P-4501A2 hemoproteins and their associated microsomal monooxygenases, which include aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin O-deethylase (EROD) activities. In addition, several PCB and PBB congeners induce other forms of cytochrome P-450 (in the rat) and these include cytochromes P-450IIB1 (cytochrome P-450b), P-450IIB2 (cytochrome P-450e), P-450IIA1 (cytochrome P-450a), and P-450IIIA1 (cytochrome P-450p) and their associated monooxygenase enzyme activities.[189-199] Toxic halogenated hydrocarbons also induce the phase II drug-metabolizing enzymes, glucuronosyl transferases, and glutathione S-transferases. Table 6 summarizes some of the major biochemical responses elicited by PCDDs, PCDFs, and PCBs/PBBs and these include the induction of diverse enzymes, the modulation of hormone receptor-binding activities, the alteration of thyroid hormone and vitamin A levels, and enzymes which metabolize steroid hormones. However, not all of these responses have been observed for all classes of the halogenated aromatics. This is due to the extensive use of 2,3,7,8-TCDD as a prototype for investigating

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Table 5
Levels of PCDDs and PCDFs in Human Tissues[25]

Congener	Sample source and mean concentrations (ppt on fat basis)		
PCDFs			
A. Adipose tissue	Japan	Sweden	FRG
2,3,7,8-TCDF	9	3,9	0.9
2,3,4,7,8-PentaCDF	25	54	44
1,2,3,4,7,8-HexaCDF	15	6	10
1,2,3,6,7,8-HexaCDF	14	5	6.7
2,3,4,6,7,8-HexaCDF	8	2	3.8
1,2,3,4,6,7,8-HeptaCDF	nr	11	19.5
OCDF	nr	4	<1
B. Milk	FRG	Sweden	Netherlands
2,3,7,8-TCDF	2.6	4.2	2.8
2,3,4,7,8-PentaCDF	22.9	21.3	79
1,2,3,4,7,8-HexaCDF	8.2	4.7	8.9
1,2,3,6,7,8-HexaCDF	6.6	3.4	10.3
2,3,4,6,7,8-HexaCDF	3.3	1.4	6.4
1,2,3,4,6,7,8-HeptaCDF	6.4	7.4	39
OCDF	22.8	3.2	ND
PCDDs			
A. Adipose Tissue	Japan	Sweden	FRG
2,3,7,8-TCDD	9	3	150
1,2,3,7,8-PentaCDD	15	10	19.2
1,2,3,6,7,8-HexaCDD	70	15	77
1,2,3,7,8,9-HexaCDD	12	4	9.4
1,2,3,4,6,7,8-HeptaCDD	77	97	56
OCDF	230	414	267
B. Milk	FRG	Sweden	Netherlands
2,3,7,8-TCDD	<5	0.6	9.7
1,2,3,7,8-PentaCDD	10.7	6.5	44
1,2,3,4,7,8-HexaCDD	8.1	2.5	25
1,2,3,6,7,8-HexaCDD	32.7	19	251
1,2,3,7,8,9-HexaCDD	6.4	6.3	23
1,2,3,4,6,7,8-HeptaCDD	49.9	59.5	130
OCDD	181.2	302	744

Note: nr = not recorded; ND = not detected.

the effects and mechanism of action of all members of this large family of chemicals.

C. Genetic Studies

Poland, Nebert, and their colleagues have investigated the inducibility of hepatic microsomal AHH activities in genetically inbred aryl hydrocarbon (Ah) responsive and nonresponsive mice (typified by the C57BL/6 and DBA strains, respectively) and their backcrosses.[243-246] Their results clearly showed at least one order of magnitude difference in the response of these strains to the induction of AHH activity by 2,3,7,8-TCDD. In contrast, 3-methylcholanthrene was inactive as an inducer in the nonresponsive mice and genetic studies with inbred strains and their backcrosses showed that AHH inducibility is inherited as a simple autosomal dominant trait in these strains. Based on these early studies, it was suggested that the induction of AHH activity by 2,3,7,8-TCDD may be a receptor-mediated process and it was suggested that the decrease responsiveness of DBA/2 mice was due to a defect in the Ah

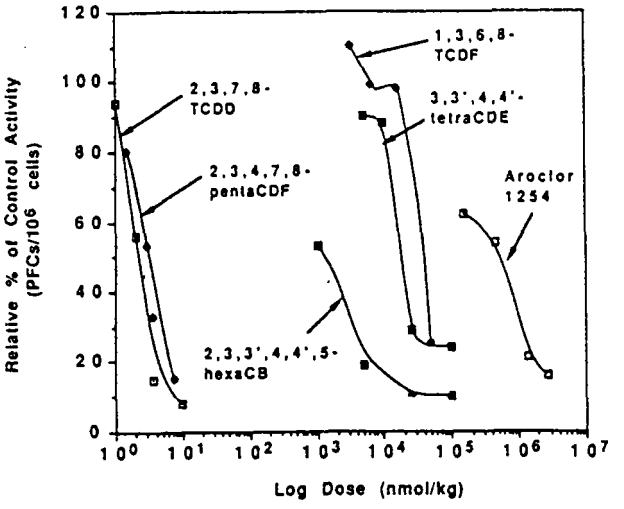


FIGURE 2. Dose-response effects of 2,3,7,8-TCDD, 2,3,4,7,8-pentaCDF, 2,3,3',4,4',5-hexaCB, 1,3,6,8-TCDF, 3,3'4,4'-tetraCDE, and Aroclor 1254 on the suppression of the splenic plaque-forming cell response to sheep red blood cells in C57BL/6 mice.

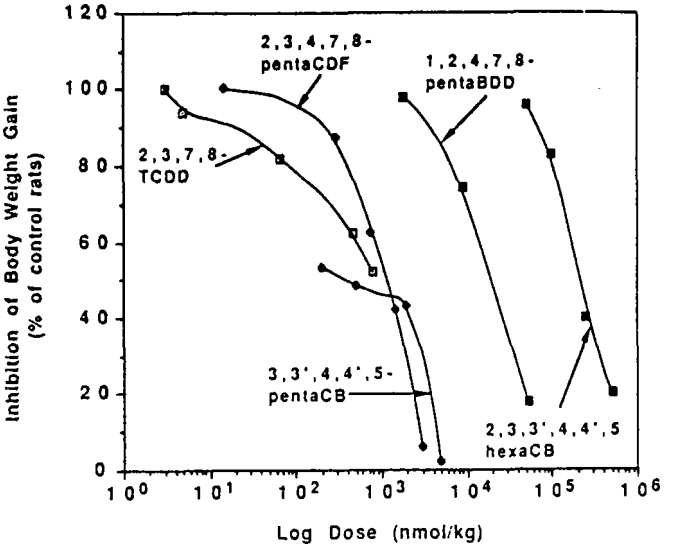


FIGURE 3. Dose-response inhibition of body weight gain by 2,3,7,8-TCDD, 2,3,4,7,8-pentaCDF, 3,3',4,4',5-pentaCB, 1,2,4,7,8-pentaBDD, and 2,3,3',4,4',5-hexa CB in immature male Wistar rats.

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Table 6
Biochemical Responses Caused by Different Class of Halogenated Aromatics

Response	Ref. PCDDs/PCDFs	Ref. PCBs/PBBs
CYP1A1 induction	181,182,192,194, 195,198,199	189—193, 196,198,199
CYP1A2 induction	181,182,192,194, 195,198,199	189—193, 196,199
Modulation of steroid-metabolizing enzymes	200—202,198,202	202
DT diaphorase induction	203,205	198
Glutathione S-transferase induction	206	207—209
UDP glucuronosyl transferase induction	210,211	212
Epidermal transglutaminase induction	213	
Aldehyde dehydrogenase induction	214	
δ-Ammolevulinic acid synthetase induction	215	216,217
Uroporphyrinogen decarboxylase inhibition	216,218—221	216,217, 219,220
Decreased EGF receptor binding	222,223,229	
Decreased progesterone receptor binding	224,225	
Increased Ah receptor binding activity	226	227
Decreased estrogen receptor levels	224,225	
Decreased glucocorticoid receptor binding	228,229	
Modulation of thyroid hormone levels/function	230—234	235,236
Decreased vitamin A levels	237,238	239—242

receptor. This explanation of differential responsiveness in inbred strains of mice has been confirmed in a recent report which showed that the hepatic Ah receptor in DBA/2 mice has a lower affinity for [^{3}H]-2,3,7,8-TCDD than the corresponding receptor in C57BL/6 mice.[247] Subsequent research with genetically inbred mice and their backcrosses has shown that for PCDDs, PCBs, and PCDFs several toxic responses including immunotoxicity, teratogenicity, body weight loss, hepatotoxicity, and porphyria segregate with the Ah locus.[248-259] Moreover, some reports indicate that other genetic loci may be important for the toxicity of 2,3,7,8-TCDD and related compounds.[251,253]

D. Identification of the Ah Receptor

Poland and co-workers[260] first identified a cytosolic protein in C57BL/6 mouse liver which exhibited saturable and high affinity binding using [^{3}H]-2,3,7,8-TCDD as a radioligand. Subsequent research in several laboratories have identified this receptor protein in both hepatic and extrahepatic tissues of several laboratory animal species, mammalian cells in culture, and in human organs and cultured cells.[261] This research has helped to confirm the role of the Ah receptor in the mechanism of action of 2,3,7,8-TCDD and structurally related toxic halogenated aryl hydrocarbons. Some of the evidence which supports a receptor-mediated response mechanism for this family of compounds is briefly summarized below. It should be noted that the development of scientifically based toxic equivalency factors is directly related to the weight of the evidence which supports the proposed receptor-mediated mechanism of action.

1. Saturable Binding

The synthesis of radiolabeled 2,3,7,8-TCDD with high specific activity triggered several important mechanistic studies; it was apparent that in the soluble fraction of hepatic and extrahepatic tissues from several species there is a protein which exhibited saturable binding with this radioligand.[260-263] Moreover, several reports have also demonstrated that other radiolabeled PCDD and PCDF congeners exhibited comparable saturable binding curves[264] (see Figure 4). In addition, radiolabeled polynuclear aromatic hydrocarbons such as 3-methylcholanthrene, benzo[a]pyrene, and dibenz[a,h]-anthracene also exhibit saturable binding with this cytosolic receptor protein.[265-267]

2. High Affinity Binding

The K_D values for the dissociation of the bound cytosolic receptor complex using both PCDD and PCDF radioligands varies from approximately 0.1 to 10 n*M*. This variability is due to a number of factors including the source of the cytosol (i.e., organ, species, sex, age, and strain of animal), the experimental conditions, and the receptor binding assay used in the studies. These variables, coupled with the apparent lower binding affinities of cytosols from human tissues and DBA/2 mice,[247,268] account for the wide range of the reported K_D values. In addition, recent studies have reported that at least two factors, namely, the high level of nonspecific binding associated with many cytosol preparations and the degradation of unbound receptors during the assay, contribute to the underestimation of K_D values for the bound cytosolic Ah receptor complex.[269,270] If the above factors are taken into account, the actual K_D values may be as low as 10^{-12} *M*.

3. Tissue or Cellular Specificity and Correlation between Ligand Binding and Biological Response

The responsiveness of a particular organ or cell to the effects of 2,3,7,8-TCDD and related compounds depends on the presence of a functional Ah receptor. This is illustrated by the effects of 2,3,7,8-TCDD on the induction of cytochrome P-4501A1 and associated monooxygenase enzyme activities in wild-type Hepa 1c1c7 cells and several mutant cell lines.[271-275] In the wild-type cells, there are relatively high levels of the Ah receptor and 2,3,7,8-TCDD is an effective

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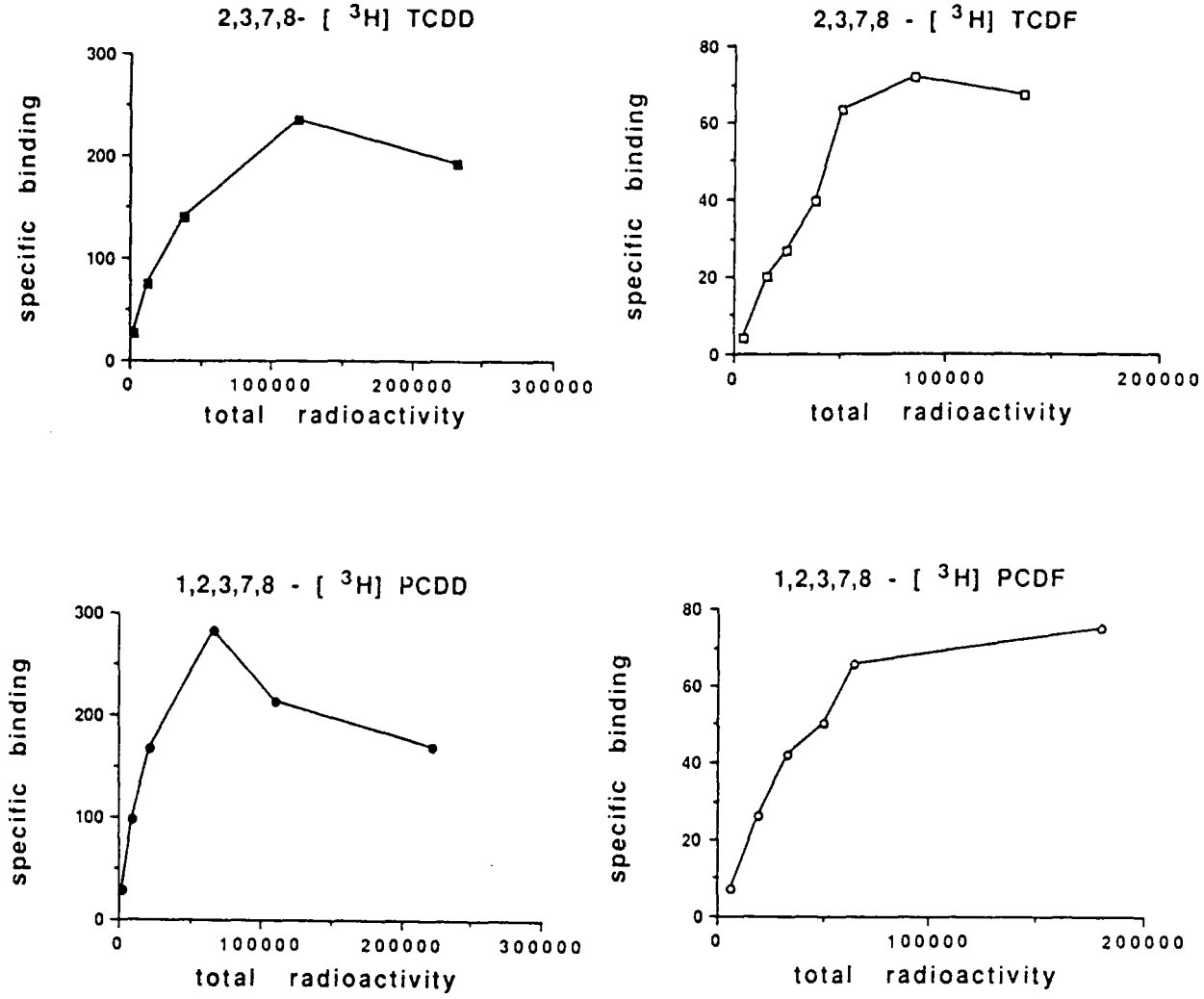


FIGURE 4. Saturation binding of several 2,3,7,8-substituted radioligands to rat hepatic cytosol. (Courtesy of R. Rosengren, unpublished results.)

inducer; whereas, class I variant cells, which contain relatively low levels of this receptor, are not inducible. Class II variant cells are also not inducible by 2,3,7,8-TCDD, however, the levels of the cytosolic Ah receptor in this cell line are comparable to those observed in wild-type cells. The nonresponsiveness of these cells is associated with a defect in the accumulation of transcriptionally active nuclear Ah receptor complexes. It is also evident from other studies that the presence of the Ah receptor is not sufficient for the induction of a biochemical response by 2,3,7,8-TCDD and related compounds. For example, 2,3,7,8-TCDD did not induce AHH activity in MDA-MB-231 human breast cancer cells even though the Ah receptor complex accumulates in the nuclear fraction of these cells.[276] Moreover, in genetically inbred haired and hairless mice, the effects of 2,3,7,8-TCDD are dependent on both the Ah and hr gene loci.[253,277,278]

A correlation between the formation of occupied nuclear receptor complexes and the expression of a biological response has also been reported in responsive target organs or cells. After administration of [^{3}H]-2,3,7,8-TCDD to C57BL/6 mice, there was a time-dependent depletion of the cytosolic Ah receptor and this was paralleled by the appearance of the nuclear 2,3,7,8-TCDD:receptor complex; the formation of the nuclear receptor complex was followed by the subsequent induction of cytochrome P-4501A1 (or P_1-450) mRNA and microsomal cytochrome P-4501A1.[279] In a recent study, it was reported that for selected radiolabeled PCDD and PCDF congeners there was a linear correlation between levels of occupied nuclear receptor complex vs. the magnitude of the induction (AHH) response in rat hepatoma H-4II E cells.[280]

These results clearly link responsivenes to 2,3,7,8-TCDD and related compounds to the accumulation of occupied nuclear

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Ah receptor complexes. Several groups have investigated the molecular biology of CYP1A1 gene expression[181,182,281-284] and their results clearly confirm the proposed receptor-mediated mechanism of action of 2,3,7,8-TCDD and related compounds, which is illustrated in Figure 5. Initial binding of the toxin to the cytosolic Ah receptor is followed by an activation or transformation step and the subsequent accumulation of occupied nuclear receptor complexes. These nuclear complexes then interact with specific DNA sequences or dioxin regulatory elements (DREs) which are located in the 5'-upstream region from the CYP1A1 gene. These interactions lead to the enhancement of CYP1A1 gene expression. It is assumed that many of the toxic effects elicited by halogenated aryl hydrocarbons are also the result of altered receptor-mediated gene expression, however, the molecular mechanisms of these responses are currently unknown.

IV. DEVELOPMENT OF TOXIC EQUIVALENCY FACTORS FOR DIFFERENT CLASSES OF HALOGENATED AROMATICS

A. Introduction

The previous section of this review noted some of the evidence which supports a receptor-mediated mechanism of action for 2,3,7,8-TCDD and related halogenated aromatic compounds. One important additional criteria for receptor-mediated responses are the steroselective ligand-receptor interactions and the corresponding structure-activity relationships (SARs). Several studies have reported the structure-receptor-binding relationships for different classes of halogenated aromatics in which the competitive *in vitro* binding EC_{50} (or IC_{50}) values for different congeners have been determined using [^{3}H]-2,3,7,8-TCDD as the radioligand and rodent hepatic cytosol as a source of the Ah receptor[183-185,260,285-289] (see Table 7). For the PCDDs, 2,3,7,8-TCDD was the most competitive ligand and the structure-binding relationships demonstrated that the most active compounds were the 2,3,7,8-substituted tetra- to hexaCDDs (see Figure 6). It was evident from the binding studies that (1) the most active congeners were substituted in all four lateral positions and the binding activity decreased with decreasing lateral substition and (2) for the 2,3,7,8-substituted congeners, there was a decrease in binding avidity with increasing non-lateral chlorine substition. The structure-binding relationship for the PCDFs showed that substituents on all four positions in the ring contributed differentially to their binding avidities[286,287] (Table 7). Like the PCDDs, the most active compounds were tetra- to hexaCDFs which are substituted in the lateral 2, 3, 7, and 8 positions (Figure 7). The dibenzofuran ring system possesses a single axis of symmetry and there are four different positions on each aromatic ring, namely, C-1 (or C-9), C-2 (or C-8), C-3 (or C-7), and C-4 (or C-6). The most active congeners, namely, 2,3,4,7,8-pentaCDF (1.5×10^{-8} *M*), 2,3,7,8-TCDF (4.1×10^{-8} *M*), 2,3,4,6,7,8-hexaCDF (4.7×10^{-8} *M*), and 1,2,3,7,8-pentaCDF (7.5×10^{-8} *M*) were all substituted in their lateral 2, 3, 7, and 8 positions. A comparision of the receptor binding EC_{50} values for the 2,3,4,7,8- (1.5×10^{-8} *M*), 1,2,4,7,8- (1.3×10^{-6} *M*), and 1,2,4,6,8-pentaCDF ($>5 \times 10^{-6}$ *M*) isomers demonstrates the importance of lateral chloro substituents since there is a decrease in receptor binding affinities with decreasing lateral substitution. Two pairs of PCDF isomers, namely, 1,3,4,7,8- (2.0×10^{-7} *M*) and 1,2,4,7,8-pentaCDF (1.3×10^{-6} *M*); 2,3,4,7- (2.5×10^{-8} *M*) and 2,3,4,8-TCDF (2.0×10^{-7} *M*) differ only with

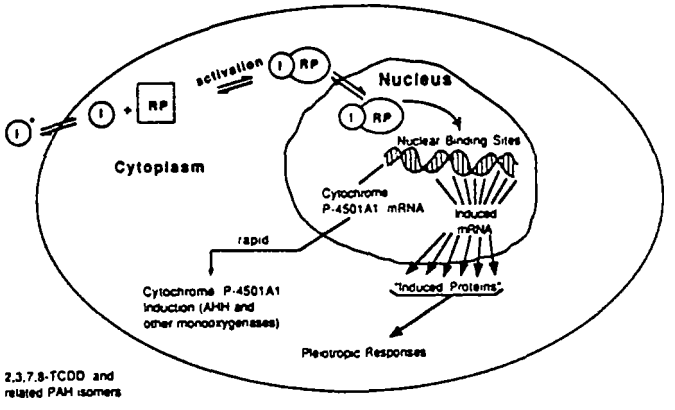


FIGURE 5. Proposed model for the mechanism of action of 2,3,7,8-TCDD and related toxic halogenated aromatic hydrocarbons. The initial formation of a cytosolic receptor complex is followed by an activation step, the formation of nuclear receptor complexes, and their interaction with specific nuclear-binding sites. This interaction contributes to an increase or induction of a 2,3,7,8-TCDD-inducible gene such as CYP1A1.

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Table 7
Halogenated Aromatic Congeners as Competitive Ligands for the Rat Hepatic Cytosol Ah Receptor[184,185]

Congeners	Receptor binding avidities (EC_{50}, M)	Congeners	Receptor binding avidities (EC_{50}, M)
PCDFs		Bromo/chloro dibenzo-p-dioxins	
Dibenzofuran	$>10^{-3}$	2,3,7,8-TBDD	1.50×10^{-9}
2-MonoCDF	2.8×10^{-4}	2,3-Dibromo-7,8-dichlorodibenzo-p-dioxin	1.48×10^{-9}
3-MonoCDF	$4.2 \pm 0.6 \times 10^{-5}$	2,8-Dibromo-3,7-dichlorodibenzo-p-dioxin	4.47×10^{-10}
4-MonoCDF	$>10^{-3a}$	2-Bromo-3,7,8-tri-chlorodibenzo-p-dioxin	1.15×10^{-8}
2,3-DiCDF	4.72×10^{-6}	2,4,6,8/1,3,7,9-TetraBDF	9.30×10^{-8}
2,6-DiCDF	2.46×10^{-4}	1,3,7,8-TetraBDD	2.00×10^{-9}
2,8-DiCDF	2.57×10^{-4}	1,2,4,7,8-PentaBDD	1.70×10^{-8}
1,3,6,-TriCDF	4.40×10^{-6}	1,2,3,7,8-PentaBDD	6.61×10^{-9}
1,3,8-TriCDF	8.50×10^{-5}	2,3,7-TriBDD	1.17×10^{-9}
2,3,4-TriCDF	1.9×10^{-5}	2,7/2,8-DiBDD	1.55×10^{-8}
2,3,8-TriCDF	$1.0 \pm 0.1 \times 10^{-6}$	2-MonoBDD	2.95×10^{-7}
2,6,7-TriCDF	4.5×10^{-7}	PCBs	
2,3,4,6-TetraCDF	3.5×10^{-7}	3,3',4,4',5-PentaCB	1.2×10^{-7}
2,3,4,8-TetraCDF	2.0×10^{-7}	3,3',4,4',5,5'-HexaCB	Insoluble
2,3,6,8-TetraCDF	2.2×10^{-7}	3,3',4,4'-TetraCB	4.3×10^{-7}
2,3,7,8-TetraCDF	$4.1 \pm 0.6 \times 10^{-8}$	2,3,3',4,4'-PentaCB	7.1×10^{-6}
1,2,4,8-TetraCDF	$>10^{-5}$	2,3,4,4',5-PentaCB	4.1×10^{-6}
1,2,3,6-TetraCDF	3.5×10^{-7}	2,3,3',4,4',5-HexaCB	7.1×10^{-6}
1,2,3,7-TetraCDF	1.1×10^{-7}	2',3,4,4',5-PentaCB	1.4×10^{-5}
1,3,4,7,8-PentaCDF	2.0×10^{-7}	2,3,3',4,4',5-HexaCB	5.0×10^{-6}
2,3,4,7,9-PentaCDF	2.0×10^{-7}	2,3,4,4'-TetraCB	2.8×10^{-5}
1,2,3,7,9-PentaCDF	4.0×10^{-7}	Aroclor 1254	6.0×10^{-6}
1,2,4,6,7-PentaCDF	6.77×10^{-8}	2,3,3',4,4',5,5'-HeptaCB	Insoluble
1,2,4,7,9-PentaCDF	2.0×10^{-5}	2,3',4,4',5-PentaCB	9.1×10^{-6}
1,2,3,4,8-PentaCDF	1.2×10^{-7}	2,3',4,4',5,5'-HexaCB	1.6×10^{-5}
1,2,3,7,8-PentaCDF	$7.45 \pm 2.04 \times 10^{-8}$	2,2',4,4',5,5'-HexaCB	7.9×10^{-5}
1,2,4,7,8-PentaCDF	1.3×10^{-6}	2,2',4,4'-TetraCB	1.3×10^{-4}
2,3,4,7,8-PentaCDF	$1.5 \pm 0.1 \times 10^{-8}$	2,3,4,5-TetraCB	1.4×10^{-4}
1,2,3,4,7,8-Hexa CDF	2.3×10^{-7}		
1,2,3,6,7,8-HexaCDF	$2.7 \pm 1.0 \times 10^{-7}$		
1,2,4,6,7,8-HexaCDF	8.3×10^{-6}		
2,3,4,6,7,8-HexaCDF	$4.7 \pm 0.4 \times 10^{-8}$		
PCDDs			
2,3,7,8-TCDD	1.0×10^{-8}		
1,2,3,7,8-PentaCDD	7.9×10^{-8}		
2,3,6,7-TetraCDD	1.6×10^{-7}		
2,3,6-TriCDD	2.2×10^{-7}		
1,2,3,4,7,8-HexaCDD	2.8×10^{-7}		
1,3,7,8-TetraCDD	7.9×10^{-7}		
1,2,4,7,8-PentaCDD	1.1×10^{-6}		
1,2,3,4-TetraCDD	1.3×10^{-6}		
2,3,7-TriCDD	7.1×10^{-8}		
2,8-TriCDD	3.2×10^{-6}		
1,2,3,4,7-PentaCDD	6.4×10^{-6}		
1,2,4-TriCDD	1.3×10^{-5}		
OCDD	$> 1.0 \times 10^{-5}$		
1-MonoCDD	$> 1.0 \times 10^{-4}$		

Note: The values for the PCDDs, PCBs and PCDFs were determined by sucrose density gradient analysis; the brominated compounds were determined using the hydroxylapatite assay.

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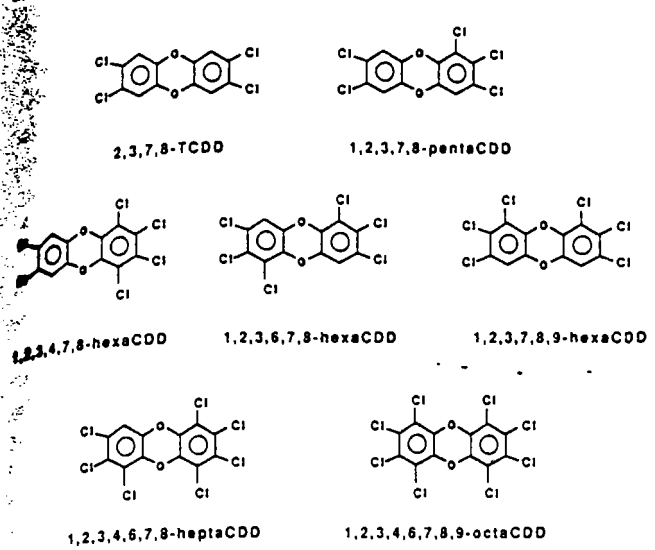


FIGURE 6. Structures of the 2,3,7,8-substituted PCDD congeners.

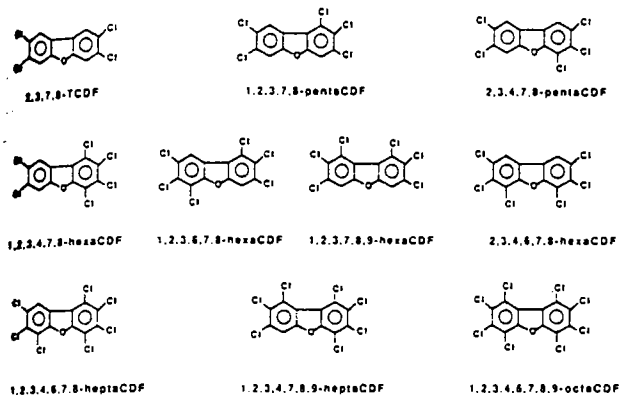


FIGURE 7. Structures of the 2,3,7,8-substituted PCDF congeners.

respect to their substitution of C-2 (or C-8) and C-3 (or C-7). In both cases, the C-3 (or C-7) -substituted compounds exhibited relatively higher competitive binding affinities than the corresponding C-2 (or C-8) -substituted isomers. A comparision of the relative binding affinities of a series of C-1 (or C-9) and C-4 (or C-6) isomer pairs illustrates the higher binding activities of the isomer which retains the C-4 (or C-6) substituent. For example, the EC_{50} values for the 2,3,4,7-, 2,3,4,7,8-, 2,3,4,7,9-, and 2,3,4,6,7,8-substituted PCDFs were 2.5×10^{-8} *M*, 1.5×10^{-8} *M*, 2.0×10^{-7} *M*, and 4.7×10^{-8} *M*, whereas the values for the coresponding C-1 (or C-9) congeners (i.e., 1,2,3,7-TCDF, 1,2,3,7,8- and 1,2,3,7,9-pentaCDF, and 1,2,3,6,7,8-hexaCDF) were 1.1×10^{-7}*M*, 7.5×10^{-8} *M*, 3.4×10^{-7} *M*, and 2.7×10^{-7} *M*, respectively.

The structure-binding relationships for the PCBs[285] have shown that the most active compounds are substituted on both para and at least 2 meta positions (Figure 8). These congeners, namely, 3,4,4',5-tetra-, 3,3',4,4'-tetra-, 3,3,',4,4',5-penta, and 3,3',4,4',5,5'-hexachlorobiphenyl (CB), are approximate isostereomers of 2,3,7,8-TCDD in their coplanar conformations. A second set of PCB congeners, namely, the monoortho coplanar analogs (Figure 9), exhibit lower competitive binding affinities for the Ah receptor (Table 7). Based on limited data, it was apparent that the structure-receptor-binding relationships for the PCBs and PBBs were comparable[290] and only minimal results have been reported for other classes of halogenated aromatics.

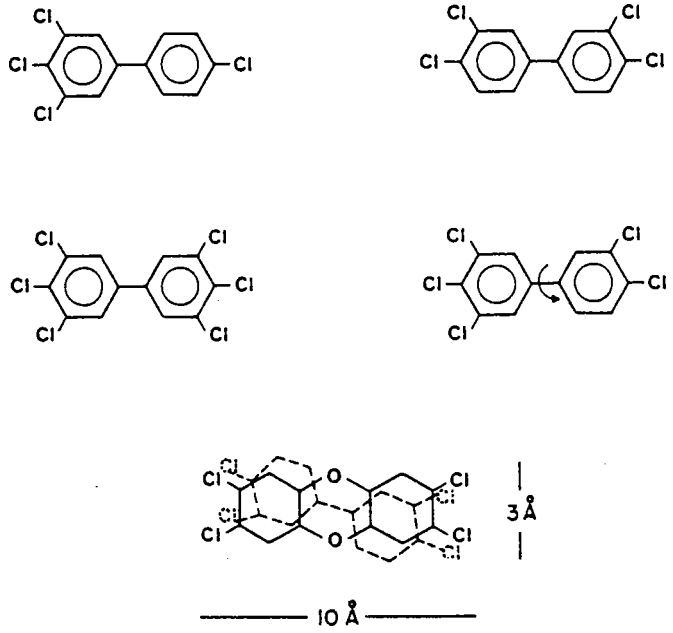


FIGURE 8. Structures of the coplanar PCB congeners substituted in both *para* and two or more *meta* positions.

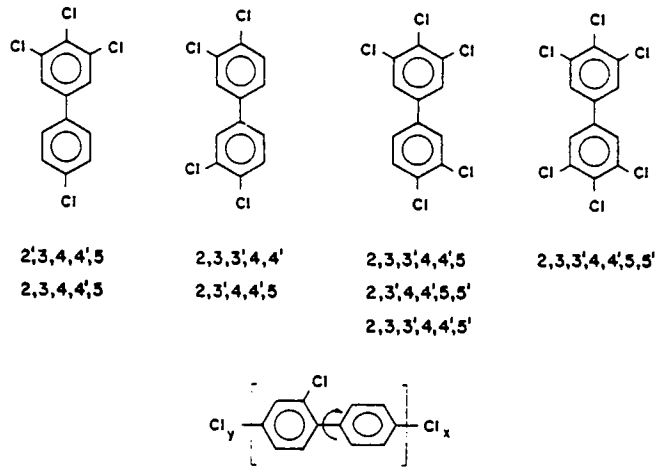


FIGURE 9. Structures of the monoortho coplanar PCB congeners.

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The structure-binding relationships observed for the different classes of halogenated aromatics provide further support for a receptor-mediated mechanism of action for these compounds. Moreover, the results of several studies have demonstrated an excellent correlation between the structure-binding and structure-activity (biochemical and toxic) relationships for several classes of halogenated aromatics.[183-186] This observation forms the mechanistic basis for the development of TEFs for individual halogenated aromatic compounds. For the sake of convenience, most TEF schemes compare the relative toxicity of different compounds to the most potent member of this family of chemicals, namely, 2,3,7,8-TCDD. It will be noted in the following sections that the activity of a congener relative to 2,3,7,8-TCDD is dependent on several factors including the specific response, the species/sex/strain and age of the animals, the duration of the feeding study, and the route of compound administration. For some compounds, the TEF will be selected from a diverse spectrum of results whereas only minimal toxicologic data will be available for other congeners. D.L. Grant (Health and Welfare Canada) has suggested that when data are available from more than one response the TEF values should be derived from the following effects in descending order of priority:

1. Results obtained from long term carcinogenicity studies
2. Data from reproductive studies
3. The results of subchronic experiments which measure Ah receptor-mediated responses such as thymic atrophy, body weight loss, and immunotoxicity
4. Acute toxicity studies
5. *In vivo* or *in vitro* biochemical responses such as enzyme induction, receptor binding, etc.

It is apparent from a review of the data that a complete set of data, including long-term carcinogenicity studies,[291-294] are available only for 2,3,7,8-TCDD and therefore the assignment of TEF values for the halogenated aromatics requires a subjective assessment of the results in which the response priorities noted above will be a contributing factor.

B. PCDDs: Development of TEFs

The relative acute and chronic toxicities of several PCDD congeners have been reported and it is apparent that the tetra- to hexachloro 2,3,7,8-substituted analogs (Figure 6) are substantially more active than those congeners which contain less than four lateral substituents.[187,188] For example, the acute LD_{50} values for 2,8-diCDD, 1,2,4,7,8-pentaCDD, and 1,3,6,8-tetraCDD are, respectively, 8,470,000, >5000, and >2,987,000 μg/kg in the mouse whereas the LD_{50}s for 2,3,7,8-TCDD were in the 114 to 284 μg/kg range. Inspection of analytical results from most environmental samples (e.g., Tables 4 and 5) and human tissues indicates that the 2,3,7,8-substituted PCDDs are the major congeners present in these extracts. Therefore, based on the structure-toxicity relationships and the analytical data, TEFs have been developed only for the 2,3,7,8-substituted compounds.

The ideal set of data from which to calculate the TEFs for PCDDs would be comprehensive cancer or multigenerational reproductive studies on all the 2,3,7,8-substituted compounds. Unfortunately, these data are available only for 2,3,7,8-TCDD[291-294] and the results from these studies have been extensively utilized to regulate the dietary intake and permissable emission levels of 2,3,7,8-TCDD. However, since 2,3,7,8-TCDD is a minor component of most environmental sources of PCDDs, TEFs for the other toxic congeners have been developed from other toxicological endpoints. The results in Table 8 summarize the various LD_{50}/ED_{50} values for several toxic endpoints in several species for the 2,3,7,8-substituted PCDD congeners. These data illustrate that (1) 2,3,7,8-TCDD is clearly the most toxic halogenated aryl hydrocarbon, (2) the guinea pig is the most sensitive species to the effects of these toxins, and (3) the SARs are comparable within each animal species for every response. These results are not surprising and they are consistent with the proposed receptor-mediated mechanism of action for the PCDDs.

The results in Table 9 summarize the relative biochemical and *in vitro* potencies of the 2,3,7,8-substituted PCDD congeners. These responses include receptor-binding affinities, keratinization, and the induction of AHH activity in rodents and in rodent cells in culture. The SARs for the biochemical responses elicited by 2,3,7,8-TCDD and related PCDDs are similar (but not identical) and the relative sensitivity of these effects are response- and bioassay-dependent. Figures 10 to 12 summarize the correlation between the $-\log EC_{50}$ values for AHH induction in rat hepatoma H-4-II E cells vs. the $-\log ED_{50}$ values for thymic atrophy, body weight loss, and hepatic microsomal AHH induction in the rat. The linear correlation coefficients for these responses were 0.73, 0.84, and 0.84 respectively. These data illustrate that the quantitative SARs for these congeners were comparable for several responses. Table 10 summarized recently proposed TEFs for the 2,3,7,8-substituted PCDDs[301-303] and compares these values with their relative potency ranges derived from the *in vivo* and *in vitro* responses summarized in Tables 8 and 9. The results show that the proposed TEFs overlap with the range of values for the relative *in vivo* and *in vitro* potencies of the 2,3,7,8-substituted PCDD congeners. Moreover, for most of these compounds, the assigned TEF values are conservative and correspond with the higher range of relative potencies.

Some previous proposals for TEFs assigned values of zero for OCDD due to the low acute toxicity of this congener. However, recent studies by Couture and co-workers[304] have reported that there was a marked increase in the absorption of this compound in rat liver and adipose tissue with an increasing number of doses. For example, animals given 65 doses of OCDD (5 doses/week for 13 weeks) exhibited marked increases in hepatic EROD activity and cytochrome P-450 levels and fatty vacuolizaton in the liver. The results of this study suggest

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Table 8
Comparative Toxic Potencies of the 2,3,7,8-Substituted PCDDs[187,188,288,295–296]

Response	Species (dose or conc.)	LD_{50}/ED_{50} Values						
		2,3,7,8-TCDD	1,2,3,7,8-PentaCDD	1,2,3,4,7,8-HexaCDD	1,2,3,7,8,9-HexaCDD	1,2,3,6,7,8-HexaCDD	1,2,3,4,6,7,8-HeptaCDD	OCDD
Acute lethality								
	Guinea pig (μg/kg)	0.6—2.0	3.1	72.5	60—100	70—100	> 600	—
	Mouse (μg/kg)	114—284	337.5	825	>1440	1250	—	$>4 \times 10^{6}$
	Rat (μg/kg)	22—45						10^{-6}
Subchronic toxicities								
Body weight loss	Rat (μmol/kg)	0.05	0.62	1.63	—	—	—	—
	Guinea pig (μmol/kg)	0.0056	—	—	—	—	—	—
Thymic atrophy	Rat (μmol/kg)	0.09	0.17	1.07				
Immunotoxicity	Mice (nmol/kg)	0.65, 0.77				7.1	85	>500
Teratogenicity	Mice (μg/kg)	3.4						

Table 9
Comparative Biochemical and *In Vitro* Potencies of 2,3,7,8-Substituted PCDDs[260,288,298–300]

Response	System (conc./dose)	ED_{50} Values						
		2,3,7,8-TCDD	1,2,3,7,8-PentaCDD	1,2,3,4,7,8-HexaCDD	1,2,3,7,8,9-HexaCDD	1,2,3,6,7,8-HexaCDD	1,2,3,4,6,7,8-HeptaCDD	OCDD
Receptor binding								
	Mouse liver (*M*)	2.7×10^{-10}	4.2×10^{-10}			1.4×10^{-9}		
	Rat liver (*M*)	1.0×10^{-8}	7.9×10^{-8}	2.8×10^{-7}				$> 10^{-5}$
AHH induction	Rat liver (μmol/kg)	0.004	0.031	0.03				
	H-4-II-E cells (*M*)	1.5, 7.2×10^{-11}	22, 1.1×10^{-8}	31, 2.1×10^{-9}	189[b]	128[b]	551[b]	2500[b]
	Guinea pig liver (μmol/kg)	0.00028						
	Chick embryo (nmol/kg)	0.2	0.9			1.0		
	Keratinization[b] (nM) (XB/3T3 cells)	0.05	0.1			10		

[a] Picomoles per plate.
[b] Minimum dose effecting keratinization.[299]

that if rats are exposed to multiple doses of OCDD, this compound is approximately 1/100 to 1/1000 times less toxic than 2,3,7,8-TCDD. Hence, the value of 0.001 has been assigned to OCDD.

C. PCDFs: Development of TEFs

The results in Table 11 summarize the acute and chronic *in vivo* toxicities of all the 2,3,7,8-substituted PCDFs. It is evident from these results that 2,3,4,7,8-pentaCDF is the most highly toxic PCDF congener and for some responses (e.g., immunotoxicity) this compound is approximately equipotent with 2,3,7,8-TCDD.[295] 2,3,4,7,8-PentaCDF also exhibits a relatively high competitive binding affinity for the Ah receptor (1.5×10^{-8} *M* compared to 1.0×10^{-8} *M* for 2,3,7,8-TCDD). The results also show that the remaining 2,3,7,8-substituted tetra-, penta, and hexaCDF congeners are less toxic than 2,3,4,7,8-pentaCDF, however, their relative toxicities are both species- and response-dependent. These differences in relative toxicities are probably related to several factors including pharmacokinetic differences which govern their uptake, distribution, and retention in laboratory animals. In additon, there was not a direct correlation between the relative toxicities of the

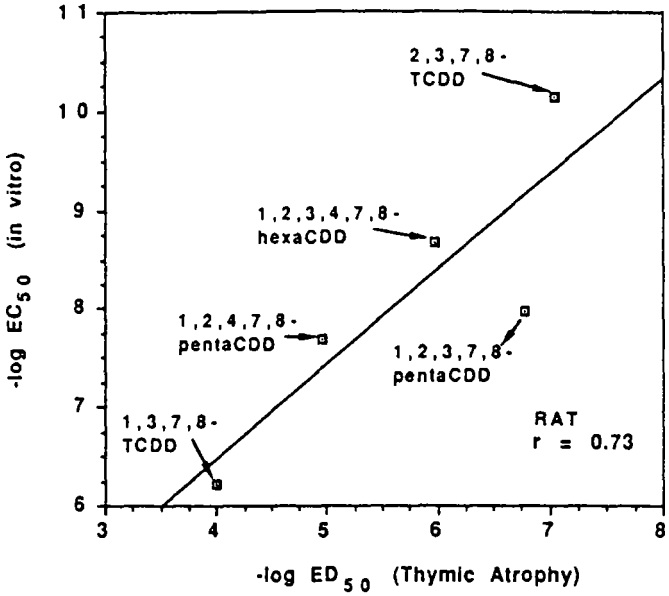


FIGURE 10. A plot of the $-\log EC_{50}$ values for AHH induction in rat hepatoma H-4-II E cells by several PCDD congeners and their corresponding $-\log ED_{50}$ values for thymic atrophy in the immature male rat.[288]

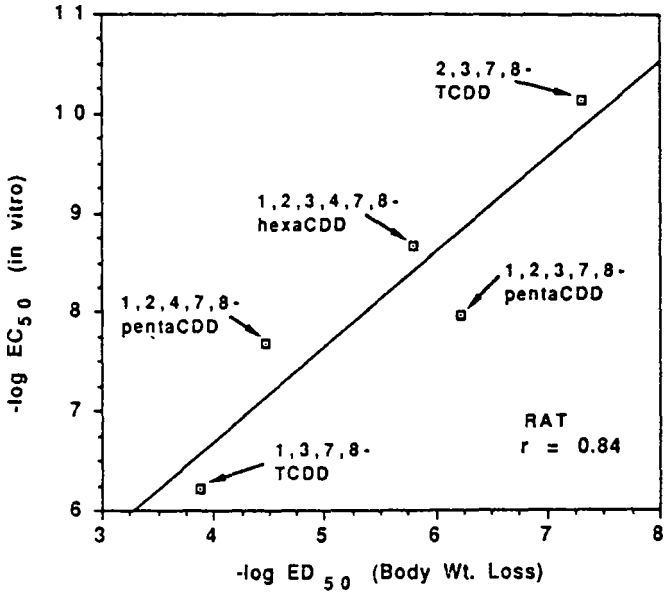


FIGURE 11. A plot of the $-\log EC_{50}$ values for AHH induction in rat hepatoma H-4-II E cells by several PCDD congeners and their corresponding $-\log ED_{50}$ values for inhibition of body weight gain in the immature male rat.[288]

2,3,7,8-substituted congeners in the rat and their competitive Ah receptor-binding avidities using rat hepatic cytosol.[287] However, for a broad spectrum of PCDFs which contained both 2,3,7,8-substituted congeners and compounds with less

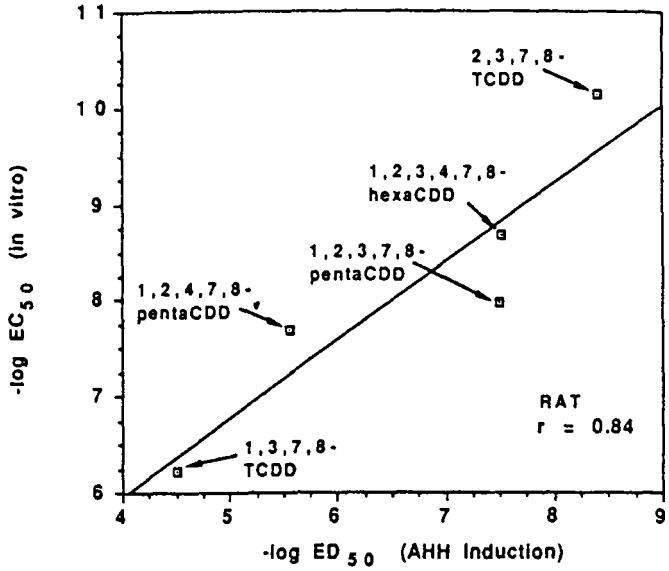


FIGURE 12. A plot of the $-\log EC_{50}$ values for AHH induction in rat hepatoma H-4-II E cells by several PCDD congeners and their corresponding $-\log ED_{50}$ values for hepatic microsomal AHH induction in the immature male rat.[288]

than four lateral substituents there was a (qualitative) correlation between their structure-binding and structure-toxicity relationships.[287] In a 13-week subchronic toxicity study, Pluess and co-workers[305,306] estimated that the relative toxicities of 2,3,7,8-TCDD:2,3,4,7,8-pentaCDF:1,2,3,7,8-pentaCDF:1,2,3,6,7,8-hexaCDF were 1.0:0.4:0.01:0.10. These results were within the ranges observed in the shorter term studies (see Table 11).

Table 12 summarized the *in vitro* and biochemical effects of the 2,3,7,8-substituted PCDFs. The structure-activity relationships for these responses show that the fully lateral-substituted (Cl_4–Cl_6) compounds are more active[287] than congeners with less than four lateral chlorine groups. The results also show that 2,3,4,7,8-pentaCDF is the most active 2,3,7,8-substituted PCDF. The dose-response effects of several structurally diverse PCDD and PCDF congeners on the induction of hepatic microsomal AHH and EROD activities, thymic atrophy, and body weight loss in the rat have been reported.[287,288] Figures 13, 14, and 15 illustrate a plot of the $-\log EC_{50}$ values for AHH induction *in vitro*, rat hepatoma H-4-II E cells) vs. the *in vivo* $-\log ED_{50}$ values for thymic atrophy, body weight loss, and hepatic microsomal AHH induction in the rat by the same compounds. The relatively high linear correlation coefficients between these responses demonstrated that the SARs for both PCDDs (Figures 10 to 12) and PCDFs are comparable for diverse responses in different biosystems. Moreover, combination of the results for both the PCDDs and PCDFs also gave good linear correlations between the *in vitro* induction

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Table 10
A Comparison of the NATO/CCMS and Nordic TEF Values and the Relative Potency Ranges for the 2,3,7,8-Substituted PCDDs

Congener	TEF values NATO/CCMS	Nordic	Relative potency ranges *In vivo* toxicities	Relative potency ranges *In vitro* results[a]
2,3,7,8-TCDD	1	1	—	—
1,2,3,7,8-PentaCDD	0.5	0.5	0.59—0.053	0.64—0.07
1,2,3,4,7,8-HexaCDD	0.1	0.1	0.24—0.013	0.13—0.05
1,2,3,6,7,8-HexaCDD	0.1	0.1	0.16—0.0152	0.5—0.005
1,2,3,4,7,8,9-HexaCDD	0.1	0.1	0.14—0.016	0.009
1,2,3,4,6,7,8-HexaCDD	0.01	0.01	0.0076	0.003
OCDD	0.001	0.001	>0.0013	0.0006

[a] Does not include receptor binding data.

Table 11
Comparative Toxic Potencies of the 2,3,7,8-Substituted PCDFs[187,188,286,287,295,297]

Response	Species (dose/conc.)	LD_{50}/ED_{50} Values 2,3,7,8-TCDF	1,2,3,7,8-PentaCDF	2,3,4,7,8-PentaCDF	1,2,3,4,7,8-HexaCDF	2,3,4,6,7,8-HexaCDF	1,2,3,6,7,8-HexaCDF	1,2,3,7,8,9-HexaCDF	1,2,3,4,6,7,8-HeptaCDF	1,2,3,4,7,8,9-HeptaCDF	OCDF	2,3,7,8-TCDD
Acute lethality												
	Guinea Pig (μg/kg)	5—10		3—10		120						0.6—2.0
	Mouse (μg/kg)	>6000										114—284
	Rat (μg/kg)	>1000		916								22—45
Subchronic toxicities												
Body weight loss												
	Rat (μmol/kg)	3.20	2.64	1.04	1.30	2.80						0.05
	Guinea pig (μmol/kg)		0.0059	0.012								0.0056
Thymic Atrophy	Rat (μmol/kg)	3.60	1.76	0.21	0.50	0.93						0.09
Immunotoxicity	Mice (μmol/kg)	0.014		0.003					0.011	0.012		0.0024
Teratogenicity	Mice (μmol/kg)	0.22	0.35	0.09	0.89							0.011

Table 12
Comparative Biochemical and *In Vitro* Potencies of 2,3,7,8-Substituted PCDFs[260,286,287,295,297–299]

Response	System	ED_{50} Values 2,3,7,8-TCDF	1,2,3,7,8-PentaCDF	2,3,4,7,8-PentaCDF	1,2,3,4,7,8-HexaCDF	2,3,4,6,7,8-HexaCDF	1,2,3,6,7,8-HexaCDF	1,2,3,7,8,9-HexaCDF	1,2,3,4,6,7,8-HeptaCDF	1,2,3,4,7,8,9-HeptaCDF	OCDF	2,3,7,8-TCDD
Receptor binding												
	Mouse liver (K_D, nM)	0.73										0.27
	Rat liver (M)	4.1×10^{-8}	7.45×10^{-8}	1.5×10^{-8}	2.3×10^{-7}	4.7×10^{-8}	2.7×10^{-7}					1.0×10^{-8}
AHH induction												
	Rat liver (μmol/kg)	0.652	1.47	0.037	0.293	0.265						0.004
	H-4-II E cells (M)	3.9×10^{-9}	2.5×10^{-9}	2.6×10^{-10}	3.6×10^{-10}	6.9×10^{-10}	1.5×10^{-9}					7.2×10^{-11}
	Chick embryo (nmol/kg)	0.46										0.2
	Mouse liver (μmol/kg)								0.11	0.70		—
	Guinea pig (μmol/kg)		0.0059	0.0012								0.00028
Keratinization	XB 3T3 cells (nM)	1										0.05

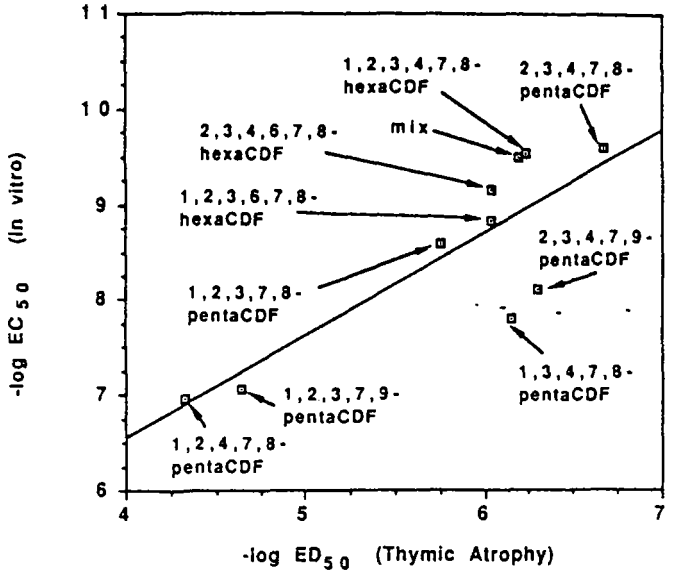


FIGURE 13. A plot of the $-\log EC_{50}$ values for AHH induction in rat hepatoma H-4-II E cells by several PCDF congeners and their corresponding $-\log ED_{50}$ values for thymic atrophy in the immature male rat.[286,287]

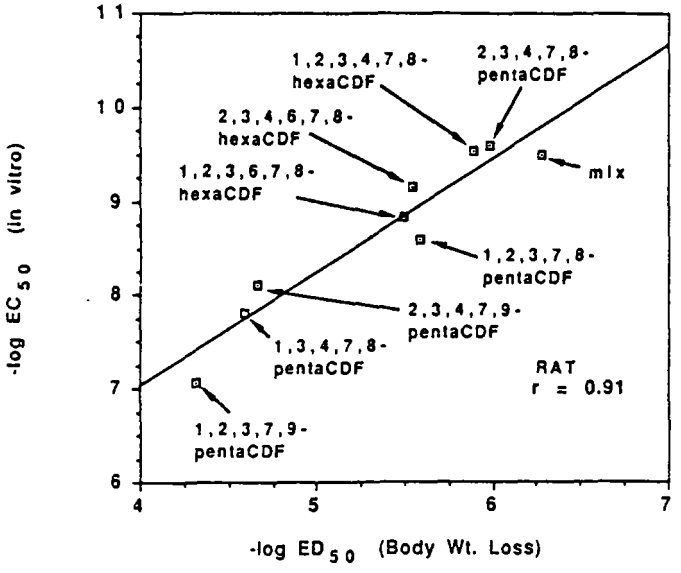


FIGURE 14. A plot of the $-\log EC_{50}$ values for AHH induction in rat hepatoma H-4-II E cells by several PCDF congeners and their corresponding $-\log ED_{50}$ values for inhibition of body weight gain in the immature male rat.[286-288]

and *in vivo* toxic responses. This is further illustrated in Figures 16 and 17 in which comparable linear correlations are observed for the *in vitro* $-\log EC_{50}$ values (AHH induction) vs. $-\log ED_{50}$ values for body weight loss and immunotoxicity in the

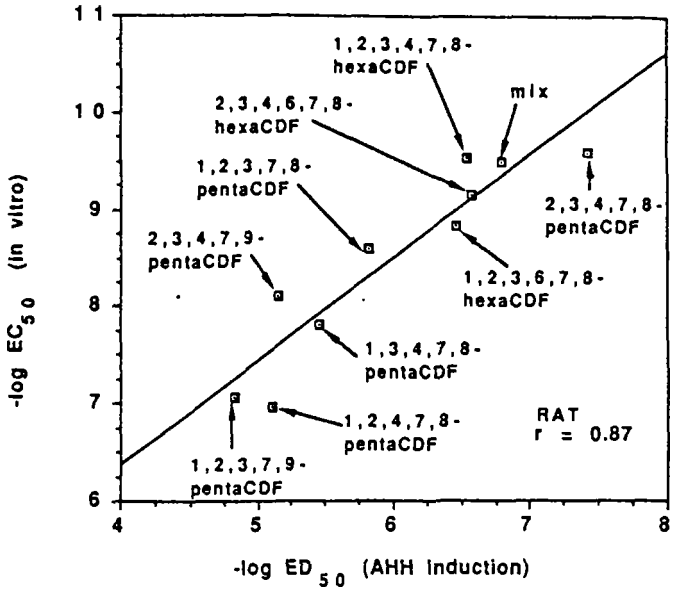


FIGURE 15. A plot of the $-\log EC_{50}$ values for AHH induction in rat hepatoma H-4-II E cells by several PCDF congeners and their corresponding $-\log ED_{50}$ values for hepatic microsomal AHH induction in the immature male rat.[286,287]

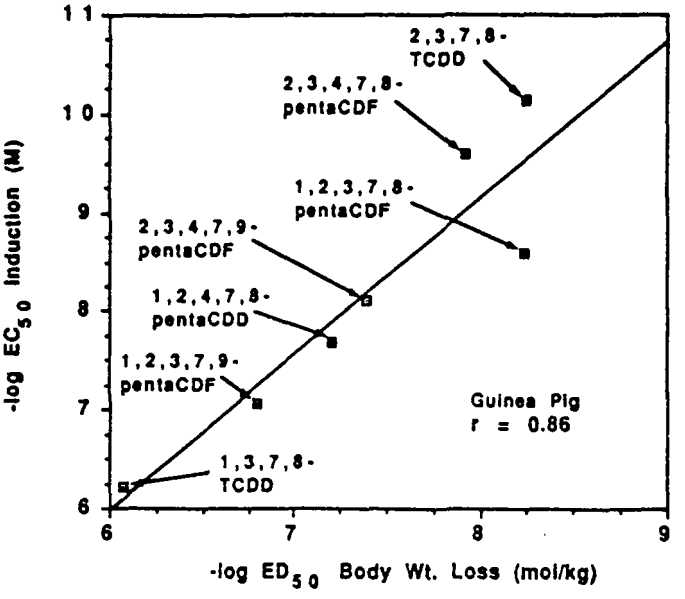


FIGURE 16. A plot of the $-\log EC_{50}$ values for the AHH induction in rat hepatoma H-4-II E cells by PCDD and PCDF congenes and their corresponding $-\log ED_{50}$ values for inhibition of body weight gain in the male guinea pig.[298]

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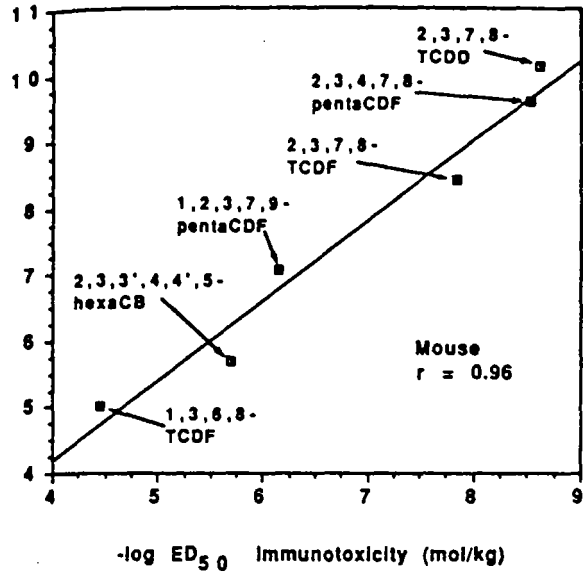


FIGURE 17. A plot of the $-\log EC_{50}$ values for AHH induction in rat hepatoma H-4-II E cells by 2,3,7,8-TCDD and related compounds and their corresponding $-\log ED_{50}$ values for immunotoxicity in C57BL/6 mice.[295,332,364,368]

male guinea pig and C57BL/6 mouse, respectively.[295,298] These results strongly support a common receptor-mediated mechanism of action of the PCDDs and PCDFs; Safe and co-workers have also used these data and other results to support the use of the *in vitro* induction (AHH and EROD activities) responses as quantitative bioassays for the hazard assessment of toxic halogenated aromatic hydrocarbons[307-311]

Table 13 summarizes the proposed TEF values for the 2,3,7,8-substituted PCDFs. Both the NATO/CCMS and Nordic values are identical except for the values of 0.05 and 0.01 which were assigned to 1,2,3,7,8-pentaCDF by the two groups, respectively. Based on the result summarized in Tables 11 and 12, the 0.01 and 0.05 values may be inappropriate. For example, 2,3,4,7,8-pentaCDF is less than four times more potent than the 1,2,3,7,8-isomer as a teratogen in C57BL/6 mice.[297] Based on this result and other comparable potency ratios of the two pentaCDFs, a more conservative TEF value for 1,2,3,7,8-pentaCDF would be 0.10.

Recent studies[387] have determined the relative potencies of the 4 heptaCDF isomers as immunotoxins and as inducers of hepatic microsomal AHH and EROD activities in C57BL/6 mice. The ED_{50} values for the suppression of the splenic plaque-forming cell response to sheep red blood cells were 0.011 and 0.012 μmol/kg for 1,2,3,4,6,7,8- and 1,2,3,4,7,8,9-heptaCDF, respectively; the corresponding ED_{50} value for 2,3,7,8-TCDD was 0.0024 μmol/kg. These results suggest that the proposed TEF value of 0.01[301-303] may underestimate the relative toxicities of the heptaCDFs and a conservative value of 0.1 may be more appropriate. The 0.001 TEF assign to OCDF was derived from the data obtained after multiple administration of OCDD to rats.[304]

D. TEFs for Other Halogenated Dibenzo-p-dioxins and Dibenzofurans

Recent studies have demonstrated that brominated/chlorinated dibenzofurans and dibenzo-p-dioxins are also formed during combustion processes.[128,169,170,311] These compounds have not been widely identified in the environment, however, analytical studies have focused on the detection and quantitation of PCDDs and PCDFs and not their chlorinated/brominated or polybrominated analogs. Most *in vitro* studies with brominated PCDDs or PCDFs or the fully chlorinated analogs suggest that these compounds are approximately equipotent. A comparative study of the dose-response effects of several bromo/chloro and polybrominated dibenzo-p-dioxins in male rats is summarized in Table 14.[289] These data show that the potencies of these congeners varies with the position and number of bromo substituents and the response which is measuresd. A direct comparision of the 2,3,7,8- and 1,2,3,7,8-substituted bromo and chloro analogs shows that there are minimal differences in their toxic potencies (Tables 8 and 14). Interestingly, 1,3,7,8-tetraCDD was more toxic in the rat than the corresponding brominated analog, whereas the reverse order of potency was observed for the 1,2,4,7,8-substituted bromo/chloro congeners.[288,289] A recent study reported that 2,3,7,8-tetrabromodibenzo-p-dioxin was at least ten times less potent than 2,3,7,8-TCDD in a 28-day rat LD_{50} and a rabbit ear dermal comedogenicity study.[312] The reduced potency of this and other brominated dibenzo-p-dioxin and dibenzofuran congeners needs to be assessed in additional studies. At present, this author suggests that the same TEF values be used for both the chlorinated, brominated, and chloro/bromo (mixed) dibenzo-p-dioxins and dibenzofurans.

E. PCBs: Development of TEFs

Several studies have confirmed a correlation between the structure-binding and structure-activity relationships for the two major classes of PCBs which exhibit Ah receptor agonist activity[184,186,191,192,198,259,285,313-327] (Figures 8 and 9). The coplanar PCBs, 3,4,4',5-tetraCB, 3,3',4,4'-tetraCB, 3,3',4,4',5-pentaCB and 3,3',4,4',5,5'-hexaCB which are substituted in both para, at least 2 meta, and no ortho positions are clearly the most toxic members of this class of halogenated aromatics. The relative toxic and biochemical potencies of the coplanar PCB congeners (class I) are summarized in Table 15 and it is apparent from these data that there are considerable variations within this group of compounds (note: very little data is available for the 3,4,4',5-tetraCB congener and this compound has been omitted from the table). The results obtained for the potencies of the co-

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Table 13
A Comparison of the NATO/CCMS and Nordic TEF Values and Relative Potency Ranges for the 2,3,7,8-Substituted PCDFs

	TEF values		Relative potency ranges	
Congener	NATO/CCMS	Nordic	*In vivo* toxicities	*In vitro* results[a]
2,3,7,8-TCDF	0.1	0.1	0.17—0.016	0.43—0.006
2,3,4,7,8-PentaCDF	0.5	0.5	0.8—0.12	0.67—0.11
1,2,3,7,8-PentaCDF	0.05	0.01	0.9—0.018	0.13—0.003
1,2,3,4,7,8-HexaCDF	0.1	0.1	0.18—0.038	0.2—0.013
2,3,4,6,7,8-HexaCDF	0.1	0.1	0.097—0.017	0.1—0.015
1,2,3,6,7,8-HexaCDF	0.1	0.1	—	0.048—0.037
1,2,3,7,8,9-HexaCDF	0.1	0.1	—	—
1,2,3,4,6,7,8-HeptaCDF	0.01	0.01	0.22	—
1,2,3,4,7,8,9-HeptaCDF	0.01	0.01	0.20	—
OCDF	0.001	0.001	—	—

[a] Does not include receptor binding data.

Table 14
The *In Vivo* Biologic and Toxic Effects of PBDDs and Brominated PCDDs in the Immature Male Wistar Rat[289]

	In vivo ED_{50} values (mol/kg)			
Compound	Body weight loss	Thymic atrophy	AHH induction	EROD induction
2,3,7,8-TBDD	6.75×10^{-8}	3.39×10^{-8}	7.59×10^{-10}	3.55×10^{-10}
2,3-Dibromo-7,8-dichlorodibenzo-p-dioxin	1.24×10^{-6}	7.25×10^{-9}	4.90×10^{-10}	3.47×10^{-10}
2-Bromo-3,7,8-trichlorodibenzo-p-dioxin	1.24×10^{-7}	3.47×10^{-8}	2.46×10^{-9}	1.15×10^{-8}
1,2,3,7,8-PentaBDD	8.71×10^{-7}	3.9×10^{-7}	2.46×10^{-8}	1.74×10^{-8}
1,2,4,7,8-PentaBDD	1.29×10^{-5}	6.17×10^{-6}	1.95×10^{-7}	2.19×10^{-7}
1,3,7,8-TBDD	2.52×10^{-4}	3.55×10^{-5}	6.50×10^{-6}	1.26×10^{-5}

highly variable and dependent on both the species and the response. The data show that 3,3′,4,4′,5-pentaCB is clearly the most toxic coplanar PCB congener and the 2,3,7,8-TCDD/3,3′,4,4′,5-pentaCB potency ratios were 66/1 (body weight loss, rat); 8.1/1 (thymic atrophy, rat); 10/1 (mouse fetal thymic lymphoid development); 125/1 (AHH induction, rat); 3.3/1 (AHH induction, rat hepatoma H-4-II E cells), and 100/1 (chick embryo hepatocytes). Based on these toxicity data, a TEF value of 0.1 is appropriate for this congener. It is clear from the data in Table 15 that both the 3,3′,4,4′-tetra- and 3,3′,4,4′,5,5′-hexaCB congeners are considerably less toxic than 3,3′,4,4′,5-pentaCB, however, their relative potencies are highly variable. It is apparent from *in vivo* studies in the rat that 3,3′,4,4′-tetraCB is >30 times less toxic than 3,3′,4,4′5,5′-hexaCB, whereas, in most of the *in vitro* assays, these compounds exhibit similar potencies or the reverse order of potency is observed. The relatively low *in vivo* toxicity of 3,3′,4,4′-tetraCB in the rat may be due to rapid metabolism which has also been observed in male ICR mice;[329,330] however, it should be noted that this congener has recently been detected in environmental and human samples[331] and these results suggest that the rapid clearance from the rat may not be observed in all animal species. Based on the *in vivo* teratogenicity and toxicity in rats, the proposed TEF value for 3,3′,4,4′,5,5′-hexaCB is 0.05 and a conservative value of 0.01 is assigned to 3,3′,4,4′-tetraCB. These assigned TEFs will change as more quantitative toxicity data becomes available from future studies.

Figure 9 illustrates the structures of all the monoortho coplanar PCB congeners which can be derived from the four coplanar congeners. These compounds all exhibit Ah receptor agonist activity; however, only limited quantitative SARs have

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Table 15
Comparative Toxic and Biochemical Potencies of Coplanar PCBs[317,323-326,328]

Response	Target species/cell	ED_{50}/EC_{50} Values			
		3,3,4,4'-TetraCB	3,3',4,4',5-PentaCB	3,3',4,4',5,5'-HexaCB	2,3,7,8-TCDD
Subchronic toxicities					
Body weight loss	Rat (μmol/kg)	>500	3.3	15	0.05
Thymic Atrophy	Rat (μmol/kg)	>500	0.95	8.9	0.09
Bursal lymphoid development	Chick embryo (μg/kg)	50	4	300	
Thymic lymphoid development	Mouse fetuses (*M*)	3×10^{-7}	2×10^{-9}	2×10^{-7}	2×10^{-10}
Immunotoxicity	Mice (μmol/kg)				.0024
Teratogenicity	Mice (μmol/kg)			.055—.110[328]	.011
AHH induction	Rat (μmol/kg)	500	0.50	1.10	0.004
AHH induction	H-4-II E cells (*M*)	3.5×10^{-8}	2.4×10^{-10}	6.0×10^{-8}	7.2×10^{-11}
AHH induction	Chick embryo hepatocytes	2.2×10^{-9}	2.0×10^{-9}	—	2.0×10^{-11}
Receptor binding	Rat cytosol (*M*)	4.3×10^{-7}	1.2×10^{-7}	Insoluble	1.0×10^{-8}

Table 16
Comparative Toxic and Biochemical Potencies of Monoortho Coplanar PCBs[317,323-326]

Response	Target species/cell	ED_{50}/EC_{50} Values								
		2,3',4,4',5-PentaCB	2,3,3',4,4'-PentaCB	2',3,4,4',5-PentaCB	2,3,4,4',5 PentaCB	2,3,3',4,4',5 HexaCB	2,3,3',4,4',5-HexaCB	2,3',4,4',5,5'-HexaCB	2,3,3',4,4',5,5'-HeptaCB	2,3,7,8-TCDD
Subchronic studies										
Body weight loss	Rat (μmol/kg)	1450	750	370	180	180	220			.0.05
Thymic atrophy	Rat (μmol/kg)	1550	1030	2790	200	180	225			0.09
Bursal lymphoid development	Chick embryos (μg/kg)	>6000	<6000			<6000	>6000	>6000		
Immunotoxocity development	Mice (μmol/kg)					2				0.0024
Teratogenicity	Mice (μmol/kg)					328				0.011
AHH induction	Rat (μmol/kg)	165	65	130	30	7	6			0.004
	Rat hepatoma (cells (*M*)	1.2×10^{-5}	8.8×10^{-6}	3.9×10^{-6}	9.7×10^{-7}	2.1×10^{-6}	1.1×10^{-5}	1.3×10^{-5}		7.2×10^{-11}
	Chick embryo hepatocytes (*M*)	5.0×10^{-8}			1.0×10^{-7}	1.4×10^{-5}				2.0×10^{-11}
Receptor binding	Rat cytosol (*M*)	9.1×10^{-5}	4.3×10^{-6}	1.4×10^{-6}	4.7×10^{-6}	7.1×10^{-6}	5.0×10^{-6}	1.6×10^{-5}		1.0×10^{-8}

these studies are summarized in Table 16. The relative potencies of this subgroup of PCBs are highly variable. 2,3',4,4',5-PentaCB is the major monoortho coplanar PCB present in Aroclors 1248 and 1254 and is routinely identified in environmental and human extracts.[13-21,44,45,331] The activity of this compound relative to 2,3,7,8-TCDD as an inducer of AHH activity is 0.004[326] (chick embryo hepatocytes), 0.000008[317] (rat hepatoma cells), and 0.000034[323] (rat liver).

