

DAVIS v. DOW

6139

ATTACHMENT TO MEMORANDUM OF NOVEMBER 13, 1991

EXHIBIT LIST

Ex. #	Date	Description
1	10/19/44	Report by B.K. Rowe of Biochemical Research Laboratory of Dow Chemical. Subject: "Preliminary Information Relative to the Toxicity of 2,4-Dichlorophenoxyacetic Acid".
2.	5/7/46	Translation from a Puerto Rican newspaper clipping entitled "Weed Killer 2,4-D Is Non-Toxic To The Human Being". Comment: This article was published in 1946 and given at an Ohio State University talk on 1/3/51 to create the thought that humans can swallow 2,4-D and no harm would be caused. If possible, getting the original copy of this would be very important; it's hard to read on the exhibit. The translation of this article would be important to obtain for us because it says "Kephart and Mitchell in the United States took doses without noxious effects!" This particular article is what got the third world countries excited about the prospects of using these new herbicides.
2a.	1946	Harrison, Jos et al. "2,4-D Toxicity-I. Toxicity Twoard Certain Species of Fish, from Am. Jour. of Pharm., Vol. 118, pp. 442-425 (1946).
3.	3/47	Hill, et al., "Toxicity of 2,4-Dichlorophenoxyacetic Acid For Experimental Animals", Journal of Industrial Hygiene And Toxicology, Vol. 29 pp. 85-95. Comment: They killed many animals doing these tests to determine the degree of toxicity of 2,4-D.
4.	6/9/48	Report by illegible name, of Biochemical Research Laboratory for the Dow Chemical Company titled: "Toxicology and Hygiene, 2,4-Dichlorophenoxyacetic Acid". Comment: This article says that 2,4-D

		toxicity only prevents minor hazards of systemic effects. There is no hazard of absorption through the skin. (A very misleading article by Dow Chemical.)
5.	10/48	Eyzaquirre, et al., "Experimental Myotonia and Repetitive Phenomenon the Veratarenic Effects of 2,4-D in the Rat", publication not indicated. Comment: It shows that 2,4-D causes rat, man and goat to have nerve/muscle twitches or spasms with the development of muscular rigidity.
6.	10/29/48	Bjorn, et al., "Effects of 2,4-D Acid on Chicks", Science, Vol. 108, pp. 479-480.
7.	5/23/49	Dow report entitled "Result of Skin Irritation Tests on Various Samples of 2,4-D Taken at Various Stages in the Manufacturing Process". Comment: This says impure 2,4-D is considerably more irritating than the purified product (for dermatitis). A damp product will produce more irritation than a dry one.
8.	12/15/49	Letter to Dr. Drill of Wayne University from Rowe of Dow re: "Studies of 2,4-D and 2,4,5-T on Dogs".
9.	Apprx 1949	Rowe, et al. of Dow: "Acute Oral Toxicity of 2,4-D to Rats, Mice, Guinea Pigs and Chicks".
10.	Apprx. 1949	Rowe, et al. of Dow: "Semi-Chronic Oral Toxicity of 2,4-D to Rats".
11.	4/11/50	Untitled chart listing ingredients of 2,4,5-T Acid and 2,4-D Acid.
11a.	8/30/50	Report to Britton of Dow from Drill of Wayne University re: "Interim Report on the Acute and Subacute Oral Toxicity of 2,4,5-T for Dogs". Comment: Says 2,4,5-T in general resembles 2,4-D in toxicity and it is greater in dogs than in rats.
12.	8/31/50	Internal Dow Memo Regarding Toxicity of 2,4-D Stating that 2,4-D is Moderately Toxic.

13.	11/20/50	French medical article on 2,4-D, no translation.
13a.	4/52	English abstract of article mentioned in exhibit 13.
14.	1950	Willard, C.J., "Indirect Effects of Herbicides", publication not indicated.
15.	10/51	Lehman, Arnold J., "Chemicals in Foods: A Report to the Association of Food and Drug Officials on Current Developments - Part II, Pesticides", from The Association of Food and Drug Officials of the United States, Vol. 15 pp. 122-129.
16.	Early 1950's	Drill, Victor, "Acute and Sub-Acute Oral Toxicity of 2,4-D for Dogs". Comment: Three of four dogs died with doses of 2,4-D.
17.	1/52	Lahman, "Chemicals in Foods: A Report to the Association of Food and Drug Officials on Current Developments, Part II, Pesticides II, Dermal Toxicity", from The Association of Food and Drug Officials of the United States, Vol. 16, pp. 3-9.
18.	4/3/52	Internal Dow memo on "Results of Skin Irritation Tests on Current Production 2,4-D As Compared With Past Production Material". Comments: It discusses the difference in cases of skin dermatitis between the old production method and the new production method.
19.	4/3/52	Internal Dow memo on "Results of Range Finding Toxilogical Tests on 2,4-D Prepared By A New Method". Comment: The document discusses hazards and proper handling procedures for 2,4-D.
20	4/52	Lahman, Arnold J., "Chemicals In Foods: A Report To The Association of Food And Drug Officials On Current Developments" from The Association of Food and Drug Officials of the Untied States, Vol. 16, pp. 47-53.

21.	10/52	Lahman, Arnold J., "Chemicals In Foods: A Report To The Association of Food and Drug Officials on Current Developments, Part II, Pesticides V, Pathology", from The Association of Food and Drug Officials of the United States, Vol. 16, pp. 126-132.
22.	Fall, 1952	"Harnessing Chemical Sprays to Serve the Sportsmen Through New Game Management Practices" from Down to Earth, pp 7-8. Comment: This is Dow hype to sell their 2,4-D and 2,4,5-T chemicals. This is their own magazine which is published quarterly to hype their products to all users.
23.	1952	Neuns, Alva A., "Woody Plant Control Along Roads in Shasta National Forest" from Down to Earth, pp. 14-15. Comment: Additional Dow hype to sell their 2,4-D and 2,4,5-T chemicals.
24.	Summer, 1953	Arend, John L., "Controlling Scrub Aspen with Basal Sprays" from Down to Earth, pp. 10-11. Comment: Explains Dow's recommended applications of 2,4-D/2,4,5-T.
25.	Winter, 1953	Leonard, Oliver A., "Chemical Control of Woody Plants in California" from Down to Earth, Vol. 9, pp. 2-4. Comment: Also contained in this issue is "a simple device for applying Esteron 2,4,5" by David Cook, page 5, and "Timber Stand Improvement on the W.G. Jones State Forest, Conroe, Texas", by Charles Stealey, pp. 12-14.
26.	11/20/53	Loustalot, A.J. et al., "2,4-D Affects Phosphorous Metabolism" from Science, Vol. 118, No. 3037, pp. 627-628. Comment: Shows 2,4-D's mode of action may be in inhibiting or interrupting the phosphate metabolism in the plant, i.e. by poisoning the enzyme or system responsible for the hydrolysis of the high energy phosphates.
27.	1953	Drill, et al, "Toxicity of 2,4-D and 2,4,5-T. A Report on Their Acute and Chronic Toxicity in Dogs" from AMA

		Archives of Industrial Hygiene and Occupational Medicine, Vol. 7, pp. 61-67.
28.	1953	Baker, et al, "Suspected Poisoning of Dogs from Eating Grasses Treated with 2,4-D", from The North American Veterinarian, Vol. not indicated, p. 194. Comment: This is the poisoning of dogs by 2,4-D with resulting liver damage. This is the same type of article that appeared on the front page of USA Today in September, 1991!
29.	Summer, 1954	Walker, A.H., "Chemicals are Effective for Woody Plant Control in the South" from Down to Earth, pp. 10-11.
30.	10/54	Rowe, et al., of Dow: "Summary of Toxilogical Information on 2,4-D and 2,4,5-T Type Herbicides and an Evaluation of Hazards to Livestock Associated With Their Use" from Am.J.Vet.Res., pp. 622-629. Comment: A very revealing article. Toxic levels of exposure are noted!
31.	Fall, 1954	Goddard, Ray E., "Killing Small Undesirable Hardwoods by the Use of Cornell Tool" from Down to Earth, Vol. 10, p. 5.
32.	1/55	Markquardt, Roland P. et al., of Dow: "Determination of 2,4-D in Grain and Seed" from Agricultural and Food Chemistry, Vol. 3, pp. 51-53.
33.	Winter, 1955	Lynn, G.E., "The Status of Pesticides Under the Miller Amendment to the Federal Food, Drug and Cosmetic Act". pp. 8-9.
34.	8/57	Dow Esteron 2,4,5 Label.
35.	1957	Lynn, G.E., "Residue Tolerances for Dow Agricultural Chemicals" from Down to Earth, pp. 7-11. Comment: Gives registered brand name products sold by Dow in 1957 and Dow tells people their products are safe to eat when residues appear on the crops.

36.	5/29/58	Hymas of Dow, "Toxicity of Herbicides to Domestic Animals".
37.	5/58	Davis, James R., "Basal Spray With 2,4,5-T for Winter Hardwood Control in East Texas" from Journal of Forestry, Vol. 56 p. 349. Comment: This was the plaintiff in Davis v. Dow. This is an example of how 2,4,5-T was used in the east Texas pines.
37a.	6/59	Stransky, John A., "Concentrated or Diluted 2,4,5-T as a Supplement to Girdling?", from Journal of Forestry, Vol. 57, pp. 432-434.
38.	1959	Dow Chemical Company Journal abstract regarding article on 2,4-D. Comment: Shows that the thyroid is effected from acute dose on in-house Dow memo. The comment is made "so what - acute dose!" They missed the whole point that 2,4-D attacks the thyroid!
39.	1959	Apffel, C.A., "The Cytostatic Action of Certain Auxins: Preliminary Review", translation from the original French article. Comment: Emphasizes that the esters of 2,4-D/2,4,5-T must be purified of any contamination to be safe to users!
40.	2/5/60	Internal Dow memo: "Results of Range Finding Toxilogical Tests on 2,4-D Acidified With Chemically Pure HCl and With HCl Recovered From the Pentachlorophenol Plant". Comment: This could be a serious introduction into the 2,4-D herbicide with this hydrochloricacid that comes out of the pentachlorophenol plant. Pentachlorophenol is another producer of the deadly dioxin 2,3,7,8-TCDD!
41.	7/60	Gibbs, Carter B., "Iso-Octyl Ester of 2,4,5-T in Hardwood Control" from Weeds, Vol. 8, pp. 462-463. Comments: This is an example of the east Texas use of this herbicide in 1960.
42.		Not used.

43.	4/62	Abstract of article on "Nervous System Effects of a Chemical Herbicide" (2,4-D) from Industrial Hygiene Digest. Comments: Observed higher nervous system acute suffering in patient with severe damage - caution is needed in use of 2,4-D. Calls for increased protection and special neurological examination of workmen in contact with 2,4-D.
44.	7/62	Florsheim, et al., "Some Effects of 2,4-D on Thyroid Function in the Rat: Effects on Iodine Accumulation" from Endocrinology, Vol. 71, pp. 1-6.
45.	1962	Foissac, Phillippe et al., "Polyneuritis After Using a Weed Killer: Containing Acid 2,4-D", from Lille Medical, Vol. 7(10), pp. 1049-51, (1962). Translation of attached French report.
45a.	1962	Desi, et al, "New Evidence Concerning the Nervous Site of Action of Chemical Herbicide Causing Professional Intoxication" from ACTA Physiol, Vol. 22, pp. 72-80. Comments: Budapest study showing that 2,4-D acts on the CNS by paralyzing its function and also damages the thyroid and other organs as secondary from CNS damage.
46.	1/15/63	"Toxicological Properties of Terdon" prepared by Biochemical Research Laboratory of Dow Chemical. Comment: Marked "Trade Secret - contains confidential information".
47.	1/62	Desi, et al., "Nervous System Effects of a Chemical Herbicide" from Archives of Environmental Health, Vol. 4, pp. 101-108. Comment: This is a budapest study that was made on 6/20/61 where it shows central nervous system damages resulting from the use of 2,4-D. This article explains that 2,4-D is designed to cause disproportionate growth and quick death of certain plants (especially the dicotyledonous weeds). It shows that 2,4-D has been known to cause myotonia, motor

disorders, paralysis in the extremities and gastrointestinal symptoms such as vomiting and diarrhea, leukopenia, atoxia and coma by the year 1948. Here they find higher CNS activity suffered by patients with severe damage and suggest there is a need for caution in the need for 2,4-D.

48. 8/15/63 — Palmer, J.S., "Chronic Toxicity of 2,4-D Alkanolamine Salts to Cattle" from Journal of the American Veterinary Medical Association, Vol. 143, pp. 398-399.

49. 8/63 — Seaberry, John H., "Toxicity of 2,4-D for Man and Dog" from The Archives of Environmental Health, Vol. 7, pp. 86-93.
Comment: This is about a doctor who tested dogs and also terminal human patients with 2,4-D and actually ended up killing one of the patients.

50. 1963 — Dolgarrd, Mikkelsen, et al., "Toxicology of Herbicides" from The Pharmaceutical Review, Vol. 14, pp. 225-250.
Comment: This document should be reviewed very carefully, it contains a variety of important revelations on the toxicity of these herbicides.

51. Jan/Feb 1964 — Clark, et al., of the US Department of Agriculture. "The Fate of 2,4-D Acid in Sheep" from Agricultural and Food Chemistry, Vol. 12, pp. 43-45.

52. 3/13/64 — Palazzolo, of Industrial Biotest Laboratories Inc., Report to the National Agricultural Chemicals Association. "Repeated Dermal Toxicity Studies on Three Formulations of 2,4-D".
Comment: It was noted that when diesel is used with the application of 2,4-D, it will immediately penetrate the animal's skin.

NOTE: This is why these herbicides are more deadly to humans through skin absorption, because of the penetrating ability of the diesel fuels that they are mixed with.

53.	3/16/64	Palazzolo, of Industrial Biotest Laboratories Inc., Addendum Report to the National Agricultural Chemicals Association. "Repeated Dermal Toxicity Studies on Three Formulations of 2,4-D". Comment: I only included cover page plus 1 & 45.
54.	1964	Kirk-Othmer, Encyclopedia of Chemical Technology, pp. 258-267 and 325-338. Comment: This is a good history of the chemistry of 2,4-D and 2,4,5-T and what they were designed to do.
55.	3/15/65	Report to Dr. Gorden of Dow from Olson of Dow re: "The Formation of Folliculitis in the Rabbit Ear Following a Single Subcutaneous Injection of Symmetrical Tetrachlorodibenzo-dioxin in Benzene". Comment: Its all about a matter of doses! Enough will injure or kill you!
56.	3/26/65	Memo to Gorden of Dow from O'Hare and Olson of Dow re: "The Ineffectiveness of Washing as a Preventative Measure in the Formation of Folliculitis in the Rabbit Ear Following Repeated Applications of TCDD in Corn Oil". Comment: This shows that soap is ineffective in getting TCDD out of the exposed area! (In an insoluble solution).
57.	6/22/65	Dow report entitled "The Determination of 2,3,7,8-TCDD in 2,4,5-T By Gas-Liquid Chromatography". Comment: This is an admission by Dow of the health effect problems caused by TCDD.
58.	4/14/66	Alexander, et al of Dow: "Acute Fish Toxicity of Daxtron, Tordon and Dursban To Three Species of Fish".
59.	5/66	"2,4-D and Related Compounds" from Wildlife, Vol. 96, pp. 61-69. Comment: This is a very good review of the chemistry of this herbicide.
60.	1966	Erne, Kurt, "Studies on the Analytical Chemistry and Toxicology of Phenoxy Herbicides".

61.	1/23/68	Letter to Rowe of Dow from Dr. Kligman re: Experiments of Adults Receiving Small and Increasing Doses of Tetrachlorodibenzo-p-dioxin (TCDD). Comments: Dow is using human guinea pigs in a blind study to determine the effects of TCDD injected into them!
61a.	1/23/68	Document entitled "Report to Dow Chemical Company on Group (1-6)" submitted by Clover Laboratories of Philedelphia. It appears to be the results of these laboratory tests. Comment: These are six groups of people who are mostly blacks that were tested with TCDD on their backs and foreheads in an apparent allergy test to see how humans would react to these deadly dioxins. There is no indication any of these knew that among the different allergies they were being tested for, someone was putting the deadly dioxin directly into their bodies.
61b.	3-4/68	Bulletin from the National Clearing House for Poison Control Centers entitled "Death from Chlorinated Phenoxyacetic Acids (2,4-D; 2,4,5-T; MCPA)". Comment:
62.	10/21/68	Leng, Marguerite of Dow, summary of an article entitled "Review on the Metabolism of Phenoxy Compounds in Plants and Animals".
63.	5/69	Table entitled "Summary of Dosages of Various Organic Herbicides that Cause Significant Weight Loss, Reduce Weight Gain, or Poisoning in Cattle, Sheep or Chickens". Production research report #106, US Department of Agriculture.
64.	12/18/69	Letter to Leng of Dow from Erne, Associate Professor of Vet. Science in Stockholm, Sweden regarding 2,4,5-T and 2,4-D.

65.	12/18/69	Table entitled "Phenoxy Acid Levels in Tissues of Broiler Chickens Fed 2,4-D and 2,4,5-T at 1,000 ppm in the Drinking Water For 104 Days".
66.	1969	Medical abstract on Russian article regarding the health in workers manufacturing 2,4-D. Comment: Shows many health problems in 51.7% of all workers studied!
67.	1969	Way, J.M., "Toxicity and Hazards to Man, Domestic Animals and Wildlife From Some Commonly Used Oxin Herbicides" from Residue Reviews, Vol. 26, pp. 37-62. Comment: This is the reply to the above letter.
67a.	1969	Way, J.M., "Toxicity and Hazards to Man, Domestic Animals and Wildlife From Some Commonly Used Herbicides", from Residue Reviews, Vol. 26, pp. 37-62. Comment: This says that because man is handling the chemicals in concentrated form at all stages from manufacturing to dilution, it follows that he is at greater risk than any other organism. Points out that "Freireich in 1966 has shown that a number of drugs on a mg/kg basis are more toxic in man than in the mouse when used as a laboratory test animal by a factor of 10 to 15!"
68.	3/9/70	Abstract from the Health Aspects of Pesticides Abstract Bulletin. Comment: This shows that if you mix 2,4-D and 2,4,5-T in esters, it can cause high mortality!
69.	4/13/70	Document entitled "The Analysis of 2,4-D Acid for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Content", publication not indicated. Comment: This document shows that 2,3,7,8-TCDD is in 2,4-D at Dow Chemical! They deny repeatedly throughout all the years they have been in business that the dioxin TCDD has ever appeared in their herbicide 2,4-D. This is clearly proof to the contrary.

70.	1970	Abstract from an article in Bio Science. Comment: Page 2 shows cancellation of 2,4,5-T in the United States for many different uses. (This is the first time it was officially cancelled by a government agency).
71.	1970	Medical abstract on "The Effect of 2,4-D on Pheasants" and attached is a Dow Chemical biology research document summarizing "The Effect of 2,4-D and Esters of the 2,4-D on Rat Embryonal, Fetal and Neonatal Growth and Development". Comment: The 2,4-D either killed or deformed alot of chickens or birds in this experiment!
72.	1970	Matsumura, Akihito, "The Fate of 2,4-D - Trichlorophenoxyacetic Acid in Man" from Japanese Journal of Industrial Health, Vol. 12, October, 1970, pp. 446-451.
73.	1970	Abstract on articles regarding "Toxic Effect of Some Herbicides on Fish and Aquatic Invertebrates" from Chemical Abstracts, Vol. 72, p. 240. Attached is a Dow memo dated 4/23/80, regarding Esteron 2,4,5 labels. Comments: Dow has started up the 2,4,5-T under a different label after it was cancelled by the EPA!
73a.	1970	Rivers, et al., "Simultaneous Gas Chromatographic Determination of 2,4-D and Dicamba in Human Blood and Urine", from J. Chromatog, Vol. 50, pp. 334-337. Comment: Suicide attempt with 2,4-D.
74.	1/23/71	Williams, C.S. of Dow, "The Status of 2,4-D; 2,4,5-T; Silvex; and MCPA Herbicides", publication not indicated. Comment: This gives the chronology of the major events effecting 2,4-D and 2,4,5-T from April, 1966, through January, 1971.
75.	3/71	Poland, et al., "A Health Survey of Workers in a 2,4-D and 2,4,5-T Plant With Special Attention to Chloracne, Porphyria, Cutanea, Tarda, and Psychologic Parameters".

76.	3/28/71	By "Our Washington Correspondent". Titled: "PSAC Hiccoughs Over 2,4,5-T" from Nature, Vol. 321, May 28, 1971, pp. 210-211. Comment: This is an interesting article on how a private government study of 2,4,5-T was accidently released to the public and thus ended up causing the herbicide 2,4,5-T to become cancelled in April, 1970.
77a.	4/27/71	Memo to Johnson, et al., of Dow from Gehring of Dow re: "Results of the Pilot Study on the Absorption and Excretion of 2,4-D and 2,4,5-T by Human Subjects". Comment: Here Dow is using human subjects to actually drink 2,4-D and 2,4,5-T!
77b.	4/27/71	Gehring, et al. of Dow, "Results of a Pilot Study on the Absorption and Excretion of 2,4-D and 2,4,5-T Following a Single Oral Dose to Human Subjects". Comment: This is referring to the same tests done in 77a.
78.	8/30/71	Sparschu, et al. of Dow, "A Probe Study of the Acute Oral Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin in the Canine". Comment: Most of these dogs died or else had very severe health effects after these tests.
79.	9/1/71	Johnson, Julius, "The Public Health Implication of Wide Spread Use of the Phenoxy Herbicides in Picloran" from Bio Science, Vol. 21, pp. 899-905. Comment: This is a white wash report written by Dow!
80.	10/25/71	Dow report on "Eye Irritation Study Conducted on: 2,3,7,8-Tetrachlorodibenzo-p-Dioxin".
81.	1971	Williams, C.S. of Dow, "The Status of 2,4-D and 2,4,5-T, Silvex and MCPA Herbicides" from Down To Earth, Vol. (not indicated), pp. 12-16.

		Comment: Dow describes the history of 2,4-D and 2,4,5-T development and their designed uses in this Dow Company magazine.
82.	1971	Kolberg, et al, "The Herbicide 2,4-Dichlorophenoxyacetic Acid I, Effects on L Cells" from ACTA Pharmacal et Toxicol, Vol. 29, pp. 81-86.
83.	2/19/71	Memo from Gehring of Dow to Gordon, et al. of Dow re: "Protocol for Consumption of 2,4-D and 2,4,5-T Study".
83a.	1971	Protocol discussed in exhibit 83 on "Human Tolerance of 2,4-D and 2,4,5-T" plus "Full Description of Analytical Standard of 2,4-D".
84.	Summer, 1971	Johnson, Julius, "Safety in the Development of Herbicides" from Down to Earth, Vol. 27, pp. 1-7. Comment: This is Dow's claim of safety in their 2,4-D and 2,4,5-T.
85.	1970-71	The records of shipments or sales in 1970 and 1971 of the products to the U.S. Forest Service, which were provided under court order by the plaintiffs in Davis v. Dow. Comment: Dow only produced these obscure records in the time period of 1970 and 1971, and ignored the full court order which stated they would produce all records of shipments or sales between the years 1954 and 1973.
86.	1/1/72	Walker, et al, "Some Effects of 2,4-D and 2,4,5-T on Ehrlich Ascites Tumor Cells In Vivo and In Vitro", from Industrial Medicine, Vol. 41, No. 1, pp. 22-27.
86a.	1970's	Abstracts on articles on the effect of herbicides on animals. Comment: Shows Dow's knowledge of the harmful effects of TCDD in causing cancers and many other defects!

87.	2/72	Hussien, et al, "Mutagenic Effects of TCDD on Bacterial Systems". Comment: This looks at the health effects of 2,4,5-T.
88.	8/72	Kimbro, R., "Toxicity of Chlorinated Hydrocarbons and Related Compounds" from ARCH Environ Health, Vol. 25, pp. 125-131.
88a.	10/6/72	"Summary Teratology" by B.E. Schwetz. Comment: Shows that TCDD is highly toxic to the developing embryo and fetus of the rat.
89.	10/18/72	Leng, Marguerite of Dow, "Summary of Teratology Studies With 2,4-D".
90.	1972	Cunningham, et al., "Effect of Tetrachlorodibenzo-P-Dioxin on Growth Rate and the Synthesis of Lipids and Proteins in Rats" from Bulletin of Environmental Contamination and Toxicology, Vol. 7, pp 45-51.
91.	2/12/73	Bosshardt, Hans, "Dioxins -Insidious Poisons in Our Environment", translation from and German article. Comment: Germany bans 2,4-D at this time because of its contaminant TCDD.
92.	4/2/73	Baughman, et al., "An Analytical Method for Detecting Dioxin", presented at the conference on Dibenzo-Dioxins and Dibenzo-Furons. Comment: Good explanation of how TCDD works.
93.	Spring, 1973	Wilson, James, "Teratological Potential of 2,4,5-T", from Down to Earth, Vol. 28, pp. 14-18.
94.	6/73	Summary of toxicology studies with 2,4-D in livestock, June 1973.
95.	6/73	Summary of metabolic studies with 2,4-D and animals, June 1973. Author not indicated.

96.	6/73	Document entitled: "Section C. Full Reports of Investigations Made With Respect to the Safety of the Pesticide Chemical".
97.	6/73	Crossland, et al., -- Title of article illegible regarding spraying of chemicals.
98.	9/73	Johnson, et al., "Chlorinated Dibenzo-Dioxins and Pentachlorophenol", from Environmental Health Perspectives, pp. 171-175. Comment: This is a good article for the defense. Dow makes improved PCP/TCDD contaminants in their product. Here they admit the older production batches of PCP/TCDD had higher values in parts per million! (This is why the actual contamination in parts per million has been so important, Dow is constantly trying to reduce the contamination level and in this case they are using a new batch.)
99.	1973	Kociba, et al., "Results of a Two Year Chronic Toxicity and Oncogenicity Study of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin in Rats", from Toxicology and Applied Pharmacology, Vol. 46, pp. 279-303.
100.	1973	Document untitled Excerpt Part I. Covers the periods 1965-1969 at a chemical plant producing pentachlorophenol and 2,4,5-T.
101.	1973	Bage, et al., "Teratogenic and Embryotoxic Effects of the Herbicides Di-and Trichlorophenoxyacetic Acid", from ACTA Pharmacal et Toxicol, Vol. 32, pp. 408-416. Comment: Note the conclusion on the combined effects of 2,4-D and 2,4,5-T.
102.	11/6/73	Crosby, et al., "Photo Decomposition of P-Chlorophenoxyacetic Acid" from Ag. Food Chem., Vol. 21, pp. 1049-1052. Comment: Talks about 2,4-D and 2,4,5-T decomposing under sunlight or laboratory ultra violet light.
102a.	1/5/73	Kanaga, "Environmental Impact of Herbicides", report prepared by Dow.

103.	1973	May, G. "Chloracne From the Accidental Production of Tetrachlorodibenzo Dioxin", from British Journal of Industrial Medicine, Vol. 30 pp. 276-283.
104.	1973	Yoder, et al., "Lymphocyte Chromosome Analysis of Agricultural Workers During Extensive Occupational Exposure to Pesticides", from Mutation Research, Vol. 21, pp. 335-340. Comment: In this test they caused mutations in the chromosomes.
104a.	1973	Karvonen, et al. of Finland: "The Health Hazards of Defoliants".
105.	1/74	Kimbrough, Renate of the Center for Disease Control, "The Toxicity of Polychlorinated Polycyclic Compounds and Related Chemicals", from CRC - Critical Reviews in Toxicology, pp. 445-498. Comment: Overall good summary of the known toxic effects at this time.
106.	2/74	Gribble, Gorden W., "TCDD: A Deadly Molecule", from Chemistry, Vol. 47, pp. 15-18. Comment: Great history of TCDD (but the concentration level in parts per million was slanted in Dow's favor).
107.	8/24/74	"Illness Associated with TCDD Contaminated Soil - Missouri", from Communicable Disease Center Morbidity and Mortality Weekly Report, p. 299.
108.	8/28/74	New York Times article: "Death of Animals Laid to Chemical". Attached is a 2/28/80 memo to the State Department of Agriculture regarding the label for Esteron 245 Herbicide.
109.	8/28/74	Washington Post article: "U.S. Scientist Disputes EPA on Toxic Herbicide".
110.	8/29/74	Detroit Free Press article: "Herbicide Killed Missouri Animals". Comment: This article is major proof of the harm that 2,4,5-T with its contaminant TCDD can cause!

111.	4/9/74	Dow requests for toxilogical studies on TCDD. Noted on the request is that the sample of TCDD is extremely toxic and chloroacengenic. Comment: Dow admits TCDD is highly toxic and chloracengenic and must be handled with extreme caution.
112.	1974	Pilinskaya, N.A., "Cytogenetic Effect of the Herbicide 2,4-D on Human and Animal Chromosomes", translation from a Russian Journal.
113.	1974	Norris, et al., "The Toxicity of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) in Guppies", from Bulletin of Environmental Contamination and Toxicology, Vol. 12, pp. 76-80.
114.	11/75	Honoroff, Ida, article from A Report to the Consumer entitled: "Dirty Tricks". Comment: This article is very hard against Dow! It is a news letter put out to consumers to wake them up on the dangers of these herbicides.
115.	1975	Archer, T.E., "Removal of 2,4-Dichlorophenoxyacetic Acid, (2,4,-D) Formulations from Non-Combustible Pesticide Containers", from Bulletin of Environmental Contamination and Toxicology, Vol. 13, pp. 44-51. Comment: This tells how to clean used 1,5,30 and 55 gallon metal pesticide drums after use.
116.	1975	Delvaux, et al., "Polychlorodibenzo-P-Dioxins", from Toxicology, Vol. 3, pp. 187-206. (Translation of a French article.) Comment: This shows another form of the deadly dioxin TCDD contained in a different chemical process.
117.	2/9/76	Abstract and attached report entitled: "The Fate of 2,4-D Following Oral Administration to Man", by Sauerhoff, et al.

118.	5/5/76	"Possible Toxic Effects of TCDD in Humans Listed by Dioxin Group", from Pesticide Chemical News, pp. 20-23. Comment: This shows that this dioxin causes a loss of libido as one of its health effects listed. 2,4,5-T is formally banned in North Carolina. (This is where all the research is done in the United States on the effects of dioxins and especially TCDD.)
119.	10/76	Poland, et al., "2,3,7,8-Tetrachlorodibenzo-P-Dioxin: Environmental Contaminant and Molecular Probe", from Federation Proceedings, Vol. 35, pp.2404-2411.
120.	1976	Courtney, K. Diane, "Mouse Teratology Studies With Chlorodibenzo-P-Dioxin", from Bulletin of Environmental Contamination and Toxicology, Vol. 16, pp. 674-681.
121.	1/24/77	Index and report on information on chloroacne. Comment: This is a great history of human health effects from 2,4,5-T exposure in Czechoslovakian plants from 1965-1969.
121a.	5/26/77	Draft of EPA's dioxin working group document entitled: "Dioxin: Position Document." Comment: This does major damage to Dow!
122.	7/9/77	Crow, K.D., "Letter to the Editor - Effects of Dioxin Exposure", from The Lancet, Vol. not indicated, p. 82.
123.	8/77	"IARC Monographs of the Evaluation of the Carcinogenic Risks of Chemicals to Man, Some Fumigants, the Herbicides 2,4-D and 2,4,5-T Chlorinated Dibenzo Dioxins and Miscellaneous Industrial Chemicals", from The World Health Organization. Comment: This contains a lot of excellent background information on the history of 2,4-D and 2,4,5-T.
124.	9/26/77	Abstract of Reports, Department of HEW, Division of Cancer Cause and Prevention,

5th Meeting of the Data Evaluation/Risk Assessment Subgroup of the Clearing house on Environmental Carcinogens.
Comment: This abstract indicates that picloram is a carcinogen because of liver lesions. (Picloram contains 2,4-D.)

125. 9/28/77 Kociba, et al., "Results of a Two-Year Toxicity and Onogenicity Study of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) in Rats", produced by the Dow Chemical Company.
Comment: I only copied the first four pages which summarizes this very lengthy report. It was designed as a white wash for Dow to prove that their 2,4,5-T does not hurt anyone.

126. 11/77 Vos, et al., "TCDD Accident at a Chemical Factory in the Netherlands", publication not indicated.
Comment: Shows knowledge of TCDD health effects in 1963! Chloroacne and cancer!

127. 1977 Ahlling, et al., "Formation of Polychlorinated Dibenzo-P-Dioxin and Dibenzofurans During Consumption of a 2,4,5-T Formulation", from Chemosphere, Vol. 8, pp 461-468.
Comment: Swedish study on how you get TCDD and PCDF (furans) when manufacturing 2,4,5-T as a natural by product.

128. 6/77 Article from the Washington Post entitled: "Deadly Poison - Dioxin - Is Showing Up in Mother's Milk".
Comment: Women living in or near the areas where 2,4,5-T was routinely sprayed had dioxin in their breast milk.

129. 1977 Zetterberg, et al., "The Influence of pH on the Effects of 2,4-D on Saccharomyces Cerevisiae and Salmonella Typhimurium", from Mutation Research, Vol. 42, pp. 3-18.
Comment: **KEY DOCUMENT:** Says that the toxic effects of 2,4-D are a function of a low pH levels when more of the 2,4-D is in its undissociated form. At low pH's, toxic and mutagenic effects occur. To

stop these effects, you only have to raise the pH, such as happens when administered orally (like they do in all animal tests).

130. 1977 — Tung, et al., "The Problem of Mutagen Effects on the Second Generation After Exposure to Herbicides", from a Vietnamese publication.
Comment: The Vietnamese made a connection between TCDD of 2,4,5-T and birth defects and a high frequency of brain defects.

131. 1977 — Allen, et al., "Morphological Changes in Monkey Consuming a Diet Containing Low Levels of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin", from ED. Cosmet. Toxicol, Vol. 15, pp. 401-410.
Comment: Caused diseases in monkeys' bone marrow and lymphs. (This leads to lymphatic cancer and leukemia!)

132. 1977 — Abstract of article entitled "Electron-acceptor Properties of Chlorinated-P-Dioxins, from Bull. Environ. Contam. Toxicol, Vol. 18(5), pp. 611-616.
Comment: This is a good explanation of how chlorine binds TCDD together.

133. 1977 — Kenaga, Eugene of Dow, "Acute and Chronic Toxicity of 75 Pesticides to Various Animal Species".

134. 1977 — Leskowski, et al., "The Pharmacology of Experimental Myopathies", from Ann. Rev. Pharmacal Toxicol, Vol. 17, pp. 387-409.
Comment: 2,4-D produces sustained contraction of muscles.

135. 1/7/78 — Franco, Berino, "Preliminary Report on Mortality in the Ceveso Area, 1975-77". Stamped "strictly confidential".

136. 1/10-11/78 — Agenda for the World Health Organization Program on Coordination of Epidemiological Studies on the Long Term Hazards of Chlorinated Dibenzodioxins and Chlorinated Dibenzofurans.
Comment: This goes together with exhibit #170.

137.	1/10-11/78	Moore, John A., "Comparative Toxicity of Dibenzodioxins and Dibenzofurans", from IRAC Meeting on Dibenzodioxins and Dibenzofurans. Comment: Excellent article (goes with exhibit 163).
138.	1/10-11/78	Moore, John A., "Comparative Toxicity of Dibenzodioxins and Dibenzofurans", presented at the IRAC Meeting on Dibenzodioxins and Dibenzofurans. Comment: This says that a result of TCDD is to attack the liver as one of its target organs! Same document as exhibit #137, but marked "Strictly Confidential" by Dow.
139.	1/78	"Cymptomatology Morbidity and Mortality Experience of Chlorinated Phenoxy Acid Herbicide (2,4-D; 2,4,5-T) Sprayers in Finland - A Clinical and Epidemiological Study". Working paper for IARC working group meeting. Comment: Marked "Strictly Confidential" by Dow.
140.	1/78	Poland, Allen, "Chlorinated Dibenzo-P-Dioxins and Dibenzofurans: Review of Toxicology and Enzyme Induction and Consideration of Mechanism of Toxicity", presented at IARC. Comment: Marked "Strictly Confidential" by Dow.
140a.	5/78	Dost, Frank, "Toxicology of Phenoxy Herbicides and Hazard Assessment of Their Use in Reforestation", U.S. Department of Agriculture Forest Service. Comment: Oregon State University study that evaluates for Region (5) in California, the hazards to human health of 2,4-D and 2,4,5-T of U.S. Forest Workers (all based on what literature and research reports have said).
141.	6/16/78	Dow analytical report "Determination of Phenoxy Herbicides in Blood Samples from 2,4,5-T Plant Personnel".

142.	7/78	Yoshida, et al., "Occupational Exposure Measurement of an Aerial Spray Crew to a Mixed Spray of 2,4-D and 2,4,5-T", pp. 1-26.
143.	7/28/78	Dow analytical report "Determination of PHenoxy Herbicides in Urine and Blood Samples from 2,4,5-T Plant Personnel - II".
144.	10/21/78	Rehder, et al., "Pathological - Embryological Investigations in Cases of Abortion Related to the Seveso Accident", from Journal of Swiss Medicine, Vol. 108, pp. 16, 17-25.
145.	5/4/77	Memo from Johnson of the EPA, to Dioxin Implementation Task Force re: Dioxin: Position Document.
145a.	12/13/78	"Human Birth Defects Not Caused By 2,4,5-T or 2,4-D Exposure, Report Says, from Pesticide and Toxic Chemical News, pp. 23-24. Comment: White wash cover up on herbicides by Australian government.
146.	12/78	"Of Mice and Men -- The Troubles of 2,4,5-T", from Journal of Forestry, pp. 787-790. Comment: Reveals the scams pulled by industry with the use of 2,4,5-T.
147.	1978	Ruber, M.D., "Carcinogenicity Testing of Chemicals With Particular Reference to Organochlorine Pesticides", from The Science of The Total Environment, Vol. 10, pp. 105-115. Comment: Great article - connects cancer with herbicides!
148.	1978	Medical Article. Kreeftenberg, et al., "Studies on 2,3,7,8-Tetrachlorodibenzo-P-Dioxin-Induced Immune Suppression and Decreased Resistance to Infection: Endotoxin Hypersensitivity, Serum Zinc Concentrations and Effect of Thymosin Treatment", from Toxicology, Vol. 9, pp. 75-86.

149.	1978	Reggiani, "Medical Problems Raised By the TCDD Contamination in Ceviso, Italy", from ARCH. Toxicol., Vol. 40, pp. 161-188. Comment: One page only was copied which is the abstract of this very lengthy article.
150.	1978	HEW Report: "Bioassay of Picloram For Possible Carcinogenicity", from The National Cancer Institute, Carcinogenesis Technical Report Series, #23. Comment: Pages i-viii only were copied.
151.	1978	Working group of NIEHS and the IARC "Coordination of Epidemiological Studies on the Long Term Hazards of Chlorinated Dibenzodioxin", publication not indicated. Comment: This is a study of the 11/13/53 German accident of a chemical plant exposing workers to the deadly dioxin TCDD. Marked "Strictly Confidential" by Dow.
152.	1978	Buser, et al., "Identification of Substitution Patterns in Polychlorinated Dibenzo-P-Dioxins (PCDDs) by Mass Spectrometry", from Chemosphere, pp. 199-211.
153.	1978	Abstract of article regarding examination of female rat liver after TCDD exposure. Comment: Discusses cytochrome (transporters of electrons to oxygen).
153a.	1978	Wassom, et al., "A Review of the Genetic Toxicology of Chlorinated Dibenzo-P-Dioxins, from Mutation Research, Vol. 47, pp. 141-160.
154.	1978-1979	Document entitled: "Chapter 6, Comparison of the Health Effects of Aquatic Herbicides". Also attached is Milwaukee Centennial article 11/7/80 "Pesticide Use is Still Hot Issue". Comment: This shows that 2,4-D is rapidly absorbed through membranes of the skin, lung and gastrointestinal tract and up to 32% percent of absorbed 2,4-D found in cell nuclei. Makes point that LD50 is

not relevant to low-dose chronic exposure to humans. This article shows that the chemical companies perform a scam on the EPA and the public in the way that they do their tests. Published reviews of this subject are extremely unreliable, quoting other reviews with no original data and thus giving the appearance that many more studies have been done than in fact have.

NOTE: Even though Dow has this document in their files, they carefully did not provide any clue as to date, author or source of publication.

155. 1978-1979 Document entitled: "Chapter 2, Health Effects of 2,4-D and its Derivatives", publication not indicated.
Comment: This shows that 2,4-D is a skin carcinogen or at least a co-carcinogen with benzene which is not carcinogenic by itself in this system. This is a major break through in proving that 2,4-D causes harm and this fact has been hidden by the chemical companies.

156. 3/79 Report entitled: "Assessment of Human Health Risk Associated With the Risk of 2,4-D in Forestry Management", from The Minnesota Department of Health.
Comment: This also explains how both 2,4-D and 2,4,5-T are chemically manufactured. It is a very good explanation.

156a. 3/29/79 Packee, E.C., "2,4-D in the Urban and Suburban Environment in Human Health". Presented to the Nanaimo Parks and Recreation Commission.
Comment: Local government speech to convince people that the key to using herbicides is to minimize one's exposure. Concludes 2,4-D is a safe and necessary substance to use in forests and parks.

157. 5/79 Attached are news articles on the ban of spraying herbicides and a 1/31/80 telegram from Dow regarding the 2,4,5-T Esteron trademark.
Comment: Spraying of 2,4-D in park caused seizure of 21 month old baby in Madison, Wisconsin on 10/10/80 and a 2,4-D ban was

		sought by the public. In another article on 10/6/80, Citizens Pesticide Control Committee in Wisconsin said "The burden of proving safety should be on the industry rather than putting the burden of proving harm on the victim".
158.	after 6/4/79	Dow production specification for 2,4,5-T, mostly illegible. Comment: This shows everything in 2,4,5-T, but it is very hard to read.
159.	6/7/79	Leng, Marguerite of Dow, "Comparative Toxicology of Various Chlorinated Dioxins as Related to Chemical Structure", presented at the Symposium of Collaborative International Pesticide Advisory Counsel. Comment: This is a very damaging document to Dow because it shows how TCDD and PCDD cause cancer!
160.	6/12/79	Ruber, Melvin, "Carcinogenicity of 2,4-D". Comment: Shows that 2,4-D causes cancer in rats!
161.	9/79	Lamberton, et al., "The Determination of Polychlorodibenzo-P-Dioxins in Pentachlorophenol and Wood Treatment Solutions", from Am. Ind. Hyg. Assoc. J., Vol. 4, pp. 816-822.
162.	9/79	Advertisement entitled: "Debating Scientists of Eight Nations Agree That 2,4,5-T is Safe!", from the Dow Chemical Company located in Science Magazine, Vol. 205, pp. 1330. Comment: This is the advertisement that was published after Dow Chemical Company financed and sponsored this conference. They only said 2,4,5-T was safe but failed to mention anywhere in their advertisement that it is the contaminant TCDD that makes 2,4,5-T deadly!
163.	10/20/79	Abstract and attached report entitled: "Reversal of Immunologic and Toxicologic Effects of a Single Exposure of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin in Mice".

Comment: This exhibit goes with exhibit #137.