In vivo studies in the rat show that the corresponding TEFs for 2,3',4,4',5-pentaCB are 0.000035 and 0.00006 for body weight loss and thymic atrophy, respectively.[323] Some of the higher cholorinated hexaCB isomers are more toxic than 2,3',4,4',5-pentaCB and the differences in potency may be related to different rates of *in vivo* metabolism. For example, the 2,3,3',4,4',5-hexaCB is one of the most active monoortho coplanar PCBs in the rat and in the C57BL/6 mouse. In the latter species, the 2,3,7,8-TCDD/2,3,3',4,4',5-hexaCB potency ratios for immunotoxicity and teratogenicity were 0.0012 and 0.00003, respectively.[297,332] This represents a 40-fold difference in the relative potency of 2,3,3',4,4',5-hexaCB for 2 different responses in the same animal species. It is apparent from the data in Table 16 that this variability is also observed for other congeners. Based on the results obtained for one of the more active congeners, namely, 2,3,3',4,4',5-hexaCB, a

TEF value of 0.001 for the monorotho coplanar PCBs would be highly conservative.

Figure 18 illustrates the structures of several PCB congeners which represent six different structural groups: the coplanar PCBs (class I), monoortho coplanar PCBs (class II), monoortho coplanar PCBs minus a single p-chloro substituent (class III), diortho coplanar PCBs (class IV), triorthocoplanar PCBs (class V), and tetraortho-substituted PCBs (class VI). The structure-immunotoxicity relationships for these compounds have been determined in C57BL/6 mice by measuring their dose-response effects on the splenic plaque-forming cell response to sheep red blood cells.[332] Previous studies have confirmed the immunotoxicity of class I and II structural groups[258,333] and this study also showed that class III and IV congeners were also active. For example, the ED_{50} values for the immunosuppressive activities of 2,3,3′,4,5,5′- and 2,3′,4,4′,5′,6-hexaCB were 200 and 120 μmol/kg, respectively. Previous studies have reported that, with the exception of 2,2′,4,4′,5,5′-hexaCB,[184,191] several diortho coplanar PCBs exhibit weak Ah receptor agonist activity.[184,191,332,334] Based on the immunotoxicity results, the potencies (relative to 2,3,7,8-TCDD) of the class III and IV PCBs would be 0.000012 and 0.00002, respectively. Since diortho coplanar PCBs are major components of the commercial products and extracts from environmental samples, more research on their relative toxicities should be carried out. However, at present a provisional TEF value of 0.00002 is assigned. Based on the results presented in Tables 15 and 16 a proposed set of TEF values for the coplanar monoortho coplanar and dioortho coplanar PCB congenes is summarized in Table 17.

F. PBBs: Development of TEFs

Several studies have demonstrated that SARs for PBBs are comparable to those reported for the corresponding PCBs.[184,186,191,192,334-345] The coplanar and monoortho coplanar congeners all elicit a typical spectrum of Ah receptor-mediated responses which include a wasting syndrome, hepatoxicity, immunotoxicity and thymic atrophy, carcinogenesis, dermal toxicity, hepatotoxicity, and porphyria. In contrast to the PCBs, quantitative SARs are not available for individual PBB congeners. Therefore, it is recommended that the TEF values for the PCBs should also be utilized for the corresponding PBBs (i.e., Table 17).

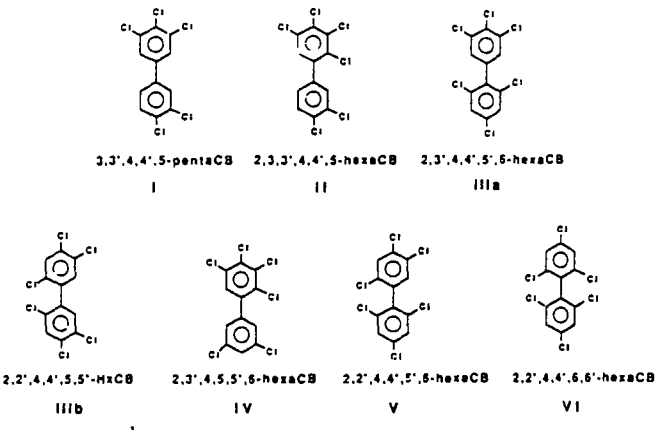


FIGURE 18. Classification of PCB congeners into six different structural groupings.[332]

Table 17
Proposed TEF Values for the Coplanar and Monoortho Coplanar PCB Congeners

Congener	TEF values	Relative potency range[a]
1. Coplaner PCBs		
3,3′,4,4′,5,5′-PentaCB	0.1	0.3—0.0006
3,3′,4,4′,5,5′-HexaCB	0.05	0.1—0.0012
3,3′,4,4′-TetraCB	0.01	0.009—0.00008
2. Monortho coplaner PCBS		
2,3′,4,4′,5-PentaCB	0.001	0.0004—0.000006
2,3,3′,4,4′-PentaCB	0.001	0.0008—0.00006
2′,3,4,4′,5-PentaCB	0.001	0.00013—0.000018
2,3,4,4′,5-PentaCB	0.001	0.00045—0.000074
2,3,3′,4,4′,5-HexaCB	0.001	0.0005—0.0000014
2,3,3′,4,4′,5′-HexaCB	0.001	0.0004—0.0000065
2,3′,4,4′,5,5′-HexaCB	0.001	0.0000055
2,3,3′,4,4′,5,5′-HeptaCB	0.001	No data available
3. Dioortho coplanar PCBs	0.00002	0.00002

[a] Does not include receptor binding data.

G. Polychlorinated Diphenyl Ethers (PCDEs): Development of TEFs

Only a limited amount of toxicologic data is available for the PCDEs and the results suggest that the SARs for the PCDEs and PCBs are comparable.[346-348] Recent studies in my laboratory have investigated the dose-response effects of several PCDE congeners on the suppression of the splenic plaque-forming cell response to sheep red blood cells and the induction of hepatic microsomal AHH and EROD activities in C57BL/6 mice.[349] The immunotoxic potencies for these PCDE congeners (ED_{50} values for the suppression of PFCs/spleen and PFCs/10^6 cells) followed the respective order 2,3,3′,4,4′,5-hexaCDE (0.5 and 2.2 μmol/kg) > 3,3′,4,4′,5-pentaCDE (8.8 and 5.1 μmol/kg) > 2,3′,4,4′,5-pentaCDE (21.8 and 14.2 μmo/kg) > 3,3′,4,4′-tetraCDE (50.6 and 28.7 μmol/kg) > 2,2′,4,4′,5,5′-hexaCDE (81.2 and 56.5 μmol/kg) > 2,2′,4,5,5′-pentaCDE (258 and 228 μmol/kg) > 2,2′,4,4′,5,6′-hexaCDE (>400 μmol/kg for both responses). The potencies of the PCDE congeners as inducers of hepatic microsomal AHH and EROD activities were similar to their immunotoxicities. The structure-activity relationships for the PCDEs and PCBs differed significantly. For example, the coplanar 3,3′,4,4′-tetraCDE and 3,3′,4,4′,5-pentaCDE congeners were less immunotoxic than their monoortho 2,3′,4,4′,5-pentaCDE and 2,3,3′,4,4′,5-hexaCDE analogs, respectively, and similar results were also observed for their enzyme induction potencies. For the cor-

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responding PCB congeners, the coplanar compounds were significantly more active than their monoortho analogs. In addition, two dioortho substituted compounds, 2,2',4,5,5'-pentaCDE and 2,2',4,4',5,5'-hexaCDE, were also immunotoxic at a dose of 400 μmol/kg, whereas their PCB analogs were not active. These studies clearly demonstrate that, for the PCDE congeners, increasing ortho-chloro substitution is less effective in reducing the activity of these compounds compared to the well-recognized ortho effects reported for the PCBs. The ED_{50} values for the immunotoxicity and AHH induction activity of the coplanar and monoortho coplanar PCDEs in the C57BL/6 mice varied from 2.2 to 28.7 μmol/kg and 26.2 to 160 μmol/kg, respectively. Since the corresponding ED_{50} value for the immunotoxicity of 2,3,7,8-TCDD was 0.0024 μmol/kg, a TEF value of 0.001 would be a conservative number for both the coplanar and monoortho coplanar PCDEs.

H. Summary

The development of TEFs for the toxic halogenated aromatics has been necessitated by the identification of complex mixtures of these compounds in almost every compartment of the global ecosystem. Although extensive toxicologic and carcinogenic data are available for one member of this family, namely, 2,3,7,8-TCDD, very little information is available for the remaining congeners. There is good evidence that the toxic halogenated aromatic hydrocarbons elicit common toxic responses through a common mechanism of action as illustrateed in Figure 5. Moreover, the SARs observed for the different classes of halogenated aromatics provide support for both the proposed receptor-mediated mechanism of action and the development of TEFs for this family of chemicals. Based on the current data available, Table 18 summarizes the proposed TEFs for the halogenated aromatics. The TEF values for the PCDDs and PCDFs are similar to those described in the Nodic and NATO/CCMS documents and in a recent update by the Risk Assessment Forum of the U.S. Environmental Protection Agency;[301-303] however, values of 0.1 have been assigned to 1,2,3,4,6,7,8- and 1,2,3,4,7,8,9-heptaCDF and 1,2,3,7,8-pentaCDF. The TEF values given to the PCBs, PCDEs, and brominated or bromo/chloro aromatic compounds have not previously been assigned by regulatory agencies. All the values reported in Table 18 are provisional and should be modified as more data become available.

V. FACTORS WHICH EFFECT THE VALIDITY OF THE PROPOSED TEFs FOR 2,3,7,8-TCDD AND RELATED TOXIC HALOGENATED AROMATICS

A. Interactive Effects — Additivity

The simple interactive effects of relatively potent Ah receptor agonists and complex mixtures of these compounds have been investigated utilizing diverse Ah receptor-mediated responses. Pregnant mice were treated with different doses of 2,3,4,7,8-pentaCDF (0 to 30 μg/kg) or 1,2,3,4,7,8-hexaCDF (0 to 300 μg/kg) or a combination of the two toxins on days 10 to 13 of gestation.[297] Based on probit analysis of the data, the combination of these two compounds as teratogens was additive. Similarly, the combination of 2,3,4,7,8-pentaCDF and 2,3,3',4,4',5-hexaCB (0 to 60 mg/kg) was also additive and this result contrasted to previous interactive studies with this PCB congener and 2,3,7,8-TCDD which gave apparent synergistic interactions for teratogenicity.[350] However, based on the more comprehensive studies noted above,[297] it is likely that interaction of 2,3,7,8-TCDD and 2,3,3',4,4',5-hexaCB is also additive.

Previous studies for several individual PCDD, PCDF, PBDD, and PCB congeners and a mixture of PCDFs have shown that there was linear correlation between their *in vitro* activity as inducers of AHH activity in rat hepatoma H-4-II E cells and their toxicities in at least three animal species[287-289,323] (see Figures 10 to 17). This research has confirmed the utility of the *in vitro* bioassay for quantitatively estimating the *in vivo* toxicities on individual congeners, and several reports also confirm the utility of the induction bioassay system for complex mixtures of halogenated aromatics.[307-311,351] In addition, it has been shown that there was also a correlation between the *in vitro* induction activity of a mixture of six PCB congeners (2,3',4,4',5-pentaCB, 2,2',4,4',5,5'-hexaCB, 2,2',3,4,4',5'-hexaCB, 2,3,3',4,4',5-hexaCB, 2,2',3,4,4',5,5'-heptaCB, and 2,2',3,3',4,4',5-heptaCB) and five PCDF congeners (2,3,7,8-TCDF, 1,2,4,7,8-pentaCDF, 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,4,7,8-hexaCDF) and their corresponding *in vivo* toxicities in the rat.[352,353] The PCB and PCDF mixtures were reconstituted from synthetic compounds and reconstituted to resemble the relative composition of PCBs and PCDFs identified in the liver of a victim of Yusho poisoning. Table 19 summarizes the observed and calculated EC_{50} values for *in vitro* induction of AHH and EROD activity by these two mixtures. The calculated values were derived from the known *in vitro* AHH induction activities of the individual congeners and their relative concentrations in the mixtures. The results showed that there was a good correspondence between the calculated and observed data and these studies suggest that the interactions of these compounds as inducers of AHH and EROD activities were additive. In additon, previous studies have demonstrated the linear correlations between the *in vitro* $-\log EC_{50}$ values for AHH induction vs. the $-\log ED_{50}$ values for *in vivo* toxicity for this mixture and other individual halogenated aromatic congeners[287-289] and this result indicates that the toxicities of the individual PCDF congeners in this mixture are also additive.

Scientists from the New York State Department of Health have utilized a number of *in vivo* and *in vitro* bioassays for assessing the hazard of toxic halogenated aromatic mixtures associated with contaminants from PCB fires (e.g., PCDFs and PCBs) and extracts of samples from Love Canal.[354-361] For

Table 18
Proposed TEFs for the Toxic Halogenated Aromatics

Congener	TEF
A. PCDDs	
2,3,7,8-TCDD	1.0
1,2,3,7,8-PentaCDD	0.5
1,2,3,4,7,8-HexaCDD	0.1
1,2,3,6,7,8-HexaCDD	0.1
1,2,3,7,8,9-HexaCDD	0.1
1,2,3,4,6,7,8-HeptaCDD	0.01
OCDD	0.001
B. PCDFs	
2,3,7,8-TCDF	0.1
2,3,4,7,8-PentaCDF	0.5
1,2,3,7,8-PentaCDF	0.1
1,2,3,4,7,8-HexaCDF	0.1
2,3,4,6,7,8-HexaCDF	0.1
1,2,3,6,7,8-HexaCDF	0.1
1,2,3,7,8,9-HexaCDF	0.1
1,2,3,4,6,7,8-HeptaCDF	0.1
1,2,3,4,7,8,9-HeptaCDF	0.1
OCDF	0.001
C. Brominated and bromo/chloro Dibenzo-p-dioxin and dibenzofurens	Same as described above in A and B
D. PCBs	
(a) Coplanar	
3,3',4,4',5-PentaCB	0.1
3,3',4,4'-5,5'-HexaCB	0.05
3,3',4,4'-TetraCB	0.01
(b) Monoortho coplanar	
2,3',4,4',5-PentaCB	0.001
2,3,3',4,4'-PentaCB	0.001
2',3,4,4',5-PentaCB	0.001
2,3,4,4',5-PentaCB	0.001
2,3,3',4,4',5-HexaCB	0.001
2,3,3',4,4',5'-HexaCB	0.001
2,3',4,4',5,5'-HexaCB	0.001
2,3,3',4,4',5,5'-HeptaCB	0.001
(c) Dioortho coplanar PCBs	0.00002
E. PBBs Same values as described above for the PCBs	
F. PCDFs Coplanar and monoortho coplanar congeners	0.001

example, the teratogenic and immunotoxic effects of an organic phase of a leachate (OPL) from Love Canal was investigated in genetically inbred mice.[354,355] The OPL was a complex mixture of organic compounds which included chlorinated hydorcarbons, toluenes, benzenes, benzaldehyde, dibenzofurans, isomeric hexachlorocyclohexanes, and 2,3,7,8-TCCD (0.003% of the administered sample). The results of the immunotoxicity studies showed that in the Ah-responsive BALB/cByJ mice the immunosuppressive effects were primarily due to 2,3,7,8-TCDD and the interactive effects with the other organic constituents in the OPL were minimal. In contrast, the other chemical components in the OPL decreased the 2,3,7,8-TCDD-mediated immunotoxicity in the Ah-nonresponsive DBA/2J mice, and the thymic atrophy and hepatomegaly observed in these mice was associated not with 2,3,7,8-TCDD but the other organic components. In the teratogenicity studies, the development of fetal cleft palate and hydronephrosis in C57BL/6J mice treated with OPL was primarily due to the 2,3,7,8-TCDD in this mixture and minimal interactions with the other chemicals were observed. However, the maternal toxicity observed in this study was primarily due to the other compounds. It was also concluded that the effects of Love Canal soil extracts on the maternal health and fetal development in rats was also due to 2,3,7,8-TCDD which was present as a trace component of this mixture.[356]

Table 19
Summary of the Observed and Calculated EC_{50} Values for the Induction of AHH and EROD Activities in Rat Hepatoma H-4-II E Cells by PCB and PCDF Mixtures[353]

Treatment	EC_{50} (M) EROD	AHH
Reconstituted liver PCBs (observed)	6.86×10^{-6}	1.11×10^{-5}
Reconstituted liver PCBs (calculated)	7.01×10^{-6}	5.98×10^{-6}
Reconstituted liver PDCFs (observed)	1.02×10^{-10}	3.23×10^{-10}
Reconstituted liver PCDFs (calculated)	3.07×10^{-10}	4.43×10^{-10}

Eadon and co-workers summarized the results of several studies on the biochemical and toxic effects of a complex mixture of PCDDs (minor) and PCDFs (major) from soot samples which originated from the PCB transformer fire in the Binghamton State Office building.[357] Their *in vivo* data also showed a good correlation between bioassay-derived "2,3,7,8-TCDD equivalents" in the extracts and the toxic equivalents calculated from the chemical analysis of these samples (see Section VI). Gierthy *et al.* have also investigated the *in vitro* activities of soot extracts from the Binghamton PCB fire using a keratinization assay with XB cells.[360] The toxic equivalents data obtained from the bioassays were comparable to the "2,3,7,8-TCDD equivalents" which were calculated from the analytical (chemial) data using TEFs for the 2,3,7,8-substituted conge-

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ners. It was evident that the results not only confirm the utility of the TEF approach, but also indicate that the interactions of the PCDD/PCDF components in the soot mixture are primarily additive.

B. Interactive Effects — Nonadditivity

Safe and co-workers have reported the interactions of 2,3,7,8-TCDD and halogenated aromatic compounds which result in the partial antagonism of several biochemial and toxic responses elicited by 2,3,7,8-TCDD.[295,361-368] The compounds which exhibit 2,3,7,8-TCDD antagonist activity generally exhibit moderate competitive binding affinities for the Ah receptor but are very weak Ah receptor agonists. For example, the EC_{50} value for the competitive binding of 1,3,6,8-TCDF to the murine cytosolic Ah receptor was 1.25×10^{-7} *M* and the results of a double-reciprocal plot analysis of the saturation binding of [^{3}H]-2,3,7,8-TCDD in the presence of different concentrations of 1,3,6,8-TCDF (1 to 100 n*M*) suggested that 1,3,6,8-TCDF was a competitive anatagonist.[295]

In contrast, 1,3,6,8-TCDF was approximately 1.5×10^4 times less active than 2,3,7,8-TCDD as an immunosuppressive agent in C57BL/6 mice.[195] Interactive studies showed that 1,3,6,8-TCDF partially antagonized 2,3,7,8-TCDD-mediated AHH and EROD induction activities in rat hepatoma H-4-II E cells[362] and immunotoxicity in male C57BL/6 mice.[295] The results in Table 20 illustrate the interactions of 1,3,6,8-TCDF with both 2,3,7,8-TCDD and 2,3,4,7,8-pentaCDF and the partial antagonism of the decrease in the splenic plaque-forming cell response to sheep red blood cells caused by the two potent Ah receptor agonists. It should be noted that these effects are dependent on both the concentration of the agonist and antagonist, and in these studies the "window of antagonism" was observed at 1,3,6,8-TCDF/2,3,7,8-TCDD ratios between 1000/1 to 13,000/1. It is unlikely that the interactions of putative PCDD/PCDF antagonists and agonists will play a significant role in hazard or risk asessment of PCDDs and PCDFs since the relative levels of the potential antagonists are relatively low in extracts which contain PCDD and PCDF mixtures.

The commercial PCB mixture Aroclor 1254 is also a weak Ah receptor agonist compared to 2,3,7,8-TCDD. For example, the EC_{50}/ED_{50} values for the immunotoxicity of 2,3,7,8-TCDD in C57BL/6 mice and the AHH induction activity in rat hepatoma H-4-II E cells are 2.4 nmol/kg and 7.2×10^{-11} *M*, respectively;[364] the corresponding values for Aroclor 1254 are 350 μmol/kg and $>10^{-5}$ *M*, respectively. In contrast, the competitive binding EC_{50} values for 2,3,7,8-TCDD and Aroclor 1254 differ by approximately 600-fold (1×10^{-8} *M* and 6.0×10^{-6} *M*, respectively).[285] The interaction of subeffective doses of Aroclor 1254 and an ED_{80-100} dose of 2,3,7,8-TCDD has demonstrated that Aroclor 1254 can partially antagonize several 2,3,7,8-TCDD-induced responses, including AHH and EROD induction in rat hepatoma H-4-II E cells and C57BL/6 mice, teratogenicity, and immunotoxicity in C57BL/6 mice.[362-364,367] Table 21 summarizes the interactive effects on the splenic plaque-forming cell response to sheep red blood cells in C57BL/6 mice. Aroclor 1254 at doses from 5 to 150 μmol/kg inhibited the immunosuppressive activity of 2,3,7,8-TCDD (3.7 nmol/kg) and this inhibitory effect was partially reversed by a higher dose (11.2 nmol/kg) of 2,3,7,8-TCDD. The results of these and other interactive studies suggest that

Table 20
Effects of 2,3,7,8-TCDD and 2,3,4,7,8-PentaCDF on the Splenic Plaque-Forming Cell (PFC) Response to Sheep Red Blood Cells in C57BL/6J Mice: Interaction with 1,3,6,8-TCDF[a] [295]

Treatment	Dose	Spleen cellularity × 10^7	PFCs/spleen × 10^5	PFCs/10^6 viable cells
Corn oil	—	—	1.50 ± 0.19	704 ± 120
2,3,4,7,8-PentaCDF	3.7 nmol/kg	14.0 ± 2.2	0.53 ± 0.05	252 ± 48
2,3,4,7,8-PentaCDF	3.7 nmol/kg			
+ 1,3,6,8-TCDF	3.3 μmol/kg	16.2 ± 1.0	0.60 ± 0.07	298 ± 87
2,3,4,7,8-PentaCDF	3.7 μmol/kg			
+ 1,3,6,8-TCDF	6.6 μmol/kg	19.8 ± 5.4	0.93 ± 0.19[b]	384 ± 16[b]
2,3,7,8-TCDD	3.7 nmol/kg	24.0 ± 2.7	0.29 ± 0.08	125 ± 46
2,3,7,8-TCDD	3.7 nmol/kg			
+ 1,3,6,8-TCDF	6.6 μmol/kg	19.7 ± 1.5	0.55 ± 0.14[c]	284 ± 81[c]
2,3,7,8-TCDD	3.7 nmol/kg			
+ 1,3,6,8-TCDF	16.5 μmol/kg	20.4 ± 4.9	0.57 ± 0.08[c]	261 ± 36[c]
2,3,7,8-TCDD	3.7 nmol/kg			
+ 1,3,6,8-TCDF	49.3 μmol/kg	20.7 ± 4.6	0.59 ± 0.06[c]	216 ± 29[c]

[a] The control group contained 8 animals and all other groups contained 5 animals; data presented as means ± SD.
[b] Significantly different ($p < 0.05$ from 2,3,4,7,8-pentaCDF-treated mice.
[c] Significantly different ($p < 0.05$ from 2,3,7,8-TCDD-treated mice.

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Table 21
Effects of 2,3,7,8-TCDD, Aroclor 1254, and 2,3,7,8-TCDD Plus Aroclor 1254 on the Plaque-Forming Cell Assay in Mice Pretreated with Sheep Red Blood Cells[364]

Treatment (dose)	PFCs/spleen × 10^5	PFCs/10^6 viable spleen cells	% Control (PFCs/spleen × 10^5)
Corn oil[a]	1.61 ± 0.17	562 ± 76	100
2,3,7,8-TCDD			
(3.72 nmol/kg)[b]	0.38 ± 0.03[c]	156 ± 40[c]	24
(11.2 nmol/kg)	0.20 ± 0.03[c]	62 ± 12[c]	12
Aroclor 1254			
(5 μmol/kg)	1.57 ± 0.11	561 ± 140	98
(15 μmol/kg)[b]	1.60 ± 0.87	465 ± 114	100
(75 μmol/kg)	1.57 ± 0.23	594 ± 35	98
(150 μmol/kg)	1.20 ± 0.22	394 ± 35	75
2,3,7,8-TCDD (3.72 nmol/kg) plus Anoclor 1254 (5 μmol/kg)	1.70 ± 0.30	491 ± 62	106
2,3,7,8-TCDD (3.72 nmol/kg) plus Aroclor 1254 (15 μmol/kg)[b]	1.49 ± 0.08	480 ± 48	93
2,3,7,8-TCDD (3.72 nmol/kg) plus Aroclor 1254 (75 μmol/kg)	1.66 ± 0.28	558 ± 52	103
2,3,7,8-TCDD (3.72 nmol/kg) plus Aroclor 1254 (150 μmol/kg)	0.92 ± 0.08[c]	372 ± 81[c]	57
2,3,7,8-TCDD (11.2 nmol/kg) plus Aroclor 1254 (75 μmol/kg)	0.58 ± 0.10[c]	170 ± 10[c]	36

[a] Eleven animals
[b] Eight animals; all others, four animal/group.
[c] Significantly different ($p < 0.01$) from the corn oil controls.

Aroclor 1254 acts as a competitive antagonist. In addition, several other commercial Aroclors exhibit similar antagonist activities.[368] As noted previously for 1,3,6,8-TCDF, the antagonist activity of Aroclor 1254 is dependent on the antagonist/agonist ratios and their absolute concentrations. Table 22 summarizes the results obtained for the interactions of Aroclor 1254 and 2,3,7,8-TCDD in C57BL/6 mice; it is evident that the % maximum antagonism is response-dependent and ratios of Aroclor 1254/2,3,7,8-TCDD, <20,000/1 and >1670/1, are required to observed partial antagonism. Analytical studies of extracts from human tissues have shown that the ratio of PCBs/PCDDs plus PCDFs is in the range within which PCBs partially antagonize the 2,3,7,8-TCDD-mediated responses in laboratory animal studies; however, the significance of PCB/PCDD plus PCDF interactions in humans is unknown. Nevertheless, it is possible that the TEFs assigned to individual PCDD and PCDF congeners are conservative values when these compounds are present in extracts which contain 10^3 to 10^4 times higher levels of PCBs.

Table 22
Aroclor 1254 as a 2,3,7,8-TCDD Antagonist in C57BL/6 Mice — Summary[363,364,368]

Response	% Maximum antagonism	Antagonist/agonist window
AHH induction	20	1,667—10,000/1
EROD induction	23	1,667—10,000/1
Thymic atrophy	0	No antagonism observed
Immunotoxicity	100	1,340—20,160/1
Teratogenicity	80	±12,100/1

C. Responses Which Are Not Mediated through the Ah Receptor

The TEF values for halogenated aromatics which are summarized in Table 18 can be utilized for the hazard and risk assessment of those congeners which elicit Ah receptor-mediated responses. This sub-group of compounds (e.g., Figures 6 to 9) constitutes only a small fraction of the total number of

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halogenated aromatic congeners (e.g., see Table 1). This limitation may not be important for PCDDs and PCDFs since most environmental samples primarily contain the 2,3,7,8-substituted compounds. In contrast, the "2,3,7,8-TCDD-like" PCBs are only a minor component of the total PCBs which are routinely identified in environmental extracts.[44,45] Therefore, the proposed TEFs for PCBs do not account for the potential toxicity of the "non-2,3,7,8-TCDD"-like congeners or their interactive effects. Although it is unlikely that any of these PCBs elicit acutely toxic effects, there are several reports which show that some members of this class of PCBs elicit biochemical and toxic responses.[184] For example, several PCB congeners resemble both phenobarbital (PB) and dexamethasone as inducers of hepatic microsomal cytochrome P-450 isozymes[191,197] (i.e., cytochromes b/e and cytochrome p, respectively). Both 2,2',4,4',5,5'-hexachloro- and hexabromobiphenyl have been characterized as PB-type inducers of hepatic drug-metabolizing enzymes.[191,192,319,322,338,341] Moreover, like PB, 2,2',4,4',5,5'-hexabromobiphenyl promoted diethylnitrosamine-initiated enzyme altered foci in Sprague-Dawley rats using a two-stage hepatocarcinogenesis protocol.[371] In addition, a combination of both 2,2',4,4',5,5'- and 3,3',4,4',5,5'-hexabromobiphenyl gave an apparent synergistic interaction in the hepatocarcinogenesis bioassay.[372] These data suggest that the corresponding PCB congeners may also exhibit comparable tumor-promoting activities, and it is clear that future studies should focus on the development of hazard/risk assessment approaches for those PCB/PBB congeners which are not covered in the current TEF approach.

VI. APPLICATIONS OF TEFs FOR HAZARD ASSESSMENT OF TOXIC HALOGENATED AROMATICS

TEFs are specifically designed to assess the potential hazard and risk of complex mixtures of halogenated aromatics. Utilization of one or more of the TEF schemes[301-303,357,373,374] requires high-resolution chemical analysis of specific analytes so that each individual congener can be unambiguously identified and quantitated. Tanabe, Tatsukawa, and coworkers[44,375-382] and other groups[383-385] have utilized TEF approaches to determine the relative toxicities of halogenated aromatic mixtures and the relative contributions of the different classes of compounds. The concentration of a specific congener times the TEF gives the concentration in terms of "2,3,7,8-TCDD equivalents". The results in Table 23[382] summarize the concentrations of the individual congeners present in the PCDD, PCDF, and PCB fractions from Kanechlor 400, a commercial PCB mixture, and Yusho oil which was originally derived from Kanechlor 400. The concentration of "2,3,7,8-TCDD equivalents" (in part-per-million) was determined for the major toxic PCDD, PCDF, and PCB congeners using the TEF values de-

Table 23
2,3,7,8-TCDD Equivalents (ppm) in Kanechlor 400 and Yusho Oil[382]

		Kanechlor 400		Yusho Oil #2	
Congener	TEF	Conc. (ppm)	2,3,7,8-TCDD eq. (ppm)	Conc. (ppm)	2,3,7,8-TCDD eq. (ppm)
2,3,7,8-TCDD	1.0	—	—	ND	0
1,2,3,7,8-PentaCDD	0.5	—	—	0.00075	0.00038
1,2,3,4,7,8-HexaCDD	0.1	—	—	0.00057	0.000057
1,2,3,6,7,8-HexaCDD	0.1	—	—	0.038	0.0038
1,2,3,7,8,9-HexaCDD	0.1	—	—	0.022	0.0022
1,2,3,4,6,7,8-HeptaCDD	0.01	—	—	0.190	0.00190
OCDD	0.001	—	—	0.110	0.000110
Total		0	0		0.00747
2,3,7,8-TCDF	0.1	1.7	0.17	0.69	0.069
1,2,3,7,8-PentaCDF	0.1	0.23	0.023	0.76	0.076
2,3,4,7,8-PentaCDF	0.5	0.68	0.34	1.4	0.7
1,2,3,4,7,8-HexaCDF	0.1	0.37	0.037	1.2	0.12
2,3,4,6,7,8-HexaCDF	0.1	0.033	0.0033	0.16	0.016
1,2,3,6,7,8-HexaCDF	0.1	0.16	0.016	0.23	0.023
1,2,3,7,8,9-HexaCDF	0.1	0.006	0.0006	ND	—
1,2,3,4,6,7,8-HeptaCDF	0.1	0.130	0.0130	0.20	0.020
1,2,3,4,7,8,9-HeptaCDF	0.1	0.038	0.0038	0.011	0.0011
OCDF	0.001	0.2	0.0002	0.076	0.000076
Total			0.6069		1.025176
3,3',4,4',5-PentaCB	0.1	89	8.9	0.73	0.073
3,3',4,4',5,5'-HexaCB	0.05	0.57	0.0285	0.03	0.0031
3,3',4,4'-TetraCB	0.01	8,500	85.0	12	0.12
2,3,3',4,4'-PentaCB	0.001	17,000	17.0	32	0.032
2,3',4,4',5-PentaCB	0.001	28,000	28.0	37	0.037
2,3,3',4,4',5-HexaCB	0.001	1,200	1.2	3.2	0.0032
Total			140.1285		0.2685

Note: ND = not detected.

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rived in Table 18. In Kanechlor 400, the concentrations of "2,3,7,8-TCDD equivalents" for the PCB and PCDF fractions were 140.13 and 0.6069 ppm, respectively, and it was apparent that greater than 99% of the "dioxin-like" activity of the commercial product was associated with the PCB fraction. In contrast, the concentrations of "2,3,7,8-TCDD equivalents" for the PCB and PCDF fractions in the Yusho oil were 0.2683 and 1.025 ppm, respectively. These results demonstrate that 79% of the "dioxin-like" activity in the Yusho oil was associated with the PCDF fraction and these calculations correspond to previous *in vitro* and *in vivo* toxicology studies with Yusho oil fractions in which the PCDFs were shown to be more potent than the PCBs.[352,386] Tanabe and co-worker derived their TEFs for individual PCDF and PCB congeners from their relative potencies as inducers of AHH activity in rat hepatoma H-4-II E cells in culture.[286-288,317] Their results for the analysis of Yusho oil showed that the PCDF fraction contributed 77% of the "dioxin-like" activity of Yusho oil. Thus, the concentrations of "2,3,7,8-TCDD equivalents" in Yusho oil were comparable using the TEFs in Table 18 or the TEF values derived from the AHH induction activities of the individual congeners. Therefore, these data not only confirm the utility of the TEF approach for the hazard assessment of complex mixtures of toxic halogenated aromatics but also show that the *in vitro* AHH induction assay provides a reliable bioanalytical method for quantitating the "dioxin-like" activity of these mixtures.

Eadon and co-workers[375] have investigated the toxicity of a complex mixture of PCDFs (Table 24) which were derived from soot samples formed during the PCB fire in the Binghamton State Office Building. The total concentration of "2,3,7,8-TCDD equivalents" in the soot was 15.371 ppm using the TEF scheme summarized in Table 18; using slightly modified values for the TEFs, Eadon and co-workers[357] estimated that the concentration of 2,3,7,8-TCDD equivalents was 19 ppm. The comparative toxicities of this mixture and 2,3,7,8-TCDD were determined in a series of *in vivo* studies in guinea pigs; the respective calculated "2,3,7,8-TCDD equivalents" concentrations in the soot were 19, 21, 5, 18, 10, and 2 ppm for relative thymus weight decrease, % initial body weight decrease, the increase in serum triglycerides, the decrease in serum ALT, the formation of hepatocellular cytoplasmic inclusion bodies, and mortality (LD_{50}). With the exception of acute toxicity data, it was apparent that there was an excellent overlap between the calculated and observed "2,3,7,8-TCDD equivalents" for these studies. Therefore, these results also validate the use of the TEF approach for 2,3,7,8-TCDD and related toxic halogenated aromatics.

Several groups have utilized the TEF approach to determine the relative contribution of PCB, PCDD, and PCDF residues to the overall "dioxin-like" activity of halogenated aromatic contaminants in environmental and human samples.[375-385] The results shown in Table 25 summarize the average concentrations (parts-per-thousand) of the toxic 2,3,7,8-substituted PCDDs and

Table 24
2,3,7,8-TCDD Equivalents in Soot Samples Derived From the PCB Fire in Binghamton State Office Building[357]

Congener	TEF	Conc. (ppm)	2,3,7,8-TCDD eq. (ppm)
2,3,7,8-TCDD	1.0	0.5	0.50
1,2,3,7,8-PentaCDD	0.5	0.5	0.25
1,2,3,4,7,8-HexaCDD	0.1	0.2	0.02
1,2,3,6,7,8-HexaCDD	0.1	0.2	0.02
1,2,3,7,8,9-HexaCDD	0.1	0.2	0.02
1,2,3,4,6,7,8-HeptaCDD	0.01	0.7	0.007
OCDD	0.001	1.0	0.001
Total		3.3	0.818
2,3,7,8-TCDF	0.1	17	1.7
1,2,3,7,8-PentaCDF	0.1	22	2.2
2,3,4,7,8-PentaCDF	0.5	12	6.0
1,2,3,4,7,8-HexaCDF	0.1	26	2.6
2,3,4,6,7,8-HexaCDF	0.1	1.2	0.12
1,2,3,6,7,8-HexaCDF	0.1	7.7	0.77
1,2,3,7,8,9-HexaCDF	0.1	1.5	0.15
1,2,3,4,6,7,8-HeptaCDF	0.1	10	1.0
1,2,3,4,7,8,9-HeptaCDF	0.1	—	—
OCDF	0.001	13	0.013
Total		110.4	14.553

PCDFs in human adipose tissue from 12 individuals and these levels are similar to those observed in human milk samples.[57-61] The concentrations of "2,3,7,8-TCDD equivalents" in the PCDD and PCDF fractions were 12.01 and 8.38 ppt, respectively. High resolution analysis of these samples for the coplanar PCB congeners showed that the concentration of "2,3,7,8-TCDD equivalents" was 41.0 ppt which was approximately two times higher than the concentration of the PCDDs plus PCDFs. The results of several studies by Tanabe and co-workers have reported that for extracts from diverse fish and wildlife samples the concentration of "2,3,7,8-TCDD equivalents" for the coplanar PCBs is significantly greater than the corresponding values observed for the 2,3,7,8-substituted PCDDs plus PCDFs.[375-384] These data indicate that future research on the potential hazards and risks associated with 2,3,7,8-TCDD and related compounds should not focus exclusively on the PCDDs an PCDFs but should also evaluate the contribution of toxic PCB congeners and other potential toxic halogenated aromatics.

VII. CONCLUSIONS

1. Toxic halogenated aromatics have been identified as complex mixtures in almost every component of the global

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Table 25
2,3,7,8-TCDD Equivalents in Human Adipose Tissue Samples[362]

Congener	TEF	Conc. (ppt)	2,3,7,8-TCDD eq. (ppt)
2,3,7,8-TCDD	1.0	3.7	3.7
1,2,3,7,8-PentaCDD	0.5	6.4	3.2
1,2,3,4,7,8-HexaCDD	0.1	3.9	0.39
1,2,3,6,7,8-HexaCDD	0.1	34	3.4
1,2,3,7,8,9-HexaCDD	0.1	5.7	0.57
1,2,3,4,6,7,8-HeptaCDD	0.01	33	0.33
OCDD	0.001	510	0.510
Total			12.010
2,3,7,8-TCDF	0.1	3.1	0.31
1,2,3,7,8-PentaCDF	0.1	0.5	0.05
2,3,4,7,8-PentaCDF	0.5	11.0	6.5
1,2,3,4,7,8-HexaCDF	0.1	5.6	0.56
2,3,4,6,7,8-HexaCDF	0.1	1.4	0.14
1,2,3,6,7,8-HexaCDF	0.1	5.3	0.53
1,2,3,7,8,9-HexaCDF	0.1	—	—
1,2,3,4,6,7,8-HeptaCDF	0.1	2.9	0.29
1,2,3,4,7,8,9-HeptaCDF	0.1	—	—
OCDF	0.001	—	—
Total			8.38
3,3',4,4',5-PentaCB	0.1	330	33.0
3,3',4,4',5,5'-HexaCB	0.05	90	4.5
3,3',4,4'-TetraCB	0.01	350	3.5
2,3,3',4,4'-PentaCB	0.001		
2,3',4,4',5-PentaCB	0.001		
2,3,3',4,4',5-HexaCB	0.001		
Total			41.0

ecosystem including fish, wildlife, and human adipose tissue, serum, and milk.

2. 2,3,7,8-TCDD and related toxic compounds elicit comparable biochemical and toxic responses in human, laboratory animals, and mammalian cells in culture. There is good evidence that the toxic halogenated aromatic hydrocarbons cause their responses through a common (Ah) receptor-mediated mechanism of action.
3. Not surprisingly, the activity of different halogenated aromatics is structure-dependent and several studies have reported the SARs among the different classes (e.g., PCDDs, PCDFs, and PCBs) of halogenated aromatics.
4. The existence of well-defined SARs for 2,3,7,8-TCDD and related compounds provides the mechanistic basis for the development of TEFs for halogenated aromatics.
5. The TEF values proposed in this review, and other similar TEFs, have been utilized to predict the toxicity of several different halogenated aromatic mixtures. The calculated "2,3,7,8-TCDD equivalents" using TEFs were similar to the observed *in vivo* toxicities of selected halogenated aromatic mixtures and these results validate the use of TEFs in the hazard and risk assessment of toxic halogenated aromatics.

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A NEW REVIEW OF THE DIOXIN LITERATURE

IN THE CONTEXT OF COMPENSATION FOR VIETNAM VETERANS

By Richard W. Clapp and James R. Olson

EARLY LAST YEAR, A TASK FORCE OF environmental scientists produced a report (1) updating and summarizing the scientific literature on the health effects of exposure to phenoxyacetic acid herbicides and/or their associated contaminants (chlorinated dioxins). The work was done at the request of three veterans' organizations — the American Legion, the Vietnam Veterans of America, and the National Veterans Legal Services Project — to be included in the debate in Congress about compensation of Vietnam veterans for service-connected health problems. The review was done primarily to see how much evidence had accumulated that would support a veteran's claim that his or her health problem might be related to these particular chemical exposures. It was done with the same overall goal as had been set for the Department of Veterans Affairs' (formerly the Veterans Administration or VA) own scientific advisory committee, but the task force came to different conclusions and policy recommendations.

The report by the Agent Orange Scientific Task Force was released in April, 1990, and immediately became a part of the consideration of the Congressional Committee on Veterans Affairs in hearings it held on extending compensation to Vietnam veterans suffering from a variety of cancers and other health effects. The Secretary of Veterans Affairs, Edwin Derwinski, previously had recommended extending benefits to Vietnam veterans who had been diagnosed with non-Hodgkin's lymphoma. The hearings involved consideration of this and several other cancers and non-malignant diseases. One of the strongest proponents of extending compensation to other diseases was Admiral Elmo Zumwalt, who had been Chief of Naval Operations in Vietnam. His son had been diagnosed with both Hodgkin's Disease and non-Hodgkin's lymphoma and had died after much publicity about his military service in Vietnam.

THE DEBATE OVER THE EVIDENCE

The debate about the degree of scientific evidence connecting health problems to exposure to phenoxyacetic acid herbicides and/or chlorinated dioxins continues. New information about occupationally exposed workers continues to accumulate. Most recently, a cancer mortality study of 5,172 workers exposed to TCDD found statistically significant excess mortality from all cancers combined, cancers of the respiratory tract, and soft tissue sarcoma, and concluded that the results were consistent with the status of TCDD as a carcinogen. (2) Nevertheless, the paper and pulp industry simultaneously is challenging the dioxin potency index proposed by the Environmental Protection Agency (EPA), and has offered testimony that dioxin effluent standards for paper and pulp mills in Georgia, Maine and several other states are too stringent. The following summary of the *Report of the Task Force on Human Health Effects Associated with Exposure to Herbicides and/or their Associated Contaminants* (1) is offered as an example of how to reach defensible scientific conclusions and policy recommendations, even under conditions of imperfect human epidemiologic evidence and a politically charged environment.

The task force on Agent Orange was established in 1989-'90 by the aforementioned three veterans organizations. The members of the task force were invited to contribute to a summary of the evidence of the health effects of Agent Orange that would be used to support legislation introduced in the U.S. Congress in the Spring of 1990. The primary authors were two individuals with previous experience in scientific studies of Agent Orange or dioxin (Dr. Olson and Dr. Clapp), and other members of the task force and other scientists active in this field were asked to review draft reports and suggest substantive and editorial changes. The work of

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producing the final report was coordinated by the National Veterans Legal Services Project. The full list of members included several other researchers familiar with Agent Orange and its health effects or with other environmental exposures and health effects.

The issue the task force was asked to address is one of the most controversial of contemporary public health issues: the health effects of Agent Orange, specifically its components 2,4,-D, 2,4,5-T (two phenoxyacetic acid herbicides) and the dioxin contaminants. There have been numerous scientific reviews of the voluminous literature on this subject. Some were for regulatory purposes such as establishing allowable environmental contamination levels or conditions under which the herbicides may be used in the U.S. (2,4,5-T was banned more than 10 years ago). Likewise, there have been several reviews of the health effects of Agent Orange exposure in Vietnam veterans, some by government commissions in the U.S. and Australia, which usually found that the evidence was too weak or contradictory to conclude that anything except a serious skin condition called chloracne is caused in humans by exposure to dioxin or Agent Orange.

"The issue the task force was asked to address is one of the most controversial of contemporary public health issues: the health effects of Agent Orange, specifically its components 2,4,-D, 2,4,5-T (two phenoxyacetic acid herbicides) and the dioxin contaminants."

The U.S. Veterans Administration also has conducted reviews of the literature on Agent Orange and has an advisory committee of scientists whose responsibility is on-going review of new evidence. This advisory committee had used the standard in many scientific committees which can be summarized as a "beyond-a-reasonable-doubt" approach to the studies it reviewed. It is an extremely conservative approach, which other contributors to this journal have characterized as "safe but overly critical." (3) The Agent Orange task force, on the other hand, took the approach that there must be some evidence from relevant scientific studies that reached the level of statistical significance widely accepted in the field (usually less than a 5 percent probability that the finding was due simply to chance). But the overall impression of the full range of studies, both those which showed a strong association between exposure to the components of Agent Orange and some health effect and those which did not, should be that the health effect was "more likely than not" or "at least as likely as not" due to the exposure. The latter criteria is important for disability compensation purposes under the VA where a statistically significant association will be deemed to exist if the evidence for and against the association is at least equally balanced. (4)

This approach to interpreting the results of scientific studies in order to make recommendations to policy-makers differs from the traditional approach. It was primarily in response to the general intent of the Congress and the courts to give the benefit of the doubt to the veteran. But it has broader relevance to standard-setting or compensation policies in other areas. Given the difficulty of conducting scientific studies in the human populations specifically affected by some toxic exposure, and the difficulties in extrapolating from animal experiments to humans, it seems humane to give the benefit of the doubt to the victims. The further implication of such an approach is that those in a position to prevent toxic exposures, when some scientific evidence of potential health effects is available, should act early to reduce or avoid such exposures and thereby prevent disease and eventual compensation costs later.

This latest review, completed in the Spring of 1990, was directed primarily at assessing adverse health effects in Vietnam veterans which may be associated with exposure to Agent Orange, a phenoxyacetic acid herbicide which was contaminated with dioxin. (References in this report to "Dioxin," "TCDD," and "2,3,7,8 - TCDD" all refer to 2,3,7,8 - tetrachlorodibenzo - p - dioxin.) The purpose of the new review was to determine the extent of the evidence in the literature in support of veterans' claims of adverse health effects.

This review included all studies and publications reviewed up until 1990 by the Advisory Committee on Environmental Hazards of the Department of Veterans Affairs, and additional pertinent studies not reviewed by the advisory committee. It was aimed primarily at the policy decision of compensation for specific illnesses because they might be service-related, and was not simply another compilation of all the articles in the scientific literature. What follows is a shortened and adapted version of the task force report.

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STANDARD USED BY THE AUTHORS

This literature review focused on epidemiologic studies of exposed humans and used the standard "significant statistical association" because that is the standard used by the VA's advisory committee. The task force did not take this to mean that every study or even the majority of studies had to meet some minimum test of statistical significance such as less than a 5 percent probability that the result was due to chance. Likewise, the task force did not attempt to combine the results from all the reviewed studies into some overall result that could be subjected to the same kind of statistical test. Instead, the task force took into account the aggregate evidence of a variety of different studies showing an association between phenoxyacetic acid herbicide (and/or dioxin) and certain diseases or conditions. The members then weighed the evidence in a subjective way, taking into account the strengths and weaknesses of the individual studies, and made an overall determination of the likelihood that the association was strong enough to be compensable.

There were several reasons for this approach. Most important was that in order for epidemiologic studies to find statistically significant associations between exposure and health effects like cancers, these studies must have sufficient power and sensitivity. This generally requires large numbers of exposed subjects available for study and follow-up for many decades (if not for the lifetime of the subjects). A major problem with the various epidemiologic studies of people exposed to phenoxyacetic acid herbicides is that there have not been many large populations with known exposures available for study and follow-up over long periods, or, where such populations exist, like Vietnam veterans and the Vietnamese, these have not been adequately studied.

PRIMARY CONCLUSIONS OF THE AUTHORS

After review of the scientific literature, the task force concluded there is a significant statistical association between exposure to phenoxyacetic acid herbicides and/or their associated contaminants (chlorinated dioxins) and the following diseases: non-Hodgkin's lymphoma, soft tissue sarcoma, skin disorders/chloracne, subclinical hepatotoxic effects (including secondary coproporphyrinuria and chronic hepatic porphyria), and porphyria cutanea tarda (most likely only in individuals with inherited uroporphyrinogen decarboxylase deficiency). The aggregate interpretation of several sound studies showing a statistically significant association for each of these conditions makes this conclusion inescapable.

Further, the scientific evidence supporting the existence of a significant statistical association between exposure to phenoxyacetic acid herbicides and/or their associated contaminants (chlorinated dioxins) and disease is at least as strong as the scientific evidence of a lack of the association for the following adverse health effects: Hodgkin's disease, neurologic effects, and reproductive and developmental effects. For each of these health effects, there are sound scientific studies showing statistically significant evidence of an association between exposure and effect. However, the association is not as consistent for these effects in the various studies as for the first group of health effects.

There are other adverse health effects for which there is sound scientific evidence of an association with exposure, but the evidence does not reach the level of formal statistical significance. Were the task force a policy-making body concerned with disability compensation for veterans, it might conclude that compensation was due. However, the limited data available at this time show an association, but not a significant statistical association, between exposure to phenoxyacetic acid herbicides and/or dioxin and the following diseases: leukemias, cancers of the kidney, testis, pancreas, stomach, prostate, colon, hepatobiliary tract, and brain, psychosocial effects, immunological abnormalities, gastrointestinal ulcer, and altered lipid metabolism. Further research should be directed at these associations, and policy-makers, cognizant of the limitations on finding significant statistical associations and of the obligation to give the veteran the benefit of the doubt, would be justified in viewing these disorders in a more conclusive light for the purpose of establishing entitlement to disability compensation.

Finally, the epidemiologic evidence on the associations between exposure and the adverse health effects described above is strongly supported by a wide range of experimental animal studies. (5)

Evidence in support of the above conclusions is found in the summary statements which follow.

CANCER

After review of the scientific literature, it is concluded that there is a significant statistical association between exposure to phenoxyacetic acid herbicides and/or their associated contaminants (chlorinated dioxins) and non-

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TABLE 1 - SELECTED EPIDEMIOLOGIC STUDIES: SOFT TISSUE SARCOMA

AUTHORS (Year, Reference)	TYPE	NO. CASES OR COHORT SIZE	EXPOSURE	RR EST.	COMMENTS
Hardell & Sandstrom (1979) (a)	c-c	52	Phenoxy Herbicides	5.3*	Original study
Eriksson, et al. (1981) (b)	c-c	110	Phenoxy Herbicides	6.8*	
Smith, et al (1984) (c)	c-c	82	Phenoxy Herbicides	1.3	
Lynge (1985) (d)	cohort	4,459	Phenoxy Herbicides	3.96*	
Coggan, et al. (1986) (e)	cohort	5,754	Phenoxy Herbicides	1.04	
Wiklund & Holm (1986) (f)	cohort	354,620	Mixture	0.9	
Holmes, et al. (1986) (g)	PMR	41,059	Vietnam Agent Orange	4.29*	PMR study of 615 deaths
Hoar, et al. (1986) (h)	c-c	200	Mostly 2, 4-D	1.4	No dioxin Contamination
Kang, et al. (1986) (i)	c-c	234	Vietnam Agent Orange	0.83	VA Hospital Cases
Woods, et al. (1987) (j)	c-c	128	Phenoxy Herbicides	0.8	
Vineis, et al. (1987) (k)	c-c	68	Phenoxy Herbicides	2.7*	Women only
Kogan & Clapp (1988) (l)	c-c	10	Vietnam Agent Orange	5.16*	SMOR within PMR Study
Eriksson, et al (1990) (m)	c-c	237	Phenoxy Herbicides	1.80*	Specific for Dioxin
CDC (1990) (n)	c-c	346	Vietnam Agent Orange	1.00	1.6 in I Corps
Wingren et al. (1990) (o)	c-c	96	Phenoxy Herbicides	1.6	
Clapp, et al. (in press) (p)	c-c	17	Vietnam Agent Orange	3.08*	C-C linkage study
Fingerhut et al. (1991) (q)	cohort	5,172	Phenoxy	3.38	9.22* for those employed ≥ 1 year & 20 yrs. latency

* Statistically Significant ($p<.05$)
c-c means case control study
PMR means Proportionate Mortality Ratio
SMOR means Standardized Mortality Odds Ratio
NHL means Non-Hodgkin's Lymphoma

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TABLE 2 - SELECTED EPIDEMIOLOGIC STUDIES: NON-HODGKIN'S LYMPHOMA

AUTHORS (Year, Reference)	TYPE	NO. CASES OR COHORT SIZE	EXPOSURE	RR EST.	COMMENTS
Hardell (1981) (r)	c-c	169	Phenoxy Herbicides	4.8*	
Burmeister, et al. (1983) (s)	cohort	6,402	Mixture	1.29	
Pearce, et al. (1986) (t)	c-c	83	Chlorophenol	1.4	
Hoar, et al. (1986) (h)	c-c	200	2,4-D Primarily	1.6	6.0* for those spraying >20 days/yr.
Woods, et al. (1987) (j)	c-c	576	Phenoxy Herbicides	1.07	1.71* for those employed 15 yrs. & 15 yrs. latency
Bond, et al. (1988) (u)	cohort	878	Phenoxy Herbicides	3.1*	NHL in a grouped Category
Breslin, et al. (1988) (v)	PMR	24,235 (deaths)	Vietnam Agent Orange	2.10*	Marines only
CDC (1990) (n)	c-c	1,157	Vietnam Agent Orange	1.47*	2.25* in I Corps
Zahm, et al. (1990) (w)	c-c	201	2,4-D Primarily	1.5	3.3 in those spraying >21 days/year
Wigle, et al. (1990) (x)	cohort	69,513	Mixture	0.92	1.34 in those on farms with 250+ acres sprayed

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Hodgkin's lymphoma and soft tissue sarcoma, and that the evidence supporting the existence of such an association is at least as strong as the evidence of a lack of the association for Hodgkin's disease. A representative group of the studies which were reviewed in order to reach this conclusion is summarized in the accompanying tables.

The Centers for Disease Control (CDC) Selected Cancers Study (6), released in April, 1990, found that veterans who served in Vietnam had a significantly increased risk of non-Hodgkin's lymphoma over veterans who served elsewhere during the same period. This study actually may add support to the evidence that exposures in Vietnam caused increased risk of non-Hodgkin's lymphoma, even though its authors deny that their data support the contention that Agent Orange was the cause. Although the study did not focus on any direct measure of Agent Orange exposure, and the proposed blood sampling to determine dioxin levels in patients was never carried out, the authors made conclusions concerning Agent Orange exposure.

First, the CDC authors contend that Vietnam veterans who served in III Corps were most likely to be exposed to Agent Orange, and the results of their study indicate that they were at decreased risk of non-Hodgkin's lymphoma. Second, the CDC study found excessive risk of non-Hodgkin's lymphoma in "blue-water" Navy veterans whom the authors presume had no exposure to Agent Orange. Finally, the CDC authors state that the responses to questions about spraying, handling or being around Agent Orange were similar among veterans with non-Hodgkin's lymphoma and veterans with no cancer of any type.

Critics have challenged these CDC interpretations of the data because the study did not contain any direct measure of exposure to Agent Orange. In addition, some indirect exposure criteria not emphasized by CDC do lead to the conclusion of adverse health effects in exposed veterans. For example, one of the heavily sprayed regions in Vietnam was I Corps. If the CDC data on veterans in I Corps and III Corps are taken together, they show an increased risk of both non-Hodgkin's lymphoma and soft tissue sarcoma. These two cancers have been found elevated in other studies of veterans done by state health departments in Massachusetts (7), West Virginia (8) and Wisconsin (9). At a minimum, the CDC findings should be put in the context of these other studies of veterans, as well as studies of agricultural workers exposed to herbicides, several of which are summarized in the tables.

> ***"There are other adverse health effects for which there is sound scientific evidence of an association with exposure, but the evidence does not reach the level of formal statistial significance. Were the task force a policy-making body concerned with disability compensation for veterans, it might conclude that compensation was due."***

The CDC Selected Cancers Study and other proposed studies of Agent Orange-exposed veterans were the subject of a Congressional hearing on July 11, 1989. This and subsequent hearings on the CDC dioxin studies were summarized in a strongly worded report by the Human Resources and Intergovernmental Relations Subcommittee of the Committee on Government Operations, House of Representatives (August 9, 1990). (10)

Other conditions also were found to be associated with exposure to phenoxyacetic acid herbicides. A full discussion of the evidence for these associations is found in the report of the task force and is not presented here. Copies are available from the National Veterans Legal Services Project.

CONCLUSIONS

This review of the scientific literature led the task force to the following conclusions:

1. There is a significant statistical association between exposure to phenoxyacetic acid herbicides and/or their associated contaminants (chlorinated dioxins) and non-Hodgkin's lymphoma, soft tissue sarcoma, skin disorders/chloracne, subclinical hepatotoxic effects (including secondary coproporphyrinuria and chronic hepatic porphyria), and porphyria cutanea tarda (most likely only in individuals with inherited uroporphyrinogen decarboxylase deficiency). The aggregate interpretation of several sound studies showing a statistically significant association for each of these conditions makes this conclusion inescapable.

2. The scientific evidence supporting the existence of a significant statistical association between exposure to phenoxyacetic acid herbicides and/or their associated

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contaminants (chlorinated dioxins) is at least as strong as the scientific evidence of a lack of the association for the following adverse health effects: Hodgkin's disease, neurologic effects, and reproductive and developmental effects. For each of these health effects, there are sound scientific studies showing statistically significant evidence of an association between exposure and effect. However, a statistically significant association is not as consistently supported for these effects as for the first group of health effects.

3. The above eight adverse health effects satisfy the VA's test for an association which qualifies for disability compensation, that is, where the scientific evidence shows that a significant statistical association is "at least as likely as not." (4).

DISCUSSION

The conclusions of the Agent Orange Scientific Task Force were different from the VA Advisory Committee and other scientific reviews such as one produced for the pulp and paper industry. (11) The task force conducted a comprehensive review of epidemiological data available as of early 1990 which included some pertinent peer-reviewed publications not reviewed at that time by the other two bodies. Furthermore, the review was focused on the issue of identifying possible health problems for which Vietnam veterans may receive compensation and used an approach which varied from that used by the VA Advisory Committee.

The task force conclusions, if they were implemented as policy, also would have significant economic implications for the affected veterans and their families. The actual number of veterans with the diseases or conditions listed in conclusion 1, above, cannot be estimated with precision, but the overall cost to the U.S. Treasury would be relatively modest by comparison to the total amount of veterans benefits paid annually.

Since the task force report was submitted to Congress in May, 1990, the Secretary of Veterans Affairs has recommended that soft tissue sarcoma in Vietnam veterans be considered a service-related condition. Earlier, he had recommended compensating Vietnam veterans who had non-Hodgkin's lymphoma, saying that it was a decision not based on science but as a matter of public policy. The implications of these two policy decisions are that there may be a shift toward giving the benefit of the doubt in areas of scientific controversy to the victims who have these two cancers. If this is so, then the Agent Orange Scientific Task Force may have played a role in justifying a new approach to such policy decisions. ▲

EDITOR'S NOTE: On January 29, 1991, the U.S. Congress passed H.R. 556, the Agent Orange Act of 1991. It provides for compensating Vietnam veterans diagnosed with non-Hodgkin's lymphoma, soft tissue sarcoma, and chloracne. It was passed by the Senate and signed by President Bush in the following two days.

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Calculation of 2,3,7,8-TCDD Equivalent Concentrations of Complex Environmental Contaminant Mixtures

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Sufficient toxicological data are now available to permit use of conventional risk assessment techniques to estimate the hazards associated with human exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). However, many real-world exposures involve complex mixtures of dibenzodioxins, dibenzofurans, and related compounds. Historical approaches to risk assessment on such mixtures have ranged from ignoring all compounds except 2,3,7,8-TCDD itself to assuming that all compounds have potencies equal to 2,3,7,8-TCDD. An alternative approach which uses existing literature data and analytical results to calculate the "2,3,7,8-TCDD equivalent" concentration of a mixture in order to "predict" its biological potency relative to 2,3,7,8-TCDD itself is advanced here. Previously reported *in vivo* acute and subchronic studies and some recently obtained analytical chemistry data are integrated here to clarify the utility of this important approach and to assess the uncertainties associated with its use. This predictive approach, and various conceptually similar ones, have now found wide applicability to the risk assessment process associated with exposure to complex mixtures of dioxins, dibenzofurans, and related compounds.

Introduction

Sufficient toxicological data are now available to permit use of conventional risk assessment techniques to estimate the hazards associated with human exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) (*1*). In a few incidents, 2,3,7,8-TCDD has been the only congener present at toxicologically significant concentrations. More commonly, however, real-world exposures involve complex mixtures of compounds with dioxin-like activity containing up to 75 different chlorinated dibenzo-*p*-dioxin (PCDD), 135 chlorinated dibenzofuran (PCDF), and 75 chlorinated biphenylene (PCBE) isomers and congeners. For example, commercial polychlorinated biphenyls (PCBs) (*2*), emissions from garbage-burning resource recovery plants (*3*), and chemical waste incinerators (*4*), foodstuffs (*5*), human milk (*6*), and pyrolyzed PCBs or chlorobenzenes (*7,8*) all consist of complex mixtures in which 2,3,7,8-TCDD is a minor component relative to the total PCDD, PCDF, and PCBE concentration.

Such mixtures present a difficult problem for estimating human health hazards for at least three reasons. First, analytical chemistry has not yet progressed to the point that reliable isomer-specific quantitation or even qualitative identification is possible for the full range of dioxin-like compounds. For example, the unlabeled standards needed both for qualitative identification and for determination of response factors are not generally available. In addition, only a very limited set of ^{13}C- or ^{37}Cl-labeled internal standards, which are necessary to correct for run-to-run variations in recovery, have been prepared. Even modern high resolution capillary gas chromatographic techniques do not permit resolution of all possible congeners. Second, insufficient toxicological data are available to permit a rigorous risk assessment for most congeners. For example, two-year carcinogenesis data are available only for 2,3,7,8-TCDD itself, for 2,7-dichlorodibenzodioxin, and for a mixture of two 2,3,7,8-substituted hexachlorodibenzodioxins (hexaCDDs) (*9*). Single oral dose guinea pig LD_{50} values have been reported for 16 different dioxins and five different furans (*10*). Fewer than 25 of the 75 possible dioxin and 20 of the 135 possible furan congeners have been subjected to *in vitro* determination of biological activity (*10*). Finally, there exists a third confounding factor. The biological potency of the mixture may not

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simply equal the summation of the activities of the individual congeners present, if synergistic of antagonistic interactions among the mixture's components are significant.

On February 5, 1981, a fire involving a transformer containing 60% Aroclor 1254 and 40% tri- and tetrachlorobenzenes extensively dispersed a fine oily soot throughout a 17 story office building in Binghamton, New York. Chemical analysis demonstrated the presence of substantial concentrations of PCDDs, PCDFs and PCBPs in the soot and on various surfaces (*11–13*). The necessity of establishing reoccupancy criteria for the building focused attention on the need to estimate the biological potency of this complex mixture. Two conceptually opposed methods can be discerned in pre-1981 governmental approaches to this problem. The commonly used early approach (probably encouraged by lack of analytical chemistry data) was explicitly or implicitly to ignore all compounds other than 2,3,7,8-TCDD itself. Alternatively, all compounds detected were assumed equal in potency to 2,3,7,8-TCDD (*14*). Either approach ignores substantial biological evidence suggesting that the potencies of particular congeners vary widely, but are, in some cases, negligible when compared to 2,3,7,8-TCDD. Any realistic attempt to estimate a mixture's biological potency must therefore take into account the relative potencies of its individual components.

The approach eventually developed to estimate the hazard associated with exposure to the Binghamton mixture is a fairly general one. Published short-term biological data are used to estimate the ratio of the potency of a particular congener relative to 2,3,7,8-TCDD. Then, the observed or estimated concentration of that congener is multiplied by this ratio to estimate the "2,3,7,8-TCDD equivalent" concentration of that congener. This 2,3,7,8-TCDD-equivalent concentration of a particular congener would thus be equated with a hypothetical concentration of 2,3,7,8-TCDD capable of producing the same biological effect as the actual concentration of that congener. The overall biological potency of the mixture could then be estimated by summing the TCDD-equivalent concentrations of all congeners present. Such an approach is only applicable when the components of the mixture exhibit biological effects similar to those of 2,3,7,8-TCDD.

When first advanced (*15*), the TCDD-equivalent approach was novel; more recently, conceptually similar approaches have been used in assessing other PCB-related incidents, e.g., the One Market Plaza incident in San Francisco (*16*), and in assessing other sources of environmental exposure, e.g., emissions from resource recovery plants (*17*), and these have gained increasing acceptance as general solutions to the PCDD/PCDF mixture problem (*18*). However, it must be emphasized that the only direct experimental support for this approach rests on work performed in connection with the Binghamton incident. Here alone was sufficient contaminated material (i.e., "soot") and appropriate laboratory resources available to allow *in vivo* comparisons of an environmental mixture's biological potency relative to 2,3,7,8-TCDD itself, and thus verification of the TCDD-equivalents calculation. The intent of this paper is to present an integrated picture of the previously reported biological experiments (Table 1) and some recently obtained analytical chemistry data (Table 2) to clarify the utility of this important approach.

Table 1. Influence of structure and chlorination pattern on guinea pig oral LD_{50} (male, Hartley, 200–250 g).

Compound	LD_{50}, μg/kg	Ratio LD_{50} compound / LD_{50} 2,3,7,8-TCDD
2,3,7,8-TetraCDD	2.5[a]	1
2,3,7,8-TetraCDF	5–10[b]	2–4
2,3,4,7,8-PentaCDF	<10[c]	<4
1,2,3,7,8-PentaCDD	3.1[c]	1.2
1,2,3,4,7,8-HexaCDD	73[c]	29
1,2,3,7,8,9-HexaCDD	60–100[c]	24–40
1,2,3,7,8-HexaCDD	70–100[c]	28–40
2,3,4,6,7,8-HexaCDF	120[e]	48
1,2,3,4,6,7,8-HeptaCDD	<600[c]	<240
1,2,4,7,8-PentaCDD	1,125[c]	450
2,3,7-TriCDD	29,444[c]	1,200
2,8-DiCDD	730,000[c]	300,000
1,3,6,8-TetraCDD	>15,000,000[d]	600,000

[a] Data of Silkworth et al. (*19*).
[b] Data of Moore et al. (*20*).
[c] Data of McConnell et al. (*21*).
[d] Data of Kawamura et al. (*22*).
[e] Data of McKinney et al. (*23*).

Calculation of TCDD Equivalents from Chemical Data

Chemical data can be used to estimate the 2,3,7,8-TCDD equivalent concentration present in a mixture, provided that the concentrations of the relevant compounds are known and that their biological activities can be estimated. A biological end point of special concern for these compounds is carcinogenesis (*1*); unfortunately, only five dibenzodioxins and no dibenzofurans have been the subject of lifetime animal bioassays (*10*). Thus, short-term bioassay data, which are substantially more numerous, must be utilized as the basis of the equivalence calculation. The end point used in the present study to estimate relative congener potency is the single-dose oral LD_{50} in the guinea pig. This is advantageous, since the LD_{50} of the mixture in question (soot) has been experimentally determined (*24*), thus allowing direct verification of the calculation. However, since there is good correlation among various short-term assays for these compounds (*25–29*) any differences between results from particular short-term assays should have only minor effects on the calculation (*18*).

The issue of the relevance of short-term exposure data to carcinogenic potency is less clear. However, the very limited experimental data now available supports the concept that short- and long-term effects are roughly proportional. Thus, a mixture of two 2,3,7,8-substituted hexaCDDs exhibited carcinogenic potency

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Table 2. Concentrations of chlorinated dibenzo-*p*-dioxins and dibenzofurans in the Binghamton soot sample used in animal toxicology experiments.[a]

Compound	Concentration, ppm
Total diCDD	0.5
Total triCDD	0.5
Total tetraCDD	1.5
2,3,7,8-tetraCDD	0.5
Total PeCDD	1.4
1,2,3,7,8-PeCDD[b]	0.5
Total HxCDD	1.2
1,2,3,4,7,8-HxCDD	0.2
1,2,3,6,7,8-HxCDD	0.2
1,2,3,7,8,9-HxCDD	0.2
Total HpCDD	1.4
1,2,3,4,6,7,8-HpCDD	0.7
Total OCDD	1
Total diCDF	5.9
Total triCDF	27
Total tetraCDF	120
2,3,7,8-tetraCDF	17
Total PeCDF	160
1,2,3,7,8-PeCDF	22
2,3,4,7,8-PeCDF	12
Total HxCDF	64
1,2,3,4,7,8-HxCDF	26
2,3,4,6,7,8-HxCDF	1.2
1,2,3,6,7,8-HxCDF	7.7
1,2,3,7,8,9-HxCDF	1.5
Total HpCDF	26
1,2,3,4,6,7,8-HxCDF	10
Total OCDF	13

[a] Unless otherwise indicated, concentrations listed are based on a recent redetermination of the PCDD/PCDF constituents in the soot using contemporary analytical methodology. This redetermination is considered more reliable than that previously reported (*32*), particularly in view of recent advances in the availability of labeled and unlabeled standards and chromatography.
[b] Estimated from data of Silkworth et al. (*32*).

estimated to be 0.04 times that of 2,3,7,8-TCDD (*18*); the ratio of corresponding guinea pig acute oral LD_{50} values is 0.03. Similarly, in two-year studies of unsubstituted dibenzodioxin (*30*) and 2,7-dichlorodibenzodioxin (*31*), doses up to 10,000 ppm in the diet elicited no carcinogenic response in either species. Unchlorinated and dichlorinated dibenzodioxins are essentially inactive relative to 2,3,7,8-TCDD in a battery of short-term bioassays (*10*).

Table 1 presents the currently available data on guinea pig acute oral LD_{50} values for dibenzodioxins and dibenzofurans. Use of this data set to predict the 2,3,7,8-TCDD equivalent concentration in a mixture (such as the Binghamton soot) is complicated by certain limitations, particularly the absence of any data on PCDFs other than 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and 2,3,4,6,7,8-HxCDF. The following assumptions will therefore be made about LD_{50} values not yet determined.

(1) The ratio of the LD_{50} values of a particular PCDF congener and 2,3,7,8-TCDF will be the same as the ratio of the LD_{50} values of the correspondingly substituted PCDD congener and 2,3,7,8-TCDD. There is no direct experimental data to support this assumption.

(2) The LD_{50} values of PCDFs and PCDDs lacking chlorines at any of the four lateral positions will be sufficiently high that their influence can be ignored for most environmental mixtures. This assumption is based on the guinea pig LD_{50} values of 2,8-diCDD, 2,3,7-triCDD, and 1,3,6,8-tetraCDD and 1,2,4,7,8-pentaCDD. All have LD_{50} values more than 450 times higher than 2,3,7,8-TCDD itself (Table 1). This simplification may be invalid for mixtures containing only very small percentages of 2,3,7,8-substituted isomers.

(3) Introduction of a single additional chlorine substituent on a 2,3,7,8-substituted congener has essentially no effect on the congener's guinea pig LD_{50}. This assumption is based on comparison of the LD_{50} values of 2,3,7,8-TCDD versus 1,2,3,7,8-pentaCDD, and 2,3,7,8-TCDF versus 2,3,4,7,8-pentaCDF (Table 1).

(4) Introduction of two additional chlorine substituents on a 2,3,7,8-chlorinated congener raises its LD_{50} by a factor of 30. This assumption is based on comparison of the LD_{50} values of 1,2,3,4,7,8-, 1,2,3,6,7,8-, and 1,2,3,7,8,9-hexaCDD versus 2,3,7,8-TCDD (Table 1).

(5) The LD_{50} values of compounds with more than six chlorines will be sufficiently high that their influence can be ignored for most environmental mixtures. This assumption is based on comparison of the LD_{50} values of 1,2,3,4,6,7,8-heptaCDD and 2,3,7,8-TCDD (Table 1).

These assumptions require that attention be focussed only on 2,3,7,8-substituted PCDDs and PCDFs. The concentration of 2,3,7,8-TCDF in a bulk soot sample used in the animal toxicology experiments described later has recently been measured at 17.1 ppm (Table 2); since the data in Table 1 indicate that the LD_{50} value of 2,3,7,8-TCDF is about three times that of 2,3,7,8-TCDD, this is equivalent in terms of acute toxicity to a 2,3,7,8-TCDD concentration of about 6 ppm. Based on assumption (2), other tetraCDFs can be neglected in this calculation. The two pentaCDF isomers with 2,3,7,8 substitution together total 34 ppm. If, as is required by assumption (3) and as is consistent with experimental data for 2,3,4,7,8-pentaCDF, the toxicity of 2,3,7,8-substituted pentaCDFs is considered equal to that of 2,3,7,8-TCDF, this concentration corresponds to a 2,3,7,8-TCDD equivalent concentration of about 11 μg/g. The hexaCDFs were measured at 64 μg/g. However, based on assumption (2), only 2,3,7,8-substituted congeners (1,2,3,4,7,8-, 1,2,3,6,7,8-, and 2,3,4,6,7,8-hexaCDF) need be considered. Table 1 suggests that 2,3,4,6,7,8-hexaCDF is about 16 times less potent than 2,3,7,8-TCDF itself. Thus, the sum of observed 2,3,7,8-hexaCDF concentrations (36 μg/g) corresponds to a 2,3,7,8-TCDD equivalent concentration of about 1 ppm (Table 3). Hepta and octaCDFs have insufficient concentrations and biological potencies to contribute to this calculation. The concentrations of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD have each been measured at 0.5 μg/g, corresponding to a "2,3,7,8-TCDD" equivalent concentration of 1 ppm. The concentration of hexaCDDs (1.2 μg/g), when adjusted for biological potency by dividing by 30, makes a negligible contribution to the calculation. Similarly, hepta and octaCDDs can be ignored.

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Table 3. Calculation of 2,3,7,8-TCDD equivalents due to various chlorinated dibenzofurans, dibenzodioxins, and biphenylenes in Binghamton soot using chemical data.[a]

C Concentration	A Relative activity compound class vs. dibenzodioxins	B Relative activity due to chlorine substitution	2,3,7,8-TCDD equivalents, ppm
2,3,7,8-TCDF/17.1 ppm	1/3	1	6
1,2,3,7,8- and 2,3,4,7,8-PeCDF/33.8 ppm	1/3	1	11
1,2,3,4,7,8-, 2,3,4,6,7,8-, 1,2,3,6,7,8-, and 1,2,3,7,8,9-hexaCDFs/36 ppm	1/3	1/16	1
2,3,7,8-TCDD/0.5 ppm	1	1	0.5
1,2,3,7,8-PeCDD/0.5 ppm	1	1	0.5
2,3,6,7-TCPB/1.1 ppm	1	1	1
1,2,3,6,7-PeCPB/2.3 ppm	1	1	2

[a] 2,3,7,8-TCDD calculated as the product $C \times A \times B$.

Together, the dibenzodioxins and dibenzofurans account for about 19 μg/g "2,3,7,8-TCDD equivalents." Stalling's demonstration (*12*) that the soot contains a substantial concentration of PCBEs complicates the calculation substantially. The only data available relevant to the biological potency of these derivatives are measurements of the cytosolic receptor binding affinity and the cell keratinization activity of 2,3,6,7-tetraCBE (analog of 2,3,7,8-TCDD) (*33*). These results suggest that 2,3,6,7-tetraCBE may have a biological potency similar to that of its structural analog, 2,3,7,8-TCDD. Unfortunately, nothing is known about the rate of metabolic detoxification of these compounds. However, in the absence of more rigorous data, it will be assumed that biphenylenes exhibit the same biological potency as the corresponding dibenzodioxins, and three times the potency of the corresponding dibenzofurans.

Even today, quantitative determination of the PCBE concentration in the soot is complicated by the absence of any reliable labeled or unlabeled biphenylene standards. As a crude estimate of their concentration, Stalling's observation that the PCBEs are present at approximately 1/15 the concentration of the PCDFs can be used. This factor, coupled with the foregoing assumption that their biological potency is three times that of the dibenzofurans implies a contribution of about 3.4 ppm from the biphenylenes. Thus, the best estimate of the overall potency of the mixture of PCDDs, PCDFs and PCBEs is ca. 22 ppm (Table 3).

Calculation of 2,3,7,8-TCDD Equivalents from Biological Data

The best test of the various assumptions underlying the calculation of TCDD equivalents from chemical data is to perform an empirical determination of the mixture's biological potency versus 2,3,7,8-TCDD itself. Since the estimate of biological potency was based on acute guinea pig oral LD_{50} data, the most direct point of comparison requires experimental measurement of this parameter for the mixture. Female guinea pigs were administered a single oral dose of a benzene extract of the soot equivalent to 4, 20, 100, 500, or 1000 mg of soot/kg (*24*). The soot extract's LD_{50} was determined to be 327 mg/kg, based on a 42-day observation period and calculated by a modification of the method of Bliss (*34*). For purposes of comparison, female guinea pigs were similarly given single oral doses of 0.1, 0.5, 2.5, 12.5, or 20 μg/kg 2,3,7,8-TCDD (*24*). The corresponding calculated LD_{50} value for 2,3,7,8-TCDD was 19 μg/kg in an aqueous vehicle. Thus, by using the relationship (1),

$$\text{2,3,7,8-TCDD-equivalent concentration } (\mu g/g) = \frac{LD_{50} \text{ for 2,3,7,8-TCDD } (\mu g/g)}{LD_{50} \text{ for soot (mg/kg)}} \quad (1)$$

it can be calculated that the soot has a 2,3,7,8-TCDD equivalent concentration of (19 μg/kg)/(327 × 10^3) μg or 58 ppm.

A more demanding test of the TCDD-equivalent concept is to calculate the TCDD equivalent concentration of the soot based on a variety of exposure end points induced subchronically. Thus, Binghamton soot was incorporated into feed at concentrations of 0, 0.2, 1.9, 9.3, 46.3, and 231.5 ppm and fed to guinea pigs for 90 days (*34*). In another study, 2,3,7,8-TCDD was incorporated into feed at concentrations of 0, 2, 10, 76, and 431 ppt and similarly fed to guinea pigs for purposes of comparison (*35*). End points chosen were those in which significant, dose-related differences from control values were observed after subchronic exposure in both studies. These endpoints included relative (to body) thymus weights (males), percent of initial body weight (males), triglyceride levels (males) serum ALT levels (females), and incidence of hepatocellular cytoplasmic inclusion bodies (females). For the continuous data parameters, linear regression analysis was performed to obtain the best straight-line fit for exposure (expressed as ppt 2,3,7,8-TCDD or log ppt 2,3,7,8-TCDD in the feed) versus response using data from the 2, 10, and 76 ppt dose groups in the present study. Correlation coefficients were obtained for each line. Response data were normalized by expressing each value as a percentage of the corresponding control value. This was necessary to correct for small variations between the control values in each study.

Response data for a particular dose level in the Binghamton soot study were then compared to the standard curves generated above to obtain a hypothetical 2,3,7,8-TCDD concentration in the feed which would have been expected to produce the same degree of response. The "2,3,7,8-TCDD equivalent" concentration of the soot was then calculated by using Equation (2). The particular dose levels of Binghamton soot chosen for this comparison were those in which the degree of response fell

within the limits of the corresponding standard curve (1.9 ppm soot level was used).

$$\text{2,3,7,8-TCDD-equivalent concentration } (\mu g/g) = \frac{\text{hypothetical 2,3,7,8-TCDD concentration in feed (pg/g)}}{\text{concentration of Binghamton soot in feed } (\mu g/g)} \quad (2)$$

A different method was employed for analysis of the quantal data obtained for the incidence of hepatocellular cytoplasmic inclusion bodies. In this case, use was made of the ED_{50} (dose level in feed expected to produce a 50% incidence) in order to facilitate comparison of pure 2,3,7,8-TCDD with the Binghamton soot. The ED_{50} following 90-day exposure to the soot was calculated using previously reported incidence data (*39*) by a modification of the method of Bliss (*36*). A corresponding ED_{50} for subchronic 2,3,7,8-TCDD exposure could not be calculated from results of the present study since a range of incidences was not obtained. For purposes of comparison, 76 ppt was assumed to be the ED_{50} dose level for 2,3,7,8-TCDD, since 50% of female animals at this dose level exhibited inclusions at a severity grade of +1 or greater as previously defined (*34*,*35*). The 2,3,7,8-TCDD equivalent concentration (ppm) of the soot was then calculated as:

$$\text{2,3,7,8-TCDD-equivalent concentration } (\mu g/g) = \frac{ED_{50} \text{ for 2,3,7,8-TCDD (ppt)}}{ED_{50} \text{ for soot (ppm)}} \quad (3)$$

Finally, an effective LD_{50} value for prolonged 2,3,7,8-TCDD exposure was similarly derived by using total dose versus mortality data from a recovery experiment in the previous investigation (*34*). This was compared with corresponding calculations from the soot study to yield an equivalent concentration (ppm) by using Equation 1.

Table 4 summarizes the results of calculations of the 2,3,7,8-TCDD equivalent concentration of Binghamton soot based on dose-response data from previous investigations (*34*,*35*). Excellent dose-response correlations ($r > 0.950$) were obtained for the continuous data endpoints (relative thymus weight, % of initial body weight, serum triglycerides, and serum ALT) after 2,3,7,8-TCDD exposure. Log transformation of the 2,3,7,8-TCDD feed concentrations was required for a good fit only in the case of serum ALT levels. Calculated "2,3,7,8-TCDD-equivalent" concentrations for the Binghamton soot were in good relative agreement for three of the four end points, with values ranging from 18 to 21 ppm (μg 2,3,7,8-TCDD/g soot). Calculations based on serum triglyceride levels resulted in a somewhat lower value of 5 ppm.

Data on the incidence of hepatocellular cytoplasmic inclusion bodies in female guinea pigs fed Binghamton soot were employed to determine a value of 7.6 ppm (2.7 to 21.6 ppm, 95% confidence range) as the ED_{50} dose level of soot for this lesion. Using a value of 76 ppt for the ED_{50} dose level following exposure to pure 2,3,7,8-TCDD, the "2,3,7,8-TCDD-equivalent" concentration of the soot was determined to be 10 ppm (4 to 28 ppm, 95% confidence limits). A similar approach was employed for calculation of an effective LD_{50} for soot exposure, except that total soot consumption was used instead of soot concentration in the feed. This LD_{50} dose was determined to be 383 mg soot/kg (186 to 790 mg/kg, 95% confidence range). Comparison with the effective LD_{50} of 0.8 μg 2,3,7,8-TCDD/kg (0.6 to 1.1 μg/kg, 95% confidence limits) yielded a value of 2 ppm (1 to 3 ppm, 95% confidence limits) for the 2,3,7,8-TCDD-equivalent concentration of the Binghamton soot.

Table 4. Calculated 2,3,7,8-TCDD-equivalent concentrations of the Binghamton soot for various dose-related endpoints following subchronic exposure.[a]

End point	Sex	Method[b]	r^c	2,3,7,8-TCDD equivalent concentration in soot, ppm
Relative thymus weight (decrease)	M	Linear	0.951	19[d]
% of initial body weight (decrease)	M	Linear	0.994	21[d]
Serum triglycerides (increase)	M	Linear	0.998	5[d]
Serum ALT (decrease)	F	Log	0.960	18[e]
Hepatocellular cytoplasmic inclusion bodies	F	ED_{50}	—	10 (4–28)[f]
Mortality	M	LD_{50}	—	2 (1–3)[f]

[a] Response data from the previously reported Binghamton soot (*34*), and 2,3,7,8-TCDD (*35*) subchronic studies 2,3,7,8-TCDD-equivalent concentrations for the soot.

[b] Method by which 2,3,7,8-TCDD dose-response standard curve was constructed: Linear = linear regression analysis of dose level (ppt) vs. response; LOG = linear regression analysis of log dose level (ppt) vs. response; ED_{50} = calculation of ED_{50} dose level by method of Carmines et al. (*36*); LD_{50} = calculation of LD_{50} for subchronic exposure by method of Carmines et al. (*36*).

[c] Correlation coefficient from linear regression analysis.

[d] Calculated using response data from 1.9 ppm Binghamton soot dose level.

[e] Calculated using response data from 3.9 ppm Binghamton soot dose level.

[f] 95% confidence limits.

Comparison of Chemical and Biological Calculations

The most direct test of the assumptions underlying the calculation of 2,3,7,8-TCDD equivalents from chemical data and published acute oral LD_{50} values is comparison with the biological calculation based on the acute oral LD_{50} of the mixture and of 2,3,7,8-TCDD itself. The chemical data-based 2,3,7,8-TCDD equivalents estimate of 22 ppm is in reasonable agreement with the experimentally determined value of 58 ppm.

From a practical perspective, however, the predictions of magnitude of acute toxicity expressed by a mixture is of less interest than prediction of the mixture's subchronic and chronic toxicity. The six subchronic endpoints listed in Table 4, although not necessarily representing adverse effects of great clinical importance, nevertheless serve as sensitive indicators of exposure.

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The calculated equivalent concentration values varied over an approximately tenfold range (2 to 21 ppm), depending upon the end point chosen. Such a range is not unexpected, since various soot components might be more or less effective than 2,3,7,8-TCDD in producing a specific end point. While the soot appeared to be very effective in causing alterations in thymus weight, body weight, and serum triglyceride levels, it was less effective at causing death. Taken together, these findings suggest that biologically based calculations of 2,3,7,8-TCDD equivalent concentrations are best based on a range of biological end points.

It is notable that for the Binghamton soot, use of the acute oral LD_{50} as the end point resulted in higher apparent toxicity (i.e., the highest 2,3,7,8-TCDD equivalent concentration) than the various subchronic endpoints. Thus, use of the acute LD_{50} or use of the chemical data to perform a calculation may provide a conservative estimate of the acute or subchronic activity of the mixture. However, the observation that the 2,3,7,8-TCDD equivalent concentration varied by about an order of magnitude when calculated by using various subchronically induced end points is an indication of the uncertainty associated with the use of any single biological end point or of chemical analysis data alone to estimate a mixture's potency.

Conclusions

The acute oral LD_{50} of a soot sample containing a complex mixture of PCDDs, PCDFs, and PCBEs has been shown to correspond to that of a hypothetical material containing only 2,3,7,8-TCDD at 58 ppm. The corresponding 2,3,7,8-TCDD equivalent concentration calculated by using various dose-related subchronic end points ranged from 2 to 19 ppm. Conversely, using existing literature data to estimate the potency of individual congeners and the results of chemical analyses on the soot, it can be "predicted" that the soot contains ca. 22 ppm 2,3,7,8-TCDD equivalents. In light of uncertainties in the chemical analyses and toxicological determinations, as well as uncertainties in the sample congener toxicity data upon which this calculation relies, the agreement between observed and predicted concentrations is good. However, the substantial variation in the results of the "equivalents" calculation depending on endpoint chosen clearly shows that any determination based on a single end point or any calculation based on chemical concentrations has a substantial uncertainty associated with it. Nevertheless, these uncertainties will often be small in comparison to the many uncertainties and approximations inherent in a risk assessment for a single compound, such as 2,3,7,8-TCDD. It appears likely that this approach, or a conceptually similar one, will find increasing use in the evaluation of the hazard posed by complex mixtures.

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Immunosuppression without Liver Induction by Subchronic Exposure to 2,7-Dichlorodibenzo-*p*-dioxin in Adult Female B6C3F1 Mice

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Immunosuppression without Liver Induction by Subchronic Exposure to 2,7-Dichlorodibenzo-*p*-dioxin in Adult Female B6C3F1 Mice. HOLSAPPLE, M. P., MCCAY, J. A., AND BARNES, D. W. (1986). *Toxicol. Appl. Pharmacol.* 83, 445–455. The overall objective of this investigation was to begin to characterize the structure–activity relationship associated with dioxin-induced suppression of humoral immunity. Subchronic exposure (14 days) to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the prototype of the class, produced a suppression of the antibody responses to both sheep erythrocytes, a T-dependent antigen, and dinitrophenyl–Ficoll, a T-independent antigen. Surprisingly, similar results were observed with 2,7-dichlorodibenzo-*p*-dioxin (DCDD), a dioxin congener lacking affinity for the *Ah* receptor. In contrast, subchronic exposure to octachlorodibenzo-*p*-dioxin (OCDD), another dioxin congener without affinity for the *Ah* receptor, was devoid of activity. Subchronic exposure to 2,3,7,8-TCDD, but not 2,7-DCDD, produced an induction of several liver parameters including: liver weight, amount of microsomal protein, amount of cytochrome *P*-450, activity of aminopyrine-*N*-demethylase and activity of aryl hydrocarbon hydroxylase. Subchronic exposure to 2,3,7,8-TCDD or 2,7-DCDD produced no marked changes in thymus weight. Acute exposure to 2,3,7,8-TCDD also produced suppression of the antibody response in the absence of effects on the thymus. © 1986 Academic Press, Inc.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) represents the prototype and most potent member of a family of low-molecular-weight halogenated aromatic hydrocarbons. The mechanism of action by TCDD is at present unknown, although many of the actions associated with exposure to TCDD are suspected to be receptor mediated. The *Ah* receptor has been implicated in a number of actions produced by TCDD and related congeners (Greenlee and Neal, 1984). Our interest in the chlorinated dibenzo-*p*-dioxins is in their effects on immunocompetence. We have recently reported that subchronic exposure (14 days) to 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin was capable of suppressing humoral immunocompetence (Holsapple *et al.*, 1984a). Surprisingly, this was the first report of immunosuppression by any dioxin congener other than TCDD. However, it is important to emphasize that other laboratories have tested nondioxin halogenated aromatic hydrocarbons. These results demonstrated that 2,3,7,8-dibenzofuran could suppress the antibody response (Vecchi *et al.*, 1983) and that Aroclor 1254, a commercial polychlorinated biphenyl (PCB) mixture, or 3,3',4,4'-tetrachlorobiphenyl, but not 2,2',4,4',6,6'-hexachlorobiphenyl, could suppress the generation of cytotoxic T cells (Clark *et al.*, 1983). The objective of the present investigation was to determine the effects of 2,7-dichlorodibenzo-*p*-dioxin (DCDD) on the humoral antibody response and to compare these effects with

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TCDD. Previous work by Greenlee *et al.* (1984) and by Poland *et al.* (1976) has shown that 2,7-DCDD was devoid of affinity for the *Ah* receptor and of the activities attributed to binding to that receptor. In this investigation, we also determined the effects of exposure to octachlorodibenzo-*p*-dioxin (OCDD) on the antibody response. Poland *et al.* (1976) showed that OCDD also lacked affinity for the *Ah* receptor. These results are significant because the positive effects by 2,7-DCDD indicate that a chlorinated dibenzodioxin can produce effects (at least on immunocompetence) which are not associated with the *Ah* receptor.

METHODS

Animals. Female (C57BL/6 × C3H)F1 (B6C3F1) mice were purchased from Litton Bionetics, Inc. (Frederick, Md.). Mice arrived at 5–6 weeks of age, were quarantined for at least 1 week, and were used for experimentation when they were 17–20 g in weight. Upon arrival, mice were randomized, weighed, and housed four mice per cage. Individual mice within a cage were identified by ear-punching. The animal quarters were maintained at 21–24°C and 40–60% relative humidity. A 12-hr light/dark cycle was used.

Chemicals. 2,3,7,8-TCDD was obtained through the National Toxicology Program and Bio-Rad Laboratories (Rockville Centre, N.Y.). 2,7-DCDD was a gift from Dr. William F. Greenlee (CIIT. Research Triangle Park, N.C.). OCDD was a gift from Dr. Anne N. Tucker (NIEHS, Research Triangle Park). Corn oil was previously demonstrated to be an effective vehicle for the studies with 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HCDD) (Holsapple *et al.*, 1984a) and was the vehicle for this investigation. The route of exposure was by oral gavage. Range-finding studies determined that 14 daily exposures to 1 μg/kg TCDD consistently produced a significant suppression of the antibody response (data not shown), so a concentration of 0.1 μg/ml TCDD was used in these studies. It is important to emphasize that the dose relatedness of the suppression of immunocompetence by TCDD has been reported by other investigators (Vecchi *et al.*, 1983; Dean and Lauer, 1984). Our rationale for using a single concentration of TCDD was based on the fact that TCDD was to serve as the prototype of the class for comparative purposes to the other dioxin congeners. The concentrations of 2,7-DCDD were 0.01, 0.1, and 1 μg/ml and of OCDD were 0.1 and 1 μg/ml. For the subchronic studies, mice were gavaged daily with 0.1 ml/10 g body wt of the vehicle or one of the dioxin congeners for 14 consecutive days unless otherwise stated. The various parameters were measured 24 hr after the final exposure to the vehicle or dioxins, except for the acute studies with TCDD as described below. The number of animals exposed to each treatment is indicated in the appropriate tables describing the results.

Body and organ weights and histopathology. Twenty-four hours after the final chemical (or vehicle) treatment, mice were weighed and body weights recorded. Gross pathological examinations were performed on all mice. The organs (brain, liver, spleen, lungs, thymus, and kidneys) were removed, trimmed, and weighed. Material for histological analysis consisted of organs removed at the time of necropsy and consisted of liver, spleen, thymus, kidney, and lung. These organs were fixed in 10% neutral buffered Formalin. The tissues were sectioned at 6 μm and stained with hematoxylin and eosin. The slides were studied by a pathologist who was unaware of the treatments.

Hepatic microsomal parameters. Twenty-four hours after the final exposure to the vehicle, TCDD, or DCDD, the animals were killed and the livers were removed, weighed, rinsed, and homogenized at 4°C in 9 vol of 1.15% KCl containing 0.1 M potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 9000*g* for 20 min, and the supernatant fraction was recentrifuged at 100,000*g* for 1 hr in a Beckman Model L5-50 ultracentrifuge (Beckman Instruments Inc., Fullerton, Calif.). The microsomal pellet was layered with 1.5% KCl and frozen immediately at −70°C. Within 24 to 48 hr after freezing, the microsomes were resuspended in 0.05 M potassium phosphate buffer, pH 7.4 (0.5 g of liver/ml), and samples were taken for study. Microsomal protein was assayed by the method of Lowry *et al.* (1951). The content of cytochrome(s) *P*-450 was determined by measuring the carbon monoxide difference spectrum in dithionite-reduced microsomes (Omura and Sato, 1964). An extinction coefficient of 91 cm^{-1} mM^{-1} was used to calculate *P*-450 content. Aminopyrine-*N*-demethylase activity was determined by measuring formaldehyde production by the Nash reaction as described by Cochin and Axelrod (1959). Aryl hydrocarbon[benzo[*a*]pyrene] hydroxylase activity was evaluated spectrofluorometrically as described by Yang *et al.* (1978) using 3-hydroxybenzo[*a*]pyrene as the standard.

Antibody response to a T-dependent antigen. On Day 11 of subchronic exposure to the various treatments, mice were given injections of 5×10^8 SRBC, ip. On Day 15, the animals were killed, and the spleens dissociated into single cell suspensions by pressing the spleen between two frosted microscope slides. Spleen cells from individual animals were assessed for the number of anti-sheep red blood cells (SRBC) AFC-producing IgM in a modified Jerne plaque assay (Bullock and Moller, 1972) as described previously (Holsapple *et al.*, 1984a). AFC (plaques) were counted at 6.5× magnification using a Bellco plaque viewer (Bellco Glass, Inc., Vineland, N.J.). Spleen cell suspensions were counted with a Coulter Counter (Model ZBI; Coulter Electronics, Inc., Hialeah, Fla.) and AFC results were calculated as AFC/10^6 recovered cells and AFC/spleen.

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It is important to emphasize that we have traditionally determined the effects of xenobiotics on the IgG response as well as IgM antibody production and on both Day 4 and Day 5 after immunization. Our decision to measure only the Day 4 IgM response is based on previous results which showed that subchronic exposure to 1,2,3,6,7,8-HCDD suppressed all parameters, but that the Day 4 IgM response was the most sensitive indicator (Holsapple *et al.*, 1984a).

Antibody response to a T-independent antigen. This response was determined as previously reported (Bick *et al.*, 1984). The thymic-independent antigen, dinitrophenyl (DNP)–Ficoll, was prepared using CNBr–Ficoll (400,000 M_r) and 2,4-dinitrophenyl-lysine (all from Sigma Chemicals) following the method of Klaus *et al.* (1976). On Day 11 of subchronic exposure to either the vehicle, TCDD, or DCDD, mice received 20 μg of DNP–Ficoll intravenously in a volume of 0.2 ml. On Day 15, the animals were killed and the Day 4 IgM antibody response was measured basically as described above. The anti-DNP antibody-forming cell response was determined for each animal in a modified Jerne plaque assay using trinitrophenyl (TNP)-coupled SRBC as indicator cells. The SRBCs were densely coupled with trinitrophenyl following the method of Rittenberg and Pratt (1969).

Polyclonal antibody response to lipopolysaccharine. This response was determined as previously reported (Holsapple *et al.*, 1984b). Animals were exposed to either the vehicle, TCDD, or DCDD daily for either 14 days (Experiment 1) or 5 days (Experiment 2). Spleen cells were collected from individual animals 24 hr after the final treatment and assayed in quadruplicate cultures (5×10^6 cells/1.0 ml/culture well) in 24-well cluster plates (Falcon). Spleen cells were cultured with 200 μg/ml lipopolysaccharide (LPS) for 48 hr. It is important to emphasize that these animals receive no immunological stimuli during the duration of the chemical exposure. Following culture, the cells were harvested using gentle resuspension with a Pasteur pipet, centrifuged, and resuspended to a suitable volume for the determination of antibody-forming cells. The antibody-forming cell response was determined using a modified Jerne plaque assay and TNP-coated sheep erythrocytes as target cells.

Effects of acute exposure to TCDD on the antibody response to a T-dependent antigen and thymic weights. In light of the traditional involvement of the *Ah* receptor in dioxin-induced thymic atrophy, the lack of effect on the thymus by subchronic exposure to TCDD was surprising. In order to rule out the possibility that the repeated exposures (i.e., daily for 14 consecutive days) may have contributed to the lack of effect, a study was conducted to determine the effects of a single exposure to TCDD. Mice were gavaged with either corn oil (vehicle) or 0.005, 0.01, 0.05, 0.1, or 0.2 μg/ml TCDD (i.e., to give doses from 0.05 to 2.0 μg/kg). Seven days later, all mice were immunized with SRBCs as described above. Four days later, all mice were killed and the spleens were removed, weighed, and assayed for the number of IgM antibody-producing cells as described above. In addition, the thymuses were removed and weighed.

Statistics. For results of all studies, a Dunnett's *t* test was performed if a one-way analysis of variance of the means showed treatment effects.

RESULTS

Body and organ weights and histopathology. The results in Table 1 indicate that 14 daily exposures to 2,7-DCDD were devoid of any effects on body weights or selected organ weights. For the sake of brevity, we have only shown the effects on the thymus and the liver. Subchronic exposure to 2,3,7,8-TCDD, but not 2,7-DCDD, produced a significant increase in liver weight. It is important to note that neither dioxin congener caused much of a change in thymus weight, although there was a significant reduction by TCDD, but only when reported as a percent of body weight. The histopathology of the thymus, spleen, lungs, and kidneys of mice receiving TCDD was normal. In agreement with the effects on liver size, the livers of TCDD-treated mice showed focal areas of hydropic degeneration.

Hepatic microsomal parameters. As suggested by the increase in liver weight shown in Table 1 and previous results described in the discussion, subchronic exposure to 2,3,7,8-TCDD produced a marked induction of a number of microsomal parameters (Table 2). In contrast, subchronic exposure to 2,7-DCDD was devoid of any changes.

Antibody response—T-dependent antigen. Two experiments were performed to determine the effects of select dioxin congeners on the Day 4 IgM antibody response to a T-dependent antigen, SRBC. In the first experiment (Table 3, Section I), subchronic exposure to 2,3,7,8-TCDD and to 2,7-DCDD produced a significant suppression at all concentrations tested. These effects occurred in the absence of any effects on spleen cell number and were therefore comparable when expressed as either IgM AFC/spleen or IgM AFC/10^6 recovered cells. The lack of effect on spleen cell number

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TABLE 1

BODY AND SELECT ORGAN WEIGHTS OF B6C3F1 MICE EXPOSED TO 2,7-DCDD OR 2,3,7,8-TCDD FOR 14 DAYS

Parameter *N*	Vehicle (corn oil) (9)	2,7-DCDD (μg/kg) 0.1 (5)	1.0 (5)	10.0 (9)	2,3,7,8-TCDD (μg/kg) 1.0 (9)
Body weight (g)	17.5 ± 0.3	18.4 ± 0.4	17.6 ± 0.2	18.6 ± 0.3	18.6 ± 0.3
Liver (mg)	879.0 ± 35.8	866.6 ± 33.0	837.2 ± 15.0	944.3 ± 26.9	1252.8 ± 36.8**
% Body weight	5.08 ± 0.15	4.70 ± 0.14	4.98 ± 0.08	5.04 ± 0.14	6.68 ± 0.12**
Thymus (mg)	65.2 ± 2.7	62.0 ± 4.6	63.6 ± 4.1	73.3 ± 1.6	54.6 ± 4.6
% Body weight	0.38 ± 0.01	0.34 ± 0.02	0.36 ± 0.03	0.39 ± 0.01	0.29 ± 0.02**

Note. B6C3F1 female mice were administered either vehicle (corn oil) or 2,7-DCDD at one of the designated concentrations, or 2,3,7,8-TCDD daily for 14 days by gavage. Twenty-four hours after the last treatment, mice were necropsied, and the organs listed above removed, trimmed of fatty tissue, and weighed. Brain, spleen, lungs, and kidney were similarly examined and there were no treatment-related effects. The values represent the $\bar{x} \pm$ SE derived from the number of animals indicated in parentheses. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significantly different at $p < 0.01$ are noted by a double asterisk, as determined by Dunnett's t test.

TABLE 2

HEPATIC MICROSOMAL PARAMETERS IN FEMALE B6C3F1 MICE EXPOSED TO 2,7-DCDD AND 2,3,7,8-TCDD FOR 14 DAYS

Parameter	Vehicle	2,7-DCDD (μg/kg) 0.1	1.0	10.0	2,3,7,8-TCDD 1.0 μg/kg
N	9	5	5	9	9
Liver weight (mg)	874.4 ± 40.3	866.6 ± 33.0	873.2 ± 15.0	945.1 ± 26.8	1253.0 ± 36.9**
Microsomal protein (mg/g liver)	16.0 ± 0.2	14.3 ± 0.6	15.2 ± 0.3	15.4 ± 0.6	21.7 ± 0.5**
Cytochrome *P*-450 (nmol/mg protein)	0.932 ± 0.03	0.934 ± 0.02	0.865 ± 0.02	0.903 ± 0.04	2.263 ± 0.09**
(nmol/g liver)	15.0 ± 0.6	13.3 ± 0.6	13.2 ± 0.6	13.9 ± 0.7	49.1 ± 2.1**
Glutathione μmol/g liver	8.27 ± 0.26	8.59 ± 0.38	8.30 ± 0.20	8.20 ± 0.32	9.69 ± 0.28*
Aminopyrine-*N*-demethylase					
nmol/min/mg prot	9.09 ± 0.33	9.28 ± 0.54	7.30 ± 0.47	8.41 ± 0.56	13.99 ± 0.46**
nmol/min/g liver	145.8 ± 6.42	131.7 ± 4.82	110.9 ± 6.16	130.0 ± 10.5	303.3 ± 9.56**
Arylhydrocarbon hydroxylase					
nmol/min/mg prot	0.54 ± 0.02	0.63 ± 0.03	0.61 ± 0.03	0.52 ± 0.02	2.33 ± 0.14**
nmol/min/g liver	8.62 ± 0.35	8.95 ± 0.32	9.21 ± 0.46	8.04 ± 0.34	50.15 ± 2.25**

Note. B6C3F1 female mice were administered DCDD or TCDD or corn oil daily for 14 days. On Day 15, the livers were aseptically removed. The values represent the $\bar{x} \pm$ SE derived from the number of animals indicated at the top of each column. *$p < 0.05$ and **$p < 0.01$ as determined by Dunnett's t test.

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TABLE 3

DAY 4 IgM ANTIBODY RESPONSE TO A T-DEPENDENT ANTIGEN, SRBC, BY FEMALE B6C3F1 MICE EXPOSED TO DCDD, TCDD, OR OCDD FOR 14 DAYS

Treatment	Number of spleen cells ($\times 10^7$)	IgM AFC/spleen ($\times 10^3$)	IgM AFC/10^6 recovered cells
I. Vehicle (corn oil)	13.72 ± 0.57	343.8 ± 41.0	2510.1 ± 282.3
1.0 μg/kg TCDD	12.44 ± 0.84	69.2 ± 22.3**	533.1 ± 157.3**
0.1 μg/kg DCDD	13.98 ± 1.26	169.4 ± 45.4**	1194.4 ± 270.2**
1.0 μg/kg DCDD	15.47 ± 0.67	157.9 ± 34.7**	1015.9 ± 199.3**
10.0 μg/kg DCDD	15.38 ± 0.96	129.1 ± 16.5**	843.8 ± 99.2**
II. Vehicle (corn oil)	16.01 ± 0.97	205.7 ± 29.2	1269.4 ± 132.7
1.0 μg/kg TCDD	14.14 ± 1.14	34.9 ± 7.7*	278.7 ± 54.8**
1.0 μg/kg OCDD	16.44 ± 0.94	220.3 ± 36.6	1349.4 ± 216.4
10.0 μg/kg OCDD	17.85 ± 0.99	192.8 ± 17.3	1082.4 ± 82.8

Note. Female B6C3F1 mice (five per treatment group) were gavaged with vehicle (corn oil), TCDD, or DCDD in corn oil daily for 14 days in Experiment I and with vehicle (corn oil), TCDD, or OCDD in corn oil daily for 14 days in Experiment II. In both experiments, the animals were immunized with SRBC on Day 11 of exposure. On Day 15, the spleen cell IgM anti-SRBC AFC response was determined. Values represent the $\bar{x} \pm$ SE derived from five animals per treatment group. Values significantly different from vehicle control at $p < 0.01$ are indicated by a double asterisk as determined by Dunnett's t test.

also indicates that neither dioxin congener produced a decrease in the distribution of lymphocytes to the spleen. The possibility for alterations in the relative subpopulations of immunocompetent cells within the spleen cannot be estimated by the present results. In the second experiment (Table 3, Section II), subchronic exposure to 2,3,7,8-TCDD produced an almost identical suppression of the Day 4 IgM response (i.e., compare a 79% suppression in Section I with a 78% suppression in Section II) even though the vehicle responses were different. It is important to emphasize that both vehicle responses are within our historical controls as generated over the last 4 years, although the response in Section II is at the low end of this range. In contrast with the positive effects by 2,3,7,8-TCDD and 2,7-DCDD, subchronic exposure to OCDD was devoid of activity.

Antibody response—T-independent antigen. As shown in Table 4, subchronic exposure to either 2,3,7,8-TCDD or 2,7-DCDD produced a significant suppression in the Day 4 IgM antibody response to a T-independent antigen, DNP-Ficoll. In contrast with the results in Table 3, there was a significant change in spleen cell number as the low dose of 2,7-DCDD produced a 24% increase. We have no explanation for this effect, but it is important to emphasize that the average spleen weight of this group of mice was 129.3 ± 9.6, as compared to 91.2 ± 3.9 for the vehicle animals.

Polyclonal antibody response—lipopolysaccharide. As shown in Table 5, the suppression by subchronic exposure (14 days) to either TCDD or DCDD can be detected even when spleen cells are removed from treated animals and immunized in culture. Both the TCDD and the high dose of DCDD produced a greater than 50% suppression of the response of LPS (middle column), which suggests functional impairment of the B lymphocyte. In contrast with the results shown in Tables 3 and 4, exposure to 1.0 μg/kg DCDD for 14 days did not produce a significant suppression.

In separate animals, we attempted a preliminary study with 5 days of exposure to either TCDD or the high dose of DCDD. These results are shown in the right-hand column of

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TABLE 4

DAY 4 IgM ANTIBODY RESPONSE TO A T-INDEPENDENT ANTIGEN, DNP-FICOLL, BY FEMALE B6C3F1 MICE EXPOSED TO DCDD OR TCDD FOR 14 DAYS

Treatment	Number of spleen cells ($\times 10^7$)	IgM AFC/spleen ($\times 10^3$)	IgM AFC/10^6 recovered cells
Vehicle (corn oil)	13.99 ± 0.72	574.2 ± 29.5	4117.9 ± 142.3
1.0 μg/kg TCDD	11.78 ± 0.57	53.4 ± 8.5**	451.3 ± 59.2**
1.0 μg/kg DCDD	17.41 ± 0.78*	516.6 ± 47.8	3152.9 ± 274.7**
10.0 μg/kg DCDD	14.56 ± 1.19	411.8 ± 33.6**	2856.6 ± 180.4**

Note. Female B6C3F1 mice (six per treatment group) were gavaged with vehicle (corn oil), TCDD, or DCDD in corn oil daily for 14 days. On Day 11 of exposure, all mice were immunized with 20 μg DNP-Ficoll. On Day 15, the spleen cell IgM anti-TNP AFC response was determined against TNP-haptenated SRBCs. Values represent the $\bar{x} \pm$ SE derived from six animals per treatment group. Values significantly different from vehicle control at $p < 0.05$ are indicated by a single asterisk, while those significantly different from vehicle control at $p < 0.01$ are indicated by a double asterisk, as determined by Dunnett's t test.

Table 5. TCDD produced a suppression after only 5 days of exposure (62%) which was comparable to that observed after 14 days of exposure (52%). In contrast, the suppression by DCDD was less after 5 days when compared to the effect by 14 days of exposure (i.e., 23 and 56%, respectively) which suggested a possible temporal relationship for the immunotoxicity by DCDD.

TABLE 5

POLYCLONAL ANTIBODY RESPONSE TO LIPOPOLYSACCHARIDE BY SPLEEN CELLS FROM FEMALE B6C3F1 MICE EXPOSED TO DCDD OR TCDD FOR 14 OR 5 DAYS

Treatment (μg/kg)	IgM AFC/10^6 recovered cells (14 days)	IgM AFC/10^6 recovered cells (5 days)
Vehicle (corn oil)	840 ± 19	542 ± 21
1.0 TCDD	402 ± 11**	203 ± 28**
1.0 DCDD	737 ± 80	ND
10.0 DCDD	372 ± 18**	416 ± 20**

Note. Female B6C3F1 mice (five per treatment group) were gavaged with vehicle (corn oil), TCDD, or DCDD in corn oil daily for either 14 days (middle column) or 5 days (right column). Twenty-four hours after the final exposure, mice were killed and spleen cell suspensions from individual mice were aseptically prepared and cultured (5×10^6 cells/1.0 ml/culture well) in quadruplicate wells with 200 μg LPS in the presence of 10% fetal calf serum. Cultures were harvested after 48 hr and the number of IgM antibody-forming cells was determined. Values represent the mean IgM AFC/10^6 recovered spleen cells ± SE.

ND—not done.

Immunosuppression—acute exposure to TCDD. In light of the marginal effects that subchronic exposure to 2,3,7,8-TCDD produced on the thymus weights (Table 1), a study was conducted with acute exposure to TCDD. These results are shown in Table 6. As shown

TABLE 6

DAY 4 IgM ANTIBODY RESPONSE TO A T-DEPENDENT ANTIGEN AND THYMUS WEIGHT OF FEMALE B6C3F1 MICE EXPOSED TO A SINGLE INJECTION OF TCDD

Treatment (μg/kg)	IgM AFC/10^6 recovered cells	Thymus weight
Vehicle (corn oil)	1495 ± 304	51 ± 3
0.05 TCDD	1558 ± 230	60 ± 5
0.1 TCDD	1570 ± 253	49 ± 5
0.5 TCDD	1104 ± 47	57 ± 5
1.0 TCDD	826 ± 82*	53 ± 7
2.0 TCDD	505 ± 78*	54 ± 2

Note. Female B6C3F1 mice were gavaged with vehicle (corn oil) or TCDD in corn oil on Day 1. On Day 8, the animals were immunized with SRBC. On Day 12, the spleen cell IgM anti-SRBC AFC response was determined and the spleen and thymuses were removed, trimmed, and weighed. Values represent the $\bar{x} \pm$ SE derived from five animals per treatment group. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, as determined by Dunnett's t test.

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in the last column, doses of TCDD up to 2.0 μg/kg had no effect on the thymus weights. This same concentration range did produce a dose-related suppression in the Day 4 IgM antibody response to a T-dependent antigen with significance noted at 1.0 and 2.0 μg/kg (45 and 66%, respectively). These results indicate that the marginal effects on the thymus shown in Table 1 are not an effect of the repeated exposures and suggest that the dioxin-induced suppression of the T-dependent response can be completely separated from the dioxin-induced thymic atrophy.

DISCUSSION

As recently reviewed by Greenlee and Neal (1984), 2,3,7,8-TCDD represents the prototype ligand for the *Ah* receptor. The *Ah* receptor is suspected to be a protein which regulates the expression of a gene battery which includes the structural genes for cytochrome(s) P_1-450 (i.e., involved in the TCDD-induced liver induction) and a second regulatory locus which controls a broader pleiotropic response (i.e., involved in actions where TCDD is suspected as producing a change in cell proliferation and/or differentiation). This receptor has been implicated in a number of actions associated with exposure to TCDD and related congeners including induction of cytochrome(s) P_1-450 (Poland and Glover, 1974, 1975), thymic atrophy (Poland and Glover, 1980), impairment of the ability of thymic epithelial cells to support the maturation of T lymphocyte precursors (Greenlee *et al.*, 1985), suppression of humoral immunity (i.e., generation of antibody producing cells) (Vecchi *et al.*, 1983), and suppression of cell-mediated immunity (i.e., generation of cytotoxic T cells) (Nagarkatti *et al.*, 1984). The studies which have demonstrated a possible role by the *Ah* receptor in the profile of activity of TCDD have capitalized on two important characteristics of this locus.

Nebert *et al.* (1972) reported that the *Ah* locus is inherited as a simple autosomal dominant trait. The genetic characteristics of the locus have been particularly well worked out in mice where the prototype responsive strains have been demonstrated to be C57BL/6 and C3H, while the prototype nonresponsive strain is the DBA/2 (Poland *et al.*, 1976, 1979). Crosses (F1) between the various strains have an activity which is intermediate to that of the parents. Therefore, the B6C3F1 mouse used in the present investigation would be expected to be *Ah* responsive, since both parents were responsive (C57BL/6 and C3H). The B6C3F1 mouse was selected for these studies because it represents the mouse approved for study by the National Toxicology Program and because we have had 5 years of experience investigating the effects of xenobiotics on its immunocompetence. When one considers all the effects previously described for TCDD (this list is by no means intended to be all inclusive), the following have been demonstrated to be produced by lower concentrations of TCDD in *Ah*-responsive mice compared to *Ah*-nonresponsive mice: liver induction (Poland and Glover, 1974, 1975), thymic atrophy (Poland and Glover, 1980), and suppression of both humoral (Vecchi *et al.*, 1983) and cell-mediated immunity (Nagarkatti *et al.*, 1984). In other words, the only parameter not to be shown to possess the genetic differential sensitivity is the recent effects on thymic epithelium (Greenlee *et al.*, 1985). It is important to emphasize that in this report, Greenlee *et al.* only measured the effects on primary cultures of cells derived from C57BL/6 mice, an *Ah*-responsive strain.

The second feature of the *Ah* receptor which has been used in the experimental designs of these studies is based on a clear structure–activity relationship regarding the ability of halogenated aromatic hydrocarbons (i.e., dibenzodioxins, dibenzofurans, azo[*x*, *y*]benzenes and biphenyls) to bind to the *Ah* receptor and produce the associated effects. In general, structures which have affinity for the *Ah* receptor must be planar or near planar and must be halogenated in the lateral ring positions. For the dibenzodioxins, halogen atoms must

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occupy at least three (and for maximal potency, four) of the lateral ring positions (2, 3, 7, and 8) and at least one ring position must be unsubstituted—i.e., octachlorodibenzo-*p*-dioxin (OCDD) is inactive. Of particular importance to the present investigation were the results which indicated that neither 2,7-DCDD nor the completely substituted OCDD could bind to the *Ah* receptor. Parameters for which these congeners have been demonstrated to be devoid of effect include liver induction (Poland and Glover, 1974, 1975), thymic atrophy (Poland and Glover, 1980), and effects on thymic epithelium (Greenlee *et al.*, 1985). Noticeably absent from this list are parameters reflecting the dioxin-induced changes in immunocompetence. As described in the introduction, Vecchi *et al.* (1983) demonstrated that 2,3,7,8-dibenzofuran could suppress the antibody response and Clark *et al.* (1983) demonstrated that planar PCBs could suppress the generation of cytotoxic T cells. Our recent report that subchronic exposure to 1,2,3,6,7,8-HCDD could suppress the antibody response (Holsapple *et al.*, 1984a) was the first report to describe immunotoxicity by any chlorinated dibenzodioxin other than TCDD, the prototype of the class. The positive effect by 1,2,3,6,7,8-HCDD supports the structure–activity relationship associated with the *Ah* receptor since this congener possesses a measurable affinity for this locus (Poland *et al.*, 1976) and is halogenated in the key lateral positions.

The positive activity presently demonstrated for 2,7-DCDD in a number of different immunological models is the first major evidence that an effect produced by the chlorinated dibenzodioxins need not be associated with the *Ah* receptor. That we were not producing the immunosuppression by contaminants of TCDD (or another *Ah* active congener) in our batch of DCDD is indicated by the studies on liver induction. TCDD produced marked induction of a variety of liver parameters (Table 2) at the doses producing immunosuppression (Tables 3–5). In contrast, 2,7-DCDD was totally devoid of effects on the liver at effective immunotoxic concentrations. While it is important to emphasize that the majority of reports on 2,7-DCDD have indicated no activity, this is not the first report for a positive effect by this congener. As described by Kociba (1984), in conventional lifetime 2-year carcinogenic studies sponsored by the National Toxicology Program (NTP) 2,7-DCDD did not elicit any response in the rat, but there was a suggestive effect in the form of increased liver tumors at the high dose in the mouse.

Due to the paucity of results for other dibenzodioxins, it is impossible to predict how much the structure–activity relationship (SAR) for immunosuppression might differ from the SAR reported for effects on the liver or thymus. We have begun to screen the activity of a number of chlorinated dibenzodioxins varying in the number and position of the chlorine substituents. One particularly interesting congener is 2,8-DCDD. Luster *et al.* (1984) have recently reported that this congener was devoid of activity when added directly to cultured spleen cells from B6C3F1 mice. We have recently demonstrated that both 2,3,7,8-TCDD and 2,7-DCDD were directly immunosuppressive when added to cultured spleen cells (Holsapple *et al.*, 1985).

In addition to indicating that immunotoxicity by the chlorinated dibenzo-dioxins need not be mediated through the *Ah* receptor, the present results also suggest that the effects on the antibody response can be completely dissociated from the effects on the thymus. This interpretation is based on the ability of TCDD and DCDD to suppress the response to a thymic independent antigen, DNP–Ficoll (Table 4), as well as the response to the polyclonal activator, LPS (Table 5). These results are in agreement with previous results which indicated that in adult mice, TCDD suppresses humoral immunity at doses which do not alter cell-mediated immunity (Mantovani *et al.*, 1980; Vecchi *et al.*, 1980). The results in Tables 3, 4, and 5 indicated that subchronic exposure to TCDD could suppress antibody responses in the complete absence of effects on the thymus (Table 1). Because of the classical association between exposure to TCDD and

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thymic atrophy, we were somewhat surprised by these results and questioned whether the lack of effect on the thymus may be a consequence of repeated exposures. Since most of the earlier investigations have utilized single exposure to TCDD, it was important to determine the effects of acute exposure. As shown in Table 6, acute exposure to TCDD at doses lower than 2 μg/kg was capable of suppressing the antibody response in the absence of effects on the thymus. We have carefully considered the available literature and have summarized comparable effects in Table 7 from four previous reports plus the present results. The first observation is the consistency of effect by TCDD—these results were generated over the last 6 years by four different laboratories—the suppression by exposure to ~1.0 μg/kg TCDD has been 54, 78, 57, 40, and 45%, respectively. The second observation is that significant effects on the thymus were only reported for doses of TCDD which were 5–10-fold higher—5–10 μg/kg.

To summarize, these results indicate differential contributions by the *Ah* receptor in mediating the effects of exposure to chlorinated dibenzodioxins. The liver and immunity are affected by doses which are devoid of activity on the thymus. The lack of effect of 2,7-DCDD on the liver is in agreement with previous results which have implicated the *Ah* receptor in this dioxin-induced action. The immunosuppression by 2,7-DCDD indicates that this effect by the dioxins is not necessarily a consequence of the *Ah* receptor and indicates the importance for further study into the SAR of dioxin-induced immunotoxicity. These studies are currently underway. While it is tempting to speculate that the activity by 2,7-DCDD is either suggestive of multiple dioxin receptors

TABLE 7

COMPARISON OF THE EFFECTS OF ACUTE EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZODIOXIN (TCDD) ON THYMUS WEIGHT AND T-DEPENDENT ANTIBODY PRODUCTION IN FOUR LABORATORIES

Dose of TCDD (μg/kg)	Thymus weight (% of control)	IgM AFC/10^6 spleen cells (% of control)	Reference (mouse strain)
1.2	ND	46%**	Vecchi *et al.* (1980)
6.0	ND	14%**	(C57BL/6)
30.0	ND	5%**	
1.2	89%	22%**	Vecchi *et al.* (1983)
6.0	56%	16%**	(C57BL/6)
30.0	36%**	3%**	
0.1	84%	97%	Dean and Lauer (1984)
1.0	94%	43%**	(B6C3F1)
10.0	61%*	19%**	
0.2	94%	35%	Luster *et al.* (1984)
1.0	100%	40%*	(B6C3F1)
5.0	77%*	47%**	
10.0	57%**	ND	
0.1	96%	105%	Summary from Table 6
0.5	117%	74%	(B6C3F1)
1.0	104%	55%*	
2.0	106%	34%**	

* $p < 0.05$; ** $p < 0.01$.
ND—not determined.

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or a nonreceptor effect, we consider this interpretation to be premature in the absence of any binding studies which demonstrate specific affinity for 2,7-DCDD on one of the subpopulations of immunocompetent cells.

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COMPARATIVE TOXICOLOGY AND MECHANISM OF ACTION OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS

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INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are members of a chemical family (polyhalogenated aromatics) that also includes the polychlorinated biphenyls, naphthalenes, azobenzenes, and azoxybenzenes, and the polybrominated biphenyls. In contrast to the polychlorinated biphenyls and naphthalenes and the polybrominated biphenyls, the PCDDs and PCDFs are not primary industrial products. PCDFs are found as by-products (< 1 ppm) in commercial polychlorinated biphenyls and naphthalenes and are probably derived from dibenzofuran impurities in the industrial hydrocarbons that are subsequently chlorinated (1–3). There is also circumstantial evidence that the effects of heat or arcing may produce PCDFs from polychlorinated biphenyls during use (4). This is evidenced by the relatively high levels of PCDFs (ca 100 ppm) detected in the polychlorinated biphenyl–containing heat transfer fluids that were the toxic agents in the Yusho disasters in Japan and Taiwan (3, 5, 6). PCDDs and PCDFs are also found as impurities in chlorinated phenols and their derived products (3, 7, 8), and it is apparent that the combustion of chlorinated aromatics and diverse types of chemical, industrial, and municipal waste results in the formation and release of these toxic chemicals into the environment (9–14). The potential for the

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formation of PCDDs and PCDFs from nonindustrial sources, i.e. energy-derived combustion and forest fires, led to the "trace chemistries of fire" hypothesis, which speculated that the origins of PCDDs and PCDFs in the environment were nonanthropogenic (15, 16). However, analysis of aquatic sediment cores from the Saginaw River and Bay and from Lake Huron does not support the trace chemistries of fire hypothesis (17, 18). The PCDD and PCDF congener composition of dated sediment cores demonstrates that the concentrations of these compounds in sediments have greatly increased since the 1940s and that "this historical increase is similar to trends for the production, use, and disposal of chlorinated organic compounds" (17, 18).

Human exposure to PCDDs and PCDFs has occurred via three major pathways: occupational, accidental, and environmental. Industrial workers engaged in the manufacture or use of polychlorinated biphenyls, chlorinated phenols, and their derived products are exposed to PCDDs and/or PCDFs in combination with their associated major commercial product (19–21). Accidents in which PCDDs have been released into the workplace or into the environment (e. g. the Seveso accident in Italy) have also resulted in human exposure to mixtures of the industrial chemicals and their PCDD/PCDF toxic contaminants (22–24). The Yusho poisoning in Japan and Taiwan involved the exposure of several thousand individuals to PCBs and their PCDF contaminants (5, 6, 25–27). The uptake of environmental residues of PCDDs and PCDFs into higher trophic levels of the food chain is only now being investigated, and trace levels (parts per trillion) have been detected in fish, wildlife, and human tissues (19, 28–32). In common with accidental and industrial exposures to PCDDs and PCDFs, exposure levels to these toxins represent only a small fraction of the total bioavailable lipophilic environmental pollutants. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the major by-product formed from 2,4,5-trichlorophenol and its derived products; this highly toxic compound has been the focus of most biologic and toxic studies on the PCDDs. However, all other human and environmental exposures to PCDDs and PCDFs involve a complex mixture of isomers and congeners in combination with other chemicals.

The scientific, regulatory, and media attention focused on PCDDs, PCDFs, and particularly 2,3,7,8-TCDD has continued unabated; moreover, with the recent identification of trace levels of these toxins in human tissue, domestic animals, the environment, and toxic chemical waste dumpsites, the scientific and societal concern about this class of compounds will no doubt continue. It is apparent from the scientific literature that one member of this class of compounds, namely 2,3,7,8-TCDD, ranks with benzo[a]pyrene as one of the most thoroughly studied toxins. Unfortunately, the biologic and toxic effects of the remaining 74 PCDD and 135 PCDF congeners have not been thoroughly

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investigated, and the interactive effects of these compounds or their activities in combination with polychlorinated biphenyls and chlorinated phenols have also not been addressed. This article (*a*) briefly reviews the species-dependent toxic and biologic effects of PCDDs and PCDFs, (*b*) demonstrates the parallel modes of action of PCDDs and PCDFs and endogenous cellular hormones, (*c*) summarizes the data that support the proposed receptor-mediated mechanism of action, including the structure-activity relationships (SARs) that have been developed for both PCDD and PCDF congeners, and (*d*) discusses the few interactive studies published.

TOXIC AND BIOLOGIC EFFECTS OF PCDDs AND PCDFs

Several review articles (33–45) have summarized the toxic and biologic effects elicited by PCDDs, PCDFs, and related toxic halogenated aryl hydrocarbons. The toxic effects resulting from exposure to this group of chemicals are dependent on a number of factors which include the dose of the toxin, and the age, strain, species, and sex of the animals used. The complete spectrum of toxicity is not usually observed in any single animal species; however, the limited data available indicate that the toxic PCDDs, PCDFs, and related compounds elicit the same qualitative pattern of responses within each species. The differences in species susceptibility to this group of chemicals are illustrated by the LD_{50} values for 2,3,7,8-TCDD, which vary over 5000-fold (33, 34) from the highly sensitive guinea pig to the resistant hamster [LD_{50}s (μg/kg): guinea pig (0.6–2.0), rat (22–45), chicken (25–50), monkey (70), rabbit (115), dog (100–200), mouse (114–284), bullfrog (>1000), hamster (1157–5051)]. The quantitative differences in the toxicity of PCDF congeners have recently been demonstrated (47) for a series of ten congeners (see structure-activity section, below). The 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) ED_{50} values for thymic atrophy and body weight loss in the rat were 0.21 and 1.04 μmol/kg; the 1,2,4,7,8-PeCDF isomer elicited the same toxic effects, but the ED_{50} values were 220 and 47 times higher, respectively (47). The toxic responses observed in several animal species by PCDDs and PCDFs include dermal toxicity, teratogenicity, reproductive problems, body weight loss, hepatotoxicity, gastric lesions, lymphoid involution, immunotoxicity, and carcinogenicity. The two most characteristic toxic effects observed in all laboratory animals are lymphoid involution and/or immunotoxicity and body weight loss. Chloracne and related dermal lesions are the most frequently noted signs of PCDD and PCDF toxicosis in humans; dermal lesions are also observed in rhesus monkeys, hairless mice, and rabbits that have been exposed to this group of toxins. In contrast, rats, most strains of mice, guinea pigs, and hamsters do not develop chloracne and related dermal toxic lesions after



Figure 1 Structure of the polychlorinated dibenzofurans and dibenzo-p-dioxins.

exposure to 2,3,7,8-TCDD. Poland & Knutson (38) have noted that many of the observed toxic lesions are either hyperplastic/metaplastic or hypoplastic, and primarily affect epithelial tissues. The mechanisms by which PCDDs and PCDFs elicit this diverse group of species-dependent toxicities remain unexplained although several hypotheses have been advanced and are discussed in this review.

PCDDs and PCDFs cause diverse biological responses in mammals and mammalian cells in culture including the highly characteristic induction of microsomal benzo[a]pyrene hydroxylase (aryl hydrocarbon hydroxylase, AHH) and several related cytochrome P-450-dependent monooxygenases (37, 38, 44, 47–53). In the rat, these activities are associated with the preferential induction of cytochrome P-450c, P-450d, and P-450a, with the former isozyme responsible for most of the induced monooxygenase enzyme activities (54). In the mouse, 2,3,7,8-TCDD induces cytochromes P_1-450 and P_3-450 (55–57), and the former isozyme exhibits antigenic and enzymatic similarities with the rat cytochrome P-450c (58). 2,3,7,8-TCDD induces two cytochrome P-450 isozymes (forms 4 and 6) in the rabbit, and their inducibility is highly tissue-specific (59, 60). 2,3,7,8-TCDD and related compounds also induce glutathione S-transferases (61) and glucuronosyl transferase (62, 63) and several other enzymes including DT-diaphorase (64), ornithine decarboxylase (65), δ-aminolevulinic acid synthetase (48), epidermal transglutaminase (66), and hepatic DNA polymerase B (67). Detailed summaries of these and other biochemical effects of 2,3,7,8-TCDD have recently been reviewed (38, 42).

The remarkably broad spectrum of biologic and toxic responses observed in animals exposed to 2,3,7,8-TCDD and related toxic halogenated aromatics has stimulated research on the mechanism or mechanisms of action of these chemicals. Unlike many toxins, the most active halogenated aryl hydrocarbons do not appear to require metabolic activation into presumed "toxic" intermediates that alkylate specific cellular acceptors (e. g. DNA, RNA, and protein) or initiate cellular lipoperoxidation. On the contrary, the most toxic halogenated aryl hydrocarbons are highly resistant to oxidative metabolic degradation and exhibit minimal metabolically mediated alkylation of cellular macromolecules (68–70); moreover limited data suggest that PCDD and PCDF metabolites are much less toxic than their parent hydrocarbons (71). Many of the effects of this

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class of environmental toxins are comparable to those associated with modulation of several hormone-mediated responses. For example, there are many similarities between animals that exhibit thyroid dysfunction and those treated with toxic halogenated aryl hydrocarbons. Daily injections of the active thyroid hormone, triiodothyronine (T_3) to male mice treated with a lethal dose (200 μg/kg) of 2,3,7,8-TCDD did increase their mean surival times; however, all the animals in the 2,3,7,8-TCDD and 2,3,7,8-TCDD + T_3-treated groups died (43). More recent studies (71, 72) have demonstrated that thyroid hormones may play a more important role in modulating the toxicity of 2,3,7,8-TCDD; radiothyroidectomy protected rats against 2,3,7,8-TCDD-mediated T-cell immunotoxicity (as measured by the spleen anti-SRBC plaque-forming cell assay), mortality, and body weight loss (73). Like the glucocorticoids, PCDDs, PCDFs, and related toxic halogenated aryl hydrocarbons cause lymphoid involution (33, 34, 37, 38, 40, 41, 74, 75), are teratogens in mice (76–80), and induce cytochrome P-450-dependent monooxygenases (37, 38, 44, 46–54). However the mechanisms of action of glucocorticoids and 2,3,7,8-TCDD are not directly linked since the latter compound is toxic to adrenalectomized rats, does not bind to the glucocorticoid receptor, and does not induce tyrosine aminotransferase (43) or the dexamethasone-induced cytochrome P-450 isozyme (81). Other adrenal steroids resemble the toxic halogenated aryl hydrocarbons since they also induce several hepatic drug-metabolizing enzymes, including monooxygenases and glucuronosyl transferases (81–84).

It has been proposed that 2,3,7,8-TCDD and related toxic isostereomers, like the steroid hormones, elicit their responses via the initial noncovalent interaction with a cytosolic receptor protein in target tissues (37, 38, 75). The synthesis of radiolabelled [^{3}H]-2,3,7,8-TCDD with high specific activity (52.5 Ci/mmol) resulted in the identification of a specific binding protein in hepatic cytosol of responsive C57BL/6J mice, whereas minimal binding activity was observed in nonresponsive DBA/2J hepatic cytosol (85). The role of this Ah receptor protein in the mechanism of action of toxic halogenated aryl hydrocarbon has been thoroughly investigated and satisfies most of the specific criteria that support a receptor mediated cellular process. These criteria include: (*a*) the existence of a finite number of binding or receptor sites and therefore saturable binding, (*b*) high affinity ligand binding that is commensurate with the usually low levels of circulating hormones, (*c*) stereoselective binding capacity for the receptor, (*d*) tissue or organ response specificity for the receptor ligand, and (*e*) a correlation between binding affinities, receptor occupancy, and the magnitude of the response. This review focuses on research that supports the role of the Ah receptor in the mechanism of action of PCDDs and PCDFs and highlights the detailed structure activity relationships (SARs) that have been developed for this group of environmental and industrial toxins.

PCDD AND PCDF ACTIVITIES: EVIDENCE THAT SUPPORTS THE ROLE OF THE CYTOSOLIC Ah RECEPTOR PROTEIN

High Affinity Saturable Binding

The saturable binding of [^{3}H]-2,3,7,8-TCDD with hepatic and extrahepatic cytosolic receptor protein from several species has been demonstrated using the following receptor assay procedures: charcoal/dextran absorption, protamine sulfate precipitation, hydroxylapatite absorption, isoelectric focusing in polyacrylamide gels, gel permeation chromatography, sucrose density gradient centrifugation, and gel permeation high performance liquid chromatography (85–110). Scatchard plot analysis of [^{3}H]-2,3,7,8-TCDD specific binding to hepatic cytosolic receptor protin gives dissociation consants (K_D) that are dependent on a number of factors including the animal species and strain, the receptor binding assay used, and the age of the animal. The K_D values for responsive C57BL/6 mice and rats vary from 0.27–3.0 nM (85, 95) and 0.13–1.2 nM (91, 95, 102), respectively, and the value for cynomolgus monkeys was approximately 3 nM (108). The concentration of hepatic cytosolic receptor was also highly variable, but the upper limit for most studies was less than 110 fmol/mg cytosolic protein (85, 91, 95). One study demonstrated that hepatic receptor levels in the rat varied with age and that these levels were endocrine independent since hepatic receptor levels were virtually unaltered by orchiectomy, ovariectomy, adrenalectomy, or hyposectomy (106). This observation was consistent with the inactivity of several steroid hormones as competitive ligands for this receptor protein (85, 86, 89, 99, 102).

Since certain polycyclic aromatic hydrocarbons resemble 2,3,7,8-TCDD in their mode of induction of AHH and related cytochrome P-450 isozymes in responsive strains of mice and mammalian cells in cultures, it is not surprising that many of these compounds competitively displace [^{3}H]-2,3,7,8-TCDD from the receptor protein. Moreover, [^{3}H]-3-methylcholanthrene, benzo[a]pyrene, and dibenzo[a,h]anthracene, three active AHH inducers, exhibit saturable binding with the rat hepatic receptor protein and the ligand-receptor complex sediments at 8-9 S under low ionic strength conditions using the sucrose density gradient technique (97, 105). The radioactive binding peaks were eliminated after competition with a 200-fold molar excess of 2,3,7,8-TCDD. Similar results were also observed with responsive C57BL/6 mouse hepatic cytosol. However, [^{3}H]-benzo[a]pyrene unexpectedly does not yield a radiolabelled ligand-receptor binding peak that is eliminated after competition with a 200-fold molar excess of unlabelled 2,3,7,8-TCDD.

Tissue/Organ, Strain, and Cell Culture Response Specificity

The 2,3,7,8-TCDD receptor levels in several organs and tissues in Sprague-Dawley rats, C57BL/6J and DBA/2J mice have been reported (96, 103, 104,

106). The cytosolic Ah receptor concentrations (fmol/mg cytosolic protein) in the C57BL/6J mice and Sprague-Dawley rat organs and tissues were: liver, 32 ± 1.5 and 39 ± 1.9; lung, 23 ± 6.4 and 47 ± 4.3; kidney, 10 ± 0.4 and 1.2 ± 0.9; intestine, 8 ± 2.3 and 15 ± 1.7; thymus, 8 ± 2.2 and 54 ± 3.9 (The Ah receptor was not detectable in the adrenals, heart, brain, skeletal muscle, and testis). Although the Ah receptor was not detected in the cytosol of DBA/2J mice, 18 hr after administration of [^{3}H]-2,3,7,8-TCDD to these animals levels of the receptor-ligand complex could be measured in nuclear protein extracts from liver (5.4 ± 0.3 fmol/mg), lung (7.4 ± 0.3 fmol/mg), and kidney (4.7 ± 0.1 fmol/mg). The appearance of hepatic nuclear radiolabeled ligand-receptor protein complexes and the elimination of this radioactivity by preinjection with a large excess of unlabeled 2,3,7,8-TCDD has been reported by several groups (89, 91, 96, 99, 102, 106); however, Mason & Okey (96) demonstrated that lung, liver, and kidney nuclear Ah receptor levels were higher in the responsive C57BL/6J mice than in the nonresponsive DBA/2J strain. This observation is consistent with the fact that 2,3,7,8-TCDD and other toxic halogenated aryl hydrocarbons elicit biologic and toxic responses in both strains of mice but at different dose levels. Unfortunately there are insufficient data available to correlate tissue/organ receptor levels with the magnitude of specific responses in these target sites.

The criteria for receptor response specificity are supported by numerous studies with genetically inbred responsive and nonresponsive strains of mice and with some mammalian cells in culture. For example, there is an excellent rank order correlation between the maximum AHH inducibility in several inbred strains of mice and F_1 hybrids and the number of Ah receptor molecules per liver cell (109). Nebert and co-workers have also shown a linear correlation ($r = 0.99$) between the amount of 2,3,7,8-TCDD-receptor complex appearing in hepatic nuclei of C57BL/6 and DBA/2 mice and the percentage of maximally induced cytochrome P_1-450 mRNA (104). Hudson and co-workers have demonstrated that for several human squamous cell carcinoma lines, the relative amount of receptor measured in each cell line correlated well with the 7-ethoxycoumarin 0-deethylase inducibility in these cells by 2,3,7,8-TCDD (110).

These data that support the receptor-mediated response specificity are in contrast to data in several other studies with animals and cell cultures. Hepatic 2,3,7,8-TCDD receptor levels in guinea pigs, rats, mice, hamsters, and nonhuman primates vary less than tenfold (10–100 fmol/mg cytosolic protein) and exhibit comparable K_D values for [^{3}H]-2,3,7,8-TCDD binding (93); these levels show no correlation between their maximal hepatic AHH inducibility or susceptibility to the toxic effects of 2,3,7,8-TCDD and related halogenated aryl hydrocarbons (93, 97, 108). For several mammalian cells in culture there is no correlation between receptor levels and their AHH inducibility (98, 99, 111–113). Recent studies by Whitlock and co-workers indicate "that transcription of

the cytochrome P_1-450 gene is under both positive and negative control by at least two trans-acting regulatory factors" (113). The factors that control cytochrome P_1-450 in variant mouse heptoma cells may also play a role in some animal species and requires further investigation. It is apparent that response specificity to Ah receptor ligands is a highly complex process that depends not only on receptor levels but also on many other factors, an observation not unique to the Ah receptor protein (113, 114).

Structure-Activity Relationships

RECEPTOR BINDING AFFINITIES OF PCDDs AND PCDFs: SUBSTITUTION EFFECTS Poland, Glover & Kende first reported the relative binding affinities of 23 halogenated dibenzo-p-dioxins and dibenzofurans using the dextran charcoal receptor assay and [^{3}H]-2,3,7,8-TCDD as the competing radioligand (85). This study included 10 PCDD congeners and 7 PCDF congeners that differed only with respect to their degree of chlorination and substitution pattern. Table 1 summarizes results from a more recent study of the effects of structure on the receptor binding affinities of 14 PCDDs and 14 PCDFs using rat hepatic cytosol and the sucrose density gradient assay procedure (47, 51; G. Mason, J. Pikorska-Pilszczynska, B. Keys & S. Safe, unpublished results). 2,3,7,8-TCDD and 1,2,3,7,8-pentachlorodibenzo-p-dioxin were the most avid PCDD competitive binding ligands for displacement of [^{3}H]-2,3,7,8-TCDD from the receptor protein, and their EC_{50} values were 1.0×10^{-8} and 7.9×10^{-8} M, respectively. Inspection of these data clearly demonstrated the importance of the lateral Cl substituents in facilitating the interaction between the PCDD ligands and the cytosolic receptor protein. The relative receptor binding EC_{50} values for a series of tetrachloro isomers were 2,3,7,8- > 2,3,6,7- > 1,3,7,8- > 1,2,3,4-, in the order of decreasing number of lateral substituents. The fivefold difference in the receptor binding activities of the 2,3,6,7- and 1,3,7,8-TCDD isomers illustrates a more subtle structural feature that affects binding. The increased affinity of the former compound must be due to the receptor binding site preference for a vicinal 6,7- (or, 1,2) group over a meta 1,3-dichloro functionality. The data also illustrate that the degree of chlorination of non-lateral sites is an important structural determinant for interaction with the receptor protein. The 2,3,7,8-tetra-, 1,2,3,7,8-penta-, 1,2,3,4,7,8-hexa-, and 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxins all contain four lateral Cl substituents; however, there is a marked decrease in their receptor binding avidities with increasing Cl substitution at the nonlateral 1, 4, 6, and 9 positions. The stepwise addition of Cl groups at 1, 4, 6, and 9 would result in several structural changes in the more highly chlorinated PCDDs including increased molecular size and volume, increased lipophilicity, a possible decrease in PCDD coplanarity associated with steric crowding, and decreased

Table 1 The effects of structure on the rat hepatic cytosolic receptor binding affinities and AHH/EROD induction potencies of PCDDs and PCDFs

	In Vitro EC_{50} (M)				In Vitro EC_{50} (M)		
PCDD	Receptor Binding	AHH	EROD	PCDF	Receptor Binding	AHH	EROD
2,3,7,8-	1.0×10^{-8}	7.2×10^{-11}	1.9×10^{-10}	2,3,4,7,8-	1.5×10^{-8}	2.6×10^{-10}	1.3×10^{-10}
1,2,3,7,8-	7.9×10^{-8}	1.1×10^{-8}	1.7×10^{-8}	2,3,4,7-	2.5×10^{-8}	1.8×10^{-8}	1.5×10^{-8}
2,3,6,7-	1.6×10^{-7}	6.1×10^{-8}	1.1×10^{-8}	2,3,7,8-	4.1×10^{-8}	3.9×10^{-10}	2.0×10^{-10}
2,3,6-	2.2×10^{-7}	—	—	2,3,4,6,7,8-	4.7×10^{-8}	6.9×10^{-10}	5.8×10^{-10}
1,2,3,4,7,8-	2.8×10^{-7}	2.1×10^{-9}	4.1×10^{-9}	1,2,3,7,8-	7.5×10^{-8}	2.5×10^{-9}	3.1×10^{-8}
1,3,7,8-	7.9×10^{-7}	5.9×10^{-7}	3.2×10^{-7}	1,2,3,7-	1.1×10^{-7}	2.7×10^{-5}	6.3×10^{-5}
1,2,4,7,8-	1.1×10^{-6}	2.1×10^{-8}	1.1×10^{-8}	1,3,4,7,8-	2.0×10^{-7}	1.6×10^{-9}	1.4×10^{-9}
1,2,3,4-	1.3×10^{-6}	3.7×10^{-6}	2.4×10^{-6}	2,3,4,7,9-	2.0×10^{-7}	7.9×10^{-9}	5.8×10^{-9}
2,3,7-	7.1×10^{-8}	3.6×10^{-7}	1.4×10^{-7}	2,3,4,8-	2.0×10^{-7}	4.1×10^{-8}	3.8×10^{-8}
2,8-	3.2×10^{-6}	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$	1,2,3,4,7,8-	2.3×10^{-7}	3.6×10^{-10}	3.8×10^{-10}
1,2,3,4,7-	6.4×10^{-6}	6.6×10^{-7}	8.2×10^{-7}	1,2,3,6,7,8-	2.7×10^{-7}	1.5×10^{-9}	1.2×10^{-9}
1,2,4-	1.3×10^{-5}	4.8×10^{-5}	2.2×10^{-6}	1,2,3,7,9-	4.0×10^{-7}	8.6×10^{-8}	8.6×10^{-8}
OCDD	$>1.0 \times 10^{-5}$	3.1×10^{-7}	7.0×10^{-7}	1,2,4,7,8-	1.3×10^{-6}	1.1×10^{-7}	1.5×10^{-7}
1-	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$	1,2,4,6,8-	3.1×10^{-6}	1.0×10^{-5}	1.2×10^{-5}

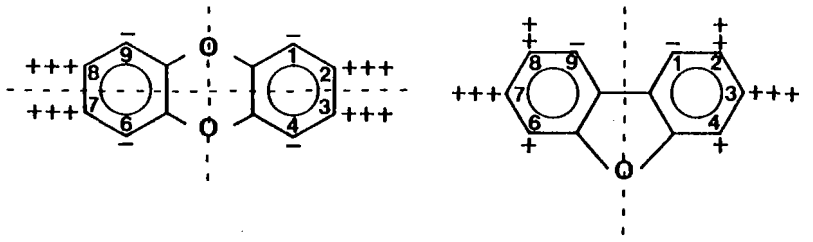


Figure 2 The differential effects of chlorine substituents at different positions in the dibenzo-p-dioxin and dibenzofuran rings on the relative receptor binding affinities of PCDD and PCDF congeners.

aromatic ring electron density (due to the additional electronegative Cl groups). One or more of these changes may be related to the decrease in binding affinities of the more highly chlorinated 2,3,7,8-substituted PCDDs. Figure 2 summarizes the differential effects of Cl substituents on the affinities of PCDDs for the cytosolic receptor protein and illustrates the importance of lateral chloro groups. It has also been suggested that the other critical structural factors that contribute to the high binding affinities of 2,3,7,8-TCDD include the planar ring structure and an ideal ligand area, 3 × 10 Å (37, 38).