164.	12/7/79	Letters to the editor: Westing of Hampshire College with rebuttal letter from Etcyl Blair of Dow Chemical. "The safety of 2,4,5-T", from Science, Vol. 206, pp. 1135-1136. Comment: One of "the top 59 world experts responds to Dow's full page add that 59 top world experts found that 2,4,5-T safe! He says that due to the TCDD, it is not safe! (And as far as he is concerned, everyone at the conference of these top 59 world experts stated the same".
165.	1979	Abstractof article on "Study of 121 Dioxin-Exposed Workers Shows No Excess Deaths Over 30 Years". Comment: Monsanto does their own study and says TCDD won't hurt you based on findings of their 1949 chemical plant explosion.
166.	1979	Raggiani, "Estimation of the TCDD Toxic Potential in the Light of the Seviso Accident", from Arch. Toxicol., Vol. 2, pp. 291-302.
167.	1979	Helder, Theo, "Effects of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) on Early Life Stages of the Pike", publication not indicated.
168.	1979	Barsotti, et al., "Hormonal Alterations in Female Rhesus Monkeys Fed a Diet Containing 2,3,7,8-Tetrachlorodibenzo-P-Dioxin", from Bulletin of Environmental Contamination and Toxicology, Vol. 21, pp. not indicated.
169.	1979	Kociba, et al., "Long Term Toxilogic Studies of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) in Laboratory Animals", from New York Academy of Sciences, Vol. not indicated, pp. 397-404.
170.	1979	Article entitled: "Twelve Wise Men Give 2,4-D and 2,4,5-T a Clean Bill of Health", publication not indicated.

Comment: This is a major defense article! This goes with exhibit 136.

171. 1979 — IARC Monographs on the "Evaluation of the Carcinogenic Risk of Chemicals to Humans", Vol. 20, pp. 303, 347-353.
Comment: Shows chemical and physical data of 2,4-D and 2,4,5-T with their histories.

172. 1979 — Kirk, Othmer, Encyclopedia of Chemical Technology, Third Edition, Vol. 5, pp. 797-808 and 866-867.
Comment: Explains chlorophenols as applied to 2,4-D and 2,4,5-T.

173. 1979 — Gregory, Arthur on NIOSH "Occupational Exposure to Herbicides", publication not indicated.

Comment: Great article explaining how TCDD effects you.

174. 1979 (or later) — Wassom, et al., "A Review of the Genetic Toxicology of Chlorinated Dibenzo-P-Dioxins".

174a. 1979? — Homberger, "The Seveso Accident, Its Nature, Extent and Consequences", publication not legible.
Comment: Page 1 only was copied.

174b. Late 1970's — Document entitled: "Section C. Toxicology-Vol. IV".
Comment: It appears to be a list of toxicology studies of herbicides in birds.

174c. 1979? — Poland, et al., "2,3,7,8-Tetrachlorodibenzo-P-Dioxin: Studies on the Mechanism of Action", from McArdle Laboratory for Cancer Research at the University of Wisconsin.
Comment: This is a another great explanation of how TCDD gets to you.

174d. late 1970's — Report entitled: "Section II, 2,4-D in the Aquatic Environment", by Halter of the University of Washington.
Comment: This report explains why 2,4-D kills things!

174e.	late 1970's	Summary prepared by O'Hare "The Effects of Repeated Applications of Symmetrical Tetrachlorodibenzo-P-Dioxin on Guinea Pig Ears". Comment: They killed guinea pigs in this test with TCDD! (Their response is the closest there is to humans.)
174f.	late 1970's	Abstracts of various medical articles.
175.	1/80	Specimen Label and Attached Product Information Insert and Direction for Use on Dow Esteron 2,4,5 Herbicide. Comment: Here, Dow has started marketing Esteron 2,4,5 under another label after 2,4,5-T was banned and then reinstated by the EPA for limited use only. This is apparently Agent Orange being sold under a new Dow trademark (Esteron 2,4,5 Herbicide) in a form that is N-Butyl esters glycol butyl esters. Apparently what happened here is that Dow got caught with a large stock pile of banned Agent Orange herbicide and simply turned around and resold it under a new trade name to unsuspecting users.
176.	1/80	Zack, et al., "The Mortality Experience of Workers Exposed to Tetrachlorodibenzo-P-Dioxin in a Trichlorophenol Process Accident", from Journal of Occupational Medicine, Vol. 22, pp. 11-14. Comment: This is a study of the 1949 Monsanto Chemical Plant accident. The majority of the workers at this plant were exposed directly to TCDD and contracted many ill health effects from the acute exposure. This article is an after the fact attempt to go back and cover up the seriousness of this accident.
177.	1/80	Shearer, et al., "Literature Reviews of Four Selected Herbicides: 2,4-D, Dichlobenal, Diquat and Endothall". Report by the Municipality of Metropolitan Seattle. Comment: This is an excellent study, however in the Dow production documents, they pulled many key pages. A check

		should be made with Seattle authorities to get this publication and determine the full document. Obviously Dow does not want this released and I am sure that it would be very strong against them.
178.	1/3/80	Memo to all Dow 2,4,5-T and Silvex Distributors from McCoy, Dow District Sales Manager, re Esteron Brush Killer, Esteron 2,4,5 and Kuron. Comment: Here Dow is pushing Esteron 2,4,5 (Agent Orange) after 2,4,5-T was banned. They bend in the interpretation from the EPA on what the term "rangeland" uses means. They decide to interpret it in their favor and sell this deadly toxin to unsuspecting users.
179.	1/8/80	MSDS (Material Safety Data Sheet) for Esteron 2,4,5 Brush and Weed Killer.
180.	1/8/80	Dow Material Safety Data Sheet on Esteron 2,4,5.
181.	1/80	Esteron 2,4,5 Herbicide Label.
182.	2/4/80	Memo to Cook from Bond of Dow re: "A Critical Review of: Case Control Study of Malignant Mesenchymal Soft-Tissue Tumors and Exposure to Chemical Substances". Comment: This is a defense oriented article with bias towards the Hardell Swedish studies by Dow Chemical people.
183.	2/8/80	Document regarding Material Safety Data Sheet on Esteron 2,4,5.
184.	2/15/80	Memo from Marguerite L. Leng, D.L. Costillo, et al., of Dow Chemical, regarding Esteron 2,4,5, Esteron Brush Killer and Kuron.
185.	2/18/80	Letter from Garner of EPA to Leng of Dow regarding revised 2,4,5-T registration. Comment: This shows how Agent Orange is hidden in this product (see label!).

186.	2/20/80	Article from Toxic Materials News entitled "California Lawsuits Challenge State Pesticide Rules". Comment: See the 2,4-D article on the miscarriages caused in 9 out of 10 women!
187.	4/80	Article entitled: "Environmental Protection Agency Review and Conclusions Concerning Potential Health Effects of the Herbicide 2,4-D", from The Bureau of National Affairs, Inc., pp. 134-136.
188.	4/22/80	2,4-D Fact Sheet.
189.	4/22/80	Document entitled: "2,4-D Fact Sheet", author not indicated, but appears to be Dow. Comment: This document that was produced from Dow production documents under court order states under section I (background) that 2,4,5-T causes cancer and miscarriages because of the TCDD (dioxin) manufacturing contaminant. If Dow didn't write this, then it had to be the EPA sending this to Dow outside the normal public channels telling them why they were cancelling their 2,4,5-T. In any event, it is a very damaging document to Dow.
190.	5/5/80	Document entitled: "Product Discontinuation - For the Product Esteron 245 Herbicide". Comment: Dow Chemical stops the use of Esteron 245 in the United States in PL and bulk sizes".
191.	5/16/80	Dow R&D Report: "Picloram: Results of a 32-Day Toxicity Tolerance in Feed in Mice", with attached report.
192.	5/30/80	J.W. Henck, et al., Dow R&D Report on 2,3,7,8-Tetrachlorodibenzo-P-DioxinAcute Oral Toxicity in Hamsters". This is a report summary.
193.	6/2/80	Letter to Stone of the EPA from Morgan of Dow re: Esteron 245 Application for Amended Registration.

194.	6/5/80	Dow Quality Assurance printout on Esteron 2,4,5 Brush and Weed Killer. Comment: Esteron 245 has been declared obsolete on 6/2/80.
195.	6/24/80	Dow form of medial information from Esteron 245. Comment: See exhibit 195a below.
195a.	6/24/80	The back page of the document in exhibit 195 which is entitled: "Composition Listing Chemical Ingredients". Comment: This states that Esteron 245 can cause serious illness (from a Dow medical sheet). If gives all chemicals in this product and they contain no inerts! (In other words, nothing is hidden here in what Dow is actually putting in their product. They usually hide the deadly ingredients under inerts and then list some percentage like 35% inerts. In this case we are getting to see for the first time everything that Dow puts in their Agent Orange product.)
196.	6/80	No author, apparent summary of report "TCDD Oral and Dermal NIC Bioassay Summary", June, 1980, no page number. Comment: Concludes that TCDD is a carcinogen from both oral and skin absorption.
197.		Document entitled "Appendix E": All registrants (by company name) with registered products containing 2,4-D. Comment: This shows the manufacturer's name and the use of these 2,4-D products.
198.	7/14/80	This is a copy of the previous document (exhibit 197) regarding "List of Your Company's Products Containing Some Form of 2,4-D". Comment: This is Dow's 43 different versions that they manufacture the 2,4-D product with many of them containing 2,4,5-T as an active ingredient (up to a 50/50 mixture of 2,4-D and 2,4,5-T which was Agent Orange).

NOTE: The EPA had already suspended 2,4,5-T at the time of this document in the United States except for specified limited uses. The point here is that you would believe that you were buying a 2,4-D product which is perfectly safe only to find it has within it a 50/50 mixture of the ingredients that make up the deadly Agent Orange. This document also lists the toxicity level of these various products and some of them are high toxic levels that would cause serious health effects.

198a. 9/23/80 Department of the Interior Transmittal sheet with the attached Pesticide Use Policy.
Comment: This prohibits the use of 2,4,5-T! No explanation is given as to why they did this.

199. 9/8/80 Transmittal memo at Dow for information received from the EPA regarding their 2,4-D study.

200. 9/12/80 The Carcinogen Assessment Groups Risk Assessment on 2,4,5-T, Silvex and TCDD.
Comment: These are summary pages from the document giving the bottom line evaluation by this industry based group.

201. 10/14/80 Ramsey, et al., "Dose Levels of 2,4-D in Forest Workers", report prepared for Dow.
Comment: Dow had a slanted test conducted at the University of Arkansas on forest workers being exposed to the herbicide 2,4-D through aerial sprays. This is nothing like a real use environment where the workers were sprayed by both 2,4-D and 2,4,5-T and had their clothes completely soaked and wet and after working all day in this environment. These were merely short term exposures in a very limited environment set up to prove that 2,4-D was safe.

202. 10/23/80 News release from Agriculture Canada entitled: "2,4-D Tests".
Comment: This shows that the Canadian government discovered a dioxin contaminant in 2,4-D and they were considering

		suspension of this product throughout Canada. It is a less toxic form of dioxin than the one found in TCDD, however it is still a deadly poison that his harmful to humans.
203.	10/27/80	Transcript of "Radio Noon" show entitled: "Dioxin Levels in 2,4-D and 2,4,5-T". Comment: This is an ongoing debate taking place in the news media regarding the concern of the Canadian government over the dioxin levels in these herbicides. There is a major public outcry to ban both of these products.
204.	10/80	Document regarding Wayne Omerod's press release. Comment: This was prepared by the Canadian government trying to decrease the level of alarm being felt in the public regarding the disclosure of these deadly dioxins in the herbicides 2,4-D and 2,4,5-T.
205.	10/80	Coversheet entitled: "Determination of 2,4-D Exposure Received by Forestry Applicators, Spring, 1980" by Lavy of the University of Arkansas in cooperation with Dow. The report is not attached.
206.	11/14/80	Letter to Edwin Johnson of the EPA from John Hall of the National Forest Products Association re: The research report entitled "Determination of 2,4-D Exposure Received by Forestry Applicators, Spring 1980".
207.	11/24/80	Short article entitled: "Park Service Halts Use of 2,4-D", from C&EN Publication.
208.	1980	Van Logten, et al., "Role of the Endocrine System in the Action of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) on the Thymus", from Toxicology, Vol. 15, pp. 135-144.
209.	1980	Murakami, et al., "Incorporation of Labelled Pesticides and Environmental

		Chemicals Into Nuclear Fraction of Cultured Human Cells", from Bulletin of Environmental Contamination and Toxicology, Vol. 24, pp. 27-30. Comment: Carefully look at the attached last page of this document from a news clipping out of Science magazine.
210.	1980	Section from exhibit by Domenico, et al., "Accidental Releases of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) at Seviso, Italy", from Eco-Toxicology and Environmental Safety, Vol. IV, pp. 327-328. This particular document is entitled "TCDD Levels in Atmospheric Particles".
211.	1980	Newspaper article entitled "Dioxins Found in 2,4-D Alarm Canada".
212.	1980	Various newspaper articles including one entitled: "Massive Rise in Cancer Deaths Predicted" (from the dioxin found in 2,4-D in Canada).
213.	1980	Canadian newspaper articles regarding dioxins being found in 2,4-D. Comment: Talks of a man who had his arm amputated (after the use of 2,4-D/2,4,5-T) for cancer plus another article on "Doctored Chemical Tests and Wide-Ranging Dishonesty in Labs".
214.	1980	Document from Health and Safety Alert entitled "Beware of the Weed Killer 2,4,5-T and 2,4,5-TP". Comment: The Canadian Union is warning its workers to not use 2,4,5-T because it is a health hazard with increased cancer risks of the muscle and the tissue.
215.	1980	Lamb, et al., "Evaluation of 2,4-D, 2,4,5-T and 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) Toxicity in Mice: Reproduction and Fertility in Treated Male Mice and Evaluation of Congenital Malformations in Their Offspring", publication not indicated. Comment: This is supposed to be a study for the effects of "Agent Orange", but Dow

provided the samples for the test and removed all TCDD impurities!

216.	1980	Sencuzk, Watold, translation by Lilienheim in November, 1981, "Absorption of 2,4-D Through the Skin", from Warsaw Panstwowy Zakled Higieny. Roczniki., Vol. 31, pp. 611-614. Comment: This shows that the period of exposure determines the amount of 2,4-D that will be absorbed through a given individuals skin, i.e. its all a question of the duration of use of this product in a given period of time.
217.	1980	Marcel, Bickel & Stefan, Muhlebch, "Pharmacokinetics and Ecodisposition of Polyhalogenated Hydrocarbons: Aspects and Concepts", from Drug and Metabolism Reviews, Vol. 11 #2, pp. 149-190, 1980. Comment: Selected pages--with the conclusion page showing a whole new dimension in the ecotoxicology that has been opened by the use of the herbicide 2,4,5-T with its contaminant TCDD through the food chain of the global ecosystem.
218.	1980	Arstila, et al., "An Epidemiological Feasibility Study on the Effects of 2,3,7,8-Tetrachlordibenzo-P-Dioxin(TCDD) on Finnish Forestry Road and Railway Workers", publication not indicated. Comment: This document is marked "strictly confidential" by Dow Chemical.
219.	1980	Editorial entitled: "The Burial of the Two Million Dollar Teaspoon", publication not indicated.
220.	-0-	VACANT
221.	1/7/81	R & D report from Dow re: "Technical Grade 2,4-D: Results of a 13-Week Sub-Chronic Dietary Toxicity Study in CDF Fisher 344 Rat", by Gorzinski, et al.
222.	1/7/81	A copy of the report referenced in exhibit 221.

223.	1/27/81	Text of press release by Dow in Ottawa, Canada outlining measures to phase out 2,4-D herbicide products.
224.	1/28/81	EPA weekly report of the Office of Pesticides and Toxic Substances entitled: "Canada to Phase Out Some 2,4-D Products".
225.	Jan/Feb 1981	Pazderova-Vejlupkova, et al., "The Development and Prognosis of Chronic Intoxication By Tetrachlorodibenzo-P-Dioxin in Men", from Archives of Environmental Health, Vol. 36, pp. 5-11.
226.	1/81	S. Libich, "A Study to Assess the Occupational Exposure to 2,4-D Herbicides". Ontario Hydro Safety Services Department Booklet. Comment: This study shows "at least 35 times more 2,4-D is absorbed through the skin than is inhaled with the breathing of the air". It says to change clothes as soon as possible after using 2,4-D and wash all exposed skin areas immediately.
227.	3/81	Hayes, Mary Catherine, American Counsil on Science and Health, "The Health Effects of Herbicide 2,4,5-T". Comment: This is a white wash job done by the industry to save 2,4,5-T.
228.	3/81	Four page document including chronology of events involving 2,4,5-T and herbicide reduction statistics. Comment: This says that the 2,3,7,8 TCDD impurity in the 2,4,5-T was made known (to the public!). It also says "2,4,5-T kills plants because it interferes with the cell division (mitosis) and cell enlargement, food utilization and various other processes necessary to stabilize life.
229.	4/30/81	Allatair, Hay, "Secrecy at Coalite" supertitled: "Dioxin Hazards". Also abstract "Dioxin, A Sarcoma Risk", from Nature, Vol. 290, April 30, 1981, p. 729. Comment: This document connects TCDD with Sarcomas!

230.	4/81	"Worker's Exposure to 2,4-D Study", from Research News of the Department of Agriculture. Comment: The results shown here are that absorption is directly related to the type of job, the amount of herbicide applied and the length of time it is handled. Those who mixed and loaded these herbicides have the highest total absorption, i.e. the results are based on the time of exposure, the amount of chemicals handled and the type of job.
231.	6/2/81	Dow Quality Assurance print out for Esteron 2,4,5 Herbicide.
232.	10/16/81	Document entitled: "Manufacturing Specification", apparently a routine slip, Esteron 2,4,5 BE Herbicide, handwritten notes, mostly illegible.
233.	12/81	Dow document entitled: "Medical Information Regarding Esteron 2,4,5 BE Herbicide, Esteron 2,4,5 Brush and Weed Killer and Esteron 2,4,5 Concentrate Brush and Week Killer".
234.	1981	Report to the Council on Scientific Affairs of the AMA on "Health Effects 'Agent Orange' and Dioxin Contaminants" presented by Dr. Dolan. Comment: This is a review of how Agent Orange was made and states that TCDD is the direct and sole cause of chloracne (see page 3 of document).
235.	1981	Abstract from the Chemical Regulation Reporter entitled: "Dioxins: Second Dow Sarcoma Case Found; Company Suggests Smoking as Factor". Comment: Four chemical workers had died of sarcoma, two were employees of Dow and were exposed to TCDD resulting in chloracne immediately. The other two workers were employees of Monsanto. They tried to white wash this exposure of their own workers who died of cancer and blame it on cigarette smoking.

236.	1981	Crow, K.D., "Chloroacne and Its Potential Clinical Implications", from Clinical and Experimental Dermatology (1981), Vol. 6, pp. 243-257. Comment: "It is essential to recognize that chloroacne appears to be the most sensitive indicator of poisoning in man". The document goes on to say that chronic exposures are the most serious to man.
237.	4/2/81	Two pages of handwritten notes regarding Esteron 2,4,5-T and comparing its acid equivalent with other weed killers.
238.	?1/27/81	Mostly illegible teletype regarding 2,4-D.
239	?1981	News article entitled: "Dioxin Haunts EPA Refuse Plans".
240.	1/12/82	Letter from M.D. Black, California State Department of Food and Agriculture. To: R.W. Colby of Dow Chemical re: Esteron Brush Killer and Esteron 2,4,5 Herbicide and Sub-Registration to Union Carbide. Comment: It appears that Dow got rid of its excess 2,4,5-T by subregistering it under a Union Carbide product!
241.	3/19/82	Letter from B.T. Buzelli to A.E. Schober, both of Dow Chemical, regarding Esteron 2,4,5 BE-TCDD analysis. Comment: THIS IS A SMOKING-GUN DOCUMENT! Here Dow knowingly sells a defective Esteron 2,4,5 BE product to consumers and hides the defects from them! This shows TCDD contamination in a 635,000 pound tank plus an additional 16,500 pound shipment that had already been packaged in 55 gallon drums.
242.	3/23/82	Document titled: "Dow Chemical Company Quality Assurance Production Specification for Esteron 245 BE Herbicide".
243.	3/24/82	Letter from Authur Schober of Dow Chemical to T.E. Adamczyk of the EPA regarding Esteron 245 BE.

244.	3/82	Several letters between Dow Chemical and the EPA regarding Esteron 245 BE. Also attached is a certification statement regarding Esteron 245 BE. Comment: Dow is pulling something here! They are also selling a banned product under a different label!
245.	4/8/82	Dow Chemical documents, "Production Specifications Regarding Esteron 245 C Herbicide".
246.	4/13/82	Document titled: "Dow Chemical Company Quality Assurance Production Specification for Esteron 245 BE Herbicide, Rough Draft.
247.	4/16/82	Document titled: "Manufacturing Specification", cover routing slips, Esteron 245 BE Herbicides, handwritten notes.
248.	5/19/82	Material Safety Data Sheet on Esteron 245 BE Herbicide.
249.	5/21/82	Memo of Dow Chemical to R.L. Eisher, et al., from Jim Havel re: Meeting Regarding Esteron 245 BE Quality and Options. Comment: Here sludge is found in Esteron 245 BE cans. Dow concludes "There is no way to burn or dispose of the technical ester. It must be sold". This was sludge found in 55,000 gallons of their Esteron 245 BE product.
250.	5/24/82	Document titled: "Dow Chemical Company Quality Assurance Production Specification for Esteron R 245 BE Herbicide". Comment: This is the final version of document 242 where it shows that 245 BE is the same as 2,4,5-T and is being sold "only to deplete current stock of this technical ester" (2,4,5-T)!
251.	8/3/82	Report by Dow Chemical, Latin American on Esteron 245 C Herbicide. Comment: This shows that 2,4,5-T is being distributed to Latin America after being banned in the U.S.! There is no mention made here cautioning the user of the TCDD

		contaminants in this. It is noted that they make many precautionary safety statements for all of the other ingredients in this product but fail to mention the deadly TCDD!
252.	9/82	Letters between EPA and Dow Chemical regarding 2,3,7,8 TCDD and Esteron 245 BE. Comment: Dow is hiding the TCDD results from the EPA here. (see exhibit 241)
253.	1982	Labels for Dow Esteron 245 and Esteron 245 BE. Comment: This is the banned 2,4,5-T with a changed name and slight modification of ingredients on the label to get around the EPA ruling.
254.	1982	Corte, et al., "2,4-D Induced Clastogenicity and Elevated Rates of Sister Chromatid Exchanges in Cultured Human Lymphocytes", from Journal of Heredity, Vol. 73, pp. 224-226. Comment: This may be a very significant document. You need to have Dr. Teitelbaum review this and determine its significance.
255.	1982	Draper, et al., "Applicator Exposure to 2,4-D, Dicamea and a Dicamba Isoxer", J. Environ. Sci. Heath, Vol. 4, pp. 321-339.
256.		VACANT
257.	1982	Michigan State Department of Agriculture Research Grant entitled: "Effects of Pesticides on the Kidney" by Hook and Serbia, at The Center For Environmental Toxicology at Michigan State University; East Lancing, Michigan. Comment: This is a study of 2,4,5-T and TCDD effects.
258.	1982	EPA Form 8570-10, November, 1974, titled: "Label Technical Data" regarding Esteron 245 BE.

259.	2/21/83	Series of letters between Jim Havel and Ralph Antz of Dow Chemical regarding use directions for Esteron 245 B. Comment: This document is very damaging to Dow because it shows them selling the defective Esteron 245 BE and not wanting any of it returned to Midland, Michigan. A suggestion is made to "blend it back into a straight 245-T product at the user level".
260.	4/22/83	Dawn Giaradin, document titled: "Customer Request", refers to determination of 2,3,7,8 TCDD. This exhibit also contains a letter of 5/19/82 regarding Esteron 245 BE and TCDD analysis and also contains a letter from the EPA regarding TCDD analysis and a memo dated 8/5/81 headed "Distribution Emergency" referring to Esteron 245. Comment: There is something very unusual happening here, but it is hard to figure it all out!
261.	5/4/83	Melvin Dwane Ruber, "Carcinogenicity and Toxicity of 2,4-D", from The Science of the Total Environment, Vol. 31, 1983, pp. 203-218. Comment: This document states "2,4-D is carcinogenic in male and female rats; 2,4-D is also mutenagenic and teratogenic in animals and causes poisoning in animals and human beings". (Underlining was mine.)
262.	5/83	Kenneth G. Torrington, M.D., letters to the editor titled: "Herbicide Exposure and Pulminary Disease", from Journal of Occupation Medicine, May 1963, Vol. 25, #5. Comment: This is an unusual exposure case; a freight train was pushing 2,4-D tank cars while performing railroad foliage clearing into a strong headwind. The spray got blown back all over the train engineer and the conductor causing serious acute health effects in them!

263.	6/83	Incident report on Esteron 2,4,5 by Mrs. Gerald Grove regarding her husband; message taken by an M.S. Betty Schmidt.
264.	7/28/83	Document was a Dow R&D report that I only used to copy the distribution page on the back to show the worldwide offices of Dow Chemical Company.
265.	8/83	No author, "Dioxin and Human Health", apparently the full report referred to in exhibit #267. Comment: This is the full report of the white wash put out apparently by Dow to create the illusion that human health was not being affected by these dioxins.
266.	1983	Easley, Carol Bryan, et al., "Laundering Procedures for Removal of 2,4-Dichlorophenoxyacetic Acid Ester and Amine Herbicides From Contaminated Fabrics", from Archives of Environmental Contamination and Toxicology, Vol. 12, pp. 71-76. Comment: This is a very important document in that it shows how 2,4-D and 2,4,5-T molecules are more readily absorbed through the skin, especially in wet herbicide soaked clothes! The major route of pesticide exposure in absorption is through the skin based on this study and not the respitory system because only small particles can enter the lungs, but larger more numerous particles settle on the surfaces of the skin and your clothing. Hence, attention has focused on clothing items which cover and protect skin surfaces. In this document they study the laundering procedures for the removal of 2,4-D.
267.	1983	Bob Charlton, Dow Chemical, "Dioxin and Human Health Executive Summary". Comment: Major Dow white wash on the public presenting a distorted and misstated version of the truth.
268.	1/84	Libich, et al., "Occupation Exposure of Herbicide Applicators to Herbicides Used

		on Electrical Power Transmission Line Right of Way", from American Industrial Hygienist Association Journal, Vol. 45, pp. 56-62. Comment: This shows that exposure through the skin is the major absorption route creating up to 50 times greater than exposure than by the inhalation route when using a hand gun sprayer. During the handling and mixing of the concentrated chemical, **A VERY SMALL SKIN EXPOSURE CAN BE EQUIVALENT TO A FULL DAY OF FIELD EXPOSURE.**
269.	4/84	Dow report entitled: "Dioxin, Agent Orange and Human Health". Comment: This is how Dow will defend the technical issues in a lawsuit. (71 pages) It was published one month prior to the start of the Agent Orange trial litigation!
270.	4/84	Puhvel, S.M., et al., "Increased Epidermal Transglutaminase Activity Following 2,3,7,8-Tetrachlorodibenzo-P-Dioxin In Vivo and In Vitro Studies With Mouse Skins", from Toxicology and Applied Pharmacology, Vol. 73, pp. 311-322, 1984. Comment: This document says "in humans one of the first signs of exposure to TCDD is the development of a skin condition known as chloroacne".
271.	1984	Hosman, et al., "Results From 86 Two-Year Carcinogenicity Studies Conducted By the National Toxicology Program", from The Journal of Toxicology and Environmental Health, Vol. 14, pp. 621-639. Comment: This document shows how misleading many of the tests have been in showing us the true picture of these health hazards!
272.	9184	Section from The Clinical Toxicology of Commercial Products, Williams and Wilkens publishers, 5th Edition, with a page on the chemical components of Esteron. Comment: This shows the 1984 marketing of the Dow 2,4-D and 2,4,5-T products under the trade name of "Esteron"!

273.	11/15/85	Elvira Spill, translated by Anita Knight and L.P. McCarthy, "The Lingering (Insidious) Contamination", subtitled: "Dioxins Have Left Their Mark on Children from Seveso Nine Years Ago". The original German article is attached.
274.	1985	John D. Constable and Maureen C. Hatch, "Reproductive Effects of Herbicide Exposure in Vietnam: Recent Studies by the Vietnamese and Others". Tetratogenisis Carcinogenesis and Mutagenesis, Vol. 5, pp. 231-250. Comment: This reports increases in miscarriages, still births, molar pregnancy and birth defects from the Vietnamese exposed during the war.
275.	1986-1987	Thompson, W.T., Agricultural Chemicals Book II - Herbicides. Section entitled: "2,4,5-T; Dacmine, 4-T; Envert-T; Esteron 245; Phortox; Reddon; Tippon; Trioxone; Veon". Comment: This gives the chemical makeup and use applications of the various herbicides.
276.	9/7/88	This letter talks about research done at Texas Tech University Health Sciences Center on labels and product information for the Dow Esteron 245 (1980 time frame) and Esteron 99 concentrate (1982 time frame). Comment: This is a good background on 2,4-D and 2,4,5-T, plus their chemical makeups, the EPA cancellation of 2,4,5-T and various health data sheets.
277.	12/87	Draft for Toxilogical Profile for 2,3,7,8-Tetrachlorodibenzo-P-Dioxin from the U.S. Public Health Service. Comment: This document is 122 pages long and is a good overview of what TCDD is.

EXHIBIT NO.	IDENTIFIED	ADMITTED	DATE	DESCRIPTION
1			1978	Medical article. Kreeftenberg, et al. "Studies on 2,3,7,8-Tetrachlorobibenzo-p-dioxin-Induced Immune Suppression and Decreased Resistance to Infection: Endotoxin Hypersensitivity, Serum Zinc Concentrations and Effect of Thymosin Treatment". Toxicology 9 (1978) 75-86.
2			1979	Medical article. Luster, et al. "Effects of Brief In Vitro Exposure to 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) on Mouse Lymphocytes". Journal of Environmental Pathology and Toxicology 2:956-977.
3			1978	Medical article. Carere, et al. "Microbiological Mutagenicity Studies of Pesticides In Vitro" Mutation Research 57 (1978) 277-286.
4			8/18/80	Dow Chemical Company Certificate of Analysis of Esteron 245 Brush and Weed Killer for Customer Imperial, Inc.
5			10/12/81	Computer printout entitled: "Dow Chemical Medical Department Chemical Exposure History by DR-Number".
6			6/14/73	R &D Report from Dow Chemical transmitting an attached copy of a report entitled: "Picloram-Impact on the Environment (EPA PR Notice #70-15)" by Lawrence Southwick of Dow Chemical.
7			10/13/71	Report from Pierce and Ens of the Organic Chemicals Production Research Department of Dow titled: "Origin and Fate of the Chlorodioxins. Abstracts From a Symposium at the 162nd National ACS Meeting, Washington DC, September 16-17, 1971"
8			00/00/00	Untitled document appearing to be the report referenced in Exhibit No. 7.
9			12/18/72	Cover memo and abstract written by Marguerite Leng. Attached is report entitled: "Fate and Safe Use of Tordon in the Environment". Paper is dated July 1969 and was submitted to the EPA.
10			10/20/79	Abstract and attached report entitled: "Reversal of Immunologic and Toxicologic Effects of a Single Exposure of 2,3,7,8-Tetrachlorobibenzo-p-dioxin in Mice".

		Authored by Sharma, et al., of Dow.
11	8/20/79	R & D Report abstract and attached report entitled: "Immuno-Toxicologic Studies Involving 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) In Mice and Rabbits" by Sharma, et al., of Dow.
12	1962	Translation of attached French documents entitled: "Polyneuritis After Using a Weed Killer: Containing Acid 2-4-D" by Foissac, et al., Lille Medical.
13	4/ 2/81	Handwritten notes entitled: "Total 2,4,5-T Acid Plus Estern (1982)".
14	00/00/00	Two pages of hand-written notes regarding Esteron 2,4,5-T and comparing its acid equivalent with other weed killers.
15	1/22/80	Information on Esteron 245 from Wolfe of Quality Assurance.
16	8/26/81	Article entitled: "Important Issues Remain Unresolved in Avian Environmental Standards" from the Pesticide and Toxic Chemical News. Also included is an article entitled: "2,4-D and Spontaneous Abortions: Questions Raised by Survey".
17	4/80	Article entitled: "Environmental Protection Agency Review and Conclusions Concerning Potential Health Effects of the Herbicide 2,4-D", from the Bureau of National Affairs, Inc., pp. 134-136.
18	1976	Smith, et al. "Teratogenicity of 2,3,7,8-Tetrachlorobibenzo-p-dioxin in CF-1 Mice" Toxicology and Applied Pharmacology, Vol. 38. 517-523.
19	1970	Norris, Logan. "Degradation of Herbicides in the Forest Floor" reprinted from Tree Growth and Forest Soils, Oregon State University Press, pp. 397-411.
20	1/27/81	Text of press release by Dow in Ottawa outlining measures to phase out 2,4-D Herbicide Products.
21	00/00/00	Mostly illegible teletype regarding 2,4-D.
22	1979	Aitio, et al. "Different Effect of 2,3,7,8-Tetrachlorobibenzo-p-dioxin On Glucuronide Congugation of Various Aglycones. Studies in Wistar and Gunn Rats" Toxicology and Applied

		Pharmacology, 47, 55-60.
(23)	12/14/70	Dow report entitled "The Determination of 2,4,5-Trichlorophenol and 2,4,5-Trichlorophenoxyacetic Acid in Blood and Urine by Gas Liquid Chromatography".
(23A)	5/19/82	Material Safety Data Sheet on Esteron 245 BE Herbicide.
(24)	1978	Somers, et al. "Reproductive Success of Hens and Cockerels Originating Sprayed with 2,4-D, 2,4,5-T and Picloram Followed by Early Performance of Their Progeny After a Comparable In Ovo Exposure". Journal name illegible.
(25)	2/9/76	Abstract and attached report entitled: "The Fate of 2,4-Dichlorophenoxyacetic Acid (2,4-D) Following Oral Administration to Man". By Sauerhoff, et al.
26	July/ August, 1973	Feung, et al. "Metabolism of 2,4-Dichlorophenoxyacetic Acid. V. Identification of Metabolites in Soybean Callus Tissue Cultures". Agricultural and Food Chemistry, Vol. 21, pp. 637-640.
(a)	5/29/58	Hymas of Dow. "Toxicity of Herbicides to Domestic Animals".
27	00/00/00	Cook, et al., of Dow. "Mortality Experience of Employees Exposed to 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD)". Publication not indicated.
(28)	1980	Fanelli, et al. "Presence of 2,3,7,8-Tetrachlorobibenzo-p-dioxin in Wildlife Living Near Seveso, Italy: A Preliminary Study". Bulletin of Environmental Contamination and Toxicology. 24, 460-463.
(29)	00/00/00	Editorial entitled: "The Burial of the $2,000,000 Teaspoon". Publication not indicated.
(30)	5/ 6/80	Bezuglyi, et al. "Clinic for Delayed Consequences of Acute Contamination From 2,4-Dichlorophenoxyacetic Acid". Translation of attached Russian Article.
(31)	3/79	Abstracts from the Society of Toxicology in New Orleans. Papers entitled: "Toxicological Effects Produced in Non-Human Primates Chronically Exposed to 50 Parts Per Trillion 2,3,7,8-

		Tetrachlorobibenzo-p-dioxin (TCDD)", and "Toxicological Responses of Non-Human Primates to 2,3,4,6,7-H-Hexachlorodibenzo-p-dioxin".
32	1/28/81	EPA Weekly Report of the Office of Pesticides and Toxic Substances entitled: "Canada to Phase Out Some 2,4-D Products".
33	1/23/81	Document entitled: "Fact Sheet on 2,4-D", author and source not indicated.
34	00/00/00	News article entitled: "Dioxin Haunts EPA Refuse Plans".
35	11/24/80	Short article entitled: "Park Service Halts Use of 2,4-D", from C&EN Publication.
36	8/26/80	Article from the Maine Times entitled: "Picloram's Turn: A Basic Philosophical Difference is Revealed".
37	5/ 5/80	Product Discontinuation-Record of Approval Form on Esteron 245 Herbicide.
38	5/ 5/80	Product Discontinuation-Record of Approval Form on Esteron 245 Herbicide.
39	1980	Gasiewicz, et al. "The Effect of Total Parenteral Nutrition on the Toxicity of 2,3,7,8-Tetrachlorobibenzo-p-dioxin in the Rat". Toxicology and Applied Pharmacology 54, 469-488.
40	1/80	Specimen label and attached product information insert and direction for use on Dow Esteron 245 Herbicide.
41	1974	Poland, et al. "Comparison of 2,3,7,-Tetrachlorobibenzo-p-dioxin, A Potent Inducer of Aryl Hydrocarbon Hydroxylase, with 3-Myethylcholanthrene". Molecular Pharmacology, 10, 349-359.
42	8/25/76	Poland, et al. "Stereospecific, High Affinity Binding of 2,3,7,8-Tetrachlorobibenzo-p-dioxin by Hepatic Cytosol". The Journal of Biological Chemistry, 251, 4936-4946.
43	10/76	Poland, et al. "2,3,7,8-Tetrachlorobibenzo-p-dioxin: Environmental Contaminant and Molecular Probe". Federation Proceedings, 35, 2404-2411.

		(2,4-D and 2,4,5-T)" Vetenskaplig Skriftseryie 17, 1-24.
53	1979	English summary of Exhibit No. 52.
54	1970	Matsumura, Akihito. "The Fate of 2,4,5-Trichlorophenoxyacetic Acid in Man" Japanese Journal of Industrial Health, Vol. 12, October 1970. 446-451.
55	7/78	Yoshida, et al. "Occupational Exposure Measurement of an Arial Spray Crew to a Mixed Spray of 2,4-D and 2,4,5-T" Pages 1-26.
56	1980	Thunberg, et al. "Effects of 2,3,7,8-Tetrachlorobibenzo-p-dioxin on the Hepatic Storage of Retinol in Rats with Different Dietary Supplies of Vitamen A (Retinol)" Arch Toxicol 45, 273-285.
57	1/ 7/81	R & D Report from Dow re: "Technical Grade 2,4-Dichlorophenoxyacetic Acid (2,4-D): Results of a 13 Week Sub-Chronic Dietary Toxicity Study in the CDF Fischer 344 Rat" by Gorzinski, et al.
58	00/00/00	A copy of the report referenced in Exhibit 57.
59	1978	Reggiani. "Medical Problems Raised by the TCDD Contamination in Seveso, Italy". Arch Toxicol. 40, 161-188.
60	00/00/00	Document entitled "Section C. Toxicology-Volume IV". It appears to be a list of toxicology study of herbicides and birds.
61	6/73	Document entitled: "Summary of Toxicology Studies with 2,4-D in Birds".
62	7/73	Document entitled: "Section D. The Results of Tests on the Amount of Residue Remaining, Including a Description of the Analycial Method Used".
63	8/15/63	Palmer, J. S. "Chronic Toxicity of 2,4-D Alkanolamine Salts to Cattle". Journal of the American Veterinary Medical Association, Vol. 143, page 398-399.
4	1963	Dolgarrd, Mikkelsen, et al. "Toxicity of Herbicides" Pharmiceutical Review 14, 225-250.
65	1978	HEW Report: "Bioassay of Picloram for Possible Carcinogenicity" from the

		National Cancer Institute, Carcinogenesis Technical Report Series, No. 23.
66	7/27/65	"Report to the Dow Chemical Company Two Year Chronic Oral Toxicity of Tordon-Beagle Dogs" Industrial Bio-Test Laboratories.
67	10/21/65	"Report to the Dow Chemical Company. Chronic Oral Toxicity of Tordon-Albino Rats" Industrial Bio-Test Laboratories, Inc.
a	1/15/63	"Toxicological Properties of Tordon" prepared
68	10/23/64	"Results of 90 Day Dietary Feeding Studies of Triisopropanolamine Salt of 4-amino-3,5,6-trichloropicolinic Acid in Rats" by Dow.
69	4/18/62	Bucek, et al. of Dow. "Effect of 4-amino-3,5,6-trichloropicolinic Acid and of 4-amino-3,5-dichloropicolinic Acid in Growing-Fattening Swine Rations".
70	2/27/63	Briscoe of Pittman, Moore Research Laboratories. "Acute Pharmacotoxicologic Tests with Tordon Acid".
71	11/ 8/63	"The Metabolism of Tordon Herbicide by the Dog", by Dow.
72	1/28/64	"The Metabolism of Tordon Herbidice by the Rat", by Dow.
73	12/14/64	US Department of Interior, Fish and Wildlife Service. "Procedure for Evaluation of Acute Toxicity of Pesticides to Fish and Wildlife".
74	1/ 8/80	MSDS for Esteron 245 Brush and Weed Killer.
75	1967	Youngson, et al. "Factors Influencing the Decompositions of Tordon Herbicide in Soils". Down to Earth (A Dow Chemical Company Publication) Vol. 23, pp. 3-11.
76	1969	Kutschinski, et al. "Residues in Various Tissues of Steers Fed 4-amino-3,5,6-trichloropicolinic Acid" Publication not indicated. Could be a Dow publication.
77	12/10/69	Bjerke, et al. "Residues of Picloram in Sheep Tissues" from Dow.

78	1/64	"Elimination of 2-Methyl-4-Chlorophenoxyacetic Acid and 4-(2-Methyl-4-Chlorophenybutyric) Acid in the Urine from Cows". Journal of Dairy Science, Vol 47, pp. 93-95. Author not indicated.
a	1/ 4/80	Kimble, et al. "Tetrochlorodibenzo-p-dioxin Quantitation in Stack-Collected Coal Fly Ash" Science Vol. 207, pp. 59-61
79	1979	Raggiani. "Estimation of the TCDD Toxic Potential in the Light of the Seveso Accident". Arch Toxicol. Vol. 2, page 291-302.
80	6/28/79	Dialog search.
a	11/ 6/73	Crosby, et al. "Photo Decomposition of P-chlorophenoxyacetic Acid" Agr. Food Chem. Vol. 21, pp. 1049-1052.
81	illegible	Homberger. "The Seveso Accident, Its Nature, Extent and Consequences". Publication--not legible.
8	9/23/60	Dept. of the Interior Transmittal Sheet with the attached pesticide use policy.
83	9/30/80	Telex to: Skinner of the Honolulu sales office. From: Ytzen. Re: prices on various Dow herbicides.
84	1970	Rivers, et al. "Simultaneous Gas Chromatograpic Determination of 2,4-D and Dicamba Ir. Human Blood and Urine". J. Chromatog, 50, pp. 334-337.
85	1/74	Somers, et al. "Effect of External Application of Pesticides to the Fertile Egg on Hatching Success and Early Chick Performance 1. Princubation Spraying with DDT and Commercial Mixtures of 2,4-D, Picloram and 2,4-D, 2,4,5-T". Bulletin of Environmental Contamination and Toxicology, Vol. 11, pp. 33-38.
86	1978	German Medical Article. See Exhibit 87.
87	10/21/78	Rehder, et al. "Pathological-Embryological Investigations in Cases of Abortion Related to the Seveso Accident". Journal of Swiss Medicine, Vol. 108, pp. 16, 17-25.
88	1978	Buser, et al. "Identification of Polychloronated Dibenzo-p-dioxin Isomers

		Found in Fly Ash". Chemosphere No. 2, pp. 165, 172.
89	1978	Buser, et al. "Identification of Substitution Patterns in Polychloronated Dibenzo-p-dioxins (PCDDS) by Spectrometry". Chemosphere No. , pp. 199-211.
90	5/ 4/77	Memo from Johnson of the EPA, to dioxin implimentation task force. Re: Dioxin: Position Document.
91	5/26/77	Draft of EPA's dioxin working group document entitled: "Dioxin: Position Document".
92	5/26/77	Last three pages of Exhibit 91.
93	9/ 1/71	Johnson, Julius. "The Public Health Implication of Widespread Use of the Phenoxy Herbicides and Picloram" Bioscience, Vol. 21, pp. 899-905.
94	1976	Courtney, K. Diane. "Mouse Teratology Studies with Chlorodibenzo-p-dioxins" Bulletin of Environmental Contamination and Toxicology, Vol 16, pp. 674, 681.
95	1977	Alie, et al. "Chlorodibenzo-p-dioxins, and Chlorodibenzo-furans are Trace Components of Fly Ash and Flue Gas of Some Municipal Incinerators in the Netherlands". Chemosphere, No. 8, pp. 455-459.
96	1978	Buser, et al. "Identification of Substitution Patterns in Polychloronated Dibenzo-p-dioxins (PCDDS) by Spectrometry". Chemosphere No. , pp. 199-211.
97	12/13/78	"Human Birth Defects Not Caused by 2,4,5-T or 2,4-D Exposure, Report Says". Pesticide and Toxic Chemical News, pp. 23-24.
98	1979	Helder, Theo. "Effects of 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) On Early Life Stages of the Pike" Publication not indicated.
99	8/28/74	New York Times Article. "Death of Animals Laid to Chemical". Attached is a 2/28/80 Dow memo to the State Dept. of Agriculture regarding the label for Esteron 245 Herbicide.