The direct binding of radiolabelled 2,3,7,8-tetrachlorodibenzofuran (TCDF) and other PCDFs to the Ah receptor protein has not been demonstrated. However, the competitive binding affinities of three 2,3,7,8-substituted compounds demonstrated their relatively high binding affinities for the receptor protein (85). The development of new procedures for the synthesis of PCDFs (115) has resulted in the preparation of over 40 congeners that have been used to develop detailed SARs for this series of halogenated aryl hydrocarbons (47, 51). The dibenzofuran ring system possesses a single axis of symmetry (Figure 2); therefore there are four geometrically different positions on each aromatic ring, namely C-1 (or C-9), C-2 (or C-8), C-3 (or C-7), and C-4 (or C-6). A complete SAR for PCDFs as ligands for the receptor protein must distinguish between the differential contributions of all four positions on the dibenzofuran ring. (See Table 1 for compounds selected for this study.) Inspection of these data confirms that the most active congeners, 2,3,4,7,8-pentachlorodibenzofuran (PeCDF, 1.5×10^{-8} M), 2,3,7,8-TCDF (4.1×10^{-8} M), 2,3,4,6,7,8-hexachlorodibenzofuran (HCDF, 4.7×10^{-8} M), and 1,2,3,7,8-PeCDF (7.5×10^{-8} M) were all fully substituted in their lateral 2, 3, 7, and 8 positions. Moreover, a comparison of the receptor binding EC_{50} values for the 2,3,4,7,8-, 1,2,4,7,8-, and 1,2,4,6,8-PeCDF isomers demonstrates the importance of lateral chloro substituents since there is a decrease in receptor binding affinities with decreasing lateral substitution. Two pairs of PCDF isomers, namely 1,3,4,7,8- and 1,2,4,7,8-PeCDF, 2,3,4,7- and 2,3,4,8-TCDF, differ only with respect to their substitution of C-2 (or C-8) and C-3 (or

C-7). In both cases the C-3 (or C-7) substituted compounds were 6.5–8 times more active than the corresponding C-2 (or C-8) isomers as competitive ligands for the rat hepatic cytosolic receptor protein. A comparison of the relative binding affinities of a series of C-1 (or C-9) and C-4 (or C-6) isomer pairs illustrates the higher binding activities of the isomer that retains the C-4 (or C-6) substituent. For example the EC_{50} values for the 2,3,4,7-, 2,3,4,7,8-, 2,3,4,7,9-, and 2,3,4,6,7,8-substituted PCDFs were 2.5×10^{-8} M, 1.5×10^{-8} M, 2.0×10^{-7}, and 4.7×10^{-8} M whereas the values for the corresponding C-1 (or C-9) isomers (i.e. 1,2,3,7-TCDF, 1,2,3,7,8-, and 1,2,3,7,9-PeCDF, and 1,2,3,6,7,8-HCDF) were 1.1×10^{-7} M, 7.5×10^{-8} M, 3.4×10^{-7} M, and 2.7×10^{-7} M, respectively.

Figure 3 illustrates an overlay of 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF, the two most active PCDD and PCDF ligands for the Ah receptor. The molecular areas and volumes of the dibenzofuran and dibenzo-p-dioxin ring systems are similar, but the spatial orientations of their substituents exhibit marked differences. The C-3 (or C-7) substituents occupy a position between the lateral 2,3 (or 7,8) groups in 2,3,7,8-TCDD and clearly occupy the dominant lateral position in the dibenzofuran ring system. The spatial orientations of the C-4 (and C-6) and C-2 (and C-8) substituents are comparable and exhibit less overlap with the lateral positions of 2,3,7,8-TCDD; the C-1 (or C-9) PCDF-substituents exhibit the least overlap with the lateral positions of 2,3,7,8-TCDD. These observations on the molecular orientations of the dibenzofuran Cl substituents are consistent with the observed SARs for PCDF receptor binding affinities and illustrate the stereospecific nature of the receptor protein-ligand interactions.

RECEPTOR BINDING AFFINITIES OF PCDDs AND PCDFs: A QSAR ANALYSIS The receptor binding avidities of PCDDs and PCDFs summarized in Table 1 and in other studies (37, 38, 47, 51) not only demonstrate the importance of Cl substitution patterns on ligand-receptor protein complex formation but also show that substituents are important structural determinants for these interactions. For example, the receptor binding EC_{50} values for 2,3,7-trichlorodibenzo-p-dioxin is 7.1×10^{-8} M; replacement of the 7-Cl substituent with H gives 2,3-dichlorodibenzo-p-dioxin, which exhibits a greatly diminished receptor binding EC_{50} value ($> 10^{-5}$ M). It is clear that these substituent effects at this lateral C-7 position must be related to differences in their physicochemical characteristics which in turn influence ligand-receptor avidities. A series of substituted PCDD, PCDF, and polychlorinated biphenyl analogs have been synthesized (Figure 4) as probes for delineating the effects of substituent structure on ligand-receptor binding affinities (116–118). Each series of analogs contains a variable substituent group at a single lateral position, and it is apparent that substituent structure has a remarkable effect on

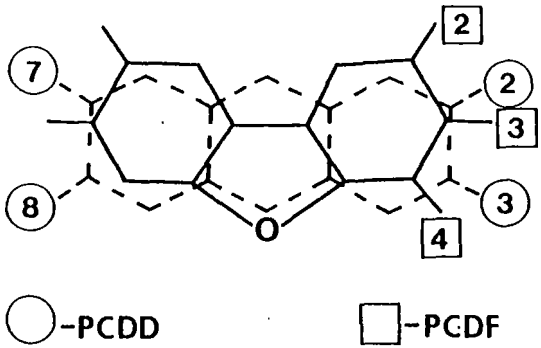


Figure 3 Overlay of 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF structures.

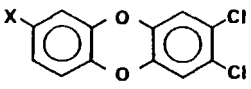
the receptor binding avidities of these compounds. For example the EC_{50} value for 7-trifluoromethyl-2,3-dichlorodibenzo-p-dioxin (1.95×10^{-8} M) was 1000 times lower than the value for 7-amino-2,3-dichlorodibenzo-p-dioxin (2.88×10^{-5} M). The effects of different substituents on the activity of a series of analogs can be analyzed quantitatively by correlating the differences in a biological effect (e.g. receptor binding) with known substituent physicochemical parameters (116–119), such as lipophilicity (π), electronegativity (σ), hydrogen bonding capacity (HB), and substituent width (ΔB_5). Multiparameter linear regression analysis of the receptor binding data for sixteen 7-substituted-2,3-dichlorodibenzo-p-dioxins gave the following equation (1):

$$\log (1/EC_{50}) = 1.24\pi + 6.11 \qquad 1.$$

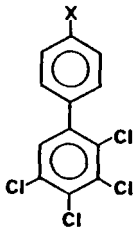
$$(n = 14,\ s = 0.29,\ r = 0.950),$$

where π is the substituent lipophilicity, s is the standard deviation, and r is the correlation coefficient. The only substituents treated as outliers for the derivation of this equation were the bulky C_6H_5 and t-C_4H_9 groups, which possess van der Waals volumes of 48.5 and 41.8 cm^3/mol, respectively. This suggests that substituent molecular volumes are also important structural determinants for determining ligand affinities for the receptor protein binding site. Previous studies with the 4'-substituted-2,3,4,5-tetrachlorobiphenyls indicated that the maximum molecular volume for lateral substituents was < 35 cm^3/mol (118). However it is apparent that if substituent molecular volume requirements are satisfied, the the receptor binding affinities of these analogs are directly related to the lipophilicity of the 7-substituents.

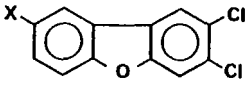
The competitive receptor binding affinities of a series of thirteen 8-substituted-2,3,4-trichlorodibenzofurans and ten 8-substituted-2,3-dichlorodibenzofurans (Figure 4) have also been determined, and the competi-



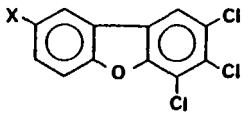
X=CF_3, Br, I, Cl, F, OH, CH_3, OCH_3, NO_2, CN, NH_2



X=H, OH, CH_3, F, OCH_3, $COCH_3$, CN, Cl, CH_2CH_3, Br, I, $CH(CH_3)_2$, CF_3



X=t-C_4H_9, F, Br, I, i-C_3H_7, Cl, CH_3, OCH_3, OH, H



X=Cl, Br, CF_3, I, F, CH_3, i-C_3H_7, C_2H_5, t-C_4H_9, H, OCH_3, OH, CH_2Br

Figure 4 Structures of substituted PCDDS, PCDFs, and polychlorinated biphenyls used for QSAR studies.

tive displacement binding data results have been analyzed by multiparameter linear regression analysis to give Equations 2 and 3, respectively.

$$\log (1/EC_{50}) = 1.09\pi + 5.77 \quad 2.$$

$$\log (1/EC_{50}) = 1.10\pi + 5.19 \quad 3.$$

For the 8-substituted-2,3,4-trichlorodibenzofurans, both the t-C_4H_9 and i-C_3H_7 were outliers, whereas only the t-C_4H_9 substituent was not included in the derivation of Equation 3. Analysis of the collective data for 33 substituted polychlorinated dibenzofurans and dibenzo-p-dioxins (Figure 5) has demonstrated the excellent linear correlation between the log ($1/EC_{50}$) receptor binding data and lipophilicity (π). Moreover the slopes and intercepts for Equations 1–3 were not significantly different. These data are consistent with a receptor protein that binds the PCDDs and PCDFs at a common binding site(s) on the protein; this site must accommodate the molecular area and volume encumbered by these ligands, and the QSAR results are consistent with a binding site that is highly hydrophobic.

The effects of substituent structure on the rat hepatic cytosolic receptor binding affinities of 4-substituted-2,3,4,5-tetrachlorobiphenyls have also been

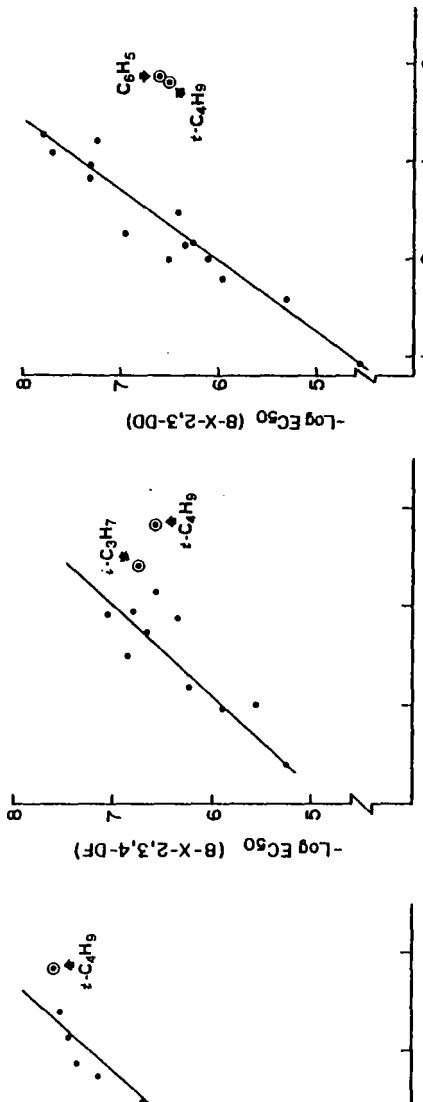


Figure 5 Correlation between receptor binding avidities for the substituted PCDDs and PCDFs vs the substituent lipophilicity (π) values.

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reported (118). Multiparameter linear regression analysis of the results of these analogs gave Equation 4, which suggests that substituent lipophilicity, electronegativity (σ) and hydrogen bonding capacity (HB) are factors that influence receptor-ligand affinities:

$$\log (1/EC_{50}) = 1.39\,\sigma + 1.31\pi + 1.12\,HB + 4.20 \qquad 4.$$

The bulky t-C_4H_9 and C_6H_5 substituents were also treated as outliers for the derivation of Equation 4. These results demonstrate that substituent molecular volumes play a role in ligand-receptor interactions for the substituted PCBs, PCDDs, and PCDFs. However, the data analysis for the former group of analogs suggests that the critical 4'-substituent for the PCBs interacts with both polar and hydrophobic regions of the receptor binding site. The differences between Equations 1–3 and Equation 4 are somewhat paradoxical since molecular overlap of the four sets of substituted ligands does not indicate that there are major differences in their spatial orientation (Figure 4). Therefore it is likely that the differences observed for the substituted PCBs are due to the free rotation about the Ph-Ph bond and a limited population of the coplanar conformers. Chlorinated biphenylenes that possess a fixed coplanar ring structure exhibit binding affinities comparable to those of 2,3,7,8-TCDD and related isostereomers; this emphasizes the importance of a planar ring system. It is also possible that the receptor binding sites for the substituted PCBs and PCDDs/PCDF are not identical, a problem currently being investigated in my laboratory.

AHH INDUCTION ACTIVITIES OF PCDDs AND PCDFs The in vivo and in vitro SARs for PCDDs and PCDFs as inducers of hepatic and extrahepatic AHH have been reported by several groups (37, 38, 47–49, 51–53). The most active PCDDs were substituted in their 2,3,7, and 8 position; inspection of the data in Table 1 indicates that there were comparable SARs for PCDDs as ligands for the receptor protein and as AHH inducers; however, there is not a linear correlation between these two bioassays. SARs for several PCDF congeners as in vitro AHH inducers were comparable to those already discussed for receptor binding. Moreover, for the PCDFs summarized in Table 1 a comparison of in vitro EC_{50} values for AHH induction in rat hepatoma H-4-II E cells and in vivo ED_{50}s for AHH induction in male Wistar rats showed a linear correlation between these two values. Like the PCDD congeners, however, there was not a strong correlation between AHH induction potencies and receptor binding avidities for the PCDF congeners. A comparison of the AHH and EROD induction potencies of the 7-substituted-2,3-dichlorodibenzo-p-dioxins with their rat hepatic cytosolic receptor binding avidities also showed that there was not a linear correlation between the two in vitro activities for this series of

analogs (116). Multiple parameter linear regression analysis of the AHH induction results for these compounds gave the following equation:

$$\log (1/EC_{50})_{AHH} = 1.60\,\pi - 0.33(\Delta B_5)^2 + 5.85 \qquad 5.$$

Like the receptor binding avidities for these substituted PCDDs, their AHH induction potencies were dependent on substituent lipophilicity; however, a second parameter, STERIMOL (ΔB_5) has also been included in the derivation of this equation. The STERIMOL parameter (119) is a measure of the maximum width of the substituents (compared to H) from the axis connecting the 7-substituent to the rest of the molecule and has previously been used in QSAR studies that involve the interaction of substituted organic ligands and macromolecules. The dependence of AHH induction activities by the substituted PCDDs on both ΔB_5 and π suggests that substituent-dependent effects such as conformational changes in the ligand-receptor complex occur after the initial receptor-ligand binding process.

The AHH and EROD induction EC_{50} values for the 8-substituted-2,3-di- and 2,3,4-trichlorodibenzofurans in rat hepatoma H-4-II E cells were also subjected to multiple parameter linear regression analysis to give Equations 6 and 7, respectively (117).

$$\log (1/EC_{50})_{AHH} = 0.80\pi + 0.87\Delta B_5 - 0.35(\Delta B_5)^2 + 4.63 \qquad 6.$$

$$\log (1/EC_{50})_{AHH} = 0.76\pi + 1.11\Delta B_5 + 2.23\,\sigma_p + 6.78 \qquad 7.$$

Both Equations 5 and 6 showed that AHH induction potencies for the 8-substituted-2,3-dichlorobenzofurans and 2,3-dichlorodibenzo-p-dioxins were dependent on substituent and ΔB_5 parameters. The correlation for the more highly chlorinated set of analogs, the 8-substituted-2,3,4-trichlorodibenzofurans, also includes a Hammett substituent parameter (σ_p); presumably the requirement for σ_p must be due to the effects of the C-4 chlorine group, which constitutes the only structural difference between the two sets of substituted PCDF analogs.

Equations 8–10 were developed from the AHH and EROD induction data for the 8-substituted-2,3-dichlorodibenzo-p-dioxins, -2,3-dichlorodibenzofurans, and -2,3,4-trichlorodibenzofurans, respectively.

$$\log (1/EC_{50})_{EROD} = 0.99 \log (1/EC_{50})_{AHH} - 0.07 \qquad 8.$$

$$\log (1/EC_{50})_{EROD} = 0.90 \log (1/EC_{50})_{AHH} + 0.83 \qquad 9.$$

$$\log (1/EC_{50})_{EROD} = 0.92 \log (1/EC_{50})_{AHH} + 0.28\pi + 0.27 \qquad 10.$$

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For a total of twenty-five 7-substituted-2,3-dichlorodibenzo-p-dioxins and 8-substituted-2,3-dichlorodibenzofurans and fifteen 4'-substituted-2,3,4,5-tetrachlorobiphenyls there was a linear correlation between the EC_{50} values for AHH and EROD induction, and the slopes for these equations were not significantly different from that of equation one. These data suggest that both ethoxyresorufin and benzo[a]pyrene are catalyzed by the same cytochrome P-450 isozyme(s). In contrast, Equation 10 required a π term to correlate the effects of the substituted 2,3,4-trichlorodibenzofurans; the rationale for these differences between the two sets of substituted PCDF analogs is unknown.

OTHER BIOLOGIC EFFECTS OF PCDDs AND PCDFs: STRUCTURE-ACTIVITY RELATIONSHIPS PCDDs and PCDFs elicit a broad spectrum of species-dependent biologic effects, but because pure standards have been unavailable few studies report qualitative or quantitative SARs for these compounds. The SARs for PCDDs as inducers of ALA synthetase were comparable to the effects of structure on their activities as AHH inducers (48). Knutson & Poland have used cultured XB cells derived from a mouse teratoma as an in vitro model for halogenated aryl hydrocarbon toxicity (120). 2,3,7,8-TCDD and related toxic isostereomers produce a dose-dependent keratinization response which in part resembles the in vivo dermal toxicity that develops in some animals after exposure to these toxins. The most active PCDD congeners in this in vitro assay possessed three or four lateral substituents, and the SARs were similar to those reported for their receptor binding affinities. 2,3,7,8-TCDD causes comparable dermal toxicity in cultures of newborn foreskin keratinocytes (121).

Several human squamous cell carcinoma (SCC) lines have been utilized as model systems for investigating the mechanism of action of toxic halogenated aryl hydrocarbons (110, 122). SCC cells possess variable Ah receptor levels, and the relative amount of receptor in several cell lines correlates with the maximal 7-ethoxycoumarin 0-deethylase inducibility in these cell lines. 2,3,7,8-TCDD causes down-regulation of the epidermal growth factor (EGF) receptor in the SCC-12F cell line, and this effect is dose- and structure-dependent. Both 2,3,7,8-TCDD and 2,3,7,8-tetrabromodibenzofuran, which exhibit a high affinity for the Ah receptor, decrease EGF receptor binding, whereas 2,7-dichlorodibenzo-p-dioxin is inactive (123). Comparable results have been observed in keratinocyte strains derived from normal neonatal foreskin; the authors report that 2,3,7,8-TCDD acts (in part) through the Ah receptor in epidermal basal cells to enhance terminal differentiation (123). 2,3,7,8-TCDD also down-regulates EGF receptor activity in hepatic plasma membranes in several animal species and cultured mouse hepatoma cells (124, 125); however, structure-activity effects in the rat do not necessarily support the role of the Ah receptor in mediating this process (125).

TOXICOLOGY OF PCDDs AND PCDFs: STRUCTURE-ACTIVITY RELATIONSHIPS

The dose-response acute toxicities of nine PCDD isomers and congeners in the guinea pig and responsive mouse have been reported (33, 34, 126). The relative LD_{50} values in both species were highly dependent on the number of lateral Cl substituents and the degree of substitution; their rank order of toxic potencies was similar to their in vitro receptor binding and AHH induction activities as discussed above. A comparative study (127) of the toxicity of 2,3,7,8-TCDF, 2,3,7,8-tetrabromodibenzofuran, and 2,3,4,7,8-pentachlorodibenzofuran in guinea pigs, mice, and rhesus monkeys confirms that these compounds elicit the characteristic broad spectrum of toxic effects observed for 2,3,7,8-TCDD and related isostereomers.

Poland & Glover (75) reported the effects of several PCDD congeners and other toxic halogenated aryl hydrocarbons on genetically inbred strains of mice. Although dose-response studies were carried out only with 2,3,7,8-TCDD, the relative toxicities (i. e. thymic atrophy) of these compounds correlated with their in vitro binding and induction activities. The dose-response toxicities (thymic atrophy and body weight loss) of the PCDFs listed in Table 1 have been determined in the immature male Wistar rat (47). Inspection of the ED_{50} data for the toxic effects showed that the potencies of these congeners were structure-dependent and that the in vivo SARs for toxicity were identical to those observed for their in vitro AHH induction potencies. Figure 6 summarizes a plot of the $-\log ED_{50}$ values for thymic atrophy and body weight loss in immature male Wistar rats vs their in vitro AHH induction activities. The linear correlation constant (r) and slope for the plots of the reciprocal log values for AHH induction vs body weight loss were 0.96 and 1.30 (slope), respectively, and values of 0.88 (r) and 1.16 (slope) were obtained for the comparable plot of the reciprocal log values for AHH induction vs thymic atrophy. The linear correlation was observed only for those compounds that do not contain vicinal unsubstituted carbon atoms and are not significantly metabolized. For example, the toxicity of 1,2,3,7- or 2,3,4,8-TCDF in the rat was lower than predicted by the in vitro AHH (or EROD) induction data (not shown) owing to in vivo metabolism. Current research in my laboratory (S. H. Safe, unpublished results) indicates that for several PCDD isomers and congeners there is a linear correlation between $-\log EC_{50}$ (AHH induction) and $-\log ED_{50}$ (thymic atrophy and body weight loss in the rat). These results suggest that the rat hepatoma cell monooxygenase induction bioassay may serve as a short-term test system for predicting the toxicities of PCDDs, PCDFs, and related halogenated aryl hydrocarbons.

Poland and co-workers have investigated the effects of 2,3,7,8-TCDD, several PCDD congeners, and related halogenated aryl hydrocarbons in the skin of inbred HRS/J hairless mice segregating for the *hr* locus (128, 129). The homozygous *hr/hr* hairless and heterozygous *hr/+* haired mice exhibit identical

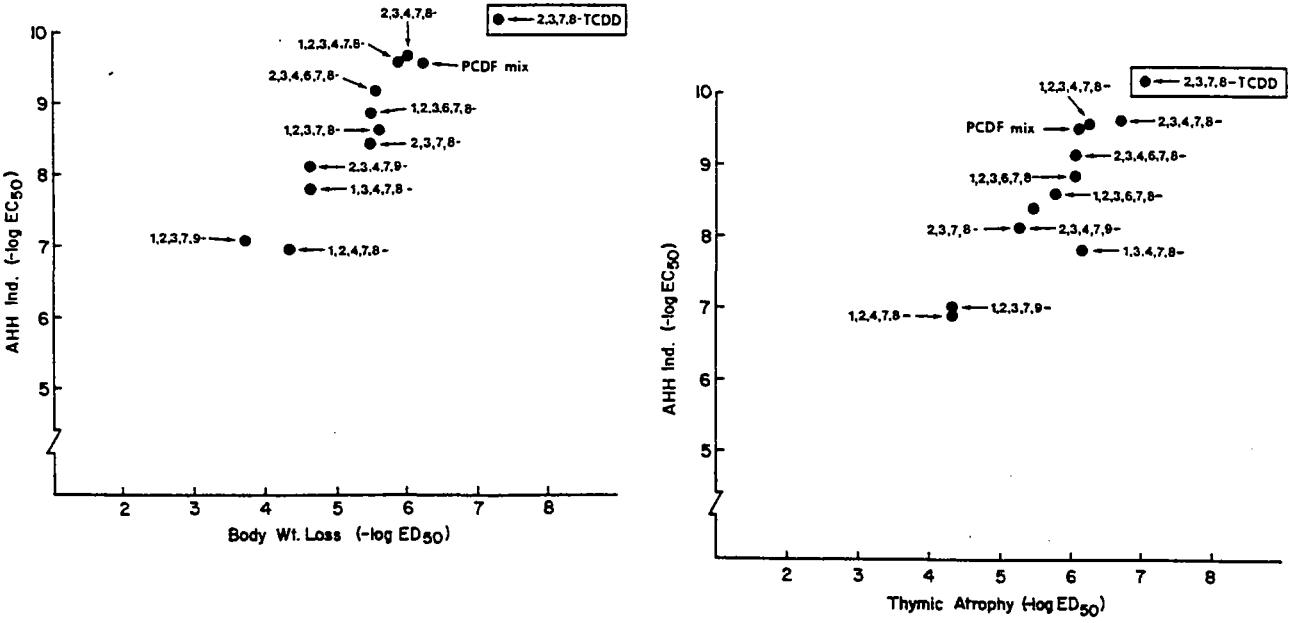


Figure 6 A plot of the $-\log EC_{50}$ values for in vitro AHH induction vs the $-\log ED_{50}$ values for thymic atrophy (right) and body weight loss (left) in the male Wistar rat for several PCDF congeners and 2,3,7,8-TCDD.

genetics except for one allele at the *hr* locus. Topical application of 2,3,7,8-TCDD to the dorsal skin of hairless mice resulted in epidermal hyperplasia, sebaceous gland metaplsia, and hyperkeratosis, but these histological lesions are not observed in *hr*/+ haired mice. The development of a graded epidermal response by several PCDD congeners, 2,3,7,8-TCDF, and related toxic aryl hydrocarbons was structure-dependent and correlated with rank order of receptor binding affinities for these compounds (128). For example, the total dose (nmol/mouse) required to produce a 2^+ response was 0.36, 1.76, 1.2 > 360 and > 360 for 2,3,7,8-TCDD, 1,2,3,6,7,8-HCDF, 2,3,7,8-TCDD, 1,3,6,8-TCDD, and 2,7-DCDD, respectively. These results are consistent with the involvement of both the *Ah* and *hr* loci in the development of murine epidermal lesions after exposure to PCDDs, PCDFs, and related toxins. 2,3,7,8-TCDD can also act as a potent tumor promoter in HRS/J mice, and the results of this study confirm the segregation of the activity with the *Ah* and *hr* loci. A qualitative structure-activity study also suggests a possible role for the Ah receptor in mediating the tumor promotion activities of the toxic halogenated aryl hydrocarbons (130).

The SARs for PCDDs and PCDFs clearly support an Ah receptor-mediated mechanism of action for these compounds; comparable studies have been reported for other classes of halogenated aryl hydrocarbons. It is assumed that the persistent effects elicited by these toxins are related to a sustained receptor-ligand occupancy of nuclear binding sites, but this has not yet been demonstrated experimentally. The identity and role of any endogenous ligand(s) for the Ah receptor have not been determined; however, lumichrome, a riboflavin metabolite, does bind to the receptor (131).

Genetic Evidence

Pharmacogenetic studies with genetically inbred strains of mice typefied by the Ah-responsive C57BL/6 and nonresponsive DBA/2 mice have provided strong evidence in support of the role of the Ah receptor in mediating the biologic and toxic effects of toxic halogenated aryl hydrocarbons. Nonresponsive DBA/2 mice contain relatively low levels of hepatic or extrahepatic cytosolic or nuclear Ah receptor (< 1 fmol/mg cytosolic protein), whereas much higher levels of the receptor are detected in responsive strains of mice. The ED_{50} for 2,3,7,8-TCDD mediated hepatic microsomal AHH induction in C57B1/6J mice was 1 nmol/kg whereas this value is at least tenfold higher in DBA/2J mice. In genetic crosses and backcrosses between C57BL/6J and DBA/2J mice the trait or responsiveness to AHH induction is inherited in a simple autosomal mode (50, 132–134). The responsive backcross animals also had detectable hepatic receptor levels (85, 89, 97, 103, 109). The segregation of the toxicity of PCDDs and PCDFs with the Ah locus has been determined primarily with 2,3,7,8-TCDD using both responsive and nonresponsive genetically inbred mice and their

crosses and backcrosses. The results illustrate that several toxic effects including teratogenicity, porphyria and hepatotoxicity, immunotoxicity, and body weight loss segregate with the Ah locus (75–80, 135–139). Dermal toxic lesions appear to be dependent on the interaction between the Ah and hr locus as previously noted (128–130). It has also been suggested that additional genetic loci may also be involved in the hepatotoxic effects of 2,3,7,8-TCDD, however this observation requires further substantiation (140).

PCDDs, PCDFs, AND RELATED COMPOUNDS—INTERACTIVE EFFECTS

Although the SARs and toxicology of PCDDs and PCDFs have been extensively studied the interactive effects of PCDD/PCDF mixtures and related compounds are not well understood. 2,3,7,8-TCDD can act as a tumor promoter for several initiators in the rodent liver (141), mouse skin (130), and C3H/10T1/2 cells (142), and as a cocarcinogen causing 3-methylcholanthrene-initiated subcutaneous tumors in nonresponsive DBA/2 mice (143). In contrast, 2,3,7,8-TCDD exhibits anticarcinogen activity in female CD-1 mice (144). These effects are related to the agent's induction of drug-metabolizing enzymes that alter rates of metabolic activation of polynuclear aromatic hydrocarbon initiators. Several studies (51, 53, 120, 145, 146) report the application of in vitro bioassays as short-term tests for assessing the potential toxicity of PCDD/PCDF mixtures; a comparison of the in vitro AHH induction activity of a reconstituted mixture of PCDFs identified in Yusho patients (147) and the toxicity of this mixture (Figure 6) suggest that the effects of the individual PCDFs in this mixture are additive (148). In contrast, the immunotoxicity and AHH induction activity of 2,3,7,8-TCDD in C57BL/6 mice were decreased by coadministering a nontoxic or noninducing dose of 2,3,7,8-TCDF (10 μg/kg) (149). A rational explanation for the antagonistic effects of 2,3,7,8-TCDF is not apparent.

Birnbaum and co-workers (77) have reported that treatment of pregnant mice with a combination of 2,3,7,8-TCDD (3 μg/kg) and a nontoxic dose of 2,3,3',4,4',5-hexachlorobiphenyl (20 μg/kg) resulted in a tenfold increase in incidence in cleft palate compared to those animals receiving only 2,3,7,8-TCDD. A second PCB congener, 2,2',4,4',5,5'-hexachlorobiphenyl, at dose levels of 50 or 25 mg/kg in combination with 2,3,7,8-TCDD (3μg/kg), did not effect the teratogenic potency of the latter compound. Although 2,3,3',4,4',5-hexachlorbiphenyl is less toxic than 2,3,7,8-TCDD, this monortho coplanar PCB congener elicits several receptor-mediated biologic and toxic effects (74). It is conceivable that the interactive effects of 2,3,3',4,4',5-hexachlorobiphenyl and 2,3,7,8-TCDD may be additive if the dose-response curve for the former compound is steep and if the 20 mg/kg dose level is just

below the minimum observable teratogenic dose. Current research in my laboratory has demonstrated that administration of several compounds, including several polychlorinated biphenyl congeners, increase hepatic 2,3,7,8-TCDD receptor levels in rats and C57BL/6 mice. Pretreatment of rats and mice with these receptor modulators followed by administration of 2,3,7,8-TCDD results in markedly increased hepatic AHH and EROD induction activities. Both positive and negative modulators and antagonists of the hepatic and extrahepatic cytosolic receptor protein are currently being investigated as probes for delineating the mechanism of action of PCDDs and PCDFs and the role of the receptor protein in mediating these effects. These interactive studies will also be important for assessing the effects of polyhalogenated aromatic environmental pollutant mixtures and their potential human health impact.

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pound, it does not vaporize and thus will only be found in the atmosphere when carried on dust particles. Dioxin has a very low solubility in water and, when released in rivers or lakes, eventually settles to the bottom and accumulates in the sediment.

Dioxin binds tightly to soil. This tendency keeps dioxin from migrating through soil, making contamination of ground water highly unlikely.[1] Soil particles protect the compound from the ultraviolet rays of the sun, which would otherwise cause the dioxin to break down rapidly.[2] In most cases of environmental dioxin contamination, the vast majority of the chemical has remained on or near the soil surface.

The half-life, or time required for the breakdown of half the dioxin residue present, has been estimated at one to three years.[1] However, if the dioxin does manage to settle deep beneath the surface, its half-life may exceed ten years because of the additional protection against the sun's ultraviolet rays.

In the sediment of a river or lake, dioxin undergoes a slow yet significant degree of microbiological degradation. The half-life in this environment has also been estimated to be one to three years.[1]

If dioxin is generated in tiny amounts, why is there so much concern about a health threat?

Animal experiments have shown dioxin to be extremely toxic to some animals, even in small amounts. The compound has caused a number of adverse effects in laboratory animals including severe blood chemistry changes, liver damage, skin disorders, lung lesions, loss of weight and death.

Animal species vary greatly in their susceptibility to dioxin poisoning. Guinea pigs are the most sensitive of the species tested. Indeed, dioxin's reputation as being extremely toxic is based primarily on tests done on these highly susceptible animals. Hamsters, in contrast, are approximately 5,000 times more resistant than guinea pigs to a single dose of dioxin. The variability of dioxin sensitivity is presented in Table 1, which lists the dose necessary to kill half of the test animals (called LD_{50} value) for a number of species.

Dioxin is also an animal carcinogen, causing many different tumor types at a number of sites including the liver and thyroid. The specific roles which dioxin plays in cancer development, however, are still being explored. Depending on the test species, dose and other experimental conditions, dioxin may act as a co-carcinogen with another cancer-causing agent or even, surprisingly enough, an anti-carcinogen, meaning that the presence of dioxin inhibits some carcinogens from

TABLE 1
DIOXIN LD_{50} VALUES

Animal	LD_{50} (μg/kg body weight)
Guinea Pig	1
Rat-male	22
Rat-female	45
Monkey	<70
Mouse	114
Rabbit	115
Dog	>300
Bullfrog	>500
Hamster	5,000

Source: Poland and Knutson, *Annual Review of Pharmacology & Toxicology*, 1982.

inducing cancer in laboratory animals.[3] Evidence that it is an actual *initiator* of cancer remains weak. Reproduction studies (three generations) with dioxin have shown that it is not mutagenic in mammals.[4]

Maternal exposure to relatively high levels of dioxin during critical periods of fetal development causes birth defects and fetal death in many animal species. While the defects observed vary from species to species, the high frequency of adverse effects leaves no doubt that dioxin can cause serious reproductive problems, at least at doses above some minimum level, in the female test animal. There is no scientific evidence that male sperm cells are affected by dioxin.

In what kinds of situations are people exposed to dioxin?

Human exposure to dioxin can be separated into four distinct groups:

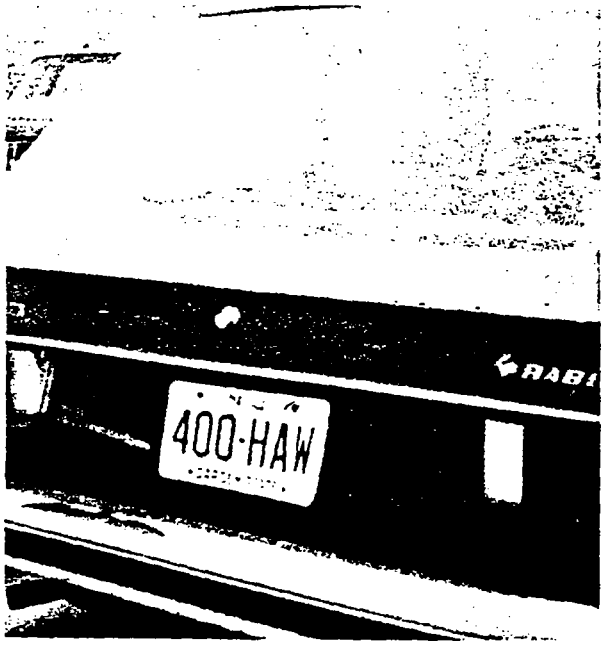
1) Accidents at chemical manufacturing plants have exposed workers to large single doses (and in the case of Seveso, Italy, local residents as well);

2) Occupational exposure to herbicides has exposed applicators over long periods, including U.S. Air Force personnel who were involved in Agent Orange spraying, and others who work with dioxin-containing products;

3) Contamination of the general environment may expose local residents to low doses over potentially lengthy periods, primarily from improper disposal of contaminated industrial waste;

4) It is believed that very, very low levels of dioxin con-

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ACSH: J. Jones

Exhaust systems of vehicles driven by diesel engines have been shown to contain trace amounts of dioxin, suggesting that dioxin is formed during the combustion of diesel fuel.

tamination are present everywhere, at least in the developed countries, due to the use of herbicides and solvents and the burning of wastes. Dioxin has been detected in pristine lakes, in freshwater fish and in normal human tissues.

What are the known health effects in humans of dioxin exposure?

Only two health effects have so far been repeatedly observed following dioxin exposure; and both are reversible. The first is a skin disorder called chloracne and the second is a general "not feeling well" syndrome including sleeplessness, headache, nausea and irritability. Other effects have been reported in single studies at borderline levels of significance and have thus far failed to be confirmed in any second study. These included: soft-tissue sarcoma (cancer) in Swedish herbicide applicators, increased incidence of stomach ulcers, some birth defects among the offspring of Vietnam veterans and some small changes in certain metabolic measurements. Conclusions are difficult to draw from the single-study effects because any random sample from an unaffected population is likely to display *some* statistically improbable results if many different properties are measured and tested for significance.

Despite the frightening image which the word "dioxin" has come to represent, there are no known human deaths from exposure to this substance. Some contamination episodes are described below:

Monsanto Workers

An accident at Monsanto Chemical's plant in Nitro, West Virginia on March 8, 1949 exposed more than 200 workers to dioxin. Fumes containing dioxin were emitted when a relief valve opened as a result of an explosion. One hundred twenty-two workers developed chloracne within a few weeks. Other immediate but transient effects were also reported, including headache, dizziness, nausea and general weakness. It is impossible to know what symptoms were specifically caused by dioxin, as many of the observed symptoms can also result from exposure to the other chemicals contained in the fumes.

A 1980 epidemiological study[5] of 121 of the 122 chloracne victims, 30 years after the incident, permits some judgment about the long-term effects of dioxin. Fewer of these workers had died from cancer than would be expected in a control (unexposed) population. Only 32 of the workers had died from all causes, where 46 deaths would be expected in a comparable sample of that age and size. The authors noted, "It is important that no apparent excess in total mortality or in deaths from malignant neoplasms or diseases of the circulatory system were observed in a group of workers with a high peak exposure to tetrachlorodibenzodioxin who were followed over a period of nearly 30 years." Unfortunately, we do not have data on the dose of dioxin to which the workers were exposed. But we do know that it was sufficient to cause chloracne and other immedi-



ACSH: J. Jones

Guinea pig. Dioxin's reputation as being extremely toxic is based primarily on tests done on guinea pigs. Hamsters, in contrast, are approximately 5,000 times more resistant than guinea pigs to a single dose of dioxin.

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Following the explosion of a chemical reaction chamber at an ICMESA chemical plant in Seveso, Italy, a section of the community was dusted with an estimated one to four pounds of dioxin. There were no deaths among humans, but plants, birds, rabbits and chickens in the area died soon after the accident.

ate symptoms. Although the total number of people exposed was not large, the results are reassuring for other exposed populations, especially those whose exposure was less than the amount needed to cause chloracne, since no long-term effects have yet been documented among the exposed Monsanto workers who did have chloracne.

Dow Workers

In 1964, 61 Dow Chemical employees were exposed to dioxin levels potentially reaching 10,000 parts per million (ppm), or ten micrograms dioxin per kilogram body weight. After a plant mishap in Michigan, 49 of the exposed employees developed chloracne. As of 1980 (15 years later), no excess in total deaths had occurred among these workers.[6]

Seveso, Italy

One of the most publicized chemical accidents in recent years was the July 1976 accident in Seveso, Italy. Following the explosion of a chemical reaction chamber at the ICMESA chemical plant, a section of the Seveso community was dusted with an estimated one to four pounds of dioxin. In this case, the dioxin was produced during the manufacture of trichlorophenol, a material which was being used to make hexachlorophene.

Some 37,000 people were potentially exposed to the dioxin and several hundred did develop symptoms of acute poisoning. Children and adults complained of nausea, nervous symptoms and chloracne, which was severe in some cases. The relative contribution of other chemicals released in the explosion towards producing these symptoms is unknown. There were no deaths among humans caused by acute poisoning.

Plants, birds, rabbits and chickens in the area died soon after the accident. The animal mortality was proportional to the dioxin levels in the soil and grass, which were as high as 5.5 ppm in the most heavily contaminated, 180- acre zone.[6] This finding illustrates a point concerning the relative hazard posed by dioxin in soil. Animals are, for the most part, at a much greater risk, as they are likely to ingest significant and repeated doses of the compound during their normal feeding activities, unlike humans who are unlikely to ingest dirt or plants tainted with dioxin residue.

In the days following the accident, a number of public health measures were instituted by the Italian authorities. Entry into the area of highest contamination was prohibited and 736 inhabitants were evacuated two to three weeks after the accident. Young children, pregnant women and the elderly who resided in the surrounding region that had less, but still significant, contamination were also evacuated. Those remaining were forbidden from consuming fruits, vegetables, meat and dairy products from the area, and domestic animals and livestock were destroyed.

An extensive health surveillance system was put into effect to record any continuing and long-term effects of dioxin exposure on the population. Medical examinations and laboratory tests were performed, pregnant women were closely monitored to record rates of miscarriage and birth defects, and a cancer registry was created to track new cases of cancer.

Chromosomal studies of humans at Seveso who were exposed to high concentrations of dioxin show no evidence of chromosome abnormalities due to dioxin exposure.[7]

A thorough analysis of the health data from the Seveso population shows that affected residents developed chloracne and minor, reversible nerve damage. Clinical studies revealed some impaired liver function, the long-term effects of which are as yet unknown. No other organs or body functions appear to have been affected, nor was reproduction adversely affected. No cases of cancer related to the incident have been observed but study will continue for many years so that long-term effects can be monitored.

Does dioxin cause cancer in humans?

Experiments on laboratory animals have shown that dioxin can cause cancer at very low doses, and this of

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course suggests that dioxin could cause cancer in humans. On the other hand, studies of people who have been exposed to dioxin have not shown a relationship between dioxin and cancer and this offers us considerable assurance that traces in the environment do not constitute a cancer hazard. However, the actual number of exposed persons is too small to rule out the possibility of such a hazard. A chromosomal study done at a plant where dioxin-contaminated 2,4,5-T was manufactured showed no chromosomal abnormalities.[8]

Much of the research on dioxin and human carcinogenesis has centered around the compound's alleged ability to cause a rare form of cancer called soft tissue sarcoma (STS). STS is a group of tumors that occur in the muscles, tendons and other connective tissues, fat tissues, blood vessels and nerves.

Researchers in Sweden concluded that development of STS was more likely to occur among workers occupationally exposed to dioxin-contaminated phenoxy acids in agricultural chemicals. Between 1970 and 1976 there were 19 cases of STS among such workers compared with 11 expected cases. Another Swedish study found that railroad workers who handled dioxin-contaminated herbicides suffered an elevated rate of cancer mortality, with six cases observed compared with three cases expected in the sample of 207 men.[1] Since no specific tumor type predominated among the six cases,[1] it is highly questionable whether dioxin was, in fact, related to the cancer mortality.

The dioxin/STS hypothesis was tested by researchers in Finland and New Zealand. The results there differed from those in Sweden. In neither case was any association seen between occupational dioxin exposure and STS, despite the fact that these workers used the same herbicides during the same time period as did the workers in Sweden. Dioxin content in commercial 2,4,5-T used in Finland ranged from 0.1 to 0.9 ppm during the 1960s.[1] The daily dose of dioxin absorbed by an applicator has been calculated to be some 500 times less than the highest level failing to produce a carcinogenic effect (called the no-effect level) in humans based on extrapolation from experimental rodent data.[1] Soft tissue sarcomas are relatively rare, thus few pathologists have been able to achieve a high degree of consistency in their diagnosis. Most experts believe that if dioxin was acting as a carcinogen it would cause an increase of one type of soft tissue sarcoma. In the Swedish studies there was no documented evidence either to intensity, frequency or time of duration of exposure. The results can be confused by exposure to other chemicals, such as amitrole, which is a known carcinogen.

In short, while the Swedish conclusions suggest a link



E. O. Jones

When dioxin contamination occurs, pets and livestock are more likely to be in danger than humans are, because the animals may ingest tainted soil and plants during their normal feeding activities, while humans are unlikely to do so.

between dioxin and STS, there has been no confirmatory evidence to support it, despite an effort by researchers to specifically monitor STS rates in comparable sample populations.

Studies on workers exposed to dioxin during occupational accidents (between 1949 and 1968) failed to establish a dioxin/cancer association. Monsanto, BASF, Boehringer, Philips, Dow, Coalite and Spolana workers all suffered some degree of acute dioxin toxicity symptoms. But of the seven groups, only the BASF group showed any excess cancer mortality. There, the three cases of stomach cancer seen in one age bracket meant that the rate for that cancer was nine times higher than normal. The Monsanto and Dow studies did reveal a few cases of STS, although the cancer rate for all tumor types was not elevated. While the doses to these workers are unknown, the dioxin levels in waste residues were very high, ranging from 140 to 2,400 ppm.[1]

Does dioxin cause birth defects?

Laboratory studies show that dioxin causes birth defects when female test animals are exposed to the compound. However, as with cancer, no solid evidence exists linking dioxin with birth defects in humans. Claims have been made to the contrary, but they have been based largely on medically unvalidated and statistically unrepresentative anecdotal reports lacking the sound data base necessary to establish a cause-and-effect relationship.

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Typical of these are the Alsea studies released by the Environmental Protection Agency (EPA) in 1979. EPA scientists concluded that there was a relationship between the use of a dioxin-containing herbicide, 2,4,5-T, and an increase in miscarriages among women living in a sprayed region in Alsea, Oregon.

The Alsea II report was understood by many in the lay community as proving that dioxin in 2,4,5-T caused reproductive abnormalities. However, after the report was made public, many scientists challenged the EPA's conclusions. More than 18 reviews of this study have uncovered numerous flaws in the study design, statistical analysis, and interpretation of the results. Criticism of the study was extensive, consistent and practically unanimous. For example, EPA concluded that a seasonal peak in miscarriages followed 2,4,5-T spraying. Close examination of the data by other scientists showed that this peak did not exist.

A number of epidemiological studies have been done to determine what, if any, relationship exists between paternal exposure to dioxin and birth defects. Children fathered by Australian veterans of the Vietnam War who served during the period when dioxin-contaminated defoliants such as Agent Orange were sprayed have not experienced elevated numbers of birth defects. Mothers of these children have not had unusually high numbers of miscarriages. Reproductive outcome of the offspring of male applicators of 2,4,5-T with an average dioxin content of 0.1 ppm in New Zealand has been normal.[1] Similarly, all children born to men affected by the Spolana accident in Czechoslovakia were free of birth defects; miscarriage incidence among their wives was below expected levels.[1]

Two major epidemiological studies were completed in 1984 on Vietnam veterans exposed to dioxin in the period 1963 to 1971.[9,10] The first, the so-called "Ranch Hand Study," because of the code name for the defoliation operations, was conducted by the Air Force and compared the 15 to 20 year health histories of 1,045 herbicide applicators with 19,000 other veterans believed to have had no exposure. The study stressed investigation of chloracne and any incidence of soft tissue sarcoma. In all cases, no adverse health effects were found. Some borderline incidence of increased infant deaths and minor birth defects was observed and noted for follow-up in future studies. The investigators concluded, "current evidence is insufficient to support a causal relationship between herbicide exposure and adverse health."

The second study was conducted by the federal Centers for Disease Control (CDC) on the family health histories of 7,000 children born with birth defects in the Atlanta



ACSH: J. Jones

Dioxin has a very low solubility in water, and when released in rivers or lakes, eventually settles to the bottom and accumulates in the sediment.

area. The parents with Vietnam experience (about 10 percent of the total sample) composed the contrast group for comparison with the others as the control group. Reassuringly, no significant correlation of birth defects or changes in fertility was found to be associated with the Vietnam experience. Weak indications were observed at the level of statistical significance for two specific birth defects: spina bifida (a spinal deformation) and congenital tumors. Again, these results were deemed inconclusive, and will be watched in the future.

Dow Chemical has a large herbicide manufacturing plant in Midland, Michigan. The Michigan Department of Public Health looked at the number of birth defects among Midland County residents and found no excess of any kind.

Researchers in Hungary studied the relationship between 2,4,5-T use and birth defects from 1966 to 1977, a period which coincided with a 30-fold increase in use of the herbicide in that country.[1] No increase in the national frequency of birth defects was detected.

Reproductive patterns were monitored closely in Seveso in the years following the 1976 dioxin exposure. Had the dioxin contamination induced miscarriage, rates would have been highest during the remainder of 1976, the period when women already pregnant in July (the time of the accident) would still be carrying their unborn offspring. Yet the frequency of miscarriage did not deviate from expected levels during these six months. A small decrease in fertility (defined as the

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number of new pregnancies) was observed during the months following the accident, possibly suggesting that there may have been an adverse effect from the dioxin or other chemicals released into the environment. More likely though, this reflected a conscious decision by many parents not to have children for fear of dioxin exposure.

In summary, the epidemiological evidence collected to date does not implicate dioxin as a cause of birth defects or miscarriages in humans. Among exposed populations which have been studied, dioxin exposures were not found to adversely affect reproduction. These findings should be a source of comfort to residents of places like Times Beach and Newark, where exposure levels have been substantially lower than in the various industrial accidents.

What happened at Times Beach, Missouri?

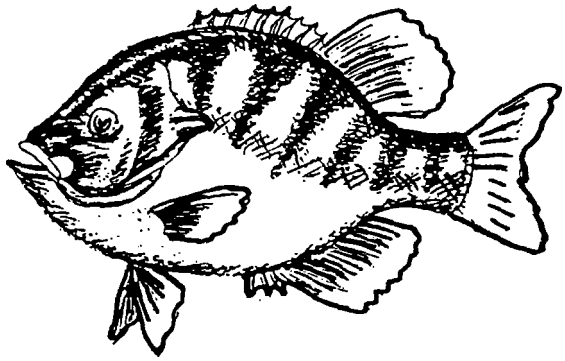
The dioxin contamination of Times Beach, Missouri was perhaps the most widely publicized episode of chemical contamination in the U.S. since the Love Canal story of 1978. Times Beach, a small town of approximately 2,000 residents located 25 miles southwest of St. Louis, drew national attention in December, 1982 after flood waters swept through the area. Concern was raised that contaminated soil which had been present along the roadside had been dispersed widely throughout the town. The incident resulted in a federal buyout and relocation of the Times Beach residents.

How did the dioxin get there?

In 1971, a private waste hauler sprayed dioxin-contaminated waste oil on unpaved roads as a dust retardant. In addition to the roads, two horse arenas were sprayed with the oil. A number of horses and small animals died and two children playing in one of the arenas developed chloracne.[11]

Investigations were conducted by state officials and the federal government's Centers for Disease Control (CDC). By 1974, CDC scientists concluded that dioxin poisoning was the cause of the horse and other animal deaths. Monitoring of soil samples revealed dioxin concentrations of 31 to 33 ppm in the horse arenas. Dioxin levels in Times Beach soil taken after the 1982 flood ranged from non-detectable levels to 0.3 ppm.[12] The highest level measured in town then was 100 times less than that found in the horse arenas some ten years earlier and approximately 15 times less than the highest level measured in the soil at Seveso, Italy after the 1976 accident.

During the autumn of 1982, EPA tested residues at the chemical plant which was the source of the dioxin



Surveys of Great Lakes fish show that the average levels of dioxin residues in various species range from 40 ppt in Lake Ontario eel and smelt to less than 10 ppt in most species in Lake Erie. Comparisons show that consumers of fish with 25 ppt of dioxin residue would be exposed to less than 1/70th the no-effect level and less than 1/7000th of the carcinogenic level in test animals.

sprayed on roads in 1971. Results showed dioxin present in concentrations of up to 0.3 ppm.

After the December floods, officials feared that the dioxin along the roads might have dispersed, increasing the chances that the population would be exposed. The news hit the front pages across the country. Many Times Beach residents began to fear for their health.

The EPA had never dealt with a comparable dioxin contamination situation. There was no tolerance level for dioxin to use as a criterion upon which to base risk assessment and strategy. The CDC was called in and after extensive epidemiological and statistical modeling, a limit or action level of one ppb (one part per billion) dioxin in soil was announced. CDC calculated, on the basis of hypothetical, yet currently accepted, models of risk estimation, that a 70-year exposure to soil concentrations of one ppb dioxin might cause one excess case of cancer per million people. A major consideration was the possibility that children would be most heavily exposed by ingestion of dirt while playing.[13] New evidence suggests that laboratory animals exhibit symptoms of dioxin poisoning after ingestion of dioxin-contaminated soil.[14] With Times Beach soil contaminated at levels reaching 0.3 ppm in some samples, CDC recommended evacuation. Times Beach was placed on top of the national priority list of hazardous waste sites. A state of emergency was declared and

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federal funds in excess of $30 million and a $3 million state contribution were allocated to buy the houses and resettle the residents.

Do the residents of Times Beach exhibit any symptoms of dioxin poisoning?

A pilot epidemiological study of Times Beach residents and of controls conducted by the CDC, Missouri Division of Health, St. Louis University School of Medicine and St. Joseph's Hospital in Kirkwood, Missouri was completed in October 1983. The project consisted of three phases—a questionnaire survey to obtain information on medical histories and risk of exposure to dioxin, a clinical screening for chloracne, and extensive examinations comparing persons with high potential of dioxin exposure to persons with little or no risk of dioxin exposure.

The results of the study were encouraging. In a letter to all participants, the Missouri Division of Health concluded that "we have not been able to identify meaningful ill-health effects related to potential exposure to dioxin. . . . The results show that the high risk group of participants had the same rate of health problems as the low risk group . . . every participant had a skin examination, but no case of chloracne was found. . . . To summarize, this pilot investigation did not turn up evidence suggesting that dioxin has caused any serious health problems in the 104 persons who were studied."[15]

Extensive clinical studies of the Times Beach residents reported in 1986[16] were similar to the earlier work in that no conclusive evidence of damaging health effects was found. The study compared 154 exposed persons with an unexposed control group of 155 people having similar lifestyles and medical histories. In an extensive battery of over fifty tests, the differences found between the two groups were some subclinical changes in liver function and some depression of the cellular immune function in the exposed group. In neither case, after whatever exposure took place between 1971 and 1983, had there been any evidence of increased liver disease or general illness in the exposed group as of 1986. Researchers will follow up on the effects noted in future years.

Has the area been cleaned up?

Not yet. Authorities in Missouri have yet to determine how best to dispose of the wastes. An interim disposal site at some Missouri location will be selected while the search for a permanent facility continues.

Is the average American being exposed to dioxin today and, if so, at what levels?

Large scale surveys to answer these questions have never been done but are in the planning stage by the EPA at the present time. The CDC recently announced plans for a $57 million study of dioxins, "its most complex, ever." With the advent of instruments capable of detecting extremely low concentrations (parts per trillion) of specific chemicals, it comes as no surprise that evidence is accumulating that dioxins, like many other contaminants, are present in extremely low quantities nearly everywhere in the developed world.



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When compared with large-scale and indisputable public health hazards such as cigarette smoking, drug abuse and drunk driving, the damage to society and individuals caused by dioxin exposure is negligible.

In spite of the widespread, low level contamination, based on the facts which have been accumulated to date it is highly unlikely that the health of Americans is being affected adversely by dioxin. The symptom-causing exposures of the past have exclusively consisted of herbicide-related accidents and occupational exposures. The EPA severely restricted use of 2,4,5-T in 1979 (inappropriately, in the opinion of many scientists) and is moving towards complete elimination of the use of the herbicide in this country (presently, it is permitted

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on rice paddies and rangelands). The 2,4,5-T that is currently used has an average dioxin concentration of only 0.01 ppm, a one hundred-fold reduction compared to the 1 ppm level often present in the 2,4,5-T manufactured twenty years ago. Indeed, some 2,4,5-T made 20 years ago contained 25 ppm or more.[17]

The Food and Drug Administration (FDA) has set guidelines for dioxin concentration in edible fish at 25 parts per trillion (ppt). This regulatory decision was arrived at by extrapolating data from rodent laboratory experiments. A simplified explanation of this complex procedure follows.

Surveys of Great Lakes fish determined that the average levels of dioxin residues in various species of fish ranged from 40 ppt in Lake Ontario eel and smelt to less than 10 ppt in most species in Lake Erie.[18] By comparing the amount of dioxin ingested by fish eaters with the no-effect level of dioxin in rodents, it was shown that consumers of fish with 25 ppt of dioxin residue would be exposed to less than 1/70th the no-effect level and less than 1/7000th of the carcinogenic level in test animals.[18] These guidelines are believed to provide a more than adequate margin of safety.

How effective are epidemiological studies and how much of this type of investigation is enough?

It is frustrating to the concerned public to find so many large epidemiological studies ending with "inconclusive results" on the main questions and then reporting weak statistical signals suggesting that some new and previously unsuspected health effect may be present. Unfortunately, it is in the nature of these studies that the two dilemmas exist. They must be expected from future epidemiological studies as well.

Suppose that in fact there is no health effect from a particular exposure. The epidemiological study, or indeed, even a perfectly controlled experiment, will never be able to "prove" that fact, for to do so would be to prove a negative proposition. The best that scientists can hope to do is investigate more and more cases to strengthen their conviction that "no evidence can be found" that the effect in question exists.

At some point we must decide when the investigation has gone far enough. The CDC study of Vietnam-related birth defects in Atlanta cost $2.8 million; the Air Force Ranch Hand Study cost $11 million; the future CDC study was planned to cost $57 million.

On the other hand, as was pointed out earlier, it is likely that *something* unusual will show up in every one of these studies through the workings of chance alone. It is like a bridge hand which, while it is unlikely to hold any specific combination of cards, is quite likely to contain *some* unusual combination.

Again, scientists must make a judgment about the likely significance of the newly discovered effect and responsible lay people must decide whether to allocate more scarce resources to further investigation.

Epidemiological studies of dioxin have certainly reached a high degree of sophistication and are receiving the benefit of continuing public concern. For example, the Veterans Administration periodically reviews all literature on dioxin with a team of independent medical specialists as mandated by Public Law 96-151, passed in 1979. The 1985 review covered 250 documents published in 1984, two-thirds of which were primary sources.[19] Five major epidemiological studies were reviewed for all of the suspected effects of dioxin exposure. No significant new results were found and the overall conclusion was that present information "was not sufficiently advanced for definitive conclusions on human health effects of low-level exposure."

The great value of epidemiological studies is that where a strong cause-and-effect relationship exists, it will almost certainly show up conclusively in a large sample. This will be true even when the cause (high dioxin exposure) and thus the effect (chloracne) are quite rare in the general population. Only such strong relationships can be conclusively established. It can be very useful, however, to know through epidemiological studies that a strong relationship *probably* does not exist if the studies keep coming in with inconclusive *results.*

We may never know whether or not weak effects exist. It might be argued that at some point we have enough information to be able to say, "The effect, if any, from this cause is lost in the background of the effect from all other causes." Then we would set prudent limits on future contamination by the target substance and turn our scientific attention, which is a limited resource, to other concerns. In the judgment of many scientists, this time has come for dioxin.

Dioxin Contamination: A Perspective

The lack of any lingering effects among the heavily exposed citizens (including the young and elderly) of Seveso, Italy is comforting for every civilian population exposed to environmental dioxin, since the doses at Seveso were higher than in any other civilian exposure situation, except the Missouri horse arenas. Knowledge about any possible long-term effects based on the Seveso experience is limited because a relatively short time (only ten years) has passed since the accident.

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However, the Monsanto workers who were exposed to enough dioxin to cause chloracne and other acute symptoms in 1949 have been studied for more than thirty years. Epidemiological studies of this group have yet to demonstrate any long-term effect of dioxin on the people who were exposed.

At the Scientific Dispute Resolution Conference in Arlington, Virginia[20] it was concluded that "phenoxy herbicides containing TCDD have not been shown to be carcinogenic in humans in retrospective epidemiologic studies to date. . . . Analysis of the available data leads this group to the conclusion that no adverse effects on human reproduction have yet been demonstrated after exposure to 2,4,5-T or TCDD." These data came from the United States, Sweden, New Zealand, Australia, Vietnam and Italy.

Possibilities that dioxin may yet cause long-term health problems do remain. The low exposure over many years in a Newark neighborhood adjacent to a chemical manufacturing plant may affect health differently than the large exposure at the Monsanto plant. Conclusions drawn from study of workers may not be able to provide information about the general population, which includes people of all age groups with the complete scope of human illnesses, in addition to healthy working men. Nevertheless, thus far we have no evidence suggesting that either short-term or long-term health effects will be seen in populations exposed to low levels of environmental dioxin such as in Times Beach and Newark.

For further reading, the 1985 book, *Dioxins in the Environment,* by Michael A. Kamrin, published by Harper & Row, is recommended.

In perspective, it should be recalled that the evidence suggesting dioxin causes cancer or birth defects is based on animal experiments. Genuine conflict exists in the scientific community about the validity of direct extrapolation of laboratory data to estimates of human risk. Beyond that, in interpreting the laboratory data on dioxin, one should be aware of the fact that a number of naturally occurring chemicals have also been shown to be potent animal carcinogens and teratogens. For example, aflatoxin (present in peanut butter and grain-based foods) is a potent animal carcinogen. However, there is no evidence that the tolerated trace levels in peanut butter and other foods pose any hazard to human health. Further, while there are some 600 to 800 agents known to be teratogenic in laboratory animals, only about 25 or 30 are known to be responsible for human malformations. It is interesting that the first compounds which showed high teratogenic activity were such essential materials as the vitamins A, D, and E, when administered in excessive quantities.

Public health policy must incorporate both scientific and nonscientific considerations. Medical, ecologic, economic, and legislative input, combined with common sense, is necessary for the framing of future public policy on hazardous waste issues such as dioxin.

No one likes to learn that chemicals are polluting the environment. As a health-conscious society, we strive to minimize health risks whenever possible. That potential health damage resulting from dioxin exposure has received so much media attention and caused enormous public concern is indeed ironic. When compared with large-scale and indisputable public health hazards such as cigarette smoking, drug abuse and drunk driving, the damage to society and individuals caused by dioxin exposure is negligible. Based on laboratory animal studies, however, some suspicion of adverse health effects persists. Fortunately, that potential has not been realized in humans, nor is there good reason to expect it to do so in the years ahead.

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PART V. CARCINOGENICITY

LONG-TERM TOXICOLOGIC STUDIES OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) IN LABORATORY ANIMALS

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INTRODUCTION

The compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a highly toxic impurity that may be formed as an unwanted contaminant under certain conditions during the production of 2,4,5-trichlorophenol. TCDD has been considered one of the causes of chloracne, which has been associated historically with the industrial production of 2,4,5-trichlorophenol and other products made from 2,4,5-trichlorophenol.

Over the past decade, a number of toxicologic studies with TCDD were conducted to assess the potential for acute toxicity and teratogenesis. Results of these earlier studies have been summarized in a previous publication by Kociba *et al.*[1] In that publication, we also reported the results of a subchronic study in which rats were given 1.0, 0.1, 0.01, 0.001 or 0 μg TCDD/kg 5 days/week for 13 weeks. Doses of 1.0 μg TCDD/kg/day caused multiple toxicologic effects, with significant morphologic changes in the liver, thymus and reproductive organs. A dose level of 0.1 μg TCDD/kg/day caused lesser degrees of toxicity, and rats given 0.01 or 0.001 μg TCDD/kg/5 days/week for 13 weeks had no alterations considered of any toxicologic significance.

Since that time, studies have been in progress to assess the potential for chronic toxicity associated with long-term exposure to TCDD. Allen *et al.*[2] reported on a subchronic study in which monkeys consumed a diet containing 500 ppt of TCDD for up to 9 months. It was calculated that these monkeys ingested 2–3 μg TCDD/kg over the course of the 9-month study. Clinically, these monkeys had changes similar to those described by McConnell *et al.*[3] as well as some hematologic depression and hemorrhages in various tissues. Hypertrophy, hyperplasia and/or metaplasia were noted in the epithelium of the bile ducts, salivary glands, bronchi, pancreatic ducts, sebaceous glands, skin, gastric lining and urinary tract of these monkeys given diets containing 500 ppt of TCDD.

In 1969, Innes *et al.*[4] reported on a long-term carcinogenic study conducted in mice given 2,4,5-trichlorophenoxyacetic acid contaminated with a level of TCDD sufficient to supply approximately 0.27 μg TCDD/kg/day. Neither of the two strains of mice had an increase in tumors as a result of the treatment. DiGiovanni *et al.*[5] reported on a study in which TCDD was reported to be only a weak tumor initiator in the two-stage system of mouse skin carcinogenesis with 7,12-dimethylbenz(a)anthracene (DMBA).

Van Miller and Allen[6] issued a preliminary report on a study of small groups of male rats fed diets containing TCDD for 65 weeks. All 10 rats of each group receiving 1.0, 0.5 or 0.05 ppm TCDD in the diet died within 4 weeks, with acute toxic effects.

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Groups of male rats on diets containing 5000 or 1000 ppt of TCDD had increased mortality, decreased weight gain and liver toxicity. In a more recent publication by this same group of researchers, Van Miller *et al.*[7] reported lung and/or liver tumors in those rats given 1000 or 5000 ppt of TCDD. Neoplasms of various organs, but not of lung or liver, were reported in rats given diets containing 500, 50 or 5 ppt of TCDD. Van Miller *et al.* reported a zero incidence of tumors in the group of rats given 1 ppt of TCDD and in a group of 50 control rats.

In view of the need for an evaluation of the chronic toxicity and potential for carcinogenicity of TCDD, the study summarized herein was conducted. For the sake of brevity, only the more salient data are included in this presentation. The full report can be found in a recent paper by Kociba *et al.*[8]

Materials and Methods

The TCDD sample was prepared by the Dow Chemical Company, Midland, Michigan, USA, and had a purity exceeding 99%. On the basis of results from a previous 13-week long toxicity study[1] in rats dosage levels of 0.1, 0.01 and 0.001 μg of TCDD/kg/day were given in the diets. The test diets were adjusted at intervals to provide these dose levels of TCDD as required by body weight and food comsumption determinations. Groups of 100 rats (50 male, 50 female) of the Sprague-Dawley strain were maintained on diets supplying these dose levels for two years. The control group consisted of 85 males and 86 females.

Samples of the prepared diets were analyzed periodically to ascertain if the dietary levels of TCDD were being maintained as scheduled.

Parameters monitored during the course of the study included body weights, food consumption, hematologic parameters including total erythrocyte count (RBC), total and differential leukocyte counts (WBC), thrombocyte and reticulocyte counts, packed cell volumes (PCV) and hemoglobin concentration, routine urinalyses, and urinary excretion of creatinine, coproporphyrin, uroporphyrin and delta-amino levulinic acid (delta-ALA). Serum samples were collected preterminally or terminally for determination of urea nitrogen (BUN), glutamic pyruvic transaminase (SGPT), bilirubin (total, direct and indirect), cholesterol, triglycerides, alkaline phosphatase (AP), gamma-glutamyl transferase (GGT), total protein, albumin and globulin.

All rats killed after two years, as well as those dying or culled during the study, were subjected to a complete necropsy examination by a veterinary pathologist. Representative tissues from all organ systems of the body of each rat were preserved in formalin fixative, with subsequent preparation of paraffin-embedded sections for light microscopy examination. Additional sections of livers collected at terminal necropsy from three female rats per group were processed for transmission electron microscopy. The weights of the liver, kidney, brain, heart, thymus, spleen and testes or ovaries/uterus were recorded at the time of terminal necropsy.

Liver and fat samples collected from three females per group at terminal necropsy were subsequently analyzed for TCDD content, using gas chromatography low-resolution spectrometry.

The various data were statistically analyzed according to the procedures listed in the previously cited publication by Kociba *et al.*,[8] which includes the full details of this research.

Results

Analyses of the test diets indicated that rats given 0.1, 0.01 and 0.001 μg TCDD/kg/day were ingesting an average of 2,193, 208 and 22 ppt of TCDD, respectively, in the diet for two years.

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females given 0.01 μ
were not affected at

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appeared to be sligh
WBC differentials at
in rats given 0.01 or
limited only to fem
increased urinary exc
delta-ALA was incre

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	0	0.
Emaciation (M, F		
Icterus (Fe)		
Liver toxicity (M,		
Liver nodules (Fe)		
Thymic atrophy (F		
Splenic atrophy (M		
Anemic paleness o hemorrhages (F		
Increased incidenc periarteritis (M,		
Pulmonary congest effusion and mas		
Decrease in incide enlargement, en plasia, uterine p cutaneous masse		
Decrease in severit renal disease (M		
Increased incidenc proliferative lesi (M, Fe)		

*(—) Indicates obs
parable to control data
Tabulation exclude

Of all the clinic
changes were noted
SGPT, AP, and GG

For the sake of t
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The rate of mortality was increased in female rats given 0.1 μg TCDD/kg/day. The body weights of male and female rats given 0.1 μg TCDD/kg/day and also the females given 0.01 μg TCDD/kg/day were decreased. However, these parameters were not affected at the lower dose levels.

Hematologic data indicated some effects at the high dose level of 0.1 μg TCDD/kg/day: this included slight decreases in PCV and Hgb of males and decreases in PCV, total RBC and WBC counts and Hgb values of females; reticulocyte counts appeared to be slightly increased. No effect was noted on thrombocyte counts and WBC differentials at all the dose levels studied. No hematologic changes were noted in rats given 0.01 or 0.001 μg TCDD/kg/day. Changes in urinary parameters were limited only to females given 0.1 or 0.01 μg TCDD/kg/day; these groups had increased urinary excretion of coproporphyrin and uroporphyrin. Urinary excretion of delta-ALA was increased only in females given 0.1 μg TCDD/kg/day.

TABLE 1

MAJOR GROSS NECROPSY FINDINGS IN RATS ATTRIBUTED TO INGESTION OF DIETS CONTAINING TCDD FOR TWO YEARS

Dose Level of TCDD (μg/kg/day)		
0.1	0.01	0.001
Emaciation (M, Fe)	—*	—
Icterus (Fe)	—	—
Liver toxicity (M, Fe)	Liver toxicity (M, Fe)	—
Liver nodules (Fe)	Liver nodules (Fe)	—
Thymic atrophy (Fe)	—	—
Splenic atrophy (M, Fe)	—	—
Anemic paleness of tissue and focal hemorrhages (Fe)	—	—
Increased incidence of mesenteric periarteritis (M, Fe)	Mesenteric periarteritis (Fe)	—
Pulmonary congestion, edema, effusion and mass formation (Fe)	—	—
Decrease in incidence of pituitary enlargement, endometrial hyperplasia, uterine polyps, and subcutaneous masses (Fe)	—	—
Decrease in severity of chronic renal disease (M)	—	—
Increased incidence of keratinized proliferative lesions near pharynx (M, Fe)	—	—

*(—) Indicates observation was not noted at that dose level, or incidence was comparable to control data.

Tabulation excludes voluminous data considered unrelated to treatment with TCDD.

Of all the clinical chemical parameters evaluated, the only treatment-related changes were noted in females given 0.1 μg TCDD/kg/day, which had increases in SGPT, AP, and GGT activities.

For the sake of brevity, treatment-related gross and microscopic observations on tissues have been summarized in TABLES 1 and 2. Gross necropsy examination indicated that the target organs of chronic TCDD toxicity in rats were primarily the liver, lymphoid organs, vascular and respiratory systems (TABLE 1). Light microscopy examination revealed the liver to be the organ most consistently affected, and rats

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TABLE 2

MAJOR HISTOPATHOLOGIC FINDINGS IN RATS ATTRIBUTED TO INGESTION OF DIETS CONTAINING TCDD FOR TWO YEARS

Dose Level of TCDD (μg/kg/day)		
0.1	0.01	0.001
Liver		
Hepatic degenerative inflamatory, necrotic and proliferative changes.	Livers had much lesser degree of change.	No changes. Livers of females had statistical increase in foci or area of hepatocellular alteration (swollen hepatocytes), and livers of males had statistical decrease in area of hepatocellular alteration (swollen hepatocytes).
Cytomegaly, distortion of lobular pattern and atropy of cords, cytoplasmic vacuolation, fatty change, altered tinctorial properties, hepatic necrosis and inflammation, multinucleated hepatocytes, foci or areas of hepatocellular alteration (swollen hepatocytes), pigment aggregates, bile duct hyperplasia, periportal inflammation, fibrosis		
Hepatocellular carcinoma (Fe)		
Hepatocellular hyperplastic (neoplastic) nodules (Fe)	Hepatocellular nodules (Fe)	—
Reproductive		
Decreased incidence of uterine hyperplasia, cyst formation and polyp formation (Fe)	Questionable decreased incidence of uterine polyps (Fe)	—
Mammary Tissue		
Decreased incidence of mammary tumors	—	—
Endocrine		
Pituitary—decreased incidence of pituitary hemangiectasis and adenoma formation (Fe)	—	—
Adrenal—decreased incidence of medullary hyperplasia (M, Fe) and pheochromocytoma (M)	—	—
Increased incidence of cortical necrosis and hemorrhage (Fe), cortical adenoma and hematocyst (M)	—	—
Pancreas—decreased incidence of acinar adenoma (M)	—	—
Thyroid—occurrence of various isolated follicular changes of questionable significance (M)	—	—
Parathyroid—decreased incidence of parathyroid hyperplasia, secondary to chronic renal disease (M)	—	—
Lymphoid		
Isolated cases of thymic/splenic atrophy (Fe)	—	—
Respiratory		
Focal alveolar hyperplasia, pigment aggregates, accumulation of alveolar macrophages and cholesterol clefts, pulmonary edema, focal interstitial inflammation and fibrosis	Focal alveolar hyperplasia (Fe)	—

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TABLE 2 (*Continued*)

Dose Level of TCDD (μg/kg/day)		
0.1	0.01	0.001
Keratinizing squamous metapiasia (Fe)	—	—
Keratinizing squamous cell carcinoma (Fe)	—	—
Cardiovascular		
Isolated cases of hemorrhage in CNS (Fe)	—	—
Increased incidence of mesenteric and thoracic periarteritis, with cases of thrombosis or hematoma formation (M, Fe)	Increased incidence of periarteritis (M)	—
Increased incidence of myocardial degenerative changes (Fe)	—	—

*(—) Indicates observation was not noted at that dose level, or incidence was comparable to control data.

Tabulation excludes voluminous data considered unrelated to treatment with TCDD.

given 0.1 or 0.01 μg TCDD/kg/day had a dose-dependent incidence of hepatic degenerative, inflammatory and necrotic changes listed in TABLE 2. Female rats given 0.1 μg TCDD/kg/day also had a statistical increase in lesions described morphologically as hepatocellular carcinomas (no metastasis) and hepatocellular nodules; females given 0.01 μg TCDD/kg/day had an increase in hepatocellular nodules.

Histopathologic examination of livers from rats given 0.001 μg TCDD/kg/day revealed none of the treatment-related effects that were noted at the higher dose levels. On a statistical basis, the incidence rate of a slight hepatocellular alteration (focal swollen hepatocytes) was increased in females and decreased in males of the group given 0.001 μg TCDD/kg/day; these statistical variations were not considered indicative of any toxicologic significance.

Ultrastructural examination of hepatocytes from females of the 0.1 μg TCDD/kg/day revealed the most consistent change to be in the rough endoplasmic reticulum (RER) which showed proliferation, distortion and fragmentation. Smooth endoplasmic reticulum (SER) and mitochondrial structures were within control limits. Other changes in hepatocytes from this group given 0.1 μg TCDD/kg/day included focal cytoplasmic vacuolization, increased lysosomal activity with residual body formation and an occasional multinucleated hepatocyte. In hepatocytes from females given 0.01 μg TCDD/kg/day, the most notable changes included a lesser degree of proliferation of the RER and some proliferation of the SER. This was accompanied by a slight increase in the number of hepatocytes with lipid droplet accumulation. The hepatocytes from females given 0.001 μg TCDD/kg/day were within the limits of variation seen in the hepatocytes of the control group. There was no general increase in lipid droplet content of the hepatocytes, although an occasional hepatocyte had some lipid droplet content.

As predicted from previous studies, gross and histopathologic examination of some rats given 0.1 μg TCDD/kg/day had isolated cases of thymic and/or splenic atrophy. Lower dose levels did not produce this effect.

The respiratory system of both male and female rats was affected by ingestion of 0.1 μg TCDD/kg/day, with focal alveolar hyperplasia, pigment aggregates, alveolar macrophages, cholesterol clefts, edema, focal interstitial inflammation, fibrosis,

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keratinizing squamous metaplasia, and keratinizing squamous cell carcinomas. At 0.01 μg TCDD/kg/day, these effects were not noted, except for focal alveolar hyperplasia in female rats. At the dose level of 0.001 μg TCDD/kg/day, there were no treatment-related changes in the respiratory tract.

Examination of the reproductive organs of females given 0.1 (and possibly those given 0.01 μg TCDD/kg/day) revealed a decreased incidence of the uterine changes typically encountered as spontaneous lesions in this strain of rat. The high dose group of females also had decreased incidences of both mammary and pituitary tumors. Adrenal changes noted only at the high dose level of 0.1 μg TCDD/kg/day included a

TABLE 3
INCREASED INCIDENCE OF TUMOR AND TUMOR-LIKE LESIONS ATTRIBUTED TO INGESTION BY RATS OF DIETS CONTAINING TCDD FOR TWO YEARS

	Dose Level of TCDD (μg/kg/day)			
	0	0.1	0.01	0.001
	Number of Males with Lesion/Number in Group			
Hard Palate/Nasal Turbinates				
Stratified squamous cell carcinoma	0/85	4/50*	0/50	0/50
Tongue				
Stratified squamous cell carcinoma	0/85	3/50*	1/50	1/50
Lung				
Squamous cell carcinoma	0/85	1/50	0/50	0/50
	Number of Females with Lesion/Number in Group			
Hard Palate/Nasal Turbinates				
Stratified squamous cell carcinoma	0/86	4/49*	1/50	0/50
Tongue				
Stratified squamous cell carcinoma	1/86	2/49	0/50	0/50
Lung				
Squamous cell carcinoma	0/86	7/49*	0/50	0/50
Liver				
Hepatocellular carcinoma	1/86	11/49*	2/50	0/50
Hepatocellular nodule(s)	8/86	23/49*	18/50*	3/50

*Statistically increased above control data, $p < 0.05$.

Table excludes additional data on incidence of tumors considered unrelated to treatment with TCDD.

decreased incidence of medullary hyperplasia and pheochromocytoma formation, and an increased incidence of cortical necrosis, hemorrhage, hematocyst and adenoma formation.

The incidence of pancreatic acinar adenoma was decreased in males given 0.1 μg TCDD/kg/day. This group of males also had a decrease in the incidence rate of secondary parathyroid hyperplasia; this was due to a decrease in the severity of chronic renal disease noted in males given this high dose level of 0.1 μg TCDD/kg/day.

Examination of the cardiovascular system revealed several effects assumed to be due to ingestion of 0.1 μg TCDD/kg/day. These effects included isolated cases of hemorrhage in different organs of the body, and an increase in the incidence of periarteritis and myocardial degenerative changes, both of which occur spontaneously

TABLE 4

DECREASED INCIDENCE OF TUMORS ATTRIBUTED TO INGESTION BY RATS OF DIETS CONTAINING TCDD FOR TWO YEARS

	Dose Level of TCDD (μg/kg/day)	
	0	0.1
	Number of Males with Lesions/Total Number in Group	
Pancreas		
Acinar adenoma	14/85	2/50*
Pheochromocytoma	28/85	4/50*
	Number of Females with Lesion/Total Number in Group	
Uterus		
Benign tumor	30/86	8/49*
Mammary Gland		
Benign tumor	73/86	24/49*
Pituitary		
Adenoma	43/86	12/49*

*Statistically decreased from control data, $p < 0.05$.

Data at lower dose levels comparable to controls, except for possible decrease in incidence of benign uterine tumors in females given 0.01 μg TCDD/kg/day.

in this strain of rat. None of these observations were noted at the two lower dose levels, except for an increase in incidence of periarteritis in male rats given 0.01 μg TCDD/kg/day.

Other treatment-related effects included an increase above the background incidence of squamous cell carcinomas of the tongue and hard palate/nasal turbinate region of rats given 0.1 μg TCDD/kg/day. Historically, squamous cell carcinomas have occurred in these organs of this strain of rat at a spontaneous incidence rate of 1–3%. Only the high dose level of 0.1 μg TCDD/kg/day increased the incidence of this type of neoplasm.

All the data on incidence rates of neoplasms that were increased or decreased by treatment with the high dose level of 0.1 μg TCDD/kg/day are summarized in TABLES 3 and 4.

Review of organ weights recorded at the terminal necropsy indicated the following effects considered related to treatment (a) an increase in absolute and/or relative liver weight of rats given 0.1 or 0.01 μg TCDD/kg/day and (b) a decrease in weight of the thymus of females given 0.1 μg TCDD/kg/day. There were no organ weight changes at the dose level of 0.001 μg TCDD/kg/day.

TABLE 5 lists the TCDD content of liver and fat samples collected from female rats at terminal necropsy after two years of ingesting diets containing TCDD.

TABLE 5

TCDD CONTENT IN RAT LIVERS AND FAT SAMPLES COLLECTED AFTER TWO YEARS INGESTION OF DIETS CONTAINING TCDD

Dose Level of TCDD (μg/kg/day)	TCDD Content (ppt)	
	Fat	Liver
0.1	8100	24000
0.01	1700	5100
0.001	540	540

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Discussion

The results of this study of rats ingesting TCDD for a lifetime serve as a basis for assessing the long-term chronic toxicity of TCDD. Continuous ingestion of a high dose level of 0.1 μg TCDD/kg/day (approximately 2200 ppt in diet) predictably caused multiple toxicologic effects. Liver toxicity was the most consistent observation, and this was accompanied by morphologic changes of the lymphoid, respiratory, and vascular tissues of the body. The incidence of hepatocellular carcinomas of the liver and squamous cell carcinomas of the lung, hard palate/nasal turbinates or tongue was increased at this dose level. Conversely, the incidence of tumors of the pituitary, uterus, mammary gland, pancreas and adrenal medulla was decreased at this high dose level of treatment. Similarly, the incidence of other spontaneous lesions such as chronic renal disease was also decreased at this high dose level.

Lifetime ingestion by rats of 0.01 μg TCDD/kg/day (approximately 210 ppt in diet) caused a lesser degree of toxicity, primarily of the liver. However, there was no increase in the incidence of neoplasia at this dose level.

Rats ingesting 0.001 μg TCDD/kg/day for two years had no adverse effects in spite of the fact the liver and fat each contained 540 ppt of TCDD at termination of the study.

Thus, these data indicate a good dose response for the long-term toxicity of TCDD. Whereas higher doses were predictably toxic and did alter the incidence of tumors, an intermediate dose level caused lesser toxicity but no neoplasia. A lower dose was tolerated for a lifetime with no adverse effects noted in any of the parameters evaluated.

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CLASSIFICATION OF SOFT TISSUE TUMORS AND TUMOR-LIKE LESIONS

Site	Non-Neoplastic Lesions	Benign Neoplastic Lesions	Malignant Neoplastic Lesions
TUMORS OF FIBROUS TISSUE	Fasciitis Keloid Elastofibroma Fibromatosis	Benign fibroblastic fibrous histiocytoma Benign histiocytic fibrous histiocytoma Benign pleomorphic fibrous histiocytoma Fibroma	Malignant fibroblastic fibrous histiocytoma Malignant histiocytic fibrous histiocytoma Malignant pleomorphic fibrous histiocytoma Desmoid tumor Fibroblastic fibrosarcoma Pleomorphic fibrosarcoma
TUMORS OF TENDOSYNOVIAL TISSUE	Tendosynovial cyst Tendosynovitis Synovial chondromatosis	Fibroma Lipoma Villonodular synovitis Benign giant cell tumor	Malignant giant cell tumor Biphasic tendosynovial sarcoma Monophasic spindle cell type tendosynovisl sarcoma Epithelioid sarcoma Clear cell sarcoma Chordoid sarcoma
TUMORS OF ADIPOSE TISSUE	Fat necrosis Lipogranuloma Lipodystrophia Piezogenic papules Adiposis dolorosa Steatopygia	Well-differentiated lipoma Myxoid lipoma Fibroblastic lipoma Pleomorphic lipoma Angiolipoma Angiomyolipoma Myelolipoma Lipoblastoma Hibernoma	Well-differentiated liposarcoma Myxoid liposarcoma Lipoblastic liposarcoma Fibroblastic liposarcoma Pleomorphic liposarcoma
TUMORS OF MUSCLE	Atrophy Dystrophy Polymyositis Rhabdomyolysis Proliferative myositis Fibromatosis Myositis ossificans	Leiomyoma Angiomyolipoma Rhabdomyoma	Leiomyosarcoma Leiomyoblastoma Embryonal rhabdomyosarcoma Pleomorphic rhabdomyosarcoma Rhabdomyoblastoma
TUMORS OF VESSELS	Pyogenic granuloma Angioblastic lymphoid hyperplasia Hereditary hemorrhagic telangiectasia Arteriovenous fistula	Capillary hemangioma Cavernous hemangioma Venous hemangioma Papillary endothelial hyperplasia Hypertrophic hemangioma Angiomatosis Angiofibroma Angiolipoma Angiomyolipoma Hemangioblastoma Glomus tumor Lymphangioma Lymphangiomyoma	Hemangiopericytoma Hemangiosarcoma Kaposi's sarcoma Lymphangiosarcoma

CLASSIFICATION OF SOFT TISSUE TUMORS AND TUMOR-LIKE LESIONS (continued)

	Non-Neoplastic Lesions	Benign Neoplastic Lesions	Malignant Neoplastic Lesions
TUMORS OF PERIPHERAL NERVE	Degeneration Hypertrophy Traumatic neuroma	Neurofibroma Neurofibromatosis Angioneuroma Plexiform neuroma Pacinian neurofibroma Glandular schwannoma Pleomorphic schwannoma Cystic schwannoma Storiform schwannoma Epithelioid schwannoma	Malignant collagenous schwannoma Malignant non-collagenous schwannoma Primitive neuroectodermal tumor
EXTRASKELETAL BONE TUMORS	Chondroid metaplasia Osteoid metaplasia Myositis ossificans	Chondroma Osteochondroma Osteoma	Osteogenic sarcoma Chondrosarcoma Ewing's sarcoma
MISCELLANEOUS SOFT TISSUE TUMORS		Benign granular cell tumor Benign mesenchymoma	Malignant granular cell tumor Alveolar soft part sarcoma Malignant lymphoma Granulocytic sarcoma Plasmacytoma Postirradiation sarcoma Malignant mesenchymoma Unsifferentiated soft tissue sarcoma

From: Hajdu, S.I.: Pathology of Soft Tissue Tumors, Lea & Febiger, Philadelpia, 1979

GROWTH PATTERN

1. Arranged pattern
2. Spreading pattern
3. Lacy pattern
4. Epithelioid pattern
5. Alveolar pattern
6. Disarranged pattern

CELL MORPHOLOGY

1. Slender spindle cells
2. Plump spindle cells
3. Granular epithelioid cells
4. Clear epithelioid cells
5. Isomorphic giant cells
6. Pleomorphic giant cells

APPEARANCE OF STROMA

1. Fibrillar stroma
2. Sclerosed stroma
3. Myxoid stroma
4. Vascular stroma
5. Inflamed stroma
6. Necrotic stroma
7. Chondrified stroma
8. Ossified stroma
9. Calcified stroma

PRODUCT OF CELLS

1. Collagen
2. Glycogen
3. Polysaccharides
4. Fat
5. Melanin
6. Secretory granules
7. Crystals
8. Fine structure
9. Tissue antigens

From: Hajdu, S.I.: Differential Diagnosis of Soft Tissue and Bone Tumors, Lea & Febiger, Philadelphia, In Press

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STATUS OF ANALYTICAL SYSTEMS FOR THE DETERMINATION OF PCDDs AND PCDFs

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During the past decade the development of analytical methodology for the determination of chlorinated dibenzo-p-dioxins (CDDs) in many and varied materials has been extraordinarily comprehensive in scope, selectivity, and sensitivity. The state of the art is now such that analytical methodology protocols can be custom-made to generate data having any desired degree of certainty at concentration levels down to sub parts per trillion.

The rigor required to achieve assurance of the quality of data varies with the many factors which govern the separation and measurement processes. Although these factors often do not need to be optimized, they must be understood, controlled, and monitored in order to understand the certainty of the results.[1,12] This has been emphasized by a National Research Council of Canada[42] panel who wrote a comprehensive review of PCDD analysis with emphasis on limitations.

The choice of methodology depends on the matrix to be analyzed, the sensitivity, specificity and the degree of certainty required in the data. Thus, analytical systems suitable for the determinations of the CDDs in commercial products do not need to be as sophisticated as those applied to the environmental materials.[15] Various analytical systems have been previously classified[6,14,15] according to the degree of resolution achieved in each of three stages: (1) sample preparation, (2) sample introduction into the mass spectrometer, and (3) mass spectrometry.

Sample preparation consists of (1) initial sample treatment, (2) liquid-liquid partition, (3) column chromatography, and (4) high pressure liquid chromatography. These are used in numerous combinations to isolate the PCDDs from most of the interferences. References to the early work have been given in a previous report.[15] Recent publications which are representative of state-of-the-art technology and present details of the techniques are given in the following table:

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TABLE 1: Analytical System - Stage I: Extraction-Cleanup Techniques

	RECENT REFERENCES
INITIAL SAMPLE TREATMENT	
Dissolution	COCHRANE et al. (1981)
Digestion	
In Base	JENSEN et al. (1981)
In Acid	LANGHORST & SHADOFF (1980)
	ZABIK & ZABIK (1980)
Direct Extraction	COCHRANE et al. (1981)
	HARLESS et al. (1980)
LIQUID-LIQUID PARTITION	
Against Conc. H_2SO_4	JENSEN et al. (1981)
Against Aqueous Base	PFEIFFER et al. (1980)
COLUMN CHROMATOGRAPHY	
Alumina	COCHRANE et al. (1981)
Silica Gel	COCHRANE et al. (1981)
Florisil	RYAN & PILON (1980)
Ion Exchange Resin	BLASER et al. (1976)
Charcoal	HUCKINS (1978)
Gel Permeation	DOUGHERTY et al. (1980)
Reagent Modified Silica	LAMPARSKI & NESTRICK (1980)
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY	
Normal Phase	LAMPARSKI & NESTRICK (1980)
	LANGHORST & SHADOFF (1980)
Reversed Phase	RYAN & PILON (1980)
Reversed + Normal Phase	LAMPARSKI & NESTRICK (1980)
	LANGHORST & SHADOFF (1980)

These sample preparation techniques have been used in many and varied combinations to successfully separate PCDDs and PCDFs from a wide variety of matrices including plant and animal tissues and products, chemical products, sediment, soil, combustion products, and waste materials. A great deal is known about the relative effectiveness for doing this assuring that measurable well-defined signals are obtained.

The power of high performance liquid chromatography to separate isomers has been demonstrated by the separation of all 22 isomers of T_4CDD[47] and the 10 isomers of H_6CDD[33] using reversed phase and normal phase HPLC consecutively. Although collaborative studies using these techniques have not yet been done, several laboratories[50,57,59] have reported success using these and similar modified approaches.

Very few direct comparisons of the efficiencies of the various extraction-cleanup procedures have yet been done. One of the most elucidating has been that of the U.S. Food and Drug Administration[5] who examined six extracts of fish samples (3 spiked and 3 unspiked) prepared by each of six different laboratories using procedures in use at that time in their respective laboratories. Under the GC/MS conditions used by the FDA the procedures of Lamparski et al.[35] and Huckins et al.[28] were the most effective.

SAMPLE INTRODUCTION

Introduction of the sample into the mass spectrometer is accomplished by (1) direct probe, (2) packed column gas chromatography, (3) capillary column gas chromatography, or (4) a second mass spectrometer. The various types of these techniques are summarized in the following tables.

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TABLE 2: Analytical System - Stage II: Mass Spectrometer Sample Introduction

TECHNIQUE	SELECTED RECENT REFERENCES
Direct Probe	BAUGHMAN & MESELSON (1973)
Packed Column GC	
OV-1	FANELLI et al. (1980)
OV-101	PHILLIPSON & PUMA (1980)
OV-3	LAMPARSKI et al. (1978)
OV-7	ERK et al. (1979)
OV-17	LIBERTI et al. (1981)
OV-105	LAMPARSKI et al. (1978)
OV-210	HASS et al. (1978)
OV-225	CHAE et al. (1977)
OV-17/Poly S-179	CHESS & GROSS (1980)
	LAMPARSKI & NESTRICK (1981)
SE-30	COCHRANE et al. (1981)
XE-60	MIEURE et al. (1977)
Carbowax 20M	EICEMAN et al. (1980)
SP-2100	PHILLIPSON & PUMA (1980)
Dexil 300	VAN NESS et al. (1980)
Capillary Column GC	
OV-17 WCOT	LIBERTI et al. (1981)
OV-61 WCOT	BUSER (1975)
OV-101 WCOT	CAVALLARO et al. (1982)
OV-17/Poly S-179	NESTRICK et al. (1980)
Silar 10C	BUSER & RAPPE (1980)
SE-30 WCOT	HARLESS et al. (1980)
SE-54	COCHRANE et al. (1981)
SP-2100	MITCHUM et al. (1980)
Methyl Silicone WCOT	NORSTROM et al. (1982)

As can be easily seen from Table 2, there are many ways whereby samples can be introduced into the mass spectrometer. Although no direct comparison of these various methods has been made, many investigators become strong advocates of the latest technology which presently is capillary column gas chromatography. The main advantage of these columns is their capability to separate isomers. For example, Buser and Rappe[7] demonstrated that 2,3,7,8-TCDD could be separated from all its 21 isomers by use of a Silar 10C capillary column using OV-17 and OV-101 capillaries for confirmation. Harless et al.[24,25] emphasize "the total enhancement of the high resolution mass spectrometry detection techniques" produced by the use of capillary columns.

MEASUREMENT BY MASS SPECTROMETRY

Mass spectrometry in the electron impact mode was first reported for the identification of PCDDs by Ress (1970). Since then it has had extensive development. As shown in the table below, at least a dozen models operating at low resolution ($M/\Delta M$ <3000) have been used to both identify and determine PCDDs after separation by Stages I and II.

TABLE 3: Stage III: Mass Spectrometry (Low Resolution Electron Impact) M/ΔM <2000

MS MODEL	T_4CDD LIMIT OF DETECTION pg	M/ΔM	REFERENCE
LKB-9000	6	600	CRUMMETT & STEHL (1973)
	5-10	400	HUMMEL (1977)
	2-10	400	SHADOFF & HUMMEL (1978)
	50	---	ADAMOLI et al. (1978)
LKB-9000S	20	---	DI DOMENICO et al. (1979)
LKB-2091	250	400	FANELLI et al. (1980)
FINNIGAN 3000	50	UNIT	ADAMOLI et al. (1978)
FINNIGAN 3200	20	UNIT	DI DOMENICO et al. (1979)
	40-80	UNIT	CAVALLARO et al. (1980)
FINNIGAN 4023	1-2	UNIT	TOSINE et al. (1981)
HEWP-5984A	20	UNIT	DI DOMENICO et al. (1979)
-5992A	40-60	UNIT	LAMPARSKI & NESTRICK (1980)
-5992	5-10	UNIT	NORSTROM et al. (1981)
-	1-5	UNIT	REES et al. (1981)
EXTRA-NUC.	1000	UNIT	TIERNAN et al. (1980)
KRATOS 30	5	1000	LANGHORST & SHADOFF (1980)
VARIAN - 311A	10	1000	RYAN & PILON (1980)

Very little work has been done at medium resolution (M/ΔM = 2500 to 9000) as shown below. Although this approach would be expected to have some advantage in the sensitivity over that of the same instrument operated at higher resolution.

TABLE 4: Stage III: Mass Spectrometry - Medium Resolution Electron Impact

MS MODEL	T_4CDD LIMIT OF DETECTION pg	M/ΔM	REFERENCE
KRATOS-30	5-10	3000	HUMMEL (1977)
KRATOS-50	50	2000	CHESS & GROSS (1980)

Although high resolution mass spectrometry (M/ΔM >9000) was used in the early work of Baughman and Meselson (1973), it has only recently been emphasized by some mass spectroscopists as being important. This is primarily due to the work of Harless et al. (1980). The experience with this approach is summarized below:

TABLE 5: Stage III: Mass Spectrometry - High Resolution Electron Impact

MS MODEL	T_4CDD LIMIT OF DETECTION pg	M/ΔM	REFERENCE
VARIAN 311A	5-10	9000	HARLESS et al. (1980)
AEI MS-9	5	10000	BAUGHMAN & MESELSON (1973)
KRATOS MS-30	100	12000	TIERNAN et al. (1980)
KRATOS MS-30	2-10	9000	SHADOFF & HUMMEL (1978)
KRATOS MS-50	1-10	10000	SMITH et al. (1981)

Chemical ionization mass spectrometry has been under investigation as a possible detector and monitor for PCDDs. The quantitative results of this work are summarized in the following table:

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TABLE 6: Stage III: Mass Spectrometry - Chemical Ionization

MS MODEL	T_4CDD LIMIT OF DETECTION pg	M/ΔM UNIT	REFERENCE
FINNIGAN 3300	50-500	UNIT	HASS et al. (1978)
EXTRA-NUC.	10-30	UNIT	MITCHUM et al. (1980) CAIRNS et al. (1980)

More recently[59] (MS/MS) has been used as a detector. Using a Finnigan triple stage quadrupole mass spectrometer the limit of detection for 2,7-DCDD and 1,3,7-T_3CDD was found to be about 10 picograms.

It is evident from the tables that the limit of detection for T_4CDD is very much the same on high resolution instruments as on low resolution ones. The advantage of high resolution lies in its ability to separate interferences in the mass spectrometer.

Criteria for the confirmation of the homologue and specific isomer is essential to any analysis. Harless et al. (1980) have proposed the following:

HARLESS CRITERIA FOR HOMOLOGUE AND SPECIFIC ISOMER CONFIRMATION

1. Correct HRGC-HRMS retention time for 2,3,7,8-T_4CDD
2. Correct HRGC-HRMS multiple ion response for $^{37}Cl_4$-T_4CDD and T_4CDD masses (simultaneous response for elemental composition of m/z 320, m/z 322, m/z 328).
3. Correct chlorine isotope ratio for the molecular ions (m/z 320 and m/z 322).
4. Correct responses for the co-injection of sample fortified with $^{37}Cl_4$-T_4CDD and T_4CDD standard.
5. Response of the m/z 320 and m/z 322 must be greater than 2.5 times the noise level.

SUPPLEMENTAL CRITERIA PROPOSED BY HARLESS

(A) COCl loss indicative of T_4CDD structure, and

(B) HRGC-HRMS peak matching analysis of m/z 320 and m/z 322 in real time to confirm the T_4CDD elemental composition.

These criteria are useful and are agreeable to almost all analytical scientists. However, it is not necessary to specify capillary gas chromatography (HRGC) in either criterion 1 or 2 since it is not needed if high resolution liquid chromatography (HPLC) is used. Also, the limit of detection in criteria 5 would be better defined as 3 times the noise level. The supplemental criteria are nice to have but usually cannot be obtained at concentration levels near the limit of detection where such information is most needed.

When HPLC is used in a manner to separate all 22 TCDD isomers, the following criteria pertains:

NESTRICK AND LAMPARSKI CRITERIA FOR SPECIFIC ISOMER CONFIRMATION

1. Correct reverse-phase HPLC retention time for TCDD isomers
2. Correct normal phase HPLC retention time for TCDD isomers
3. Correct GC (packed or capillary) retention time for TCDD isomers
4. Multiple ion detection at m/z 320, 322, and 324 with correct Cl-isotope ratio
5. Response of m/z 320 and m/z 322 greater than 3 times the noise level

THE ANALYTICAL PROCESS

All three stages of the analytical process may be thought of as consisting of three categories of resolving power: low (L), medium (M), and high (H). The categories of sample preparation are described as follows:

TABLE 7: Stage I - Sample Preparation

RESOLVING POWER	DESCRIPTION
L	CHEMICAL TREATMENT AND/OR EXTRACTION NO CHROMATOGRAPHY
M	L + COLUMN CHROMATOGRAPHY
H	M + HIGH PRESSURE LIQUID CHROMATOGRAPHY

The sample introduction into the mass spectrometer may be described as follows:

TABLE 8: Stage II - MS Sample Introduction

RESOLVING POWER	DESCRIPTION
L	DIRECT PROBE (NO GAS CHROMATOGRAPHY)
M	PACKED COLUMN GC
H	CAPILLARY COLUMN GC

Likewise, the operation of the mass spectrometer may be assigned rankings as follows:

TABLE 9: Stage III - Mass Spectrometry (MS)

RESOLVING POWER	DESCRIPTION
L	LOW RESOLUTION (M/ΔM <2500)
M	MEDIUM RESOLUTION (M/ΔM = 2500 to 9000)
H	HIGH RESOLUTION (M/ΔM > 9000)

Obviously, these can be put together in all possible combinations to creat various methods. Thus, methods could be developed which could be described by each of these acronyms.

TABLE 10: Possible Analytical Methods For The Determination of PCDDs and PCDFs

LLL	MLL	HLL
LLM	MLM	HLM
LLH	MLH*	HLH
LML	MML*	HML*
LMM	MMM	HMM
LMH	MMH*	HMH
LHL	MHL	HHL*
LHM	MHM	HHM
LHH	MHH*	HHH*

At least seven of these (marked with asterisks) have been used recently to determine dioxins in environmental samples. Some of the studies are summarized in Table 11 along with the limits of detection and estimated ability to resolve TCDD isomers.

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TABLE 11: Performance of PCDD Analytical Methods on Environmental Samples

ANALYTICAL SYSTEM	LIMIT OF DETECTION, ppt	NUMBER OF T_4CDD ISOMERS SEPARATED	SAMPLE MATRIX	REFERENCE
MLH	0.2-3	0	HUMAN MILK	MESELSON & O'KEEFE (1977)
MMH	0.1-6	4-12	HUMAN MILK	GROSS (1980)
MMH	3-6	4-12	HUMAN MILK	TIERNAN (1981)
MHH	1-6	15-22	HUMAN MILK	SHADOFF et al. (1977)
MML	0.5-1	15-22	HUMAN MILK	LOOMIS et al. (1980)
MMH	2-4	4-12	HUMAN ADIPOSE TISSUE	GROSS (1980)
MHH	3-5	15-22	HUMAN ADIPOSE TISSUE	
MML	1	4-12	BOVINE MILK	MAHLE et al. (1977)
MML	2-10	4-12	BOVINE TISSUES	JENSEN et al. (1981)
MML	10	4-12	FISH	FUKUHARA et al. (1975)
MML	3-5	4-12	GULL EGGS	NORSTROM et al. (1982)
MML	4-20	15-22	FISH	LAMPARSKI et al. (1979)
MHL	0.6-2	22	FLYASH	NESTRICK & LAMPARSKI (1982)
MHL	1-2	22	FISH	TOSINE et al. (1981)
MHH	4-13	15-22	FISH	HARLESS & LEWIS (1982)
HHH	1-5	22	FISH	SMITH et al. (1981)

Of course for most of these studies the specificity of the method for 2,3,7,8-TCDD is the most important consideration and many of these methods are specific for this isomer.

Even though each step in the methodology just described is well understood, results generated by its use need to be carefully interpreted and documented if they are to be compared with other similar data. This is made clear by the ACS guidelines[1] and reaffirmed at the IUPAC Conference in Helsinki[12]. A Canadian panel[42] applied the ACS guidelines to rate publications and found only two that came close to meeting the guidelines. However, the references which are cited in the following tables all contain essential elements which permit the data to be compared even though collaborative studies have not always been done. To be included in the tables the data must meet the following criteria:

TABLE 12: Criteria For Intercomparability

1. Isomer specificity defined and similar
2. Sufficient recovery studies reported
3. Interferences removed or their role explained
4. Appropriate analytical standards used
5. Detection limits defined and reported
6. Sampling protocol documented

It is of interest to look at the results of some of these methodologies in more detail especially as they relate to a particular matrix. Table 13 summarizes data taken from the determination of 2,3,7,8-TCDD in fish. Positive results were reported for fish from bodies of water close to industrial sites but not found in those accummulating water from areas heavily sprayed with 2,4,5-T. The positive results, however, have not been systematically

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traced to a particular source causing Harless (1982) to conclude that "the source or sources of the contamination is unknown". Data taken by different laboratories on fish from the same body of water appear to be in reasonable agreement although the methodology is considerably different.

Data taken on various species of fish shown in Table 14 are generally higher on bottom feeders. This would be predicted and gives further confidence in the reliability of the data.

TABLE 13: Search For 2,3,7,8-TCDD In Fish

LABORATORY	ANALYTICAL SYSTEM	SAMPLE SOURCE	YEAR SAMPLED	NUMBER OF SAMPLES	NUMBER APPARENT POSITIVE RESULTS	APPARENT TCDD CONC. RANGE	REFERENCE
Dow	MML	Texas	1974				SHADOFF et al. (1977)
		Reservoir		20	0	ND(5-7)	
		Arkansas	1974				
		Rice Pond		20	1	ND(4)-6(5)	
Dow	MML	Michigan	1976				NESTRICK & LAMPARSKI (1979)
		Tittabawassee River		10	9	ND(20)-170(120)	
		Saginaw Bay		2	0	ND(10)	
MDNR-EPA	MMH	Michigan	1978				OSWALD (1978)
		Tittabawassee River		18	13	ND(2)-695	
		Saginaw River		5	4	ND(11)-105	HARLESS (1982)
		Saginaw River Mouth		3	3	11(4)-153	
		Saginaw Bay		2	1	ND(3)-4(2)	
		Grand River		7	5	ND(2)-41(8)	
		Lake Michigan		1	0	ND(7)	
Dow	MML	Michigan	1979				NESTRICK & LAMPARSKI (1979)
		Tittabawassee River		13	7	ND(3)-500	
		Saginaw River		12	11	ND(3)-310	
		Saginaw River Mouth		12	12	5(1)-32(2)	
		Saginaw Bay		18	14	ND(4)-29(5)	
		Flint River		6	5	ND(3)-11(2)	
		Cass River		5	3	ND(5)-104	
		Shiawassee River		6	1	ND(5)-9(9)	
NYS-DOH	MMH	Lake Ontario	1978-1980	10	10	4(1)-162	SMITH et al. (1981)
		Lake Huron	1978-1980	7	6	ND(2)-29	
MOE	MML	Niagara River	1980	23	0	ND(10)	NATIONAL RESEARCH COUNCIL CANADA (1981)
		Lake Ontario	1980	52	14	ND(10)-19(10)	
		Lake Erie	1980	3	0	ND(10)	
		Lake Huron	1980	18	0	ND(10)	
		Lake Superior	1980	6	0	ND(10)	
		Detroit River	1980	3	0	ND(10)	
		Grand River	1980	2	0	ND(10)	
		Canagagique Cr.	1980	6	0	ND(10)	
NYS-DOH	MMH	Lake Ontario	1978-1980	46	45	ND(3)-107	NRCC (1981)
		Lake Erie	1979	17	5	ND(1)-2	
		Lake Superior	1979	1	1	1(1)	
		Lake Michigan	1979	5	0	ND(2)-ND(5)	

TABLE 14: Relationship of 2,3,7,8-TCDD Concentration To Fish Species

SPECIES	YEAR SAMPLED	LABORATORY	NUMBER SAMPLES	NO. APPARENT POSITIVE RESULTS	APPARENT TCDD CONC. RANGE, PPT	MEAN APPARENT CONC. PPT
Channel Catfish	1976	Dow	2	2	90-170	130 (avg.)
	1978	MDNR-EPA	8	8	28(13)-695	160
	1979	Dow	17	17	9(3)-500	30
Carp	1976	Dow	1	0	ND(80)	--
	1978	MDNR-EPA	14	10	20(7)-153	55
	1979	Dow	1	0	ND(5)	--
Bullhead	1976	Dow	3	3	20(20)-140(40)	70
	1979	Dow	14	9	1(1)-14(7)	9
Crappie	1976	Dow	3	3	40(20)-90(30)	80
	1979	Dow	16	10	ND(3)-118(14)	10
Yellow Perch	1978	MDNR-EPA	6	3	ND(5)-20	13
	1979	Dow	12	9	ND(4)-10(5)	5
Lake Trout	1980	NYS-DOH	3	3	41-72	42

Although not all the data generated by the EPA Dioxin Implementation Plan have been published, some of it is shown in Table 15. These data can easily be misinterpreted and require special explanation. The Dow data were taken on the fat cut from steaks. With a limit of detection of 3 times noise only one sample gave a positive signal. The samples analyzed by EPA-Dow were a complete set of peritoneal beef fat samples taken in phase I of the Dioxin Implementation Plan while those analyzed by EPA-WSU were a part of the same set. Other analytical collaborators were EPA and the University of Utah. From the table it appears that EPA-Dow found 9 positive results on 88 samples. However, most of these same samples were shown to be negative by other investigators with lower detection limits. Only the Missouri sample showing a concentration of 60 parts per trillion at a detection limit of 25 was proven positive (EPA-WSU found 54(25) for example). Similar comments apply to the data generated by other collaborators. So the table is a good example of false positive data resulting from both the uncertainty of the methodology and interferences.

TABLE 15: Search For 2,3,7,8-TCDD In Beef Fat

LABORATORY	ANALYTICAL SYSTEM	SAMPLE SOURCE	YEAR SAMPLED	NUMBER OF SAMPLES	NUMBER OF APPARENT POSITIVE RESULTS	RANGE OF APPARENT POSITIVE 2,3,7,8-TCDD CONC. (PPT)	REFERENCE
Dow	MML/MMH	Texas	1976	19	3	3(3)-6(4)	CRUMMETT (1979)
		Oklahoma		2	0	ND(5)	
		Missouri		3	0	ND(5)	
EPA-Dow (DIP)	MML/MMH	Texas	1976	20	0	ND(6)-ND(23)	
				5 (controls)	0	ND(3)-ND(18)	
		Oklahoma		19	2	9(5)-30(20)	
				5 (controls)	0	ND(5)-ND(20)	
		Kansas		15	1	7(6)	
				10 (controls)	1	6(4)	
		Missouri		14	5	10(5)-60(25)	
EPA-WSU (DIP)	MMH	Texas/ Oklahoma/ Missouri/Kansas	1976	28	8	11(10)-54(25)	TIERNAN (1981)
		Unknown	Unknown	39	4	10(7)-33(8)	

TABLE 16: Search For 2,3,7,8-TCDD In Beef Liver

LABORATORY	ANALYTICAL SYSTEM	SAMPLE SOURCE	YEAR SAMPLED	NUMBER OF SAMPLES	NUMBER OF APPARENT POSITIVE RESULTS	APPARENT TCDD CONC. RANGE	REFERENCE
EPA-Dow (DIP)	MML	Texas	1976	20	0	ND(2)-ND(100)	CRUMMETT (1979)
				5 (controls)	0	ND(3)-ND(7)	
		Kansas	1976	10	0	ND(2)-ND(6)	
				1 (control)	0	ND(5)	
		Oklahoma	1976	5	0	ND(2)-ND(25)	
				2 (controls)	0	ND(2)-ND(5)	
		Missouri	1976	6	0	ND(2)-ND(15)	
EPA-WSU (DIP)	MMH	Texas Kansas Oklahoma Missouri	1976	37	3	13(8)-40(8)	TIERNAN (1981)

TABLE 17: Search For 2,3,7,8-TCDD In Bovine Milk

LABORATORY	ANALYTICAL SYSTEM	SAMPLE SOURCE	YEAR SAMPLED	NUMBER OF SAMPLES	NUMBER OF APPARENT POSITIVE RESULTS	APPARENT TCDD CONC. RANGE	REFERENCE
Dow	MML	Oklahoma	1976	10	2	ND(1)-1(1)	MAHLE et al. (1977)
		Arkansas		7	2	ND(1)-1(1)	
		Missouri		8	2	ND(1)-1(1)	
		Michigan (controls)		2	1	ND(1)-1(1)	

Table 16 shows some of the results on the EPA DIP beef liver sampling. Many of the samples are from the same cattle as the beef fat previously discussed. The 3 apparent positive results obtained by WSU were not detected by other collaborators at lower detection limits.

Table 17 shows the small amount of work that has been reported on bovine milk. The authors used 2.5 times noise as a detection limit. At 3 times noise all results would have been negative at a detection limit of 1 part per trillion. This clearly illustrates the importance of having well-defined and accepted criteria for the determination of chemicals in environmental samples. This point is also illustrated in Table 18. The positive results reported by Meselson would be negative at 3 times noise. The positive results being reported by Loomis are also right at the detection limit. One explanation is that the limit of detection has now been lowered by improved analytical methodology to a level corresponding to that of the background itself.

TABLE 18: Search For 2,3,7,8-TCDD In Human Milk

LABORATORY	ANALYTICAL SYSTEM	SAMPLE SOURCE	YEAR SAMPLED	NUMBER OF SAMPLES	APPARENT POSITIVE RESULTS	CONCENTRATION RANGE OF SAMPLES, PPT	REFERENCE
Dow	MMH	Texas	1974	6	0	ND(1)-ND(6)	SHADOFF et al. (1977)
Harvard	MLH	Texas	1976	6	3	0.6(0.6)-1.5(1.5)	MESELSON & O'KEEFE (1977)
		Missouri	1976	6	0	ND(0.6)-ND(3)	
		Oregon	1976	7	1	0.8(0.7)-1.4(1.5)	
		Massachusetts	1976	6	0	ND(0.6)-ND(1.6)	
Harvard	MLH	Texas	1978	9	2	0.4(0.2)-0.6(0.3)	CRUMMETT et al. (1979)
		Kansas	1978	4	1	1(0.6)-ND(1.1)	
Dow	MML	Washington	1978	46	5	ND(0.5)-2(1)	LOOMIS et al. (1980)
EPA/UN	MMH	Wash./Ore./ California	1978	72	0	ND(0.2)-ND(6)	GROSS (1980)
		Calif./Alaska	1978	31	0	ND(0.1)-ND(3)	
EPA/WSU	MMH	Wash./Oregon Calif./Alaska	1978	7	0	ND(3)-ND(6)	TIERNAN (1981)

TABLE 19: Search For Dioxins Other Than 2,3,7,8-TCDD In Human Milk

DIOXIN	LABORATORY	ANALYTICAL SYSTEM	SAMPLE SOURCE	YEAR SAMPLED	NUMBER OF SAMPLES	APPARENT POSITIVE RESULTS	CONCENTRATION RANGE, PPT	REFERENCE
ISO - TCDDs	Dow	HML	Washington	1978	46	0	ND(0.5)-ND(0.7)	LOOMIS et al. (1980)
H_6CDD	Dow	HML	Washington	1978	46	27	ND(1)-4(3)	LOOMIS et al. (1980)
1,2,3,4,6,7,9-H_7CDD	Dow	HML	Washington	1978	46	3	ND(1)-2(1)	LOOMIS et al. (1980)
1,2,3,4,6,7,8-H_7CDD	Dow	HML	Washington	1978	46	33	ND(1)-7(2)	LOOMIS et al. (1980)
OCDD	Dow	HML	Washington	1978	46	38	ND(6)-41(6)	LOOMIS et al. (1980)

TABLE 20: Search For 2,3,7,8-TCDD In Adipose From Vietnam Veterans

LABORATORY	ANAL. SYSTEM	SAMPLE SOURCE	NUMBER OF SAMPLES	NUMBER OF APPARENT POSITIVE RESULTS	RANGE OF APPARENT 2,3,7,8-TCDD CONC. PPT	REFERENCE
UNL	MMH	Heavily Exposed	3	2	ND(3)-99	GROSS (1980)
		USAF Officers	2	2	4(3)-5(3)	
		Other Exposed	5	3	ND(2)-7(3)	
		Controls	5	3	ND(4)-20	
UNL-EPA	MMH	Heavily Exposed	1	1	36	
		USAF Officers	1	1	3	
		Other Exposed	3	2	3-5	
		Controls	2	2		
EPA	MMH	Heavily Exposed	3	3	20-173	
		USAF Officers	2	2	10-24	
		Other Exposed	5	2	ND(5)-18	
		Controls	5	4	ND(3)-46	

TABLE 21: Search For 2,3,7,8-TCDD In Human Adipose From Rice Growing States

LABORATORY	ANALYTICAL SYSTEM	SAMPLE SOURCE	YEAR SAMPLED	NUMBER OF SAMPLES	NUMBER OF APPARENT POSITIVE RESULTS	RANGE OF APPARENT 2,3,7,8-TCDD CONC. (PPT)	REFERENCE
UNL	MMH	Miss/Tenn/ Arkansas	1977	41	0	ND(1)-ND(12)	Gross (1980)
				2	0	ND(17)-ND(19)*	
				1	0	ND(160)**	

*Sample size too small

**Recovery only about 1%

The Loomis study also included other selected polychlorinated dibenzodioxins - total isomers of 2,3,7,8-tetrachlorodibenzo-p-dioxin, total hexachlorodibenzo-p-dioxins, the two hepta isomers, and octachlorodibenzo-p-dioxin. The results in Table 19 are positive in a large number of cases but are generally close to the limit of detection - only two being greater than ten times noise.

Table 20 on the determination of 2,3,7,8-TCDD in Vietnam veterans shows the type of agreement collaborative studies will produce using the most recently developed methodology.

Table 21 suggests that persons living in rice-growing areas are not as likely to have detectable levels of TCDD in their fat as those used for controls in the Vietnam veterans study.

A summary of these data is attempted in Table 22 in which the ACS guidelines of 3 times noise and 10 times noise for limit of detection and limit of quantitation is applied. Only 1 beef fat sample gives a quantitative signal and no human or bovine milk. However, 84 of 233 samples of fish contain quantitative signals for TCDD.

TABLE 22: Summary of 2,3,7,8-TCDD Data From Biological Samples

MATRIX	TOTAL SAMPLES ANALYZED	NUMBER OF APPARENT POSITIVE RESULTS	NUMBER AT CONCENTRATION LEVEL	
			>3 X NOISE	>10 X NOISE
Beef Fat	179	24	17	1
Beef Liver	51	0	0	0
Bovine Milk	27	7	0	0
Fish	233	146	134	84
Human Milk	193	12	6	0
Human Adipose (non-military)	49	4	4	3

Chlorinated Dioxins In Chemical Products

The determination of chlorinated dioxins in chemical products uses the same analytical systems of LC/GC/MS methodology as used for environmental samples. However, the detection limit is higher, usually in the part per billion range. Also, it has usually already been established that the dioxin being measured is present and it's merely a matter of deciding whether or not the level is above or below a regulatory limit or a level of concern.

The proper development of such methods requires the cooperation of industrial and governmental scientists. An excellent example of collaboration between Agriculture Canada and Dow Chemical is illustrated by the data shown in Tables 23, 24, and 25. Although the methodology was considerably different, the results agree to a remarkable degree. This kind of cooperative effort must be supported and encouraged if reasonable regulations are to result.

TABLE 23: Dioxins In 2,4-D Butoxyethyl Ester

SAMPLE NUMBER	APPARENT DCDD, PPB		APPARENT T_4CDD, PPB	
	AGRICULTURE CANADA	DOW	AGRICULTURE CANADA	DOW
1	2	1	0	1
2	5	4	4	4
3	2	1	2	1
4	ND	1	ND	1
5	2	1	1	1
29	ND	3	ND	1
30	ND	1	ND	1
31	ND	1	ND	1

TABLE 24: Dioxins In Formula 40 (DEA)

SAMPLE NUMBER	APPARENT DCCD, PPB		APPARENT T_4CDD, PPB	
	AGRICULTURE CANADA	DOW	AGRICULTURE CANADA	DOW
6	ND	4	ND	3
7	2	1	2	1
8	1	ND	ND	ND
9	1	ND	ND	ND
37	5	8	6	7

TABLE 25: Dioxins In DMA 6 Unsequestered

SAMPLE NUMBER	APPARENT DCCD, PPB		APPARENT T_4CDD, PPB	
	AGRICULTURE CANADA	DOW	AGRICULTURE CANADA	DOW
11	ND	1	ND	1
12	ND	1	ND	ND
19	6	10	5	8
20	20	30	24	38
21	1	1	--	--
22	1	1	ND	ND
23	2	2	ND	1
25	1	1	ND	ND
26	2	3	2	2

This report has been concerned primarily with the use of LC/GC/MS systems for the quantitative determination of the chlorinated dibenzo-p-dioxins in environmental and biological samples and products. Other analytical methods which are at an early stage of development largely for screening purposes include radio immunoassay (McKinney et al. 1982), aryl hydrocarbon hydroxylase induction assay (Bradlaw and Casterline, 1979), cytosil-receptor assay (Hutzinger et al. 1981), and kertatization of mouse teratoma cell line XB (Knutson and Poland, 1980).

SUMMARY AND CONCLUSIONS

Many techniques are available to dissolve or extract samples containing chlorinated dioxins, to remove the bulk of the interferences, to separate homologues and isomers, and to measure isomers either specifically or as part of a homologue. These techniques can be combined in an almost infinite number of ways to yield procedures which appear to produce reliable data. Because these procedures are tedious and require considerable time to carry out, true measures of the certainty of results are often neglected. Thus, validation and collaborative studies are seldom done to the satisfaction of the scientific community.

However, the quality assurance programs now placed on much of the work are so rigorous that the certainty of the data is assured. In fact no other analysis known to me is subjected to such a complete and comprehensive control. Recent methods thus serve as models for other investigations in trace analysis. Analytical scientists can now write protocols which are based on good estimates of the sensitivity and specificity of the total analytical scheme. This approach is much preferred over the use of "best available analytical methodology" as often specified by regulators.

Of course more validation and collaborative studies are needed if regulation is indicated. However, these should not require that all participants use identical procedures but that the methodologies which are used are equivalent and are subjected to suitable quality control. Both intra- and inter-laboratory studies are required. However, regulations should be based on inter-laboratory studies. Moreover, any regulatory level should include allowance for the certainty of the method. This generally requires that regulation be attempted at levels no lower than the limit of quantitation.

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WEDNESDAY, 19 OCTOBER

8:00 [REGISTRATION AND COFFEE]

WELCOME AND INTRODUCTION

9:00 WELCOME

Joshua Lederberg, PhD (President, The Rockefeller University)

9:15 INTRODUCTION TO THE SYMPOSIUM

William Lowrance, PhD (Senior Fellow and Director, Life Sciences and Public Policy Program, The Rockefeller University)

9:30 SPECTRUM OF METABOLIC EFFECTS FOUND IN ANIMAL EXPERIMENTS

Robert Neal, PhD (President, Chemical Industry Institute of Toxicology)

10:00 GENERAL DISCUSSION

CHEMICAL ANALYSIS AND EXPOSURE ESTIMATION

10:15 POSSIBILITIES, LIMITATIONS, AND PITFALLS OF CHEMICAL ANALYSIS

Thomas O. Tiernan, PhD (Professor of Chemistry and Director, Brehm Laboratory, Wright State University)

10:45 U.S. AND CANADIAN ANALYSES OF HUMAN TISSUES

Alvin L. Young, PhD, Lt. Col. USAF (Special Assistant for Environmental Sciences, Agent Orange Projects Office, U.S. Veterans Administration)

11:00 SWEDISH ANALYSES OF HUMAN TISSUES

Christopher Rappe, PhD (Professor and Chairman, Department of Organic Chemistry, University of Umea)

11:15 PANEL DISCUSSION OF ANALYTIC AND EXPOSURE-ESTIMATION ISSUES

Charles Nauman, PhD, MPH (Environmental Scientist, Office of Health and Environmental Assessment, U.S. Environmental Protection Agency)

Michael L. Taylor, PhD (Associate Professor of Pharmacology/Toxicology, Wright State University)

Helle Tosine, PhD (Advanced Instrumentation Supervisor, Pesticides Section, Laboratory Services Branch, Canadian Ministry of the Environment)

Mary S. Wolff, PhD (Assistant Professor of Chemistry, Mt. Sinai School of Medicine)

11:40 GENERAL DISCUSSION

12:00 [LUNCH BREAK]

CARCINOGENICITY AND CANCER RISK ASSESSMENT

1:30 SUMMARY AND CRITIQUE OF RODENT CARCINOGENICITY ASSAYS

Richard J. Kociba, PhD (Senior Associate Scientist, Toxicology Research Laboratory, Dow Chemical U.S.A.)

2:10 RISK ASSESSMENT PROJECTIONS BASED ON RODENT CARCINOGENICITY ASSAYS

John Van Ryzin, PhD (Professor of Biostatistics, School of Public Health, Columbia University) [reporting calculations conducted jointly with David G. Hoel and Christopher J. Portier of the U.S. National Institute of Environmental Health Sciences]

2:30 COMMENTS ON RISK ASSESSMENT MODELS

Nancy K. Kim, PhD (Director, Bureau of Toxic Substances Assessment, New York State Department of Health)

2:45 AN APPROACH TO RISK ASSESSMENT FOR DIOXINS IN SOIL

Renate D. Kimbrough, MD (Medical Officer, Center for Environmental Health, U.S. Centers for Disease Control)

3:15 [COFFEE BREAK]

3:30 DIOXINS AS CARCINOGENIC PROMOTERS

I. Bernard Weinstein, MD (Professor of Medicine and Director, Division of Environmental Sciences, Columbia University College of Physicians and Surgeons)

3:40 DIOXINS AS ANTICARCINOGENS

John Di Giovanni, PhD (Assistant Professor of Biochemistry, Systems Cancer Center, University of Texas)

3:50 GENERAL DISCUSSION

HUMAN SOFT-TISSUE SARCOMA ISSUE

4:00 PATHOLOGICAL DEFINITION OF SOFT-TISSUE SARCOMAS

Steven I. Hajdu, MD (Attending Pathologist and Chief, Pathology Service, Memorial Sloan-Kettering Cancer Center)

4:20 REINTERPRETATION OF RECENT U.S. OCCUPATIONAL SOFT-TISSUE SARCOMA CASES

Marilyn Fingerhut, PhD (Epidemiologist, Division of Surveillance, Hazard Evaluations, and Field Studies, U.S. National Institute for Occupational Safety and Health)

4:35 DIOXINS AND SOFT-TISSUE SARCOMAS

Ralph R. Cook, MD, MPH (Director of Epidemiology, Dow Chemical U.S.A.)

4:55 GENERAL DISCUSSION

5:30-7:00 [COCKTAIL RECEPTION FOR ALL PARTICIPANTS AND OBSERVERS]

THURSDAY, 20 OCTOBER

8:00 [COFFEE]

IMMUNOTOXICITY

9:00 IMMUNOLOGICAL OBSERVATIONS IN ANIMALS

Jack H. Dean, PhD (Head, Department of Cell Biology, Chemical Industry Institute of Toxicology)

9:15 POSSIBLE HUMAN IMMUNOTOXIC EFFECTS

Albert E. Munson, PhD (Associate Professor of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University)

9:30 RECENT CLINICAL IMMUNOLOGICAL OBSERVATIONS

J. George Bekesi, MD (Professor of Medicine and Neoplastic Disease; and Director, Environmental Immunobiology Laboratory, Mt. Sinai School of Medicine)

9:45 GENERAL DISCUSSION

REPRODUCTIVE TOXICITY

10:00 OVERVIEW OF REPRODUCTIVE EFFECTS

Donald Mattison, MD (Medical Officer, Pregnancy Research Branch, U.S. National Institute for Child Health and Human Development)

10:15 TERATOGENICITY AND FETOTOXICITY

Wilbur P. McNulty, MD (Head, Division of Primate Medicine, Oregon Regional Primate Research Center)

10:30 MONITORING FOR REPRODUCTIVE EFFECTS IN POPULATIONS AT RISK

Zena A. Stein, MB, BCh (Professor of Epidemiology, School of Public Health, Columbia University; and Director, Epidemiology of Developmental Brain Disorders, New York State Psychiatric Institute)

10:45 GENERAL DISCUSSION

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BROAD METABOLIC TOXICITY

11:00 ALTERATION OF CELL MEMBRANE FUNCTION, CAUSING WIDESPREAD METABOLIC CHANGES

Fumio Matsumura, PhD (Director, Pesticide Research Center, Michigan State University)

11:20 THYROTOXIC EFFECTS

Carl Potter, PhD (Postdoctoral Trainee, Environmental Toxicology Center, School of Pharmacy, University of Wisconsin--Madison)

11:30 HORMONAL CHANGES AND "WASTING" OBSERVED IN MODERATELY-HIGH-DOSE ANIMAL EXPERIMENTS

Richard Peterson, PhD (Associate Professor of Pharmacology and Toxicology, School of Pharmacy, University of Wisconsin--Madison)

11:45 SOME OBSERVATIONS ON YUSHO DISEASE

Masanori Kuratsune, MD (Professor of Public Health, Kyushu University)

12:00 GENERAL DISCUSSION

12:30 [LUNCH BREAK]

2:00 PANEL TO COMMENT ON ASSESSMENT ISSUES [tentative]

Paul F. Deisler, Jr., PhD (Vice President for Health, Safety, and Environment, Shell Chemical Company)

Perry J. Gehring, PhD (Vice President for Agricultural Products R&D; and Director of Health and Environmental Science U.S.A., Dow Chemical Company)

Michael Gough, PhD (Senior Analyst, Office of Technology Assessment, U.S. Congress)

Robert Harris, PhD (Co-Director, Hazardous Waste and Research Division, Center for Energy and Environmental Studies, Princeton University)

Ellen Silbergeld, PhD (Chief Scientist, Environmental Defense Fund)

SUMMARIES OF CURRENT ACTIVITIES

2:45 THE NIOSH OCCUPATIONAL DIOXIN REGISTRY

Marilyn Fingerhut, PhD (Epidemiologist, Division of Surveillance, Hazard Evaluations, and Field Studies, U.S. National Institute for Occupational Safety and Health)

3:00 PROPOSED FOOD AND DRUG ADMINISTRATION APPROACH TO TOLERANCE-SETTING FOR DIOXINS IN FOODS

Robert Scheuplein, PhD (Associate Director for Toxicological Sciences, U.S. Food and Drug Administration)

3:15 CURRENT ENVIRONMENTAL PROTECTION AGENCY ACTIVITIES

Donald G. Barnes, PhD (Science Advisor to the Assistant Administrator, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency)

3:45 PANEL TO COMMENT ON BIOCHEMICAL AND CLINICAL ISSUES [tentative]

Attallah Kappas, MD (Professor and Head, Laboratory of Metabolism--Pharmacology; and Physician-in-Chief, The Rockefeller University)

Robert W. Miller, MD (Chief, Clinical Epidemiology Branch, U.S. National Cancer Institute)

Ruth Lilis, MD (Associate Professor of Occupational and Environmental Medicine, Mt. Sinai School of Medicine)

James P. Whitlock, Jr., MD (Associate Professor of Pharmacology, Stanford University School of Medicine)

4:15 PANEL TO COMMENT ON RESEARCH STRATEGY AND AGENDA [tentative]

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Anthony Cerami, PhD (Professor and Head, Laboratory of Medical Biochemistry, The Rockefeller University)

Glenn Paulson, PhD (Vice President for Science, National Audubon Society)

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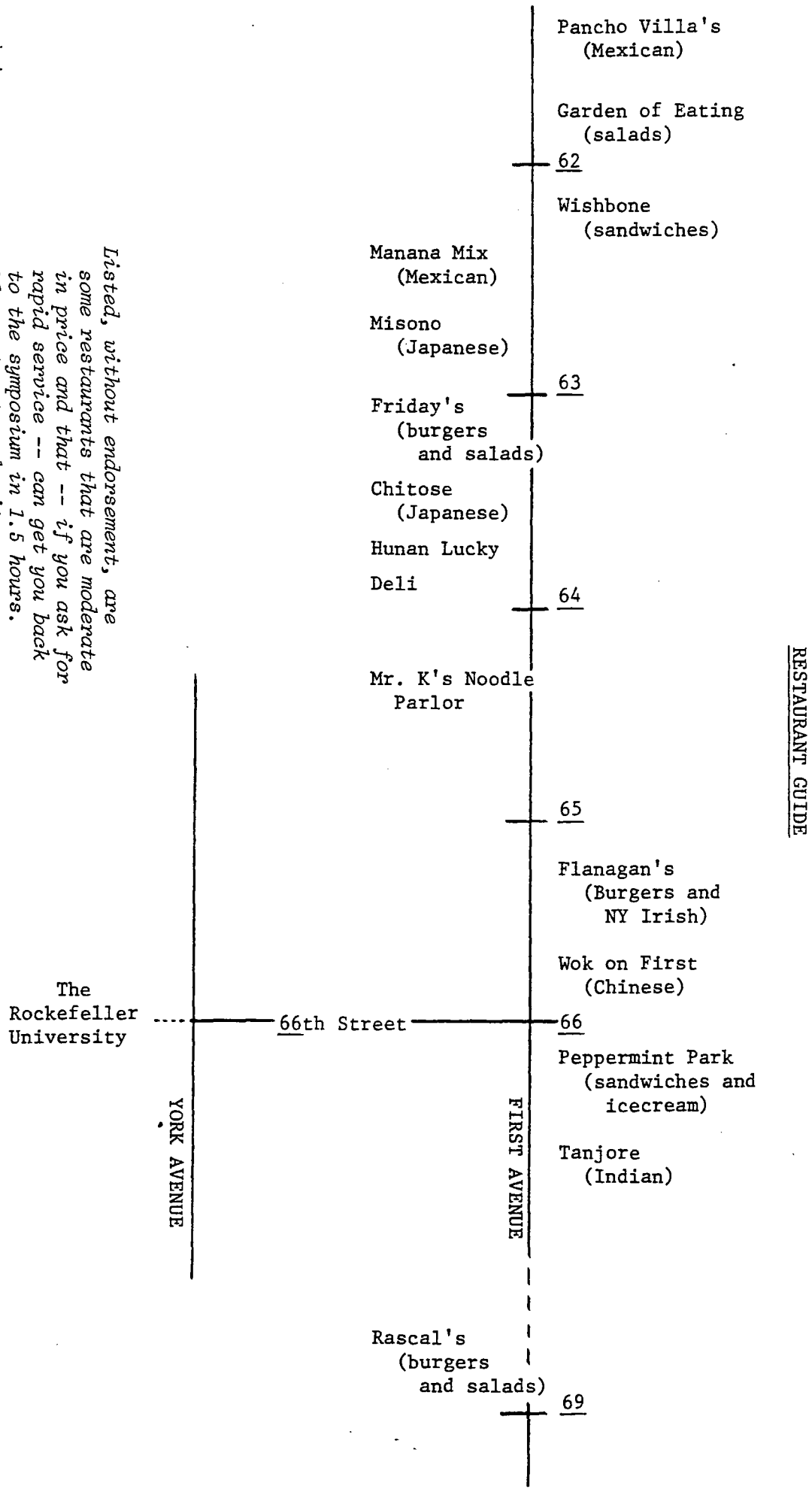
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RESTAURANT GUIDE

Listed, without endorsement, are some restaurants that are moderate in price and that -- if you ask for rapid service -- can get you back to the symposium in 1.5 hours. Please try to make it...

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Symposium Announcement
and
Call for Abstracts

SEP 1 1983

DIOXINS IN THE ENVIRONMENT

December 6 and 7, 1983
Kellogg Center for Continuing Education
Michigan State University
East Lansing, Michigan 48824

Sponsored by Center for Environmental Toxicology, Michigan State University
in cooperation with
Cranbrook Institute of Science
and
Limno-Tech, Inc., Ann Arbor

Continuing concern with dioxins in the United States, especially the Great Lakes region, has led to heightened public awareness and increased effort on the part of government, academic and industrial scientists to understand and deal with possible health and environmental problems due to these chemicals. The purpose of this symposium is to bring together scientists from these various sectors to discuss what we know at present, what we need to know for the future and what is presently being done to fill in these knowledge gaps. Symposium topics include analytical methodology, monitoring, environmental source, distribution and fate, human and animal health effects, mechanisms of action and public policy issues. Experts from U.S., Canada and Europe will deliver comprehensive talks which will place the most recent research results into an overall framework. Interested parties from State, Provincial and Federal agencies, academia and industry are invited to attend and participate. Special invitation is extended to individuals concerned with environmental quality in the Great Lakes region. Space will be provided for poster presentations. Poster abstracts on dioxins and related chemicals (single space, approximately 4½x7½) should be submitted to the Center for Environmental Toxicology by November 1, 1983. The abstracts will be reproduced and distributed at the meeting.

This symposium will be followed by two days of workshops designed to summarize the state-of-knowledge of the symposium topics, explore relevant issues, and make recommendations for future research and policy decisions. The proceedings of the symposium and the results of the workshops will be published at a later date.

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Speakers include:

D. Crosby, Department of Environmental Toxicology
University of California - Davis
W. Crummett, Analytical Laboratories
Dow Chemical Company
J. Doull, Department of Pharmacology and Toxicology
University of Kansas Medical Center
M. Gross, Chemistry Department
University of Nebraska
R. Hites, Chemistry Department
Indiana University
O. Hutzinger, Laboratory of Environmental and Toxicological Chemistry
University of Amsterdam, Netherlands
W. Lowrance, Life Sciences and Public Policy Institute
The Rockefeller University
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E. Smuckler, Pathology Department
University of California - San Francisco
D. Stalling, Columbia National Fisheries Research Laboratory
U.S. Fish and Wildlife Service
R. Suskind, Institute of Environmental Health
University of Cincinnati
A. Young, Office of Environmental Medicine
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The registration fee for this meeting will be $50. Meals and housing will be available in Kellogg Center at reasonable rates. Please use the attached form to obtain more detailed information and registration materials.

Send further information on this meeting to:

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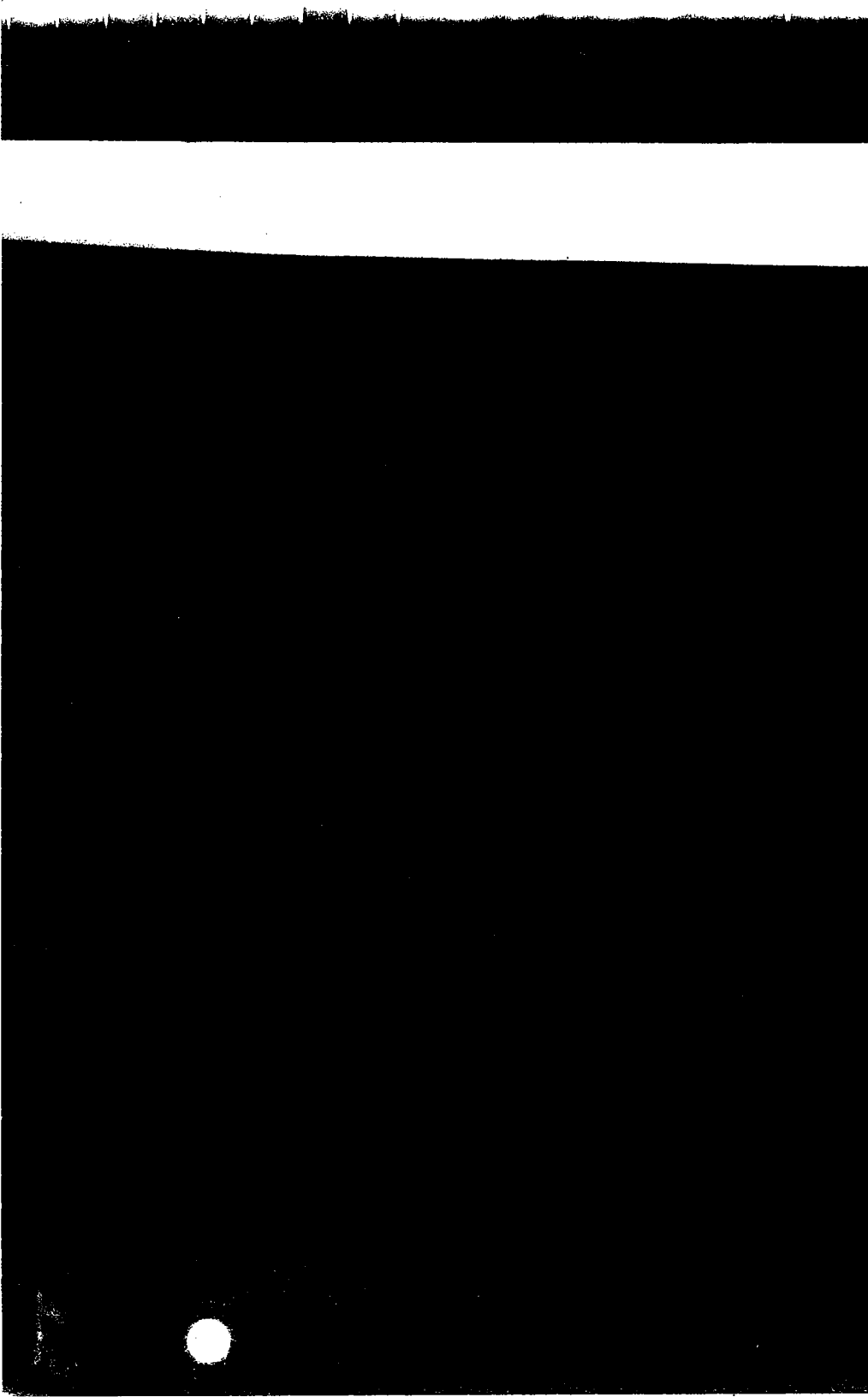
Information on Registration & Housing

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EXHIBITS TO EATON DEPO
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The Science of the Total Environment, 31 (1983) 203—218
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CARCINOGENICITY AND TOXICITY OF 2,4-DICHLOROPHENOXY-ACETIC ACID

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(Received February 9th, 1983; accepted May 4th, 1983)

ABSTRACT

2,4-Dichlorophenoxyacetic acid (2,4-D) is carcinogenic in male and female rats and probably also in mice. Male and female rats ingesting 2,4-D developed increased incidences of malignant neoplasms. Lymphosarcomas were increased in rats of both sexes, and neoplasms of the mammary gland in female rats. Male rats also had carcinomas of the endocrine organs. 2,4-D isooctyl ester was carcinogenic for the lymphoreticular system in female mice. 2,4-D and 2,4-dichlorophenol also were promoters of neoplasms of the skin in mice. Male mice given 2,4-D isopropyl ester developed an increased incidence of neoplasms of the lung.

2,4-D also is mutagenic and teratogenic in animals and causes poisoning in animals and human beings.

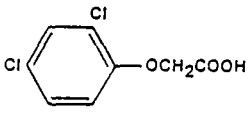
INTRODUCTION

The auxinlike, plant growth-promoting chemical, 2,4-dichlorophenoxy-acetic acid (2,4-D), has been used for may years as a plant herbicide [1]. The acid, its salts, and its esters are used to kill dicotyledonous weeds and certain other plant species. The chemical is readily absorbed from the digestive tract of animals and excreted by the kidney. Acute and subacute toxicity tests have shown that 2,4-D can cause poisoning in animals.

2,4-D is widely used as a herbicide to control certain types of vegetation. It is sprayed from airplanes on many millions of acres to kill shrub and broad-leaved plant life on specialized farms and range and pasture lands used for grazing. 2,4-D is used to kill vegetation adjacent to railroads, highways and other roads, pipe lines, power lines, and in and around lakes, ponds and irrigation ditches. It is used in aerial spraying operations of forest lands to kill broad-leaved vegetation and encourage soft wooded trees such as spruce and pine. It is present in preparations sold at retail for home gardeners and is widely used on home lawns and grass covered areas used for recreational purposes. 2,4-D is also used on cereal crops such as rice, on sugar cane and to control ripening of bananas and citrus fruits, to delay preharvest dropping of some fruits, and for the control of rot when lemons are stored. 2,4-D

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2, 4-D

was extensively sprayed together with 2,4,5-trichlorophenoxyacetic acid on millions of acres as a defoliant in Vietnam.