100	May-June, 1967	Bjerke. "Determination of Residues of 4-amino-3,5,6-trichloropicolinic Acid in Cereal Grains by Gas Chromatography". J. Agr. Food Chem., Vol. 15, pp. 469-473.
101	1969	Way, J. M. "Toxicity and Hazards to Man, Domestic Animals and Wildlife from Some Commonly Used Auxin Herbicides". Publication illegible.
102	2/13/80	Letter from Garner of the EPA to Leng of Dow re: Revised Labelling for Various Herbicides Including Esteron 245".
103	1/21/80	Letter from Leng of Dow, to Garner of the EPA regarding revised labelling.
104	8/24/74	"Illness Associated with TCDD Contaminated Soil--Missouri". Communicable Disease Center Morbidity and Mortality Weekly Report, p. 299.
105	1976	Smith, et al. "Teratogenicity of 2,3,7,8-Tetrachlorobibenzo-p-dioxin in CF-I Mice" Toxicology and Applied Pharmocology, 517-523.
106	5/78	Notice of research project, Tox-Tips. The project title is: "In Vitro Mutagenic Evaluation of the Herbicide 2,4-D" from the National Library of Medicine.
107	5/78	Tox-Tips Notice of Research Project entitled: "Carcinogenesis Bioassay of Tetrochlorodibenzo-p-dioxin" from the National Library of Medicine.
108	1/ 3/80	Letter to Garner of the EPA, from Leng, regarding: registration of various Dow Herbicides including Esteron 245.
109	3/47	Hill, et al. "Toxicity of 2,4-Dichlorophenoxyacetic Acid for Experimental Animals" Journal of Industrial Hygiene and Toxicology, Vol. 29, pp. 85-95.
110	3/13/64	Palazzolo of Industrial Biotest Laboratories, Inc. "Report to the National Agricultural Chemicals Association. Repeated Dermal Toxicity Studies on Three Formulations of 2,4-Dichlorophenoxyacetic Acid".
111	3/16/64	Palazzolo of Industrial Biotest

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4	January/ February, 1981	Pazderova-Veglupkova, et al. "The Development and Prognosis of Chronic Intoxication by Tetrachlorodibenzo-p-dioxin in Men". Archives of Environmental Health, 36, 5-11.
45	1/ 7/78	Franco, Berrino. "Preliminary Report on Mortality in the Seveso Area, 1975-77". Stamped "Strictly Confidential".
46	7/62	Florsheim, et al. "Some Effects of 2,4-Dichlorophenoxyacetic Acid on Thyroid Function in the Rat: Effects on Iodine Accumulation", Endocrinology, 71, 1-6.
47	10/80	Cover sheet entitled "Determination of 2,4-D Exposure Received by Forestry Applicators, Spring 1980", by Lavy of the University of Arkansas in cooperation with Dow. This report is not attached.
a	11/14/80	Letter to: Edwin Johnson of the EPA. From: John Hall of the National Forest Products Association. Re: The research paper entitled "Determination of 2,4-D Exposure Received by Forestry Applicators, Spring 1980"
b	8/79	Carlstedt-Duke, Jan. "Tissue Distribution of the Receptor for 2,3,7,8-Tetrachlorobibenzo-p-dioxin in the Rat". Cancer Research 39, 3172, 3176.
48	1979	Abstract of medical article entitled: "2,3,7,8-Tetrachlorobibenzo-p-dioxin Induction of Aryl Hydrocarbon Hydroxylase in Female Rat Liver. Evidence of De Novo Synthesis of Cytochrome".
49	1975	Beatty, et al. "Effects of 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) on Mammalian Cells in Tissue Cultures". Toxicology and Applied Pharmacology 31, 309,312.
50	1966	Erne, Kurt. "Studies on the Animal Metabolism of Phenoxyacetic Herbicides". Publication and page numbers illegible.
51	1976	Koschier, et al. "In Vitro Uptake of Organic Ions by Renal Cortical Tissue of Rats Treated Acutely with 2,4,5-Trichlorophenoxyacetic Acid". Toxicology and Applied Pharmacology 35, 355-364.
52	1979	Kolmodin-Hedman, et al. "Control of Occuaptional Exposure to Phenoxy Acids

		Laboratories, Inc. "Addendum Report to the National Agriculatural Chemicals Association. Repeated Dermal Toxicity Studies on Three Formulations of 2,4-Dichlorophenoxyacetic Acid".
112	10/29/48	Bjorn, et al. "Effects of 2,4-Dichlorophenoxyacetic Acid on Chicks" Science, Vol. 108, pp. 479-480.
113	00/00/00	Rowe, et al., of Dow. "Acute Oral Toxicity of 2,4-Dichlorophenoxyacetic Acid to Rats, Mice, Guinea Pigs and Chicks"
114	00/00/00	Rowe, et al., of Dow. "Semi-Chronic Oral Toxicity of 2,4-Dichlorophenoxyacetic Acid to Rats".
115	10/29/48	Bjorn, et al. "Effects of 2,4-Dichlorophenoxyacetic Acid on Chicks" Science, Vol. 108, pp. 479-480.
116	10/54	Rowe, et al., of Dow. "Summary of Toxicological Informaton on 2,4-D and 2,4,5-T Type Herbicides and an Evaluation of Hazards to Livestock Associated with their Use". Am. J. Vet. Res. pp. 622-629.
117	January-February, 1964	Clark, et al., of the US Dept. of Agriculture. "The Fate of 2,4-Dichlorophenoxyacetic Acid in Sheep". Agricultural and Food Chemistry, Vol 12, pp. 43-45.
118	1/55	Markquardt, et al., of Dow. "Determination of 2,4-Dichlorophenoxyacetic Acid (2,4-D) in Grain and Seed". Agricultural and Food Chemistry, Vol. 3, pp. 51-53
119	6/73	A table of contents entitled "Section C Safety and Toxicology", with a listing of studies regarding herbicides and animals.
120	12/63	Lisk, et al. "Elimination of 2,4-D in the Urine of Steers Fed 4-(2,4-DB) or 2,4-D". Journal of Dairy Science, Vol. 46, pp. 1435-1437.
121	6/73	Document entitled: "Section C. Full Reports of Investigations Made with Respect to the Safety of the Pesticide Chemical".
122	4/72	Abstract from the British Industrial Biological Research Association, Information Bulletin entitled: "Peace and

		Quiet for 2,4-D . . .".
123	10/18/72	Leng, Marguerite of Dow. "Summary of Teratology Studies with 2,4-D".
124	10/ 6/72	Document entitled: "Summary Teratology".
125	6/73	"Summary of Metabolic Studies with 2,4-D and Animals, June 1973". Author not indicated.
126	00/00/00	Document entitled: "Section C Toxicology-Volume 6", with a listing of articles on toxicology studies in livestock.
127	6/73	"Summary of Toxicology Studies with 2,4-D in Livestock, June 1973"
128	5/69	Table entitled: "Summary of Dosages of Various Organic Herbicides that Cause Significant Weight Loss, Reduced Weight Gain, or Poisoning in Cattle, Sheep or Chickens". Production Research Report No. 106, U.S. Dept. of Agriculture.
129	3/72	Table entitled "Summary of Dosages of Various Organic Herbicides that Cause Significant Weight Loss, Reduced Weight Gain or Poisoning in Cattle, Sheep and Chickens". Production Research Report No. 137, U.S. Dept. of Agriculture.
130	1973	Kociba, et al. "Results of a Two Year Chronic Toxicity and Oncogenicity Study of 2,3,7,8-Tetrachlorobibenzo-p-dioxin in Rats". Toxicology and Applied Pharmacology, 46, 279-303.
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132	4/72	Abstract from the British Industrial Biological Research Association, Information Bulletin entitled: "Peace and Quiet for 2,4-D . . .".
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Pharmacology, 35, 553-574.

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138 1972 Khera, et al. "Pre and Post Natal Studies on 2,4,5-Trichlorophenoxyacetic Acid, 2,4-Dichlorophenoxyacetic Acid and their Derivatives in Rats". Toxicology and Applied Pharmacology, 22, 14-28.

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142 1977 Shadoff, et al., of Dow. "The Gas Chromatographic-Mass Spectrometric Determination of 2,3,7,8-Tetrachlorobibenzo-p-dioxin in Fat From Cattle Fed Ronnel Insecticide". Annali Di Chimica, 67, pp. 583-592.

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144		00/00/00	Heikes, Eugene. "Do's and Don'ts for Proper Use of Tordon Herbicides on Non-Cropland in Colorado".
145		5/66	Jackson, J. B. "Toxicologic Studies of a New Herbicide in Sheep and Cattle". Am. J. Vet. Res., Vol. 27, pp. 821-824.
146		1/18/80	Deleted order for Esteron 245.
147		1967	Goring, et al., of Dow. "The Effect of Tordon on Micro-Organisms and Soil Biological Processes" Down to Earth, Spring, 1967, pp. 1-4.
148		00/00/00	Henck, et al., of Dow. "Acnegenic Potential of 2,4-Dichlorophenoxyacetic Acid Samples".
149		12/19/79	R & D Report from Dow on Exhibit 148.
150		1973	Yoder, et al. "Lymphocyte Chromosome Analysis of Agricultural Workers During Extensive Occupational Exposure to Pesticides". Mutation Research, 21, 335-340.
151	*	6/12/79	Reuber, Melvin. "Carcinogenicity of 2,4-Dichlorophenoxyacetic Acid".
152		5/23/78	Letter to: Anderson, from: Swerczek. Re: Orik, et al., v U.S. and AmChem
153		00/00/00	Swerczek, T. W. "Experiments on the Toxicity of the Herbicide 2,4-D in Horses".
154		6/2/78	Letter from: Anderson of the law firm of Friday, Eldredge and Clark, to: Taynor, Dow attorney. Re: Newton County Wildlife Association v. Larry Hinsen.
155		4/22/80	2,4-D Fact Sheet.
156		1978	Reuber, M. D. "Carcinogenicity Testing of Chemicals with Particular Reference to Organochlorine Pesticides". The Science of the Total Environment, 10, 105-115.
157		1974	Grnow, W. "Metabolism of 2,4,5-T and 2,4-D in Rats and Mice". Achieves of Toxicology, Vol. 32, pp. 217-225. Original article in German attached.
158	6672	5/66	"2,4-D (2,4-Dichlorophenoxyacetic Acid) and Related Compounds". Wildlife, Vol.

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159		1/21/80	Letter from: Leng of Dow, to: Garner of the EPA. Re: Revised Interim Amended Registration for various herbicides including Esteron 245, with attached 5/18/80 memo from Leng to John.
160		6/7/79	Leng, Margeurite, of Dow. "Comparative Toxicology of Various Chlorinated Dioxins as Related to Chemical Structure". Presented at the Symposium of Collaborative International Pesticide Advisory Council.
161	*	1972	Huston, Beverley. "Identification of Three Neutral Contaminants in Production Grade 2,4-D". J. Agr. Food Chem. Vol. 20, pp. 724-727.
162	*	2/74	Gribble, Gorden W. "TCDD: A Deadly Molecule". Chemistry, Vol. 47, pp. 15-18.
163		January 10-11, 1978	Agenda for the World Health Organization Program on the Coordination of Epidemiological Studies on the Long Term Hazards of Chlorinated Dibenzo Dioxins and Chlorinated Dibenzo Furans.
164		00/00/00	Arstila, et al. "An Epidemiological Feasibility Study on the Effects of 2,3,7,8-Tetrachlorobibenzo-para-dioxin (TCDD) on Finnish Forestry Road, and Railway Workers". Publication not indicated.
165		00/00/00	Pocchiari, F. "Accidental TCDD Contamination in Seveso (Italy): Epidemiological Aspects".
166		1/78	"Symptomatology, Morbidity and Mortality Experience of Chlorinated Phenoxy Acid Herbicide (2,4-D; 2,4,5-T) Sprayers in Finland. A Clinical and Epidemiological Study" Working Paper for IARC Working Group Meeting.
167		11/77	Vos, et al. "TCDD Accident at a Chemical Factory in the Netherlands". Publication not indicated.
168		00/00/00	Abstract. Greig, J. B. "NIEHS Conference on Chlorinated Dibenzo Dioxins and Dibenzo Furans Proposed Presentation".
169	6673	1978	McConnell, et al. "The Comparative Toxicity of Chlorinated Dibenzo-p-dioxins

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170	January 10-11, 1978	Moore, John A. "Comparative Toxicity of Dibenzo Dioxins and Dibenzo Furans" IRAC Meeting on Dibenzo Dioxins and Dibenzo Furans.
171	00/00/00	Abstracts of articles on the effect of herbicides on animals.
172	1972	Cunningham, et al. "Effect of Tetrachlorobibenzo-p-dioxin on Growth Rate and the Synthesis of Lipids and Proteins in Rats". Bulletin of Environmental Contamination and Toxicology, Vol. 7, pp. 45-51.
173	8/72	Kimbrough, R. "Toxicity of Chlorinated Hydrocarbons and Related Compounds". Arch Environ Health, Vol. 25, pp. 125-131.
174	1978	Tulp, et al. "Rat Metabolism of Polychlorinated Dibenzo-p-dioxins". Chemosphere, No. 9, pp. 761-768.
175	1975	Allen, et al. "Tissue Distribution, Excretion and Biological Effects of Tetrachlorobibenzo-p-dioxin in Rats". Ed. Cosmet. Toxicol., Vol. 13, pp. 501-515.
176	1977	Nelson, et al. "2,3,7,8-Tetrachlorobibenzo-p-dioxin: In Vitro Binding to Rat Liver Microsomes". Bulletin of Environmental Contamination and Toxicology, Vol. 18, pp. 9-13.
177	11/73	Goldstein, et al. "Hepatic Porphyria Induced by 2,3,7,8-Tetrachlorobibenzo-p-dioxin in the Mouse". Research Communication in Chemical Pathology and Pharmacology, Vol 6, pp. 919-928.
178	3/1/77	Federation Proceedings Abstracts of the 61st Annual Meeting of the Federation of American Societies for Experimental Biology.
179	1976	Van Miller, et al. "Tissue Distribution and Excretion of Tritiated Tetrachlorobibenzo-p-dioxin in Non-Human Primates and Rats". Ed. Cosmet. Toxicol. Vol. 14, pp. 31-34.
180	1979	Barsotti, et al. "Hormonal Alterations in Female Rhesus Monkeys Fed a Diet Containing 2,3,7,8-Tetrachlorobibenzo-p-

		dioxin". Bulletin of Environmental Contamination and Toxicology, Vol. 21, pp. not indicated.
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182	00/00/00	Pitot, et al. "Promotion by 2,3,7,8-Tetrachlorobibenzo-p-dioxin of Hepatocarinogenesis From Diethylnitrosamine". McArdle Laboratory for Cancer Research
182a	1977	Digiovanni, et al. "Tumor-Initiating Ability of 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) in Arochlor 1254 in the Two Stage System of Mouse Skin Carcinogenesis". Bulletin of Environmental Contamination and Toxicology, Vol. 18, pp. 552-557.
183	00/00/00	Courtney, K. Diane. "Prenatal Effects of Herbicides: Evaluation by the Prenatal Development Index". Publication not indicated.
184	2/72	Hussian et al. "Mutegenic effects of TCDD on bacterial systems".
185	1/72	Walker et al. "Some effects of 2,4-D and 2,4,5-T on Ehrlich Ascites Tumor Cells In Vivo and In Vitro"
186	6/14/67	Dow summary on study entitled "Residues of tordon acid in tissues from steers fed the herbaside"
187	1975	Vettorazi, G. "State of the art of the toxicological evaluation carried out by the joint SAO/WHO expert committee on pesticide residues. I. Ogranohalogenated pesticides used in public health and agriculture"
188	6-8/73	Title page "Summary sections from amendment to PP 8F0670 requesting tolerances for residues of 2,4-Dichlorophenoxyacetic Acid (2,4-D)" submitted by Dow.
189	1980	Letter from Adamson of the Office of Science and Technology policy to: Hummell of Dow requesting an article on Esteron

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190		3/71	Poland, et al. "A health survey of workers in a 2,4-D and 2,4,5-T plant with special attention to Chloracne, porphyria chtanea tarda, and psychologic parameters"
191		1977	Coutselinis et al. "Concentration levels of 2,4-D and 2,4,5-T and forensic materials"
192		8/20/79	Dow R & D report on "Reversal of Immunologic and toxichologic effects of a single exposure of 2,4,7,8-Tetrachlorobibenzo-p-dioxin in mice" the copy of the article is attached.
193		00/00/00	Rappe, Christoffer "Chemical aspects of polychlorinated dibenzo dioxins (PCDDs) and polychlorinated dibenzo furans (PCDFs)" Publication not indicated.
194		9/5-10/77	Reggiani of Hoffman-LaRoche and Co. "Medical problems raised by the PCDD contamination in Seeso, Italy". Presented at the fifth international conference on occupational health in the chemical industry.
195		5/78	Dost, Frank "Toxicology of Phenoxy herbicides and hazard assessment of their use in reforestation" U.S. of Agriculture Forest Service.
196		3/29/79	Packee, E.C. "2,4-D in the urban and suburban environment in human health" Presented to the Nanaimo Parks and Recreation Commission.
197		1973	Karvonen, et al. of Finland "The health hazards of defoliants"
198		00/00/00	To: illegible; from: Emerson re proported incidents of animal deaths and human illness associated with dioxin contamination.
199		2/78	Dost, Frank "Toxicology of Phenoxy herbicides and 2,3,7,8 Tetrachlorobibenzo-p-dioxin" Department of Agricultural Chemistry and Environmental Health Sciences Center at Oregon State University.
200	6676	10/3/77	Dow R & D report on "Ultra-structural evaluation of liver cells from female rats maintained for two years on diets

			containing 2,3,7,8-tetrachlorobibenzo-p-dioxin (TCDD)" The report is attached.
201		1/23/68	Letter to Rowe of Dow from Dr. Kligman re experiments on adults receiving small and increasing doses of Tetrachlorobibenzo-p-dioxin.
202		8/22/78	Dow R & D report on "2,3,7,8-Tetrachlorobibenzo-p-dioxin: Tissue distribution and excretion following a single oral dose in female guinea pig" That study done at Dow is attached.
203		00/00/00	Dow form "Request for toxocnological studies on 2,3,7,8-Tetrachlorobibenzo-p-dioxin"
204		7/11/79	Dow R & D report on "Urinary excretion of radio activity following oral administration of C labeled 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) to female guinea pigs" That report prepared by Dow is attached.
205		2/27/78	Dow R & D report on "Three Generation reproduction study of rats ingesting 2,3,7,8-Tetrachlorobibenzo-p-dioxin"
206		1977	Rehwolbt, et al. "Investigation into the acute toxicity and some chronic effects of selected herbicides and pesticides on several fresh water fish species" Bulletin of Environmental Contamination and Toxicology, Vol. 18 pgs. 361-365.
207		4/27/71	Memo to Johnson et al. of Dow from Gehring of Dow re results of the pilot study on the absorption and excretion of 2,4-D and 2,4,5-T by human subjects.
208		4/27/71	Gehring, et al. of Dow "Results of a pilot study on the absorption and excretion of 2,4-Dichloroophenoxyacetic Acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T) following a single oral dose to human subjects"
209		11/21/73	Dow summary of "Placental transfer of 2,4-D and 2,4,5-T in pregnant rats"
210		00/00/00	Schwetz, et al. of Dow "Placental transfer of 2,4-D and 2,4,5-T in pregnant rats"
211	6677	00/00/00	Summary prepared by O'Hare "The effects of repeated applications of semetrical Tetrachlorobibenzo-p-dioxin on Guinea pig

		ears"
212	8/25/71	Dow summary on "Excretion and tissue distribution of 2,3,7,8-Tetrachlorobibenzo-p-dioxin in the rat" With the attached report.
213	6/21/73	Memo to Ling of Dow from Schwetz re letter report of 2,3,7,8-Tetrachlorobibenzo-p-dioxin (2,3,7,8-TCDD) in guinea pigs.
214	5/13/75	Dow summary on "The fate of 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) following single and repeated oral doses to the rat" with the attached report.
215	1973	Dow request for toxichological studies on 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD)
216	9/25/73	Letter to Lynn of Dow from Rose and Ramsey of Dow re "Progress Report: The Accumulation and Elimination Kinetics of 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD)"
217	7/2/73	Dow's request for pathology on a rat which had been given TCDD
218	7/2/73	Dow's request for pathology on a rat which had been given TCDD
219	7/2/73	Dow's request for pathology on a rat which had been given TCDD
220	7/5/73	Request for pathology on a rat who had died spontaneously after receiving TCDD
221	7/5/85	Pathology analysis of TCDD infected rat
222	7/5/73	Analysis of TCDD infected rat
223	00/00/00	Dow's work progress sheet on a TCDD kinetic probe study
224	8/6/71	Medical article abstract on study of young chicken exposed to TCDD
225	8/6/71	Emmerson, et al. "Results of chick edema bioassay test using young chickens treated orally for twenty-one days with 2,3,7,8-Tetrachlorobibenzo-p-dioxin" from Dow.
226	2/12/73	Bosshardt, Hans "Dioxins-Insidious Poisons in our Environment" Translation from a German article.
227	10/21/78	Rehder, et al. "Pathological-Embryological

		Investigations in Cases of Abortion Related to the Seveso Accident" Journal of Swiss Medicine; Vol. 108, pgs. 1617-1625.
228	8/20/79	Dow R & D report on "Immunotoxological studies involving 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) in mice and rabbits" This article prepared by Dow is attached.
229	10/3/77	Dow R & D report on "An ultrastructural evaluation of liver cells from female rats maintained for two years on diets containing 2,3,7,8-Tetrachlorobibenzo-p-dioxin (PCDD)" With the attached report.
230	00/00/00	Gregory, Arthur of NIOSH "Occupational exposure to herbicides" Publication not indicated.
231	00/00/00	Poland, et al. "2,3,7,8-Tetrachlorobibenzo-p-dioxin: Studies on the mechanism of action" McArdle laboratory for cancer research at the University of Wisconsin.
232	3/3/79	Fanelli, et al. "Studies of TCDD levels in animals living in the Seveso area" Publication not indicated.
233	6/16/78	Dow analytical report "Determination of phenoxy herbicides in blood samples from 2,4,5-T plant personnel"
234	7/28/78	Dow analytical report "Determination of phenoxy herbicides in urine and blood samples from 2,4,5-T plant personnel - II"
235	2/19/71	Memo from Gehring of Dow to Gordon, et al. of Dow re protocol for consumption of 2,4-D and 2,4,5-T study.
236	00/00/00	Protocol discussed in exhibit 234
237	4/21/70	Dow document entitled phenoxy sample of record on 2,4,5-Trichlorophenoxyacetic Acid.
238	1/80	Zack, et al. "The mortality experience of workers exposed to Tetrachlorobibenzo-p-dioxin in a Trichlorophenol process accident" Journal of Occupational Medicine, Vol. 22, Pgs. 11-14.
239	00/00/00	Williams of Dow "Fate of Tordon herbicides containing Picloram in the ecosystem"

240	1968	Moffat, R.W. "Some factors effecting the disappearance of Tordon in soil" Down to Earth, Vol. 23, Pgs. 6-10.
241	00/00/00	Goring, et al. of Dow "Residue and toxicology research" Biokemia (a Dow publication) Pgs. 2-8.
242	00/00/00	Toth, et al. "Carcinogetic bioassay of the herbiside 2,4,5-Trichlorophenoxyethanol (TCPE) with different 2,3,7,8-Tetrachlorobibenzo-p-dioxin (dioxin) content in Swiss mice. Publication illegible. Pgs. 82-93.
243	9/15/79	DiGiovanni, et al. "Effects of pretreatment with 2,3,7,8-Tetrachlorobibenzo-p-dioxin on the capacity of hepatic and extra hepatic mouse tissues to convert procarcinigens to mutengens for salmonella typhimurium auxotroph" Toxicology and Applied Pharmacology 50, 229-239.
244	9/30/79	Peterson, et al. "Reversal of 2,3,7,8-Tetrachlorobibenzo-p-dioxin - induced depression of ouabain biliary excretion by pregnenolone-16-cabonitrile and spirononlactone in isolated perfused rat livers" Toxicology and Applied Pharmacology 50, 407-416.
245	00/00/00	Abstract of article on study of 121 dioxin exposed workers shows no excess deaths over thirty years.
246	1979	Gasiewicz, et al. "2,3,7,8-Tetrachlorobibenzo-p-dioxin tissue distribution, excretion and effects on clinical chemical parameters in guinea pigs" Toxicology and applied pharmachology 52, 329-339.
247	00/00/00	Notice of research project on registration of dioxin workers from the National Library of Medicine.
248	6/80	Vecchi, et al. "Effect of acute exposure to 2,3,7,8-Tetrachlorobibenzo-p-dioxin on humoral antibody production in mice" Chem-Biol. Interactions, 30, 337-342.
249	1980	Seefeld, et al. "Blood clearance tests for detecting 2,3,7,8-Tetrachlorobibenzo-p-dioxin hepatotoxicity in rats and rabbits" Archives of the Environmental

		Contamination and Toxicology Vol. 9, pgs. 317-327.
250	1980	Vanlogeton, et al. "Role of the endocrine system in the action of 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) on the thymus" Toxicology 15 135-144.
251	1980	Magazine article "Health Hazard of Dioxins Still Uncertain" Chemical and Engineering News.
252	1/31/80	Letter to the editor from Crummett of Dow to the editor of Nature Vol. 283.
252a	6/24/80	Dow form of medical information for Esteron 245.
253	00/00/00	Document entitled composition listing chemical ingredients.
254	1978	Luster, et al. "Inability of passive antibodies to reverse the effect of dioxin toxcicity" Chemosphere No. 1 pgs. 29-34.
255	00/00/00	Newton, et al. "Exposure of Forest Herbivores to 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) in areas sprayed with 2,4,5-T" Publication not indicated.
256	1980	Dominico, et al. "Accidental release of 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) at Seveso, Italy" Toxicology and Environmental Safety 4, 321-326.
257	1980	Domenico, et al "accidental release of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy" Eco-Toxicology and Environmental Safety Vol. IV, pp. 327-338.
258	1980	A section from the article from Exhibit 257, entitled "Environmental Persistence of TCDD in Soil".
259	1980	Section from Exhibit 257 entitled "TCDD Levels in Atmospheric Particles".
260	1980	Olson, et al. "Tissue Distribution, Excretion and Metabolism of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in the Golden Syrian Hamster". Toxicology and Applied Pharmacology 56, 78-85.
261	1980	Knutson, et al. "2,3,7,8-Tetrachlorodibenzo-p-dioxin: Failure to demonstrate toxicity in 23 cultured cell types". Toxicology and Applied

		Pharmacology 54, 377-383.
262	1978	Chemical abstracts, Vol. 89, p. 141.
263	9/74	Chemical abstracts on article regarding TCDD.
264	1978	Abstract on article regarding analysis of rat liver after exposure to TCDD.
265	1978	Abstract of article on study of rats exposed to TCDD.
266	1978	Abstract of article regarding examination of female rat liver after TCDD exposure.
267	1968	Abstract of article regarding exposure of TCDD in rats.
268	1977	Abstract of article regarding electron-acceptor properties of chlorinated dibenzo-p-dioxins.
269	4/2/73	Baughman, et al. "An Analytical Method for Detecting Dioxin" presented at the conference on dibenzo-dioxins and dibenzo-furons.
270	00/00/00	Illegible article in French on primary carcinoma of the liver in Vietnam with short English summary at the end.
271	6/73	Crossland, et al. -- Title of article illegible regarding spraying of chemicals.
272	1972	Illegible graph regarding normal and abnormal fetuses of mice.
273	Illeg.	Illegible medical article.
274	Illeg.	French article on polychlorodibenzo-p-dioxins with English summary.
275	2/75	Oliver R. M. "Toxic Effects of 2,3,7,8-Tetrachlorodibenzo-1,4-dioxin in Laboratory Workers", British Journal of Industrial Medicine, Vol. 32, pp.49-53.
276	1977	Miller, et al. "Increased Incidence of Neoplasms in Rats Exposed to Low Levels of 2,3,7,8-Tetrachlorodibenzo-p-dioxin" Chemosphere No. 10, pp. 625-632.

277	1979	Kociba, et al. "Long-Term Toxicologic Studies of 2,3,7,8-Tetrachlorodibenzo-p-

		dioxin (TCDD) in Laboratory Animals". New York Academy of Sciences Vol. (not indicated), pp. 397-404.
278	1979	Norstrom, et al. "Analysis of Some Older Scandinavian Formulations of 2,4-dichlorophenoxy acetic acid and 2,4,5-Trichlorophenoxy acetic acid for contents of chlorinated dibenzo-p-dioxins and dibenzo-furons. Scandinavian Journal of Work Environment and Health, Vol. V, pp. 375-378.
279	1980	Reggini, "Acute Human Exposure to TCDD in Seveso, Italy", Journal of Toxicology and Environmental Health, Vol., VI, pp. 27-43.
280	3/80	Fanelli, et al. "Presence of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Wildlife Living Near Seveso, Italy: A Preliminary Study", Bulletin of Environmental Contamination and Toxicology, Vol. 24, pp. (not indicated).
281	1/24/67	McCollister, et al. of Dow "Results of Fertility and Reproduction Studies in Rats Maintained on Diets Containing Tordon Herbicide"
282	1/24/77	Index and report on information on chloracne.
283	1976	Hilbig, "Studies of the Determination of Toxic Effects of Derivatives of 2,4-Dichloro and 2,4,5-Trichlorophenoxyacetic acid (2,4-D and 2,4,5-T) on Incubated Pheasant, Quail and Hen Eggs". Translation from the original German article with the original article attached.
284	00/00/00	Article entitled "Twelve Wise Men Give 2,4-D and 2,4,5-T A Clean Bill of Health", Publication not indicated.
285	1973	Neubert, et al. "A Survey of the Embryo Toxic Effects of TCDD in Mammalian Species". Environmental Health Perspectives, Vol. V., pp. 67-79.
286	1966	Bjorklund, et al. "Toxicological Studies of Phenoxyacetic Herbicides in Animals". Acta Vet. Scand. Vol. VII, pp. 364-390.
287	1971	Sparschu, et al. "Study of the Teratogenicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Rat".

		F.D. Cosmet. Toxicol. Vol. 9, pp. 405-412.
288	1973	Berndt, et al., "In Vitro Uptake of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T) by Renal Cortical Tissue of Rabbits and Rats". Toxicology and Applied Pharmacology 26, 559-570.
289	1974	German medical article on 2,4,5-T and 2,4-D.
290	1974	Fedorova, et al. "Inclusion of 2,4-Dichlorophenoxyacetic Acid in Organs of Animals, Paths and Dynamics of Its Excretion" Gigiena I Sanitariia 39, pp. 105-107.
291	1971	Schwetz, et al. "The Effect of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Esters of 2,4-D on Rat Embryonal, Foetal and Neonatal Growth and Development" F. D. Cosmet. Toxicol. Vol. 9, pp. 801-817.
292	1975	Delvaux, et al. "Polychlorodibenzo-p-dioxins" Toxicology 3, 187-206.
293	1975	Translation of Exhibit 292.
294	1976	Rose, et al. "The Fate of 2,3,7,8-Tetrachlorodibenzo-p-dioxin Following Single and Repeated Oral Doses to the Rat", Toxicology and Applied Pharmacology 36, 209-226.
295	8/8/73	Letter to Sehl of Dow from Bosshardt of the Swiss Federal Research Station re: dioxin content of Dow products.
296	00/00/00	Article on dioxin in German by Bosshardt.
297	1969	McCollister, et al. of Dow "Toxicology of Picloram and Safety Evaluation of Tordon Herbicides". Down to Earth, Vol. 25, pp. 5-10.
298	11/72	Kerney, et al. "Persistence and Metabolism of Chlorodioxins in Soils". Environmental Science and Technology, Vol. 6, pp. 1017-1019.
299	9/73	Harris, et al. "General Biological Effects of TCDD in Laboratory Animals", Environmental Health Perspectives, p. 101-109.
300	9/73	Zinkl, et al. "Hematologic and Clinical

		Chemistry Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Laboratory Animals. Environmental Health Perspectives, pp. 111-118.
301	9/73	Johnson, et al. "Chlorinated Dibenzo-dioxins and Pentachlorophenol", Environmental Health Perspectives, pp. 171-175.
302	6/22/65	Dow report entitled "The Determination of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in 2,4,5-Trichlorophenoxyacetic Acid by Gas-Liquid Chromatography"
303	00/00/00	Document entitled "Table of Contents".
304	12/18/69	Letter to Leng of Dow from Erne, Associate Prof. of Vet. Science in Stockholm regarding 2,4,5-D and 2,4,5-T
305	00/00/00	Table entitled "Phenoxy Acid Levels in Tissues of Broiler Chickens Fed 2,4-D and 2,4,5-T at 1000 p.p.m. in the Drinking Water for 104 Days".
306	1966	Erne, Kurt "Studies on the Analytical Chemistry and Toxicology of Phenoxy Herbicides".
307	1963	Erne, Kurt "Detection and Determination of Chlorophenoxyacetic Acid Derivatives in Water". Acta Chemica Scandinavica 17, 1663-1676.
308	1966	Erne, Kurt "Determination of Phenoxyacetic Residues in Biological Materials", Acta Vet. Scand. No. 7, 77-96.
309	1966	Erne, Kurt "Distribution and Elimination of Chlorinated Phenoxyacetic Acids in Animals", Acta Vet. Scand. No. 7, pp. 240-256.
310	1971	Bjorklund, et al. "Phenoxy-Acid-Induced Renal Changes in the Chicken", Acta Vet. Scand. No. 12, 243-256.
311	1971	Shafik, et al. "A Method for Determination of Low Levels of Exposure to 2,4-D and 2,4,5-T", Intern. J. Environ. Anal. Chem. Vol. 1, pp. 23-33.
312	1975	Almeida, Waldemar "Toxicological Aspects of 2,4,-D and 2,4,5-T Herbicides", publication not indicated.

313	2/9/78	Dow R & D report on "Acute Toxicity and Chloracne Potential of Tars from the 2,4-D Plant".
314	00/00/00	Wassom, et al. "A Review of the Genetic Toxicology of Chlorinated Dibenzo-dioxins"
315	1978	Hardell, et al. "Malignant Mesenchymal Soft-Tissue Tumors and Exposure to Phenoxy Acids of Chlorophenols", a translation from the original German article.
316	00/00/00	Document entitled "A Review on Swedish Epidemiologic Studies with Relation to Chlorinated Dibenzo-dioxins"
317	1/10-11/78	Moore, John "Comparative Toxicity of Dibenzo-dioxins and Dibenzo-furans", presented at the IARC meeting on dibenzo-dioxins and dibenzo-furans.
318	1/78	Poland, Alan "Chlorinated Dibenzo-p-dioxins and Dibenzo-furans: Review of Toxicology and Enzyme Induction and Consideration of Mechanism of Toxicity", presented at IARC.
319	6/72	Report on background information for Picloram.
320	6/72	A different version of Exhibit 319.
321	1966	Hardy, J. L. of Dow "Effect of Tordon Herbicides on Aquatic Chain Organisms", Down to Earth, Vol. 22, pp. 11-13.
322	1967	Hamaker, et al. of Dow "Prediction of the Persistence and Activity of Tordon Herbicide in Soils under Field Conditions", Down to Earth, Vol. (not indicated), pp. 30-36).
323	1967	Youngson, et al. of Dow "Factors Influencing the Decomposition of Tordon Herbicide in Soils", Down to Earth, Vol. 23, pp. 3-11.
324	1979	Seefeld, et al. "Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on Indocyanine Green Blood Clearance in Rhesus Monkeys" Toxicology, Vol. 14, pp. 263-272.
325	00/00/00	Berry, et al. "Studies with Chlorinated Dibenzo-p-dioxins, Polybrominated Byphenyls and Polychlorinated Byphenyls in a Two-Stage System of Mouth Skin Tumorigenesis: Potent Anticarcinogenic

		Effects", publication not indicated.
326	1977	Berry, et al. "Transplacental Induction of Mixed-Function Oxygenases in Extra-Hepatic Tissues by 2,3,7,8-Tetrachlorodibenzo-p-dioxin", Biochemical Pharmacology, Vol. 26, pp. 1383-1388.
327	5/5/76	"Possible Toxic Effects of TCDD in Humans Listed by Dioxin Group", Pesticide Chemical News, pp. 20-23.
328	1970	Abstract on articles regarding toxic effect of some herbicides on fish and aquatic invertebrates from Chemical Abstracts, Vol. 72, p. 240. Attached is a Dow memo dated 4/23/80, regarding Esteron 2,4,5 labels.
329	00/00/00	Handwritten notes on Esteron 2,4,5.
330	1965	Goring, et al. of Dow "Tordon Herbicide . . . Disappearance from Soils" Down to Earth, Vol. 20. Magazine also contains other articles on Tordon.
331	2/18/80	Letter from Garner of the EPA to Leng of Dow regarding revised 2,4,5-T registration.
332	2/20/80	Article from Toxic Materials News entitled "California Lawsuits Challenge State Pesticide Rules"
333	8/31/50	Internal Dow memo regarding toxicity of 2,4,-D stating that 2,4,-D is moderately toxic.
334	4/10/52	Internal Dow memo on "Results of Range Finding Toxicological Tests on 2,4-Dichlorophenoxyacetic Acid Prepared by a New Method". The document discusses hazards and proper handling procedures for 2,4-D.
335	4/10/72	Internal Dow memo on "Results of Skin Irritation Tests on Current Production 2,4-Dichlorophenoxyacetic Acid as Compared with Past Production Material". It discusses the difference in cases of skin dermatitis between the old production method and the new production method.
336	2/5/60	Internal Dow memo "Results of Range Finding Toxicological Tests on 2,4-Dichlorophenoxyacetic Acid Acidified with Chemically Pure HCl and with HCl Recovered

		from the Pentachlorophenol Plant"
337	00/00/00	Drill, Victor "Acute and Subacute Oral Toxicity of 2,4-Dichlorophenoxyacetic Acid for Dogs"
338	1/3/80	Memo to all Dow 2,4-T and Silvex distributors from McCoy, Dow District Sales Manager Re Esteron Brush Killer, Esteron 245 and Kuron.
339		Copy of Exhibit 338.
340	1978	Newton, et al., "Exposure of Forced Herbivores to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) an Area Sprayed with 2,4,5-T". Bulletin of Environmental Contamination and Toxicology, Vol. 20, pp. 743-750.
341	5/5/80	Document entitled "Produce Discontinuation - Record of Approval for the Product Esteron 245 Herbicide.
342	6/2/80	Letter to Stone of the EPA from Morgan of Dow re Esteron 245 application for amended registration.
343	1/80	Esteron 245 herbicide label.
344	1971	Hansen, et al. "Chronic Toxicity of 2,4-Dichlorophenoxyacetic Acid in Rats and Dogs". Toxicology and Applied Pharmacology, 20, 122-129.
345	1970	Medical abstract on the effect of 2,4-D on pheasants and attached is a Dow chemical biology research document summarizing "The Effect of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Esters of the 2,4-D on Rat Embryonal, Fetal and Neonatal Growth and Development"
346	3/56	Rogoff, et al. "Bacterial Decomposition of 2,4-Dichlorophenoxyacetic Acid" Journal of Bacteriology, Vol. 71, pp. 303-307.
347	8/29/74	Newspaper article from the Detroit Free Press "Herbicide Killed Missouri Animals".
348	1977	Mahle, et al. "Search for the Presence of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Bovine Milk". Bulletin of Environmental Contamination Toxicology, Vol. 18, pp. 123-130.

349	9/4-7/78	Bisanti, et al. "Experience of the Accident of Seveso". 6th Conference of the European Teratology Society.
350	1965	Lynn, G. E. "A Review of Toxicological Information on Tordon Herbicides". Down to Earth (pp. and Vol. not indicated).
351	1/79	Homberger, et al. "The Seveso Accident: Its Nature, Extent and Consequences", publication not indicated.
352	1980	Olson, et al. "Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Golden Syrian Hamster". Toxicology and Applied Pharmacology, 55, 67-78.
353	1980	Jones, et al. "Dependents of the Porphyrogenic Effect of 2,3,7,8-Tetrachlorodibenzo-p-dioxin upon Inheritants of Aryl Hydrocarbon Hydroxylase Responsiveness". Toxicology and Applied Pharmacology, 53, 42-49.
354	1978	Aitio, et al. "Organ Specific Induction of Drug Metabolizing Enzymes by 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Rat". Toxicology and Applied Pharmacology, 44, 107-114.
355	1/80	Shearer, et al. "Literature Reviews of 4 Selected Herbicides: 2,4,-D, Dichlobenal, Diquat and Endothall". Report by the Municipality of Metropolitan Seattle.
356	1977	Shadoff, et al. "The Gastromatagraphic-Mass Spectrometric Determination of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Fat from Cattle Fed Ronnel Insecticide", Annali Di Chimica, 67, 583-592.
357	9/28/77	Kociba, et al. "Results of a Two-Year Toxicity and Oncogenicity Study of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Rats" produced by Dow Chemical Company.
358	3/15/65	Report to Dr. Gordon of Dow from Olson of Dow re "The Formation of Folliculitis in the Rabbit Ear Following a Single Subcutaneous Injection of Symmetrical Tetrachlorodibenzo-dioxin in benzene.
359	00/00/00	Memo from O'Hare of Dow re: same topic as Exhibit No. 358.
360	3/26/65	Memo to Gordon of Dow from O'Hare and Olson of Dow re: "The Ineffectiveness of

		Washing as a Preventative Measure in the Formation of Folliculitis in the Rabbit Ear Following Repeated Applications of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Corn Oil".
361	5/18/70	Sparschu, et al. of Dow "Teratogenic Study of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Rat".
362	00/00/00	Document entitled "Report to Dow Chemical Company on Group (1-6)" submitted by Clover Laboratories of Philadelphia. It appears to be the results of animal laboratory tests.
363	8/30/71	Sparschu, et al. of Dow "A Probe Study of the Acute Oral Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Canine"
364	3/22/71	Dow report on "Acute Oral Lethality Studies Conducted on 2,3,7,8-Tetrachlorodibenzo-p-dioxin Using Guinea Pigs"
365	10/25/71	Dow report on "Eye Irritation Study Conducted on: 2,3,7,8-Tetrachlorodibenzo-p-dioxin"
366	5/30/74	Kociba, et al. of Dow "2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD): Results of a 13-Week Oral Toxicity Study in Rats"
367	1972	Zitko, V. "Absence of Chlorinated Dibenzo Dioxins and Dibenzo Furans from Aquatic Animals", Bulletin of Environmental and Toxicology, Vol. 7, pp. 105-110.
368	00/00/00	Dow production specification for 2,4,5-T, mostly illegible.
369	1971	Collins, et al. "Teratogenic Studies with 2,4,5-T and 2,4-D and the Hamster", Bulletin of Environmental Contamination and Toxicology, Vol. 6, pp. 559-567.
370	1973	Bage, et al. "Teratogenic and Embryotoxic Effects of the Herbicides Di- and Trichlorophenoxyacetic acid", Acta Pharmacol. et Toxicol., Vol. 32, pp. 408-416.
371	5/80	DiGiovanni, et al. "Time-Dependent Inhibition by 2,3,7,8-Tetrachlorodibenzo-p-dioxin of Skin Tumorigenesis with Polycyclic Hydrocarbons" Cancer Research,

		40, 1580-1587.
372	1973	Poland, et al. "Chlorinated Dibenzo-p-dioxins: Potent Inducers of Aminolevulinic Acid Synthetase and Aryl Hydrocarbon Hydroxylase", Molecular Pharmacology, 9, 736-747.
373	10/14/80	Ramsey, et al. "Dose Levels of 2,4-D in Forced Workers" report prepared for Dow.
374	00/00/00	Stevens, et al. "Effects of Plant Growth Regulators and Herbicides on Metabolism of C-Labelled Acetate in Pea Root Tissues", publication not indicated.
375	1/5/73	Dow summary of report entitled "Environmental Impact of Herbicides".
376	1/5/73	Kenaga "Environmental Impact of Herbicides", report prepared by Dow.
377	7/71	Author not indicated, "Residual Characteristics of Picloram in Grassland Eco-Systems".
378	4/9/74	Dow requests for toxicological studies on TCDD. Noted on the request is that the sample of TCDD is extremely toxic and chloracengenic.
379	00/00/00	Report entitled "Section II, 2,4-D in the Aquatic Environment" by Halter of the University of Washington.
380	4/13/70	Report to Lynn of Dow from Dunn of Dow re results of chloracne studies conducted on assorted 2,4-D, 2,4,5-T, Silvex samples and their esters, orange and diamonds crop rider amine 4-T-2.
381		Same as Exhibit 380 except with different handwritten markings.
382	4/11/75	Dow report entitled "An Improved Method for the Determination of 2,4-Dichlorophenoxyacetic acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) Concentrations in Plasma and Urine"
383	3-4/68	Bulletin from the National Clearing House for Poison Control Centers entitled "Death from Chlorinated phenoxyacetic acids (2,4-D; 2,4,5-T; MCPA)"
384	00/00/00	Translation from a Puerto Rican newspaper clipping entitled "Weed Killer 2,4-D is

		Non-Toxic to the Human Being"
385	1950?	Willard, C. J. "Indirect Effects of Herbicides", publication not indicated.
386	11/72	Abstract of article entitled "No Herbicide Residues In Meat" from the Health Aspects of Pesticides Abstract Bulletin from the EPA.
387	00/00/00	Drill, Victor "Acute and Subacute Oral Toxicity of 2,4,5-Trichlorophenoxyacetic Acid for Eggs"
388	10/21/68	Leng, Marguerite of Dow, summary of an article entitled "Review on the Metabolism of Phenoxy Compounds in Plants and Animals"
389	00/00/00	Kenaga, Eugene of Dow, "Acute and Chronic Toxicity of 75 Pesticides to Various Animal Species"
390	11/65	Kay, et al. "Subacute Dermal Toxicity of 2,4-D", Archives of Environmental Health, Vol. 11, pp. 648-651.
391	1979	Medical article with both author and title illegible.
392	1978	Gehring, et al. "Phenoxy Acids: Effects and Fate in Mammals", Ecol. Bull. 27, 122-133.
393	00/00/00	Abstracts of medical articles on pesticides.
394	8/78	Hilbig, V. "Studies to Determine the Toxic Effects of Derivatives of the 2,4-Dichlor and 2,4,5-Trichlorphenoxyacetic Acids (2,4-D and 2,4,5-T) on Eggs for Incubation of Pheasants, Quail and Chickens", translated from the original German.
395	00/00/00	Abstracts of medical articles.
396	11/76	Koschier, et al. "Studies on the Mechanism of Renal Transport Depression Caused by Pretreatment with 2,4,5-Trichlorophenoxyacetate", Journal of Toxicology and Environmental Health, Vol. 2, pp. 323-334.
397	00/00/00	Abstracts of medical articles.
398	12/1/75	One-page section from Toxic Materials News.

399	00/00/00	Abstract of medical articles.
400	10/72	Abstracts from the news letter of the Environmental Mutagen Society.
401	00/00/00	Abstracts from the Information Bulletin of the British Industrial Biological Research Association.
402	1970	Abstract from an article in Bioscience.
403	3/9/70	Abstract from the Health Aspects of Pesticide Abstract Bulletin.
404	4/52	Lehman, Arnold J. "Chemicals in Foods: A Report to the Association of Food and Drug Officials on Current Developments" from The Association of Food and Drug Officials of the United States, Vol. 16, pp. 47-53.
405	10/51	Lehman, Arnold J. "Chemicals in Foods: A Report to the Association of Food and Drug Officials on Current Developments -- Part II, Pesticides" from The Association of Food and Drug Officials of the United States, Vol. 15, pp. 122-129.
406	8/30/50	Report to Britton of Dow from Drill of Wayne University re: Interim Report on the Acute and Subacute Oral Toxicity of 2,4,5-Trichlorophenoxyacetic Acid for Dogs.
407	12/15/49	Letter to Dr. Drill of Wayne University from Rowe of Dow re: Studies of 2,4-D and 2,4,5-T on Dogs.
408	00/00/00	Schwetz of Dow "The Effect of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Esters of 2,4-D on Rat Embryonal, Fetal and Neonatal Growth and Development"
409	00/00/00	Abstract of reports.
410	00/00/00	Abstract of reports.
411	7/71	Summary of article entitled "Residual Characteristics of Picloram and Grassland Eco-Systems"
412	various	Dow summaries of research reports on herbicides and their toxicity.
413	4/14/66	Alexander, et al. of Dow "Acute Fish Toxicity of Daxtron, Tordon, and Dursban to Three Species of Fish"

414	00/00/00	Summary of report on Tordon.
415	11/22/65	Alexander, et al. of Dow "Acute Fish Toxicity of Daxtron, Tordon and Dursban to Three Species of Fish"
416	2/10-13/69	McCollister of Dow "Toxicity and Safety Evaluation of 4-Amino-3,4,6-Trichloropicolinic Acid (Picloram)" presented to the Reed Science of America Meeting.
417	5/9/67	Summary of report entitled "The Development of Certain Tordon Formulations in Argentina and Chile", also attached are other summaries of articles concerning herbicides.
418	8/24/65	Summary of article entitled "A Game Bird Toxicology Study -- Acute Dietary Feeding of Tordon in Bobwhite Quail", attached is the actual article.
419	12/7/62	Report from Dow entitled "Results of Food Acceptance Tests for Rodent Repellency on Certain Pyridine and Iodonium Derivatives and Pentachlorophenol"
420	1/16/63	Dow report entitled "Results of Range Finding Toxicological Tests on 4-Amino-3,4,6-Trichloropicolinic Acid (Tordon -- trade name).
421	11/15/62	Dow report entitled "Results of 90-Day Dietary Feeding Studies of 4-Amino-3,5,6-Trichloropicolinic Acid in Rats".
422	6/1/61	Dow report entitled "Results of Range Finding Toxicological Tests on 4-Amino-3,5,6-Trichloro Picolinic Acid".
423	1/74	Report entitled "Picloram: The Effects of Its Use As A Herbicide on Environmental Quality", from the Associate Committee on Scientific Criteria for Environmental Quality.
424	00/00/00	Dow R and D report on an article entitled "The Cytogenetic Effects of Picloram on the Bone Marrow Cells of Rats" and that report by Mensik of Dow is attached.
425	00/00/00	A report on the toxicity of herbicides to fish and other aquatic organisms. Author and title are not listed.

426	2/27/69	Dow summary on the article entitled "The Toxicity of 2,4-D, 2,4,5-T, Silvex, MCPA, Dalapon, Erbon, TCA, Picloram, DNBP; and Their Derivatives and Formulations to Fish and Other Aquatic Organisms".
427	8/18/64	Dow report entitled "Results of Human Irritation and Sensitization Studies Conducted on Tordon 101 Mixture (5% Aqueus Solution)" by Hilltop Research Institute, Inc.
428	Illeg.	Somers, et al. "The Effect of External Application of Pesticides to the Fertile Egg on Hatching Success and Early Chick Performance 3. Consequences of Combining 2,4,-D with Picloram and Extremes in Contamination", Bulletin of Environmental Contamination and Toxicology, pp. 511-516.
429	11/75	Honoroff, Ida article from A Report to the Consumer entitled "Dirty Tricks".
430	10/31/63	Dow report entitled "Results of Human Skin Irritation and Skin Sensitization Tests on M-2431 (A 10% Aqueus Solution of Potassium-4-Amino-3,5,6-Trichloro Picolinate) and Tordon 22K (M-2477)"
431	1976	Pegg, et al. "Effect of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on Renal Function in the Rat", Journal of Toxicology and Environmental Health, Vol. 2, pp. 55-65
432	1972	Heath, et al. "Comparative Dietary Toxicities of Pesticides to Birds", special scientific report (Wildlife No. 152 Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior, pp. 1-28).
433	8/78	Tuchmann-Dupleisis, H. "Pollution of the Environment and Offspring Apropos of the Accident of Seveso". Medecine et Hygiene, Vol. 36, pp. 1758-1766.
434	1977	Tuchmann, H. "Embryological Problems Posed By The Accident At Seveso", Concours Medical, Vol. 44, pp. 6869-6897.
435	00/00/00	Pesticide abstract on article regarding 2,4,5-T.
436	1975	Abstract on article regarding TCDD.
437	1978	Abstract on article regarding TCDD from

		Chemical Abstracts, Vol. 88.
438	1977	Mostly illegible abstract on pesticide article.
439	1978	Abstract on article regarding TCDD.
440	1979	Abstract on articles regarding TCDD.
441	1979	Abstract on article regarding TCDD.
442	1979	Abstract on article regarding TCDD in the rat.
443	00/00/00	Poland, et al. "2,3,7,8-Tetrachlorodibenzo-p-dioxin: Segregation of Toxicity with the AH Locus", publication not indicated.
444	5-6/79	Manis, et al. "Introduction of Iron Transport by A Potent Inducer of Aryl Hydrocarbon Hydroxylase, 2,3,7,8-Tetrachlorodibenzo-p-dioxin", Archives of Environmental Health, Vol. 34, pp. 141-145.
445	1977	Author not indicated, "Chloracne Due to Tetrachloro-2,3,7,8-dibenzo-p-dioxin in Children", Annals Dermatologic Venereologic, Vol. 104, pp. 825-829.
446	00/00/00	Fanelli, et al. "Degradation of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Organic Solvents by Gamma Ray Irradiation", publication not indicated.
447	1977	Van Miller, et al. "Increased Incidents of Neoplasms in Rats Exposed to Low Levels of 2,3,7,8-Tetrachlorodibenzo-p-dioxin", publication not legible.
448	10/77	Notice of research project on the toxic effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Rhesus monkeys.
449	7/77	Morita, et al. "Clearance and Tissue Distribution of Polychlorinated Dibenzofurons in Mice", Bulletin of Environmental Contamination and Toxicology, Vol. 18, pp. 61-66.
450	00/00/00	Document entitled Critique of Manuscript No. 5482, "The Comparative Toxicity of Chlorinated Dibenzo-P-Dioxin Isomers in Mice and Guinea Pigs" by E. E. McConnell, et al., attached is a document dated 4/30/77 that contains more of the critique

		of this article.
451	00/00/00	Report reference in Exhibit 450.
452	2/26/78	Report of the Consultative Council on Congenital Abnormalities in the Yarram District (Canada).
453	00/00/00	Rappe, et al. "Formation of Polychlorinated Dibenzo-p-dioxins (PCDDs) and Dibenzofurons (PCDFs) by Burning or Heating Chlorophenates", Chemosphere (no Volume listed).
454	10/79	Colon, et al. "Anticarcinogenic Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on Benzo (a) Pyrene and 7,12-Dimethylbenz(a) Anthracene Tumor Initiation and Its Relationship to DNA Binding", Cancer Research, Vol. 39, pp. 4027-4033.
455	1980	Murakami, et al. "Incorporation of Labelled Pesticides and Environmental Chemicals into Nuclear Fraction of Cultured Human Cells", Bulletin of Environmental Contamination and Toxicology, Vol. 24, pp. 27-30.
456	1976	Woodward, D. F. "Toxicity of the Herbicides Dinoseb and Picloram to Cutthroat (Salmo Clarki) and Lake Trout" J. Fish. Res. Board Can. 33, 1671-1676.
457	1978	Somers et al. "Hatching Success and Early Performance of Chicks from Eggs Sprayed with 2,4-D, 2,4,5-T and Picloram at Various Stages of Embryonic Development", Bulletin of Environmental Contamination and Toxicology, Vol. 20, pp. 289-293.
458	8/73	Lichtenstein, et al. "Synergism of Insecticides by Herbicides", Science, Vol. 181, pp. 847-849.
459	1974	Pilinskaya, M. A. "Cytogenetic Effect of the Herbicide 2,4-D on Human and Animal Chromosomes", translation from a Russian journal.
460	1973	Whitehead, C. C. "Growth Depression of Broilers Fed on Low Levels of 2,4-Dichlorophenoxyacetic Acid", Br. Poult. Sci., Vol. 14, pp. 425-247.
461	1/52	Lehman "Chemicals in Foods: A Report to the Association of Food and Drug Officials on Current Developments, Part II,

		Pesticide Section II, Dermal Toxicity", Association of Food and Drug Officials of the United States, Vol. 16, pp. 3-9.
462	10/52	Lehman, Arnold J. "Chemicals in Foods: A Report to the Association of Food and Drug Officials on Current Developments, Part II, Pesticide Section V, Pathology", Association of Food and Drug Officials of the United States, Vol. 16, pp. 126-132.
463	1973	Flick, et al. "Studies of the Chick Edema Disease: Toxicity of Chick Edema Factors in the Chick, Chick Embryo, and Monkey", Poultry Science, Vol. 52, pp. 1637-1641.
464	5/1/80	Report entitled "2,4-Dichlorophenoxyacetic acid (2,4-D) Evaluation of the Human Health Hazards", prepared by the Department of Industrial Relations.
465	1973;75	Abstracts on articles regarding pesticides.
466	1/8/80	Dow material safety data sheet on Esteron 2,4,5.
467	2/8/80	Document regarding material safety data sheet on Esteron 2,4,5.
468	1/80	Printout on Esteron 2,4,5.
469	1/80	Printout on Esteron 2,4,5.
470	1973	May, G. "Chloracne from the Accidental Production of Tetrachlorodibenzo Dioxin", British Journal of Industrial Medicine, Vol. 30, pp. 276-283.
471	1979	Kociba, et al. "Toxicologic Studies of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Rats", Toxicology and Occupational Medicine, pp. 281-287.
472	1979	Murray, et al. "Three Generation Reproduction Study of Rats Given 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in the Diet", Toxicology and Applied Pharmacology, Vol. 50, pp. 241-252.
473	5/31/79	Sharma, et al. "Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) on Splenic Lymphocyte Transformation in Mice after Single and Repeated Exposures", Annals of the New York Academy of Sciences, Vol. 320, pp. 487-497.

474	4/13/70	Document entitled "The Analysis of 2,4-D Acid for 2,3,7,8-Tetrachlorodibenzo-p-dioxin Content", publication not indicated.
475	1977	Tung, et al. "The Problem of Mutagen Effects on the Second Generation after Exposure to Herbicides", from a Vietnamese publication.
476	8/80	Cook, et al. "Mortality Experience of Employees Exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)", Journal of Occupational Medicine, Vol. 22, pp. 530-532.
477	5/79	Woodward, D. F. "Assessing the Hazard of Picloram to Cutthroat Trout", Journal of Range Management, Vol. 32, pp. 230-232. Also attached are news articles on the ban on spraying herbicides and a 1/31/80 telegram from Dow regarding the 2,4,5-T Esteron trademark.
478	1978	Abstract on article regarding TCDD.
479	1978	Abstract on article regarding pesticides.
480	1978	Abstract on article regarding TCDD.
481	1978	Abstract on article chicken egg sprayed with herbicides.
482	1978	Abstract of article regarding TCDD.
483	1978	Abstract of article regarding TCDD.
484	1977	Abstract of article regarding dioxin.
485	1977	Abstract of article on TCDD exposure in primates.
486	1977	Abstract of article on renal effects of TCDD.
487	1975	Abstract of article on effect of TCDD on pregnant rats.
488	1977	Zetterberg, et al. "The Influence of PH on the Effects of 2,4-D on Saccharomyces Cerevisiae and Salmonella Typhimurium", Mutation Research, 42, pp. 3-18.
489	6/77	Article from the Washington Post entitled "Deadly Poison -- Dioxin -- Is Showing up in Mother's Milk".

490	5/30/74	Kociba, et al. "2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD): Results of a 13-Week Oral Toxicity Study in Rats" by Dow Chemical.
491	5/30/74	Dow summary of article referenced in Exhibit 490.
416-491		
492	00/00/00	Clegg et. al. "Teratogenicity of pesticides, their Metabolities and Contaminants" publication not indicated.
493	00/00/00	Document entitled "Order and Notice" addressed Dear Registrant from Johnson, Deputy Assistant Administrator for pesticide programs regarding the EPA review of available scientific information on the potential health effects of 2,4-D.
494	11/8/80	Newspaper article from the Milwaukee Sentinel, "More Pesticide Protection Urged For Migrant Farmworkers In State."
495	11/7/80	Newspaper article from The Daily Tribune of Wisconsin Rapids, "Proposed Pesticide Control Rules Debated Again."
496	11/7/80	Newspaper article from The Milwaukee Sentinel, "Cost Of Pesticide Rule Changes Feared."
497	11/11/80 & 11/13/80	Newspaper articles from The Milwaukee Sentinel entitled, "Rules on Pesticide Use Disputed" and "Pesticide Testimony Clashes."
498	11/12/80	Newspaper article from The Milwaukee Sentinel, "Green Bay Official Sees Problem with Pesticide Proposal."
499	09/8/80	Transmittal sheet indicating receipt of information from the EPA.
500	00/00/00	Document entitled "Chapter Six, Comparison of the Health Effects of Aquatic Herbicides." Also attached is Milwaukee Sentinel article 11/7/80 "Pesticide Use is Still Hot Issue."
501	00/00/00	Document entitled "Chapter 2, Health Effects of 2,4-D (2,4-Dichlorophenoxyacetic Acid) and its derivatives" Publication not indicated.

502		1980	A variety of newspaper articles regarding Dioxins found in 2,4-D in Canada.
503		1980	Various newspaper articles including one entitled massive rise in cancer deaths predicted.
504		10/27/80	Transcript from CBS radio show regarding dioxin levels in 2,4-D and 2,4,5-T.
505		1980	Canadian newspaper articles regarding dioxins being found in 2,4-D.
506		07/04/80	Document entitled, "Appendix A, list of your companies products containing some form of 2,4-D".
507		00/00/00	Document entitled, Appendix B and Appendix C, it appears to be authored by the EPA.
508		07/11/80	Document entitled, "Appendix D. List of your company's products containing some form of 2,4-D formulated in a liquid form or emulsifiable concentrate."
509	*	00/00/00	Document entitled Appendix G all registrants (by company name) with registered products containing 2,4-D.
510		03/79	Report entitled assessment of human health risk associated with the risk of 2,4-D in forestry management from the Minnesota Department of Health.
511		1964	Wiltse of Dow "Tordon herbicide as a soil treatment for brush control" Down To Earth Volume 19, pages 3-6.
512		1971	Goring et. al. of Dow "The Degradation and Movement of Picloram in soil and water" Down To Earth Volume 27 pages 12 through 15.
513		4/22/80	Document entitled "2,4-D Fact Sheet"

		author not indicated, appears to be Dow.
514	7/14/80	Copy of previous document regarding "List of your Company's products containing some form of 2,4-D."
515	9/08/80	Transmittal memo at Dow for information received from the EPA regarding 2,4-D study.
516	02/04/80	Memo to Cook from Bond of Dow re: A Critical Review of: "Case Control Study of Malignant Mesenchymal Soft Tissue Tumors and Exposure to Chemical Substances".
517	01/74	Kimbrough, Renate of the Center for Disease Control "The Toxicity of Polychlorinated Polycyclic Compounds and Related Chemicals" CRC Critical reviews in Toxicology pages 445-498.
518	05/18	March/April 1977 Kimbrough, et. al. "Epidemiology and pathology of a tetrochloral dibenzo dioxin poisoning episode" Archives of Environmental Health, pages 77-85.
519	00/00/00	Dow label for Tordon 10K pellets.
520	00/00/00	Lamb et. al. "Evaluation of 2,4-Dichlorophenoxyacetic Acid (2,4-D), 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T), and 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) toxicity in mice: reproduction and fertility in treated male mice and evaluation of congenital malformations in their offspring", publication not indicated.
521	1981	Ericksson et. al. "Soft Tissue sarcomas and exposure to chemical substances: a case referent study". British Journal of Industrial Medicine Volume 38, pages not indicated.
522	9/73	Moore et. al. "Postnatal effects of maternal exposure to 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD)" Environmental Health Perspectives pages 81 through 85.

523	1965	Nielsen et. al. "Fatal poisoning in man by 2,4-Dichlorophenoxyacetic Acid (2,4-D) Determination of the agent in forensic materials" Acta pharmacol. et toxicol. Volume 22, pages 224-234.
524	1969	Medical abstract on Russian article regarding the health in workers manufacturing 2,4-D.
525	05/09/70	Berwick, Philip "2,4-Dichlorophenoxyacetic Acid poisoning in man" JAMA Volume 214 pages 1114 through 1117.
526	1970	Medical abstract of article regarding 2,4-D poisoning in man.
527	00/00/00	Computer information search request entitled 2,4-D.
528	00/00/00	Computer search entitled Dioxin.
529		Continuation of Exhibit 528.
530		Continuation of Exhibit 528.
531		Continuation of Exhibit 528.
532	00/00/00	Computer search entitled Dioxin, 2,4-D, 2,4,5-T.
533		Continuation of Exhibit 532.
534	1970	Paper from the Pesticide Symposia entitled "2,4,5-T in the rat: Excretion Pattern, Serum Levels, Placental Transport, and Metabolism" by K.D. Kourtney.
535	1962	Desi et. al. "New evidence concerning the nervous site of action of a chemical herbicide causing professional intoxication" ACTA Physiol Volume 22, pages 72-80.

536	05/77	Taylor et. al. "Chloracne from manufacture of a new herbicide" ARCH Dermatol 113: 616-619.
537	1972	Jones, et. al. "A technic for testing acnegenic potency in rabbits, applied to the potent acnegen 2,3,7,8-Tetrachlorobibenzo-p-dioxin. The Journal of Investigative Dermatology. Volume not indicated, pages 511-517.
538	09/73	Schwetz, et. al. "Toxicology of Chlorinated Dibenzo-P-Dioxins" Environmental Health Perspectives, page 87-93.
539		Continuation of Article reference in Exhibit 538.
540	11/80	Huff, et. al. "Long Term Hazards of Polychlorinated Dibenzodioxin and Polychlorinated Dibenzofurans" Environment Health Perspectives Volume 36, page 221-240.
541	1974	Greig, et. al. "Effects of 2,3,7,8-Tetrachlorobibenzo-p-dioxin on stimulated DNA Synthesis in the Liver and Kidney of the Rat." Chem-Biol. Interactions Volume 8 pages 31-39.
542	04/11/80	Telegram from Charlton of Dow entitled "2,4,5-T Update".
543	1980	Dominico, et. al "Accidental Release of 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) at Seveso, Italy. II TCDD distribution in the soil surface layer" Echotoxicology and Environment Safety Volume IV page 298-320.
544	1980	Part 4 of article began in Exhibit 543 entitled "Vertical distribution of TCDD in soil."
545	05/06/81	Dow customer requests from Midland Analytical Laboratories regarding TCDD in

		Esteron 245.
546	03/06/80	Dow R & D report summarizing article entitled "2,4-D formulations containing specific amounts of 2,4,5,7-Tetrachloroxanthone: chloracnegenic potential" with the attached article.
547	1977	Guarino et.al. "Fate in Distribution of the Herbicides Dichloral phenoxicedic acid (2,4-D) and 245 trichloral phenoxiacidic acid (145T) in the dogfish shark" Xenobiotica, Volume 7 pages 623-631.
548	12/23/80	News release "Dow Study Show No Correlation Between Dioxin Exposure and Pregnancy Results".
549	1973	Huff, et. al. "Hazardous Contaminants: Chlorinated Dibenzodioxins and Chlorinated Dibenzofurans" Second Annual Conference of Environment Chemicals Human and Animal Health, pages 175-197.
550	00/00/00	Poland, et. al. "An Estimate of The Maximum In Vivo Covalent Binding of 2,3,7,8-Tetrachlorobibenzo-p-dioxinto rat liver protein RNA and DNA" publication not indicated.
551	8/1976	Poland, et. al. "Stereospecific, High Affinity Binding of 2,3,7,8-Tetrachlorobibenzo-p-dioxin by Hepatic Cytosol: Evidence that the binding species is the receptor for the induction of aryl hydrocarbon hydroxylase" publication not indicated.
552	1978	Viviani, et. al. "Time course of the induction of aryl hydrocarbon hydroxylase in rat liver nuclia and microsomes by phenobarbital, 3-methylcholanthrene, 2,3,7,8-Tetrachlorobibenzo-p-dioxin, bieldrin and other inducers" Biochemical Pharmacology, Volume 27, pages 2103 through 2108.
553	1975	Nebert, et. al. "Genetic Expression of

		Ayrol hydrocarbon hydroxylase activity in the mouse" J.Cell. physiol. 85, pages 393-414.
554	1980	Poland, et. al. "2,3,7,8-Tetrachlorobibenzo-p-dioxin" segregation of toxicity with the AH locus" Molecular Pharmacology volume 17 pages 86-94.
555	1978	Gellert, et. al. "Topical Exposure of Neonates to Hexachlorophene: long standing effects on mating behavior and prostatic development in rats" Toxicology and Applied Pharmacology Volume 43 pages 339 through 349.
556	1977	Yang, et. al. "Effects of 2,3,7,8-Tetrachlorobibenzo-p-dioxin on plasma disappearance and biliary excretion of foreign compounds in rats" Toxicology and Applied Pharmacology Volume 40 pages 485-496.
557	3/80	Dow summary on reports entitled "Histopathological and Ultrastructural studies of Liver Tissue from TCDD-exposed beach mice"
558	1979	Abstract entitled "The Dioxin Saga-Monkey Reproduction"
559	1981	Cantoni, et. al. "Porphyrogenic Effect of Chronic Treatment with 2,3,7,8-Tetrachlorobibenzo-p-dioxin in Female Rats. Dose-effect relationship following urinary excretion of porphyrins" Toxicology and Applied Pharmacology, Volume 57, pages 156-163.
560	00/00/00	Khera, et. al. "Polychlorodibenzo-P-dioxins: Perinatal Effects and the Dominant Lethal Test in Wistar Rats", Publication not indicated.
561	1980	Van Logten, et. al. "Role of The Endocrine system in the action of 2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD) on the thymus" Toxicology Volume 15, pages 135-144.

562	1979	Niensedt, et. al. "Effect of 2,3,7,8-Tetrachlorobibenzo-p-dioxin in the Hepatic Metabolism of Testosterone in the Rat." Toxicology Volume 13 pages 233-236.
563	11/16/68	Higginbotham, et. al. "Chemical and Toxicological Evaluations of isolated and Synthetic Chloro Derivatives of Dibenzo-P-dioxin" Nature, Volume 220, pages 702-702.
564	1978	McDonnel, et. al "The Toxico Pathology of TCDD" Publication not indicated.
565	07/09/77	Crow, K.D. "Letter to the Editor - Effects of Dioxin exposure" The Lancet, Volume not indicated, page 82.
566	00/00/00	Working group of the NIEHS and the IARC "Coordination of Epidemiological Studies on the Long Term Hazards of Chlorinated Dibenzodioxin" Publication not indicated.
567	1978	Report entitled "Report of The Consultative Counsel on Congenital Abnormalities In the Yarram District" presented to both Houses of Parliament in Victoria, Australia.
568	11/7/78	Letter to Houghton, Minister of Health in Australia from Schroeter, California Attorney re: report of 2,4-D and 2,4,5-T investigation.
569	11/14/78	Letter to Schroeter of Los Angeles from Houghton of Australia transmitting copies of the Australian report.
570	08/77	"IARC Monographs on the Evaluation of the Carcinogenic Risks of Chemicals to Man. Some fumigants, the Herbicides 2,4-D and 2,4,5-T, chlorinated dibenzodioxins and miscellaneous industrial chemicals" from The World Health Organization.
571	1975	Archer, T. E. "Removal of 2,4-Dichlorophenoxyacetic Acid (2,4-D) Formulations from Non-combustible Pesticide Containers" Bulletin of

		Environmental Contamination and Toxicology Volume 13 pages 44-51.
572		Continuation of Article Referenced in Exhibit 570.
573	1980	Abstract of article regarding TCDD and toxicity.
574	1973	Fishbein, Laurence "Mutations and Potential Mutations in the Biosphere. 1. DDT and Its Metabolites, Polychlorinated Biphenyls, Chlorodioxins, Polycyclic Aromatic Hydrocarbons, Haloethers", The Science of the Total Environment, Vol. 4, pp. 305, 340.
575	5/4/81	Letter to the editor entitled TCDD and Hexachlorophene, Chemical and Engineering News, pp. 2 and 58.
576	1973	Schwetz, et al. "Toxicology of Chlorinated Dibenzo-p-dioxins", Advances in Chemistry Series, No. 120, pp. 55-69.
577	8/63	Seabury, John H. "Toxicity of 2,4-Dichlorophenoxyacetic Acid for Man and Dog", Archives of Environmental Health, Vol. 7, pp. 86-93.
578	1980	Outline and abstract of article. Townsend, J. C. "Survey of Reproductive Events of Wives of Employees Exposed to Chlorinated Dioxins"
579	00/00/00	Lamb, et al. "Evaluation of 2,4-Dichlorophenoxyacetic Acid (2,4-D), 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T), and 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Toxicity in Mice: Reproduction and Fertility in Treated Male Mice an Evaluation of Congenital Malformations in Their Offspring"
580	1972	Epstein, Samuel S. "Teratological Hazards Due to Phenoxy Herbicides and Dioxin Contaminants", Environmental Science Research, Vol. 2, pp. 708-729.
581	7/70	Article from the Ontario Department of Health entitled "Revised Registration Status of 2,4-T in Canada"
582	7/17/70	Dow summary of article entitled "Results of Tolerance Studies of 4-Amino-3,4,6-Trichloropicolinic Acid (Picloram) in Pregnant Rats"

582(a)	1981	Tondeur, et al. "New Screening Methods for Acetic Toxic Substances Using Negative Chemical Ionization Mass Spectrometry. Tetrachloroterephalate in Human Urines", Environmental Science and Technology, Vol. 15, pp. 216-219.
583	1/20/71	Report entitled "Determination of 2,4-Dichlorophenol and 2,4-Dichlorophenoxy-acetic Acid in Human Blood by Gas Liquid Chromatography", author not indicated.
584	10/14/80	Langghorst, M. L. of Dow "Monitoring Esteron 245 in Air"
585	5/5-7/81	Kociba, et al. of Dow "A Review of the Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) with a Comparison of Other Chlorinated Dioxin Isomers", presented at a meeting of the Central States Association of Food and Drug Officials.
586	3-4/78	Danon, et al. "Subacute Skeletal Myopathy Induced by 2,4-Dichlorophenoxyacetate in Rats and Guinea Pigs", Muscle and Nerve, Vol. 1, pp. 89-102.
587	00/00/00	Report to the Council on Scientific Affairs of the AMA on "Health Effects of 'Agent Orange' and Dioxin Contaminants", presented by Dr. Dolan.
588	1971	Williams, C. S. of Dow "The Status of 2,4-D, 2,4,5-T, Silvex and MCPA Herbicides", Down to Earth, Vol. (not indicated), pp. 12-16.
589	Various	Abstracts of articles on TCDD.
590	00/00/00	Abstract of article on 2,4,5-T, 2,4-D and the fetus.
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947	1981	Abstract and diagrams attached to article by David R. Bickers, M.D. "Treatment of Porphyrias: Mechanisms of Action", Journal of Investigative Dermatology, Volume 77, pages 107 to 113, 1981.
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950	1986	Carl L. Potter, et. al. "Relationship of Alterations in Energy Metabolism to Hypophagia in Rats Treated With 2,3,7,8-Tetrachlorobibenzo-p-dioxin" Fundamental and Applied Toxicology, Volume 6, page 89

1045	03/19/82	Letter from Thomas Adamczyk from EPA to Arthur Schober of Dow regarding Esteron 245 BE.
1046	1982	Several letters between Dow Chemical and EPA regarding Esteron 245 BE. Also attached is certification statement regarding Esteron 245 BE.
1047	00/00/00	EPA form 8570-10, November 1974 titled "Label Technical Data" regarding Esteron 245 BE.
1048	00/00/00	Labels for Dow Esteron 245 and Esteron 245 BE.
1049	00/00/00	F. Safe, et. al. Report titled "PCBB's and related compounds: Metabolism and Biochemistry.
1050	06/83	Incident report on Esteron 245 by Mrs. Gerald Grove regarding her husband taken by Betty Schmidt.
1051	12/81	Dow document titled "Medical Information Regarding Esteron 245BE herbicide, Esteron 245 brush and weed killer and Esteron 245 concentrate brush and weed killer."
1052	08/81	Documents, including a report of telephone inquiries involving Esteron 245, medical information, incident report, data sheet of properties, health hazards and precautions for safe handling of materials and material safety data sheet.
1053	08/27/82	Emergency response information sheet regarding Esteron 245 brush killer, also attached is Interoffice memo from Dow Chemical referring to attached document, State of Alabama, notice of suspension of sale.
1054	03/82	Dow specimen label for Esteron 245 BE.
1055	04/08/82	Dow chemical documents, "Production Specifications Regarding Esteron 245 C

			Herbicide.
1056		00/00/00	Report by H. Poiger and H. R. Buser, "Structure Elucidation of Manillion TCDD Metabolites".
1057		03/24/82	Letter from Arthur Schober of Dow Chemical to T.E. Adamczyk of the EPA regarding Esteron 245 BE.
1058		03/82	Copy of specimen label for Dow Esteron 245 BE.
1059		1978	Lennart Hardell and Anita Sandstrom, translation of an article titled "Malignant Mesenthyne soft tissue tumor when exposed to phenoxy acid or Cholorphenolo" Lakartibningen, Volume 75, number 40, 1978.
1060		02/15/80	Memo from Marguerite L. Ling, G. L. Costillo, et al., of Dow Chemical, regarding Esteron 245, Esteron Bush Killer and Kuron.
1061		1982	Document titled: "Dow Chemical Company Quality Assurance Production Specification for Esteron R 245BE Herbicide.
1062		5/24/82	Document titled: "Dow Chemical Company Quality Assurance Production Specification for Esteron R 245BE Herbicide".
1063		1982	Document titled: "Dow Chemical Company Quality Assurance Production Specification for Esteron R 245BE Herbicide", rough draft.
1064		10/16/81	Document titled: "Manufacturing Specifications", apparently a routing slip, Esteron 245BE Herbicide, handwritten notes, mostly illegible.
1065		4/82	Document titled: "Manufacturing Specifications", cover routing slips, Esteron 245BE Herbicides, handwritten notes.
.066		9/23/80	Document titled: "Production Specification Dow Chemical Esteron 245BE Herbicide", rough draft.
1067	5745	1984	Report "The Pharmacokinetic Evaluation of

		C-2,4-Dichlorophenoxyacetic Acid (2,4-D) in the Mouse", prepared by the Office of Quality Assurance of Hazleton Laboratories and Submitted to the Industry Task Force on 2,4-D research data in Midland, Michigan.
1068	7/22/85	Dow R & D report on the article "Molten 2,4-D Acid: Dermal Sensitization Study in Hartley Guinea Pigs" with the report attached.
1069	7/26/85	Final report "A Dietary Two-Generation Reproduction in Fischer 344 Rats with 2,4-Dichlorophenoxyacetic Acid" prepared for the Industry Task Force on 2,4-D research data.
1070	4-6/52	Zimmerman, et al. "Detection and Determination of 2,4-D as a Contaminant by Biological Methods", publication not indicated.
1071	1948	French article on 2,4-D, no translation.
1072	1983	Reuber, Melvin "Carcinogenicity and Toxicity of 2,4-Dichlorophenoxyacetic Acid", The Science of the Total Environment, Vol. 31, pp. 203-218.
1073	6/25/85	Report from Hazelton Laboratories, "Oncogenicity Study in Mice 2,4-Dichlorophenoxyacetic Acid 52 Week Progress Report" submitted to the Industry Task Force on 2,4-D research data.
1074	1953	Baker, et al. "Suspected Poisoning of Dogs from Eating Grasses Treated with 2,4-D", The North American Veterinarian, Vol. not indicated, p. 194.
1075	8/26/50	Audus, L. J. "Biological Detoxication of 2,4-Dichlorophenoxyacetic Acid in Soils: Isolation of an Effective Organism" Nature, Vol. 166, p. 356.
1076	11/20/50	French medical article on 2,4-D, no translation.
1077		English abstract of article mentioned in Exhibit 1076.
1078	4/63	Abstract of article regarding 2,4-D.
1079	10/71	Abstract of articles on 2,4-D.
1080	1970	Abstract of article on 2,4-D.

1081	3/14/47	Hsueh, et al. "Effects of 2,4-D on Seed Germination and Respiration", Science, Vol. 105, pp. 283-285.
1082	1946	Abstract of article on 2,4-D Toxicity and Fish.
1083	9/19/85	Dow R & D report on "Effect of Dissolved Oxygen Concentration on the Biodegradation of 2,4-Dichlorophenoxyacetic Acid", this article is attached.
1084	4/2/84	Dow R & D report on "Substrate Specificity of a Mixed Microbial Culture Isolated on 2,4-Dichlorophenoxyacetic Acid", report is attached.
1085	12/13/83	Dow R & D report on "High Performance Liquid Chromatography Determination of 2,4-Dichlorophenoxyacetic Acid in Waste Treatment Effluents", report is attached.
1086	12/18/83	Dow R & D report on "Effect of Bioaugmentation on the Removal of 2,4-Dichlorophenoxyacetic Acid in Waste Water Treatment" with attached report.
1087	00/00/00	Illegible document.
1088	00/00/00	Illegible document.
1089	Various	Abstracts of different articles, some are illegible.
1090	1/69	Fullerton, Pamela "Toxic Chemicals and Peripheral Neuropathy", publication illegible.
1091	Various	Abstract of articles on herbicides.
1092	3-4/68	"Effect of 2,4-D on Incorporation of C into Urinary Metabolites from Rats Receiving C-Labelled Acetate", J. Agr. Food Chem., Vol. 16, pp. 358-360.
1093	Various	Abstracts of articles on 2,4-D.
1094	11/26/80	"National Park Service Urged By AFBF, NFPA, NCA to Discontinue 2,4-D Use", Pesticide and Toxic Chemical News, pp. 18-19.
1095	12/74	Schoemaker, et al. "Direct Injection of Experimental Mammary Adenocarcinoma with Picloram", The American Journal of the Medical Sciences, Vol. 268, pp. 333-335.