With the widespread use of this herbicide, possible toxic effects are important. 2,4-D finds its way to humans through direct contact either occupationally or by spraying humans purposely or accidentally. Drift after spraying can contaminate gardens, plant nurseries, water and other areas. Residues may inadvertently be encountered in food, vegetables, meat and water.

The production and use of 2,4-D can only be estimated because the data often is considered as trade secret by the manufacturers. The latest data available are for the year 1975. An estimated 27 million kg were used in the U.S.A. The Federal Republic of Germany and the U.K. are the major producing countries of western Europe where annual production was estimated to be 3—30 million kg. In eastern Europe it was estimated to be less than 10 million kg. In Japan production amounted to 511 thousand kg.

This review includes, to the best of our knowledge, every study on the carcinogenicity of 2,4-D, or its esters, and a metabolite, 2,4-dichlorophenol in animals. The raw data and histological sections were reviewed for the FDA 2,4-D Rat Study and FDA 2,4-D Dog Study [1—3] and the results are based on this examination. The Innes et al. Mouse Studies for 2,4-D, or its esters [4], the Archipov and Kozlova 2,4-D Mouse and Rat Studies [5], and the Boutwell and Bosch 2,4-Dichlorophenol Mouse Study [6] are also included.

Statistical tests of significance were p (probability) values obtained with Fisher's exact test and tests for positive linear trend and departure from linear trend.

FDA 2,4-D RAT STUDY

Osborne-Mendel rats, three weeks of age, ingested either 0, 5, 25, 125, 625, or 1250 ppm of 2,4-D in the diet for 104 weeks [1, 3]. There were 25 males and 25 females at each dose. The rats were individually caged and were weighed weekly. Hematologic studies were done periodically.

Survivors were killed after 104 weeks, and organ weights were taken for heart, liver, spleen, kidneys, and testes. Organs were also weighed from moribund rats killed after 52 weeks; however, no organ weights were included from rats that died.

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TABLE 1
NUMBER OF MALE RATS INGESTING 2,4-D WITH CARCINOMAS AND SARCOMAS (FDA RAT STUDY)

Dose (ppm)	Carcinomas	p	Sarcomas	p	Both[a]	p
0	0/25 (0%)		1/25 (4%)		1/25 (4%)	
5	2/25 (8%)		2/25 (8%)		3/25 (12%)	
25	6/25 (24%)	0.011	4/25 (16%)		9/25 (36%)	0.0053
125	6/25 (24%)	0.011	6/25 (24%)	0.049	9/25 (36%)	0.0053
625	5/24 (21%)	0.022	4/24 (17%)		6/24 (25%)	0.043
1250	3/23 (13%)		7/23 (30%)	0.018	9/23 (39%)	0.0033
5—1250	22/122 (18%)	0.012	23/122 (19%)	0.052	36/122 (30%)	0.0038
				0.021[b]		0.039[b]
		0.057[c]				0.034[c]

[a] Some rats had both carcinoma and sarcoma and are counted only once.
[b] Test for positive trend.
[c] Departure from linear trend.

Detailed histologic examination was made of tissues from six male rats and six female rats from the 1250 ppm 2,4-D groups and the control groups. Liver, kidney, spleen, and ovary or testes, as well as tumors and other gross lesions, were sectioned histologically from the rats on all other doses of 2,4,-D.

At the end of 104 weeks the following rats were alive: 12 males, 6 females (0 ppm); 8 males, 4 females (5 ppm); 9 males, 9 females (25 ppm); 9 males, 8 females (125 ppm); 10 males, 9 females (625 ppm); and 5 males, 11 females (1250 ppm).

Body weights and the organ—body weight ratios for liver, kidney, heart and testes were reported as showing no differences among the various groups. These results have little meaning, however, since the rats that died with the most chance of being abnormal were not included. The spleen—body weight ratio was slightly elevated in rats given 625 ppm or 125 ppm 2,4-D ($p \leqslant 0.05$).

Terminally, the red blood cells of rats given 5 ppm, 625 ppm, or 1250 ppm 2,4-D showed macrocytosis, slight polychromasia, and slight to moderate hypochromasia. These changes were not present, or were of a minor degree in control rats.

Malignant neoplasms at all sites in male rats

There were increased incidences of malignant neoplasms in male rats given all doses of 2,4-D (Table 1). Nine of 25 rats (36%) given 25 or 125 ppm ($p = 0.0053$) and 9 of 23 (39%) given 1250 ppm of 2,4-D ($p = 0.0033$) developed carcinomas and sarcomas. The incidences of these neoplasms were dose related ($p = 0.039$). For some unexplained reason, the rats receiving 625 ppm, 6 of 24 (25%), had fewer malignant neoplasms than male

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TABLE 2
NUMBER OF MALE RATS INGESTING 2,4-D WITH LYMPHOSARCOMAS AND NEUROSARCOMAS (FDA RAT STUDY)

Dose (ppm)	Lymphosarcoma	p	Subcutaneous neurosarcomas
0	0/25 (0%)		0/25 (0%)
5	2/25 (8%)		0/25 (0%)
25	4/25 (16%)	0.055	0/25 (0%)
125	5/25 (20%)	0.025	1/25 (4%)
625	3/24 (13%)		1/24 (4%)
1250	6/23 (26%)	0.0082	1/23 (4%)
5—1250	20/122 (16%)	0.018 0.034[a]	3/122 (2%)

[a] Test for positive trend.

rats in other groups ($p = 0.043$). Thirty-six of 122 (30%) 2,4-D-treated male rats had carcinomas and sarcomas ($p = 0.0038$).

Sarcomas in male rats

The sarcomas in 2,4-D-treated male rats were mainly lymphosarcomas, however, there were a small number of subcutaneous neurosarcomas (Table 2). Sarcomas were present in 4 of 25 rats (16%) ingesting 25 ppm, 6 of 25 rats (24%) ingesting 125 ppm ($p = 0.049$), and 7 of 23 (30%) receiving 1,250 ppm of 2,4-D ($p = 0.018$) (Table 1). Twenty-three of 122 (19%) male rats given 2,4-D had sarcomas ($p = 0.052$).

Carcinomas in male rats

Carcinomas in male rats treated with 2,4-D were seen in the endocrine system. Six of 25 male rats (24%) ingesting 25 or 125 ppm ($p = 0.011$) and 5 of 24 rats (21%) given 625 ppm ($p = 0.022$) of 2,4-D developed carcinomas (Table 1). Twenty-two of 122 (18%) male rats given 2,4-D had carcinomas ($p = 0.012$).

Malignant neoplasms at all sites in female rats

Eight of 20 (40%) female rats given 5 ppm ($p = 0.019$), 11 of 22 (50%) given 25 ppm ($p = 0.058$), 13 of 23 (57%) on 125 ppm ($p = 0.022$), 18 of 24 (75%) receiving 625 ppm ($p = 0.00047$), and 17 of 25 (68%) female rats ingesting 1,250 ppm ($p = 0.0022$) of 2,4-D developed malignant neoplasms at all sites (Table 3). Altogether, malignant neoplasms were observed in 67 of 114 female rats (59%) ingesting 2,4-D ($p = 0.0018$).

Lesions of the lymphoreticular system in female rats

The highest incidence of lymphosarcomas was 12 of 24 (50%) female rats given 625 ppm of 2,4-D ($p = 0.00007$) (Table 4). Female rats in the other

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TABLE 3
NUMBER OF FEMALE RATS INGESTING 2,4-D WITH CARCINOMAS AND SARCOMAS (FDA RAT STUDY)

Dose (ppm)	Carcinomas	p	Sarcomas	p	Both[a]	p
0	4/22 (18%)		1/22 (5%)		5/22 (23%)	
5	8/20 (40%)		5/20 (25%)		8/20 (40%)	
25	7/22 (32%)		8/22 (36%)	0.011	11/22 (50%)	0.058
125	10/23 (43%)	0.065	8/23 (35%)	0.013	13/23 (57%)	0.022
625	14/24 (58%)	0.0059	12/24 (50%)	0.00007	18/24 (75%)	0.00047
1250	13/25 (52%)	0.017	10/25 (40%)	0.0044	17/25 (68%)	0.0022
5—1250	52/114 (46%)	0.013	43/114 (36%)	0.0017	67/114 (59%)	0.0018
		0.017[b]		0.024[b]		0.0022[b]
				0.089[c]		0.090[c]

[a] Some rats had both carcinomas and sarcomas and are counted only once.
[b] Test for positive trend.
[c] Departure from linear trend.

2,4-D treatment groups had 24—27% lymphosarcomas ($p = 0.011$—0.018). Thirty-one percent of all females ($p = 0.00073$) developed lymphosarcomas. The findings are even more significant if hyperplasia of the lymphoreticular system is considered along with lymphosarcomas, i.e., 39% ($p = 0.00005$) of female rats ingesting 2,4-D. Female rats also had sarcomas of the uterus.

Carcinomas at all sites in female rats

Carcinomas at all sites were seen in 43% (10 of 23) ($p = 0.065$) of female rats on 125 ppm, 58% (14 of 24) ($p = 0.0059$) given 625 ppm, 52% (13 of 25) ($p = 0.017$) receiving 1250 ppm and 46% (52/114) ($p = 0.013$) of female rats given 2,4-D (Table 3). Carcinomas were found in the reproductive system, particularly the mammary gland, and occasionally in endocrine organs.

Neoplasms of the mammary gland in female rats

The incidence of mammary gland tumors was higher in treated female rats, particularly on gross examination, which is generally quite reliable. The number of rats with such tumors was less in all but one group after histologic examination (Table 5).

On gross examination, 18 of 21 female rats (86%) ingesting 25 ppm ($p = 0.0064$), 20 of 24 rats (83%) ingesting 625 ppm ($p = 0.0080$) and 16 of 23 rats (70%) given 1250 ppm of 2,4-D had neoplasms of the mammary gland, compared to 10 of 22 (45%) control female rats. The findings are quite different for the numbers or rats with histological sections examined by pathologists at FDA and sections available for this examination.

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TABLE 4
NUMBER OF FEMALE RATS INGESTING 2,4-D WITH LESIONS OF THE LYMPHORETICULAR SYSTEM (FDA RAT STUDY)

Dose (ppm)	Hyperplasia	*p*	Lymphosarcoma	*p*	Hyperplasia and lymphosarcoma	*p*
0	0/22 (0%)		0/22 (0%)		0/22 (0%)	
5	0/22 (0%)		5/20 (25%)	0.018	5/20 (25%)	0.018
25	2/22 (9%)		6/22 (27%)	0.011	8/22 (36%)	0.0018
125	2/23 (9%)		6/23 (26%	0.012	8/23 (35%)	0.0023
625	4/24 (17%)	0.065	12/24 (50%)	0.00007	16/24 (67%)	< 0.00001
1250	2/25 (8%)		6/25 (24%)	0.016	8/25 (32%)	0.0034
5—1250	10/114 (9%)		35/114 (31%)	0.00073	45/114 (39%)	0.00005
						0.039[a]
						0.0004[b]

[a] Test for positive trend.
[b] Departure from linear trend.

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TABLE 5

NUMBER OF FEMALE RATS INGESTING 2,4-D WITH NEOPLASMS OF THE MAMMARY GLAND AND HISTOLOGIC EXAMINATION[a] (FDA RAT STUDY)

Dose (ppm)	Gross[b]	*p*	Histologic[c]	*p*	Histologic[d]	*p*
0	10/22 (45%)		10/22 (45%)		8/22 (36%)	
5	8/20 (40%)		7/20 (35%)		7/20 (35%)	
25	18/21 (86%)	0.0064	11/21 (52%)		11/21 (50%)	
125	8/23 (35%)		10/23 (43%)		9/23 (39%)	
625	20/24 (83%)	0.0080	14/24 (58%)		15/24 (63%)	0.070
1250	16/23 (70%)	0.091	15/23 (65%)		11/23 (45%)	
5—1250	70/111 (63%)		57/111 (52%)		53/111 (47%)	
		0.0233[e]		0.0334[e]		
		0.0008[f]				

[a] Corrected for survival time, i.e., time of appearance of first neoplasms of the mammary gland.
[b] Gross necropsy at FDA.
[c] Histological examination by FDA.
[d] Histological examination by Reuber.
[e] Test for positive trend.
[f] Departure from trend.

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Comments

It is worth noting that histological examination of tissues from this study was inadequate. Microscopic neoplasms would have been overlooked at the 5 to 625 ppm doses of 2,4-D (only gross neoplasms were sectioned histologically) and also even at the highest dose because only six rats of each sex were examined in detail. Also histological sections were not available for 19 mammary gland neoplasms described at the time of necropsy, and no explanation was given for this discrepancy.

Despite the shortcomings of this study, it must be considered as an acceptable study.

The largest numbers of rats alive were in the 25 to 1250 ppm doses; and the smallest number of rats alive was in the lowest dose, 5 ppm. These results suggest that rats in at least some of the 25 to 1250 ppm groups were not ingesting their diets because of toxicity. The differences in the incidences of neoplasms, therefore, would not be great.

Tumors were analyzed, on the basis of 25 rats per group, and the following conclusions were made by the authors: "There is a statistically significant ($p \leqslant 0.05$) linear relationship between the proportion of female rats with tumors and the level of the log dose, but not the arithmetic dose, indicating that there is a tendency for the proportion of female rats with tumors to increase with the log dosage. There is also a statistically significant ($p \leqslant 0.05$) linear relationship between the proportion of male rats with malignant tumors and the level of arithmetic and log dose. A comparison was made of the control group with each treatment group. Statistically significant ($p \leqslant 0.05$) differences were found only between the control group and the 1250 ppm dose level with respect to male rats with malignant tumors."

The authors also believed that the raw data and the pathologic interpretation did not support the statistical analyses and that "a carcinogenic effect of 2,4-D has not been shown". They also stated that "additional support for this interpretation has been given by the long-term study in mice".

Summary

Male and female rats ingesting 2,4-D developed increased incidences of malignant neoplasms. Lymphosarcomas were increased in rats of both sexes, and neoplasms of the mammary gland in female rats.

Conclusions

2,4-D is carcinogenic for male and female rats.

FDA 2,4-D DOG STUDY

Beagle dogs, 6 to 8 months old (3 males and 3 females per group) ingested 0, 10, 50, 100, or 500 ppm 2,4-D in the diet for 104 weeks [1, 2]. Organ weights were taken for brain, heart, liver, kidneys, spleen, thyroid, adrenals, and testes. Tissues from all dogs were studied grossly and microscopically.

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Many of the dogs ingesting 2,4-D lost weight. There were scattered lesions such as atrophy of the testes and prostate, interstitial nephritis, hemangioma of the adrenal, atrophic or cystic pituitary, atrophy of the thyroid, and hypoplasia of the bone marrow. Most of the lesions were seen in the endocrine organs. Control dogs generally did not have lesions.

Comments

Previous experience at FDA has shown that long-term chronic dog studies should be carried out for six years or longer in order for neoplasms to develop. Since they occurred predominantly in 2,4-D treated dogs, lesions may have progressed to neoplasms had the dogs been treated for a longer period of time.

Summary

A two-year feeding study in dogs cannot be considered a carcinogenicity study.

INNES et al. 2,4-D, OR ITS ESTERS, ORAL MOUSE STUDIES

1. 2,4-D

The maximum tolerated dose of 2,4-D was given to two hybrid strains of mice, (C57BL/6 x C3H/Anf)F_1 designated as "strain A" and (C57BL/6 x AKR)F_1 designated as "strain B" mice [4, 7]. There were 18 treated mice and 18 untreated controls of each strain and each sex. 46.4 mg/kg was given in 0.5% gelatin daily by stomach tube beginning at 7 days of age. After the mice were weaned at 28 days of age, 149 ppm of 2,4-D was mixed directly in the diet and provided ad libitum. "Strain B" male and female mice were also given 100 mg/kg, followed by 323 ppm. Treatment was continued approximately 18 months.

Postmortem included thorough external examination and internal examination of the neck glands and the thoracic and abdominal cavities, with histologic examination of major organs and of all grossly visible lesions. Thyroid glands were not examined.

There was no increase in neoplasms in the 2,4-D-treated mice.

2. 2,4-D isopropyl ester

"Strain A" and "strain B" male and female mice were given 46.6 mg/kg of 2,4-dichlorophenoxyacetic acid, isopropyl ester in 0.5% gelatin daily by stomach tube. At 28 days of age the mice received 111 ppm in the diet for approximately 18 months.

There were neoplasms in the lungs of 4 of 18 "strain A" male mice (22%) ingesting 2,4-D isopropyl ester compared to 2 of 17 matched controls (12%) and 5 of 79 pooled male control mice (6%) ($p = 0.0584$) (Table 6).

In this study, "strain A" male mice receiving 2,4-D isopropyl ester developed an increased incidence of neoplasms of the lung.

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TABLE 6
NUMBER OF MALE AND FEMALE MICE INGESTING 2,4-D ISOPROPYL ESTER WITH NEOPLASMS OF THE LUNG (INNES et al. [4, 7], MOUSE STUDY)

Strain	Dose (ppm)	Male	p	Female
"A" Matched	0	2/17 (12%)		1/18 (6%)
"A" Pooled	0	5/79 (6%)		3/87 (3%)
"A"	111	4/18 (22%)	0.0584	1/18 (6%)
"B" Matched	0	2/18 (11%)		0/17 (0%)
"B" Pooled	0	9/90 (10%)		3/82 (4%)
"B"	111	2/18 (11%)		0/17 (0%)

3. 2,4-D butyl ester

"Strain A" and "strain B" male and female mice received 46.4 mg/kg of 2,4-dichlorophenoxyacetic acid, butyl ester in 0.5% gelatin daily by stomach tube. At 28 days of age the mice received 149 ppm in the diet for approximately 18 months.

Three of 18 "strain A" female mice (17%) and 4 of 87 pooled controls (5%) had reticulum cell sarcomas ($p = 0.0955$) (Table 7).

In this study, there was an increased incidence of reticulum cell sarcoma in 2,4-D butyl ester-treated "strain A" female mice.

4. 2,4-D isooctyl ester

"Strain A" and "strain B" male and female mice received 46.4 mg/kg of 2,4-dichlorophenoxyacetic acid, isooctyl ester in 0.5% gelatin by stomach tube. After 28 days they ingested 130 ppm in the diet for approximately 18 months.

Neoplasms in treated mice were found mainly in the liver and lung. One "strain A" treated male mouse had an "angioma" of the liver and another an "angioma" of the spleen.

In this study, neoplasms of the liver were slightly increased in 2,4-D

TABLE 7
NUMBERS OF MALE AND FEMALE MICE INGESTING 2,4-D BUTYL ESTER WITH RETICULUM CELL SARCOMAS (INNES et al. [4, 7], MOUSE STUDY)

Strain	Dose (ppm)	Male	p	Female	p
"A" Matched	0	1/17 (6%)		2/18 (11%)	
"A" Pooled	0	5/79 (6%)		4/87 (5%)	
"A"	149	0/18 (0%)		3/18 (17%)	0.0955
"B" Matched	0	1/18 (6%)		2/17 (12%)	
"B" Pooled	0	1/90 (1%)		3/82 (4%)	
"B"	149	2/18 (11%)	0.0714	0/16 (0%)	

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isooctyl ester-treated "strain A" male mice and tumors of the lung in "strain A" female mice. Two male mice had rare tumors; "angiomas" of the liver and spleen.

INNES et al. 2,4-D, OR ITS ESTERS, SUBCUTANEOUS MOUSE STUDY

A single subcutaneous injection of 2,4-D, or its esters, was given in the nape of the neck to two hybrid strains of mice, (C57BL/6 x C3H/Anf)F_1 designated as "strain A" and (C57BL/6 x AKR)F_1 designated as "strain B" at approximately the 28th day of age [7]. There were 18 treated mice and 18 untreated controls of each strain and each sex. They were killed after approximately 18 months.

Mice received the following doses: 215 mg/kg or 464 mg/kg of 2,4-D in DMSO; 100 mg/kg of 2,4-D isopropyl ester, 25.5 mg/kg of 2,4-D butyl ester, or 21.5 mg/kg of 2,4-D isooctyl ester in corn oil.

Necropsy included thorough external examination and internal examination of the neck glands and the thoracic and abdominal cavities. Histological examination was done on all major organs and of all grossly visible lesions.

Reticulum cell sarcomas in "strain B" female mice

Five of 17 "strain B" female mice (29%) given 2,4-D isooctyl ester had reticulum cell sarcomas, compared to 5 of 157 control female mice (3%) ($p = 0.0009$) (Table 8).

Summary

2,4-D isooctyl ester was carcinogenic for the lymphoreticular system (reticulum cell sarcomas) in "strain B" female mice.

ARCHIPOV AND KOZLOVA MOUSE AND RAT STUDIES

Three groups, 100 each, of CBA x C57/BL hybrid mice were used [5]. Two groups of mice received one drop of a 0.5% solution of 3-methylcholanthrene

TABLE 8

NUMBER OF "STRAIN B" MALE AND FEMALE MICE GIVEN A SINGLE SUBCUTANEOUS INJECTION OF 2,4-D ISOOCTYL ESTER WITH RETICULUM CELL SARCOMAS (INNES et al. [4, 7], MOUSE STUDY)

Dose (mg/kg)	Males	Females	p
0	0/161 (0%)	5/157 (3%)	
1	0/18 (0%)	5/17 (29%)	0.0009

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in benzene on the skin for 3 weeks. Later the skin of one group of mice was painted with a 10% solution of the amine salt of 2,4-D in acetone. The skin of the third group of mice was painted with a 10% solution of the herbicide. Mice were observed for 20 months.

Neoplasms of the skin

Papillomas developed on the skin of 17.7% of the mice treated with 3-methylcholanthrene followed by 2,4-D, and none for the mice receiving 3-methylcholanthrene only or 2,4-D only.

Comments

Groups of rats also ingested 1/10th the LD_{50} of the amine salt of 2,4-D [5]. The dose was not given.

Summary

2,4-D is a promoter of neoplasms of the skin in mice.

BOUTWELL AND BOSCH 2,4-DICHLOROPHENOL MOUSE SKIN STUDY

Female Sutter mice, 2—3 months of age, were used [6]. Mice were painted on the skin with a single initiating dose of 0.3% dimethylbenzanthracene in acetone followed by 20% 2,4-dichlorophenol in benzene, benzene only, or no treatment. 2,4-Dichlorophenol was applied twice weekly. One group of mice was observed for 15 weeks and a second for 24 weeks.

Neoplasms of the skin

Mice treated with both chemicals developed an increased incidence of neoplasms of the skin. Thirteen of 27 treated mice (48%) ($p = 0.00607$) had papillomas and 3 of 27 treated mice (11%) had carcinomas of the skin, compared to 1 of 15 untreated mice (7%) with papillomas and 0 of 15 untreated mice (0%) after 15 weeks. Papillomas of the skin were observed in 12 of 16 treated mice (75%) ($p = 0.00004$) and carcinomas of the skin in 10 of 16 treated mice (62%) ($p < 0.00001$); whereas, papillomas were seen in 3 of 27 mice (11%) and carcinomas in 0 of 27 mice (0%) receiving benzene only after 24 weeks.

Conclusion

2,4-Dichlorophenol promoted the incidence of neoplasms of the skin in mice when administered after a single initiating dose of dimethylbenzanthracene.

DISCUSSION

2,4-D was found to be carcinogenic for rats in the original publication by

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Hansen et al. [1]. It was also concluded that "2,4-dichlorophenoxyacetic acid is carcinogenic in rats" in a preliminary review of the raw data, but not the histological sections, requested by Senator Edward M. Kennedy's Subcommittee on Administrative Practice and Procedure of the Committee on the Judiciary, United States Senate [8]. The examination and diagnoses of the histological sections, which was done for this review further strengthened the findings and conclusion that 2,4-D is carcinogenic in rats.

Experience has shown that it is necessary to review the raw data and histological sections of old chronic toxicity and carcinogenicity studies in animals [9, 10, 11]. The data from studies often is not adequately analyzed and the conclusions are suspect. It also is not unusual for results of such studies, which are statistically significant, to be ignored because they are described as not biologically significant. The pathology report for the FDA 2,4-D study in rats was completed in 1964; however, the results were not published until 1971 [1, 3].

2,4-D is mutagenic and teratogenic as well as carcinogenic [12]. 2,4-D and its derivatives are teratogenic in mice, rats and hamsters. 2,4-D induces skeletal malformations, cleft palats, eye malformations, subcutaneous edema and hemorrhages in the snout, abdominal cavity, liver and soft tissues in rats when administered during early pregnancy [7, 13—17]. 2,4-D is embryotoxic [18]. 2,4-D penetrates the placenta and the fetuses [19]. 2,4-D caused point mutations in animal cells without liver activation, damages DNA in a manner similar to ionizing radiation and stimulates mitosis [20—22]. Endothel-treated fruit flies developed an increase of recessive lethal mutations [23].

2,4-Dichlorophenol is a major product of the breakdown of 2,4-D by microorganisms [24]. The compound is also teratogenic in rats and carcinogenic in mice. [6, 14].

2,4-D is rapidly absorbed from the human gastrointestinal tract and distributed widely throughout the body in animals and in human beings [25—28]. It is fat soluble and rapidly absorbed through the skin and lungs [29]. Acute effects of 2,4-D poisoning in humans include headache, weakness, dizziness, nausea and vomiting, sore throat, irritation of nasal mucosa, impared sense of taste and smell, substernal chest pain and loss of consciousness [30]. Peripheral neuropathy, which begins with tingling and numbness in the extremeties, has been reported in humans hours or days after exposure to 2,4-D on the skin. The symptoms in some people increased through several weeks until pain, paresthesia, and paralysis were severe. Disability was protracted and recovery was incomplete even after a lapse of years [31, 32]. These symptoms resemble those of multiple sclerosis, and this has been confirmed by the finding of plaques of acute demyelination in all parts of the brain of a man who died after ingestion of 2,4-D [33].

The formation and occurrence of tumors in man and other mammals, such as rats and mice, is quite similar [11]. In tests undertaken to date, it has been demonstrated that virtually every chemical which has been found to be carcinogenic in man is also carcinogenic in one or more

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mammalian test animals. It has been shown that if these compounds will produce tumors in one species, they will very likely produce tumors in more than one; thus adding weight to any finding of carcinogenicity in tests using any mammalian species.

Sufficient documentation is available on qualitative extrapolation of animal data that one must conclude that a finding of carcinogenicity in one mammalian species should be deemed to have relevance for other mammalian species — including man. This threat may not be manifested for up to 30 or 40 years, given the long latent period of many known human carcinogens. Given the state of carcinogenicity testing and knowledge, there can be only one safe tolerance for a carcinogen — an absolute zero tolerance — and the only way a substance shown to be carcinogenic in test animals will not be a threat to human health is simply to prevent that substance from entering the environment. To the extent that it does enter man's environment, it will constitute a very real threat to his health.

Recent reports have described an increase of malignant neoplasms in human beings exposed to phenoxyacetic acids. Humans exposed to phenoxyacetic acids or chlorophenols showed an increased risk for the development of soft-tissue sarcomas [34, 35]. Results also have indicated that humans in contact with phenoxy acids may also develop malignant lymphomas [36].

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Sarcomas in Male Rats

The sarcomas in 2,4-D treated male rats were mainly lymphosarcomas, however, there were a small number of subcutaneous neurosarcomas (Table 2). Sarcomas were present in 4 of 25 rats (16%) ingesting 25 ppm ($p = 0.055$), 6 of 25 rats (24%) ingesting 125 ppm ($p = 0.011$), and 7 of 23 (30%) receiving 1250 ppm of 2,4-D ($p = 0.0033$). Twenty-three of 122 (19%) male rats given 2,4-D had sarcomas ($p = 0.0092$).

Carcinomas in Male Rats

Carcinomas in male rats treated with 2,4-D were seen in the endocrine system. Six of 25 male rats ingesting 25 or 125 ppm ($p = 0.011$) and 5 of 24 rats (21%) given 625 ppm ($p = 0.022$) of 2,4-D developed carcinomas (Table 1).

Malignant Neoplasms at All Sites in Female Rats

Eight of 20 (40%) female rats given 5 ppm ($p = 0.019$), 11 of 22 (50%) given 25 ppm ($p = 0.058$), 13 of 23 (57%) on 125 ppm ($p = 0.022$), 18 of 24 (75%) receiving 625 ppm ($p = 0.00047$), and 17 of 25 (68%) female rats ingesting 1250 ppm of 2,4-D developed malignant neoplasms at all sites (Table 3). Altogether, malignant neoplasms were observed in 67 of 114 female rats (59%) ingesting 2,4-D ($p = 0.0018$).

Lesions of the Lymphoreticular System in Female Rats

The highest incidence of lymphosarcomas was 12 of 24 (50%) female rats given 625 ppm of 2,4-D ($p = 0.00007$) (Table 4). Female rats in the other 2,4-D treatment groups had 24 to 27% lymphosarcomas ($p = 0.011$ to $p = 0.018$). Thirty-one percent of all females ($p = 0.00073$) developed lesions of the lymphoreticular system. The findings are even more significant if hyperplasia is considered along with lymphosarcomas, i.e., 39% ($p = 0.00005$) of female rats ingesting 2,4-D. Female rats also had sarcomas of the uterus (see Table 3).

Carcinomas at All Sites in Female Rats

Carcinomas at all sites were seen in 43% (10 of 23) ($p = 0.065$) of female rats on 125 ppm, 58% (14 of 24) ($p = 0.0059$) given 625 ppm and 52% (13 of 25) ($p = 0.017$) receiving 1250 ppm 2,4-D (Table 3). Carcinomas were found in the reproductive system, particularly the mammary gland, and occasionally in endocrine organs.

Neoplasms of the Mammary Gland in Female Rats

The incidence of mammary gland tumors were higher in treated female rats, particularly on gross examination, which is generally quite reliable. The number of rats with such tumors was less in all but one group after histologic examination (Table 5).

On gross examination, 18 of 21 female mice (85%) ingesting 25 ppm ($p = 0.0064$), 20 of 24 rats (83%) ingesting 625 ppm ($p = 0.0080$) and 16 of 23 rats (70%) given 1250 ppm of 2,4-D had neoplasms of the mammary gland, compared to 10 of 22 (45%) control female rats. The findings are quite different for the numbers of histological sections examined by pathologists at FDA and sections available for my examination.

Comments

It is worth noting that histological examination of tissues from this study was inadequate. Microscopic neoplasms would have been overlooked at the 5 to 625 ppm doses of 2,4-D (only gross neoplasms were sectioned histologically) and also even at the highest dose because only six rats of each sex were examined in detail. Also histological sections were not available for 19 mammary gland neoplasms described at the time of necropsy, and no exaplanation was given for this discrepancy.

Despite the shortcomings of this study, it must be considered as an acceptable study.

The largest numbers of rats alive were in the 25 to 1250 ppm; and the smallest number of rats alive was in the lowest dose, 5 ppm. These results suggest that rats in at least some of the 25 to 1250 ppm groups were not ingesting their diets because of toxicity. The differences in the incidences of neoplasms, therefore, would not be great.

Tumors were analyzed, on the basis of 25 rats per group, and the following conclusions were made by the authors: "There is a statistically significant ($p = <0.05$) linear relationship between the proportion of female rats with tumors and the level of the log dose, but not the arithmetic dose, indicating that there is a tendency for the proportion of female rats with tumors to increase with the log dosage. There is also a statistically significant ($p = <0.05$) linear relationship between the proportion of male rats with malignant tumors and the level of arithmetic and log dose.... A comparison was made of the control group with each treatment group. Statistically significant ($p = <0.05$) differences were found only between the control group and the 1250 ppm dose level with respect to male rats with malignant tumors."

The authors also believed that the raw data and the pathologic interpretation did not support the statistical analyses and that "a carcinogenic effect of 2,4-D has not been shown." They also stated that "additional support for this interpretation has been given by the long-term study in mice (i.e., the Innes, et al. 2,4-D Mouse Study).

Summary

Male and female rats ingesting 2,4-D developed increased incidences of malignant neoplasms. Lymphosarcomas were increased in rats of both sexes, and neoplasms of the mammary gland in female rats.

Conclusions

2,4-D is carcinogenic for male and female rats.

FDA 2,4-D DOG STUDY

Beagle dogs, 6 to 8 months old (3 males and 3 females per group) ingested 0, 10, 50, 100, or 500 ppm 2,4-D in the diet for 104 weeks (1-3). Organ weights were taken for brain, heart, liver, kidneys, spleen, thyroid, adrenals, and testes. Tissues from all dogs were studied grossly and microscopically.

Many of the dogs ingesting 2,4-D lost weight. There were scattered lesions such as atrophy of the testes and prostate, interstitial nephritis, hemangioma of the adrenal, atrophic or cystic pituitary, atrophy of the thyroid, and hypoplasia of the bone marrow. Most of the lesions were seen in endocrine organs. Control dogs generally did not have lesions.

Comments

Previous experience at FDA has shown that long-term chronic dog studies should be carried out for six years or longer in order for neoplasms to develop. Since they occurred predominantly in 2,4-D treated dogs, lesions may have progressed to neoplasms had the dogs been treated for a longer period of time.

Summary

A two-year feeding study in dogs cannot be considered a carcinogenicity study.

INNES, ET AL. 2,4-D, OR ITS ESTERS, ORAL MOUSE STUDIES

1. 2,4-D

The maximum tolerated dose of 2,4-D was given to two hybrid strains of mice, (C57BL/6 x C3H/Anf)F_1 designated as "strain A" and (C57BL/6 x AKR)F_1

designated as "strain B" mice (4-5). There were 18 treated mice and 18 untreated controls of each strain and each sex. 46.4 mg/kg was given in 0.5% gelatin daily by stomach tube beginning at 7 days of age. After the mice were weaned at 28 days of age, 149 ppm of 2,4-D was mixed directly in the diet and provided ad libitum. "Strain B" male and female mice were also given 100 mg/kg, followed by 323 ppm. Treatment was continued approximately 18 months.

Postmortem included thorough external examination and internal examination of the neck glands and the thoracic and abdominal cavities, with histologic examination of major organs and of all grossly visible lesions. Thyroid glands were not examined.

There was no increase in neoplasms in the 2,4-D-treated mice.

2. 2,4-D Isopropyl Ester

"Strain A" and "strain B" male and female mice were given 46.6 mg/kg of 2,4-dichlorophenoxyacetic acid, isopropyl ester in 0.5% gelatin daily by stomach tube. At 28 days of age the mice received 111 ppm in the diet for approximately 18 months.

There were neoplasms in the lung of 4 of 18 strain A male mice (22%) ingesting 2,4-D isopropyl ester compared to 2 of 17 matched controls (12%) and 5 of 79 pooled male control mice (6%) ($p = 0.0584$) (Table 6).

In this study, strain A male mice receiving 2,4-D isopropyl ester developed an increased incidence of neoplasms of the lung.

3. 2,4-D Butyl Ester

"Strain A" and "strain B" male and female mice received 46.4 mg/kg of 2,4-dichlorophenoxyacetic acid, butyl ester in 0.5% gelatin daily by stomach tube. At 28 days of age the mice received 149 ppm in the diet for approximately 18 months.

Three of 18 strain A female mice (17%) and 4 of 87 pooled controls (5%) had reticulum cell sarcomas (p = 0.0955) (Table 7).

In this study, there was an increased incidence of reticulum cell sarcoma in 2,4-D butyl ester-treated strain A female mice.

4. 2,4-D Isooctyl Ester

"Strain A" and "strain B" male and female mice received 46.4 mg/kg of 2,4-dichlorophenoxyacetic acid, isooctyl ester in 0.5% gelatin by stomach tube. After 28 days they ingested 130 ppm in the diet for approximately 18 months.

Neoplasms in treated mice were found mainly in the liver and lung. One strain A treated male mouse had an "angioma" of the liver and another an "angioma" of the spleen.

In this study, neoplasms of the liver were slightly increased in 2,4-D isooctyl ester-treated strain A male mice and tumors of the lung in strain A female mice. Two male mice had rare tumors, "angiomas" of the liver and spleen.

INNES, ET AL. 2,4-D, OR ITS ESTERS, SUBCUTANEOUS MOUSE STUDY

A single subcutaneous injection of 2,4-D, or its esters, was given in the nape of the neck to two hybrid strains of mice, (C57BL/6 x C3H/Anf)F_1 designated as "strain A" and (C57BL/6 x AKR)F_1 designated as "strain B" at approximately the 28th day of age (4-5). There were 18 treated mice and 18 untreated controls of each strain and each sex. They were killed after approximately 18 months.

Mice received the following doses: 215 mg/kg or 464 mg/kg of 2,4-D in DMSO; 100 mg/kg of 2,4-D isopropyl ester, 25.5 mg/kg of 2,4-D butyl ester, or 21.5 mg/kg of 2,4-D isooctyl ester in corn oil.

Necropsy included thorough external examination and internal examination of the neck glands and the thoracic and abdominal cavities. Histological examination was done on all major organs and of all grossly visible lesions.

Reticulum Cell Sarcomas in "Strain B" Female Mice

Five of 17 "strain B" female mice (3%) given 2,4-D isooctyl ester had reticulum cell sarcomas, compared to 5 of 157 control female mice (29%) ($p = 0.0009$) (Table 8). Reticulum cell sarcomas were seen in 2 of 18 male mice (11%) and 0 of 161 control mice ($p = 0.0096$).

Summary

2,4-D isooctyl ester was carcinogenic for the lymphoreticular system (reticulum cell sarcomas) in "strain B" female and male mice.

COMMENTS

Virtually every chemical which has been found to be carcinogenic in humans is also known to be carcinogenic in one or more other mammalian test animals (Reuber, 1978). It has been demonstrated that if a compound will produce neoplasms in any one species, it will likely produce them in another. We strongly feel that a finding of carcinogenicity in one mammalian species is therefore relevant to all mammals, including humans, even though direct evidence of danger to humans may not be available.

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TABLE 1. NUMBER OF MALE RATS INGESTING 2,4-D WITH CARCINOMAS AND SARCOMAS (FDA Rat Study)

Dose (ppm)	Carcinomas	Sarcomas	Both[a/]
0	0/25 (0%)	1/25 (4%)	1/25 (4%)
5	2/25 (8%)	2/25 (8%)	3/25 (12%)
25	6/25 (24%) p = 0.011	4/25 (16%) p = 0.055	9/25 (36%) p = 0.0053
125	6/25 (24%) p = 0.011	6/25 (24%) p = 0.011	9/25 (36%) p = 0.0053
625	5/24 (21%) p = 0.022	4/24 (17%) p = 0.050	6/24 (25%) p = 0.043
1250	3/23 (13%)	7/23 (30%) p = 0.0033	9/23 (39%) p = 0.0033
5-1250	22/122 (18%) p = 0.012	23/122 (19%)p = 0.0092	36/122 (30%)p = 0.0038
		0.013[b/]	0.039[b/]
	0.057[c/]		0.034[c/]

a/ Some rats had both carcinoma and sarcoma and are counted only once.

b/ Test for positive trend

c/ Departure from linear trend

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TABLE 2. NUMBER OF MALE RATS INGESTING 2,4-D WITH LYMPHOSARCOMAS AND NEUROSARCOMAS (FDA Rat Study)

Dose (ppm)	Lymphosarcoma	Subcutaneous Neurosaromas	Total
0	0/25 (0%)	0/25 (0%)	0/25 (0%)
5	2/25 (8%)	0/25 (0%)	2/25 (8%)
25	4/25 (16%) p=0.055	0/25 (0%)	4/25 (16%) p=0.055
125	5/25 (20%) p=0.025	1/25 (4%)	6/25 (24%) p=0.011
625	3/24 (13%)	1/24 (4%)	4/24 (17%) p=0.050
1250	6/23 (26%) p=0.0082	1/23 (4%)	7/23 (30%) p=0.0033
5-1250	20/122 (16%) p=0.018 0.034[a/]	3/122 (2%)	23/122 (19%) p=0.0092 0.013[a/]

a/ Test for positive trend

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TABLE 3. NUMBER OF FEMALE RATS INGESTING 2,4-D WITH CARCINOMAS AND SARCOMAS (FDA Rat Study)

Dose (ppm)	Carcinomas	Sarcomas	Both[a/]
0	4/22 (18%)	1/22 (5%)	5/22 (23%)
5	8/20 (40%)	4/20 (20%)	8/20 (40%) p=0.019
25	7/22 (32%)	8/22 (36%) p=0.011	11/22 (50%) p=0.058
125	10/23 (43%) p=0.065	8/23 (35%) p=0.013	13/23 (57%) p=0.022
625	14/24 (58%) p=0.0059	12/24 (50%) p=0.00007	18/24 (75%) p=0.00047
1250	13/25 (52%) p=0.017	10/25 (40%) p=0.0044	17/25 (68%) p=0.0022
5-1250	52/114 (46%) p=0.013	41/114 (36%) p=0.0017	67/114 (59%) p=0.0018
	0.017[b/]	0.024[b/]	0.0022[b/]
		0.089[c/]	0.090[c/]

a/ Some rats had both carcinomas and sarcomas and are counted only once.

b/ Test for positive trend

c/ Departure from linear trend

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TABLE 4. NUMBER OF FEMALE RATS INGESTING 2,4-D WITH LESIONS OF THE LYMPHORETICULAR SYSTEM (FDA Rat Study)

Dose (ppm)	Hyperplasia	Lymphosarcoma	Hyperplasia and Lymphosarcoma
0	0/22 (0%)	0/22 (0%)	0/22 (0%)
5	0/20 (0%)	5/20 (25%) p=0.018	5/20 (25%) p=0.018
25	2/22 (9%)	6/22 (27%) p=0.011	8/22 (36%) p=0.0018
125	2/23 (9%)	6/23 (26%) p=0.012	8/23 (35%) p=0.0023
625	4/24 (17%) p=0.065	12/24 (50%) p=0.00007	16/24 (67%) p=<0.00001
1250	2/25 (8%) p=0.28	6/25 (24%) p=0.016	8/25 (32%) p=0.0034
5-1250	10/114 (9%) p=0.16	35/114 (31%) p=0.00073	45/114 (39%) p=0.00005 0.039[a/] 0.0004[b/]

a/ Test for positive trend

b/ Departure from linear trend

TABLE 5. NUMBER OF FEMALE RATS INGESTING 2,4-D WITH NEOPLASMS OF THE MAMMARY GLAND ON GROSS AND HISTOLOGIC EXAMINATION[a/]
(FDA Rat Study)

Dose (ppm)	Gross[b/]	Histologic[c/]	Histologic[d/]
0	10/22 (45%)	10/22 (45%)	8/22 (36%)
5	8/20 (40%)	7/20 (35%)	7/20 (35%)
25	18/21 (86%) p = 0.0064	11/21 (52%)	11/21 (50%)
125	8/23 (35%)	10/23 (43%)	9/23 (39%)
625	20/24 (83%) p = 0.0080	14/24 (58%)	15/24 (63%) p = 0.070
1250	16/23 (70%) p = 0.091	15/23 (65%)	11/23 (45%)
625-1250	70/111(%)	57/111(%)	53/111(%)
	0.0233[e/]	0.0334[e/]	
	0.0008[f/]	--	

a/Corrected for survival time, i.e., time of appearance of first neoplasm of the mammary gland.
b/Gross necropsy at FDA.
c/Histological examination by FDA.
d/Histological examination by Reuber.
e/Trend for dose response curve.
f/Departure from trend.

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TABLE 6. NUMBERS OF MALE AND FEMALE MICE INGESTING 2,4-D ISOPROPYL ESTER WITH TUMORS OF THE LUNG

Strain	Dose (ppm)	Male	Female
"A" Matched	0	2/17 (12%)	1/18 (6%)
"A" Pooled	0	5/79 (6%)	3/87 (3%)
"A"	111	4/18 (22%) p = 0.0584	1/18 (6%)
"B" Matched	0	2/18 (11%)	0/17 (0%)
"B" Pooled	0	9/90 (10%)	3/82 (4%)
"B"	111	2/18 (11%)	0/17 (0%)

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TABLE 7. NUMBERS OF MALE AND FEMALE MICE INGESTING 2,4-D BUTYL ESTER WITH RETICULUM CELL SARCOMAS

Strain	Dose (ppm)	Male	Female
"A" Matched	0	1/17 (6%)	2/18 (11%)
"A" Pooled	0	5/79 (6%)	4/87 (5%)
"A"	149	0/18 (0%)	3/18 (17%) p = 0.0955
"B" Matched	0	1/18 (6%)	2/17 (12%)
"B" Pooled	0	1/90 (1%)	3/82 (4%)
"B"	149	2/18 (11%) p = 0.0714	0/16 (0%)

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TABLE 8. NUMBER OF "STRAIN B" MALE AND FEMALE MICE GIVEN A SINGLE SUBCUTANEOUS INJECTION OF 2,4-D ISOOCTYL ESTER WITH RETICULUM CELL SARCOMAS

Dose (mg/kg)	Males	Females
0	0/161 (0%)	5/157 (3%)
1	2/18 (11%) p = 0.0096	5/17 (29%) p = 0.0009

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air samples

Although we apply dispersion staining primarily to bulk samples it may also be applied to air samples. When air samples are examined it is necessary to separate the sample dust from the filter. A convenient method of eliminating the filter is low temperature ashing. Portions of filters, cut in the shape of sectors, may be low temperature ashed directly on microscope slides. During ashing the filter sector must adhere to the slide. Adherence can be accomplished by placing the filter sector on a slide that has a drop of the n_D^{25} 1.580 mounting liquid applied to its surface. The mounting liquid should be spread over an area slightly larger than the filter sector.

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No excess in cancer deaths found in largest group ever studied for long-term effects of dioxin exposure . . .

A standardized mortality analysis has been completed of a group of 121 workers believed to have been exposed to TCDD, one of a family of toxic impurities commonly called "dioxin," in an industrial accident 30 years ago at Monsanto Company's Nitro, West Virginia plant. The study found no apparent excess in deaths from cancer or cardiovascular disease. The total deaths observed (32) were also less than expected when compared to the national average.

The medical study was co-authored by Judith A. Zack, a Monsanto epidemiologist, and Raymond R. Suskind, M.D., Director of the Institute of Environmental Health at the University of Cincinnati Medical Center. A report of their findings will be published in the Journal of Occupational Medicine.

TCDD is sometimes formed as a trace contaminant during the production of certain industrial chemicals. One of these is the herbicide, 2,4,5-T, currently the subject of widespread controversy centering on its use in the defoliant "Agent Orange" in Vietnam and in agricultural applications in the United States and abroad.

The 121 Monsanto workers involved in this study represent all of the employees who were exposed in the accident and who subsequently developed "chloracne," a skin disorder commonly associated with dioxin exposure. All 121 people were traced through plant medical records, workmen's compensation files and, where applicable, official death certificates. Thus, a complete follow-up was achieved.

According to the authors, the acute or short-term health effects of dioxin exposure are described in the literature but until now little has been known regarding the chronic or long-term effects. They stressed that this analysis is important in that "no apparent excess of total mortality or deaths from malignant neoplasms or disease of the circulatory system was observed in a group of workers with a high peak exposure to TCDD (dioxin) followed over a period of nearly 30 years.

The 1949 Nitro plant accident is the earliest recorded incident of dioxin exposure involving a population of this size. Since then, over 200 dioxin-related industrial accidents have occurred around the world, including a widely reported 1976 accident in Seveso, Italy.

Accordingly, the authors stated that the Nitro group represents the best opportunity thus far, in terms of people exposed and duration of latency period since exposure, to study the long-term effects of dioxin or mortality. Despite the relatively small number of deaths involved in the study, the authors said that "by augmenting these data with the results of comparable mortality studies, the long-term effects of TCDD (dioxin) may be more definitely evaluated."

This study will become a part of a larger analysis of not only mortality data but also of health information gathered last June in examinations of over 400 present and former Nitro plant employees by Dr. Suskind and his medical team from the University of Cincinnati's Institute of Environmental Health.

Included in this larger study are both workers exposed during the 1949 accident and those not exposed at that time but who worked in the 2,4,5-T operation between 1948 when the unit started up and 1969 when Monsanto ceased production of the product. A control group of employees who worked in other areas of the plant during that time frame is also being studied for comparative purposes.

This extensive medical investigation is being directed by Dr. Suskind. His findings are expected to be published later this year or early next year.

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ABSTRACT

The toxicological significance of exposures to synthetic chemicals is examined in the context of exposures to naturally-occurring chemicals. We calculate that 99.99% (by weight) of the pesticides in the American diet are chemicals that plants produce to defend themselves. Only 52 natural pesticides have been tested in high-dose animal cancer tests, and about half (27) are rodent carcinogens; these 27 are shown to be present in many common foods. We conclude that natural and synthetic chemicals are equally likely to be positive in animal cancer tests. We also conclude that at the low doses of most human exposures the comparative hazards of synthetic pesticide residues are insignificant.

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Toxicological examination of synthetic chemicals such as pesticides and industrial pollutants, without similar examination of the chemicals in the natural world to use for comparison, has generated an imbalance in both data and perception about potential hazards to humans (1-6). In this and two accompanying papers (7,8), we try to redress this imbalance and discuss in detail one major group of natural chemicals in our diet — nature's pesticides.

About *half* of all chemicals (whether natural or synthetic) tested chronically in animal cancer tests at the maximum tolerated dose (MTD) are carcinogens (7,9-14). The MTD of the test chemical is a near-toxic dose that can cause chronic mitogenesis, often as a result of cell killing (7). We have argued that mitogenesis increases mutagenesis, and therefore that a high percentage of all chemicals might be expected to be carcinogenic when tested chronically at the MTD (7). A high proportion of both natural and synthetic test chemicals is positive for carcinogenicity. Natural chemicals constitute the vast bulk of chemicals in the human diet, and therefore should be used as a reference for evaluating possible carcinogenic hazards from synthetic chemicals. In recent years, we have compared the possible hazards of various rodent carcinogens, using the HERP ratio: human exposure/rodent potency (1,6,). It should be emphasized that as the understanding of carcinogenesis mechanisms improves, these comparisons can be refined but they cannot provide a direct estimate of human hazard. This paper does not extend the HERP comparisons (1) because our purpose is different and space does not allow a proper analysis.

NATURE'S PESTICIDES: MUTAGENICITY AND CARCINOGENICITY

"Plants are not just food for animals. . . The world is not green. It is colored lectin, tannin, cyanide, caffeine, aflatoxin, and canavanine" [Janzen (16)].

Dietary Pesticides are 99.99% All Natural. Nature's pesticides are one important subset of natural chemicals. Plants produce toxins to protect themselves against fungi, insects, and animal predators (5,16-23). Tens of thousands of these natural pesticides have been discovered, and every species of plant analyzed contains its own set of perhaps a few dozen toxins. When plants are stressed or damaged, such as during a pest attack, they may greatly increase their natural pesticide levels, occasionally to levels that can be acutely toxic to humans. We estimate that Americans eat about 1.5 g of natural pesticides per person per day, which is about 10,000 times more than they eat of manmade pesticide residues (see below). As referenced in this paper (see 16-21 and Legends to Table 1, 2) there is a very large literature on natural toxins in plants and their role in plant defenses. The human intake of these toxins varies markedly with diet and would be higher in vegetarians. Our estimate of 1.5 g of natural pesticides per person per day is based on the content of toxins in the major plant foods (e.g. 13 g roasted coffee per person per day contains about 765 mg of chlorogenic acid, neochlorogenic acid, caffeic acid, and caffeine; see (22,23) and Table 2). Phenolics from other plants are estimated to contribute another several hundred mg of toxins. Flavonoids and glucosinolates account for several hundred mg; potato and tomato toxins may

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contribute another hundred, and saponins from legumes another hundred. Grains such as white flour and white rice contribute very little, but whole wheat, brown rice, corn (maize) may contribute several hundred mg more. The percentage of a plant's weight that is toxin varies, but a few percent of dry weight is a reasonable estimate: e.g. 1.5% of alfalfa sprouts is canavanine and 4% of coffee beans is phenolics. However, the percentage in some plant cultivars is lower, e.g. potatoes and tomatoes.

Concentrations of natural pesticides in plants are usually measured in parts per thousand or million (16-23) rather than parts per billion (ppb), the usual concentration of synthetic pesticide residues or of water pollutants (1,24). We estimate that humans ingest roughly 5,000 to 10,000 different natural pesticides and their breakdown products (16-23). For example, Table 1 shows 49 natural pesticides (and metabolites) that are ingested when eating cabbage and indicates how few have been tested for carcinogenicity or clastogenicity. Lima beans contain a completely different array of 23 natural toxins that, in stressed plants, range in concentration from 0.2 to 33 parts per thousand fresh weight; none appears to have been tested yet for carcinogenicity or teratogenicity (19). Many Leguminosae contain canavanine, a toxic arginine analog that, after being eaten by animals, is incorporated into protein in place of arginine. Feeding alfalfa sprouts (1.5 % canavanine dry weight) or canavanine to monkeys causes a lupus erythematosus-like syndrome (44). Lupus in man is characterized by a defect in the immune system that is associated with autoimmunity, antinuclear antibodies, chromosome breaks, and various types of pathology. The toxicity of non-food plants is well known: plants are among the most commonly

ingested poisonous substances for children under five.

Surprisingly few plant toxins have been tested for carcinogenicity (10-13,45). Among 1052 chemicals tested in *at least one species* in chronic cancer tests, only 52 are naturally occurring plant pesticides (10-13). Among these, about half (27/52) are carcinogenic.‡ Even though only a tiny proportion of plant toxins in our diet has been tested so far, the 27 natural pesticides that are rodent carcinogens are present in the following foods: anise, apples, apricots, bananas, basil, broccoli, Brussels sprouts, cabbage, cantaloupe, caraway, carrots, cauliflower, celery, cherries, cinnamon, cloves, cocoa, coffee, collard greens, comfrey herb tea, currants, dill, eggplant, endive, fennel, grapes, grapefruit juice, guava, honey, honeydew melon, horseradish, kale, lentils, lettuce, mangoes, mushrooms, mustard, nutmeg, orange juice, parsley, parsnips, peaches, pears, peas, black pepper, pineapples, plums, potatoes, radishes, raspberries, rosemary, sesame seeds, tarragon, tea, tomatoes, and turnips. Thus, it is probable that almost every fruit and vegetable in the supermarket contains natural plant pesticides that are rodent carcinogens. The levels of these 27 rodent carcinogens in the above plants are commonly thousands of times higher than the levels of manmade pesticides. Table 2 shows a variety of natural pesticides that are rodent carcinogens occurring in the ppm range in plant foods.

The catechol-type phenolics such as tannins, and caffeic acid and its esters (chlorogenic and neochlorogenic acids), are more widespread in plant species than other natural pesticides (e.g., Table 1 and 2). It may be that these phenolics have an antimicrobial role analogous to the respiratory burst of

oxygen radicals from mammalian phagocytic cells. The phenolics oxidize when a plant is wounded, yielding a burst of mutagenic oxygen radicals, e.g. the browning when an apple is cut.

Caution is necessary in interpreting the implications of the occurrence in the diet of natural pesticides that are rodent carcinogens. It is not argued here that these dietary exposures are necessarily of much relevance to human cancer. Indeed, a diet rich in fruit and vegetables is associated with lower cancer rates (86,87). This may be because anticarcinogenic vitamins and antioxidants come from plants (86,87). What is important in our analysis is that exposures to natural rodent carcinogens may cast doubt on the relevance of far lower levels of exposures to synthetic rodent carcinogens.

Residues of Manmade Pesticides. A National Research Council report has discussed the regulation of synthetic pesticides that are rodent carcinogens, but ignored natural pesticides (88). The U.S. Food and Drug Administration (FDA) has assayed food for 200 chemicals including the manmade pesticide residues thought to be of greatest importance and the residues of some industrial chemicals such as polychlorinated biphenyls (PCBs) (24). FDA found residues for 105 of these chemicals: the U.S. intake of the sum of these 105 chemicals averages about 0.09 mg per person per day, which we compare to 1.5 g of natural pesticides (i.e. 99.99% natural).[§] Other analyses of synthetic pesticide residues are similar (90). About half (0.04 mg) of this daily intake of synthetic pesticides is composed of 4 chemicals (24) that were not carcinogenic in rodent tests: ethylhexyl diphenyl phosphate, chlorpropham, malathion, and dicloran (10,89). Thus, the intake of rodent carcinogens from synthetic

residues is only about 0.05 mg a day (averaging about 0.06 ppm in plant food) even if one assumes that all the other residues are carcinogenic in rodents (which is unlikely).

Cooking food. The cooking of food is also a major dietary source of potential rodent carcinogens. Cooking produces about 2 g per person per day of mostly untested burnt material that contains many rodent carcinogens, e.g. polycyclic hydrocarbons (81,91) heterocyclic amines (92,93), furfural (22,23), nitrosamines and nitroaromatics (1,94), as well as a plethora of mutagens (91-95). Thus, the number and amounts of carcinogenic (or total) manmade pesticide residues appear to be minimal compared to the background of naturally-occurring chemicals in the diet. Roasted coffee, for example, is known to contain 247 volatile chemicals (22); 11 have been tested chronically and 8 are rodent carcinogens (10-13); caffeic acid, a non-volatile rodent carcinogen, is also present (Table 2). A typical cup of coffee contains at least 9 mg (40 ppm) of rodent carcinogens (mostly caffeic acid, catechol, furfural, and hydrogen peroxide)(Table 2). The evidence on coffee and human health has been recently reviewed, and the evidence to date is not sufficient to show that coffee is a risk factor for cancer in humans (81,86). The same caution about the implications for humans of rodent carcinogens in the diet that were discussed above for nature's pesticides apply to coffee and the products of cooked food.

Clastogenicity and Mutagenicity Studies. Results from *in vitro* studies also indicate that the natural world should not be ignored and that positive results are commonly observed in high-dose protocols. For example, Ishidate *et*

al (26) reviewed experiments on the clastogenicity (ability to break chromosomes) of 951 chemicals in mammalian cell cultures. Of these 951 chemicals, we identified 72 as natural plant pesticides: 35 (48%) were positive for clastogenicity in at least one test. This is similar to the results for the remaining chemicals, of which 467/879 (53%) were positive in at least one test.

Of particular interest are the *levels* at which some of the carcinogenic plant toxins in Table 2 were clastogenic (26): a) Allyl isothiocyanate was clastogenic at a concentration of 0.0005 ppm, which is about 200,000 times less than the concentration of sinigrin, its glucosinolate, in cabbage. Allyl isothiocyanate was among the most potent chemicals in the compendium (26), and is also effective at unusually low levels in transforming (96) and mutating animal cells (30). (See also the discussion of cancer tests in Table 1.) b) Safrole was clastogenic at a concentration of about 100 ppm, which is 30 times less than the concentration in nutmeg, and roughly equal to the concentration in black pepper. The rodent carcinogens safrole and estragole, and a number of other related dietary natural pesticides that have not been tested in animal cancer tests, have been shown to produce DNA adducts in mice (97). c) Caffeic acid was clastogenic at a concentration of 260 and 500 ppm, which is less than its concentration in roasted coffee beans and close to its concentration in apples, lettuce, endive, and potato skin. Chlorogenic acid, a precursor of caffeic acid, was clastogenic at a concentration of 150 ppm, which is 100 times less than its concentration in roasted coffee beans and similar to its concentration in apples, pears, plums, peaches, cherries and apricots. Chlorogenic acid and its metabolite caffeic acid are also mutagens (Table 1).

The genotoxic activity of coffee to mammalian cells has been demonstrated (98).

The carcinogenicity and mutagenicity of many plant pesticides have been recently reviewed (45): 5- and 8-methoxypsoralen are light activated mutagens (17); benzyl acetate and ethyl acrylate mutate mouse lymphoma cells (30). Plant phenolics such as caffeic acid, chlorogenic acid, and tannins (esters of gallic acid) have been reviewed for their mutagenicity and anti-mutagenicity, clastogenicity, and carcinogenicity (99).

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Table 1. Cabbage: 49 Natural Pesticides and Metabolites

Glucosinolates: 2-propenyl glucosinolate (sinigrin)*, 3-methyl-thio-propyl glucosinolate, 3-methyl-sulfinyl-propyl glucosinolate, 3-butenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate, 4-methyl-thio-butyl glucosinolate, 4-methyl-sulfinyl-butyl glucosinolate, 4-methylsulfonyl-butyl glucosinolate, benzyl glucosinolate, 2-phenyl-ethyl glucosinolate, propyl glucosinolate, butyl glucosinolate

Indole Glucosinolates And Related Indoles: 3-indolyl-methyl glucosinolate (glucobrassicin), 1-methoxy-3-indolylmethyl (neoglucobrassicin), indole-3-carbinol*, indole-3-acetonitrile, 3,3′-diindolylmethane

Isothiocyanates And Goitrin: allyl isothiocyanate*, 3-methyl-thio-propyl isothiocyanate, 3-methyl-sulfinyl-propyl isothiocyanate, 3-butenyl isothiocyanate, 5-vinyloxazolidine-2-thione (goitrin), 4-methylthiobutyl isothiocyanate, 4-methyl-sulfinylbutyl isothiocyanate, 4-methylsulfonyl butyl isothiocyanate, 4-pentenyl isothiocyanate, benzyl isothiocyanate, phenylethyl isothiocyanate

Cyanides: 1-cyano-2,3-epithiopropane, 1-cyano-3,4-epithiobutane, 1-cyano-3,4-epithiopentane, *threo*-1-cyano-2-hydroxy-3,4-epithiobutane, *erythro*-1-cyano-2-

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hydroxy-3,4-epithiobutane, 2-phenylpropionitrile, allyl cyanide*, 1-cyano-2-hydroxy-3-butene, 1-cyano-3-methylsulfinylpropane, 1-cyano-4-methylsulfinylbutane

Terpenes: menthol, neomenthol, isomenthol, carvone*

Phenols: 2-methoxyphenol, 3-caffoylquinic acid (chlorogenic acid)*, 4-caffoylquinic acid*, 5-caffoyl quinic acid (neochlorogenic acid)*, 4-*p*-coumaroylquinic acid, 5-*p*-coumaroylquinic acid, 5-feruloylquinic acid

Legend to Table 1

* Discussed below; all others untested. *Clastogenicity:* Chlorogenic acid (25) and allyl isothiocyanate are positive (26). Chlorogenic acid and its metabolite caffeic acid are also mutagens (27-29), as is allyl isothiocyanate (30). *Carcinogenicity:* Allyl isothiocyanate induced papillomas of the bladder in male rats (a neoplasm that is unusually rare in control rats) and was classified by NTP as carcinogenic. There was no evidence of carcinogenicity in mice: however, NTP indicated "the mice probably did not receive the MTD" (31,32). Sinigrin (the glucosinolate, i.e., thioglycoside of allyl isothiocyanate) is co-carcinogenic for the rat pancreas (33). Carvone is negative in mice (34). Indole acetonitrile has been shown to form a carcinogen, nitroso indole acetonitrile, in the presence of nitrite (35). Caffeic acid is a carcinogen (36,37) and clastogen (25) and is a metabolite of its esters 3-, 4-, and 5-caffoylquinic acid (chlorogenic and neochlorogenic acid). *Metabolites:* Sinigrin gives rise to allyl isothiocyanate on eating raw cabbage (e.g., coleslaw); in cooked cabbage it also is metabolized to allyl cyanide, which is untested. Indole carbinol forms dimers and trimers on ingestion, which mimic dioxin (TCDD) (8). *Occurrence* (18,21,38,40). *Toxicology*: The mitogenic effects of goitrin (which is goitrogenic) and various organic cyanides from cabbage suggest that they may be potential carcinogens (41,42). Aromatic cyanides related to those from cabbage have been shown to be mutagens and are metabolized to hydrogen cyanide and potentially mutagenic aldehydes (43).

Table 2. Natural Pesticide Carcinogens in Food

Plant Food	Rodent Carcinogen	Concentration (ppm)
Parsley	5- and 8-methoxypsoralen	14
Parsnip, cooked	"	32
Celery	"	0.8
Celery, new cultivar	"	6.2
Celery, stressed	"	25
Mushrooms	*p*-hydrazinobenzoate	11
"	glutamyl-*p*-hydrazinobenzoate	42
Cabbage	sinigrin* (allyl isothiocyanate)	35-590
Collard greens	"	250-788
Cauliflower	"	12-66
Brussels sprouts	"	110-1,560
Mustard (brown)	"	16,000-72,000
Horseradish	"	4,500
Orange juice	limonene	31
Mango	"	40
Pepper, black	"	8,000
Basil	estragole	3,800
Fennel	"	3,000
Nutmeg	safrole	3,000
Mace	"	10,000
Pepper, black	"	100
Pineapple	ethyl acrylate	0.07
Sesame seeds (heated oil)	sesamol	75
Cocoa	α-methylbenzyl alcohol	1.3
Basil	benzyl acetate	82
Jasmine tea	"	230
Honey	"	15
Coffee (roasted beans)	catechol	100

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Apple, Carrot, Celery, Cherry, Eggplant, Endive, Grapes, Lettuce, Pear, Plum, Potato	caffeic acid	50-200
Absinthe, Anise, Basil, Caraway, Dill, Marjoram, Rosemary, Sage, Savory Tarragon, Thyme	"	>1,000
Coffee (roasted beans)	"	1,800
Apricot, Cherry, Peach, Plum	chlorogenic acid** (caffeic acid)	50-500
Coffee (roasted beans)	"	21,600
Apple, Apricot, Broccoli, Brussels sprouts, Cabbage, Cherry, Kale, Peach, Pear, Plum	neochlorogenic acid** (caffeic acid)	50-500
Coffee (roasted beans)	"	11,600

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Legend to Table 2

*Sinigrin is a co-carcinogen (33) and is metabolized to the rodent carcinogen allyl isothiocyanate, although no adequate test has been done on sinigrin itself. The proportion converted to allyl isothiocyanate or to allyl cyanide depends on food preparation (38-40). **Chlorogenic and neochlorogenic acid are metabolized to the carcinogens caffeic acid and catechol (a metabolite of quinic acid), but have not been tested for carcinogenicity themselves. The clastogenicity and mutagenicity of the above compounds are referenced in Table 1.

Carcinogen refs are in (10-13) and the following: 5-methoxypsoralen (light-activated), 8-methoxypsoralen (47), *p*-hydrazinobenzoate and glutamyl-*p*-hydrazinobenzoate (48,49), allyl isothiocyanate (31,32), D-limonene (50), estragole and safrole (45,51), ethyl acrylate, benzyl acetate (52), α-methylbenzyl alcohol (53), caffeic acid (37), sesamol (37), catechol (37). *Concentration refs.*: 5-, 8-methoxypsoralen (17,55-59), *p*-hydrazinobenzoates (in commercial mushrooms) (48,49), sinigrin (38-40,60), D-limonene (61-63), estragole and safrole (64-67), ethyl acrylate (68), benzyl acetate (69-71), α-methylbenzyl alcohol (23), caffeic acid, chlorogenic acid, neochlorogenic acid (72-80) (in coffee (81,82)), catechol (83,84), sesamol (85). *Mutagenicity and clastogenicity refs.*: see text.

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FOOTNOTES

† References to, and analyses of, individual cancer tests are in the Carcinogenic Potency Database papers (10-13). Our analyses are based on this database, which reports only results of chronic, long-term bioassays that are adequate to detect a carcinogenic effect or lack of effect. More than 4000 experiments met the inclusion criteria of the database, but thousands of others did not, e.g. tests that lack a control group, that are too short or include too few animals to detect an effect, that use routes of administration not likely to result in whole body exposure (like skin painting or subcutaneous administration), co-carcinogenesis studies, and bioassays of particulate or fibrous matters.

One third of the chemicals in the database have been tested by the National Cancer Institute/National Toxicology Program, using standard protocols that include testing in two species at the MTD (15). About half of the chemicals in the database, however, have been tested in only one species. Positivity rates and prediction between species have been analyzed (9).

We classify the results of an experiment as either positive or negative on the basis of the author's opinion in the published paper, and classify a chemical as positive if it has been evaluated as positive by the author of at least one experiment. We use the author's opinion to determine positivity because it often takes into account more information than statistical significance alone, such as historical control rates for particular sites, survival and latency, and/or dose response. Generally, this designation by author's opinion corresponds well with the results of statistical reanalyses of the significance of the dose-response effect (9).

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‡ The list of 52 natural plant pesticides includes 7 toxins from edible mushrooms because mushrooms are commonly considered a plant food. Fungal toxins are not included in this list, but are given below. *Plant pesticides. Carcinogens*: acetaldehyde methylformylhydrazone, allyl isothiocyanate, arecoline.*HCl*, benzaldehyde, benzyl acetate, caffeic acid, catechol, clivorine, cycasin and methylazoxymethanol acetate mixture, estragole, ethyl acrylate, glutamyl *p*-hydrazinobenzoic acid, *p*-hydrazinobenzoic acid, lasiocarpine, *N*-methyl-*N*-formylhydrazine, D-limonene, α-methylbenzyl alcohol, methylhydrazine, 8-methoxypsoralen, monocrotaline, pentanal methylformalhydrazine, petasitenine, reserpine, safrole, senkirkine, sesamol, symphytine. (Cycasin as well as its metabolite methylazoxymethanol are positive in numerous tests (45) that do not meet the inclusion criteria of the database.) 5- and 8-methoxypsoralen are isomeric, light activated mutagens (17,45,46). 8-methoxypsoralen is positive in an NTP Gavage study (without light) and is in the database (47). 5-methoxypsoralen has only been tested in a skin painting study (with light) and is positive (46); it is not in our database because it is a skin painting test. *Non-carcinogens:* atropine, benzyl alcohol, biphenyl, caffeine, d-carvone, deserpidine, disodium glycyrrhizinate, emetine.*2HCl*, ephedrine sulphate, eucalyptol, eugenol, *beta-N*-[γ-*L*(+)-glutamyl]-4-hydroxymethylphenylhydrazine, isosafrole, kaempferol, DL-menthol, nicotine, norharman, pilocarpine, piperidine, rotenone, rutin sulfate, sodium benzoate, vinblastine. *Uncertain*: *trans*-anethole, quercetin.

Among fungal toxins tested for carcinogenicity, 11/16 were positive. *Fungal toxins*: *Carcinogens*: aflatoxin, 5-azacytidine, azaserine, citrinin,

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griseofulvin, luteoskyrin, mitomycin-C, ochratoxin A, sterigmatocystin, streptozotocin, zearalenone. *Non-carcinogens*: erythromycin stearate, fusarenon-X, oxytetracycline.*HCl*, patulin, penicillin VK.

§ Figures here are based on males aged 25-30 in 1982-1984. Cancer test results are in (10-13). The negative test on 2-ethylhexyl diphenyl phosphate is in (89). The latest FDA figures on actual exposures do not include every known manmade pesticide, and diets vary. Nevertheless, 0.05 mg of possibly-carcinogenic pesticide residues consumed in a day seems to be a reasonable rough estimate.

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Ranking Possible Carcinogenic Hazards

BRUCE N. AMES,* RENAE MAGAW, LOIS SWIRSKY GOLD

This review discusses reasons why animal cancer tests cannot be used to predict absolute human risks. Such tests, however, may be used to indicate that some chemicals might be of greater concern than others. Possible hazards to humans from a variety of rodent carcinogens are ranked by an index that relates the potency of each carcinogen in rodents to the exposure in humans. This ranking suggests that carcinogenic hazards from current levels of pesticide residues or water pollution are likely to be of minimal concern relative to the background levels of natural substances, though one cannot say whether these natural exposures are likely to be of major or minor importance.

EPIDEMIOLOGISTS ESTIMATE THAT AT LEAST 70% OF HUMAN cancer would, in principle, be preventable if the main risk and antirisk factors could be identified (*1*). This is because the incidence of specific types of cancer differs markedly in different parts of the world where people have different life-styles. For example, colon and breast cancer, which are among the major types of cancer in the United States, are quite rare among Japanese in Japan, but not among Japanese-Americans. Epidemiologists are providing important clues about the specific causes of human cancer, despite inherent methodological difficulties. They have identified tobacco as an avoidable cause of about 30% of all U.S. cancer deaths and of an even larger number of deaths from other causes (*1, 2*). Less specifically, dietary factors, or their absence, have been suggested in many studies to contribute to a substantial proportion of cancer deaths, though the intertwined risk and antirisk factors are being identified only slowly (*1, 3, 4*). High fat intake may be a major contributor to colon cancer, though the evidence is not as definitive as that for the role of saturated fat in heart disease or of tobacco in lung cancer. Alcoholic beverage consumption, particularly by smokers, has been estimated to contribute to about 3% of U.S. cancer deaths (*1*) and to an even larger number of deaths from other causes. Progress in prevention has been made for some occupational factors, such as asbestos, to which workers used to be heavily exposed, with delayed effects that still contribute to about 2% of U.S. cancer deaths (*1, 5*). Prevention may also become possible for hormone-related cancers such as breast cancer (*1, 6*), or virus-related cancers such as liver cancer (hepatitis B) and cancer of the cervix (papilloma virus HPV16) (*1, 7*).