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1097		6/29/67	Dow summary on "Residues of Tordon Acid in Milk from Cows Fed the Herbicide", the report is not attached.
1098		00/00/00	Dow research report "Results of Additional Acute Oral Tests and Skin Absorption Studies on 4-Amino-3,5,6-Trichloro Picolinic Acid"
1099		9/26/77	Document entitled "Review of the Bioassay of Picloram for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearing House on Environmental Carcinogens".
1100		1969	Abstract on article regarding herbicide residues in milk from cows.
1101		Various	Abstracts of articles regarding Picloram.
1102		9/70	Weimer, et al. "Toxicological Studies on the Herbicide 'White' in Animals", Dept. of the Army, Edgewood Arsenal Research Laboratories.
1103		2/72	Heath, et al. "Comparative Dietary Toxicities of Pesticides to Birds", from the Division of Wildlife Research Bureau of Sport Fisheries and Wildlife.
1104		2/4/80	Dow R & D report on "Kinetics of C-Labelled Picloram in Male Fischer 344 Rats" with attached report.
1105		1983	Woodruff, et al. "Pesticide-Induced Complete Impartial Chromosome Loss in Screens with Repair Defective Females of Drosophila Melanogaster", Environmental Mutagenesis, Vol. 5, pp. 835-846.
1106		7/78	Abstract of article on Pesticides and Rats.
1107		1976	Guerzoni, et al. "Mutagenic Activity of Pesticides", translation from original Italian article.
1108		11/16/82	Dow R & D report on "The Toxicity of Picloram (4-Amino-3,5,6-Trichloro Picolinic Acid to Representative Fresh Water Organisms.
1109	3748	4/30/82	Dow R & D report on "Picloram: Results of

		A Two-Week Dietary Probe Study in CDF Fischer 344 Rats", with attached report.
1110	6/9/82	Dow R & D report "Results of a Six-Month Dietary Toxicity Study of Picloram (4-Amino-3,5,6-Trichloro Picolinic Acid) Administered in the Diet to Male and Female Beagle Dogs" with attached report.
1111	7/29/82	Dow R & D report on "Picloram: The Influence of Hexachlorobenzene Concentration on Picloram-Induced Liver Toxicity in Fischer 344 Rats" with attached report.
1112	1983	Borzelleca, Joseph F. "A Review of Volatile Organic Contaminant Data", publication not indicated.
1113	7/28/83	Dow R & D report on "Picloram: Quantitative Determination in Human Blood and Urine" with attached report.
1114	8/19/83	Dow R & D report on "Picloram: Pharmacokinetics in Human Volunteers" with attached report.
1115	10/10/83	Dow R & D report "An Acute Oral Toxicity Study in the Mallard with Picloram Technical" with attached report.
1116	1984	Libich, et al. "Occupational Exposure of Herbicide Applicators to Herbicides Used Along Electric Power Transmission Line Right of Way", American Industrial Hygienist Association Journal, Vol. 45, pp. 56-62.
1117	3/12/73	Suschetet, et al. "Physiopathological and Nutritional Effects of 4-Amino-3,5,6-Trichloro Picolinic Acid (Picloram) on the Rat" from a French article.
1118	2/84	National Toxicology Program annual Plan for fiscal year 1984 from the Department of Health and Human Services.
1119	1984	Nolan, et al. "Pharmacokinetics of Picloram in Male Volunteers", Toxicology and Applied Pharmacology, Vol. 76, pp. 264-269.
1120	8/16/84	Dow R & D report "Biological Mechanisms Involved in the Toxicity of Picloram Herbicide in Rats" with attached report.
1121	8/13/84	Dow R & D report "The Toxicity of

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			Technical Picloram to the Embryo, Larval, and Juvenile Stages of the Rainbow Trout", with attached report.
1122		6/18/84	Dow R & D report "The Acute and Chronic Toxicity of Technical Picloram (4-Amino-3,5,6-Trichloro Picolinic Acid) to Daphnia Magna Straus" with attached report.
1123		6/12/84	Dow R & D report "The Determination of 4-Amino-3,5,6-Trichloro Picolinic Acid in Water by High Performance Liquid Chromatography During Aquatic Toxicity Studies" with attached report.
1124		4/9/85	Dow R & D report "Evaluation of Picloram in the Mouse Bone Marrow Micronucleus Text", with attached report.
1125		00/00/00	Mullison, Wendell R. of Dow "A Toxicological and Environmental Review of Picloram", publication not indicated.
1126		1985	John-Greene, et al. "Teratological Evaluation of Picloram Potassium Salt in Rabbits", Fd. Chem. Toxic., Volume and page numbers not indicated.
1127		3/86	Abstract of article on Picloram.
1128		1/9/86	Dow R & D report "Picloram: A Two-Year Dietary Chronic Toxicity-Oncogenicity Study in Fischer 344 Rats", with attached report.
1129		1/6/86	Dow R & D report "Evaluation of the Toxicity of Technical Picloram to Fresh Water Green Alga", with attached report.
1130		11/20/50	French article on 2,4-D.
1131		1963	Citation of article on 2,4-D.
1132		00/00/00	Shafik, et al. "A Method for Determination of Low Level of Exposure to 2,4-D and 2,4,5-T", from the Public Health Service Division of Pesticide, Chemistry and Toxicology.
1133		1962	Cite on article on 2,4-D.
1134	*	4/13/70	Memo from Dunn of Dow to Lyn of Dow re results of Chloracne studies conducted on assorted 2,4-D, 2,4,5-T, Silvex samples and their esters, orange and diamonds crop rider amine 4-T-2.

1135	2/13/70	Dow requests for preliminary toxicological studies on 2,4-D.
1136	00/00/00	Dow report entitled "Chlorophenoxy Herbicides Drinking Water Limits"
1137	1970	Abstract on article regarding pesticide residues in natural waters.
1138	10/84	Rubinstein, et al. "Inhibition of Intercellular Communication in Cultures of Chinese Hamster V-79 Cells by 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid", Fundamental and Applied Toxicology, Vol. 4, pp. 731-739.
1139	00/00/00	Cortney, K. D. "2,4,5-T in the Rat: Excretion Pattern, Serum Levels, Placental Transport and Metabolism", Pesticides Symposia, pp. 277-283.
1140	8/84	Mortelmans, et al. "Muted Genicity Testing of Agent Orange Components and Related Chemicals", Toxicology and Applied Pharmacology, Vol. 75, pp. 137-146.
1141	1985	Singh, et al. "Inhibition of Human Glutathione S-Transferases by 2,4-Dichlorophenoxyacetate (2,4-D) and 2,4,5-Trichlorophenoxyacetate (2,4,5-T)", Toxicology and Applied Pharmacology, Vol. 81, pp. 328-336.
1142	3/82	Riihimaki, et al. "Mortality of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid Herbicide Applicators in Finland", Scandinavian Journal of Work Environment and Health, Vol. 8, pp. 37-42.
1143	1/15/84	Kawashima, et al. "Effects of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid on Peroxisomal Enzymes in Rat Liver", Biochemical Pharmacology, Vol. 33, pp. 241 through 245.
1144	1984	Vessey, et al. "Differential Activation and Inhibition of Different Forms of Rat Liver Glutathione S-Transferase by the Herbicides 2,4-Dichlorophenoxyacetic Acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T)", Toxicology and Applied Pharmacology, Vol. 73, pp. 492-499.
1145	4/73	Abstract of article on

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		Chlorodibenzodioxins in the liver.
1146	1975	Abstract of article on dioxins.
1147	11/75	Citation of article on the effects of TCDD in rats.
1148	1984	Haseman, et al. "Results From 86 Two-Year Carcinogenicity Studies Conducted by The National Toxicology Program". Journal of Toxicology and Environmental Health, Vol. 14, pp. 621-639.
1149	1/23/84	Niosh. Current Intelligence Bulletin, No. 40. "2,3,7,8-Tetrachlorobibenzo-p-dioxin (TCDD 'Dioxin')".
1150	11/84	Dow report entitled: "Dioxin, Agent Orange and Human Health".
1151	5/16/80	Dow R & D Report. "Picloram: Results of a 32 Day Toxicity Tolerance in Feed in Mice", with attached report.
1152	1/30/82	Dow R & D Report. "Comparative Toxicity of Three Samples of Technical Picloram Containing Various Amounts of N-(2,3,7,8-Tetrachloro-2-pypridinyl)guanidine 2 Bluegill", with attached report.
1153	2/7/78	Health, Education and Welfare Release regarding a report on animal tests of Picloram for carcinogenicity.
1154	8/23/65	Dow report. "A Game Bird Toxicology Study--Acute Dietary Feeding of Tordon to Wild Type Mallard Ducklings", with attached report.
1155	1/31/63	Dow report. "Fish Toxicity of Lili", with attached report.
1156	4/1/63	Dow Report. "Toxicology Evaluation of K-38, 232 (Picolinic Acid: 4-amino-3,5,6-trichloro)", with attached report.
1157	12/30/65	Dow report. "Toxicological Properties and Industrial Handling Hazards of 11-Tordon Process Samples".
1158	1979	Morpurgo, et al. "Analysis of Mitotic Non-disjunction with Aspergillus Nidulans". Environmental Health Perspectives, Vol. 31, pp. 81-95.
a 6752	1977	Bignami, et al. "Mutagenic and Recombinogenic Action of Pesticides in

		Aspergillus Nidulans". Mutation Research, Vol. 46, pp. 395-402.
1159	Various	Abstracts of various articles on herbicides.
1160	1965	Cope, F. G. "Toxicity of Tordon 101 to Rainbow Trout". Pesticide Prog, Vol. 3, pp. 89-90.
1161	1974	Somers, et al. "Effect of External Application of Pesticides to the Fertile Egg on Hatching Success and Early Chick Performance. No. 2. Commercial-Herbicides Mixtures of 2,4-D with Picloram or 2,4,5-T Using the Pheasant". Bulletin of Environmental Contamination and Toxicology, Vol. 11, pp. 339-342.
1162	1974	Suschetet, et al. "Toxicity and Nutritional of Two Herbicides in the Rat: Picloram (4-amino-3,5,6-trichloro-picolinic Acid and Atrazine 2-chloro-4-ethylamino-6-isopropylamino-s-triazine)". Translated from the French article.
1163	7/30/80	"Picloram: Results of a 13 Week Toxicity Study in Mice", with attached report.
1164	3/20/81	Dow R & D report. "Acute Toxicological Properties and Industrial Handling Hazards of Tordon Acid: 4 and 6 Isomers with Impurities, Not Washed".
1165	00/00/00	Dow data sheet of properties, health hazards and precautions for safe handling of materials for Tordon Acid.
1166	3/20/81	Dow R & D report. "Acute Toxicological Properties and Industrial Handling Hazards of Tordon Acid: 4 and 6 Isomers with Impurities, Not Washed".
1167	5/81	Reuber, Melvin. "Carcinogenicity of Picloram". Journal of Toxicology and Environmental Health, Vol. 7, pp. 207-222.
1168	Various	Various abstracts of articles on herbicide used, including one entitled: "Exposure of Forest Workers Using Herbicides Measured".
1169	1984	Libich, et al. "Occupational Exposure of Herbicide Applicators to Herbicides Used Along Electric Power Transmission Line Right of Way". American Industrial Hygienists Association Journal, Vol. 45,

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		pp. 56-62.
1170	00/00/00	Illegible article.
1171	00/00/00	Document with handwritten title: "Picloram Snarl" and printed list of participants.
1172	1972	Abstract of article on Picloram.
1173	4/88	Draft Environmental Impact Statement: "Vegetation Management in the Coastal Plain--Piedmont", by the USDA Forest Service.
1174	6/83	Draft Environmental Impact Statement on Vegetation Management for Reforestation, from the USDA Forest Service.
1175	5/22/88	Supplement Draft Environmental Impact Statement on Vegetation Management for Reforestation, USDA Forest Service.
1176	1/89	Final Draft Environmental Impact Statement on Vegetation Management in the Coastal Plains/Piedmont, USDA Forest Service.
1177	9/88	Review draft record of decision for USDA Forest Service Specific Northwest Region Environmental Impact Statement on Managing Competing and Unwanted Vegetation.
1178	2/15/79	"The Biologic and Economic Assessment of 2,4,5-T", submitted to the Environmental Protection Agency by USDA. It includes the supplement report.
1179	7/5/88	Twenty photographs of the Johnson Wash project.
1180	00/00/00	Computer printout of product information for Esteron 99, Esteron 99 concentrate and Formula 40 Herbicide.
1181	11/5/82	Document entitled: "2,4-D Herbicide", giving information about the product, prepared by Donald Reed of the FDA.
1182	3/73	Lindenmuth and Davis (plaintiffs' decedent). "Predicting Fire Spread in Arizona's Oak Chaparral", USDA Forest Service research paper.
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1187		00/00/00	Description of duties for research forester (general).
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1194		Various dates in the 1950's	Series of work plans for James R. Davis.
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1218	00/00/00	List of publications by James R. Davis.
1219	Various	Discovery papers from the Agent Orange Case (MDL 381) from the U. S. District Court, Eastern District of New York.
1220	3/13/85	Transmittal and documents of the personnel record of James R. Davis, from the US Office of Personnel Management.
1221	Various	Additional personnel records of James R. Davis, including USDA-Forest Service Performance Rating Sheets.
1222	11/23/76 and 11/9/76	Funeral bills for James R. Davis.
1223	Various	Social Security Benefit Statement, Award Certificate and Medi-Care Information provided to Ruth Davis.
1224	Various	Retirement benefit information for Ruth Davis.

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1225	Various	Tax information for James and Ruth Davis.
1226	Various	Medical records for James R. Davis.
1227	10/76	Death Certificate for James R. Davis.
1228	7/26/88	Exhibits to the deposition of Dr. Ozonoff.
1229	4/19/88	Exhibits to the deposition of Ruth Davis.
1230	1/6/89	Exhibits to the deposition of Dr. Marvin Legator.
1231	7/29/88	Exhibits to the deposition of Peter Formuzis, Ph.D.
1232	Various	10 family photographs.
1233	2/16/86	Article from Times-Colonist Newspaper: "Dow Chemical's Works at Polishing Tarnished Image".
1234	Various	Employment information for Ruth Davis.
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		of Intent to Hold a Hearing", pp. 72328-72341.
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1291	Various	Photographs of James R. Davis at work and

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1. Davis v. Dow Exhibits # 1 - # 277 (+25 sub-parts) 302 total exhibits; 2948 pages total.

2. Davis v. Dow Attachment to Memorandum of November 13, 1991- EXHIBIT LIST OF DOCUMENTS (45 Pages).

3. Davis v. Dow Legal Documents, Exhibits # I. - XVI. (203 pages).

4. Davis v. Dow Attachment to Memorandum of November 13, 1991- SUPPLEMENTAL LIST OF LEGAL DOCUMENTS, (3 pages).

5. Keister v. Dow Exhibits # 1 - # 49 (+6 sub-parts) 758 total pages.

6. Keister v. Dow Memorandum of November 28, 1991 on Review of Documents (3 pages).

7. Keister v. Dow Attachment to Memorandum of November 28, 1991 EXHIBIT LIST OF DOCUMENTS (13 pages).

8. Newman v. AT&T Exhibits # 1 - # 20 (196 pages).

9. Newman v. AT&T Memorandum of 29, 1991 on Review of Documents (3 pages).

10. Newman v. AT&T Attachment to Memorandum of November 29, 1991 EXHIBIT LIST OF DOCUMENTS (6 pages).

FEDEX PAK
(12" x 15 1/2")

1. Davis v. Dow Exhibits # 1 - # 25 on Dow's Publications and Hype on Herbicides; Supplement to Memo of November 13, 1991 (102 pages).

2. Davis v. Dow Supplemental List of Exhibits on Dow's Publications and Hype on Herbicides (2 pages).

3. Memorandum of November 30, 1991 on 2,4-D and 2,4,5-T Chemical Formulas and Key Epidemiological Studies with listing of Documents #1 - # 10 (3 pages)

DAVIS v. DOW

Exhibits 101 - 150

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cc: Kowe
Gehring
D. McCollister
G. Lynn
A. Schaber
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From the Laboratory of Teratology, Karolinska Institutet, S-104 01 Stockholm, Sweden

Teratogenic and Embryotoxic Effects of the Herbicides Di- and Trichlorophenoxyacetic Acids (2, 4-D and 2, 4, 5-T)

By

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(Received November 28, 1972; Accepted December 13, 1972)

Abstract: Commercial solutions of phenoxyacetic acids were tested for teratogenic effects in NMRI-mice. The Swedish product Hormoslyr 500-T contained only 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) while Hormoslyr 64 was a mixture of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T (2:1). Subcutaneous injections were given from day 6 through day 14 of pregnancy and the animals were sacrificed on day 18. The number of resorbed embryos, living embryos with gross malformations, as well as internal and skeletal malformations were recorded. It was found that both preparations at the high dosage (110 mg/kg/day) were teratogenic and embryotoxic. At the low dose level (50 mg/kg/day) the 2,4,5-T solution was more harmful than the mixture of 2,4-D and 2,4,5-T. The risks of teratogenicity in human civilian use and the role of dioxins are discussed.

Key-words: Teratogenesis – embryotoxicity – herbicides – 2,4-D – 2,4,5-T.

The lively discussion of the possible hazard to man of the use of large ntities of herbicides in agriculture and forestry has been focused upon chlorinated phenoxyacetic acids, even though these compounds have been sidered to be only moderately toxic in animals (ROWE & HYMAS 1954). report by COURTNEY *et al.* (1970) on teratogenic and embryotoxic effects 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and to some extent of 2,4-lorophenoxyacetic acid (2,4-D) in experimental animals was the basis the rapidly growing interest in the various adverse effects of these icides. The most significant malformations induced at high doses are t palate in mice and cystic kidneys in rats and mice. That the teratogenic ct is due not only to the 2,4,5-T but also to the high content (27 p.p.m.) he dioxin contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was irmed in a later study (COURTNEY & MOORE 1971).

tal studies on 2,4,5-T, 2,4-D and dioxins have since been performed

in different species and these essentially confirm that the two phenoxyacetic acids at high doses are teratogenic and embryotoxic and show that TCDD is teratogenic in the mouse (ROLL 1971; NEUBERT & DILLMANN 1972). It is interesting to note that even with high doses (40 mg/kg 2,4,5-T and 80 mg/kg 2,4-D), it has not been possible to produce malformations in monkeys (WILSON 1972a). However, two out of four females aborted after 2,4-D treatment (WILSON 1972b). The report that phenoxyacetic acids cause abortions in reindeers in Sweden was later not confirmed in a study by EHRNE (personal communication). Several newspaper reports of human malformations in Sweden, Vietnam and Arizona which had been attributed to the mothers' alleged exposure to the phenoxyacetic acids have been evaluated on more than one occation but it has not been possible to point at a causal relationship (LARSSON 1971).

2,4,5-T and 2,4-D as different esters, salts or acids have been commonly used as weed killers for more than 20 years. In Vietnam they have been used as defoliants and crop-destroyers in quantities 10–15 times that recommended for civilian use. The regulations for use in the U. S. A. and Sweden have recently been restricted. For example, the use of phenoxyacetic acids in forestry was temporarily banned in March, 1971, by the Swedish Poisons and Pesticides Board in Sweden. The case was to be opened for re-evaluation at the beginning of 1972 when additional data would be available. Most of the reports so far have dealt with studies on 2,4,5-T with various dioxin contents. However, since the phenoxyacetic acids used as herbicides for civilian purposes usually contain both 2,4,5-T and 2,4-D together with emulsifiers and solvents, and since mechanisms of interaction, e. g. synergism, cannot be ruled out untried, it has been considered to be of practical interest to test the teratogenic effects of commercial samples. In the following study the effects on mice of two commercial phenoxyacid formulations in Sweden, Hormoslyr 64 (2,4-D and 2,4,5-T, 2:1) and Hormoslyr 500-T (2,4,5-T) have been tested in a double blind test.

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Material and Methods

Three-month-old random-bred NMRI mice (obtained from Gl. Bomholtgaard, DK-86 Ry, Denmark) were used. The animals were kept in macrolone cages in a room with constant temperature (23°) and automatically regulated light from 6 a. m. to 6 p. m. and fed standard lab chow (Ewos, Södertälje, Sweden) and water *ad libitum*. The females were mated overnight and checked for vaginal plugs the next morning, denoted as day zero of pregnancy. The females were weighed on this day and also from days 6 to 18.

The test solutions were prepared and coded for the double blind test at the Swedish Poisons and Pesticides Board. The substances (kindly supported from Gullviks Fabriks

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Table I.

Main teratogenic and embryotoxic effect on NMRI mice after treatment with 2,4,5-T or 2,4-D/2,4,5-T (2:1) from day 6 through day 14 of gestation.

Group	Daily treatment Substance	Dose s.c. mg/kg	No. of litters	No. of implantation sites	% of resorbed implantation sites	No. of living foetuses	No. of foetuses with cleft palate	% of foetuses with cleft palate	Mean weight of living foetuses g	± S. D.
A	2,4,5-T	110	18	203	70.4	60	44	73.3	0.76	± 0.14
B	2,4,5-T	50	19	247	23.1	190	38	20.0	0.99	± 0.16
C	2,4-D+2,4,5-T	110	20	250	55.6	111	75	67.6	0.78	± 0.14
D	2,4-D+2,4,5-T	50	20	256	14.5	219	21	9.6	1.05	± 0.14
E	Vehicle	0	19	218	12.4	191	9	4.7	1.07	± 0.14

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AB, Malmö, Sweden) used for preparing the solutions were the commercially available Hormosiyr 500-T which contains the butoxyetylester of 2,4,5-trichlorophenoxyacetic acid (500 g/l), and Hormosiyr 64 which contains 2,4-dichlorophenoxyacetic acid (330 g/l) and 2,4,5-trichlorophenoxyacetic acid (170 g/l). The content of dioxins, mainly TCDD, was less than 1 p.p.m. and the most common dioxin was 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (verified at the Swedish Poisons and Pesticides Board). Dimethylsulphoxide (DMSO) was used as a vehicle of the test solutions, which also contained 19 mg/ml of the solvent (a non-specified mixture of petroleum distillates). The animals were given subcutaneous injections 0.1 ml/20 g body weight at 10 a. m. from day 6 through day 14 of gestation. The daily dose of phenoxyacetic acid for the different groups was as follows:

A: 110 mg/kg of 2,4,5-T
B: 50 mg/kg of 2,4,5-T
C: 110 mg/kg of 2,4-D/2,4,5-T (2:1)
D: 50 mg/kg of 2,4-D/2,4,5-T (2:1)
E: 0 mg/kg controls given 0.1 ml/20 g of the vehicle).

The number of animals treated in the five experimental groups are given in table 1. None of the treated dams died or showed any signs of adverse effects except for local necroses at the injection sites which appeared after a few days.

On day 18 of gestation, the animals were sacrificed by cervical dislocation. The living foetuses were removed, weighed and examined for gross malformations. The number and size of the dead and resorbed foetuses were noted. One third of the living foetuses from each litter was fixed in ethanol, cleared in potassium hydroxide and stained with Alizarin red S for examination of skeletal malformations (Dawson 1926–1928). The remainder of the living foetuses was fixed in Bouin's solution for subsequent sectioning and examination for internal malformations (Wilson 1965).

Results

Foetal mortality. Foetal mortality was significantly increased at both dose levels of 2,4,5-T and at the high dose of the 2,4-D/2,4,5-T mixture, see table 1. The most severe effect was obtained in the two groups given the highest phenoxyacid dose, see table 2. Thus, every litter in group A and C showed resorptions and 33 % and 25 % of the litters respectively were totally resorbed. In group B only 2 of 19 litters showed more than 50 % resorption. Group D showed more litters with resorptions than the control group E, even if the resorption rate was almost similar in these groups.

Isolated cleft palate. An increased frequency of cleft palate was also observed in the same groups and to the same degree as described for the incidence of foetal mortality, see table 1. Litters with cleft palate in all surviving foetuses were obtained only in the high dose groups: in A, 6 out of 12 litters and in C, 6 out of 15 litters.

Retarded foetal growth. The foetal weight was considerably reduced in animals treated with the high doses of the phenoxyacetic acids while no definite effect was registered after treatment with the low doses (table 1).

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Table 2.

ribution of resorption frequency in litters from NMRI mice after treatment with 2,4,5-T and 2,4-D/2,4,5-T (2:1) from day 6 through day 14 of gestation.

roup	Daily treatment Sub-stance	Dose s.c. mg/kg	No. of litters	Litters with at least one foetus resorbed no.	%	Litters with > 50 % resorbed foetuses no.	%	Litters with all resorbed foetuses %	no.
A	2,4,5-T	110	18	18	100	13	72	6	33
B	2,4,5-T	50	19	16	84	2	4	0	
C	2,4-D+ 2,4,5-T	110	20	20	100	12	60	5	25
D	2,4-D+ 2,4,5-T	50	20	15	75	0		0	
E	Vehicle		19	11	58	0		0	

slightly low-weight foetuses most often showed retarded skeletal develop-nt and reduced amounts of brown fat in the cervical region. Very few of foetuses in the control group showed these signs of retarded development.

keletal malformations. After the highest doses of phenoxyacetic acids the centage of foetuses with malformations of ribs and vertebrae was double t of the other three groups, including the controls (table 3). In addition, degree of malformations was much more severe in these two groups, ecially in group A.

Table 3.

letal and internal malformations in NMRI mice after treatment with 2,4,5-T and 2,4-D/2,4,5-T (2:1) from day 6 through day 14 of gestation.

roup	Daily treatment Sub-stance	Dose s.c. mg/kg	Rib and vertebral malformations no.*	%	Haemorrhages subcutaneous no.	%	renal no.	%	Dilated renal pelvis no.	%
A	2,4,5-T	110	6/24	25.0	10/60	16.7	1/36	2.8	3/36	8.3
B	2,4,5-T	50	7/70	10.0	9/190	4.7	0/120		4/120	3.3
C	2,4-D+ 2,4,5-T	110	11/44	25.0	21/111	18.9	11/67	16.4	5/67	7.5
D	2,4-D+ 2,4,5-T	50	7/82	8.5	6/219	2.7	6/137	4.4	4/137	2.9
E	Vehicle		8/68	11.8	10/191	5.2	3/123	2.4	7/123	5.7

lo. with malformations/number examined.

Table 4.

Examples of spontaneously occurring malformations in NMRI mice after treatment with 2,4,5-T and 2,4-D/2,4,5-T (2:1) from day 6 through day 14 of gestation.

Group	Daily treatment Substance	Dose s.c. mg/kg	No. of living foetuses	No. of foetuses with exencephaly	hydrocephaly	open eye	micrognatia	ectrodactyly
A	2,4,5-T	110	60	2	1	1	3	1
B	2,4,5-T	50	190	3	1	2	–	–
C	2,4-D+ 2,4,5-T	110	111	–	4	1	3	1
D	2,4-D+ 2,4,5-T	50	219	2	1	–	–	–
E	Vehicle		191	2	1	1	1	–

Haemorrhages. Subcutaneous bleeding with a large variation in location was found to be three times as common in the two high dose groups as in the other three groups (table 3). The frequency of renal haemorrhage was increased only in the group given 2,4-D/2,4,5-T in the high dose (table 3). No gastro-intestinal haemorrhages were detected.

Renal malformations. Cystic kidneys were not found. Foetuses with different degrees of dilated renal pelves were found, of which only the more pronounced ones were recorded (table 3). Only a slightly increased frequency was observed in the high dose groups. The foetal kidneys in the two high dose groups usually appeared smaller and opaque in the sections as compared with the controls which were larger and had translucent medullae.

Spontaneous malformations. In the investigated material a few malformations appeared in all the groups as shown in table 4.

Discussion

The present investigation confirms earlier studies, which have shown that high doses of phenoxyacetic acids are teratogenic, cause foetal death and retarded foetal growth in mice (COURTNEY *et al.* 1970; ROLL 1971; NEUBERT & DILLMANN 1972). The substances tested which are used as weed killers in Sweden do not, however, indicate any greater teratogenic risk than 2,4,5-T or 2,4-D compounds used in other experimental studies.

The various types of malformations found in this study were identical with those reported by other investigators. The most common defect was

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olated cleft pal Cystic kidneys which was one of the most characteristic malformations observed by COURTNEY *et al.* (1970) after 2,4,5-T treatment were not found. Nor did we observe any marked dilatation of the renal elves. Less frequent, but probably also caused by the treatment, were celetal malformations and subcutaneous haemorrhages. A frequent observation was that the skin of the living foetuses, especially the small ones, as very sensitive to handling with easily occurring subcutaneous bleeding. he phenomenon was mainly observed after treatment with the phenoxyetic acid mixture at the higher dose but it also occurred in the other groups. andling NMRI foetuses of the same age from untreated litters (unpublished servation) did not cause this type of subcutaneous bleeding. It cannot be led out that the dimethylsulphoxide or the trace quantities (less than p.p.m.) of dioxin present caused some of the subcutaneous bleeding (CAULLE *et al.* 1967). Gastro-intestinal bleeding was not detected, though it s been reported to occur after 2,4,5-T treatment or dioxin treatment OLLINS & WILLIAMS 1971; COURTNEY *et al.* 1971; SPARSCHU *et al.* 1971). ıly the mixture of 2,4-D/2,4,5-T at the 110 mg/kg dose increased the quency of renal haemorrhages.

Earlier studies indicated that 2,4,5-T was more teratogenic than 2,4-D IRAK 1969). It is interesting to note that the foetal damaging effect of the mbination of 2,4-D/2,4,5-T did not exceed the effect of 2,4,5-T at a comrable dose of phenoxyacetic acid. Concerning the teratogenic effects of two substances in combination at 110 mg/kg it seems, however, as if the 4-D contributes more than would be expected from purely additive effect. ice only few studies on 2,4-D derivatives are available the results indicate it further studies should be carried out to investigate a possible synergistic ect of the two phenoxyacetic acids.

LINDQUIST & ULLBERG (1971) suggested an interference with embryonic trition as a possible mechanism for the teratogenic action of the herbicides ,5-T and 2,4-D. In spite of a rather even distribution on whole-body oradiographs of the two substances there was a much more rapid disearance from all tissues for 2,4-D and a stronger accumulation in the k sac placenta for 2,4,5-T (LINDQUIST & ULLBERG 1971). The selective ake of 2,4,5-T in the yolk sac placenta and a lack of placental transfer in ly gestation was interpreted in favour of a similar teratogenic mode of ion for 2,4,5-T at that postulated for trypan blue (LLOYD & BECK 1969). wever, an inhibition of the embryonic nutrition seems to be a less likely se for, e. g. cleft palate since even single injections of 2,4,5-T on day 13 UMLRT & DILLMANN 1972) can cause cleft palate and at that stage the in nutritional role of the yolk sac placenta is over.

n all animal experiments the dose resulting in teratogenic effects has been remely high as compared with the dose which a pregnant woman could normally be exposed to. Thus, the results of the present and other studies do not substantiate any special risk to the human embryo from the regular use of phenoxyherbicides.

Acknowledgements

This study was supported by grants from the Swedish Medical Research Council (14X and 14P-993-07) and from the Swedish Poisons and Pesticides Board.

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Key references missing: Emerson et al, 1971; Khera & McKinley, 1972; Schwetz et al., 1971

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Protein Binding of Drugs in Plasma from Patients with Acute Renal Failure

By

Frederik Andreasen

(Received July 19, 1972; Accepted October 30, 1972)

Abstract: It was found by *in vitro* experiments that the protein binding of acetylsalicylic acid, salicylic acid, phenylbutazone, diphenylhydantoin, sulphadiazine and thiopental was decreased in the plasma of 10 surgical patients with acute renal failure. The plasma was taken 4 to 8 days after the onset of the renal failure. At a drug concentration of 400 µg/ml the decreases ranged from 21.8 to 60.3 % as compared with a normal control group. At drug concentrations in or near the normal therapeutic level, the protein binding was even more decreased for the salicylic acids and sulphadiazine but less decreased for diphenylhydantoin and thiopental. The decreased binding could only partly be explained by the lower concentration of albumin in the patients: 3.0 g/100 ml ± 0.48 (S.D.) compared to 4.1 g/100 ml ± 0.53 (S.D.) ($P < 0.01$).

Key-words: Kidney failure – acute anuria – protein binding – drug therapy.

Acute renal failure (tubulo-interstitial nephritis (BRUN 1954)) is a com plication which may occur in patients suffering from severe injuries. Beside the renal changes the patients frequently show impaired hepatic functio (cf. BEECHER *et al.* 1947; SHERLOCK 1963; THORÉN 1969). The concentratio of serum proteins can be expected to be decreased (THORÉN 1969). Valu for the serum albumin concentration in patients with acute renal failure a not given in the literature. LANGE *et al.* (1971) found in general agreeme with other investigators that the average protein breakdown in 10 patien with acute renal failure was 105 g/24 hrs during the first eight days.

KUNIN (1967) and BENNETT *et al.* (1970) have given practical guides drug usage in patients with impaired renal function and HEDGER (197 gives a similar guide for patients suffering from acute renal failure. Appa ently drug therapy in these patients is usually based on knowledge of t biological half-life of the drug in normal subjects and on the creatini clearance of the patients (cf. BERNE & BARNOUR 1971).

The purpose of the *in vitro* experiments reported here was to elucida the following questions:

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DATE: January 5, 1973

AUTHORS: E. E. Kenaga

PAGES: 38

TITLE OF REPORT: ENVIRONMENTAL IMPACT OF HERBICIDES

PROBLEM NUMBER:

Herbicide Study Group, HMAC, EPA

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INFORMATIVE SUMMARY WITH CONCLUSIONS

In April, 1972 at the request of the administrator of the Environmental Protection Agency, the Hazardous Materials Advisory Committee was asked to appoint an expert committee called a "Herbicide Study" group to determine and write a report on whether herbicides were being evaluated properly and impartially in regards to cost-benefits to man and hazards to the environment. Areas of insufficient knowledge were to be pointed out. E. E. Kenaga was subsequently appointed to the "Environmental Impact" subcommittee. Kenaga's input, but not necessarily what will be issued in the final committee report, is covered here. Subjects discussed are physical properties of widely used herbicides, herbicide residues in the environment, toxicity of herbicides to wildlife and a summary of the evaluation of the uses and hazards of herbicides in various habitats.

AUTHOR(S) SIGNATURE: Eugene E. Kenaga

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ratories, State College, Pa.). Column temperatures ranged from 150 to 220° (isothermal conditions) and the carrier gas flow was 40 ml/min (prepurified helium).

Photoreduction Rates. Methanol and *n*-butyl alcohol solutions were 10^{-4} *M*. Water solutions were 10^{-5} *M*. Samples, 10 ml each, were placed in borosilicate tubes fitted with Teflon stoppers (volume remained constant throughout the reaction period). Irradiation was carried out for periods of 1–32 hr at 300 nm at 40°. A "merry-go-round" apparatus was used to ensure equal exposure of all samples to uv irradiation. The light source used was a Rayonet reactor equipped with RUL 3000 lamps (maximum output at 300 nm) (The Southern N.E. Ultraviolet Co.).

After irradiation, 2-μl aliquots were injected in the gas chromatograph and *s*-triazine disappearance was measured (peak areas were measured by weight). From the amount reacted ($A_0 - A$) and the elapsed time (t) the rate constants (k) were calculated (Table II).

RESULTS AND DISCUSSION

As shown in Table II and Figures 1–4, the rate of photoreduction is dependent on at least three factors. The nature of the halogen substituent (Figures 1 and 2) has a definite influence on the rate. The value of k calculated decreases rapidly in the order I-Br-Cl-F. These results are in agreement with the known dissociation energies of the corresponding carbon–halogen bonds. The large difference between the rates for iodo- and bromo-substituted triazines and those for chloro- and fluoro-substituted ones may also be the result of the increased interaction between the nonbonding electrons on iodine and bromine with the π-system in the ring. Both these halogens are known to undergo valency shell expansion (Forbes, 1959) especially in the excited state. This heavy atom effect facilitates intersystem crossing.

The rate of reaction depends on the nature of the solvent employed as well. A decrease in k is observed when *n*-butyl alcohol is used instead of methanol or water. The rate of photoreaction in the latter solvents is approximately the same (Table II). This effect is probably the result of a difference in polarity of the solvents involved and of their viscosity. Polar solvents are known to decrease the energy required for π–π^* transitions (Forbes, 1960).

	First absorption band	
s-Triazine	λ (methanol)	λ (1-butanol)
Simazine	268	262
Atrazine	248	244
Propazine	237	235

s-Triazines with ethyl substituents in the 4 and 6 *N* positions show a greater k value than those with isopropyl groups (Table II, Figures 3 and 4). The main difference in these alkyl groups is their size. As shown above, the increase in chain length results in a decreased wavelength of absorption. Steric effects are known to disturb the geometry of an excited state (Ruzo *et al.*, 1973). This fact may explain our observations; however, further investigation is necessary in order to assess its importance in the case of *s*-triazine photochemistry.

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Photodecomposition of *p*-Chlorophenoxyacetic Acid

Donald G. Crosby* and Anthony S. Wong

Aqueous solutions of 4-CPA (*p*-chlorophenoxyacetic acid) decomposed readily under sunlight or laboratory ultraviolet light (300–450 nm) to provide principally *p*-chlorophenol, phenol, hydroquinone, *p*-chlorophenyl formate, phenoxyacetic acid, *p*-hydroxyphenoxyacetic acid, and humic acids. These products represent oxidative removal of the side chain, replacement of the chlorine by hydroxyl or by hydrogen, and polymerization of unstable intermediates. Formation of *p*-chlorobenzonitrile by irradiation of 4-CPA in the presence of cyanide ions substantiated that the corresponding replacement of the ring chlorine by hydroxyl was a photonucleophilic reaction.

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The effects of ultraviolet (uv) light on chlorinated phenoxyacetic acids have been reported by several investigators. Kelly and Pinhey (1964) irradiated *p*-chlorophenoxyacetic acid (4-CPA) in ethanol with a medium-pressure mercury arc lamp and recovered phenol as well as *o*- and *p*-hydroxyphenylacetic acids from the migration of the side chain. Crosby and Tutass (1966) reported that irradiation of 2,4-dichlorophenoxyacetic acid (2,4-D) in aqueous solution with a low-pressure lamp resulted in 2,4-dichlorophenol, 4-chlorocatechol, 2-hydroxy-4-chlorophenoxyacetic acid, 1,2,4-benzenetriol, and humic acid from processes involving mainly the removal of the side

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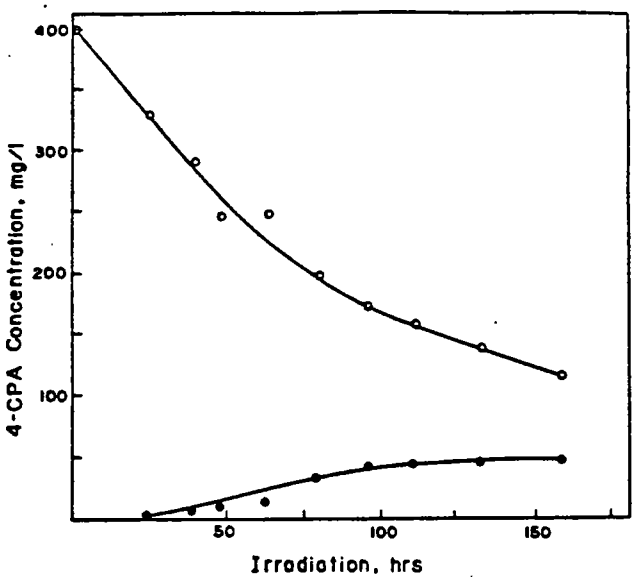


Figure 1. Photodecomposition rate of 4-CPA (○), and simultaneous formation of p-chlorophenol (●), at an initial pH of 8.0.

chain and the replacement of chlorines by hydroxyls; similar results also were obtained from irradiation of chlorophenylacetic acids (Crosby and Leitis, 1969).

Few photolysis experiments have been conducted with chlorinated phenoxyacetic acids using the low-energy uv light characteristic of natural sunlight. 4-CPA is a widely used growth regulator and the simplest of the chlorinated phenoxyacetic acids, making it an ideal model. The purpose of this investigation was to examine the effect of natural and simulated sunlight (300–450 nm) on dilute aqueous 4-CPA solutions and establish a general mechanism for the environmental photolysis of phenoxy acids.

EXPERIMENTAL SECTION

Materials. *p*-Bromophenol, *p*-chloroanisole, *p*-chlorobenzonitrile, *p*-chlorophenoxyisobutyric acid, *p*-chlorophenol, *p*-cyanophenol, hydroquinone, *p*-hydroxyphenoxyacetic acid, and phenol were commercial products used without further purification. 4-CPA was recrystallized three times from benzene, mp 157–158°. Humic acid was prepared previously by Crosby and Tutass (1966).

p-Chlorophenyl formate was prepared according to Van Es and Stevens (1965); mass spectrum *m/e* 156 (M^+), 127 (base, $M^+ - CHO$); infrared (ir) spectrum 1200, 1757 (OCHO) cm^{-1}. *p*-Cyanophenoxyacetic acid was obtained by the reaction of chloroacetic acid with *p*-cyanophenol in dilute base, mp 177–178° [Hayes and Branch (1943) reported 178.2–178.5°].

Irradiations. Standard solutions of 4-CPA (400 mg/l.), adjusted to pH 8 with aqueous sodium hydroxide, were irradiated for 65–100 hr with uv light (300–450 nm) in the presence of either air or nitrogen in a 2-l. borosilicate glass reactor (Crosby and Tang, 1969) or with natural sunlight for 7–10 days during April and May in Davis, Calif.

The brownish-yellow irradiated solutions (pH 3–4) were neutralized with dilute aqueous base and extracted three times with ether; the combined organic phases were washed with water, dried over sodium sulfate, and evaporated to a small volume on a rotary evaporator (neutral fraction). The aqueous phase was acidified to pH 2, extracted with ether, and the extract dried and evaporated as above (acidic fraction). The aqueous portion remaining after the second extraction was evaporated almost to dryness and taken up into 7:3 (v/v) methylene chloride–acetone (insoluble fraction).

detector as described previously (Moilanen and Crosby, 1972). Most separations employed 2 ft × ¼ in. stainless steel columns packed with either 1% DEGA or 6% SE30 on 60–80 mesh Chromosorb G, helium flow rate 25 ml/min, and an oven temperature programmed from 75° to 200° at 5°/min. Acids were methylated with ethereal diazomethane before chromatography.

Other aqueous solutions were irradiated as follows: (A) *p*-chlorophenol (100 mg/l.), adjusted to pH 8, irradiated for 16 hr; (B) 4-CPA (400 mg/l.) in 1% sodium bisulfite, adjusted to pH 8, irradiated for 68 hr; (C) *p*-chlorophenoxyisobutyric acid (200 mg/l.), adjusted to pH 8, irradiated for 64 hr; (D) 4-CPA (10 g/l.) in 5% potassium cyanide containing 0.5% potassium hydroxide, irradiated for 76 hr; (E) 4-CPA (400 mg/l.) in 0.5% potassium bromide, adjusted to pH 9, irradiated for 65 hr.

Rate Measurement. Aqueous solutions containing 400 mg/l. of 4-CPA and adjusted to pH 8 or 2 were irradiated for 200 hr. The temperature was maintained at a constant 35° with a heating tape, and the voltage supply to the uv light was stabilized with a regulator. No attempt was made to control pH. Aliquots (50 ml) were withdrawn at intervals, acidified to pH 2, and extracted with ether. *p*-Chlorophenol was determined by glc, while unreacted 4-CPA was similarly determined after methylation. Analytical values were estimated from a standard curve for each compound. Recovery from unirradiated standards was quantitative.

Identification. Identification of all compounds except 2,4′-dihydroxybiphenyl was based on comparisons of physical properties and spectra of fractions isolated by glc with those of authentic standards. 2,4′-Dihydroxybiphenyl eluted at 200°: mp 162–163° [Omura and Matsuura (1969) reported 162–163°]; mass spectrum *m/e* 186 (base and M^+); ir spectrum 3600, 3400 (OH), 1600, 1525 (φ), 835 (*p*-φ), 770 (*o*-φ) cm^{-1}.

RESULTS AND DISCUSSION

When dilute aqueous solutions of 4-CPA (sodium salt) were irradiated in air with either uv light in the laboratory or with natural sunlight, the neutral extract yielded primarily *p*-chlorophenol (III) along with *p*-chlorophenyl formate (II) and phenol (V). The acidic extract contained only *p*-hydroxyphenoxyacetic acid (VII) and unreacted 4-CPA. Subsequent irradiation of *p*-chlorophenol yielded phenol, hydroquinone, humic acid, and a small amount of 2,4′-dihydroxybiphenyl (Experiment A). The polymeric humic acid was insoluble.

As with 2,4-D (Crosby and Tutass, 1966), inhibition of the final autoxidation step by sodium bisulfite permitted isolation of *p*-chlorophenol, *p*-chloroanisole, hydroquinone (VI), and phenoxyacetic acid (IV) (Experiment B), although control experiments showed that 4-CPA also reacted with sodium bisulfite in the dark to form *p*-chlorophenol and the *p*-chloroanisole.

The rates of photodecomposition of 4-CPA in basic solution (pH 8) and the formation of *p*-chlorophenol are illustrated in Figure 1. The highest observed yields of *p*-chlorophenol and *p*-chlorophenyl formate at any given time were 12 and 3%, respectively, while the other products were present in much smaller quantities. The photodecomposition rate of 4-CPA in acidic solution (pH 2) was about half that in the basic solution. Under laboratory conditions, the time for total disappearance of 4-CPA was 250–300 hr, while an equal amount of *p*-chlorophenol decomposed in half that time.

These observations suggest that the photodecomposition reactions of 4-CPA in water consist of three types: oxidative side chain reactions, ionic ring reactions, and condensations.

Side Chain Reactions. The rate-determining step

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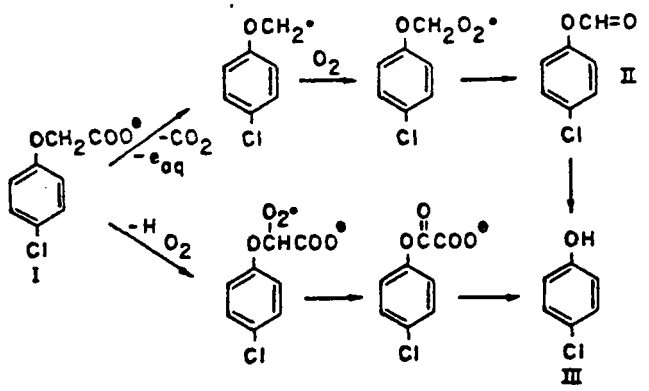


Figure 2. Mechanisms of 4-CPA photooxidation.

whose rate varied directly with pH. Two oxidative mechanisms are plausible (Figure 2): (a) the normal autoxidation of an ether (Walling, 1957) followed by rearrangement of the resulting peroxy radical and hydrolysis; and (b) oxygenation of the radical generated by loss of a hydrated electron and carbon dioxide from 4-CPA anion (Joschek and Grossweiner, 1966), again followed by rearrangement. The second mechanism is preferred: the predicted intermediate II was isolated, and replacement of the methylene hydrogens of the phenoxyacetic acid with methyl groups (*p*-chlorophenoxyisobutyric acid) still permitted the efficient formation of *p*-chlorophenol upon irradiation (Experiment C). However, the formation of traces of *p*-chlorobenzonitrile during irradiation of 4-CPA in the presence of cyanide ions (Experiment D) suggests that the *p*-chlorophenol observed in normal 4-CPA photolysis could be formed, in part, by nucleophilic displacement of the side chain by hydroxide ion.

The intramolecular rearrangement, proposed to involve a radical pair enclosed in a solvent cage, which produced the *o*- and *p*-hydroxyphenylacetic acids during irradiation of 4-CPA in ethanol at 254 nm (Kelly and Pinhey, 1964; Kelly *et al.*, 1969) was not observed in our experiments (300–450 nm in aqueous solutions), perhaps due to the presence of oxygen. An ionic process also has been suggested for the rearrangement (Coppinger and Bell, 1966) but was not in evidence.

Ring Reactions. The most characteristic ring reaction was the pH-dependent replacement of the chlorine by hydroxyl or by hydrogen. These reactions were not affected by or dependent on oxygen, although similar processes have been considered by Joschek and Miller (1966) to involve free radicals. While the intensity of short wavelength uv radiation in sunlight is low, there would be sufficient quantum energy near the atmospheric cutoff (100 kcal/mol at 286 nm) to break the carbon-chlorine bond (about 80 kcal/mol) but apparently not enough for homolytic dissociation of water (116 kcal/mol). Further, the replaced chlorine appeared as Cl^- rather than Cl_2, and our specific search failed to reveal biphenyls. Hydroxylation rationalized on the basis of some energetic oxygenating species such as those generated by the hydrated electron (Figure 3) is not consistent with the lack of an oxygen dependence. The source of hydrogen to produce the dechlorinated products IV and VII has not been identified.

The reaction is better explained as a photonucleophilic substitution (Havinga and Kronenberg, 1968) in which hydroxide ion displaced chloride from the photoexcited ring, a process already known to occur under high-energy irradiation (Nijhoff and Havinga, 1965). This mechanism is supported by our observation that 4-CPA reacted readily with cyanide ions upon irradiation (Experiment D) to form *p*-cyanophenol and *p*-cyanophenoxyacetic acid analogous to the similar reaction of *p*-chlorophenol (Omura and Matsuura, 1969); a small amount of *p*-chlorobenzonitrile resulted from photonucleophilic displacement of the oxyacetate side chain (eq 1). In the dark, 4-CPA formed traces of *p*-cyanoanisole and *p*-chlorobenzonitrile; it reacted with potassium bromide in the light (Experiment E) to give *p*-bromophenol but was unreactive in the dark.

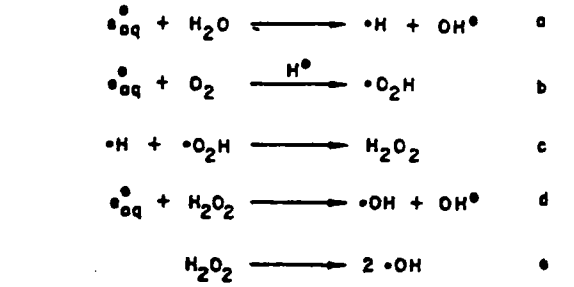


Figure 3. Radical generation from solvated electrons.

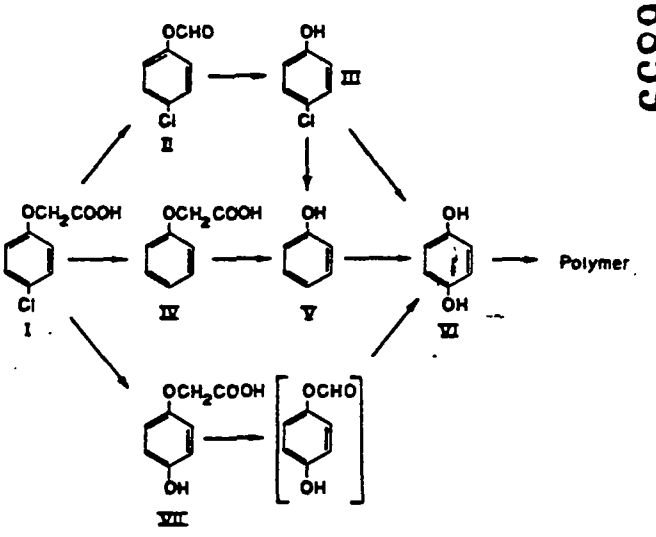
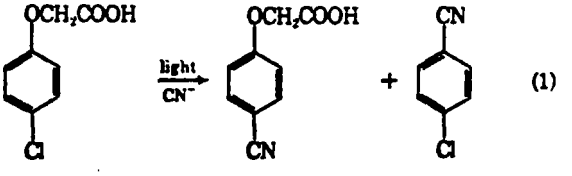


Figure 4. Proposed route of phenoxy acid photodecomposition based on 4-CPA photoproducts.



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Condensations. When the irradiation of aqueous *p*-chlorophenol with a low-pressure mercury arc lamp was conducted at sufficiently high concentrations (>1%), hydroxylated biphenyls were produced (Omura and Matsuura, 1969). However, only traces of 2,4′-dihydroxybiphenyl were detected at the low concentrations of *p*-chlorophenol in our experiments (Experiment D), and none was formed from 4-CPA. The expected 4-chloro-2,4′-dihydroxybiphenyl was not observed at all.

Formation of the polymeric humic acids represented the eventual fate of 4-CPA as it did with other compounds that can be converted to quinoid products (Crosby and Leitis, 1969; Crosby and Tutass, 1966; Moilanen and Crosby, 1972). Under field conditions, this polymerization probably would involve both ionic and oxidative combination with other substances in contact with the quinoid intermediates to form a variety of polymer types representing, in part, the carbon atoms of the original 4-CPA ring.

The products identified and the mechanisms proposed for their formation suggest the photolysis pathway for

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4-CPA shown in Figure 4. However, the close analogy between these products and those obtained from the photolysis of 2,4-D (Crosby and Tutass, 1966) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) (Crosby and Wong, 1973) indicates that the pathway will be general for the other commercial phenoxy herbicides.

ACKNOWLEDGMENT

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DOW 556860

Photodecomposition of 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T) in Water

Donald G. Crosby* and Anthony S. Wong

Photodecomposition of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in aqueous solution principally involved cleavage of the ether bond and replacement of the ring chlorines by hydroxyl and by hydrogen. The major products were 2,4,5-trichlorophenol and 2-hydroxy-4,5-dichlorophenoxyacetic acid; 4,6-dichlororesorcinol, 4-chlororesorcinol, 2,5-dichlorophenol, and a dark polymeric product also were isolated. The toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was not detected among the photodecomposition products. 2,4,5-T photolyzed very slowly compared to its 4-chloro and 2,4-dichloro analogs, but the 11-fold increase in photolysis rate caused by sensitization with acetone or riboflavin suggests that sunlight can be an important factor in the environmental degradation of 2,4,5-T.

For almost three decades, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T, I) and its derivatives have received wide use as herbicides, especially for control of brush, jungle, and aquatic weeds. For example, the 1968 U. S. production of these compounds exceeded 60 million pounds (U. S. Tariff Commission, 1970), and residues have been detected in crops, soil, the atmosphere (Bamesberger and Adams, 1966), rainwater (Cohen and Pinkerton, 1966), and surface waters (Thoman and Nicholson, 1963). The fate and persistence of 2,4,5-T in the environment have been the subject of considerable investigation (Loos, 1969; PSAC, 1971); this report deals with the influence of sunlight, natural and simulated, on aqueous solutions of 2,4,5-T and its salts.

EXPERIMENTAL SECTION

Materials. 2,4,5-T, 2,4,5-trichlorophenol (II), 4,6-dichlororesorcinol (III), 2,5-dichlorophenol (IV), and 4-chlororesorcinol (V) were purified commercial products. 2,4,5-T was recrystallized three times from benzene, mp 153.5–54.5°. The trichlorophenol was freed of persistent impurities by dissolving it in aqueous sodium hydroxide solution, extracting neutral impurities with several portions of benzene, acidification, recrystallization three times from benzene, and sublimation under reduced pressure, mp 67.5°.

Irradiation. Solutions of 2,4,5-T (100 mg/l.) in distilled water were adjusted to pH 8 with 0.1 *N* sodium hydroxide and irradiated outdoors with summer sunlight in Davis, Calif., or indoors with ultraviolet (uv) light in the wavelength region of 300–450 nm. The irradiations were conducted in 3-l. borosilicate glass flasks, those indoors mounted in a 14 in. (i.d.) × 5 ft cylindrical chamber (Figure 1) constructed of Transite pipe cut in halves longitudinally, hinged, and lined with bright aluminum sheet. The chamber was fitted with six 4-ft F40BL fluorescent uv lamps (Crosby and Tang, 1969), three clamped to the fixed half and three to the hinged door. Light intensity measured with a YSI radiometer (Yellow-Springs Instrument Co., Yellow Springs, Ohio) was 390 μW/cm^2 at the center of the chamber and 785 μW/cm^2 halfway from the center to the lamp surface. The chamber temperature remained close to 30°, and filtered air was passed through the solutions to maintain oxygen saturation and efficient agitation. The removable hood and vent for toxic compounds were not used in the present experiments.

The indoor irradiation was allowed to proceed for 200 hr, and 50-ml aliquots were withdrawn at intervals and analyzed by gas–liquid chromatography (glc). Equivalent experiments were conducted with: (A) 2,4,5-T in 0.5% aqueous sodium bisulfite; (B) aqueous 4-chlorophenoxyacetic acid (4-CPA) at pH 8; and (C) aqueous 2,4-dichlorophenoxyacetic acid (2,4-D) at pH 8, as well as in the dark. The exposure time for the outdoor preparative scale experiments amounted to about 9 hr each day for 60 days (540 hr).

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ENVIRONMENTAL IMPACT OF HERBICIDES

Herbicide Study Group, HMAC, EPA

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January 5, 1973

SUMMARY

In April, 1972 at the request of the administrator of the Environmental Protection Agency, the Hazardous Materials Advisory Committee was asked to appoint an expert committee called a "Herbicide Study" group to determine and write a report on whether herbicides were being evaluated properly and impartially in regards to cost-benefits to man and hazards to the environment. Areas of insufficient knowledge were to be pointed out. E. E. Kenaga was subsequently appointed to the "Environmental Impact" subcommittee. Kenaga's input, but not necessarily what will be issued in the final committee report, is covered here. Subjects discussed are physical properties of widely used herbicides, herbicide residues in the environment, toxicity of herbicides to wildlife and a summary of the evaluation of the uses and hazards of herbicides in various habitats.

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INTRODUCTION

In April, 1972 I was appointed as a private citizen to the Environmental Subcommittee of the "Herbicide Study" group, sponsored by the Hazardous Materials Advisory Committee (HMAC) of the Environmental Protection Agency (EPA). The task of the "group" initially was to study the chemistry, toxicology, crop and other uses, and environmental impact of the most commonly used herbicides. Upon deciding which were the most heavily used pesticides (see Table 1) in the U.S. my initial assignment was a section on residues in the environment. Assignments were given to other people and their reports were reviewed. I was not satisified with the wildlife toxicology data section, or the summary. Also needed (I thought) was a table of the physical properties, all of which I furnished. Thus, the information given here, while not a complete treatise on the subject, does represent my input to the Environmental Subcommittee. The "Herbicide Study" report is still being written and revised and there is no guarantee that my information will be recognizable.

I don't believe there is any "classified" information in my report. However, I believe that it is possibly of use to the herbicide research and development people as well as others so I am making it available.

STRUCTURE AND PHYSICAL PROPERTIES OF SOME HERBICIDES USED IN LARGE QUANTITIES IN THE U.S.

The compounds in Table 1 are those selected to have potential environmental impact because of their widespread use in the United States. Important physical properties given are those

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related to vapor pressure (volatility) and water-organic solvent partitioning ratios (solubility data) of these herbicides: acrolein, alachlor, butylate, and propachlor are most volatile. Nitralin and trifluralin are the compounds which partition most strongly to organic solvents in preference to water (a factor related to bioaccumulation). See Table 1.

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RESIDUES OF HERBICIDES IN THE ENVIRONMENT

The important use of residue data is the matching of residues in the environment with toxicity to plant and animal organisms. Persistence of residues and their decline should be related with toxicity which takes these factors into account. The level of residues of herbicides in the environment is related to dosage applied, number of applications, method of application, type of herbicide formulation, habitat, wind, sunlight, oxygen, rain, soil type (especially to organic matter, clay minerals, pH, temperature), microorganisms (aerobic or anaerobic) and time after application, etc. Relative values of these residues can be predicted to a certain degree from a correlation of the physical, chemical and biological properties of the different kinds of herbicides with the above environmental variables and with herbicide uses and application methods (Freed, 1966; Van Middelen, 1966; Kenaga, 1968).

Determination of residues of herbicides must include the possibility of molecular transformation such as the hydrolysis, oxidation and reduction products of the chemical, if any, and metabolites, degradates, etc. Also, consideration must be made of the ingredients of the formulation and impurities

present whether due to manufacture, packaging, or storage of the material. One need only to trace the development of pesticides such as DDT (and its more stable metabolite DDE), parathion (and its potent cholinesterase inhibitor paraoxon), heptachloro (and its more toxic oxidation product heptachlorepoxide), and 2,4,5-T (and its toxic tetrachlorodibenzo-p-dioxin trace impurity) to realize the importance of the above determinations. Reactions associated with microbial metabolism of herbicides include N-dealkylation of urea; ester or amide hydrolysis of carbamates; side chain degradation of the 5-triazine; dechlorination of 2,4-D and dalapon; etc. (Kearney, 1966). Not all herbicides involve metabolism directly to smaller molecules. For example, propanil converted microbially to 3,4-dichloroaniline, is thought to form a larger molecule by diazotization by the nitrate ion in the soil (Rosen, 1972).

Few pesticides, including herbicides, have been subjected to detailed analysis for product impurities in the quantitative order of 1 ppm.

Bioaccumulation of Residues

The use of the terms bioaccumulation and bioconcentration often conjures up the image of the type of residue accumulation which occurs with DDT in animal organisms, and especially in their fat tissues. The bioconcentration factor (bio-

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magnification) is the ratio of the measured residue held by animals or plants (or specific tissues thereof) compared to the residues of the pesticide in the ambient air, water, or soil environment of the organism and/or the various species of food organisms consumed, as specified (Kenaga, 1972). The highest bioconcentration factors with insecticides usually occur in water. DDT and its degradate DDE may have a bioconcentration factor of a million. Herbicides such as picloram (Hardy, 1966), silvex (Getzendaner, 1960), 2,4-D (Cope, 1970; Smith and Isom, 1970), and dichlorobenil (Cope et al., 1969) do not appear to bioconcentrate in fish or aquatic organisms more than ten-fold and often less than one-fold the concentrations found in water where fish were present. Diuron may have a bioconcentration factor of several hundred (McCraren et al., 1969). Although oysters and clams may accumulate a few ppm of 2,4-D in herbicidally treated areas such as Chesapeake Bay, they cleanse themselves of residues before harvest time (Frank, 1971). Butler (1965) exposed oysters continously for 7 days to 0.1 ppm of 2,4-D, butoxy ethanol ester. The oysters accumulated 18 ppm and cleansed themselves in 7 days with fresh seawater. However, generally not much residue data is available for invertebrates. Fish may retain residues of some herbicides for weeks in treated ponds (McCraren et al., Cope et al., 1969). There appears to be little herbicide residue data pertaining to wild mammals and birds, however domestic animal and bird tissues appear to be free of significant residues for human use.

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The important fact, as far as chronic toxicity is concerned is not how large the bioconcentration factor is, but now much actual residue (in terms of ppm) bioaccumulates. While the terms bioaccumulation and bioconcentration are often applied to animals, it may also apply to plants, although it is rarely used in such context. Generally speaking, the properties of a chemical used to penetrate and kill insects are quite different than those needed to penetrate and kill plants. Among other things, insecticides are often quite fat soluble (in order to penetrate insects) and low in water solubility, thus stable compounds may be bioaccumulated in animal fats. Most herbicides tend to have greater water solubility and/or do not partition as greatly in favor of fat tissues as does DDT and other organochlorine insecticides. Further degradation of most herbicides lead to even more water soluble products, for example, atrazine to hydroxy atrazine, and prometryne to hydroxypropazine (Frans et al., 1972). An insecticide like DDT is not systemic in plants and therefore does not accumulate inside the plant. However, many herbicides penetrate plants, and are systemic, and thus redistribute and accumulate residues, especially in the growing portions of the plants, including leaves and roots. Since residues on treated plant foliage are often determined for the whole leaf, it is not then possible to determine the differences between surface residues and internal plant residues.

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Assuming an even coverage of herbicide on the surface of various plants, the residues vary greatly in ppm since the surface area to volume ratios of the different parts of plants and different species of plants vary greatly. In a literature study of many pesticides, maximum residues of pesticides were calculated on a one pound per acre basis by dividing the residues determined by analysis, by the dosage in pounds per acre applied (Hoerger and Kenaga, 1972). Such maximum residues, immediately after application, ranged from 6.6 ppm for fruits to 240 ppm for foliage such as grass, depending on the surface area to volume ratio (size and shape of vegetation sprayed). The highest amounts of residues occurred with systemic herbicides and insecticides on foliage. Most residues were far less than the maximum residues cited above. All residues declined within days after application although some persisted for months at levels of analytical sensitivity.

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Duggan et al. (1971) reported on pesticide levels in foods and feeds in the United States from 1963 to 1969. The only herbicides found were 2,4-D, MCP, and dacthal. None were found commonly, or in amounts more than ppb quantitatively.

Residues in Soil

A major problem in working with herbicide residues in soil is the accurate measurement of actual amounts of the compound and/or its degradation products in varying time periods after application. Very few studies are made which

include a number of herbicides tested comparatively in the field. A southern state regional cooperative study (Frans et al., 1972) showed that no phytotoxic residues of atrazine, chlorpropham, DCPA, diphenamid, diuron, linuron, norea, prometryne or trifluralin persist in soil after one year when the herbicides are applied at recommended application rates, however, no analytical residue data accompanied this phytotoxicity data. Herbicide residues are known to persist longer in the colder areas of the United States. In Oregon, studies of chemical brush control with 2,4-D, amitrole; 2,4,5-T and picloram showed residues of picloram to persist for more than a year while the rest were more rapidly degraded (Norris, 1971).

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Analytical work shows that even though phytotoxicity may not occur on the target crop in soil treated year after year, residues of the herbicide (and degradates) may still be bound to soil and may be subject to release under certain circumstances. The nature and mechanism of binding and releasing of pesticide residues held by lignin, humus, cellulose, and other organic matter is uncertain and residues may sometimes be a problem in the rotation of more herbicidally sensitive crops.

Organic material (principally humus) in soil is the major soil constituent which reduces leaching because of strong adsorption. Two major components of humus may act entirely

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different. For example, sodium humate apparently sorbs and solubilizes the nonpolar compound DDT, while humic acid strongly sorbs the polar compound 2,4,5-T from solution (Wershaw et al., 1969). Organic material is capable of sorbing most organic compounds from water, thus in practice, leaching which causes phytotoxicity away from the treated area is a rare problem even when water soluble herbicides are applied to organic soil. There is no compound which does not leach to some degree. Anderson et al. (1968) discussed the various factors related to leachability, relating it to soil pH, mineral, and organic content. Two closely related compounds, trifluralin and nitralin, vary greatly in leachability. Even mineral soils are capable of strong sorption such as with phenolic and phenoxyacetic acid herbicides. Sorption of such compounds is near a maximum when the calculated ionization is about 15 to 30% and when the pH approaches the pKa of the herbicide in water (Miller, 1972).

Herbicide residues in bottom muds of cold lakes or those of low oxygen content, may persist for months (Smith and Isom, 1971).

The relative order of persistence of the subject herbicides has been reported in the Herbicide Handbook of the Weed Society of America (1970) and by Sheets and Harris (1965). The most persistent herbicides of this group are atrazine, diuron, linuron, nitralin, propazine and trifluralin, all of which may be phytotoxic for over 2 months (see Table 2).

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Residues in Water

The most common herbicides used for control of aquatic weeds are xylene, copper sulfate, 2,4-D, acrolein, endothall and diuron (Anon., 1971). Very few materials are registered for this use because of the risks that the manufacturer takes in such use.

The main sources of herbicidal residues in water are from direct application, aerial drift from application, run off from land, and from control of ditchbank vegetation (Frank, 1971).

Johnson et al. (1967) discussed the pesticide residue monitoring studies carried on by the various U. S. agencies and concluded that a number of insecticides were important residue indicators, but none were herbicides. Eleven streams in western United States were monitored for nine insecticides, 2,4-D, 2,4,5-T, and silvex. No herbicide residues were found (Brown and Nishioka, 1967).

Dosages of most herbicides applied for aquatic weed control are initially in the order 0.1 to 4 ppm in water, except for aromatic solvents (xylene) which are as high as 600 to 700 ppm. The latter residues, however, are highly volatile and diminish rapidly in water. Residues of many herbicides are detectable (>0.1 ppm) for days or weeks after application in treated pond water, but are rarely greater than 0.5 ppm

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within a few days (Table 4, Frank, 1971). Fenac, 3-amino-triazole, and dichlobenil appear to be more persistant (Grzenda et al., 1966; Van Valin, 1966). Herbicide concentrations in excess of 0.1 ppm are seldom encountered in streams close to treatment areas, even immediately after spraying (Norris, 1971).

DOW/18208

Interpretation of analytical data concerning pesticide residues in water can sometimes be misleading expecially if the water contains organic or other particulate matter. In such cases, the herbicide may be strongly adsorbed to solids suspended in water by the same mechanism by which they are sorbed on particles in soil, and thus may exaggerate the true amount of herbicide solubilized in water, if particulates are not first filtered out. In such cases there may be more herbicide on the suspended particulate than solubilized in the water.

The relative mobility of pesticides in soil leaching experiments show phenoxy and picloram herbicides to be most mobile, followed by a group of miscellaneous herbicides, then by phenyl urea, triazine and other related herbicides, and then by CIPC and toluidine herbicides. Insecticides, especially chlorinated hydrocarbons were least mobile (Norris and Moore, 1970).

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It is important to determine the half-life of herbicide residues in water. This will vary considerably depending

on temperature, ultraviolet light, pH of the water, aeration, size and nature of the particulate matter in the water, the amount of vegetation in water, and surface-depth relationships of the body of water treated, etc. The half-life of chemical residues in water is not necessarily linear with time, depending on the effects of various concentrations of the herbicide on degradative organisms and other factors.

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A number of chemicals which are persistent in soil are less persistent in water due to the action of ultraviolet light. A herbicide such as picloram which is resistant to degradation in soil is more easily decomposed by sunlight in water with half-lives ranging from 2 to 41 days depending on water depth, amount of sunlight, and water quality (Hedlund, 1970). More studies on various herbicides concerning decomposition by sunlight are needed to help calculate a half-life or a "disappearance rate" from water.

Residues in Air

Herbicide residues in air may be from three sources: (1) those caused at the time of application of the pesticide, such as occurs with spray and dust drift. Only 20-80 percent of the pesticides dispersed in air may reach the target (Norris and Moore, 1970); (2) those related to dispersal of pesticides on particles due to wind erosion after application; (3) those related to gaseous dispersion because of volatility from treated areas (soil, water, plants, etc.)

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or other areas contaminated due to the above three mechanisms. It is generally supposed that items 1 and 3 are most important with herbicides.

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Very little work has been done concerning the measurement of residues of herbicides in air except near the point of application. Airborne concentrations which are harmful to non-target crops are most frequently due to drift at the time of application of the herbicide to the target. Usually residues on plants contaminated from drift are far less than those on the target species of plants, however, sensitive plants such as cotton and grapes are particularly vulnerable to 2,4-D and may occasionally be damaged by residues as much as 15 miles from the point of application under rare weather circumstances, but are most frequently important within the nearest 500 feet (Van Middelem, 1966). Herbicide residues due to drift are most closely correlated with the type of equipment and herbicide formulation used, and wind velocity. Most serious drift problems can be solved by modification of equipment and formulations such as granules, inverted emulsions, and spray thickeners, and avoiding small particulates and windy days for application (Butler et al., 1969; Akesson et al., 1971; Akesson et al, 1972).

Occasionally during periods of great winds, the accompanying dust storms may deposit herbicides presumably carried on contaminated or treated soil particles over great distances such as from Texas to Ohio (Cohen and Pinkerton, 1966).

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Even during normal weather particulate matter suspended in air may sorb volatile herbicides as they do DDT, lindane et al., and then be redeposited on bodies of water or on the ground by rain storms some distance from the original source of the particulate or herbicide application (Abbott et al., 1965).

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Particles of soil, spray droplets, and rain droplets, which are able to be carried by normal wind velocities of 5 mph for over 100 feet vary from <5 to 100 microns in diameter (Reimer et al., 1966). Assuming particles to have nearly equal deposition of the herbicide on treated soil, a particle with a diameter of 10 microns will have a surface area 100 times greater per volume than a particle with a diameter of 100 microns and consequently about 100 times the residue in ppm, assuming no volatility. Particles over 5 microns are not normally expected to be carried very far while particles of 0.5 to 1 micron may be transported on transoceanic trade winds (Risebrough et al., 1968). In order to be carried on small particles in air, and subject to light, wind and rain, a pesticide must be very chemically stable, and low in volatility.

Analyses of particles from air have been shown to contain 2,4-D, 2,4,5-T and atrazine in amounts of 10 ppb or less. Residues of herbicides are not known to build up to high levels by air dispersal at great distances from the point of application. They may be of less consequence than DDT

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for example, since the herbicides do not appear to build up in the animal food chain of life.

To a lesser extent than concentrations caused by drift, concentrations caused by volatility may be responsible for damage of sensitive non-target plants surrounding the target plants. The presence of wide spread air concentrations in Washington has been studied with various salts and esters of 2,4-D (Adams et al., 1964). The role of volatility of herbicides is very little understood and is indicated mostly where herbicidal damage occurs on valuable crops. Volatility of 2,4-D varies greatly depending on its form. For example the vapor pressure of the methyl ester is greater than 100 fold that of 2,4-D (Hamaker and Kerlinger, 1969). Volatility of a given herbicide from various treated surfaces varies greatly depending on the weather, formulation, and sorptive capacity of the surface from which the herbicide volatilizes. Generally speaking soil and other surfaces which adsorb herbicides the strongest, allow the least volatility. Many soils lose a large percentage of a herbicide within a few hours or days by volatility.

Little is known of the fate of herbicides or other pesticides in air except that they are rapidly diluted. The dilution factor is the main reason for the difficulty in detecting herbicides in air since the lower analytical sensitivity limits for most compounds are soon reached.

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It is known that sunlight alters the structure of many pesticides especially in the presence of moisture and particulate matter as occurs normally in air, however, the techniques for simulating these effects are not well established. Compounds like 2,4-D have been shown to be reduced to polymeric humic acids similar to soil humic acids by either artificial light or sunlight (Crosby and Tutass, 1966).

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In general, the metabolites and degradates of herbicides have been shown to be less of a problem than the original compound. Knowledge of all routes and rates of degradation for all herbicides is incomplete as it is with other pesticides, however, the major facts concerning the eventual degradation of herbicides is quite well known except for soil binding and air degradation. Not much is known about minor impurities formed during the production of herbicides.

THE TOXICITY OF HERBICIDES TO MAMMALS, BIRDS, FISH AND INVERTEBRATES

The amount of data available for some herbicides is not very much, while on the other hand the data reported here in Table 3 is by no means complete. An attempt was made to find acute and especially chronic toxicity data on all four of the major zoological groups mentioned in the above title.

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Toxicity data on mammals indicates that only acrolein, copper sulfate, and possibly endothall, are acutely toxic. Apparently none of the herbicides are chronically toxic at residue levels likely to persist in the environment.

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Toxicity data on birds indicates that only endothall (no data on acrolein) may be acutely toxic. There is no indication of chronic toxicity to birds at residue levels likely to persist in the environment.

Acute toxicity data on fish indicates that several compounds have LC50's of 1 ppm or less in water. Among these are acrolein (aerates rapidly from water), alachlor, copper sulfate, certain esters of 2,4-D, certain esters and salts of endothall, and trifluralin. Acrolein, copper sulfate and endothall are applied directly to water. None or very little acute or chronic toxicity data was found on insects, shrimp, and oysters for some herbicides such as acrolein, alachlor, butylate, copper sulfate, linuron, nitralin, propachlor, propazine, or xylene. Copper sulfate and xylene were acutely toxic to Daphnia at 0.1 ppm. Trifluralin was acutely toxic to certain species of Daphnia and Gammerus below 1 ppm.

SUMMARY STATEMENT FOR THE ENVIRONMENT IMPACT SECTION REPORT, HERBICIDE STUDY, HMAC, EPA

When the members of the environmental impact subcommittee turned in their reports (including mine), the chairman (Dr. David Pimentel) assembled a summary. Pimentel's summary pinpointed many adverse effects known to have occurred with herbicides without putting these effects into perspective in regard to the use of the herbicides in crop and non-crop area (ecological niches) and the likelihood of environmental contamination. Since I did not like the chairman's summary, I wrote my own as follows:

"The earth's environment is nearly all managed in part by man, either directly through crop production, construction, mining, etc. or indirectly through pollution, elimination by carelessness such as by fires, or overuse of certain parts of the environment and by many other ways. The use of herbicides is only one of man's many technological tools which influence the environment and manage ecosystems.

Basic to the use of herbicides is the desire of man to raise essential and sufficient food crops with a minimum of effort. cost, and space. Where food is raised only food plant species are desired and the aboriginal plant and animal ecosystem has often long since been usurped. It is not reasonable to consider food croplands as part of a natural ecosystem or to expect it to maintain the diversity of life found in uncultivated areas.

The main requirement which might be made of a herbicide on such croplands is that they be used for efficient production of quality food which would not result in non-target damage such as might occur from toxic residues encountered by a diversity of organisms on or off the treated area via contact with air, water, soil or food.

Thus, if the use of herbicides result in competitive food production of crops without side effects of economic or non-target significance, the criteria for ecological evaluation are not the same as for the use of herbicides on aquatic sites, right-of-ways, parks, and forested areas, which include natural ecosystems. Even in the latter areas herbicides are rarely

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used to eliminate all vegetation, but are mainly needed to control economic pest plants.

Herbicides do not always create ecosystems containing less plant species. When used to create right-of-way strips within forest climax areas, herbicides create more overall species diversity and varied habitats within such areas because climax species may be locally eliminated and replaced by different plant species.

Man's uses for herbicides are designed to change or manage various environments and may be divided into general habitats as follows:

a. Selective plant control in row crops which are grown annually as large acreages of single species such as corn, cotton, soybeans, sugarbeets, sorghum, tobacco, peanuts, sugar cane, sweet corn and other vegetables and are subject to cultivation for weed control. Herbicides used here are selective to control grasses and/or broadleaf plants without adversely effecting the desired commercial crop. (There is no need to be concerned for herbicides which are not persistent nor leave the target area in significants amounts). Such areas are at best only temporary wildlife habitats and not the crops intended as primary food for wildlife.

b. Selective control in grains planted annually which cover the ground like wheat, barley, oats, and rice, etc. Control of weeds by cultivation in such crops would be impossible. These areas serve at best as temporary wildlife habitats and the crops are not intended as primary food for such wildlife.

c. Selective plant control to maintain a habitat dominated by grass or forage plants which annually or perennially cover the ground such as rangeland and pasture plants,

lawns, hay, alfalfa, legumes, airports, roadsides and utility rights-of-way (non-cropland). These uses while designed to eliminate trees and tall shrubs for domestic animals or for human safety purposes, furnish a great deal of food and habitat for wildlife.

d. Selective plant control in woody vegetation areas such as forest plantings, fruit and nut trees, and shrubs and ornamental plants for elimination of certain competitive plants. Wild or mature forest lands are rarely treated with herbicides. Taller plants such as trees and shrubs may serve as nesting places or homes for birds and animals. Ground areas under trees and shrubs may also be useful for wildlife habitats depending on whether the ground cover is eliminated or kept in grass, etc.

e. Non-selective pest plant control in sites where no vegetation is wanted such as parking lots, railroad beds, transformer areas, industrial areas, etc. The areas are not suitable for wildlife habitats.

f. Selective plant control in land and marsh areas managed for growing wildlife food or improving wildlife habitat for game animals such as deer, grouse, geese, ducks, etc. These herbicides are used to enlarge the food carrying capacity for individual game species which need man's aid for such directed ecological changes.

g. Pest plant control of weeds and algae in aquatic sites such as rivers, ponds, lakes, irrigation and drainage ditches, etc. Weeds such as these cause blockage of channels by boats, ships, etc., add to eutrophication of water, alter water flow rates. Such weeds change the ecosystems for wildlife. These areas are wildlife habitats unless polluted beyond suitability by man.

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Using the above description of habitats and the 1968 census of crop acreages (Ref. 1972, USDA, Crop Research, Extent and Cost of Weed Control with Herbicides and an Evaluation of Important Weeds, 1968. Table 4. ARS, USDA. In press.) the areas treated with herbicides in the USA have been grouped as shown in Table 4. Habitats "a", "b" and "e" are not considered to be reservoirs for natural wildlife and have little environmental potential except possibly for temporary food and cover. The herbicide treatments on these areas account for about 85.2% of the total U.S. acreage treated. In addition to being the largest crop habitat, the areas may receive several to many annual treatments. The major volume of the herbicides is expended in this habitat. Very few major herbicides (atrazine and 2,4-D) are used in natural environments. Most of the herbicides used on such habitats do not leach readily and are degraded in soil. Major environmental effects on non-target areas are unlikely and have not been found to be significant to date with such herbicides.

Habitats "c" and "d" are selectively managed by herbicides to allow grassy and forage vegetation to grow in preference to woody vegetation, particularly trees and tall shrubs. Wildlife is often encouraged or present in such areas. Herbicide treatments may be made much less frequently than in the previous category and represent about 14.6% of the area in U.S. treated with herbicides.

Habitats "f" and "g" represent terrestrial and aquatic areas which are commonly used by wildlife and aquatic organisms. However, these areas represent only a very small part (about 0.2%) of the U.S. area treated with herbicides.

All of the areas treated with herbicides in U.S. in 1968 represented about 5.62% of the total land area.

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Herbicides have certain benefits environmentally in that they are selective and in that plant species can be manipulated for the selection of beneficial food of game or other animals, in addition to man. Such selective plant control may result in better ground cover, and consequently less erosion and silting in rivers, and more stabilized plant and wildlife communities.

The use of herbicides results in more efficient use of farmland, which allows the use of more marginal farm land for other purposes, including wildlife areas.

The majority of herbicide usages do not appear to directly involve natural environmental habitats. Very few incidences of serious damage to wildlife and natural environments have occurred beyond that intended by man, which would not be equally damaging by use of non-chemical methods. Herbicide residues do not seem to be building up in the air, water, and soil environment. Fortunately, the chemical and physical characteristics of herbicides allow most of them to degrade within about 3 months. A few herbicides bioconcentrate temporarily in animals but the level seldom is more than 10-fold.

In spite of the beneficial uses of herbicides, their effects need to be kept under constant surveillance and in balance to prevent trends which could lead to serious environmental disruptions, disastrous to man as well as his environment.

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TABLE 1

STRUCTURE AND PHYSICAL PROPERTIES OF SOME HERBICIDES

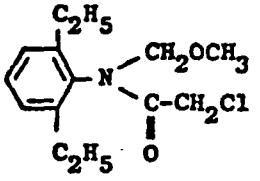
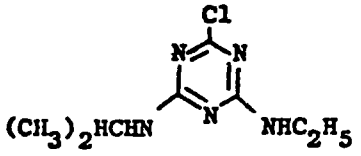
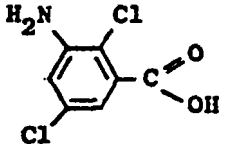
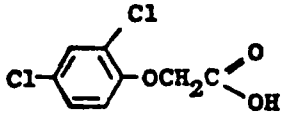
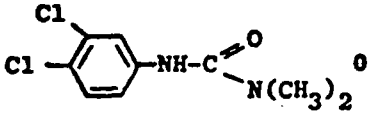
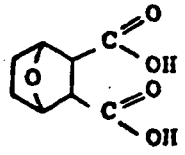
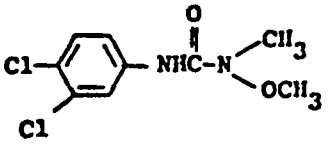
Common Name of Herbicide	Structure	Vapor Pressure mm Hg (°C)	Solubility of Herbicide in		
			Water	Acetone	Xylene (x) Benzene (b)
Acrolein	$CH_2 = CH\text{-}CHO$	325 (30)	25%	misc.	misc. (x)
Alachlor		0.02 (100)	148 ppm	sol.	sol. (b)
Atrazine		$3x10^{-7}$ (20) $1.4x10^{-6}$ (30)	33 ppm	1.8g/100g (Methanol)	360 ppm (n-Pentane)
Butylate	$CH_3\text{-}CH_2\text{-}S\text{-}C(=O)N(CH_2\text{-}CH(CH_3)\text{-}CH_3)_2$	$13x10^{-3}$ (25)	45 ppm	misc.	misc. (x)
Chloramben		?	700 ppm	23g/100g	0.02g/100g (
Copper Sulfate	$CuSO_4$	?	sol.	?	?
2,4-D		$6x10^{-7}$ (25)	600-700 ppm	45g/100cc	1.07/g100cc (
Diuron		$0.31x10^{-5}$ (50)	42 ppm	5.3g/100g	0.12g/100g (b
Endothall		?	10%	7g/100g	0.01g/100g (b
Linuron		?	75 ppm	50g/100g	13g/100g (b)

TABLE 1 (Cont.)

STRUCTURE AND PHYSICAL PROPERTIES OF SOME HERBICIDES

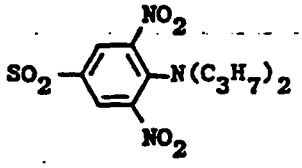
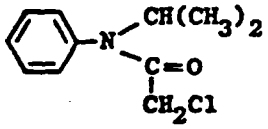
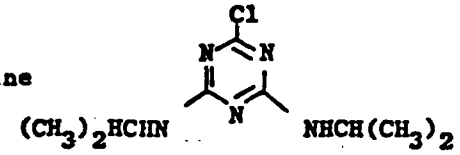
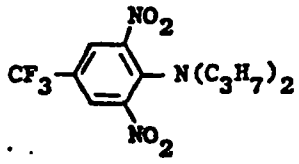
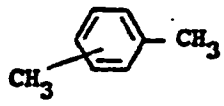
Common Name of Herbicide	Structure	Vapor Pressure mm Hg (°C)	Solubility of Herbicide in Water	Acetone	Xylene (x) Benzene (b)
Nitralin		$<1.5\times10^{-6}$ (25)	0.6 ppm	37g/100g	<12.5g/100g
Propachlor		0.03 (110)	700 ppm	30.9%	50% (b)
Propazine		2.9×10^{-8} (20)	8.6 ppm	diff.	diff. (x,b)
Trifluralin		1.99×10^{-4} (29.5)	<1 ppm	1.19 (% Molarity)	1.73 (x) (% Molarity)
Xylene		isomer o m p	6.62 (25) 8.30 (25) 8.76 (25)	196 ppm 198 ppm	inf. inf. inf.	inf. (b) inf. (b) inf. (b)

TABLE 2. PERSISTENCE OF HERBICIDES IN SOIL IN FIELD TESTS

Herbicide	lb/A Applied to Soil	Residual Phytotoxicity (Months)	Reference
Alachlor	1-4	1.5-2.5	HH
Atrazine	2-4	4-7	S&H
	2-3	4-7	S&H
	3-8	12	S&H
	2-4	<12->12	HH
Butylate	3-4	0.12-0.75*	HH
Chloramben	2-4	1.5-2	HH
2,4-D	3-4	0.25-1	HH
Diuron	3.6-4	5-7	S&H
	1-2	4-8	S&H
	2	15	S&H
	0.6-6.4	One Growing Season	HH
Endothall	12	1	S&H
Linuron	4	<4	S&H
	0.5-3	4	HH
Nitralin	0.5-1.5	"Moderately Persistent"	HH
Propachlor	3-6	1-1.5	HH
Propazine	1-4	<12	HH
	3	2	S&H
	1.8	14	S&H
Trifluralin	4	5	S&H
	0.5-1	4-6	HH

HH = Herbicide Handbook of the Weed Society of America. 1970.

S&H = Sheets, T. J. and C. I. Harris. 1965.