Animal bioassays and in vitro studies are also providing clues as to which carcinogens and mutagens might be contributing to human cancer. However, the evaluation of carcinogenicity in rodents is expensive and the extrapolation to humans is difficult (*8–11*). We will use the term "possible hazard" for estimates based on rodent cancer tests and "risk" for those based on human cancer data (*10*).

Extrapolation from the results of rodent cancer tests done at high doses to effects on humans exposed to low doses is routinely attempted by regulatory agencies when formulating policies attempting to prevent future cancer. There is little sound scientific basis for this type of extrapolation, in part due to our lack of knowledge about mechanisms of cancer induction, and it is viewed with great unease by many epidemiologists and toxicologists (*5, 9–11*). Nevertheless, to be prudent in regulatory policy, and in the absence of good human data (almost always the case), some reliance on animal cancer tests is unavoidable. The best use of them should be made even though few, if any, of the main avoidable causes of human cancer have typically been the types of man-made chemicals that are being tested in animals (*10*). Human cancer may, in part, involve agents such as hepatitis B virus, which causes chronic inflammation; changes in hormonal status; deficiencies in normal protective factors (such as selenium or β-carotene) against endogenous carcinogens (*12*); lack of other anticarcinogens (such as dietary fiber or calcium) (*4*); or dietary imbalances such as excess consumption of fat (*3, 4, 12*) or salt (*13*).

There is a need for more balance in animal cancer testing to emphasize the foregoing factors and natural chemicals as well as synthetic chemicals (*12*). There is increasing evidence that our normal diet contains many rodent carcinogens, all perfectly natural or traditional (for example, from the cooking of food) (*12*), and that no human diet can be entirely free of mutagens or agents that can be carcinogenic in rodent systems. We need to identify the important causes of human cancer among the vast number of minimal risks. This requires knowledge of both the amounts of a substance to which humans are exposed and its carcinogenic potency.

Animal cancer tests can be analyzed quantitatively to give an estimate of the relative carcinogenic potencies of the chemicals tested. We have previously published our Carcinogenic Potency Database, which showed that rodent carcinogens vary in potency by more than 10 millionfold (*14*).

This article attempts to achieve some perspective on the plethora of possible hazards to humans from exposure to known rodent carcinogens by establishing a scale of the possible hazards for the amounts of various common carcinogens to which humans might be chronically exposed. We view the value of our calculations not as providing a basis for absolute human risk assessment, but as a guide to priority setting. One problem with this type of analysis is that few of the many natural chemicals we are exposed to in very large amounts (relative to synthetic chemicals) have been tested in animals for carcinogenicity. Thus, our knowledge of the background levels of human exposure to animal carcinogens is fragmentary, biased in favor of synthetic chemicals, and limited by our lack of knowledge of human exposures.

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RISK ASSESSMENT

Ranking of Possible Carcinogenic Hazards

Since carcinogens differ enormously in potency, a comparison of possible hazards from various carcinogens ingested by humans must take this into account. The measure of potency that we have developed, the TD_{50}, is the daily dose rate (in milligrams per kilogram) to halve the percent of tumor-free animals by the end of a standard lifetime (*14*). Since the TD_{50} (analogous to the LD_{50}) is a dose rate, the lower the TD_{50} value the more potent the carcinogen. To calculate our index of possible hazard we express each human exposure (daily lifetime dose in milligrams per kilogram) as a percentage of the rodent TD_{50} dose (in milligrams per kilogram) for each carcinogen. We call this percentage HERP [*H*uman *E*xposure dose/*R*odent *P*otency dose]. The TD_{50} values are taken from our ongoing Carcinogenic Potency Database (currently 3500 experiments on 975 chemicals), which reports the TD_{50} values estimated from experiments in animals (*14*). Human exposures have been estimated from the literature as indicated. As rodent data are all calculated on the basis of lifetime exposure at the indicated daily dose rate (*14*), the human exposure data are similarly expressed as lifelong daily dose rates even though the human exposure is likely to be less than daily for a lifetime.

It would be a mistake to use our HERP index as a direct estimate of human hazard. First, at low dose rates human susceptibility may differ systematically from rodent susceptibility. Second, the general shape of the dose-response relationship is not known. A linear dose response has been the dominant assumption in regulating carcinogens for many years, but this may not be correct. If the dose responses are not linear but are actually quadratic or hockey-stick shaped or show a threshold, then the actual hazard at low dose rates might be much less than the HERP values would suggest. An additional difficulty is that it may be necessary to deal with carcinogens that differ in their mechanisms of action and thus in their dose-response relationship. We have therefore put an asterisk next to HERP values for carcinogens that do not appear to be active through a genotoxic (DNA damaging or mutagenic) mechanism (*15*) so that comparisons can be made within the genotoxic or nongenotoxic classes.

Table 1 presents our HERP calculations of possible cancer hazards in order to compare them within several categories so that, for example, pollutants of possible concern can be compared to natural carcinogens in the diet. A convenient reference point is the possible hazard from the carcinogen chloroform in a liter of average (U.S.) chlorinated tap water, which is close to a HERP of 0.001%. Chloroform is a by-product of water chlorination, which protects us from pathogenic viruses and bacteria.

Contaminated water. The possible hazards from carcinogens in contaminated well water [for example, Santa Clara ("Silicon") Valley, California, or Woburn, Massachusetts] should be compared to the possible hazard of ordinary tap water (Table 1). Of 35 wells shut down in Santa Clara Valley because of their supposed carcinogenic hazard, only two have HERP values greater than ordinary tap water. Well water is not usually chlorinated and typically lacks the chloroform present in chlorinated tap water. Water from the most polluted well (HERP = 0.004% per liter for trichloroethylene), as indicated in Table 1, has a HERP value orders of magnitude less than for the carcinogens in an equal volume of cola, beer, or wine. Its HERP value is also much lower than that of many of the common natural foods that are listed in Table 1, such as the average peanut butter sandwich. Caveats for any comparisons are given below. Since the consumption of tap water is only about 1 or 2 liters per day, the animal evidence provides no good reason to expect that chlorination of water or current levels of man-made pollution of water pose a significant carcinogenic hazard.

Pesticide residues. Intake of man-made pesticide residues from food in the United States, including residues of industrial chemicals such as polychlorinated biphenyls (PCBs), averages about 150 μg/day. Most (105 μg) of this intake is composed of three chemicals (ethylhexyl diphenyl phosphate, malathion, and chlorpropham) shown to be noncarcinogenic in tests in rodents (*16*). A carcinogenic pesticide residue in food of possible concern is DDE, the principal metabolite (>90%) of DDT (*16*). The average U.S. daily intake of DDE from DDT (HERP = 0.0003%) is equivalent to the HERP of the chloroform in one glass of tap water and thus appears to be insignificant compared to the background of natural carcinogens in our diet (Table 1). Even daily consumption of 100 times the average intake of DDE/DDT or PCBs would produce a possible hazard that is small compared to other common exposures shown in Table 1.

Nature's pesticides. We are ingesting in our diet at least 10,000 times more by weight of natural pesticides than of man-made pesticide residues (*12*). These are natural "toxic chemicals" that have an enormous variety of chemical structures, appear to be present in all plants, and serve to protect plants against fungi, insects, and animal predators (*12*). Though only a few are present in each plant species, they commonly make up 5 to 10% of the plant's dry weight (*12*). There has been relatively little interest in the toxicology or carcinogenicity of these compounds until quite recently, although they are by far the main source of "toxic chemicals" ingested by humans. Only a few dozen of the thousands present in the human diet have been tested in animal bioassays, and only some of these tests are adequate for estimating potency in rodents (*14*). A sizable proportion of those that have been tested are carcinogens, and many others have been shown to be mutagens (*12*), so it is probable that many more will be found to be carcinogens if tested. Those shown in Table 1 are: estragole (HERP = 0.1% for a daily 1 g of dried basil), safrole (HERP = 0.2% for a daily natural root beer), symphytine (a pyrrolizidine alkaloid, 0.03% for a daily cup of comfrey tea), comfrey tablets sold in health food stores (6.2% for a daily dose), hydrazines in mushrooms (0.1% for one daily raw mushroom), and allyl isothiocyanate (0.07% for a daily 5 g of brown mustard).

Plants commonly produce very much larger amounts of their natural toxins when damaged by insects or fungi (*12*). For example, psoralens, light-activated carcinogens in celery, increase 100-fold when the plants are damaged by mold and, in fact, can cause an occupational disease in celery-pickers and in produce-checkers at supermarkets (*12, 17*).

Molds synthesize a wide variety of toxins, apparently as antibiotics in the microbiological struggle for survival: over 300 mycotoxins have been described (*18*). They are common pollutants of human food, particularly in the tropics. A considerable percentage of those tested have been shown to be mutagens and carcinogens: some, such as aflatoxin and sterigmatocystin, are among the most potent known rodent carcinogens. The potency of aflatoxin in different species varies widely; thus, a bias may exist as the HERP uses the most sensitive species. The aflatoxin content of U.S. peanut butter averages 2 ppb, which corresponds to a HERP of 0.03% for the peanut butter in an average sandwich (Table 1). The Food and Drug Administration (FDA) allows ten times this level (HERP = 0.3%), and certain foods can often exceed the allowable limit (*18*). Aflatoxin contaminates wheat, corn (perhaps the main source of dietary aflatoxin in the United States), and nuts, as well as a wide variety of stored carbohydrate foodstuffs. A carcinogenic, though less potent, metabolite of aflatoxin is found in milk from cows that eat moldy grain.

There is epidemiologic evidence that aflatoxin is a human carcinogen. High intake in the tropics is associated with a high rate of liver cancer, at least among those chronically infected with the hepatitis B

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virus (*19, 20*). Considering the potency of those mold toxins that have been tested and the widespread contamination of food with molds, they may represent the most significant carcinogenic pollution of the food supply in developing countries. Such pollution is much less severe in industrialized countries, due to refrigeration and modern techniques of agriculture and storage, including use of synthetic pesticides and fumigants.

Preparation of foods and beverages can also produce carcinogens. Alcohol has been shown to be a human carcinogen in numerous epidemiologic studies (*1, 21*). Both alcohol and acetaldehyde, its

Table 1. Ranking possible carcinogenic hazards. *Potency of carcinogens*: A number in parentheses indicates a TD_{50} value not used in HERP calculation because it is the less sensitive species; (−) = negative in cancer test. (+) = positive for carcinogenicity in test(s) not suitable for calculating a TD_{50}; (?) = is not adequately tested for carcinogenicity. TD_{50} values shown are averages calculated by taking the harmonic mean of the TD_{50}'s of the positive tests in that species from the Carcinogenic Potency Database. Results are similar if the lowest TD_{50} value (most potent) is used instead. For each test the target site with the lowest TD_{50} value has been used. The average TD_{50} has been calculated separately for rats and mice, and the more sensitive species is used for calculating the possible hazard. The database, with references to the source of the cancer tests, is complete for tests published through 1984 and for the National Toxicology Program bioassays through June 1986 (*14*). We have not indicated the route of exposure or target sites or other particulars of each test, although these are reported in the database. *Daily human exposure*: We have tried to use average or reasonable daily intakes to facilitate comparisons. In several cases, such as contaminated well water or factory exposure to EDB, this is difficult to determine, and we give the value for the worst found and indicate pertinent information in the References and Notes. The calculations assume a daily dose for a lifetime; where drugs are normally taken for only a short period we have bracketed the HERP value. For inhalation exposures we assume an inhalation of 9,600 liters per 8 hours for the workplace and 10,800 liters per 14 hours for indoor air at home. *Possible hazard*: The amount of rodent carcinogen indicated under carcinogen dose is divided by 70 kg to give a milligram per kilogram of human exposure, and this human dose is given as the percentage of the TD_{50} dose in the rodent (in milligrams per kilogram) to calculate the *H*uman *E*xposure/*R*odent *P*otency index (HERP).

Possible hazard: HERP (%)	Daily human exposure	Carcinogen dose per 70-kg person	Potency of carcinogen: TD_{50} (mg/kg)		References
			Rats	Mice	
		Environmental pollution			
0.001*	Tap water, 1 liter	Chloroform, 83 μg (U.S. average)	(119)	90	*96*
0.004*	Well water, 1 liter contaminated (worst well in Silicon Valley)	Trichloroethylene, 2800 μg	(−)	941	*97*
0.0004*	Well water, 1 liter contaminated, Woburn	Trichloroethylene, 267 μg	(−)	941	*98*
0.0002*		Chloroform, 12 μg	(119)	90	
0.0003*		Tetrachloroethylene, 21 μg	101	(126)	
0.008*	Swimming pool, 1 hour (for child)	Chloroform, 250 μg (average pool)	(119)	90	*99*
0.6	Conventional home air (14 hour/day)	Formaldehyde, 598 μg	1.5	(44)	*100*
0.004		Benzene, 155 μg	(157)	53	
2.1	Mobile home air (14 hour/day)	Formaldehyde, 2.2 mg	1.5	(44)	*28*
		Pesticide and other residues			
0.0002*	PCBs: daily dietary intake	PCBs, 0.2 μg (U.S. average)	1.7	(9.6)	*101*
0.0003*	DDE/DDT: daily dietary intake	DDE, 2.2 μg (U.S. average)	(−)	13	*16*
0.0004	EDB: daily dietary intake (from grains and grain products)	Ethylene dibromide, 0.42 μg (U.S. average)	1.5	(5.1)	*102*
		Natural pesticides and dietary toxins			
0.003	Bacon, cooked (100 g)	Dimethylnitrosamine, 0.3 μg	(0.2)	0.2	*40*
0.006		Diethylnitrosamine, 0.1 μg	0.02	(+)	
0.003	Sake (250 ml)	Urethane, 43 μg	(41)	22	*24*
0.03	Comfrey herb tea, 1 cup	Symphytine, 38 μg (750 μg of pyrrolizidine alkaloids)	1.9	(?)	*103*
0.03	Peanut butter (32 g; one sandwich)	Aflatoxin, 64 ng (U.S. average, 2 ppb)	0.003	(+)	*18*
0.05	Dried squid, broiled in gas oven (54 g)	Dimethylnitrosamine, 7.9 μg	(0.2)	0.2	*37*
0.07	Brown mustard (5 g)	Allyl isothiocyanate, 4.6 mg	96	(−)	*47*
0.1	Basil (1 g of dried leaf)	Estragole, 3.8 mg	(?)	52	*48*
0.1	Mushroom, one raw (15 g) (*Agaricus bisporus*)	Mixture of hydrazines, and so forth	(?)	20,300	*104*
0.2	Natural root beer (12 ounces; 354 ml) (now banned)	Safrole, 6.6 mg	(436)	56	*105*
0.008	Beer, before 1979 (12 ounces; 354 ml)	Dimethylnitrosamine, 1 μg	(0.2)	0.2	*38*
2.8*	Beer (12 ounces; 354 ml)	Ethyl alcohol, 18 ml	9110	(?)	*23*
4.7*	Wine (250 ml)	Ethyl alcohol, 30 ml	9110	(?)	*23*
6.2	Comfrey-pepsin tablets (nine daily)	Comfrey root, 2700 mg	626	(?)	*103*
1.3	Comfrey-pepsin tablets (nine daily)	Symphytine, 1.8 mg	1.9	(?)	
		Food additives			
0.0002	AF-2: daily dietary intake before banning	AF-2 (furylfuramide), 4.8 μg	29	(131)	*44*
0.06*	Diet Cola (12 ounces; 354 ml)	Saccharin, 95 mg	2143	(−)	*106*
		Drugs			
[0.3]	Phenacetin pill (average dose)	Phenacetin, 300 mg	1246	(2137)	*51*
[5.6]	Metronidazole (therapeutic dose)	Metronidazole, 2000 mg	(542)	506	*107*
[14]	Isoniazid pill (prophylactic dose)	Isoniazid, 300 mg	(150)	30	*108*
16*	Phenobarbital, one sleeping pill	Phenobarbital, 60 mg	(+)	5.5	*50*
17*	Clofibrate (average daily dose)	Clofibrate, 2000 mg	169	(?)	*52*
		Occupational exposure			
5.8	Formaldehyde: Workers' average daily intake	Formaldehyde, 6.1 mg	1.5	(44)	*109*
140	EDB: Workers' daily intake (high exposure)	Ethylene dibromide, 150 mg	1.5	(5.1)	*55*

*Asterisks indicate HERP from carcinogens thought to be nongenotoxic.

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major metabolite, are carcinogens in rats (*22, 23*). The carcinogenic potency of ethyl alcohol in rats is remarkably low (*23*), and it is among the weakest carcinogens in our database. However, human intake of alcohol is very high (about 18 g per beer), so that the possible hazards shown in Table 1 for beer and wine are large (HERP = 2.8% for a daily beer). The possible hazard of alcohol is enormous relative to that from the intake of synthetic chemical residues. If alcohol (*20*), trichloroethylene, DDT, and other presumptive nongenotoxic carcinogens are active at high doses because they are tumor promoters, the risk from low doses may be minimal.

Other carcinogens are present in beverages and prepared foods. Urethane (ethyl carbamate), a particularly well-studied rodent carcinogen, is formed from ethyl alcohol and carbamyl phosphate during a variety of fermentations and is present in Japanese sake (HERP = 0.003%), many types of wine and beer, and in smaller amounts in yogurt and bread (*24*). Another fermentation product, the dicarbonyl aldehyde methylglyoxal, is a potent mutagen and was isolated as the main mutagen in coffee (about 250 μg in one cup). It was recently shown to be a carcinogen, though not in a test suitable for calculating a TD_{50} (*25*). Methylglyoxal is also present in a variety of other foods, such as tomato puree (*25, 26*). Diacetyl (2,3-butanedione), a closely related dicarbonyl compound, is a fermentation product in wine and a number of other foods and is responsible for the aroma of butter. Diacetyl is a mutagen (*27*) but has not been tested for carcinogenicity.

Formaldehyde, another natural carcinogenic and mutagenic aldehyde, is also present in many common foods (*22, 26–28*). Formaldehyde gas caused cancer only in the nasal turbinates of the nose-breathing rodents and even though formaldehyde is genotoxic, the dose response was nonlinear (*28, 29*). Hexamethylenetetramine, which decomposes to formaldehyde in the stomach, was negative in feeding studies (*30*). The effects of oral versus inhalation exposure for formaldehyde remain to be evaluated more thoroughly.

As formaldehyde is almost ubiquitous in foods, one can visualize various formaldehyde-rich scenarios. Daily consumption of shrimp (HERP = 0.09% per 100 g) (*31*), a sandwich (HERP of two slices of bread = 0.4%) (*22*), a cola (HERP = 2.7%) (*32*), and a beer (HERP = 0.2%) (*32*) in various combinations could provide as much formaldehyde as living in some mobile homes (HERP = 2.1%; Table 1). Formaldehyde is also generated in animals metabolically, for example, from methoxy compounds that humans ingest in considerable amounts from plants. The level of formaldehyde reported in normal human blood is strikingly high (about 100 μ*M* or 3000 ppb) (*33*) suggesting that detoxification mechanisms are important.

The cooking of food generates a variety of mutagens and carcinogens. Nine heterocyclic amines, isolated on the basis of their mutagenicity from proteins or amino acids that were heated in ways that occur in cooking, have now been tested; all have been shown to be potent carcinogens in rodents (*34*). Many others are still being isolated and characterized (*34*). An approximate HERP of 0.02% has been calculated by Sugimura *et al.* for the daily intake of these nine carcinogens (*34*). Three mutagenic nitropyrenes present in diesel exhaust have now been shown to be carcinogens (*35*), but the intake of these carcinogenic nitropyrenes has been estimated to be much higher from grilled chicken than from air pollution (*34, 36*). The total amount of browned and burnt material eaten in a typical day is at least several hundred times more than that inhaled from severe air pollution (*12*).

Gas flames generate NO_2, which can form both the carcinogenic nitropyrenes (*35, 36*) and the potently carcinogenic nitrosamines in food cooked in gas ovens, such as fish or squid (HERP = 0.06%; Table 1) (*37*). We suspect that food cooked in gas ovens may be a major source of dietary nitrosamines and nitropyrenes, though it is not clear how significant a risk these pose. Nitrosamines were ubiquitous in beer and ale (HERP = 0.008%) and were formed from NO_2 in the gas flame–heated air used to dry the malt. However, the industry has switched to indirect heating, which resulted in markedly lower levels (<1 ppb) of dimethylnitrosamine (*38*). The dimethylnitrosamine found in human urine is thought to be formed in part from NO_2 inhaled from kitchen air (*39*). Cooked bacon contains several nitrosamines (HERP = 0.009%) (*40*).

Oxidation of fats and vegetable oils occurs during cooking and also spontaneously if antioxidant levels are low. The result is the formation of peroxides, epoxides, and aldehydes, all of which appear to be rodent carcinogens (*8, 12, 27*). Fatty acid hydroperoxides (present in oxidized oils) and cholesterol epoxide have been shown to be rodent carcinogens (though not in tests suitable for calculating a TD_{50}). Dried eggs contain about 25 ppm of cholesterol epoxide (a sizable amount), a result of the oxidation of cholesterol by the NO_2 in the drying air that is warmed by gas flames (*12*).

Normal oxidation reactions in fruit (such as browning in a cut apple) also involve production of peroxides. Hydrogen peroxide is a mutagenic rodent carcinogen that is generated by oxidation of natural phenolic compounds that are quite widespread in edible plants. A cup of coffee contains about 750 μg of hydrogen peroxide (*25*); however, since hydrogen peroxide is a very weak carcinogen (similar in potency to alcohol), the HERP for drinking a daily cup of coffee would be very low [comparable to DDE/DDT, PCBs, or ethylene dibromide (EDB) dietary intakes]. Hydrogen peroxide is also generated in our normal metabolism; human blood contains about 5 μ*M* hydrogen peroxide and 0.3 μ*M* of the cholesterol ester of fatty acid hydroperoxide (*41*). Endogenous oxidants such as hydrogen peroxide may make a major contribution to cancer and aging (*42*).

Caloric intake, which could be considered the most striking rodent carcinogen ever discovered, is discussed remarkably little in relation to human cancer. It has been known for about 40 years that increasing the food intake in rats and mice by about 20% above optimal causes a remarkable decrease in longevity and a striking increase in endocrine and mammary tumors (*43*). In humans, obesity (associated with high caloric intake) leads to increased levels of circulating estrogens, a significant cause of endometrial and gall bladder cancer. The effects of moderate obesity on other types of human cancer are less clear (*1*).

Food additives are currently screened for carcinogenicity before use if they are synthetic compounds. AF-2 (HERP = 0.0002%), a food preservative, was banned in Japan (*44*). Saccharin (HERP = 0.06%) is currently used in the United States (the dose-response in rats, however, is clearly sublinear) (*45*). The possible hazard of diethylstilbestrol residues in meat from treated farm animals seems miniscule relative to endogenous estrogenic hormones and plant estrogens (*46*). Some natural carcinogens are also widely used as additives, such as allyl isothiocyanate (*47*), estragole (*48*), and alcohol (*23*).

Air pollution. A person inhales about 20,000 liters of air in a day; thus, even modest contamination of the atmosphere can result in inhalation of appreciable doses of a pollutant. This can be seen in the possible hazard in mobile homes from formaldehyde (HERP = 2.1%) or in conventional homes from formaldehyde (HERP = 0.6%) or benzene (HERP = 0.004%; Table 1). Indoor air pollution is, in general, worse than outdoor air pollution, partly because of cigarette smoke. The most important indoor air pollutant may be radon gas. Radon is a natural radioactive gas that is present in the soil, gets trapped in houses, and gives rise to radioactive decay products that are known to be carcinogenic for humans (*49*). It has been estimated that in 1 million homes in the United States the level of exposure to products of radon decay may be higher than that

received by today's uranium miners. Two particularly contaminated houses were found that had a risk estimated to be equivalent to receiving about 1200 chest x-rays a day (*49*). Approximately 10% of the lung cancer in the United States has been tentatively attributed to radon pollution in houses (*49*). Many of these cancers might be preventable since the most hazardous houses can be identified and modified to minimize radon contamination.

General outdoor air pollution appears to be a small risk relative to the pollution inhaled by a smoker: one must breathe Los Angeles smog for a year to inhale the same amount of burnt material that a smoker (two packs) inhales in a day (*12*), though air pollution is inhaled starting from birth. It is difficult to determine cancer risk from outdoor air pollution since epidemiologists must accurately control for smoking and radon.

Some common drugs shown in Table 1 give fairly high HERP percentages, primarily because the dose ingested is high. However, since most medicinal drugs are used for only short periods while the HERP index is a daily dose rate for a lifetime, the possible hazard would usually be markedly less. We emphasize this in Table 1 by bracketing the numbers for these shorter exposures. Phenobarbital (HERP = 16%) was investigated thoroughly in humans who had taken it for decades, and there was no convincing evidence that it caused cancer (*50*). There is evidence of increased renal cancer in long-term human ingestion of phenacetin, an analgesic (*51*). Acetaminophen, a metabolite of phenacetin, is one of the most widely used over-the-counter pain killers. Clofibrate (HERP = 17%) is used as a hypolipidemic agent and is thought to be carcinogenic in rodents because it induces hydrogen peroxide production through peroxisome proliferation (*52*).

Occupational exposures can be remarkably high, particularly for volatile carcinogens, because about 10,000 liters of air are inhaled in a working day. For formaldehyde, the exposure to an average worker (HERP = 5.8%) is higher than most dietary intakes. For a number of volatile industrial carcinogens, the ratio of the permitted exposure limit [U.S. Occupational Safety and Health Administration (OSHA)] in milligrams per kilogram to the TD_{50} has been calculated; several are close to the TD_{50} in rodents and about two-thirds have permitted HERP values >1% (*53*). The possible hazard estimated for the actual exposure levels of the most heavily exposed EDB workers is remarkably high, HERP = 140% (Table 1). Though the dose may have been somewhat overestimated (*54*), it was still comparable to the dose causing cancer in half the rodents. An epidemiologic study of these heavily exposed EDB workers who inhaled EDB for over a decade did not show any increase in cancer, though because of the limited duration of exposure and the relatively small numbers of people monitored the study would not have detected a small effect (*54, 55*). OSHA still permits exposures above the TD_{50} level. California, however, lowered the permitted level over 100-fold in 1981. In contrast with these heavy workplace exposures, the Environmental Protection Agency (EPA) has banned the use of EDB for fumigation because of the residue levels found in grain (HERP = 0.0004%).

Uncertainties in Relying on Animal Cancer Tests for Human Prediction

Species variation. Though we list a possible hazard if a chemical is a carcinogen in a rat but not in a mouse (or vice versa), this lack of agreement raises the possibility that the risk to humans is nonexistent. Of 392 chemicals in our database tested in both rats and mice, 226 were carcinogens in at least one test, but 96 of these were positive in the mouse and negative in the rat or vice versa (*56*). This discordance occurs despite the fact that rats and mice are very closely related and have short life-spans. Qualitative extrapolation of cancer risks from rats or mice to humans, a very dissimilar long-lived species, is unlikely to be as reliable. Conversely, important human carcinogens may not be detected in standard tests in rodents; this was true for a long time for both tobacco smoke and alcohol, the two largest identified causes of neoplastic death in the United States.

For many of the chemicals considered rodent carcinogens, there may be negative as well as positive tests. It is difficult to deal with negative results satisfactorily for several reasons, including the fact that some chemicals are tested only once or twice, while others are tested many times. The HERP index ignores negative tests. Where there is species variation in potency, use of the more sensitive species, as is generally done and as is done here, could introduce a tendency to overestimate possible hazards; however, for most chemicals that are positive in both species, the potency is similar in rats and mice (*57*). The HERP may provide a rough correlate of human hazard from chemical exposure; however, for a given chemical, to the extent that the potency in humans differs from the potency in rodents, the relative hazard would be different.

Quantitative uncertainties. Quantitative extrapolation from rodents to humans, particularly at low doses, is guesswork that we have no way of validating (*1, 5, 10, 11, 58*). It is guesswork because of lack of knowledge in at least six major areas: (i) the basic mechanisms of carcinogenicity; (ii) the relation of cancer, aging, and life-span (*1, 10, 42, 59*); (iii) the timing and order of the steps in the carcinogenic process that are being accelerated; (iv) species differences in metabolism and pharmacokinetics; (v) species differences in anticarcinogens and other defenses (*1, 60*); and (vi) human heterogeneity—for example, pigmentation affects susceptibility to skin cancer from ultraviolet light. These sources of uncertainty are so numerous, and so substantial, that only empirical data will resolve them, and little of this is available.

Uncertainties due to mechanism in multistage carcinogenesis. Several steps (stages) are involved in chemical carcinogenesis, and the dose-response curve for a carcinogen might depend on the particular stage(s) it accelerates (*58*), with multiplicative effects if several stages are affected. This multiplicative effect is consistent with the observation in human cancer that synergistic effects are common. The three steps of carcinogenesis that have been analyzed in most detail are initiation (mutation), promotion, and progression, and we discuss these as an aid to understanding aspects of the dose-response relation.

Mutation (or DNA damage) as one stage of the carcinogenic process is supported by various lines of evidence: association of active forms of carcinogens with mutagens (*61*), the changes in DNA sequence of oncogenes (*62*), genetic predisposition to cancer in human diseases such as retinoblastoma (*63*) or DNA-repair deficiency diseases such as xeroderma pigmentosum (*64*). The idea that genotoxic carcinogens might show a linear dose-response might be plausible if only the mutation step of carcinogenesis was accelerated and if the induction of repair and defense enzymes were not significant factors (*65*).

Promotion, another step in carcinogenesis, appears to involve cell proliferation, or perhaps particular types of cell proliferation (*66*), and dose-response relations with apparent thresholds, as indicated by various lines of evidence: (i) The work of Trosko *et al.* (*67*) on promotion of carcinogenesis due to interference with cell-cell communication, causing cell proliferation. (ii) Rajewsky's and other work indicating initiation by some carcinogenic agents appears to require proliferating target cells (*68*). (iii) The work of Farber *et al.* (*69*) on liver carcinogenesis supports the idea that cell proliferation (caused by partial hepatectomy or cell killing) can be an important aspect of hepatocarcinogenesis. They have also shown for several chemicals that hepatic cell killing shows a toxic threshold with dose. (iv) Work on carcinogenesis in the pancreas, bladder and stomach

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0), and other tissues (*58*) is also consistent with results on the liver (*71, 72*) though the effect of cell proliferation might be different in tissues that normally proliferate. (v) The work of Mirsalis *et al.* (*71*) suggests that a variety of nongenotoxic agents are hepatocarcinogens in the B6C3F1 mouse (commonly used in cancer tests) because of their toxicity. Other studies on chloroform and trichloroethylene also support this interpretation (*72, 73*). Cell proliferation resulting from the cell killing in the mouse liver shows a threshold with dose (*71*). Also relevant is the extraordinarily high spontaneous rates of liver tumors (21% carcinomas, 10% adenomas) in the male B6C3F1 mouse (*74*). These spontaneous tumors have a mutant *ras* oncogene, and thus the livers in these mice appear to be highly initiated (mutated) to start with (*75*). (vi) Oncogenes: As Weinberg (*62*) has pointed out, "Oncogene-bearing cells surrounded by normal neighbors do not grow into a large mass if they carry only a single oncogene. But if the normal neighbors are removed . . . by killing them with a cytotoxic drug . . . then a single oncogene often suffices." (vii) Cell killing, as well as mutation, appears to be an important aspect of radiation carcinogenesis (*76*).

Promotion has also been linked to the production of oxygen radicals, such as from phagocytic cells (*77*). Since chronic cell killing would usually involve inflammatory reactions caused by neutrophils, one would commonly expect chemicals tested at the maximally tolerated dose (MTD) to be promoters because of the chronic inflammation.

Progression, another step in carcinogenesis, leading to selection for invasiveness and metastases, is not well understood but can be accelerated by oxygen radicals (*78*).

Chronic cell toxicity caused by dosing at the MTD in rodent cancer bioassays thus not only could cause inflammation and cell proliferation, but also should be somewhat mutagenic and clastogenic to neighboring cells because of the release of oxygen radicals from phagocytosis (*12, 79, 80*). The respiratory burst from phagocytic neutrophils releases the same oxidative mutagens produced by radiation (*77, 79*). Thus, animal cancer tests done at the MTD of a chemical might commonly stimulate all three steps in carcinogenesis and be positive because the chemical caused chronic cell killing and inflammation with some mutagenesis. Some of the considerable human evidence for chronic inflammation contributing to carcinogenesis and also some evidence for and against a general effect of inflammation and cytotoxicity in rodent carcinogenesis have been discussed (*81*).

Another set of observations may also bear on the question of toxicity and extrapolation. Wilson, Crouch, and Zeise (*82*) have pointed out that among carcinogens one can predict the potency in high-dose animal cancer experiments from the toxicity (the LD_{50}) of the chemical, though one cannot predict whether the substance is a carcinogen. We have shown that carcinogenic potency values are bounded by the MTD (*57*). The evidence from our database suggests that the relationship between TD_{50} and MTD has a biological as well as a statistical basis (*57*). We postulate that a just sublethal level of a carcinogen causes cell death, which allows neighboring cells to proliferate, and also causes oxygen radical production from phagocytosis and thus chronic inflammation, both important aspects of the carcinogenic process (*57*). The generality of this relationship and its basis needs further study.

If most animal cancer tests done at the MTD are partially causing cell killing and consequent cell proliferation and phagocytic oxygen radical damage as steps in the carcinogenic process, one might predict that the dose-response curves would generally be nonlinear. For those experiments in our database for which life table data (*14*) were available, a detailed analysis (*83*) shows that the dose-response relationships are more often consistent with a quadratic (or cubic) model than with a linear model.

Experimentally, it is very difficult to discriminate between the various extrapolation models at low doses (*11, 58*). However, evidence to support the idea that a nonlinear dose-response relationship is the norm is accumulating for many nongenotoxic and some genotoxic carcinogens. Dose-response curves for saccharin (*45*), butylated hydroxyanisole [BHA (*84*)], and a variety of other nongenotoxic carcinogens appear to be nonlinear (*85*). Formaldehyde, a genotoxic carcinogen, also has a nonlinear dose response (*28, 29*). The data for both bladder and liver tumors in the large-scale study on acetylaminofluorene, a genotoxic chemical, could fit a hockey stick–shaped curve, though a linear model, with a decreased effect at lower dose rates when the total dose is kept constant (*86*), has not been ruled out.

Carcinogens effective at both mutating and killing cells (which includes most mutagens) could be "complete" carcinogens and therefore possibly more worrisome at doses far below the MTD than carcinogens acting mainly by causing cell killing or proliferation (*15*). Thus, all carcinogens are not likely to be directly comparable, and a dose of 1/100 the TD_{50} (HERP = 1%) might be much more of a carcinogenic hazard for the genotoxic carcinogens dimethylnitrosamine or aflatoxin than for the apparently nongenotoxic carcinogens trichloroethylene, PCBs, or alcohol (HERP values marked with asterisks in Table 1). Short-term tests for mutagenicity (*61, 87*) can have a role to play, not only in understanding mechanisms, but also in getting a more realistic view of the background levels of potential genotoxic carcinogens in the world. Knowledge of mechanism of action and comparative metabolism in rodents and humans might help when estimating the relative importance of various low-dose exposures.

Human cancer, except in some occupational or medicinal drug exposures, is not from high (just subtoxic) exposures to a single chemical but is rather from several risk factors often combined with a lack of antirisk factors (*60*); for example, aflatoxin (a potent mutagen) combined with an agent causing cell proliferation, such as hepatitis B virus (*19*). High salt [a possible risk factor in stomach cancer (*13*)] and high fat [a possible risk factor in colon cancer (*4*)] both appear to be effective in causing cell killing and cell proliferation.

Risk from carcinogenesis is not linear with time. For example, among regular cigarette smokers the excess annual lung cancer incidence is approximately proportional to the fourth power of the duration of smoking (*88*). Thus, if human exposures in Table 1 are much shorter than the lifetime exposure, the possible hazard may be markedly less than linearly proportional.

A key question about animal cancer tests and regulatory policy is the percentage of tested chemicals that will prove to be carcinogens (*89*). Among the 392 chemicals in our database that were tested in both rats and mice, 58% are positive in at least one species (*14*). For the 64 "natural" substances in the group, the proportion of positive results is similar (45%) to the proportion of positive results in the synthetic group (60%). One explanation offered for the high proportion of positive results is that more suspicious chemicals are being tested (for example, relatives of known carcinogens), but we do not know if the percentage of positives would be low among less suspicious chemicals. If toxicity is important in carcinogenicity, as we have argued, then at the MTD a high percentage of all chemicals might be classified as "carcinogens."

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The Background of Natural Carcinogens

The object of this article is not to do risk assessment on naturally occurring carcinogens or to worry people unduly about an occasional raw mushroom or beer, but to put the possible hazard of man-made carcinogens in proper perspective and to point out that we

lack the knowledge to do low-dose "risk assessment." We also are almost completely ignorant of the carcinogenic potential of the enormous background of natural chemicals in the world. For example, cholinesterase inhibitors are a common class of pesticides, both man-made and natural. Solanine and chaconine (the main alkaloids in potatoes) are cholinesterase inhibitors and were introduced generally into the human diet about 400 years ago with the dissemination of the potato from the Andes. They can be detected in the blood of almost all people (*12, 90*). Total alkaloids are present at a level of 15,000 μg per 200-g potato with not a large safety factor (about sixfold) from the toxic level for humans (*91*). Neither alkaloid has been tested for carcinogenicity. By contrast, malathion, the main synthetic organophosphate cholinesterase inhibitor in our diet (17 μg/day) (*16*), is not a carcinogen in rodents.

The idea that nature is benign and that evolution has allowed us to cope perfectly with the toxic chemicals in the natural world is not compelling for several reasons: (i) there is no reason to think that natural selection should eliminate the hazard of carcinogenicity of a plant toxin that causes cancer in old age past the reproductive age, though there could be selection for resistance to the acute effects of particular carcinogens. For example, aflatoxin, a mold toxin that presumably arose early in evolution, causes cancer in trout, rats, mice, and monkeys, and probably people, though the species are not equally sensitive. Many of the common metal salts are carcinogens (such as lead, cadmium, beryllium, nickel, chromium, selenium, and arsenic) despite their presence during all of evolution. (ii) Given the enormous variety of plant toxins, most of our defenses may be general defenses against acute effects, such as shedding the surface lining of cells of our digestive and respiratory systems every day; protecting these surfaces with a mucin layer; having detoxifying enzymes that are often inducible, such as cytochrome P-450, conjugating enzymes, and glutathione transferases; and having DNA repair enzymes, which would be useful against a wide variety of ingested toxic chemicals, both natural and synthetic. Some human cancer may be caused by interfering with these normal protective systems. (iii) The human diet has changed drastically in the last few thousand years, and most of us are eating plants (such as coffee, potatoes, tomatoes, and kiwi fruit) that our ancestors did not. (iv) Normal metabolism produces radiomimetic mutagens and carcinogens, such as hydrogen peroxide and other reactive forms of oxygen. Though we have defenses against these agents, they still may be major contributors to aging and cancer. A wide variety of external agents may disturb this balance between damage and defense (*12, 42*).

Implications for Decision-Making

For all of these considerations, our scale is not a scale of risks to humans but is only a way of setting priorities for concern, which should also take into account the numbers of people exposed. It should be emphasized that it is a linear scale and thus may overestimate low potential hazards if, as we argue above, linearity is not the normal case, or if nongenotoxic carcinogens are not of very much concern at doses much below the toxic dose.

Thus, it is not scientifically credible to use the results from rodent tests done at the MTD to directly estimate human risks at low doses. For example, an EPA "risk assessment" (*92*) based on a succession of worst case assumptions (several of which are unique to EDB) concluded that EDB residues in grain (HERP = 0.0004%) could cause 3 cases of cancer in 1000 people (about 1% of all U.S. cancer). A consequence was the banning of the main fumigant in the country. It would be more reasonable to compare the possible hazard of EDB residues to that of other common possible hazards. For example, the aflatoxin in the average peanut butter sandwich, or a raw mushroom, are 75 and 200 times, respectively, the possible hazard of EDB. Before banning EDB, a useful substance with rather low residue levels, it might be reasonable to consider whether the hazards of the alternatives, such as food irradiation, or the consequences of banning, such as increased mold contamination of grain, pose less risk to society. Also, there is a disparity between OSHA not regulating worker exposures at a HERP of 140%, while the EPA bans the substance at a HERP of 0.0004%. In addition, the FDA allows a possible hazard up to a HERP of 0.3% for peanut butter (20 ppb), and there is no warning about buying comfrey pills.

Because of the large background of low-level carcinogenic and other (*93*) hazards, and the high costs of regulation, priority setting is a critical first step. It is important not to divert society's attention away from the few really serious hazards, such as tobacco or saturated fat (for heart disease), by the pursuit of hundreds of minor or nonexistent hazards. Our knowledge is also more certain about the enormous toll of tobacco—about 350,000 deaths per year (*1, 2*).

There are many trade-offs to be made in all technologies. Trichloroethylene and tetrachloroethylene (perchloroethylene) replaced hazardous flammable solvents. Modern synthetic pesticides displaced lead arsenate, which was a major pesticide before the modern chemical era. Lead and arsenic are both natural carcinogens. There is also a choice to be made between using synthetic pesticides and raising the level of plants' natural toxins by breeding. It is not clear that the latter approach, even where feasible, is preferable. For example, plant breeders produced an insect-resistant potato, which has to be withdrawn from the market because of its acute toxicity to humans due to a high level of the natural plant toxins solanine and chaconine (*12*).

This analysis on the levels of synthetic pollutants in drinking water and of synthetic pesticide residues in foods suggests that this pollution is likely to be a minimal carcinogenic hazard relative to the background of natural carcinogens. This result is consistent with the epidemiologic evidence (*1*). Obviously prudence is desirable with regard to pollution, but we do need to work out some balance between chemophobia with its high costs to the national wealth, and sensible management of industrial chemicals (*94*).

Human life expectancy continues to lengthen in industrial countries, and the longest life expectancy in the world is in Japan, an extremely crowded and industrialized country. U.S. cancer death rates, except for lung cancer due to tobacco and melanoma due to ultraviolet light, are not on the whole increasing and have mostly been steady for 50 years. New progress in cancer research, molecular biology, epidemiology, and biochemical epidemiology (*95*) will probably continue to increase the understanding necessary for lengthening life-span and decreasing cancer death rates.

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96. A national survey of U.S. drinking water supplies identified the concentrations of about 20 organic compounds. The mean total trihalomethane concentration was 117 μg/liter, with the major component, chloroform, present at a mean concentration of 83 μg/liter (83 ppb). Raw water that is relatively free of organic matter results in drinking water relatively free of trihalomethanes after chlorination. These studies are reviewed in S. J. Williamson, *The Science of the Total Environment* 18, 187 (1981).
97. Public and private drinking water wells in Santa Clara Valley, California, have been found to be contaminated with a variety of halogenated hydrocarbons in small amounts. Among 19 public water system wells, the most commonly found contaminants were 1,1,1-trichloroethane (TCA), and 1,1,2-trichloro-1,2,2-trifluoroethane (Freon-113). TCA was found in 15 wells generally at concentrations of less than 30 ppb, though one well contained up to 8800 ppb, and Freon-113 was found in six wells at concentrations up to 12 ppb. Neither chemical has been adequately tested for carcinogenicity in long-term bioassays. In addition to these compounds, three wells also contained carcinogenic compounds at low concentrations. Water from public supply wells may be mixed with treated surface water before delivery, thus the concentrations of these compounds that people actually receive may be somewhat reduced. Thirty-five private drinking water supply wells were examined; the major contaminant was the carcinogen trichloroethylene (TCE), at levels up to 2800 ppb. TCA and Freon-113 were also found in some wells, at maximum levels of 24 ppb and 40 ppb, respectively. Though fewer people drink from private water wells, the contaminant concentrations may be higher because the water is not mixed with water from other sources [California Department of Health Services, California Regional Water Quality Control Board 2, Santa Clara County Public Health Department, Santa Clara Valley Water District, U.S. Environmental Protection Agency, *Ground Water and Drinking Water in the Santa Clara Valley: A White Paper* (1984), table 8]. Trichloroethylene may not be a carcinogen in humans at low doses [R. D. Kimbrough, F. L. Mitchell, V. N. Houk, *J. Toxicol. Environ. Health* 15, 369 (1985)].
98. Contaminated drinking water in the area of Woburn, Massachusetts, was found to contain 267 ppb trichloroethylene, 21 ppb tetrachloroethylene, 12 ppb chloroform, 22 ppb trichlorotrifluoroethane, and 28 ppb 1,2-*trans*-dichloroethylene [S. W. Lagakos, B. J. Wessen, M. Zelen, *J. Am. Stat. Assoc.* 81, 583 (1986)].
99. The amount of chloroform absorbed by a 6-year-old child in a chlorinated freshwater swimming pool has been estimated [J. A. Beech, *Med. Hypotheses* 6, 303 (1980)]. Table 1 refers to the chloroform in an average pool (134 μg/liter) and for a 37-kg child. Three other trihalomethanes were identified in these freshwater pools: bromoform, bromodichloromethane and chlorodibromomethane. U. Lahl, J. Vondusze, B. Gabel, B. Stachel, W. Thiemann [*Water Res.* 15, 803 (1981)] have estimated absorption in covered swimming pools.
100. J. McCann, L. Horn, J. Girman, A. V. Nero, in *Short-Term Bioassays in the Analysis of Complex Environmental Mixtures*, V. S. Sandhu, D. M. De Marini, M. J. Mass, M. M. Moore, J. L. Mumford, Eds. (Plenum, New York, in press). This estimate (Table 1) for formaldehyde in conventional homes, excludes foam-insulated houses and mobile homes. The figure is a mean of the median or mean of the reported samples in each paper. For benzene, the figure is a mean of all reported median or mean samples. The level of benzene in Los Angeles outdoor air is similar (U.S. EPA Office of Air Quality Planning and Standards, EPA 450/4-86-012, 1986).
101. The average adult daily PCB intake from food estimated by the FDA in fiscal years 1981/1982 was 0.2 μg/day (*16*). Many slightly different PCB mixtures have been studied in long-term animal cancer bioassays; the calculation of TD_{50} was from a test of Aroclor 1260 which was more potent than other PCBs (*14*).
102. The average consumption of EDB residues in grains has been estimated by the EPA for adults as 0.006 μg kg^{-1} day^{-1} and for children as 0.013 μg kg^{-1} day^{-1} [U.S. EPA Office of Pesticide Programs, *Ethylene Dibromide (EDB) Scientific Support and Decision Document for Grain and Grain Milling Fumigation Uses* (8 February 1984)].
103. The leaves and roots of Russian comfrey are widely sold in health food stores and are consumed as a medicinal herb or salad plant or are brewed as a tea. Comfrey leaf has been shown to contain 0.01 to 0.15%, by weight, total pyrrolizidine alkaloids, with an average level of 0.05% for intermediate size leaves [C. C. J. Culvenor, J. A. Edgar, J. L. Frahn, L. W. Smith, *Aust. J. Chem.* 33, 1105 (1980)]. The main pyrrolizidine alkaloids present in comfrey leaves are echimidine and 7-acetylvcopsamine, neither of which has been tested for carcinogenicity. Almost all tested 1,2-unsaturated pyrrolizidine alkaloids have been shown to be genotoxic and carcinogenic [H. Mori *et al.*, *Cancer Res.* 45, 3125 (1985)]. Symphytine accounts for 5% of the total alkaloid in the leaves and has been shown to be carcinogenic [C. C. J. Culvenor *et al.*, *Experientia* 36, 377 (1980)]. We assume that 1.5 g of intermediate size leaves are used per cup of comfrey tea (Table 1). The primary alkaloids in comfrey root are symphytine (0.67 g per kilogram of root) and echimidine (0.5 g per kilogram of root) [T. Furuya and M. Hikichi, *Phytochemistry* 10, 2217 (1971)]. Comfrey-pepsin tablets (300 mg of root per tablet) have a recommended dose of one to three tablets three times per day. Comfrey roots and leaves both induce liver tumors in rats [I. Hirono, H. Mori, M. Haga, *J. Natl. Cancer Inst.* 61, 865 (1978)], and the TD_{50} value is based on these results. Those pyrrolizidine alkaloids tested have been found to be at least

as potent as carcinogens such as symphytine. If the other pyrrolizidine alkaloids in comfrey were as potent carcinogens as symphytine, the possible hazard of a daily cup of tea would be HERP = 0.6% and that of a daily nine tablets would be HERP = 7.3%.

104. *Agaricus bisporus* is the most commonly eaten mushroom in the United States with an estimated annual consumption of 340 million kilograms in 1984–85. Mushrooms contain various hydrazine compounds, some of which have been shown to cause tumors in mice. Raw mushrooms fed over a lifetime to male and female mice induced bone, forestomach, liver, and lung tumors [B. Toth and J. Erickson, *Cancer Res.* 46, 4007 (1986)]. The 15-g raw mushroom is given as wet weight. The TD_{50} value based on the above report is expressed as dry weight of mushrooms so as to be comparable to other values for TD_{50} in Table 1; 90% of a mushroom is assumed to be water. A second mushroom, *Gyromitra esculenta*, has been similarly studied and found to contain a mixture of carcinogenic hydrazines [B. Toth, *J. Environ. Sci. Health* C2, 51 (1984)]. These mushrooms are eaten in considerable quantities in several countries, though less frequently in the United States.
105. Safrole is the main component (up to 90%) of oil of sassafras, formerly used as the main flavor ingredient in root beer [J. B. Wilson, *J. Assoc. Off. Anal. Chem.* 42, 696 (1959); A. Y. Leung, *Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics* (Wiley, New York, 1980)]. In 1960, safrole and safrole-containing sassafras oils were banned from use in foods in the United States [*Fed. Regist.* 25, 12412 (1960)]. Safrole is also naturally present in the oils of sweet basil, cinnamon leaf, nutmeg, and pepper.
106. Diet cola available in a local market contains 7.9 mg of sodium saccharin per fluid ounce.
107. Metronidazole is considered to be the drug of choice for trichomonal and *Gardnerella* infections [AMA Division of Drugs, *AMA Drug Evaluations* (American Medical Association, Chicago, IL, ed. 5, 1983), pp. 1717 and 1802].
108. Isoniazid is used both prophylactically and as a treatment for active tuberculosis. The adult prophylactic dose (300 mg daily) is continued for 1 year [AMA Division of Drugs, *AMA Drug Evaluations* (American Medical Association, Chicago, IL, ed. 5, 1983), pp. 1766–1777].
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Perception of Risk

PAUL SLOVIC

Studies of risk perception examine the judgments people make when they are asked to characterize and evaluate hazardous activities and technologies. This research aims to aid risk analysis and policy-making by (i) providing a basis for understanding and anticipating public responses to hazards and (ii) improving the communication of risk information among lay people, technical experts, and decision-makers. This work assumes that those who promote and regulate health and safety need to understand how people think about and respond to risk. Without such understanding, well-intended policies may be ineffective.

THE ABILITY TO SENSE AND AVOID HARMFUL ENVIRONMENTAL conditions is necessary for the survival of all living organisms. Survival is also aided by an ability to codify and learn from past experience. Humans have an additional capability that allows them to alter their environment as well as respond to it. This capacity both creates and reduces risk.

In recent decades, the profound development of chemical and nuclear technologies has been accompanied by the potential to cause catastrophic and long-lasting damage to the earth and the life forms that inhabit it. The mechanisms underlying these complex technologies are unfamiliar and incomprehensible to most citizens. Their most harmful consequences are rare and often delayed, hence difficult to assess by statistical analysis and not well suited to management by trial-and-error learning. The elusive and hard to manage qualities of today's hazards have forced the creation of a new intellectual discipline called risk assessment, designed to aid in identifying, characterizing, and quantifying risk (*1*).

Whereas technologically sophisticated analysts employ risk assessment to evaluate hazards, the majority of citizens rely on intuitive risk judgments, typically called "risk perceptions." For these people, experience with hazards tends to come from the news media, which rather thoroughly document mishaps and threats occurring throughout the world. The dominant perception for most Americans (and one that contrasts sharply with the views of professional risk assessors) is that they face more risk today than in the past and that future risks will be even greater than today's (*2*). Similar views appear to be held by citizens of many other industrialized nations. These perceptions and the opposition to technology that accompanies them have puzzled and frustrated industrialists and regulators and have led numerous observers to argue that the American public's apparent pursuit of a "zero-risk society" threatens the nation's political and economic stability. Wildavsky (*3*, p. 32) commented as follows on this state of affairs.

> How extraordinary! The richest, longest lived, best protected, most resourceful civilization, with the highest degree of insight into its own technology, is on its way to becoming the most frightened.
>
> Is it our environment or ourselves that have changed? Would people like us have had this sort of concern in the past? . . . Today, there are risks from numerous small dams far exceeding those from nuclear reactors. Why is the one feared and not the other? Is it just that we are used to the old or are some of us looking differently at essentially the same sorts of experience?

During the past decade, a small number of researchers has been attempting to answer such questions by examining the opinions that people express when they are asked, in a variety of ways, to evaluate hazardous activities, substances, and technologies. This research has attempted to develop techniques for assessing the complex and subtle opinions that people have about risk. With these techniques, researchers have sought to discover what people mean when they say that something is (or is not) "risky," and to determine what factors underlie those perceptions. The basic assumption underlying these efforts is that those who promote and regulate health and safety need to understand the ways in which people think about and respond to risk.

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Red Herrings Don't Cause Cancer

END PAPER

BY BRUCE N. AMES



The cancer-causing hazard of a fish highly contaminated with DDT/DDE or PCB—that is, 100 times the average level—is comparable to the hazard of the average peanut butter sandwich.

The carcinogens and pesticide residues currently being found in California water supplies, such as in Silicon Valley, are present in tiny amounts that, except in rare cases, are trivial relative to the background level of carcinogens in nature. Therefore, I am convinced that such water pollution is irrelevant as a cause of human cancer.

The main current fallacy in our approach to such pollution consists in believing that carcinogens — cancer-causing substances — are rare and that they are mostly man-made chemicals. Quite the contrary is the case. My estimate is that more than 99.99 percent of the carcinogens Californians ingest are from natural sources (that is, substances normally present in food) or traditional sources (that is, cigarets, alcohol and chemicals formed by cooking food).

Every meal has many carcinogens, and when one compares the level of carcinogens in contaminated water or pesticide residues in food with the level of natural carcinogens also present in the diet, it is clear that water pollution or pesticide residues represent a trivial exposure by comparison.

Water pollution and pesticide residues are almost always present in the ppb (parts per billion) range. One part per billion (i.e., one person in all of China) is an extraordinarily small amount. By comparison, the carcinogens in a few common drinks are listed below. Every common drink contains carcinogens.

■ Coffee contains the known natural carcinogens hydrogen peroxide and methylglyoxal, each at about 4000 ppb.

■ Tap water, as a consequence of chlorination, contains the carcinogen chloroform at a national average of 83 ppb.

■ Cola drinks contain the carcinogen formaldehyde at 7900 ppb, though this level is not much higher than that in human blood, which averages about 3000 ppb in formaldehyde from normal metabolism.

■ Beer contains nitrosamines, formaldehyde (700 ppb) and alcohol (50 million ppb, or 5 percent), all known carcinogens. Alcohol consumption is a known cause of human cancer (3 percent of U.S. cancer), and ethyl alcohol is a carcinogen in rats.

■ Milk contains a high percentage of fat, and high fat consumption has been implicated in human breast and colon cancer and rodent cancer (though milk is an important source of calcium, which may be important as an anti-carcinogen).

■ Fruit juices may have various amounts of carcinogenic mold toxins, depending on how many moldy fruits were processed.

Calculating a possible hazard to humans from information obtained from a cancer test on rats must take into account the potency of the carcinogen in rats as well as the human dose. Some colleagues and I are just completing a study in which we compare possible hazards for humans due to typical daily intake of carcinogens, after adjusting for the potency of each carcinogen from the animal data. This adjustment is necessary because the potency of carcinogens varies more than a millionfold. For example, aflatoxin, a mold carcinogen present in small amounts in peanut butter (2 ppb is the U.S. average) and in corn products such as tortillas, requires about a million times smaller dose to cause the same incidence of cancer in test animals as trichloroethylene, which was the main contaminant in Silicon Valley wells. Consider the following comparisons.

■ Contaminated water: The level of carcinogens in contaminated well water (for example, trichloroethylene in Silicon Valley or Woburn, Mass.) only rarely involves a possible hazard greater than that of ordinary chlorinated tap water. Of 35 private wells shut down in Silicon Valley because of their supposed carcinogenic hazard in an EPA study, only two were of greater possible hazard than ordinary tap water (well water usually lacks the chloroform present in chlorinated tap water), and the most polluted well (2800 ppb trichloroethylene) is still at least 1000 times less of a possible hazard than an equal volume of cola, beer or wine. This is because trichloroethylene is an extremely weak carcinogen. It is comparable to saccharin and only 10 times more potent than alcohol, which is present at about 50 million ppb in a beer. Given that the consumption of tap water is only about one or two liters per day, it seems unlikely that man-made water pollution in the trace amounts typically seen is causing more than a minimal hazard.

■ Pesticide residues: Manmade pesticide residues present in our food amount to about 100 ppb on the average; most of these residues are composed of noncarcinogenic compounds. The manmade carcinogenic residue of most interest in food is likely to be DDT and its metabolite (or breakdown product), DDE. The possible hazard of the DDT/DDE in the average U.S. daily intake is equivalent to that of the chloroform in a glass of tap water and is insignificant compared with natural carcinogens in our diet. Even an occasional fish highly contaminated with DDT/DDE or PCB — that is, 100 times the average level — would contribute a possible hazard that is comparable to the average peanut butter sandwich and is small compared with other very common minimal risks, such as a glass of beer.

■ Nature's pesticides: We are ingesting natural pesticides in our diet in amounts at least 10,000 times greater than manmade pesticide residues. Natural pesticides are natural toxic chemicals present in all plants, usually making up 5 to 10 percent of a plant's weight. They have an enormous variety of chemical structures, though only a few are present in each plant species. Their function is to protect against fungi, insects and animal predators. Thus, a major aspect of the evolution of plants is chemical warfare. There has been relatively little research on the toxicology or carcinogenicity of these compounds until quite recently, and thus very few of the large number present in the human diet have been tested for their ability to cause cancer in animals.

We must ignore the trivia if we wish to deal with the important causes of cancer.

A fair percentage of those few natural pesticide materials from plants that have been tested have turned out to be carcinogens in rats or mice. They include estragole (in basil), safrole (in herbs), symphitine (in comfrey tea), psoralens (in parsley and celery), hydrazines (in mushrooms), and allyl isothiocyanate (in mustard). The possible carcinogenic hazards of nature's pesticides completely overshadow the traces of manmade pesticide residues found in the daily diet. Plants also contain anti-carcinogens and valuable nutrients, so I believe that even these possible hazards seem too small to worry about, particularly given my skepticism about extrapolating risks from rodents.

All calculations of human risk based on rat and mouse cancer tests, both from natural and manmade carcinogens, are hypothetical. Thus, they should be taken with a great dose of skepticism, unlike known human carcinogens such as smoking (which causes 400,000 deaths from cancer, heart disease and other illnesses a year in the United States) or alcoholic beverages (100,000 deaths from cancer and other diseases each year).

There are many new reasons for being skeptical of uncritically extrapolating animal data — obtained by feeding enormous doses of carcinogens — to human risks from low doses of a carcinogen, but these cannot be discussed here. This contention is reinforced by the studies of cancer epidemiologists who are making considerable progress in understanding the smoking, dietary, hormonal, viral and occupational contributions to human cancer, but who are finding remarkably little solid evidence for any significant contribution from pollution. Even in the well-publicized cases of Love Canal, Contra Costa County, Santa Clara County and Woburn, Mass., they found no convincing evidence that pollution was the cause of the hazard.

Since we now know that carcinogens are common, not rare — more than half of the chemicals tested in rats or mice have been judged carcinogens — we must of necessity ignore the trivia if we wish to deal with the important causes of cancer. We might possibly eliminate every trace of manmade carcinogen from our water or food supply, but it would cost an enormous amount of California's wealth, be of minimal relevance to causes of human cancer and distract health workers from real, more important risks.

Thus, one can either chase after parts per billion of every manmade carcinogen that turns up or have some sensible regulations about pollution. ■

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REVIEW

Perspectives on Comparing Risks of Environmental Carcinogens[1]

Frederica Perera, Paolo Boffetta*[2,3]

In 1987, investigators (Ames et al.) concluded that the risks of man-made industrial carcinogens and pesticides (outside of the workplace) are trivial compared with the risks of naturally occurring carcinogens found mostly in the diet. They used a ranking system based on human exposure and rodent potency (HERP) data to arrive at this conclusion. As a result, they recommend that regulatory agencies, such as the Environmental Protection Agency and the Food and Drug Administration, base their priorities in this area on their HERP system. We analyzed the assumptions and data set upon which the HERPs were based, concluding that such a simplified approach to set public health policy is inappropriate given the underlying uncertainties. However, we note that when comparisons are consistently based on estimates of average daily exposure to common carcinogens, the HERP scores of many man-made pollutants are comparable to those of naturally occurring carcinogens in the diet. [J Natl Cancer Inst 1988;80:1282-1293]

Background

The majority (an estimated 60%-90%) of human cancer is considered to be attributable to environmental factors, broadly defined to include cigarette smoking, industrial pollutants, radiation, diet, and perhaps other life-style factors and viruses (*1*). Thus, in theory most cancer is preventable through the identification and control of causative factors, including exposure to carcinogens. For decades, policymakers concerned with the assessment and regulation of environmental carcinogens have searched for a systematic way to set priorities among the many candidates. This paper critically evaluates the most recent proposal for such a ranking scheme (*2*).

Identification of specific etiologic factors and estimation of their relative importance constitute a formidable task. Few cancers are attributable to single factors or exposures; rather, complex interactions between environmental and host factors are generally involved (*3-5*). Moreover, because of the limitations of epidemiology (*6*), only rarely are human data available that directly link an environmental agent to human cancer. For example, epidemiological studies strongly suggest, although they do not conclusively establish, an association between organic chemical carcinogens in drinking water (such as chloroform) and cancers at several sites, including the rectum, colon, and bladder (*7-11*). Certain dietary and nutritional factors (such as dietary fat and fiber) have been implicated in cancer of the breast, colon, rectum, and stomach (*12,13*), but here too a direct causal association has not been established for specific dietary constituents. In addition to active cigarette smoking and a significant number of pollutants in the workplace, established to be human lung carcinogens (*14*), there is growing evidence that passive smoking (*15*) and pollutants in the ambient air (*16,17*) contribute significantly to lung cancer mortality. However, because of cost and feasibility constraints and the difficulty in identifying an appropriate study population, the vast majority of animal carcinogens, both naturally occurring and man-made, have neither been the subject of epidemiological investigation nor are they likely to be (*18*). Thus, for practical purposes, as a matter of long-standing policy, regulatory agencies accept the use of positive animal data as predictive of carcinogenic hazard in human beings (*19*). The alternative, awaiting positive evidence of carcinogenicity in humans,

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traditionally has been rejected as morally and socially unacceptable.

A major limitation of epidemiology (and risk assessment) is that reliable data on human exposure to specific chemicals are frequently lacking. Therefore, by necessity, most epidemiological studies have relied on crude or indirect measures of exposure. A significant number of carcinogens have been detected in drinking water, ambient air, and the food supply; however, reliable monitoring data exist for only a small fraction of these chemicals. For example, while dozens of pesticides and industrial chemical carcinogens have been measured routinely in surface water, groundwater, and drinking water, they represent only a small percentage of chemical pollutants present (*10,20–23*). Over 700 organic chemicals have been found to be present in the U.S. drinking water supply, including 40 known or suspected carcinogens (*24*). Numerous carcinogenic air pollutants (trace metals, polycyclic aromatic hydrocarbons, and volatile organic chemicals) have been detected in ambient air; again, there are little or no reliable monitoring data on the majority of airborne carcinogens (*25*). Similarly, many carcinogenic pesticide residues have been identified in the food supply, but reliable exposure data are lacking for most (*26*). Testifying to the pervasiveness of environmental contamination are studies showing significant concentrations of synthetic organic chemicals in the blood, urine, and/or adipose tissue of the U.S. population. These include 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), dieldrin, heptachlor epoxide, polychlorinated biphenyls (PCBs), and dioxin (*27,28*). Again, data are far from comprehensive; however, they do show a decline in the concentrations of DDT and PCBs as a result of regulation.

Despite their limitations, available exposure and epidemiologic data have served as the basis for a number of widely varying estimates of the proportion of human cancer in the U.S. population that can be attributed to life-style, occupational exposures, or other environmental pollution. These exercises have generated significant debate, as much over the underlying assumptions as the data used to generate them (*17,29–35*). Unfortunately, various such estimates (ranging, for example, from 4% to >20% for occupational exposures) have been cited as a basis for setting priorities for public health protection. This approach ignores both the underlying uncertainties, the relative preventability of various risk factors (*36*), and the disproportionate impact on some segments of the population. For example, once recognized, most chemical pollutant hazards can be reduced or eliminated by practical means. Moreover, the involuntary nature of these exposures necessitates control at the source, in contrast to exposures related to life-style (e.g., diet and smoking), which can be addressed more effectively through public education regarding personal behavioral choices. Another inherent problem with the approach of estimates is that it obscures the much higher risks to certain subpopulations. For example, if the contribution of occupational carcinogens to all cancer deaths in the United States were as low as 3%, for male industrial workers as a group, workplace carcinogens would account for at least 25% of all identified causes of cancer (*33*).

Another tool that has been used increasingly by regulatory agencies to set priorities and even to determine acceptable levels of exposure to individual environmental contaminants has been quantitative risk assessment. Here, also, the lack of good information on human exposure as well as the usual paucity of epidemiological data are compounded by uncertainties regarding the proper way to extrapolate from high to low dose and from experimental animals to humans (*37*). To offset these uncertainties, the four major U.S. regulatory agencies, including the Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA), the Consumer Product Safety Commission, and the Food and Drug Administration (FDA), have traditionally preferred conservative models that incorporate an assumption of low-dose linearity, regardless of the presumed mechanism of action of the chemical carcinogen (*19*). However, in certain instances, these conservative models may underestimate cancer risk. For example, the widely accepted linearized multistage model (*38*), considered to be one of the most conservative of the biologically plausible risk-assessment models, works on the assumption that the exposed population is of uniform susceptibility and that interactions do not occur between chemical exposures and other risk factors. Yet significant intraindividual variability has been demonstrated for human metabolism and binding of drugs and carcinogens (*39–45*) as well as for repair of DNA damage (*46*). Moreover, a number of epidemiological studies have demonstrated synergism between chemical exposures and host factors, such as cigarette smoking and air pollutants in the workplace and urban air (*47,48*). To further complicate the situation, although nonlinear (both superlinear and sublinear) dose-response relationships have been observed experimentally and epidemiologically, the available data do not allow low-dose linearity to be ruled out in any of these cases (*49*). Given these uncertainties, it is reassuring that, in a number of cases, risks observed in humans have been consistent with those calculated from high-dose animal experiments with the use of models that incorporated linearity at low dose. These include benzene, ethylene dibromide (EDB), gasoline, asbestos, and ethylene oxide (*50–56*). Therefore, there is general agreement that the use of quantitative risk assessment, performed with appropriate and consistent assumptions and models, affords the possibility of comparing risks for the purpose of setting priorities among selected candidates for regulation. However, most scientists do not view quantitative risk assessment as capable of providing precise estimates of human risk from individual chemicals; general sources of chemical exposures are considered even less likely candidates for risk estimation by this method.

Human Exposure/Rodent Potency (HERP) Index

Most recently, researchers at the University of California at Berkeley and Lawrence Berkeley Laboratory have suggested still another approach to priority setting (*2*). They have calculated a possible hazard index for selected carcinogens by expressing the human exposure (in milligrams/kilogram) as a percentage of the rodent TD_{50} dose

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(also in milligrams/kilogram).[4] They have compared the resultant HERP indices for four pollutants found in drinking water and indoor air, three man-made pesticides and other residues, 10 natural pesticides and dietary toxins, two food additives, five drugs, and two occupational exposures (*see* table 1). The authors conclude that man-made environmental pollutants, such as pesticide residues and contaminants in drinking water, are "likely to be of minimal carcinogenic hazard" relative to the background of natural carcinogens (found largely in the diet). They recommend that regulatory agencies that traditionally have emphasized control of exposures to man-made or industrial carcinogens (in addition to those in the occupational setting) revise their priorities.

The authors acknowledge several major limitations of the HERP system, such as the possibility of interspecies (rodent and human) variation in susceptibility to carcinogens and quantitative uncertainties regarding the general shape of the dose-response curve, including the possibility of synergistic effects and thresholds for nongenotoxic carcinogens, such as promoters (*see* discussion below). They caution that it would be a mistake to use the HERP index as a direct estimate of human hazard, but they conclude that the scale provides "a way of setting priorities for concern."

Although this is an innovative approach, it suffers from several inherent flaws. First, as we will show in table 2, the results are influenced strongly by the selection of chemicals and whether one classifies them as "man-made" or "natural." The rationale for selection of the individual compounds in table 1 was not provided by the authors, but presumably it was dictated by the nature and availability of both exposure and rodent potency data. As mentioned, the rodent potency data base is not comprehensive. For example, it omits a number of carcinogenic pesticides including alachlor, which is of current concern as a food contaminant (*26*) and has been found in water supply wells at significant concentrations (*61*). Certainly, the four selected drinking water and air pollutants and the pesticides listed cannot represent the large number of industrial chemicals and pesticides that have been detected frequently in the U.S. drinking water, air, and food supply and that also have evidence of carcinogenicity in humans and/or laboratory animals (*62,63*).

Moreover, although we are aware that there are many potential dietary hazards, the majority of which also are not well characterized (*2,64*), the 10 natural dietary carcinogens in table 1 include a number of exotic foods to which the U.S. general population has limited exposure (sake, comfrey

Table 1. Possible carcinogenic hazards, as ranked by Ames et al. (2)*

Possible hazard (HERP %)	Carcinogenic exposure
	Man-made chemicals in foods and beverages
0.0002	PCBs,† U.S. average daily dietary intake
0.0003	DDE/DDT,† average daily dietary intake
0.0004	EDB, average daily dietary intake from grains/grain products
0.0002	Furylfuramide in 2-fluorenamine, daily dietary intake before banning
0.06	Saccharin† in 12-oz diet cola
	Natural carcinogens in foods and beverages
0.003	DMN in 100 g of cooked bacon
0.006	DEN in 100 g of cooked bacon
0.003	Urethane in 250 mL of sake
0.03	Symphytine in 1 cup of comfrey herb tea
0.03	Aflatoxin in 1 peanut butter sandwich
0.06	DEN in dried squid, broiled in gas oven
0.07	Allyl isothiocyanate in 5 g of brown mustard
0.1	Estragole in 1 g of dried basil leaf
0.1	Hydrazines in 1 raw mushroom
0.2	Safrole in natural root beer, before ban
0.008	DMN in 12-oz beer, before 1979
2.8	Ethyl alcohol† in 12-oz beer
4.7	Ethyl alcohol† in 250 mL of wine
6.2	Comfrey root in comfrey-pepsin tablets, 9 daily
1.3	Symphytine in comfrey-pepsin tablets, 9 daily
	Indoor air pollutants
0.6	Formaldehyde in conventional home air, 14 hr/day
0.004	Benzene in conventional home air, 14 hr/day
2.1	Formaldehyde in mobile home air, 14 hr/day
	Water pollutants
0.001	Chloroform† in tap water, 1 L U.S. average
0.004	Tetrachloroethylene† in well water, 1 L, highly contaminated
0.0002	Chloroform† in well water, 1 L, contaminated
0.0003	Tetrachloroethylene† in well water, 1 L, contaminated
0.008	Chloroform† in average swimming pool, 1 hr
	Drugs
0.3	Phenacetin, average dose
5.6	Metronidazole, therapeutic dose
14	Isoniazid, prophylactic dose
16	Phenobarbital, 1 sleeping pill
17	Clofibrate,† average daily dose
	Occupational exposure
5.8	Formaldehyde, worker's average daily exposure
140	EDB, worker's daily intake, high exposure

*DMN = *N*-nitrosodimethylamine, and DEN = *N*-nitrosodiethylamine.
†Carcinogens characterized by Ames et al. as nongenotoxic and likely to have thresholds.