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TABLE 3. TOXICITY OF HERBICIDES TO MAMMALS, BIRDS, FISH, AND INVERTEBRATES

Common Name (Trade Name) of Herbicide	Name of Organism	Type of Test Exposure	Dosage a.i.	Effect	Reference
Acrolein (Aqualin, Hydrothal)	Rat	Acute Oral	46 mg/kg	LD_{50}	Herbicide Handbook
	Rabbit	Acute Oral	7 mg/kg	LD_{50}	
	Rat	Water Dietary, 90 Day	200 ppm	No Effect	
	Bluegill	Acute, 96 hr	0.1 ppm	LC_{50}	Lawrence, 1963
	Chinook Salmon	Acute, 24 hr	0.08 ppm	LC_{50}	
	Coho Salmon	Acute, 0.25 hr	2.0 ppm	100% Mort.	
	Rainbow Trout	Acute, 24 hr	0.14 ppm	LC_{50}	Pimentel
	Largemouth Bass	Acute, 96 hr.	0.16 ppm	LC_{50}	Lawrence, 1963
	Mosquito Fish	Acute, 48 hr	0.06 ppm	LC_{50}	
	Fathead Minnow	Acute, 48 hr	0.015 ppm	LC_{50}	
	Bluegill	Acute*, 24 hr	0.079 ppm	LC_{50}	Batelle
	Brown Trout	Acute*, 24 hr	0.046 ppm	LC_{50}	
*Continuous Flow					
Alachlor (Lasso)	Rat	Acute Oral	774 mg/kg*	LD_{50}	Herbicide Handbook
		90 Day Dietary	200 ppm	No Effect	
	Pheasant	5 Day Dietary ?	>4300 ppm	LC_{50}	
	Rainbow Trout	Acute, 96 hr	1.0 ppm*	LC_{50}	
	Bluegill	Acute, 96 hr	5.8 ppm*	LC_{50}	
*Corrected for 4 lb/gal emulsifiable composition, probably 43% a.i.					
Atrazine	Rat	Acute Oral	3080 mg/kg	LD_{50}	Herbicide Handbook
	Mice	Acute Oral	1750 mg/kg	LD_{50}	
	Rat	2 yr Dietary	100 ppm	No Effect	
	Cattle	Acute Oral	50 mg/kg	Signif Effect[b]	Palmer et al
		2 CDOD[a]	25 mg/kg	Signif Effect	
		10 CDOD	10 mg/kg	No Effect	
	Sheep	Acute Oral	100 mg/kg	Signif Effect	
		3 CDOD	50 mg/kg	Signif Effect	
		8 CDOD	25 mg/kg	Signif Effect	
	Bobwhite	5 Day Dietary	>5000 ppm	LC_{50}	Heath
	Mallard	5 Day Dietary	>5000 ppm	LC_{50}	
	Japanese Quail	5 Day Dietary	>5000 ppm	LC_{50}	
	Ring-necked Pheasant	5 Day Dietary	>5000 ppm	LC_{50}	
	Chicken	10 CDOD	50 mg/kg	Signif Effect	Palmer et al
		10 CDOD	25 mg/kg	No Effect	
	Spot	Acute, 48 hr	1 ppm	No Mortality	Pimentel
	Harlequin Fish	Acute, 24 hr	0.55 ppm	LC_{50}	
	Rainbow Trout	Acute, 96 hr	4.5 ppm	LC_{50}	EPA
	Bluegill	Acute, 48 hr	26 ppm	LC_{50}	
	E. Oyster	Acute, 96 hr Flowing Sea Water	1 ppm	No Effect	Pimentel
	Brown Shrimp	Acute, 48 hr	1 ppm	30% Mortality or Loss of Equilibrium	
	Water Flea (Daphnia magna)	Acute, 48 hr	3.6 ppm	LC_{50}	Pimentel

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TABLE 3. TOXICITY OF HERBICIDES TO MAMMALS, BIRDS, FISH, AND INVERTEBRATES (CONT.)

Common Name (Trade Name) of Herbicide	Name of Organism	Type of Test Exposure	Dosage a.i.	Effect	Reference
Butylate (Sutan)	Rat	Acute Oral	4659-5431 mg/kg	LD_{50}	Herbicide Handbook
	Guinea Pig	Acute Oral	1659 mg/kg	LD_{50}	
	Rat	90 Day Dietary	32mg/kg/day	No Effect	
	Dog	90 Day Dietary	40mg/kg/day	No Effect	
	Bobwhite	7 Day Dietary	40,000 ppm	LC_{50}	
	Bluegill	Acute, 96 hr	6.9 ppm	LC_{50}	
	Rainbow Trout	Acute, 96 hr	4.2 ppm	LC_{50}	
	Gammarus fasciatus	Acute, 96 hr	10.0 ppm	LC_{50}	Sanders
Chloramben (Amiben)	Rat	Acute Oral	5620 mg/kg	LD_{50}	Herbicide Handbook
		2 yr Dietary	10,000 ppm	No Effect	
	Cattle	10 CDOD	250 mg/kg	Signif Effect	Palmer
		10 CDOD	175 mg/kg	No Effect	
	Sheep	2 CDOD	50 mg/kg	Signif Effect	
		10 CDOD	25 mg/kg	No Effect	
	Bobwhite	Acute Oral	>700 mg/kg	LD_{50}	EPA
	Chicken	7 CDOD	500 mg/kg	Signif Effect	Palmer
		10 CDOD	375 mg/kg	Signif Effect	
		10 CDOD	250 mg/kg	No Effect	
	Rainbow Trout	Acute, 96 hr	4.2 ppm	LC_{50}	EPA
	Bluegill	Acute, 96 hr	1000 ppm	LC_{50}	
Copper Sulfate*	Sheep	Dietary	25 mg/day	Jaundice	Pimentel
	Mallard	Acute Oral	>2000 mg/kg**	LD_{50}	
	Ring-necked Pheasant	Acute Oral	>2000 mg/kg**	LD_{50}	
	Bluegill	Acute, 24 hr	1.5 ppm	LC_{50}	
	Striped Bass	Acute, 48 hr	0.15 ppm	LC_{50}	
	Bluegill	Acute, 96 hr	3.0 ppm	Tolerated	Lawrence, 1962
	Bass	Acute, 96 hr	3.0 ppm	Tolerated	
	Goldfish	Acute, 96 hr	1.0 ppm	Tolerated	
	Fathead Minnow	Acute, 96 hr	1.0 ppm	Tolerated	
	Daphnia	Acute, 16 hr	0.1 ppm	EC***$_{50}$	
	Daphnia	Acute, 2 hr	100 ppm	Killed	
2,4-D (Acid)*	Rat	Acute Oral	666 mg/kg	LD_{50}	Pimentel
	Mouse	Acute Oral	375 mg/kg	LD_{50}	
	Rabbit	Acute Oral	800 mg/kg	LD_{50}	
	Dog	Acute Oral	100 mg/kg	LD_{50}	
	Guinea Pig	Acute Oral	1000 mg/kg	LD_{50}	
	Mule Deer	Acute Oral	400-800 mg/kg	LD_{50}	
	Rat	2 yr Dietary	1250 ppm	No Effect	Hansen et al
	Dog	2 yr Dietary	500 ppm	No Effect	
	Rat	3 Generation Reproduction	500 ppm	No Effect	
	Cattle	Acute Oral	250mg[c]/kg	Signif Effect	Palmer
		25 CDOD	200mg[c]/kg	Signif Effect	
		86 CDOD	100mg[c]/kg	Signif Effect	
		112 CDOD	50mg[c]/kg	No Effect	

* Toxicity varies greatly, with pH and turbidity.
** Bordeaux mixture.
***Dead or immobilized.

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TABLE 3. TOXICITY OF HERBICIDES TO MAMMALS, BIRDS, FISH, AND INVERTEBRATES (CONT.)

Common Name (Trade Name) of Herbicide	Name of Organism	Type of Test Exposure	Dosage a.i.	Effect	Reference
2,4-D Cont.	Sheep	7 CDOD	500mg[c]/kg	Signif Effect	Palmer
		10 CDOD	250mg[c]/kg	Signif Effect	
		8 CDOD	100mg[c]/kg	No Effect	
	Mallard	Acute Oral	>1000mg/kg	LD_{50}	Pimentel
	Ring-necked Pheasant	Acute Oral	472 mg/kg	LD_{50}	
	Japanese Quail	Acute Oral	668 mg/kg	LD_{50}	
	Pigeon	Acute Oral	668 mg/kg	LD_{50}	
	Mallard	5 Day Dietary	>5000*ppm	LC_{50}	
	Ring-necked Pheasant	5 Day Dietary	>5000*ppm	LC_{50}	
	Japanese Quail	5 Day Dietary	>5000*ppm	LC_{50}	
	Chicken	10 CDOD	500mg[c]/kg	Signif Effect	Palmer
		10 CDOD	250mg[c]/kg	Signif Effect	
		10 CDOD	100mg[c]/kg	No Effect	
	Chorus Frog Tadpole	Acute, 24 hr	100 ppm	LC_{50}	Pimentel
	Bluegill	Acute, 24 hr	5 ppm	No Mortality	Applegate
	Rainbow Trout	Acute, 24 hr	5 ppm	No Mortality	
	Daphnia magna	Acute, 48 hr	>100 ppm	LC_{50}	Sanders
	Gammarus fasciatus	Acute, 48 hr	3.2 ppm	LC_{50}	

*Very little variation in toxicity occurs between various salts and esters of 2,4-D in mammals and birds, however, certain esters are much more toxic than the acid to fish (around 1 ppm).

Diuron (Karmex)	Rat	Acute Oral	3400 mg/kg	LD_{50}	Herbicide Handbook
	Rat (Low Chronic Toxicity)				
	Cattle	10 CDOD	100 mg/kg	Signif Effect	Palmer et al
		10 CDOD	50 mg/kg	No Effect	
	Sheep	Acute Oral	250 mg/kg	Signif Effect	
		2 CDOD	100 mg/kg	Signif Effect	
		10 CDOD	50 mg/kg	No Effect	
	Bobwhite	5 Day Dietary	1730 ppm	LC_{50}	Heath
	Japanese Quail	5 Day Dietary	>5000 ppm	LC_{50}	
	Ring-necked Pheasant	5 Day Dietary	>5000 ppm	LC_{50}	
	Mallard	5 Day Dietary	>5000 ppm	LC_{50}	
	Chicken	10 CDOD	10 mg/kg	Signif Effect	Palmer et al
		10 CDOD	5 mg/kg	No Effect	
	Bullfrog Tadpole	Acute	10 ppm	All Killed	Lawrence, 1963
	Rainbow Trout	Acute, 48 hr	4.3 ppm	LC_{50}	Pimentel
	Coho Salmon	Acute, 48 hr	42 ppm	LC_{50}	
	Largemouth Bass	Acute, 48 hr	16 ppm	LC_{50}	
	Bluegill	Acute, 24 hr	9.7-27 ppm*	LC_{50}	
	White Crappie	Acute, 24 hr	8.75 ppm	50% Mortality	
	Striped Bass	Acute, 96 hr	3.1 ppm	LC_{50}	
	Daphnia pulex	Acute, 48 hr	1.4 ppm	LC_{50}	
	Gammarus	Acute, 48 hr	0.38 ppm	LC_{50}	
	E. Oyster	Acute, 96 hr Flowing Salt Water	1.8 ppm	Shell Growth Decreased	Batelle
	Brown Shrimp	Acute, 48 hr Flowing Salt Water	1.0 ppm	No Effect	

*Different temperatures.

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TABLE 3. TOXICITY OF HERBICIDES TO MAMMALS, BIRDS, FISH, AND INVERTEBRATES (CONT.

Common Name (Trade Name) of Herbicide	Name of Organism	Type of Test Exposure	Dosage a.i.	Effect	Reference
Endothall (Endothal)	Rat	Acute Oral	38-51 mg/kg	LD_{50}	Herbicide Handbook
		2 yr Dietary	>300 ppm*	No Effect	
	Dog	2 yr Dietary	>300 ppm*	No Effect	
	Cattle	2 CDOD	25 mg[d]/kg	Signif Effect	Palmer
		10 CDOD	10 mg[d]/kg	No Effect	
	Sheep	2 CDOD	50 mg[d]/kg	Signif Effect	
		2 CDOD	25 mg[d]/kg	Signif Effect	
		10 CDOD	10 mg[d]/kg	Signif Effect	
		10 CDOD	5 mg[d]/kg	No Effect	
	Chicken	10 CDOD	10 mg[d]/kg	Signif Effect	
	Bluegill	Acute, 96 hr	125 ppm**	LC_{50}	Pimentel
	Carp	Acute, 96 hr	175 ppm**	LC_{50}	
	Largemouth Bass	Acute, 96 hr	120-200 ppm**	LC_{50}	
	Fathead Minnow	Acute, 96 hr	320-610 ppm**	LC_{50}	Lawrence, 196
	Daphnia magna	Acute, 24 hr ?	46 ppm	EC***$_{50}$	Pimentel
	Gammarus lacustris	Acute, 24 hr	2 ppm	LC_{50}	Sanders
Linuron (Lorox)	Rat	Acute Oral	1500 mg/kg	LD_{50}	Martin
		Acute Oral	4000 mg/kg	LD_{50}	
	Dog	Acute Oral	500 mg/kg	LD_{50}	
	Cattle	6 CDOD	100 mg/kg	Signif Effect	Palmer et al
		10 CDOD	50 mg/kg	Signif Effect	
		10 CDOD	25 mg/kg	No Effect	
	Sheep	4 CDOD	100 mg/kg	Signif Effect	
		1 CDOD	50 mg/kg	Signif Effect	
		10 CDOD	25 mg/kg	No Effect	
	Chicken	10 CDOD	10 mg/kg	Signif Effect	
	Carp	Acute, 48 hr	>10 ppm	LC_{50}	EPA
Nitralin (Planavin)	Rat	Acute Oral	>2000 mg/kg	LD_{50}	Herbicide Handbook
	Mouse	Acute Oral	>2000 mg/kg	LD_{50}	
	Rat	2 yr Dietary	2000 ppm	No Effect?	
	Cattle	2 CDOD	250 mg/kg	Signif Effect	Palmer
		10 CDOD	175 mg/kg	No Effect	
	Sheep	4 CDOD	500 mg/kg	Signif Effect	
		10 CDOD	250 mg/kg	No Effect	
	Chicken	Acute Oral	>1000 mg/kg	LD_{50}	Martin
		10 CDOD	500 mg/kg	No Effect	Palmer
	Bluegill	Acute, 48 hr	20 ppm	No Mortality	Martin
	? Trout	Acute, 48 hr	20 ppm	No Mortality	
	Goldfish	Acute, 48 hr	20 ppm	No Mortality	
	Silver Salmon	Acute, 48 hr	20 ppm	No Mortality	

* Disodium Salt.
** Cocoamine, dimethylamine and copper salts may be toxic to some fish at less than 1 ppm.
***Immobilization.

TABLE 3. TOXICITY OF HERBICIDES TO MAMMALS, BIRDS, FISH, AND INVERTEBRATES (CONT

Common Name (Trade Name) of Herbicide	Name of Organism	Type of Test Exposure	Dosage a.i.	Effect	Reference
Propachlor (Ramrod)	Rat	Acute Oral	710 mg/kg	LD_{50}	Herbicide Handbook
		90 Day Dietary	133mg/kg/day	No Effect	
	Cattle	3 CDOD	25 mg/kg	Signif Effect	Palmer
		10 CDOD	10 mg/kg	No Effect	
	Sheep	4 CDOD	50 mg/kg	Signif Effect	
		9 CDOD	10 mg/kg	Signif Effect	
		10 CDOD	5 mg/kg	No Effect	
	Ring-necked Pheasant	Acute Oral	735 mg/kg	LD_{50}	Herbicide Handbook
	Chicken	3 CDOD	1000 mg/kg	Signif Effect	Palmer
		10 CDOD	10 mg/kg	Signif Effect	
		10 CDOD	5 mg/kg	No Effect	
	Bluegill	Acute, 96 hr	1.3 ppm	LC_{50}	Herbicide Handbook
	Fathead Minnow	Acute, 96 hr	0.49 ppm	LC_{50}	
Propazine (Milogard)	Rat	Acute Oral	>5000 mg/kg	LD_{50}	Herbicide Handbook
	Mouse	Acute Oral	>5000 mg/kg	LD_{50}	
	Rat	130 CDOD	250 mg/kg	No Effect	
	Cattle	2 CDOD	250 mg/kg	Signif Effect	Palmer et al
		3 CDOD	25 mg/kg	Signif Effect	
		10 CDOD	10 mg/kg	No Effect	
	Sheep	Acute Oral	500 mg/kg	Signif Effect	
		10 CDOD	100 mg/kg	Signif Effect	
		5 CDOD	25 mg/kg	Signif Effect	
		10 CDOD	10 mg/kg	No Effect	
	Bobwhite	(Low Toxicity)			Herbicide Handbook
	Mallard	(Low Toxicity)			
	Chicken	6 CDOD	250 mg/kg	Signif Effect	Palmer et al
		10 CDOD	100 mg/kg	Signif Effect	
		25 CDOD	25 mg/kg	No Effect	
	Rainbow Trout	Acute, 96 hr	>100 ppm	LC_{50}	EPA
		Acute, 48 hr	7.8 ppm	LC_{50}	Pimentel
	Goldfish	Acute, 48 hr	>32 ppm	LC_{50}	EPA
	Bluegill	Acute, 48 hr	>100 ppm	LC_{50}	
Trifluralin (Treflan)	Rat	Acute Oral	>10,000 mg/kg	LD_{50}	Pimentel
	Mouse	Acute Oral	5000 mg/kg	LD_{50}	
	Dog	Acute Oral	>2000 mg/kg	LD_{50}	
	Rabbit	Acute Oral	>2000 mg/kg	LD_{50}	
	Rat	2 yr Dietary	>2000ppm/day	No Effect	Herbicide Handbook
	Dog	2 yr Daily Dietary Capsule	>1000 ppm	No Effect	
	Cattle	2 CDOD	175 mg/kg	Signif Effect	Palmer et al
		10 CDOD	100 mg/kg	No Effect	
	Sheep	2 CDOD	175 mg/kg	Signif Effect	
		10 CDOD	100 mg/kg	No Effect	
	Ring-necked Pheasant	Acute Oral	>2000 mg/kg	LD_{50}	Pimentel
	Chicken	Acute Oral	>2000 mg/kg	LD_{50}	
	Mallards	Acute Oral	>2000 mg/kg	LD_{50}	
	Chicken	10 CDOD	500 mg/kg	Signif Effect	Palmer et al
		10 CDOD	250 mg/kg	Signif Effect	
		10 CDOD	100 mg/kg	No Effect	

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TABLE 3. TOXICITY OF HERBICIDES TO MAMMALS, BIRDS, FISH, AND INVERTEBRATES (CONT.)

Common Name (Trade Name) of Herbicide	Name of Organisms	Type of Test Exposure	Dosage a.i.	Effect	Reference
Trifluralin Cont.	Rainbow Trout	Acute, 96 hr	0.01-0.086ppm	LC_{50}	EPA
	Fathead Minnow	Acute, 96 hr	0.093	LC_{50}	Batelle
	Bluegill	Acute, 96 hr	0.019-0.089ppm	LC_{50}	EPA
	Channel Catfish	Acute, 96 hr	0.254 ppm	LC_{50}	
	Goldfish	Acute, 96 hr	0.252 ppm	LC_{50}	
	Daphnia magna	Acute, 48 hr	0.56 ppm	LC_{50}	Sanders
	Daphnia pulex	Acute, 48 hr	0.24 ppm	LC_{50}	Pimentel
	Gammarus lacustris	Acute, 48 hr	5.6 ppm	LC_{50}	
	Gammarus fasciatus	Acute, 48 hr	1.0 ppm	LC_{50}	Sanders
	Crayfish (Orconectes nais)	Acute, 48 hr	50.0 ppm	LC_{50}	
Xylene	Rat	Acute Oral	4300 mg/kg	LD_{50}*	McKee and Wolf
	Various Species of Fish	Acute	10-90 ppm	Will Kill Fish**	
	Bluegill	Acute, 24 hr	5 ppm	Sick	
	Rainbow Trout	Acute, 24 hr	5 ppm	No Mortality	
	Sea Lamprey	Acute, 24 hr	5 ppm	No Mortality	
	Orange-spotted Sunfish	Acute, 1 hr	47 ppm	Kill	Lawrence, 1962
	Daphnia magna	Acute, 48 hr	0.01-0.1 ppm	Kill	

* Usually a mixture of o, m, and p xylene.
** m-Xylene - usually most toxic of isomers.

a/ CDOD = Consecutive daily oral doses each at the given mg/kg.

b/ Signif Effect = Significant effect (weight loss, reduced weight gain or illness).

c/ Alkanol amine salts.

d/ Potassium salt.

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TABLE 4. RELATIVE ACREAGE OF HERBICIDAL USE CLASSIFIED BY HABITAT

Habitat or Crop Classification	No. of 1000 Acres Treated 1968	% of Herbicidally Treated Acres for Each Crop or Group of Crops	% of Total U.S. Treated with Herbicides
a. Row Crops	72,693	56.91	3.20
1. Corn	39,302	30.77	1.73
2. Soybeans	17,167	13.44	0.76
b. Grain Cover Crops	36,157	28.30	1.65
1. Wheat	20,915	16.37	0.92
c. Grass and Forage Dominated Areas	15,475	12.12	0.68
1. Rangeland	4,373	3.42	0.19
2. Pastures	4,525	3.54	0.20
d. Woody Plant Crops	3,188	2.50	0.14
e. Land Bare of Vegetation	?	<0.1	<0.01
f. Wildlife Management Areas	?	<0.1	<0.01
g. Aquatic	216	0.17	0.01
U.S. Total	127,729	100.00	5.62

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Chloracne from the accidental production of tetrachlorodibenzodioxin

GEORGE MAY[1]
General Practice, Bolsover, Derbyshire and Coalite and Chemical Products Limited

May, G. (1973). *British Journal of Industrial Medicine*, 30, 276-283. **Chloracne from the accidental production of tetrachlorodibenzodioxin.** Following the accidental production of 2,3,7,8-tetrachlorodibenzodioxin (dioxin) as the result of an exothermic reaction at a chemical plant in Derbyshire, 79 cases of chloracne were recorded, many of them severe. Contrary to the usual experience they have responded very favourably to treatment and there were no cases of contact chloracne among relatives or domestic animals in the initial outbreak. However, two cases of contact chloracne were recorded three years later.

Similar incidents are known to have occurred in both Europe and the United States of America, almost invariably accompanied by widespread severe illness and with fatalities. Apart from one death due to an explosion which followed the exothermic reaction the more serious sequelae, which may range from depression and loss of weight to liver, kidney, and cardiac failure as well as malignant disease, have not occurred.

A quick and reliable method of biological assay for the presence of dioxin in produced trichlorophenol was developed based on oral dosage to rabbits with assessment of liver function at fixed time intervals thereafter. This test has already been superseded by instantaneous gas-liquid chromatography.

An entirely new plant with suitable modifications and multiple safety features has now been in satisfactory operation for three years.

Production of 2,4,5-trichlorophenol (2,4,5-TCP) began in August 1965 at Bolsover, Derbyshire at the Fine Chemicals Unit of Coalite and Chemical Products Limited.

1,2,4,5,-Tetrachlorobenzene and ethylene glycol were charged to the reactor together with ortho dichlorobenzene and 100°Tw caustic soda solution (Fig. 1).

The vessel was heated by oil and reaction took place at about 180°C with an arbitrary upper limit of 200°C. The progress of the reaction was monitored by sampling, and on completion the vessel contents were cooled to 140°C. Steam was then injected into the reaction vessel in order to recover the ortho dichlorobenzene which was used purely as a solvent to prevent blocking of the condenser by solid tetrachlorobenzene. After the ortho dichlorobenzene ceased to be evolved, the reaction mixture was run into water in the separator. The contents of the separator were acidified with concentrated sulphuric acid at a temperature of about 70 to 80°C. After the separation of the organic layer containing 2,4,5-TCP from the aqueous phase, agitation was continued for a further 15 minutes and the contents of the vessel were allowed to stand for two hours. The aqueous phase was then syphoned off and transferred to a storage vessel, and a second waterwash was added to the organic layer. A third wash was also required, and finally a wash of town water was run to the effluent treatment drain. Since about 1966-67 caustic soda flake has been charged directly to the reactor and no water has been added at this stage.

The aqueous phase consisting of the washings, which contained sodium chloride, ethylene glycol, and digol was distilled under vacuum to remove

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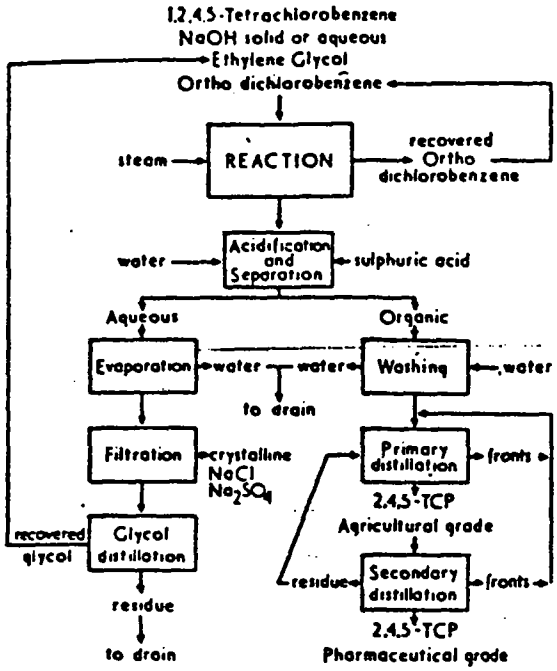


FIG. 1. Schematic representation of the reaction in 2,4,5-trichlorophenol production.

water. When the base temperature of the still reached 120°C distillation was discontinued and the still contents were transferred to a centrifuge where the precipitated salts were removed by filtration. The liquor remaining after filtration, which consists essentially of ethylene glycol, was fractionated and the glycol thus recovered was recycled to the first stage of the process.

Distillation

The organic material which was separated off was distilled at a vacuum of 20 mm of mercury. The residues from the still were run to waste, the fronts were recycled to the organic layer from the washing process, and the main fraction produced was agricultural grade 2,4,5-TCP. In the Coalite process a proportion of the pure or pharmaceutical grade of 2,4,5-TCP was produced by a further fractionation of the agricultural grade. The residues from this second distillation were fed back into the primary distillation and the fronts were added to the agricultural grade material for sale. The main distillation was the pharmaceutical grade material.

The agricultural grade was reacted with monochloroacetic acid to give 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and the pharmaceutical grade was condensed with formaldehyde to form hexachlorophane.

Production had been maintained for three years on the above basis. If the arbitrary temperature deadline was reached the process was cooled by lowering the temperature of the heating oil. This regulation was manually controlled.

Uses of finished products

The most important application of 2,4,5-T is for killing woody plants, several species of which have been shown to be more susceptible to 2,4,5-T than to 2-methyl, 4-chlorophenoxyacetic acid (MCPA) or 2,4-dichlorophenoxyacetic acid (2,4-D). Both amine and ester derivatives are available commercially, the latter being the most important. The esters are applied either in emulsions for overall foliage sprays or in solution in a mineral oil such as vaporizing oil or diesel oil for local application to frill girdles, or to stumps, or by the 'basal bark' technique. Commercial products often contain a mixture of 2,4-D and 2,4,5-T esters for reasons of economy and the greater range of effectiveness on different plant species gained by including the two chemicals.

Like the other auxin type herbicides, 2,4,5-T is of low mammalian toxicity. It is generally less effective on herbaceous species than MCPA and 2,4-D but it is much more persistent in the soil.

Hexachlorophane has for many years been used as a bacteriostatic agent in operating theatres and it is most likely that this use will continue. It is also used in topical applications in dermabrasion, and in a large range of toilet preparations ranging from soap to vaginal sprays and baby powders. Recently some baby powders have contained hexachlorophane in a concentration of 6% instead of the recommended maximum of 0·6%, and its use in toilet preparations is under review.

Incident

At midnight on 23 April 1968, the reaction temperature reached 175°C and thereafter it rose continuously for 50 minutes. When it had reached a point somewhere in excess of 250°C an explosion of considerable violence occurred and the supervising chemist was killed by falling masonry. Subsequent analysis indicates that the explosion was of a double nature. When the temperature had reached 225°C the process began to generate its own heat and the initial rupturing of the vessel was due to the advance of this exothermic reaction. Ethylene glycol and orthodichlorobenzene vapours were released in quantity into the atmosphere. In the presence of atmospheric oxygen these constituted an explosive mixture which appears to have been detonated by an overhead electric lamp. Extensive local damage was done to both plant and building and a con-

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siderable part of the wall in the proximity of the explosion was demolished. By far the greater part of all four floors, however, had apparently been completely unaffected by blast or residue.

The entire Fine Chemicals Unit was closed down pending clinical and laboratory investigation on 14 employees who were in the building at the time of the incident or who had had to enter in its aftermath.

Clinical and laboratory findings

Two men complained of tiredness, and three of slight tightness of the chest, but clinical examination revealed nothing of note. The investigations included liver function tests, full blood counts, and urinalysis.

Two men, the works engineer and a senior laboratory technician, both of whom had been closely associated with process development and with continuing pilot plant research, did not enter the building until some hours after the explosion. These two men were the most likely to show any work-induced condition which pre-existed the explosion but neither of them had any abnormality.

Of the remaining 12 men, only one showed no abnormality in any test. Three men showed one abnormality, four showed two, and another four showed three abnormalities. Among these were 13 abnormal liver function tests: $ZnSO_4$ turbidity in six men, thymol turbidity in two men, and serum transaminase in five men were abnormally high (Table). One man had albuminuria and one had an abnormal serum protein level. White cell counts in five men were regarded by the haematologist as being somewhat too high or too low and there were three men with glycosuria.

These findings persuaded us to treat the incident with extreme caution and to wait and see whether the changes were progressive. Fortunately, all the men reported fit very shortly after, and 10 days later practically all the tests were within normal limits. One man with 1% glycosuria was known to have had this before the explosion.

In view of these satisfactory findings the building was re-opened. All damaged and obviously dirty areas were sealed off to be decontaminated properly and at leisure, while the remainder of the building was subjected to a thorough spring clean. Production was then resumed in the greater part of the plant.

For a time work continued normally. Process operators were watched particularly carefully but they all remained well. However, certain men, all of whom had been in the Fine Chemicals Unit, developed a mild but peculiar skin condition. This consisted of a slight malar erythema and the eruption of tiny vesicles around the borders of the pinnae (Fig. 2). Occasionally there was evidence of mild conjunctivitis. These men were not continuously occupied in the same building as operators who always wore gloves, but were mostly fitters, plumbers, and electricians who only entered the unit on occasion to do specified jobs with bare hands. Obviously, therefore, it was something which had been deposited or embedded in the matrix of the building itself which was causing the reaction and which was being absorbed by contact. The diagnosis quickly established itself as chloracne and it became widespread. Later it was established that the toxic material was 2,3,7,8-tetrachlorodibenzodioxin (dioxin) (Fig. 3), a reaction product of the sodium salt of trichlorophenol (Milnes, 1971).



FIG. 2. Typical established chloracne of pinna showing cysts and comedones.

From 8 May to 8 December 1968, 79 cases of chloracne were recorded. Not all individuals presented with malar erythema, etc., but those who did generally became severe and extensive cases. Some men appeared

TABLE

LIVER FUNCTION TESTS IN 14 MEN IN BUILDING AT TIME OF EXPLOSION OR WHO ENTERED IT SOON AFTERWARDS

	Patient													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Thymol turbidity (normal upper limit 2·5 units)	1·2	0·7	0·5	0·7	0·5	1·2	0·8	0·6	1·8	1·1	1·2	2·5	3·8	3·05
Zinc turbidity (normal upper limit 10 units)	7	8	17	8	13·5	12·8	9·9	12·7	7·5	13·5	5	12·5	5·5	9·4
SGPT (normal upper limit 35 units)	18	20	32	16	18	36	42	22	40	6	28	40	6	36

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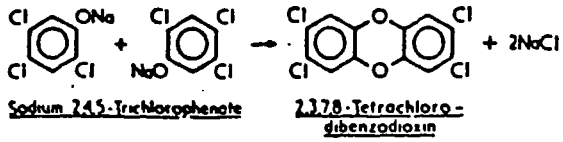


FIG. 3. Formation of one molecule of 2,3,7,8-tetrachlorodibenzodioxin from two molecules of sodium 2,4,5-trichlorophenol. (This is the International Union of Pure and Applied Chemistry nomenclature; in some earlier literature the compound has been referred to as 2,3,6,7-tetrachlorodibenzodioxin.)

to react within days while others did not present with established symptoms until between two and three months after the last known exposure. Younger men, particularly those with fair complexions, were first affected, while the reaction was most persistent in sallow-skinned men in the 25–40 age group, particularly if their personal hygiene was neglected.

The established clinical condition was present on the face in all cases. Inclusion type cysts, ranging from 1 to 7 mm in diameter, were diagnostic (Fig. 4). The malar



FIG. 4. Facial chloracne of the multiple comedone and small cystic type showing well the most common distribution.

areas were worst affected, the cysts diminishing in number as the condition extended on to the cheeks, maxillary areas, and sides of the neck. The ear lobes, post-auricular triangles, and suboccipital hair-line were also involved. In some men the condition was predominantly cystic, while in others there was a liberal smattering of comedones, even to the extent that not a pore had been missed and the whole side of the face bore a dusky grey hue. The facial condition varied from very mild and just diagnosable to severe and obvious.

The other parts of the body most often involved were the extensor aspects of the arms, the lateral aspects of thighs and calves, the back, and the sternum in that order. In five cases the antecubital fossae were involved (Fig. 5),

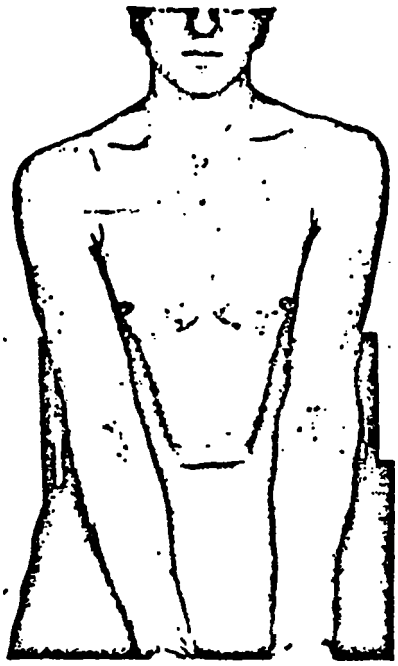


FIG. 5. Chloracne affecting the antecubital fossae.

a condition which has not previously been described, and in two cases the medial aspects of the thighs were affected.

Postular folliculitis, presumably from secondary infection, was present in a moderate number of cases.

Treatment

In the early stages it was found that the use of ointments, creams, pastes, and steroids tended to aggravate the condition. Oral oxytetracycline, 250 mg, two to four times daily, was used for pustular follicles, depending on the severity and extent of the infection. Otherwise, in the more severe cases a 1% solution of cetrimide was applied after the daily bath; in moderately severe cases zinc sulphide lotion, BPC, was used while mild cases were given no local medication whatever. Oxytetracycline and cetrimide were often used together. Severe to moderate cases were also treated with ultraviolet light at the works. Treatment was reinforced by the most rigorous campaign of works and personal cleanliness. A system for laundering overalls was introduced and the men were advised to use the showers regularly, and to keep the garments they wore under their overalls clean. They were also asked to change into clean clothing before leaving the works. Hair oils were discouraged and thorough rinsing of the skin after washing, particularly shaving, was recommended. Finger nails were kept short and clean and steaming of the face

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at home, followed by gentle squeezing of comedones and cysts with subsequent application of eau-de-cologne or astringent lotion, was advised. The men were also recommended to get as much sunshine as possible.

Within the next four to six months the great majority of cases made an almost complete recovery, in reverse order, with the younger fairer individuals leading. Ten men who had shown disappointing progress under the general regime were given intensive steam bathing followed by ultraviolet radiation, after which even their chloracne began to resolve.

Treatment of the building

The Fine Chemicals Unit was again closed and no one was allowed into it without special protective clothing, including full face mask, gloves, and boots. Anyone coming out of the building had to do so through a special decontamination room where he had assistance to change. The presence of dioxin on the walls, roof, and other parts of the plant was shown by painting a solution prepared from rubbings taken from different parts of the building on the inner side of rabbits' ears, as first demonstrated by Adams, Irish, Spencer, and Rowe (1941). The results were so consistent that they were checked by testing the plain solvent and even rubbings from new asbestos to demonstrate that these, on their own, could not produce chloracne. As the rabbit's ear test took eight weeks a method of oral dosage and control was developed with estimation of the serum alanine aminotransferase at fixed time intervals. Not only was this method faster but it was more reproducible and reliable.

It was obvious that the entire building would have to be thoroughly cleaned and resurfaced. Heavy and expensive equipment which had been affected by blast or was heavily contaminated was buried 150 feet (45 metres) deep at the base of an open-cast coal mine. Dioxin is water insoluble and will not be leached out.

After the most extensive and vigorous decontamination, swabs from the areas previously contaminated were tested on rabbits and all were found to be negative. Rabbits were also exposed to the atmosphere of the building itself for one week with negative results. Production began once more and since then no new case of chloracne has developed in that building, nor has chloracne appeared among the decontamination squad.

Discussion

Manufacturing procedure at Coalite did not utilize either pressure or agitation in the autoclave stage. Explosions elsewhere, of which the research staff were aware, used both but at a lower operating temperature. It was believed that the Coalite method could not produce an explosion and, furthermore, in the event of an unforeseen temperature rise, that there would be fewer and less dangerous toxic byproducts. As far as is known, the Coalite method was unique in that it used glycol (boiling point 197°C) while other methods utilized methanol (boiling point 64°C). Owing to the lower boiling point of the latter, high pressures were required before a suitable reaction temperature could be reached in the liquid phase. The Coalite reaction took place at little more than atmospheric pressure. Attempts to produce explosions in grossly overheated pilot plants had failed and it was not until after the incident that the more toxic byproducts were isolated in such pilot plants. Neither has it since been possible to reproduce an explosion.

The only available reference to dangerous by-products from the interaction of similar chemicals at explosive temperature and force (Quarterly Safety Summary of the Association of British Chemical Manufacturers, 1960) briefly described two incidents. In the first, a charged autoclave was heating up when the pressure and temperature increased rapidly and an explosion ensued, resulting in fatal injuries. In the second incident, which occurred in a similar process, the temperature rose unexpectedly to 400°C with escape of vapours. After 10 days all 50 persons exposed fell ill and had more or less severe liver damage. It was thought that spontaneous decomposition had taken place and that pentachlorooxydiphenyloxide was formed. The residues were very toxic, very sticky, and difficult to clean up.

When a plant in Germany was told the reason for the cessation of their supplies of another chemical, normally produced in the Fine Chemicals Unit, they provided detailed information about a similar experience some years earlier in which they had identified dioxin as the toxic material. Dioxin is a natural, very minor contaminant in the routine manufacture of trichlorophenol. It does not appear to any great extent unless the reaction temperature exceeds 200°C and it is normally removed in the purification process. Thereafter it becomes an increasing constituent of the reaction products. In the German incident there had been some months of chronic overheating during which all the workers and many of their wives, children, and domestic animals had developed chloracne. It was only after production was finally terminated by an explosion that the gravity of the situation was fully realized and intensive research was carried out to isolate the toxin.

Exact figures of individuals involved or of morbidity or mortality rates were not given, but there were a number of cases of severe liver disorder, some

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of them fatal. Fifteen years later one individual was expected to die soon from this and others were still under treatment. One death from pulmonary carcinoma had been rejected as being attributable while one of intestinal sarcoma had been accepted. The works medical officer considered that dioxin passed into the system by inhalation as well as orally and transdermally. He was indeed surprised that our chloracne cases had developed no other symptoms.

The German literature on the subject of dioxin and its toxicity makes no specific reference to this particular plant. There can be little doubt, however, that this incident along with two others forms the basis of a series of articles (Kimmig and Schulz, 1957; Bauer, Schulz, and Spiegelberg, 1961; and Schulz, 1968) which describe in great detail not only chloracne but all the toxic reactions attributed to dioxin, which include liver damage, emphysema, myocardial degeneration, raised blood pressure, renal damage, reflex irregularities, depression, disturbances of memory and concentration, etc. Oettal (Hofmann, 1957) reported liver necrosis in rabbits kept in cages adjacent to those exposed to dioxin. Furthermore, unexposed animals which were put into cages previously occupied by exposed animals also suffered liver necrosis so that a virus infection was at first suspected.

Crow (1970) defined chloracne as the 'production in the human subject of a type of acne with characteristic clinical features, so far produced only by aromatic chlorinated hydrocarbons of varying structures'. It is quite distinct from all the other acnes of external origin.

The unchlorinated dioxin molecule does not cause acne and is also non-toxic. It has been argued that the toxicity is related to the degree of chlorination. This is demonstrably untrue because the 2,3,7,8-tetrachlorinated dioxin has often been shown to be not only the most toxic but also the most powerful cause of acne. The interrelationship or positioning of chlorine atoms in dioxin molecular space is, therefore, of paramount importance. As in the dioxins, some members of the chlornaphthalenes and the diphenyloxides cause chloracne. There is a strong school of thought which believes that it is not only the position of the chlorine atoms in molecular space but their symmetry which is responsible for their biological activity. Whether it be one or both, we have a whole series of compounds whose unchlorinated molecules are inert but which, when chlorinated, and depending on the number and position of their chlorine atoms, cause an identical clinical condition. They have, therefore, a common factor and I suggest that this should be called 'the chloracne factor'.

The literature shows that chloracne is extremely resistant to all forms of treatment. We saw cases in Germany which were still active after 15 years' treatment and freedom from contact. At the present time, four years after the incident at Bolsover, chloracne is just diagnosable in seven cases. At no time did we have to resort to the incision of cysts, neither has there been any legacy of severe scarring. Whether this is due to the comparatively short period of exposure to dioxin, to the comprehensive personal and environmental hygiene measures or to a mixture of both, is difficult to say.

Though chloracne was the presenting symptom, the possibility of underlying serious disease was not ignored. No other ailment presented which could reasonably have been held attributable to dioxin exposure and continuous monitoring of liver function tests and urinalysis was satisfactory.

The German company had indicated that the bromsulphthalein test of liver function and serum gamma-globulin levels were also important. These were checked in some of the more severe cases without revealing any abnormality. Their recommendation that we should carry out a few liver biopsies was discarded, for the time being at any rate, owing to the continued fitness of the men, the normality of the other investigations, and our desire not to create undue alarm. Time appears to have proved this decision to be correct. During the months that followed no contact, such as wife, child or domestic pet, was reported to have chloracne.

Rabbits were used to demonstrate the presence of dioxin as Hofmann and Neumann (1952), dealing with a similar incident, had indicated that rats introduced to the autoclave room had shown no reaction and guinea-pigs only non-specific reactions. Cats, however, suffered diffuse loss of hair after a lengthy latent period, while rabbits died of acute liver necrosis after a latent period of one to three weeks. Obviously, rabbits are extremely sensitive to dioxin as a liver intoxicant.

When the German company had resumed its own production in the early 1950s they had not thereafter released a single batch of trichlorophenol without submitting a sample to an eight-week biological assay against the rabbit's ear. If chloracne was then produced the dioxin content was too high.

Trichlorophenol is very important commercially and it was therefore necessary to carry out toxicity tests on Coalite's own commercial product before going into production once more. A great deal of information was available on animal exposure (Anderson, Arndt, Goldbey, and Jones, 1949; Farquharson, Gage, and Northover, 1958; McCollister, Lockwood, and Rowe, 1961) which indicated that trichlorophenol was not toxic unless given in massive dosage. Indeed, McCollister *et al.* (1961) had shown that the rabbit, though not as resistant as the rat, did have a high tolerance to trichlorophenol. It was shown that a dose of the

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Coalite commercial product of about 1 g/kg had to be given before it was lethal.

In the incident described here, the chlorine atoms in dioxin were situated at points 2,3,7, and 8, which appears to be the most toxic of the dioxin group. There are, however, eight points onto which a chlorine atom can be hooked and therefore there can be over 60 different chlorinated dioxins, depending on the number and position of the chlorine atoms. Very little is known about the other chlorinated dioxins in which the biological activity is also dictated by the number and position of the chlorine atoms.

Although Coalite chemists failed to produce an explosion experimentally using the old process, one did take place eventually in the production plant, despite the absence of pressure and agitation. Whether the sheer size of the commercial plant could excite an exothermic reaction into becoming explosive or whether there was any other constituent is not possible to say. An exotherm has been apparent experimentally at 400°C, and dioxin has been recovered and purified from this production (Milnes, 1971).