[4]Here the TD_{50} is the average daily dose rate to halve the percent of tumor-free animals by the end of a standard lifetime (*57*). The average TD_{50} is calculated by taking the harmonic mean of the TD_{50}s of the positive tests in the most sensitive species. From each test, the target site with the lowest TD_{50} value was used. In general, the harmonic mean and the lowest TD_{50} differ by a factor of ≈2 (*58*). The source of TD_{50} values is the Carcinogenic Potency Data Base (CPDB) (*57-60*). The data base is a compilation of results from >3,500 experiments on 975 chemicals. It includes results from the Carcinogenesis Bioassay Program of the National Cancer Institute/National Toxicology Program (through May of 1986) as well as studies published in the literature (through December of 1984). The data base is restricted to tests that meet very stringent methodologic criteria. Thus certain human carcinogens (such as asbestos and tobacco smoke) are excluded; seven chemicals regarded by the International Agency for Research on Cancer (IARC) as having sufficient evidence of carcinogenicity in ar s (cadmium chloride, cadmium sulfate, epichlorohydrin, glycidaldehy sosafrole, mestranole, and 2-nitropropane) are recorded in the CPDB as having only negative tests. The CPDB is a useful tool, but its limitations should be kept in mind.

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herb tea, dried squid, brown mustard, and comfrey-pepsin tablets). Therefore, comparisons between drinking 1 L of water containing average concentrations of chloroform and eating a daily serving of dried squid ignore the fact that the average American adult ingests an estimated 2 L or more of water a day (*65*)[5] and rarely, if ever, eats dried squid.

An additional problem is that several "natural pesticides and dietary toxins" in table 1 are misclassified in that they can result from harvesting, manufacturing, or cooking processes and therefore cannot be considered to be strictly natural. For example, aflatoxin in nuts and grains is partially attributable to improper harvesting and storage procedures, whereas, as the authors acknowledge, nitrosamines are formed in cured meats through the reaction of secondary amines with nitrites added as preservatives. Carcinogenic nitropyrenes and nitrosamines occur in browned or burned meats as a result of cooking with gas flames that generate NO_2 (*2*).

Moreover, a number of natural substances or food additives in table 1 have been banned (safrole in natural root beer and AF-2, a Japanese food additive never used in the United States) (*67*), so that there is no current exposure to the U.S. population. Several of the environmental pollutants have been regulated (chloroform, PCBs, and EDB) or even banned (DDT), so that postregulatory exposures (and HERPs) are predictably low, testifying to the effectiveness of regulation.

A second major limitation of the approach of Ames et al. derives from the fact that, as can be seen in table 1, varying exposure indices were used. For waterborne and airborne contaminants, daily exposure was calculated; for pesticides and other residues in food, daily average dietary intake was provided; for "natural" carcinogens in food, one serving was assumed to occur daily; for food additives and drugs, several different measures were used.

To illustrate the effect of chemical selection and of assumptions regarding levels of exposure, we have constructed table 2; it includes all of the chemicals/exposures in table 1, except for those dietary constituents not widely consumed in the United States and those that have been banned and have no current U.S. exposure. We have also omitted drugs because exposure is generally of short duration; drugs are a special case because they are prescribed when benefits are thought to outweigh risks to the individual. Finally, we have included in table 2 several chemicals or sources of exposure that are encountered commonly by the U.S. population and for which rodent potency (*58–60*) and exposure data are available. Unfortunately, in several cases environmental chemicals of concern were in the rodent potency data base, but we could not find reliable exposure data for specific media. This was true for dioxin or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). However, according to the EPA, a crude estimate of total daily intake of dioxin by sizeable segments of industrialized populations is 1 pg/kg (*28*), which corresponds to a HERP of 0.004.

To avoid the problems of inconsistent exposure indices, we have adopted in table 2 the standard approach of uniformly providing average daily dose to the U.S. adult. We recognize both the uncertainties in available exposure data (*143*) and the fact that the average estimates mask wide interindividual variation in exposure depending on geographical, cultural, economic, social, and host factors. For example, a child's exposure to pollutants in drinking water is proportionally greater than exposure of the adult, because children ingest an estimated 1 L of water per 10 kg of body weight compared with 2 L or more per 70 kg of body weight for the adult (*65*). Children may also consume more of a contaminated food than adults. In the case of the pesticide daminozide, the daily dose to the U.S. child (1–6 yr) from consumption of apples, apple juice, and peanut butter is from fivefold to 15-fold higher than the daily dose to the U.S. adult (*70,71.*)

Thus, there are obvious drawbacks to using each of the possible exposure indices (average, worst case, general population, or sensitive subpopulation). However, it is imperative in making comparisons that the same measure of exposure be used consistently. This is demonstrated by table 3 in which we compared HERPs from tables 1 and 2 for the same compound.

As mentioned above, postregulatory exposures (and HERPs) for environmental carcinogens such as DDT/DDE (1,1-dichloro-2,2′-bis(*p*-chlorophenyl)ethylene) are low. Therefore, in a number of cases we have included preregulatory and postregulatory values for purposes of comparison.

Unfortunately, any listing of chemicals such as in tables 1–3 cannot convey the reality of cumulative exposures to different carcinogens in the same medium. Also, it does not reflect the possibility of interactions among them or the need to consider exposures to the same chemical via several different media. For example, the individual has exposure to synthetic volatile organic compounds in the drinking water from ingestion, from dermal absorption while bathing or showering, and from inhalation of the volatilized compound (*144*). Humans may be exposed concurrently to the same carcinogenic substance via a number of different sources and media. For example, in considering the risk of EDB in grain, the New York Department of Health reasonably chose to sum the potential risks of the pesticide in food, ambient air (from use of unleaded gas), and drinking water (*145*).

Finally, an important distinction not conveyed by either table 1 or table 2 is that between voluntary and involuntary exposure. As discussed in the introduction, individuals are capable of voluntarily reducing exposure to substances in diet and cigarette smoke that have been identified as carcinogenic hazards. By contrast, individuals cannot feasibly control their exposure to air, water, and workplace pollution.

In tables 2 and 3, we have attempted to demonstrate the susceptibility of the HERP system (or any such simplified approach) to the effects of selection of both chemicals and exposure estimates. As is clear from table 3, the differences between tables 1 and 2 are largely because of these two factors. In contrast to that of Ames et al. (*2*), our approach, incorporating a representative set of exposures to the U.S.

[5]In fact, the results of a recent water consumption survey show that for 5% of adults 20–64 yr old, the average daily consumption of tap water is 2.71 L/day, whereas the average daily total water intake is 3.79 L (*66*).

Table 2. Ranking possible carcinogenic hazards with the use of the methodology of Ames et al. (2)*

Possible hazard (HERP %)	Carcinogenic exposure	Average daily carcinogen dose (70-kg adult)	Potency of carcinogen TD_{50} (mg/kg)	Comment†
	Man-made chemicals in foods/beverages			
0.02	Daminozide in treated apples and apple juice (1987)	20 μg	1.2	(1)
0.002	Daminozide in peanuts and peanut butter (1987)	1.9 μg	1.2	(1)
0.03	DBCP in treated carrots (preregulatory, 1976)	5.1 μg	0.24	(2)
0.003	DDT, DDD, and DDE in food (preregulatory, 1968–1969)	29.0 μg	13	(3)
0.0003	DDT, DDD, and DDE in food (postregulatory, (1980–1982)	2.3 μg	13	(3)
0.002	Dieldrin in food (preregulatory, 1968–1969)	1.5 μg	1.1	(4)
0.001	Dieldrin in food (postregulatory, 1980–1982)	1.1 μg	1.1	(4)
0.004	EDB in treated apples (preregulatory)	4.1 μg	1.5	(5)
0.0004	EDB in grain products (preregulatory, 1983)	0.42 μg	1.5	(5)
0.01	PCBs in food (preregulatory, 1971)	15 μg	1.7	(6)
0.0002	PCBs in food (postregulatory, 1980–1982)	0.2 μg	1.7	(6)
0.003	Sodium saccharin in diet soda (1977–1978)	4.9 ng	2,100	(7)
	Natural carcinogens in foods and beverages			
0.003	Aflatoxins in peanuts and peanut butter (1977)	5.8 ng	0.0026	(8)
<0.0001	Estragole in basil	<3.8 μg	52	(9)
1.6	Ethyl alcohol in beer (1981)	10.2 g	9,100	(10)
0.4	Ethyl alcohol in wine (1981)	2.7 g	9,100	(10)
1.3	Ethyl alcohol in hard liquor (1981)	8.1 g	9,100	(10)
0.01	Hydrazines in mushrooms (1977)	0.16 g	20,000	(11)
0.001	DMN in cured meat and bacon (1980)	0.12 μg	0.16	(12)
0.002	DEN in cured meat and bacon (1980)	0.034 μg	0.021	(12)
	Ambient air pollutants			
0.03	Benzene (Los Angeles, preregulatory, 1968)	1.0 mg	53	(13)
0.009	Benzene (Los Angeles, postregulatory, 1984)	0.32 mg	53	(13)
0.0005	Carbon tetrachloride (U.S. urban and surburban areas, 1973–1974)	48 μg	140	(14)
0.0004	Carbon tetrachloride (U.S. urban areas, 1980)	42 μg	140	(14)
0.0002	DDT (U.S. rural areas, preregulatory, 1972)	2.0 μg	13	(15)
0.00003	DDT (U.S. rural areas, postregulatory, 1974)	0.24 μg	13	(15)
0.004	EDB (U.S. urban areas, 1980–1981)	4.3 μg	1.5	(16)
1.8	Formaldehyde (Los Angeles, 1966)	1.9 mg	1.5	(17)
0.4	Formaldehyde (Los Angeles, 1979)	370 μg	1.5	(17)
0.002	PCBs (U.S. suburban areas, preregulatory, 1975)	2 μg	1.7	(18)
0.0001	PCBs (U.S. urban areas, postregulatory, 1979)	150 ng	1.7	(18)
0.003	Tetrachloroethylene (Bayonne, NJ, 1973)	220 μg	100	(19)
0.001	Tetrachloroethylene (Bayonne, NJ, 1983)	92 μg	100	(19)
0.001	Toxaphene (U.S. rural areas, 1972)	5.2 μg	5.8	(20)
	Indoor air pollutants			
0.005	Benzene (personal average, New Jersey, 1981)	173 μg	53	(21)
0.0002	Carbon tetrachloride (personal average, New Jersey, 1981)	16.2 μg	140	(22)
0.01	Chlordane (average in treated homes, 1976–1982)	20.5 μg	2.4	(23)
0.6	Formaldehyde in conventional homes (average of all reported U.S. data)	600 μg	1.5	(24)
2.1	Formaldehyde in mobile homes (U.S. average, 1984)	2.2 mg	1.5	(24)
0.02	Heptachlor (average in treated homes, 1982)	13.9 μg	1.2	(25)
0.001	Tetrachloroethylene (personal average, New Jersey, 1981)	80 μg	100	(26)
	Water pollutants			
0.0001	Chlordane (Kansas City drinking water, preregulatory, 1965–1967)	0.14 μg	2.4	(27)
0.003	Chloroform (average U.S. drinking water, 1976)	170 μg	90	(28)
0.01	DBCP (California, postregulatory, 1984)	2.0 μg	0.24	(29)
0.007	EDB (Florida, groundwater, 1983)	7.8 μg	1.5	(30)
0.03	Heptachlor (South Carolina rural drinking water, preregulatory, 1977)	24 μg	1.2	(31)
0.0003	PCBs (U.S. surface water, preregulatory, 1971–1974)	0.4 μg	1.7	(32)
0.0002	Tetrachloroethylene (New Jersey water supplies, 1985)	12 μg	100	(33)
0.00002	TCE (U.S. water supplies, 1985)	14 μg	940	(34)
0.0002	Vinylidine chloride (New Jersey water supplies, 1985)	4 μg	24	(35)

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Table 2. Continued

Possible hazard (HERP %)	Carcinogenic exposure	Average daily carcinogen dose (70-kg adult)	Potency of carcinogen TD_{50} (mg/kg)	Comment†
	Occupational exposures			
32.3	Benzene (rubber industry, preregulatory, 1942)	1.2 g	53	(36)
0.06	Benzene (rubber industry, postregulatory, 1980s)	2.4 mg	53	(36)
105.0	Formaldehyde (resin and paper manufacture, 1961)	110 mg	1.5	(37)
3.0	Formaldehyde (resin and plastic manufacture, 1980s)	3.2 mg	1.5	(37)
6.2	TCE (small factories, preregulatory, 1940s)	4.1 g	940	(38)
0.2	TCE (postregulatory, 1980s)	0.1 g	940	(38)

*The selection of chemicals and the estimates of exposure differ somewhat from those in Ames et al., as described in the text. To calculate average daily dose over an individual lifetime, we assumed: *a*) food consumption according to nationwide surveys; *b*) water consumption: 2 L/day; *c*) ambient air: inhalation of 20,000 L/day; *d*) indoor air: inhalation of 10,800 L/14-hr day; *e*) workplace air: inhalation of 9,600 L/day, 5 days/wk, 50 wk/yr, 40/70 yr (i.e., 3,768 L/day over an average lifetime) (*68*). For carcinogens listed as ambient and indoor air pollutants, the respective HERPs cannot be considered additive, since the 20,000 L/day may include both types of exposure. We also calculated exposure for a 70-kg male adult, although a 60-kg adult is more reasonable (*69*). When only a range of values was reported in the literature, their geometric mean was used as the average exposure. The HERP is derived by dividing the daily carcinogen dose by 70 kg to provide a milligram-per-kilogram value, which is then given as the percentage of the TD_{50} dose in the rodent (also in mg/kg).

†*See* appendix for comments.

Table 3. Comparison of possible carcinogenic hazards (HERPs) as estimated by Ames et al. and with the use of average exposure estimates*,†

Carcinogenic exposure	Ames et al. estimate		Our estimate, average exposure
	Average exposure	Worst-case exposure	
	Man-made chemicals in foods and beverages		
DDE/DDT in food			
Preregulatory	—	—	0.003
Postregulatory	0.0003	—	0.0002
EDB in grains	0.0004	—	0.0004
PCBs in food			
Preregulatory	—	—	0.01
Postregulatory	0.0002	—	0.0002
Sodium saccharin in diet sodas	—	0.06	0.003
	Natural carcinogens in foods and beverages		
Aflatoxins in peanuts and peanut butter	—	0.03	0.003
DMN in cured meat and bacon	—	0.003	0.001
DEN in cured meat and bacon	—	0.006	0.002
Estragole in basil	—	0.1	<0.0001
Ethyl alcohol in beer	—	2.8	1.6
Ethyl alcohol in wine	—	4.7	0.4
Hydrazines in mushrooms	—	0.1	0.01
	Ambient air pollutants		
Formaldehyde in conventional home air	0.6	—	0.6
Formaldehyde in mobile home air	2.1	—	2.1
	Water pollutants		
Chloroform in water	0.001	—	0.003
Tetrachloroethylene in water	—	0.0003	0.0002‡
TCE in water	—	0.004	0.00002§
	Occupational exposures		
Formaldehyde in workplace air	5.8	—	3.0

*DMN = *N*-nitrosodimethylamine, DEN = *N*-nitrosodiethylamine, and TCE = trichloroethylene.

†*See* tables 1 and 2 for details on daily carcinogenic dose and TD_{50}.

‡Worst-case assumption: HERP % = 0.007.

§Worst-case assumption: HERP % = 0.1.

population, shows that the selected man-made or industrial pollutants generally are comparable in terms of HERP scale to naturally occurring carcinogens in the diet. Because of the limitations in the HERP approach, however, we stress that regulatory agencies would be unwise to base public health policies principally on comparisons such as these.

Finally, Ames et al. (*2*) asserted that nine of the 26 carcinogens listed in table 1 "are thought to be nongenotoxic" and are therefore likely to have nonlinear dose-response curves or a decreased risk at lower dose. This subject has been discussed frequently (*146–149*). The general consensus on the part of regulatory agencies and expert groups has been that such policy distinctions are premature because they are supported inadequately by scientific data (*19,63,147,150,151*).

There are few, if any, clear-cut promoters and initiators; rather, there is evidence that under different conditions the same carcinogen can operate as a complete carcinogen, an initiator, or a promoter (*147*). For example, TCDD has demonstrated the ability to act both as a complete carcinogen and a promoter (*152–155*). It is just as difficult to distinguish between genotoxic and nongenotoxic agents because in most cases short-term tests for genetic toxicity have generated a mixture of positive and negative results. This phenomenon has been observed with a variety of chemicals regarded up to this time as model "epigenetic, late stage" carcinogens: asbestos, the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate, diethylstilbestrol, and DDT. These compounds have induced a variety of genetic effects, either indirectly or directly, in experimental systems or in humans (*149*).

The nine so-called nongenotoxic carcinogens in table 1 illustrate the difficulty of making such categorical distinctions. Although in most instances the majority of short-term test results have been negative, each of the compounds (with the exception of clofibrate) has tested positive in at least one assay for each of several different genetic toxicity end points

(*156*).[6] For eight of the nine chemicals, although the evidence of genetic toxic effects is generally limited, it cannot be dismissed. Therefore, it is not possible to conclude definitively that these are nongenotoxic carcinogens that do not act at some stage and under some conditions, either directly or indirectly, by damaging the genetic material. Viewed in a larger context, the proposed distinction among carcinogens on the basis of presumed mechanism or stage at which they act is belied by the observation that control of late-stage carcinogens that may not be genotoxic may lead to the most rapid reduction in risk, as has been seen with postmenopausal estrogen therapy and cigarette smoking (*158*).

In summary, there is no question that both occupational and food carcinogens are real concerns. However, while the hazards of the workplace are relatively well characterized (*68*), there is clearly a need for more research on dietary carcinogens. At the same time, there is evidence from epidemiologic, experimental, and monitoring sources that the cumulative risks of environmental pollution are important. Despite its limitations, the HERP analysis for a selection of exposures prevalent in the U.S. environment tends to support this conclusion. Although it is not possible to estimate the magnitude of these risks with certainty, it is prudent to continue to reduce involuntary exposures to carcinogens. The dramatic decrease in estimated cancer risk following regulation of a number of industrial chemicals illustrates this point. Controlling exposures to carcinogens such as those listed in table 2 has important side benefits in that many carcinogens are mutagenic, teratogenic, reproductive, or neurological toxicants (*36*).

[6]As summarized by the IARC, chloroform has been positive in only one of many assays for gene mutation in bacteria and has been largely negative in other systems used. However, it has tested positive in lower eukaryotic systems (inducing differential toxic effects in DNA repair-deficient strains, gene conversion, and/or recombination and reverse mutation). Trichloroethylene has been positive in a number of assays. It has induced genetic toxic effects in bacteria (mutation), in yeast (gene conversion or recombination and mutation), in *Tradescantia* (mutation), in rodent cells (transformation), in human cells in vitro [unscheduled DNA synthesis (UDS) and sister chromatid exchanges (SCEs)], in animals in vitro (DNA damage), and in animal cells in vivo (mutation and micronuclei). Tetrachloroethylene, PCBs, and DDT have been largely negative in assays for genetic toxic effects; however, for tetrachloroethylene there have been positive results in yeast (recombination or gene conversion), in *Tradescantia* (mutation), and in animal cells (transformation and DNA damage). PCBs have induced DNA damage in animal cells in vitro and UDS in rat primary hepatocytes. DDT has been positive in insect systems (dominant lethal mutation and aneuploidy), animal cells in vitro (chromosome aberrations), and animal cells in vivo (chromosome aberrations and dominant lethal mutation). Ethyl alcohol has been studied extensively; a significant number of positive results were found. Evidence of genetic toxic effects includes gene conversion or recombination, mutation, and aneuploidy in lower eukaryotic systems; SCE, micronuclei, and chromosome aberrations in plant systems; SCE, chromosome aberrations, and aneuploidy in animal cells in vitro; SCE, chromosome aberrations, micronuclei, dominant lethal mutations, and aneuploidy in animal cells in vivo; and SCE and chromosome aberrations in human cells in vivo. Alcoholism is associated with increased incidence of chromosome aberrations (*157*). For phenobarbitol, the preponderance of results has been negative. However, there is evidence for the induction of gene mutation in bacteria and aneuploidy in yeast; gene mutation, SCE, and chromosome aberrations in rodent cells in vitro; cell transformation in rodent cells; as well as gene mutation and chromosome aberrations in human cells in vitro. Clofibrate has only been tested in two assays, both of which were negative. Finally, sodium saccharin has evidence of genetic toxic effects in lower eukaryotic systems (gene conversion or recombination and mutation), in insects (mutation), in animal and human cells in vitro (SCE and chromosome aberrations), and in animals in vivo (SCE and mutation).

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APPENDIX: TABLE 2 COMMENTS

(1) Daminozide is a plant growth retardant. In 1986, the EPA adopted interim measures to reduce its use, and a ban has been proposed. The estimated residues are 0.86 ppm in peanuts, 0.47 ppm in peanut butter, 1.02 ppm in apples, and 0.38 ppm in apple juice (*70*). The estimated average adult daily consumption of these foods is: apples, 18.1 g; apple juice, 4.4 g; and peanuts and peanut butter, 2.9 g (*71*).

(2) 1,2,-Dibromo-3-chloropropane (DBCP), a soil fumigant for the control of nematodes, was widely used on a variety of vegetable, fruit, and nut crops until the ban in 1979. Concentrations were highest in root crops. The DBCP concentration in peeled raw carrots 9 wk after treatment at seeding was 0.607 mg/kg; the concentration of DBCP was 60% lower after 5 min of boiling in water (*72*). The average daily consumption of carrots is 8.4 g/day (*71*).

(3) The dose calculation for DDT, 1,1-dichloro-2,2′-bis(*p*-chlorophenyl)ethane (DDD), and DDE is based on FDA's Total Diet Study, which yielded estimates of daily intakes of 29 μg/day in 1968–1969 (*73*). DDD and DDE are the major metabolites of DDT and are therefore present mainly in meat, fish, and dairy products (*74*). The TD_{50}s of the three chemicals are similar. DDT was phased out from use on many food crops in the late 1960s and was banned in 1972. According to an FDA study on pesticide intake from the diet in 1980–1982, the average daily intake of DDT was <40 ng/day; for DDE it was 2.2 μg/day (*75*). The higher levels of DDE reflect the fact that long after the DDT ban, significant residues of DDT were available for metabolic conversion to DDE by animals used for food consumption.

(4) Dieldrin is a cyclodiene pesticide that was banned in 1974, as was aldrin, another pesticide that was oxidized in the environment to yield residues of dieldrin. The estimates of average daily intake of dieldrin are derived from Duggan and Corneliussen (*73*) and Gartell et al. (*75*).

(5) EDB residue (0.23 ppm) was estimated 12 days after fumigation, the average interval between spraying and consumption (*76*). Daily apple consumption is estimated at 18 g/day for a 70-kg adult (*71*). Citrus fruits, plums, peaches, and cherries were also fumigated with EDB prior to its being banned for this use in 1984. In 1983, EPA estimated the average adult consumption of EDB in grain products at 0.006 μg/kg/day (*77*). The EDB residues in these foods are lower than in fruits, reflecting the relatively long interval between fumigation and consumption of grain and grain products. Fumigation of grains with EDB was banned in 1984.

(6) The dose calculation is based on FDA's Total Diet Study, which yielded estimates of average daily PCB intake of 15.0 μg/day in 1971, falling to 8.7 μg/day in 1975 (*78*). Although PCBs were used in pesticide manufacture, the main source of dietary exposure was the widespread environmental contamination from industrial uses. Fish, cheese, and eggs were the foods most significantly contaminated with PCBs. A number of regulatory and other actions were taken in 1971–1972 to reduce food contamination, and PCBs were banned from use in nonclosed systems in 1979. Although manufacture was discontinued in 1977, residues persist in the environment. The 1980–1982 estimate of daily intake was 0.196 μg (*75*).

(7) Consumption of saccharin increased substantially after cyclamates were banned in 1970. In recent years, other artificial sweeteners have become more popular. In 1976, an estimated 45% of all saccharin consumed in the United States was in soft drinks (*79*). A U.S. Department of Agriculture nutritional survey in 1977–1978 reported an average intake of artificially sweetened soft drinks at 14.7 g/day for males aged 19 and over (*80*). The average concentration of sodium saccharin is 33.5 mg/100 g of diet soda (*79*). A survey during 1977–1978 indicated an average daily intake of saccharin from all sources of 9.24 mg for males aged 18 and over (*81*).

(8) Aflatoxins are substances produced by molds that contaminate nuts, cereals, and other foods. Although they are naturally occurring, their concentration is inversely proportional to the quality of food storage techniques. Thus, they are, in part, man-made carcinogens, and exposures can be appreciably controlled by improvements in commercial processes. The average consumption of peanuts and peanut butter is 2.9 g/day (*71*), and the U.S. average concentration of aflatoxins in peanuts and peanut butter is assumed to be 2 ppb (*82,83*).

(9) The average concentration of estragole in dried basil is 3.8 mg/g (*84*). The EPA reports "zero" average daily consumption of basil (*71*). However, since EPA reports no daily intake <1 mg, we are assuming basil intake to be <1 mg. Other herbs (anise, fennel, bay, and marjoram) are considered to be the most important sources of estragole. They are also reported to have zero average daily consumption in the United States (*71*).

(10) Ethyl alcohol is the best known human dietary carcinogen. It has been associated with cancers of the oral cavity, larynx, esophagus, and liver (*85*). Health and societal effects of alcohol consumption other than carcinogenesis are more important in terms of mortality, morbidity, and costs. Valid estimates of alcoholic beverage consumption are difficult to obtain. Data from household surveys, estimated consumption based on tax revenues, and estimates by producers do not cover all aspects of drinking patterns. The dose calculation is based on estimates by the Distilled Spirits Council of the United States and by the Economic Department of the Wine Institute (*86*): 116.56 L/yr of beer (3.2% alcohol by weight), 10.36 L/yr of wine (9.6% alcohol by weight), and 9.26 L/yr of hard liquor (32% alcohol by weight).

(11) The TD_{50} is derived from data on dry *Agaricus bisporus* (*2,87*). The average daily consumption of mushrooms has been estimated as 1.6 g (*71*). The HERP is calculated assuming 90% of mushroom weight to be water and that all eaten mushrooms have the carcinogenic potency of *A. bisporus*, the most commonly eaten mushroom in the United States (*88*). No data are available concerning possible transformation of hydrazines, the principal carcinogens, during the cooking process.

(12) N-nitrosamines constitute an important group of carcinogens formed by the reaction of amines with a nitrosating agent, usually nitrous acid. Human exposure to nitrosamines has a complex pattern with four major aspects: *a*) endogenous formation of precursors (from saliva and gastric juice) and endogenous formation of N-nitrosamines, *b*) endogenous formation after uptake of precursors (mainly nitrite and nitrate in foods), *c*) uptake of N-nitrosamines present in food, and *d*) uptake from other sources (tobacco smoke, drugs, cosmetics, and various occupations) (*89*). It has been estimated that 94% of exposure to *N*-nitrosodimethylamine (DMN) is from exogenous sources (*90*). N-nitrosamines are present in cooked bacon, mainly from the frying process, whereas they occur in cured meat from added nitrate (*89,91*). The DMN content of beer, formerly high, has been significantly reduced through technological changes. Levels of N-nitrosamines in cured meat are also decreasing over time. The estimated daily intake is based on average concentrations in both cured meat and bacon of 3.4 μg/kg (DMN) and 1 μg/kg [*N*-nitrosodiethylamine (DEN)] (*91*) and a total consumption of 34 g/day (bacon and cured meat) (*92*). Daily intake of DEN from meat in the Federal Republic of Germany in 1976 was estimated to be 0.1 μg/day (*89*).

(13) Benzene is mainly used as an intermediate in the synthesis of numerous chemicals, as a solvent, and as a gasoline component. The major source of ambient air concentration is from gasoline, both from refueling and motor operation (*93*). The average concentration of benzene in Los Angeles ambient air in 1968 was 0.05 mg/m^3 (*94*), corresponding to a daily intake of 1.0 mg. In 1980, the average intake from the ambient air of a number of U.S. cities was estimated to be 0.6 mg/day (*95*). The 1984 Los Angeles estimate is 16 μg/m^3 (*96*). The average 1979–1982 concentration from five urban areas was 12.3 μg/m^3 (*25*).

(14) Carbon tetrachloride is used as a solvent and in the production of freons. It was used as a grain fumigant until banned for that use in 1985. The major sources of ambient air contamination are solvent emissions; the use of carbon tetrachloride is declining because of the concern about its toxic effects (*97*). The mean of eight measurements in urban and suburban areas in the Northeastern United States in the early 1970s was 2.4 μg/m^3 (*98*). Other measurements during the same period ranged between 0.45 and 9.1 μg/m^3 (*99–101*). A decade later, the average concentration in five urban areas (2.1 μg/m^3) was comparable (*25*).

(15) The estimates derive from a 1972–1974 ambient air survey in the Mississippi Delta area where DDT was widely used on cotton crops until its ban in January of 1973. The average concentrations were 0.1 μg/m^3 in 1972 and 0.012 μg/m^3 in 1974 (*102*). A previous estimate from nine urban and agricultural areas in the United States had an upper estimate of 1.56 μg/m^3, corresponding to a HERP% of 0.003 (*103*).

(16) The average ambient air concentration of EDB in 10 U.S. cities was 213 ng/m^3 (*104,105*), largely from its use as a scavenger in leaded gas. Regulations to reduce the use of leaded gas have been in effect since 1975.

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(17) Formaldehyde is an important industrial chemical used as an intermediate in the production of resins for the construction, automotive, and appliance industries. It is also used in the manufacture of fungicides and bactericides, various textiles, paper, and a number of consumer products, such as cosmetics, air fresheners, and drugs (*93*). Formaldehyde is a component of tobacco smoke. There is no major environmental (other than occupational) regulation of formaldehyde on the basis of its carcinogenicity. Data from 1966 are derived from a survey that showed a range of daily average concentrations in Los Angeles ambient air between 60 and 148 $\mu g/m^3$ (geometric mean, 94 $\mu g/m^3$) (*106*). Other estimates in the same period were similar (*107*). The 1979 estimate (18.5 $\mu g/m^3$) is considerably lower (*108*). Other investigators have also reported a decline in formaldehyde levels over time (*109*).

(18) The average ambient air concentration of PCBs in suburban areas of Florida, Mississippi, and Colorado in 1975 was 100 ng/m^3 (*110*). The most significant reduction in PCB air concentrations followed the production ban in 1979. The average urban air concentration in 1979 was estimated to be 5–10 $\mu g/m^3$ (*111*); the HERP is based on the midpoint. A 1979 study in Minneapolis reported an average ambient air concentration of 7.1 $\mu g/m^3$ (*112*).

(19) Tetrachloroethylene is used largely in the textile (processing and finishing) and dry cleaning industries, in vapor degreasing and cold cleaning of metals, and as an intermediate in the chemical industry. In 1975, it constituted >65% of the total dry cleaning solvent usage (*113*). Both exposure estimates refer to Bayonne, NJ, ambient air. The average daily ambient concentrations were 11.1 $\mu g/m^3$ in 1973 (*98*) and 4.6 $\mu g/m^3$ in 1983 (*23*). Studies of other urban areas reported similar average daily concentrations: Los Angeles 1976, 4.6 $\mu g/m^3$ (*114*); Baltimore 1978–1983, 4.0 $\mu g/m^3$; and Philadelphia 1979–1982, 5.9 $\mu g/m^3$ (*25*).

(20) Use of toxaphene has increased in the last decade, partly because of the ban on DDT. It is mainly used for the control of cotton insect pests. The average concentration measured in 1972 in the Mississippi Delta area was 0.26 $\mu g/m^3$ (*102*).

(21) The dose calculation for benzene is based on personal air samplers carried by ≈600 New Jersey residents in the fall of 1981. These samplers would have integrated outdoor and indoor exposure, but in all cases outdoor concentrations were lower than personal average concentrations; hence, the personal samples would have underestimated indoor exposure. Tobacco smoke was thought to be the main source of indoor concentrations of benzene (*23*).

(22) The dose calculation for carbon tetrachloride is based on personal air sampling in New Jersey (*23*). *See* comment 21.

(23) Chlordane and heptachlor are chlorinated cyclodiene insecticides, structurally similar to dieldrin. Chlordane was widely applied to the soil below and around the foundations of buildings to control subterranean termites until this use was terminated by agreement with the EPA in 1987. The dose calculation is based on a survey of military housing (*115*). Data for nonmilitary housing in the same period suggested higher average doses of 28–48 μg/day (*116,117*). Chlordane is a complex mixture. The methods of measurement used in the cited studies would not have included the more volatile fractions and hence would have underestimated total exposures. Local data for 1983–1985 suggest a substantial reduction in exposure levels, perhaps resulting from improved application techniques introduced in the 1980s (*118*).

(24) The release of formaldehyde from urea-formaldehyde foam insulation in mobile homes as well as in conventional homes containing particle board and plywood are the most important sources of indoor exposure to formaldehyde, but it has widespread use in construction materials, wood products, and furniture, textiles, and paper. Sources of indoor contamination for benzene include tobacco smoke and solvents (*93*). The estimate of average daily uptake of formaldehyde in the air in conventional homes is based on the means of all reported median or mean concentrations, as estimated by Ames et al. (*2*). The mean concentration of formaldehyde in the air of mobile homes (0.205 mg/m^3) is derived from Connor et al. (*119*).

(25) Heptachlor is a component of technical chlordane (5.8%) and is often applied with it in a 1:2 mixture. The dose calculation is based on measurements in homes treated with either this mixture or with technical chlordane (*116*). Local data for 1983–1985 suggest a substantial reduction in exposure levels, perhaps resulting from improved application techniques introduced in the 1980s (*118*).

(26) The dose calculation for tetrachloroethylene is based on personal air sampling in New Jersey (*see* comment 21). Dry-cleaned clothes were thought to be a source of indoor concentrations of tetrachloroethylene (*23*).

(27) Chlordane was used on many commercial fruit, vegetable, and grain crops, as well as in private gardening. Chlordane was detected in 21 out of 50 samples of Kansas City drinking water between 1965 and 1967 (*120*). Because only concentrations higher than 0.1 $\mu g/L$ were listed separately in this study, a level of 0.05 $\mu g/L$ was assumed for the remaining positive samples; the average (0.07 $\mu g/L$) is used in the table. The maximum chlordane concentration reported in the same study was 8 $\mu g/L$ (HERP%, 0.01).

(28) The average drinking water concentration of chloroform estimated in a 1976 nationwide study of 113 public water systems was 84 $\mu g/L$ (*121*). It is mainly formed through the reaction of chlorine with organic chemicals in water (*122*). The risk posed by chloroform must be compared with the benefits of chlorination, and its benefits should be compared with those of other water-disinfection technologies.

(29) DBCP concentrations in positive groundwater samples from five states in the 1980s ranged between 0.02 and 20 $\mu g/L$ (*123*). The upper value corresponds to a HERP% of 0.2. A concentration of 137 $\mu g/L$ (HERP% 1.6) has been reported for a single polluted well in Arizona (*10*). Monitoring of wells in both small domestic and large water systems in California showed that ≈30% were contaminated with DBCP. The majority had concentrations >1 $\mu g/L$; hence this value is used in the table as an average estimate (*124*).

(30) An EPA groundwater EDB contamination study in four states found >100 contaminated wells. Eighty-six of them were in Florida; the concentration ranged between 1.0 and 15.0 $\mu g/L$. The HERP is based on the geometric mean (3.9 $\mu g/L$). The highest concentration in this study was 100 $\mu g/L$, corresponding to a HERP% of 0.2, and was found in Georgia (*125*).

(31) Before being banned in 1983, heptachlor was mainly used to treat corn crops and seeds. A 1977 study of drinking water in two U.S. rural counties showed mean levels of heptachlor in positive samples of 15 and 9 $\mu g/L$, respectively (*126*). The HERP is based on the average value of 12 $\mu g/L$.

(32) The estimated surface water concentration of PCBs in 17 major U.S. drainage basins during 1971–1974 was 0.1–3.0 $\mu g/L$ (*127*). The HERP is based on the average of positive results for 1974: 0.2 $\mu g/L$.

(33) The median concentration of tetrachloroethylene in NJ public water supplies that tested positive from July to December of 1985 was 6 $\mu g/L$; maximum concentration was 46 $\mu g/L$ (*128*) (HERP%, 0.001). Concentrations as high as 1,500 $\mu g/L$ (HERP%, 0.04) have been detected in polluted wells (*10*).

(34) Trichloroethylene (TCE) is used in the vapor degreasing of lubricated metal parts, as a solvent in the textile, adhesive, and lubricants industry, as well as in some consumer cleaning products. In 1977, FDA prohibited its use as an extraction solvent in the food (coffee and spices) industry (*129*). The major source of environmental contamination by TCE is the metal industry (*129*). The median contamination of TCE in New Jersey public water supplies that tested positive from July to December 1985 was 7 $\mu g/L$ (*128*). The same study showed a maximum concentration of 190 $\mu g/L$ (HERP%, 0.0006). Levels as high as 35,000 $\mu g/L$ (HERP%, 0.1) have also been found in contaminated private drinking water supply wells (*130*).

(35) In the New Jersey study described in comment 33, the median concentration of vinylidine chloride was 2 $\mu g/L$, and the maximum was 9 $\mu g/L$ (HERP%, 0.001). Vinylidene chloride is the basis for the production of copolymers, which are used in food packaging, coatings for paper, fibers, tubes, and pipes (*131*). Vinyl chloride, whose polymers are also used in the production of plastics, is a known human and animal carcinogen and is strictly regulated both in the workplace and in the environment. It has been found in polluted wells at concentrations as high as 20 $\mu g/L$ (*132*) and 10 $\mu g/L$ (*128*). Inasmuch as the TD_{50} is 8.0 mg/kg, the respective HERP% values are 0.007 and 0.004.

(36) Concentrations of 100 ppm of benzene or more were common in the rubber, printing, leather, and shoe industries until the 1970s (*93,133*). The 1942 exposure corresponded to 100 ppm. In 1980, a permissible exposure limit (PEL) of 100 ppm was issued by OSHA; today the limit is 1 ppm or 3.2 mg/m^3. The estimated average postregulatory benzene concentration in the rubber industry is 0.20 ppm (*134*).

(37) The estimated workplace air concentration (28.3 mg/m^3) is the midpoint of values obtained in a 1961 U.S. study on resin manufacture and paper production (*135,136*). In 1980, OSHA set the PEL at 3.7 mg/m^3 (HERP%, 13.3) on the basis of noncarcinogenic effects. In 1987, it was lowered to 1.2 mg/m^3 (HERP%, 4.3). Estimates of the concentration of formaldehyde (0.86 mg/m^3) in the resin and plastic industry during the 1980s indicates a 33-fold decrease (*137–139*).

(38) The HERP is based on an estimated workplace concentration of 200 ppm, which was not uncommon before regulations were imposed (*140*). Concentrations as high as 8,000 ppm, corresponding to a HERP% of 256, have also been measured (*141*). Today, the OSHA PEL is 100 ppm or 538 mg/m^3 (HERP% 3.2), and the HERP is based on an estimated workplace concentration of 5 ppm (*142*).

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standards of scientific validity without significant improvements in the state of the science. Thus, a tension was established, which exists to this day, between the need to act and the availability of knowledge.

Even at the outset as the environmentalist idea moved, in Winston Churchill's phrase, "from the wonderful cloudland of aspiration to the ugly scaffolding of attempt and achievement," it confronted a problem that would plague EPA henceforth: the basic disjunction between the reality of science and the assumption, inherent in the mission of all governmental protective agencies, that it is always possible, and therefore obligatory, to provide swift and sure regulation of all substances and situations that threaten public health and the environment.

This disjunction is hardly ever acknowledged by the general public, so completely has science been accepted as the handmaiden of its material desires. The relationships among basic science, applied science, and improvements in daily life are usually regarded as simple, being much like those among growing trees, cutting lumber, and building houses. This concept feeds the notion that when we want something from science, we can order it, as we order lumber to build a house. If there is not enough lumber, we can grow and cut more trees. It follows that there is a way to "manage" this orderly process so as to make it more efficient, or more suitable to our current needs.

Science is only orderly after the fact; in process it is chaotic and fiercely controversial.

Even though a scientific explanation may appear to be a model of rational order, we should not infer from that order that the genesis of the explanation was itself orderly. Science is only orderly after the fact; in process, and especially at the advancing edge of some field, it is chaotic and fiercely controversial. Thus, the expectation built into environmental law, that science can provide definitive answers to the kinds of questions that policymakers are obliged to ask under the terms of that law, will be disappointed to the degree that such answers derive from the forward edge of research.

We can direct our resources in various directions with the expectation that talented researchers will work on questions that bear on important policy issues, but we cannot order answers as efficiently as the public would like. As Louis Pasteur said, "There is no such thing as applied science, there are only applications of science." Even in those scientific establishments specifically funded to explore policy questions for federal protective agencies, we find scientists behaving rather more like scientists in "basic" research than their budget justifications would suggest.

Nor can we order a consensus in the areas of greatest interest to environmental policy: pollutant exposure and effects. Policymakers, including me, have often deplored the tendency of scientific panels to engage in interminable debate rather than reach the agreement that was clearly indicated on the invitation. *Of course* scientists will disagree on issues involving the advancing edge of research: that is what they do for a living. And even if we could somehow get a group of scientists to endorse a consensus position, it would be, in the first place, only tentative and subject to revision with the arrival of new discoveries; and in the second place, it may be entirely wrong.

In science, the majority does not rule, as the history of science amply demonstrates. Everybody but Semmelweiss was wrong on childbed fever. Everybody but Wegener was wrong on continental drift. Scientists outside the

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consensus, who may not be averse to voicing their objections in public, may be cranks or they may be right. Public officials cannot make this distinction; only the slow mills of science can grind out the truth.

Public officials, though, do not have that kind of time. From its earliest days EPA was often compelled to *act under conditions of substantial scientific uncertainty*. The full implications of this problem were partially masked at the beginning because the kind of pollution we were trying to control was so blatant. Although scientists were often in the forefront of the early struggles against pollutants, most people did not need a scientific panel to tell them that air is not supposed to be brown, that streams are not supposed to ignite and stink, that beaches are not supposed to be covered with raw sewage. When I left EPA in 1973, the means for ending the worst forms of pollution were fairly well in hand. If emission controls were put on cars, if the requisite sewage treatment plants were built, and if industrial firms installed readily available control technologies, pollution would be essentially eliminated as a national problem. While I had some reservations about the scientific basis of some of the criteria we had established for health protection and about the ability of industry to meet congressional deadlines, and while we were probably not protecting as efficiently as we might have, all in all it did not seem to matter much. I was certain that Congress would adjust the more obvious infirmities in our environmental laws once it realized the social and economic distortion inherent in pursuing zero-risk environmental goals. We had made a start and had demonstrated that the federal government could move effectively against major polluters in such a way that the general public could literally see through its windows.

IV

The events of the past dozen or so years have demonstrated that my opinion was in one sense quite correct and in another far too sanguine. Many of the grosser sorts of pollution are indeed under control. But the level of controversy about environmental protection has not diminished. On the evidence of my more recent experience as administrator of EPA, as indicated by what regulatory issues were of greatest concern, what the various congressional committees were interested in hearing about, press coverage, and so on, environmental controversy is now largely focused on the carcinogenic risk to human health from toxic chemicals, and on the removal of ever smaller increments of conventional pollution from the air and water.

"Risk" is the key concept here. It was hardly mentioned in the early years of EPA, and it does not have an important place in the Clean Air or Clean Water Acts passed in that period. Of the events that contributed to this change, the most important were the focus of public attention on PCBs and asbestos (two substances that are ubiquitous in the American environment and that are capable of causing cancer) and the realization that exposure to a very large number of unfamiliar and largely untested chemicals is universal. The discovery by cancer epidemiologists that cancer rates vary with environment suggested that pollution might play a role in causing this disease. And finally, the cancer risk was pushed to the forefront by the emergence of abandoned

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dumps of toxic chemicals as a consuming public issue. As a direct result of this shift in attention, the relation of EPA to its science base was altered; the problem of uncertainty was moved from the periphery to the center.

This shift occurred because the risks of effects from typical environmental exposures to toxic substances—unlike the touchable, visible, and malodorous pollution that stimulated the initial environmental revolution—are largely constructs or projections based on scientific findings. We would know nothing at all about chronic risk attributable to most toxic substances if scientists had not detected and evaluated them. Our response to such risks, therefore, must be based on a set of scientific findings. Science, however, is hardly ever unambiguous or unanimous, especially when the data on which definitive science must be founded scarcely exist. The toxic effects on health of many of the chemicals EPA considers for regulation fall into this class.

"Risk assessment" is the device that government agencies such as EPA have adopted to deal with this quandary. It is the attempt to quantify the degree of hazard that might result from human activities—for example, the risks to human health and the environment from industrial chemicals. Essentially, it is a kind of pretense; to avoid the paralysis of protective action that would result from waiting for "definitive" data, we assume that we have greater knowledge than scientists actually possess and make decisions based on those assumptions.

Of course, not all risk assessment is on the controversial outer edge of science. We have been looking at the phenomenon of toxic risk from environmental levels of chemicals for a number of years, and as evidence has accumulated for certain chemicals, controversy has diminished and consensus among scientists has become easier to obtain. For other substances—and these are the ones that naturally figure most prominently in public debate—the data remain ambiguous.

In such cases, risk assessment is something of an intellectual orphan. Scientists are uncomfortable with it when the method must use scientific information in a way that is outside the normal constraints of science. They are encroaching on political judgments and they know it. As Alvin Weinberg has written:

> Attempts to deal with social problems through the procedures of science hang on the answers to questions that can be asked of science and yet which cannot be answered by science. I propose the term *trans-scientific* for these questions. . . . Scientists have no monopoly on wisdom where this kind of trans-science is involved; they shall have to accommodate the will of the public and its representatives.

However, the representatives of the public, in this instance policy officials in protective agencies, have their problems with risk assessment as well. The very act of quantifying risk tends to reify dreaded outcomes in the public mind and may make it more difficult to gain public acceptance for policy decisions or push those decisions in unwise directions. It is hard to describe,

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say, one cancer case in 70 years among a population of a million as an "acceptable risk" when such a description may too easily summon up for any individual the image of some close relative on his deathbed. Also, the use of risk assessment as a policy basis inevitably provokes endless arguments about the validity of the estimates, which can seriously disrupt the regulatory timetables such officials must live by.

Despite this uneasiness, there appears to be no substitute for risk assessment, in that some sort of risk finding is what tells us that there is any basis for regulatory action in the first place. The alternative to not performing risk assessment is to adopt a policy of either reducing all *potentially* toxic emissions to the greatest degree technology allows (of which more later) or banning all substances for which there is any evidence of harmful effect, a policy that no technological society could long survive. Beyond that, risk assessment is an irreplaceable tool for setting priorities among the tens of thousands of substances that could be subjects of control actions—substances that vary enormously in their apparent potential for causing disease. In my view, therefore, we must use and improve risk assessment with full recognition of its current shortcomings.

In my view, we must use and improve risk assessment with full recognition of its current shortcomings.

This accommodation would be much easier from a public policy viewpoint were it possible to establish for all pollutants the environmental levels that present zero risk. This is prevented, however, by an important limitation of the current technique; the difficulty of establishing definitive no-effect levels for exposure to most carcinogens. Consequently, whenever there is any exposure to such substances, there is a calculable risk of disease. The environmentalist ethos, which is reflected in many of our environmental laws, and which requires that zero-risk levels of pollutant exposure be established, is thus shown to be an impossible goal for an industrial society, as long as we retain the no-threshold model for carcinogenesis.

V

This situation has given rise to two conflicting viewpoints on protection. The first, usually proffered by the regulated community, argues that regulation ought not to be based on a set of unprovable assumptions, but only on connections between pollutants and health effects that can be demonstrated under the canons of science in the strict sense. It points out that for the vast majority of chemical species, we have no evidence at all that suggests effects on human health from exposures at environmental levels. Because many important risk assessments are based on assumptions that are scientifically untestable, the method is too susceptible to manipulation for political ends and, the regulated community contends, it has been so manipulated by environmentalists.

The second viewpoint, which has been adopted by some environmentalists, counters that waiting for firm evidence of human health effects amounts to using the nation's people as guinea pigs, and that is morally unacceptable. It proposes that far from overestimating the risks from toxic substances, conventional risk assessments underestimate them, for there may be effects from chemicals in combination that are greater than would be expected from the

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sum effects of all chemicals acting independently. While approving of risk assessment as a priority-setting tool, this viewpoint rejects the idea that we can use risk assessment to distinguish between "significant" and "insignificant" risks. Any identifiable risk ought to be eliminated up to the capacity of available technology to do so.

It is impossible to evaluate the merits of these positions without first drawing a distinction between the assessment of risk and the process of deciding what to do about it, which is "risk management." The arguments in the form sketched here are really directed at both these processes, a common confusion that has long stood in the way of sensible policymaking.

Risk assessment is an exercise that combines available data on a substance's potency in causing adverse health effects with information about likely human exposure, and through the use of plausible assumptions, it generates an estimate of human health risk. Risk management is the process by which a protective agency decides what action to take in the face of such estimates. Ideally the action is based on such factors as the goals of public health and environmental protection, relevant legislation, legal precedent, and application of social, economic, and political values. *Risk Assessment in the Federal Government*, a National Research Council (NRC) document, recommends that regulatory agencies establish a strict distinction between the two processes, to allay any confusion between them. In my view Congress should do the same in all statutes seeking to deal with risk.

Returning now to the opposing viewpoints we see that both reflect the fear that risk assessment may be imbued with values repugnant to one or more of the parties involved. That is, some people in the regulated community believe that the structure of risk assessment inherently exaggerates risk, while many environmentalists believe that it will not capture all the risk that may actually exist. As we have seen, this disagreement is not resolvable in the short run through recourse to science. Risk assessment is necessarily dependent on choices made among a host of assumptions, and these choices will inevitably be affected by the values of the choosers, whether they be scientists, civil servants, or politicians.

The NRC report suggests that this problem can be substantially alleviated by the establishment of formal public rules guiding the necessary inferences and assumptions. These rules should be based on the best available information concerning the underlying scientific mechanisms. Adoption of such guidelines reduces the possibility that an EPA administrator may manipulate the findings of some risk assessment so as to avoid making the difficult, and perhaps politically unpopular, choices involved in a risk-management decision. Both industry and environmentalists fear this manipulation—from different brands of administrator, needless to say. Although we cannot remove values from risk assessment, we can and should keep those values from shifting arbitrarily with the political winds.

The explicit and open codification suggested by the NRC will also ensure that the assumptions used in risk assessment will at least be uniform among all agencies that adopt them, will be plausible scientifically, and will reflect a predictable and relatively constant policy amid this complex and chaotic hybrid discipline. It also offers the possibility that one day all the protective

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agencies of government will speak with one voice when they address risks, so that estimates of risk will be comparable among agencies and the public at last will be able to make a fair comparison of the individual risk-management decisions of separate agencies.

The remaining points of both positions are really about risk management and on this issue both are flawed. At its extreme, the first position—that regulation should be based solely on scientifically provable connections between pollutants and health effects—would allow the release of unlimited quantities of substances that cause cancer in animals, on the assumption that there will be no analogous effect on people and that there must be thresholds for carcinogenesis. I expect that most Americans would reject that assumption as imprudent, given our current knowledge about carcinogenesis (for example, the similarity of cancer-causing genes across species). At some level we have to regard the possibility that we are controlling somewhat in excess of the true risk as a kind of insurance, with the cost of control as its premium. The effort to reduce apprehension, even so-called unreasonable apprehension, about the future results of current practices is a valid social function. Risk-management agencies such as EPA could be chartered to do precisely that. If so, we had better make clear what we are doing, and establish rules for doing it.

In confronting any risk there is no way to escape the question "Is controlling it worth it?"

The weakness of the second viewpoint, that any identifiable risk ought to be eliminated up to the capacity of available technology to do so, lies in the concept of a best available technology that must invariably be applied where risk is discovered. "Best" and "available" are terms as infinitely debatable as the assumptions of risk assessment. There is always a technology conceivable that is an improvement on a previous one, and as the last increments of pollution are removed, the cost of each successive fix goes up very steeply. Because, according to the no-threshold assumption, even minute quantities of carcinogens can be projected out to cause cases of disease, arguments about technology reduce in the end to arguments about risk and cost: technology A allows a residual risk of 10^{-5} and costs $1 million; technology B allows a residual risk of 10^{-6} and costs $10 million, and so on ad infinitum. It is specious to pretend that costs do not matter, because it is always possible to show that at a certain level of removal, costs in fact do matter: technology Z allows residual risks of 10^{-15} and costs $1 trillion.

Once this is admitted, as it almost always is when we come down to debating actual regulations, the position is reduced to arguments about affordability. This too is treacherous ground. Firms vary in their ability to pay, and what is affordable for one may bankrupt another. If requirements are adjusted so as not to cripple the poorest firms, the policy amounts to an environmental subsidy to the less efficient players in our economy. In the end, discussions about available technology and affordability develop into discussions of corporate management: "If they can't afford pollution control, how come their executives got such a big bonus?" This may be a fascinating topic to some, but it distracts from the main issue.

My point is that in confronting any risk there is no way to escape the question "Is controlling it worth it?" We must ask this question not only in terms of the relationship of the risk reduced and the cost to the economy but also as it applies to the resources of the agency involved. Policy attention is the

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most precious commodity in government, and a regulation that marginally protects only 20 people may take up as much attention as a regulation that surely protects a million.

"Is it worth it?" That this question must be asked and asked carefully is a token of how the main force of the environmental idea has been modified by the recent focus on toxic risk to human health. In truth this question should always have been asked, but because the early goals of environmentalism were so obviously good, the requirement to ask, "Is it worth it?" was not firmly built into all our environmental laws. Who would dare to question the worth of saving Lake Erie? Environmentalism at its inception was a grand vision, one that nearly all Americans willingly shared. Somehow that vision of the essential unity of nature and of the need for bringing industrial society into harmony with it has been lost among the parts per billion, and with it we have lost the capacity to reach social consensus on environmental policy.

That vision of the essential unity of nature has been lost, and with it the capacity to reach social consensus on environmental policy.

VI

Why has this happened? I believe it is because environmentalism, like many another social movement, is suffering from the excesses of its own youthful vigor. In the early legislation promises were made that could not reasonably be kept; expectations were raised that were bound to be disappointed. A case in point is the language of Section 112 of the Clean Air Act, which requires that EPA establish "an ample margin of safety" in the control of hazardous air pollutants. We can achieve this margin only when it is possible to determine a level at which there are no apparent effects on humans, as we may be able to with such substances as carbon monoxide and nitrogen oxides. It then becomes possible to roll that level back to one presumed to protect the most sensitive individuals in the population, which is, of course, the point of the "ample margin" language. Most of the air pollutants now under consideration, however, are suspected carcinogens. Because, as already noted here, we have assumed that it is impossible to establish no-effect levels for carcinogens, we cannot establish the margin of safety demanded by the law without banning these substances or the processes that produce them, and such action is nearly always unwise for social and economic reasons.

The Clean Air Act promised absolute protection from airborne carcinogens, but we cannot keep that promise. The American people were also promised under the clean air and water laws that the air would be pure and water pollution would be eliminated by a specific date; we have not kept those promises either. Few things are more corrupting to a free society such as ours than grand promises unfulfilled. Senator Daniel Patrick Moynihan (D-N.Y.) has written of this:

> The malaise of overpromising derives almost wholly, in my experience, from the failure of executives and legislators to understand what is *risked* when promises are made.... When things don't work out as promised it is all too easy to suspect that someone *intended* they should not.

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Many of the promises made or implied by federal law as part of the triumph of environmentalism have indeed had this sad effect. Since the beginning of EPA, there has been insufficient appreciation of the difficulties involved in the successive tasks the agency has been assigned by Congress. As a result many people on Capitol Hill assume that if unrealistic promises have not been kept, some people are not doing their jobs—and that the way to ensure that they do is to write ever more stringent prescriptions into environmental law, with appropriate deadlines. Thus more promises are added to unrealistic promises, and the cycle of disappointment and growing mistrust is accelerated.

The foregoing should in no way be taken as implying that the control of toxic substances is unimportant or that EPA could not be better managed. My point is that EPA will not do an effective job in either case unless we realize that the debate about risk in its present form leads us ever deeper into a blind alley. We must revisit that crucial stage of problem solving in government at which one defines what kind of problem it is. We will emerge from the blind alley and reforge a practical consensus on the environment only when we are able to redefine the problem of environmental protection as being "the management of risk."

When we adopt this definition, we specifically will abandon the impossible goal of perfect security and accept the responsibility for making difficult and painful choices among competing goods. We can then move forward from the present impasse and begin to look at a more productive set of questions.

VII

What then is risk management? As management in general can be defined as the distribution of current resources to shape some desirable future state, risk management in its broadest sense means adjusting our environmental policies to obtain the array of social goods—environmental, health-related, social, economic, and psychological—that forms our vision of how we want the world to be.

In practical terms, risk management means giving the protective agencies flexibility comparable to that which managers have traditionally exercised in other spheres. This suggestion, of course, runs counter to the inclination to further restrict the discretion accorded those agencies. Administrations do vary in their priorities and competence, so how can we ensure that this flexibility will not be misused? Obviously, flexibility cannot be taken to mean carte blanche for the agencies. Flexibility in risk management must be bounded by evidentiary rules of the type described in the NRC report, as well as by rules to assess the adequacy and competence of information. Most important, flexibility should be limited by broad public acceptance, including acceptance by individuals subject to risk, and also by more sensible and appropriate congressional oversight.

It may be argued that the administrator of EPA already has sufficient flexibility. Did we not, for example, attempt to get around the impossible standard in Section 112 of the Clean Air Act by adopting a best-available-

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technology approach that is not mentioned in the act? The answer is that this is the wrong kind of flexibility. Agency staffs can always finagle their way toward some workable solution out of almost any legislative language. This does not mean that we should not strive for clear and realistic language in our laws. Once the law is written, Congress has the responsibility to provide oversight to ensure that the reasonable goals of the law are carried out (an activity that Congress now performs very poorly). This is not the same as telling the EPA administrator what to do on specific issues. I believe that the tendency of Congress to give such direction will diminish over the long run if the protective agencies are able to redefine their missions and to accept the responsibility for showing what they are doing as clearly as possible; that is, what they are doing in terms of risk management. This approach requires

FIGURE 2:
DISTRIBUTION OF HAZARDOUS WASTE SITES IN THE UNITED STATES



much more flexibility to address specific pollution problems than many of the most important laws now permit.

Two characteristics of such problems make it imperative that we begin to address them in this way. The first of these has to do with the distribution of toxic substances; we typically find large variations in risk in different parts of the country or even in different parts of the same community. The second characteristic is that toxic risk engenders extraordinary fear, which may paralyze rational public policy and lead to unproductive or even perverse actions on the part of government. Let us deal with each of these characteristics in turn.

The distribution of pollutants is obviously not uniform across the country. We find abandoned hazardous waste dumps only in particular places, and we find the same sort of localization in connection with industrial-process releases of toxic substances. Receiving waters, whether on the surface

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or in the ground, differ in their vulnerability to pollution, as do air sheds. Further, public exposure, and hence risk, varies greatly depending on the location of pollution sources in relation to population centers. Finally, different populations may differ greatly in their opinions about what risks they are willing to endure in return for what benefits.

Current law makes it hard to recognize such distinctions. For example, the Clean Air Act requires EPA to impose expensive automobile inspection and maintenance programs in communities where certain pollutant criteria have been exceeded twice a year. EPA must do this even though the violations may be a consequence of the placement of the air-quality monitoring devices and may not reflect the general quality of the air, even though there is no discernible health effect, and even though the people in the community strongly oppose the action. The law does not allow the federal government to distinguish between (for example) Los Angeles and Spokane, Washington, in this regard—a restriction that defies common sense. In the same way, we cannot distinguish between a plant discharging pollutants into a highly stressed river in Connecticut and one discharging into Alaskan waters that bear no other pollutant burden. In other words, the law does not permit us to act sensibly.

In my view, sound public policy would give the EPA the flexibility to confront and deal with risks in the local context.

VIII

In my view, sound public policy would give EPA the flexibility to confront and deal with risks in the local context. This flexibility would entail, first, balancing risks against the local economic impacts of controlling them; second, ensuring that our national programs that attempt to deal with local risks operate according to risk-management principles; and third, involving the local public in a meaningful way in the decisionmaking process.

A typical example of the localization of risk is that in which an industrial plant imposes some local risks despite the installation of advanced pollution controls. Local economic interests then confront local health interests in reducing risk. A paradigm of such cases was the situation in Tacoma, Washington, where until recently a copper smelter processed arsenic-rich ore and released quantities of this carcinogen into the ambient air. Even after the plant was heavily controlled, it appeared impossible to eliminate the carcinogenic risk from this release; that is, eliminating the risk meant eliminating the plant. As EPA administrator, I believed EPA had a responsibility to explain to the people who would be most directly affected by the decision what we knew about the risks from the smelter.

True public involvement meant forcing the public to confront the trade-offs involved in this risk-management decision. When we began to examine the Tacoma situation in detail, we discovered that most of the local people were willing to view the problem in terms of the risk-management choice, although their positions on the arsenic risk depended (understandably) on where they lived in relation to the distribution pattern of the emissions, and on whether they had a personal economic stake in the plant remaining open. As the public discussions continued and we refined our data on both the risk and the means of reducing it, the citizens of Tacoma began to come up with

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helpful ideas about how we could minimize the actual impact of the arsenic emissions and keep the smelter operating.

Although the plant's owners eventually decided to close it (for reasons not directly connected to the pollution issue), we learned something valuable from the experience. Despite initial fears, it is possible for people subject to toxic risk to think rationally about it. It is possible for them to confront the hard truth that solutions to such problems necessarily involve an uneven distribution of risks and benefits. This rational thinking involves plunging into the uncertainties involved in the analyses we use to define the issues. How sure are we about exposure? How sure are we about the effects of the substance? How certain are the economic impacts?

Such rational thinking requires a kind of democratic citizenship that is willing to dig deeper than the glib headlines and the usual invitations to panic. It also requires much more from the regulatory agency providing the facts: The agency must be willing to explain and able to communicate, and most of all, it must admit the uncertainties buried in its calculations. Only then can the appropriate balancing decisions take place. Our environmental laws should specifically permit EPA to manage a localized risk in this manner.

Risk management thus provides us with the flexible approach needed to deal with the way toxic substances are actually distributed. However, it must also enable us to deal with the second characteristic of the current situation with respect to toxic substances: public fear and the mistrust often associated with it.

How can risk management, a logical and rational process, cope with emotionally based public reactions? While we do not yet have a perfect solution to this problem, we do know enough, I think, to identify some nonsolutions. Reliance on experts is not a solution. Psychologist Paul Slovic writes of this:

> Since even well-informed laypeople have difficulty judging risks accurately, it is tempting to conclude that the public should be removed from the risk assessment process. Such action would seem to be misguided on several counts. First, we have no assurance that experts' judgments are immune to biases once they are forced to go beyond hard data. . . . Second, in many if not most cases, effective hazard management requires the cooperation of a large body of laypeople. These people must agree to do without some things and accept substitutes for others; they must vote sensibly on ballot measures and for legislators who will serve them as surrogate hazard managers; they must obey safety rules and use the legal system responsibly. Even if the experts were much better judges of risk than laypeople, giving experts an exclusive franchise for hazard management would mean substituting short-term efficiency for the long-term effort needed to create an informed citizenry.

Burying quantitative estimates of risk within some procedural regulatory framework, an abstract scoring system perhaps, is also a nonsolution. In

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debates surrounding any environmental decision, however, the subject of risk is bound to arise, and then the agency will have to explain not only the risk itself but also why it was trying to "hide" it. The regulatory agencies must accept the fact that the public is part of the regulatory process. Given the atmosphere of mistrust that has characterized governmental relations with the public since Vietnam and Watergate, it is vital that the agencies be forthcoming with *all* the information involved in their decisions.

Furthermore, as we have seen, regulatory approaches not based on quantified risk must eventually confront the economic realities and become risk management in all but name. It is thus impossible for modern environmental policymakers to avoid dealing directly with named, quantified risks and the public fear these engender.

IX

I believe that we can mitigate this fear only by adopting as policy the full disclosure of the risks involved in regulatory decisions. This belief is not universally shared within the environmentalist or the regulated communities or even at the protective agencies. It is not pleasant to tell people that some residual risk is nearly always associated with practical levels of pollution control. It becomes less and less pleasant as one gets closer to the most affected locality. In a way, the great national debates on environmental policy are resolvable only in high school auditoriums across the country and in terms of what public officials are able to say to worried citizens. The questions that these audiences naturally ask (Can we drink the water? Will my child get cancer?) can hardly ever be answered unequivocally. The uncertainties involved in risk assessment must be explained, and people must be brought to understand that "safety" is a social construct, the definition of which is, or ought to be, a part of each citizen's duty in our society.

I believe that we can mitigate this fear only by adopting as policy the full disclosure of the risks involved in regulatory decisions.

Our skill at developing such constructs for new apparent dangers is not yet what it should be. Communication and public education must become a much more important part of the work of EPA and the other protective agencies, on a level with their scientific, engineering, policymaking, and legal functions. Risk management is not merely a set of techniques for arriving at the correct answers. It must include communication to the public about how we arrive at environmental protection decisions. The values and assumptions that underlie all such decisions must be made manifest. Transparency is the object of the whole process, and public trust is the ultimate goal.

But it is not easy, for we must confront one of the inevitable dilemmas of leadership in a democracy, that of having to choose between telling people what they want to hear and telling them what they ought to know. A good deal of our environmental legislation has bent toward the former, which has made things more difficult for the officials who have to stand up on the stages of those high school auditoriums. If people have been told in statutes that risk can be made to vanish through governmental action, they will be satisfied with nothing less, and they will not treat kindly the messenger who has to tell them it cannot be done.

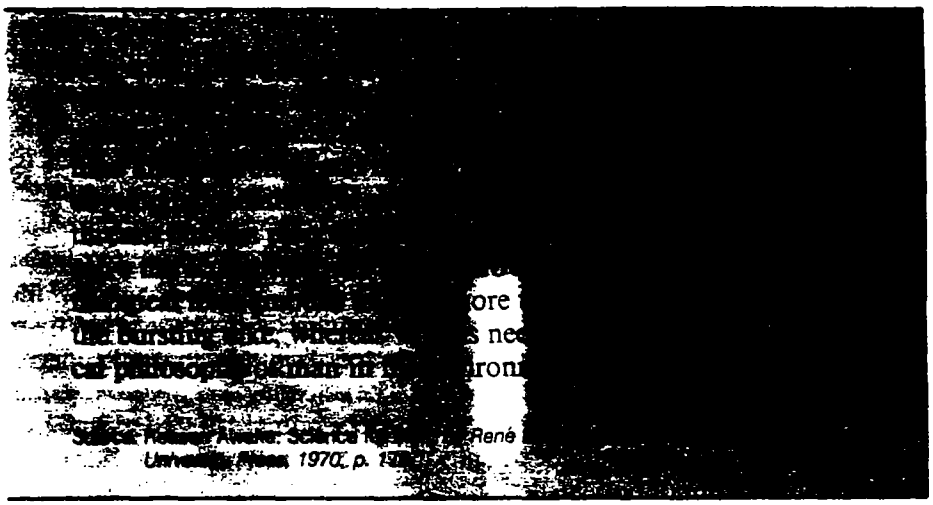
If risk management is to be used as a means of coming to decisions at spe-

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cial locales, the principle must be specifically established in environmental law. Our statutes should reflect the reality that environmental protection in an imperfect world means the assessment of risks and the ability to manage them as particular situations warrant. Note that this should go beyond health risk in the strict sense. We should learn to look at all the impacts of pollution from the perspective of balancing some definable improvement against our always finite resources, whether control expenditures or governmental attention.

This is always a hard case to make, especially to Congress, where the prizes seem to go for new programs and ever more stringent ones. It sometimes seems that those who write our protective statutes would rather have EPA pretend to be doing a thousand tasks than have it select the hundred most important tasks and do them well. I am not saying that our present laws

REASON AWAKE: SCIENCE FOR MAN, BY RENÉ DUBOS.



have not worked. There is no question that we have mounted a successful national effort against the more gross sorts of pollution. Those laws, however, will become increasingly ineffective if they force us to deal with toxic risk, or even with the final remaining increments of conventional pollutants, in the wrong way. Our laws need less pious hope and more determination to strike at the sources of actual harm. The management of EPA would surely benefit from such legislative changes.

For example, in the air program we would allocate resources not to the inevitably slow process of listing and controlling a handful of the immense number of chemical species that could cause trouble but rather to those actual situations in which some unacceptable risks can be found. We would work with industry and the affected communities to develop acceptable levels, which would naturally vary with conditions specific to different areas. Where appropriate, we would have the flexibility to establish ambient, source-specific, or technology-based standards tailored to the particularities of each risk situation.

In the water program we would pay more attention to the impact of

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pollutants on aquatic ecosystems, and invest time and attention where it promises to do the most good. We would question whether it pays to go from 95 to 99 percent removal of toxic substances and conventional pollutants at industrial sources where we know that the bulk of the pollution load on the water comes from urban and agricultural nonpoint runoff. By now it is clear that we are not going to eliminate all pollutant discharges into our waterways by the date specified in the Clean Water Act. Should we therefore wring our hands and cry "failure"? No, we should decide what kind of water quality we can live with, in which bodies of water, and what we are really prepared to pay to get it, and then we should put that kind of flexibility into law.

In groundwater protection we should start relating our concern for this resource to its presumptive use. Groundwater is a resource like any other. Obviously we cannot allow our groundwater to be carelessly fouled, but let us cultivate a sense of proportion as well as a sense of outrage. There is a great difference between the careless and casual destruction of a supply of drinking water and the demand that a vast pool of groundwater lying beneath an industrial metropolis be scrubbed as clean as it was when a dozen farmers lived above it.

We accept risks from chlorinated drinking water in most places in the United States right now, risk levels that many communities have declared to be unacceptable when presented by way of groundwater. Most chlorinated drinking waters contain chloroform, a product of the reaction between chlorine and the humic acids occurring naturally in many water sources. We have established an acceptable level for chloroform in drinking water, based on a balancing of the carcinogenic risk of this substance against the risk of pathogenic diseases that could result from insufficient chlorination. We ought to eventually establish the same kind of acceptable levels for the potential carcinogens that may show up in groundwater.

The hazardous waste problem boils down to specifying where we want the waste to go: the land, the air, or the water.

The hazardous waste problem boils down to specifying where we want the waste to go. In the last analysis, after we have reduced production and have recycled and treated as much as we can of our waste, we have three choices as to where to put it: the land, the air, or the water. Congress has never analyzed the total risk associated with a certain hazardous waste policy and the total cost of eliminating it and then used the analysis as the basis for legislation. However, that is what we will increasingly have to do if our national environmental policies are to retain any contact at all with the day-to-day realities they are supposed to influence.

In the control of pesticides, we have relied heavily on the banning of chemicals that entail unacceptable risks. We must continue to do so, of course, but we must go beyond the single-substance ban as our major weapon. The industrial laboratory will always outpace the regulatory agency in providing substitutes for banned chemicals, and some of those substitutes in field use may prove as troublesome as the ones they replace. We must start looking at the long-term risks posed by whole systems of agriculture—land management, tillage practices, and herbicide and pesticide applications—and not just at the health risks associated with chemical residues on food or applicator contact.

What is the impact of all this chemical loading over the years on the

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ecological systems in which human culture is embedded? After decades of so-called pesticide control, we have not even begun to ask this question. Indeed, it is odd how little time is spent at the upper levels of EPA thinking about such things and how much time is spent worrying about tiny increases in the risk of a single human disease. We need a return to the vision of environmentalism embodied in the documents that created EPA.

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Some years ago, when pollution control seemed a far more overwhelming task than it does now, René Dubos said that the way to cope with such massive problems was to "think globally and act locally." It is still good advice, and may serve as a general prescription for successful management of technological risk in a democratic society as big and diverse as ours. Global thinking in the present case means dealing explicitly with the central questions of risk management: how to reconcile technological systems with social values; how to develop the consensus about potentially dangerous technologies that is necessary for continued growth; and how to establish and maintain trust in our protective institutions. We do not yet know how to deal very well with any of these questions, although I have tried to suggest some promising directions to follow.

This is why local action—a diversity of local actions—is necessary as well. I suspect that the most efficient way for our society to learn how to cope with risk is to enable hundreds or thousands of locally based risk-management endeavors to take place. Local risk-taking preferences could then be expressed, under broad limits set by higher levels of government. This will inevitably change public perceptions of risk, for in such perceptions familiarity breeds, not contempt, but the ability to discriminate between trivial and important risks. Fear is, after all, what has tended to paralyze public policy on these issues, and all our research shows that familiar risks engender less fear than unfamiliar ones. As our people begin to assess and manage risks at the local level, they will be preparing themselves to cope as citizens of a democratic society moving into a future dominated by barely imaginable technologies and fraught with unfamiliar risks. ■

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