An entirely new plant to produce 2,4,5-trichlorophenol in which all processes, including temperature control and other new techniques, are automated has been in operation for three years without the occurrence of chloracne or production of dioxin. All batches are now submitted to gas liquid chromatographic (GLC) analysis by a technique specially developed to test for dioxin and which is sensitive to 0·2 p.p.m. This level is considered to be well within the limits of acceptability.

In the process for trichlorophenol production currently in use orthodichlorobenzine has been replaced by petroleum ether. The former process involved a total condenser and external separator (glass condenser). The petrol ether process incorporates a reflex condenser fabricated from steel and reflex divider which is much simpler and safer. Additionally, each reactor has five fail-safe functions built into the instrumentation independent of the programme control. These functions are designed to shut down a reactor automatically in the event of a fail-safe condition being reached and to shut down that reactor in such a way that an exothermic reaction is not possible. The programming computers memorize the slightest deviation towards a fail-safe situation and are therefore continuously updated. The temperature of the reaction is held at 180°C until tests show that there is no dioxin in the reaction mixture.

A fine powder such as purified dioxin could obviously be absorbed by inhalation and oral ingestion. In our own case, the contaminant was trapped in a heavy greasy deposit so we are inclined to rule out inhalation completely. Early liver function tests appeared to indicate the presence of a toxin which might have been inhaled but these departures from normal were transient and none of the individuals concerned developed chloracne. In the event the workers who did develop chloracne were those who had to get down to handling pipes, joints, cables, and the like with their bare hands, and this suggests that these men absorbed the dioxin through the skin. In our opinion, there must then be some excretion of absorbed dioxin or its products by the facial pores. It is suggested that in these light-exposed areas, such as pinnae and malar region, a chemical reaction takes place, probably itself light-induced, causing oedema and obstruction of the follicles with subsequent formation of retention cysts and comedones. It is not until the follicles re-open and function normally that chloracne begins to clear, and it is fully accepted that the methods by which this becomes possible were not, in our case at least, those necessarily applicable to ordinary acne. At the Coalite Refinery approximately 500 men are employed and no new case of chloracne has appeared among them since December 1968.

In March 1971, construction began of an entirely new building for an unrelated product using, almost throughout, factory fresh materials. This building was erected by outside contractors using their own employees. After only three weeks at this work two of these employees developed a skin condition which they reported to their own doctors and thereafter to separate dermatologists. The son of one (Fig. 6) and the wife of the other also developed this condition which was undoubtedly chloracne (Jensen and Walker, 1972). The only piece of equipment with which they had any authority to come in contact, which could possibly have been contaminated with dioxin, was a large metal vessel which had been subject to the most rigorous cleaning. Coalite's own employees had not only welded new collars to it after burning new vents but had actually been inside it. Later tests (GLC and double biological assay) on this vessel failed to reveal the presence of dioxin. The only contact these individuals had was when they connected up new equipment to the new collars. Furthermore, they only worked together for a day or two and yet they developed the most persistent chloracne of all; it is believed they are still undergoing intensive treatment, and they were the only people to pass the condition on at home. We have been unable to satisfy ourselves where or how this contamination occurred. Coalite's own workers must have been at very much greater risk over many months before. Even if it could be postulated that tolerance can develop to dioxin, which is not so, examples should have been seen in newcomers to the Company staff. It is certainly the case that the men affected did not avail themselves of the high-grade personal hygiene facilities at the

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FIG. 6. Case of contact chloracne showing fairly typical comedone distribution but no cysts. There is some malar pigmentation, a condition frequently described elsewhere but one which did not develop among the original cases.

works for either their persons or their clothing and this may explain the home transference. The wife developed symptoms nine months after her husband, and eight months after his last employment here. It was not possible to demonstrate dioxin by GLC in the contents of his facial cysts, something which had not been tried on the original cases.

Recently, environmentalists have been watching dioxin contamination of 2,4,5-T very carefully. Many of the early batches of this herbicide had been produced at excessive temperatures and, on release, contained up to 40 or even 60 p.p.m. of dioxin. Regulations, quality control, and cessation of production by companies without adequate technical knowledge have now eliminated dioxin contamination. The United States Agricultural Research Service (Cherry, 1972) has analysed samples from Florida experimental plots that had received for eight years massive 2,4,5-T sprays with dioxin traces up to 40 p.p.m. without finding residues. Dioxin residues have not been found at the lower limit of detection (0·05 p.p.m.) in tissues of the bald eagle collected in 15 widely separated states.

I wish to thank the Directors of Coalite and Chemical Products Limited for permission to publish this article, the Coalite Research Laboratory, where pure dioxide was isolated, the late Dr. Herbert Bondy, chemical adviser, and Dr. Ken Payne, research director, for comments on the technical aspects of the paper, and The Huntingdon Research Institute where extensive animal investigations were carried out, largely by Ronald E. Davies. Dr. Doris Fletcher, formerly consultant dermatologist at Chesterfield Royal Hospital, advised on the treatment of the patients.

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LYMPHOCYTE CHROMOSOME ANALYSIS OF AGRICULTURAL WORKERS DURING EXTENSIVE OCCUPATIONAL EXPOSURE TO PESTICIDES*

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SUMMARY

Lymphocyte cultures from 42 pesticide applicators and 16 controls were scrutinized for chromosomal aberrations during the midwinter ebb in pesticide spraying operations, and again during the peak summer period of intense spraying activity. Cultures prepared from the exposed individuals during the heavy spraying period showed a marked increase in the frequency of chromatid lesions. This trend was especially noticeable among workers exposed primarily to herbicides. A few chromatid exchange figures were also noted among the exposed group. Also discussed is the need for better methods of estimating the potential mutagenic threat, if any, of such observed clastogenicity.

INTRODUCTION

The Idaho Community Study on Pesticides is one of thirteen similar research facilities throughout the United States in areas where pesticides are extensively used. Sponsored by the Environmental Protection Agency, the primary task of this unified investigative effort is to evaluate the effects of pesticide exposure on human health. The Idaho program is now in its sixth year of intense clinical, biochemical and epidemiological scrutiny of a core group of carefully selected volunteers having extreme occupational exposure to various pesticides. An additional parameter currently being examined in detail is the possibility of using cytogenic analysis of lymphocyte chromosomes as an index of pesticide mutagenicity in man.

Cytogenetic analysis of chromosomal aberrations in somatic cell cultures is one of the major methods of testing currently recommended for evaluating chemical mutagenesis in mammalian systems[4,11,14]. Most investigators agree that the dominant lethal test and the host-mediated assay are also important test procedures, and that

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all three should be utilized in confirming mutagenicity in mammals. However, the latter two techniques are not suited for use with human subjects, and somatic cell cultures thus remain the only practical method of assaying morphological evidence of apparent damage to the human genetic material, *per se*. With respect to such cultures, *in vivo* techniques are preferred over *in vitro* tests because they provide the added advantage of taking metabolism into account[6,13]. Although such an approach by itself cannot provide unassailable proof of concurrent gametic involvement, it appears likely that autosomal aberrations may indicate parallel gonosomal events of a similar nature[14]. Even as a purely somatic indicator, analysis of lymphocyte chromosomes would seem highly pertinent when one considers that many spontaneously occurring diseases, certain neoplasms included, are now believed to be caused by environmental pollutants[3].

The tremendous array of agricultural xenobiotics now in common use constitutes a large portion of the various chemical agents to which man is environmentally exposed. The indiscriminate use of such substances would logically present the possibility of a genetic legacy of increased mutation frequency and subsequent human hazard. Increased aberrations of lymphocyte chromosomes have been recently reported[9] in a group of Michigan fruit producers involved in heavy pesticide spraying. Accordingly, we have examined chromosomes of peripheral lymphocytes (leukocytes) cultured from persons selected from our occupationally exposed study group. The prevalence of chromatid lesions among these volunteers has then been compared with that of a control group, both prior to and during the heavy spray season.

MATERIALS AND METHODS

It was initially hoped that sufficient numbers of persons having exclusive occupational exposure to a single chemical series could be obtained. However, because of the great agricultural diversity of this region, such a sampling technique proved to be impossible. Within Canyon County alone, in which most of the exposed participants reside, more than one million pounds of pesticides were applied to crops during 1972 (ref. 10). This figure, irrespective of other agricultural chemicals such as fertilizers, amounts to approx. 4 pounds of pesticides for each acre of farmland. Persons highly exposed to pesticides in this area usually apply a large variety of chemicals throughout the growing season, and multiple exposure is the rule rather than the exception. For these reasons, dose or duration of exposure is another variable that cannot be rigidly defined by such an approach.

Because of their local prevalence in pesticide-related occupations, only male Caucasians were sampled. Persons with recent histories of viral infections, chemotherapy or X-ray treatment were rejected, as were those with histories of malignancies. A total of 16 control and 42 exposed persons, matched as closely as possible as to ages and physical characteristics, were chosen. High exposure was defined as spraying with pesticides for at least 4 h per day on three or more days per week throughout the peak spray season of June 15th through August 30th. The control group was composed of businessman, students, health professionals and teachers who had no history of involvement with pesticides. Ages of the control group ranged from 19 to 64 (mean 39.8). Persons in the exposed group ranged in age from 18 to 70 (mean 38.6) and had from 1 to 25 years (mean 8.5) of prior occupational exposure to pesticides. As shown

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TABLE I

PESTICIDES MOST COM
ORDER OF FREQUENCY

Group A *Insecticide-exposed*	
Common name	
Demeton	
Ethyl parathion	
Trichlorofon	
Naled	
Malathion	
Dicofol	C
Diazinon	C
Endosulfan	C
DNOC	F
Methyl parathion	C
Toxaphene	C
Dimethoate	O
DDT	O
Chlordimeform	O
Oxydemetonmethyl	O
TEPP	O
Carbaryl	C

[a] Nomenclature after Berg
[b] OP, organic phosphorous series.

in Table I, persons in th
accordance with their p
age, 42.3; mean years of
spray rig operators and f
chemicals most frequent
pounds demeton, ethyl pa
applied less frequently b
36.5; mean years of prior
of local weed control ag
herbicides; especially 2,4-

Blood samples (10
during the off-season, Ja
pationally exposed for a
obtained from all persons
the peak season of pestic
daily exposure among the
extensively prior to both
fully noted.

Lymphocyte cultur
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in common use consti-
man is environmentally
gically present the pos-
and subsequent human
have been recently re-
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then been compared with
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TABLE I

PESTICIDES MOST COMMONLY USED BY EXPOSED SUBJECTS IN THIS STUDY[a] (LISTED IN DECREASING ORDER OF FREQUENCY)

Group A Insecticide-exposed		*Group B Herbicide-exposed*
Common name	*Type*[b]	*Common name*
Demeton	OP	2,4-D
Ethyl parathion	OP	Amitrole
Trichlorofon	OP	Atrazine
Naled	OP	Zineb (fungicide)
Malathion	OP	DCPA
Dicofol	OC	Dicamba
Diazinon	OP	Chlorpropham
Endosulfan	OC	Alachlor
DNOC	PH	Metiram (fungicide)
Methyl parathion	OP	Trifluralin
Toxaphene	OC	Maneb (fungicide)
Dimethoate	OP	Picloram
DDT	OC	Diquat
Chlordimeform	OC	2,4,5-T
Oxydemetonmethyl	OP	
TEPP	OP	
Carbaryl	C	

[a] Nomenclature after Berg[4] and Frear[6].
[b] OP, organic phosphorous series; OC, organic chlorine series; PH, phenolic series; C, carbamate series.

in Table I, persons in the exposed group were further divided into two subgroups in accordance with their predominant type of occupational exposure. Group A (mean age, 42.3; mean years of prior exposure, 12.0) consisted of 16 cropdusters, formulators, spray rig operators and farmers who were chiefly exposed to insecticides. The specific chemicals most frequently dispersed by Group A were the organic phosphorous compounds demeton, ethyl parathion, trichlorofon and naled. These and other insecticides applied less frequently by the group are summarized in Table I. Group B (mean age, 36.5; mean years of prior agricultural exposure, 6.3) was comprised of 26 employees of local weed control agencies. These persons were all predominantly exposed to herbicides; especially 2,4-D, amitrole and atrazine (Table I).

Blood samples (10 ml heparinized) were first obtained from all volunteers during the off-season, January–February, 1971, when none of them had been occupationally exposed for a period of at least 30 days. A second blood sample was then obtained from all persons during the period of July through August, which is well into the peak season of pesticide application and represents a common interval of intense daily exposure among the agricultural workers selected. Each person was interviewed extensively prior to both samplings and all pesticide-related information was carefully noted.

Lymphocyte cultures were prepared by means of Difco TC chromosome culture kits (Difco Laboratories, Detroit, Michigan). The technique is based on the procedure previously reported by MOORHEAD *et al.*[12]. Our only modification of their standard procedure was to increase incubation temperature from 37° to 38°, which seemed to facilitate the technique. The samples were cultured for 48 h to obtain cells in the first mitotic division in order to minimize the variability in aberration yield that is some-

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times associated with longer culture times[1]. Flame-dried slides were stained with Giemsa and rearranged and coded by a person other than the scorer to avoid bias. 25 metaphase spreads from each person were then examined microscopically for chromosomal aberrations. Only karyotypable cells having widespread chromatids with minimal overlapping were selected for scoring. Photographs were made of all abnormal metaphase figures and karyotyping was performed whenever there appeared to be evidence of obvious chromosomal rearrangement. The criteria of GEBHARDT[7] were followed in the determination of chromatid achromatic lesions (gaps), breaks and chromatid exchange figures.

RESULTS AND DISCUSSION

Other than random loss of chromosomes, heteroploidy was not demonstrated in any of the 1450 mitotic bursts examined. With respect to chromosomal lesions, the majority of achromatic areas (gaps) and breaks were confined to single chromatids. Although isochromatid lesions were occasionally encountered in both control and exposed individuals, this apparent trend toward chromatid aberrations rather than chromosome aberrations would appear to merit further study.

The incidence of chromatid lesions on a per person basis is presented in Table II. The mean number of gaps and breaks per person per 25 cells examined did not appreciably change for the control group at either sampling period. Among the exposed group, however, the mean number of gaps per person nearly doubled in cultures from the midsummer samples, and the mean number of breaks observed per person increased by a factor of 10. The maximum number of gaps found in any single exposed individual (Table II) increased from 2 in the slack season cultures to 5 in the midseason cultures. Maximum breaks for any single exposed volunteer rose from 2 to 6 during the same period. (Different persons were involved in each of the four cases cited.)

TABLE II

SEASONAL VARIATION IN THE OCCURRENCE OF CHROMATID LESIONS

Group	*Number*		*(per person per 25 cells scored)*			
			Chromatid gaps		*Chromatid breaks*	
			Off-season	*Mid-season*	*Off-season*	*Mid-season*
Controls	16	Mean ± S.E.	0.63 ± 0.22	0.69 ± 0.24	0.31 ± 0.12	0.44 ± 0.22
		Range	0–3	0–3	0–1	0–3
Total exposed	42	Mean ± S.E.	0.62 ± 0.11	1.14 ± 0.17	0.17 ± 0.08	1.71 ± 0.24
		Range	0–2	0–5	0–2	0–6
Group A	16	Mean ± S.E.	1.00 ± 0.22	0.75 ± 0.27	0.31 ± 0.18	1.56 ± 0.29
(Insecticide-exposed)	16	Range	0–2	0–3	0–2	0–3
Group B	26	Mean ± S.E.	0.38 ± 0.10	1.38 ± 0.22	0.07 ± 0.05	1.81 ± 0.35
(Herbicide-exposed)		Range	0–1	0–5	0–1	0–6

Those exposed primarily to insecticides (Group A) exhibited a 25% reduction in the mean number of gaps per person in cultures from the high exposure period. However, the mean number of breaks increased 5-fold in these same samples. Members of the group frequently involved with herbicide spraying (Group B) showed an in-

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0.31 ± 0.18 0–2	1.56 ± 0.29 0–3
0.07 ± 0.05 0–1	1.81 ± 0.35 0–6

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crease of nearly 4 times the mean number of gaps per person during the peak spraying season. Mean breaks per person for Group B increased by a factor of 25 during this period (Table II). Also noteworthy is that the two subjects having the most gaps and breaks, respectively, during the high exposure period were members of the herbicide-exposed group. On the other hand, the markedly low frequency of breaks (0.07 ± 0.05) noted in Group B workers during the slack season invites speculation as to the possibility of induced compensatory protection occurring at this time in the form of enhanced chromosomal repair.

During the July–August scoring of the 42 highly exposed individuals (1050 cells examined), a small number of chromatid exchange figures were noted. These included one Group A quadriradial, two Group C triradials, two Group F quadriradials and a possible Group G triradial. Such interchanges have been found to occur in the general population at a frequency of about one per 3000 cells[8]. The frequency noted in our exposed group approaches one per 200 cells. None of the controls exhibited any of these configurations, but, unfortunately, fewer individuals (400 cells examined) were sampled in the control group. One exposed individual also had a pericentric inversion in the A 2 chromosome. This was confirmed by karyotyping but did not appear in any of the other 25 cells scored for that person.

From these results, it is apparent that the extent of visible chromosomal damage in lymphocyte cultures prepared from the exposed group is considerably greater during the heavy spray season. Without additional correlative studies, however, the possibility of concurrent mutagenic involvement must remain only speculative. Because of the tremendous limiting factors imposed by long generation times, the direct phenotypic surveillance of human populations is restricted to only a few retrospective techniques. One such method would logically include the analysis of existing health statistics. Unfortunately, contemporary vital records are not usually compiled with the geneticist in mind. We have recently attempted to examine the incidence of such parameters as stillbirths, teratisms, retardation etiologies, neoplasms, *etc.*, among agricultural workers in this state, and have had little success because of insufficient data. On a more personal level, another aid in seeking to estimate mutagenicity in suspected populations is the large scale use of detailed interview techniques. However, a major obstacle here is the extent of credibility between researcher and subject.

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"ASSORTED PESTICIDE COMMITTEE REPORTS"

THE HEALTH HAZARDS OF DEFOLIANTS

Investigations and Recommendations of the Committee

THE Ralph McElroy COMPANY, INC.
2102 RIO GRANDE
P.O. BOX 7552, AUSTIN, TEXAS 78705

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To: Ministery of Social and Health Affairs

The working group on health hazards and labor safety of defoliants has learned that the safety rules concerning defoliants have been neglected. It is also known that the defoliants are toxic for man and animal if taken in large quantities. Therefore, it is possible that people exposed to the defoliants - in particular those spraying them - have been in danger of their health.

The suspected health hazards may be occupational poisonings or the defoliants might have chronic effects such as severe types of tumor, chronic lung illnesses or other chronic diseases.

The Institute has planned the enclosed medical investigation to begin with. Based on the data from this investigation one can conclude if further investigation is necessary. Funding for these further possible investigations will be requested separately.

The present goals of the investigation are:

1. To determine the health of the sprayers of defoliant in Oulu area (30 persons) in order to get statistics about various symptoms and diseases. This part will be called the preliminary investigation.

2. To determine the cause of death among workers using defoliants in the largest possible span of time. The investigation will concern at least one thousand workers. The results will be compared with a control group of the same size. The goal is to find out if the distribution of the cause of death among workers,

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who are exposed to defoliants, differ from those who are not. This part will be called the cause of death investigation.

3. To classify the symptoms among those workers which have contacted the Institute because of the use of defoliants (50 persons). The purpose of this part of the investigation is to identify the symptoms caused by the defoliants and to determine the irregularities in various organs. This part will be called the health investigation.

Based on the results from the above investigation one can conclude if a more extensive investigation is necessary.

Since the planning of activities of the Institute for the year 1972 does not include the cost of this investigation, it is necessary to hire additional staff to replace those that will do this investigation. There are also other direct and indirect expenses as indicated in the enclosed estimate of cost (Appendix 1).

Referring to the above reasons the Institute of Labor Health asks for the special grant of 79,690 FMk for the investigation from the Ministery of Social and Health Affairs.

M. J. Karvonen, professor
Chairman of the Institute of Labor Health

S. Hernberg, M.D.
Chairman of Department

V. Riihimäki, M.D.
Assistant physician

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Appendix 1

Estimate of cost on the health investigation of people exposed to defoliants.

I.	Planning	5,000
II.	Preliminary investigation 30 VR workers from Oulun area according to plan 1, 523 FMk/person	15,690
	Possible additional costs	1,500
	Computer costs, summary and report to the workers	4,000
	Incidental (heating, electricity, cleaning, phone, typing, mailing,...)	5,000
III.	Cause of death investigation for about 1,000 persons exposed to defoliants and a control group of equal size. Collection of data, computer cost and summary	5,000
IV.	Health investigation The medical check-up of the 50 persons reported to the Institute for symptoms of defoliant poisonings, 550 FMk/person	27,500
	Computer cost, summary and report to the participants	8,000
	Incidental (heating, electricity, cleaning, phone, typing, mailing, etc.)	8,000
	Total FMk	**76,690**

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Plan 1

THE PLAN OF THE PRELIMINARY INVESTIGATION IN THE INSTITUTE OF LABOR HEALTH

Medical check-up (1 hour)			30
Neurological test			67
Neurophysiological special test		ENMG	97
		EEG	48
Lab test:	LA		4
	PVK		9
	GOT		10
	GPT		10
	Alkalic phosphatase		10
	Creatinine of the serum		9
	Urine albumin		3
	Urine glucose		3
	2 hour sugar test		37
	SPJ		16
	T_3 test		33
	Cholesterol		10
The test for the lung functioning:		spirometry	31
Heart investigation:		EKG	18
X-ray investigation:		thorax X-ray (heart thorax)	40
		neck X-ray	38
Total			FMk 523

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THE HEALTH HAZARDS OF DEFOLIANTS

Investigations and Recommendations of the Committee

Helsinki 1973

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In March 1972, there were unusually frequent complaints of sickness among employees of the State Railway System who have been working to clear out bushes using herbicides. That is why the General Director of the Health Department of the State, Professor Leo Noro, asked a group of experts to investigate the health hazards and labor safety connected with the use of herbicides of that kind.

Dr. Juhana Idänpään-Heikkilä, M.D., from the Health Department was appointed as the chairman of the working group. Other members were Ms. Kirsti Arjanne and Mr. Heikki Loppi, inspectors of the State Health Department, Dr. Holger Törnroth, M.D., from the State Railway Department, and Dr. Sven Hernberg, M.D., from the Institute of Labor Health. The secretaries were Mr. Vesa Riihimäki from the Institute of Labor Health and Mr. Pekka Hillman from the State Railway Department. Dr. Törnroth was later replaced by Dr. Matti Romo, also from the State Railway Department. On the first of May the group was enlarged to include two more members: Mr. Oiva Lyytinen to represent the State Forestry Department and Mr. Kaarlo Laaksonen to represent the State Highway Construction Department. As an specialist involved in poisons, Dr. Jorma Rautapää attended the meetings of the group. The task of the working group was changed in May 1972 by excluding the matters concerning labor safety, which were committed to another working group set up by the Health Department.

The working group has further heard as specialists Dr. Pekka Nuortevaa, Professor Marja Sorsa, and Mr. Ukko Rummukainen, all from the Institute of Forestry, Professor Pekka Koivistonen from the Food Research and Technology Department of the Helsinki University and Professor Jaakko Mukkula from the Institute of Plant Ecology. The chemical analyses were done in the State Institute of

Agricultural Chemistry and in the Institute of Labor Health. The working group has met with the other working group on labor safety, as well as, with a working group of the Institute of Plant Ecology.

From Sweden the working group has been in contact with Dr. Åke Swensson, from the Labor Health Department, Karolinska Institute, Dr. Lennart Sundell, Labor Health Department, from the Regional Hospital in Örebro, and Dr. Sten Rosenqvist from the Swedish State Railway System.

The working group has met 20 times.

Upon completion of the job, the working group submitted this report to the State Health Department.

Helsinki, February 23, 1973

Juhana Idänpään-Heikkilä

Kirsti Arjanne
K. J. Laaksonen
Oiva Lyytinen
Sven Hernberg
Heikki Loppi
Matti Romo
Vesa Riihimäki
Pekka Hillman

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1. DEFINING THE TASK AND PRELIMINARY RESEARCH

1.1. Preliminary investigations and analysis

First, the working group tried to relate the suspected cases of illness and their possible connection to the defoliants, to find out statistics about persons working with herbicides, either spraying or handling them in the factory, and to verify how completely the existing health regulations were followed. The statistics were gathered immediately among the State Railway System, the State Highway Construction Department, the Forestry Department and some private Power Companies. The Labor Health Institute issued a questionnaire to investigate the known health hazards that occurred to workmen during and after spraying the herbicides.

The working group procured the health reports and documents related to the suspected cases of illness or death. Based on these documents, six cases of illness were reviewed in detail by the working group. Five of these cases were from Haukipudas, and one was from Jyväskyla. The working group became familiar with the working methods, spraying equipment and safety procedures in Oulu and Haukipudas on April 7, 1972. At the same time, information from the workers was collected on the use of defoliants and their possible health hazards for workers. The known cases of sickness were reviewed and the patients interviewed in detail.

The Committee on Toxic Materials issued a memorandum in March 1972 for the working group (referenced under Myrkkyasiain Neuvottelukunta).

The working group also obtained the memoranda from a corresponding committee in Sweden. To complete the information from Sweden, Dr. Idänpään-Heikkilä and Dr. Hernberg visited Stockholm and drew up a plan of cooperation concerning task assignments for finding out about health hazards of the defoliants concerned.

1.2. Conclusions

Based on preliminary research, it was concluded that it was highly improbable that defoliants were the cause of serious illness

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(e.g. tumor or emphysema), although the case histories reviewed could not give reliable data about the causality. It was concluded in the preliminary report that some workers had shown symptoms of poisoning obviously due to defective safety methods and substandard equipment. Further, it was noted that toxic impurities (e.g. dioxin) has been used earlier in Finland in herbicides from 1950 to 1960. The total number of workers in contact with herbicides was estimated to be a couple of thousand.

2. PROPOSALS FOR THE PROJECT

Based on the preliminary research, the working group divided its project into three phases in order to find out about the current situation and facts concerning defoliants and about their alleged health hazards (see Appendix 1 for the project proposal). The working group submitted the project proposal and cost evaluation to Mr. Osmo Kaipainen, the Minister of Health and Social Affairs, on April 22, 1972, and he considered the research urgent. Mr. Matti Louekoski and Mr. Reino Lindroos, representing the Government, supported this opinion on April 28, 1972. The Government granted 80,000 FMk for the preliminary investigation. The Labor Health Institute initiated the urgent parts of the project according to the recommendations of the working group.

On May 25, the working group proposed to halt temporarily spraying from a backpack and to use other methods until further information about the health hazards had been gathered. This method of spraying was considered the most dangerous.

3. BACKGROUND INFORMATION ABOUT THE LABOR GROUPS UNDER INVESTIGATION AND THE REPORT OF THEIR EMPLOYER'S DEFOLIATION METHODS

3.1. Defoliation in Finland

The herbicides to clear out tree shoots and bushes appeared commercially in Finland from the beginning of the 1950's and, since 1960, the main method to clear out bushes has been by the use of defoliants. The main users of herbicides have been the Government Departments for Forestry, Agriculture, Highway Maintenance and

Construction, the State Railway System as well as private power companies. Private owners of farms or forests have used insignificant amounts of herbicides compared to the above users.

In the beginning of 1973, about 50 herbicides were granted permission to be sold. The active ingredients were

- 2,4-D amine salt and ester
- 2,4,5-T amine salt and ester
- MCPA salt and ester
- pichlorame

The use of MCPA has been very insignificant for clearing out tree shoots since it has no effect on the most frequent types of trees, aspen and mountain ash. In 1973 there were three herbicides containing pichloram and 2,4-D that were approved for clearing out aspen and mountain ash shoots. According to statistics on its use, the amounts of pichloram is rather small. So, over the past 20 years, the main ingredients for these kinds of herbicides have been 2,4-D and 2,4,5-T. According to the statistics gathered by the Institute of Plant Ecology, the sale of these two compounds for the past three years has been as follows:

Compound	Sales in metric tons (of the active ingredient)		
	1969	1970	1971
2,4-D	53.4	55.9	42.2
2,4,5-T	69.8	52.1	48.2

3.2. Defoliation methods used by the State Railway System

The use of herbicides was started in 1953, when 16,400 litres of commercial products were purchased. During the peak years of 1962-63 the quantity of herbicides was 27,000 litres annually. In 1971, only 5,200 litres were used. In 1972, no defoliants were sprayed or purchased.

Spraying has annually employed about 90 persons, railroad

workers and others (e.g. students). The herbicides are commercial products, whose active ingredients have been the phenoxy compounds 2,4-D and 2,4,5-T in a ratio of 2:1. One commercial product contained DCA, TCA, and MCPA. The concentration of active ingredients has been 500 g/ℓ with a spraying concentration of 1-2%.

The herbicides have been sprayed primarily on the leaves (85%) using hand-operated backpack type sprayers. Some experience has been collected concerning spraying from a train coach.

For visibility, it is necessary to clear out bushes in the railway. Tree shoots that are too long also make communication difficult. In winter these bushes collect snow drifts and block the railway tracks. The use of herbicides is necessary since no good mechanical methods for clearing out the bushes have been developed so far. The mechanical methods to clear out railway shoulders are not developed due to the inaccessibility of the terrain. Use of manual labor is impossible since it is expensive and not available.

The backpack type sprays will be replaced by train coaches. The backpack type sprays will be used only when other methods fail. Near lakes and rivers each tree is treated separately. The same is true for overly long tree shoots. The use of train coaches will reduce the number of people used for spraying. The allergic reactions will be confined to a smaller number of people. The use of safety regulations will be strictly enforced.

3.3. Defoliation methods used by the State Highway Construction Department

For traffic safety clearing out bushes from the highway shoulders is necessary, mainly to improve visibility and to prevent snow drifts from blocking the roads.

The herbicides are used because they destroy the roots as well as other parts of the tree shoots. The mechanical methods only increase the amount of shoot growing. Experimentally, the spraying was started in 1951 and was adopted as the primary method in 1953. The peak year was 1969 when about 10,000 ha were

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sprayed. In 1972 the total area sprayed was 4,500 ha, which is about 30% of the total area cleared out.

Annually this project employs about 150 persons in the country.

The active ingredients are phenoxy compounds in which the ratio of 2,4-D to 2,4,5-T is 2:1, which corresponds to about 500 g/ℓ of pure acid since there is difficulty in evaporating the esters. The commercial product is diluted in water to give a spraying emulsion of 2.5%.

The spraying is done from the highways using car or truck sprayers. Very infrequently the backpack type sprayer is used.

It is estimated that according to the prices in 1972 it would cost an additional 2,000,000 FMk annually to give up entirely the use of herbicides. Until another solution is found, spraying of herbicides will continue since it is fast, effective, and economic.

3.4. Defoliation methods used by the State Forestry Department

The defoliation was experimented with first in 1955 over an area of a few acres. Larger areas were used in 1958. Chemical defoliation was first used in soil renewal. Only a few acres each year were sprayed. The activity really started in 1960 when the airplane was also adopted. In 1969 the use of the airplane was temporarily halted as a method of spraying. Consequently, during ten years the area defoliated totalled about 60,000 ha. Up until 1971, the area sprayed from the ground totalled 28,000 ha. In addition, other methods have been used in an area of 6,000 ha. In 1972 defoliation by herbicides was completely stopped.

For the current year about 2,000 ha will be treated using a tree by tree method, 3,000 ha will be sprayed. Annually, the total area defoliated is about 50,000 ha.

A tree by tree method would employ about 50 men for two months. The time for spraying is one month and employs 200 men using motor sprayers.

In the future the tree by tree method will be employed as much as it has been until today. Where it can be used, it is the

cheapest way to defoliate. Spraying from an airplane or from the ground is by far cheaper than any mechanical method. Depending on circumstances, mechanical methods may be 2-3 times more expensive than the chemical methods. Chemical methods will be used on an area that is just 10-20% of the total area to be cleared out, because the question of selectivity is not well known according to present knowledge concerning the spraying method.

Safety in spraying will be considered in the future. That would favor spraying from an airplane.

3.5. Defoliation methods used by Imatran Voima Co.

The use of herbicides in clearing out tree shoots under open power lines was started in 1958. From 1965-1969 3,000-4,500 ℓ of herbicides were used annually. In 1971 only 1,800 ℓ was used. In 1972 no chemicals were used. Annually, the area treated has been about 1,000-2,000 ha.

The spraying has been subcontracted. The company employs about 10 persons to supervise and plan the activity.

The herbicides are commercially available, with the main active ingredients being the phenoxy compounds 2,4-D and 2,4,5-T in a ratio of 2:1.

The spraying has been done almost exclusively using backpack type sprayers. Some tractors and airplanes have also been used.

For the time being it appears that power lines cannot be protected by any other methods. Mechanical methods would add 50% to the cost in the long run. From terrain vehicles the spraying cannot be done extensively enough. This is due to the location of the power lines in the terrain. Spraying from the air, however, is possible and this method is employed to some extent.

4. THE PROPERTIES AND EFFECTS OF THE HERBICIDES USED TO DEFOLIATE (PHENOXY COMPOUNDS)

4.1. General

The phytotoxic chemicals used in defoliation are chlorinated phenoxy acids, mainly 2,4-D and 2,4,5-T. To a lesser extent it

also contains an important weed killer MCPA. In addition to phenoxy acids some defoliants also contain pichloram. Since this chemical is in frequent use in Finland and since there is current knowledge about the toxicity of pichloram [e.g. it is known that the acute toxicity of pichloram is small (Aamisepp et al. 1970, Johnson 1971)], this compound will not be considered in this discussion.

The literature on 2,4-D or 2,4,5-T does not always indicate what kind of chemical compound is being discussed (the compound may be a water soluble amine or a Na-salt or one of the several esters that are commercially available). Furthermore, the additives or impurities are seldom mentioned. It is important to note that the composition of the phenoxy acid as well as its additives and contaminants can drastically change its effect on metabolism and health (e.g. acutic toxicity, chronic toxicity or teratogenicity) as will be indicated later on. In particular, the contaminant of 2,4,5-T, called 2,3,7,8-tetrachlorodibenzo-p-dioxine (TCDD), has been an important factor and made it difficult to evaluate the results; it will be treated in a separate chapter. The survey of the literature on the properties and effects of these compounds (2,4-D and 2,4,5-T) does not necessarily discuss pure compounds only. It appears that the purity of the chemical compounds has become important only after 1969-1970.

4.2. Metabolism of 2,4-D and 2,4,5-T in animals and men

Experiments with animals (rats, pigs, cows, hens) has shown that the water solutions or free acids of 2,4-D and 2,4,5-T will be absorbed effectively and almost completely in the digestive system. The absorption of esters, however, has been slower and less complete and the absorption has obviously been preceded by hydrolyzation into a free acid (Erne 1966a). The concentration in plasma reached a peak in 3-10 hours, when high concentrations were observed in the kidney, liver, and lungs. Pigs also showed high concentrations in their metabolic glands. The excretion of 2,4-D and 2,4,5-T was mainly via the kidney in the urine (pigs showed only minor amounts in their feces or gall liquid) and the

process was rapid (e.g. the half times of 2,4-D were, depending on the dose, 5-10 hours in rats, 10-30 hours in pigs and cows). The half time of 2,4,5-T was somewhat longer. The corresponding process for esters was slower than that for water soluble salts. Seventy-two hours after intake there were only insignificant amounts of these compounds in the animal tissues. Experiments in pigs indicated that 2,4-D can go through the placenta and reach the embryo. On the other hand, the amounts in the brain were very low, indicating the slow penetration through the blood-brain barrier (Erne 1966a). Erne (1966b) noted that the metabolites of 2,4-D in the urine of the pig were mainly free acids but were also to some extent protein conjugates. The experiments in 2,4-D metabolism in sheep, using isotopes (Clark et al., 1964), and rats (Khanna and Fang 1966) indicated that the compound showed up unchanged in the urine; the experiment using rats further indicated that larger quantities slowed down the absorption and secretion times. Contradicting the previously mentioned observations, Clark and Palmer (1971) report that 86% of the 2,4,5-T ester given to sheep was still an ester in the urine whereas 1.4% had changed to a free acid. Even though it is evident that the major part of 2,4-D and 2,4,5-T that enters an animal will pass through unchanged, small quantities of metabolites can be found. Wilson (1971) observed that pregnant rhesus monkeys under 2,4,5-T feeding showed an occurrence of trichlorophenol equal to about 1% of the amount of 2,4,5-T in the urine. Several 2,4-D and 2,4,5-T experiments on the metabolism of animals indicate no storage of these compounds in fatty tissues. There is no theoretical likelihood for this storing property due to the polaric nature of the compounds.

If the daily dose that gets into the organism is large enough, there is some slow accumulation, since the half time of 2,4-D and 2,4,5-T varies in different mammals from 10-30 hours according to Erne (1966a) if the daily dose is high (over 50 mg/kg). The chronic feeding experiments have indicated that the animals will die of an acute type poisoning due to the accumulation of the phenoxy compounds.

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According to Erne (1966a) the esters will be transformed into their corresponding acids in the digestive system and will be absorbed in that form. There are no papers about the water soluble phenoxy compounds or esters to indicate how they will be absorbed through the lungs or the skin and what happens subsequently. It is supposed that the absorption through the lungs is almost total and that the absorption through the skin is less significant. Ester compounds can be expected to be absorbed through the skin more easily than the water soluble and polar acid or salt.

Little data is available on the metabolism of 2,4-D and 2,4,5-T in men and mostly comes from blood, urine, and tissue tests in suicide and attempted suicide cases. Observations [e.g. Nielsen et al. (1965)] indicate no essential difference between human and animal metabolism in this case.

Conclusion

There is data about the metabolism in various mammals. Experiments have been conducted almost entirely concerning absorption through the digestive system, excluding absorption through the lungs or skin. The metabolic change of the compounds into more toxic products, even in lesser quantities, cannot be excluded at the present. The metabolism of the compounds may indicate significant differences between different animal species as well as between animals of the same species with different genetic properties. The flow of the phenoxy compounds in various organs and their access to various important organs (e.g. genital glands) has not been adequately investigated.

The metabolism of phenoxy compounds in man is nearly unknown and the estimates and beliefs are based on animal investigations.

4.3. The toxic mechanism of 2,4-D and 2,4,5-T in animals

The effect of 2,4-D and 2,4,5-T on cellular metabolism is hardly known at all. Stanosz (1969) noticed reduction in the consumption of oxygen in the heart, liver, and kidney when rats were given 10 mg/kg/day of 2,4-D over a period of 120 days. Kuhn

and Stein (1965) observed acute poisoning causing amounts of 2,4-D to induce in the muscles of rats an increase in glycolycis. They also observed the occurrence of traces of oxydative phosphorylation. Furthermore, Philleo and Fang (1967) discovered that the results were dependent on the amounts and that, even with small doses of 2,4-D, there were occurrences of disturbances in the acetate metabolism.

Based on current theoretical knowledge of the chemical structure of the phenoxy compounds, they seem to have no influence on the hereditary material changes of the cell (ability to chemically react with DNA) (Giftnämnden, 1971). Desi et al. (1962) observed EEG-irregularities by using very large amounts (200 mg/kg), when they studied the neurotoxicity of 2,4-D in various animals. No microscopic changes in the neurons could be shown in animals that died by poisoning. The same authors thought that 2,4-D caused changes in the thyroid glands originating from the brain or the thalamus, but later on Florsheim et al. (1963) showed that the actual cause was the inability of thyroxine to bind in the plasma of the carrier protein.

Conclusion

The mechanism of the toxic effects of the phenoxy compounds (enzyme inhibition) in animal cells and tissue is virtually unknown according to the current literature.

4.4. Toxicity of phenoxy compounds

4.4.1. Acute toxic effects

The acute toxic effects on animals of the most common phenoxy compounds are known, when the chemical has entered their digestive system (see Table I). Acute toxicity is expressed in units of LD_{50} which gives that amount of poison in milligrams per kilogram of the weight of the animal that kills 50% of the animal population under investigation. Some defoliants and chemicals go through the skin to the organism and, if the compound is very toxic, even small amounts on the skin can be dangerous for the health of a person

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who is handling them. In the case in which the chemical comes in contact with skin, acute toxicity is expressed as dermal LD_{50}. According to the current available literature, the dermal toxicity of phenoxy compounds is scantily investigated. According to Aamisepp et al. (1970), the dermal LD_{50} of 2,4-D for rats is fairly low, about 1,500 mg/kg.

Various evaluations for LD_{50} give different results even for animals of the same species. Some mammals, in particular dogs and pigs, seem to be particularly sensitive to the toxicity of the phenoxy compounds. For the dog the oral 2,4-D LD_{50} value is about 100 mg/kg (Drill and Hirazka, 1953).

TABLE I. The acute toxicity of defoliants and TCDD contaminants contained, when given once orally to rats and gerbils.

	LD_{50} per kg of body weight	
	rat	gerbil
2,4-D	375 - 700	1 000
2,4,5-T	500	380
MCPA	600 - 800	
pichloram	8 200	3 000
2,3,7,8-tetrachlorodibenzo-p-dioxine (TCDD)	0.022 - 0.045	0.0006

The symptoms of acute poisoning are very similar for different mammals. A few hours after the introduction of the phenoxy compounds in the digestive system, a reduction in appetite, vomiting and diarrhea appear, as well as, motor disturbances, stiffness of the muscles and ataxia. There has been observed in dogs (Drill and Hirazka 1953) after death some non-specific congestive and toxic degenerative changes in their digestive system, liver, and lungs. The cause of death among apes (Hill and Carlisle 1947) was supposed to be cardiac arrythmia. Brandt (1971) has done a survey of the literature on poisoning cases in human beings and gives one case

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of his own observations. All poisonings (total 9) have been caused by swallowing some phenoxy compounds and were primarily in connection with suicide attempts. In six cases the compound was 2,4-D and in three cases MCPA. In Finland only one suicide has been committed with a 2,4-D preparation (ALHA, private communication). The symptoms of acute poisoning among men resemble those obtained in animal experiments. Non-specific toxicity and changes has been observed in the liver and kidney. Nielsen et al. (1965) indicated some pathological changes in the central nervous system due to poisoning, but these may have been due to a lack of oxygen. Brandt's (1971) own description of a case, when a person recovered after having taken at least 300 mg/kg of 2,4-D, showed the only permanent damage to be mono-neuropathy in one leg. Based on the above case histories the estimated dangerous dose of 2,4-D for man is 50-100 mg/kg.

4.4.2. Chronic toxic effects

When animals were fed with 2,4-D and 2,4,5-T over several weeks and even months, the symptoms of poisoning were observed among dogs for doses of 20 mg/kg/day (Drill and Hirazka 1953) and among pigs for doses of 50 mg/kg/day (Björklund and Erne 1966). Other mammals have tolerated repeated intake of much larger quantities. In the above investigations the chronic symptoms resemble the acute symptoms. It appears that the accumulation of poison in tissues causes "acute poisoning" and there is essentially no different mechanism for chronic toxicity. Some animals have shown toxic degenerative changes in the liver and kidney as observed in some feeding experiments.

4.4.3. Embryotoxic and teratogenic effects

The teratogenic and mutagenic effects of 2,4-D and 2,4,5-T have been extensively discussed in the publications by Giftnämnd (1971) and Myrkkyasiain Neuvottelukunta (1972). It seems evident that 2,4-D and 2,4,5-T prevent the development of the embryo and cause deformed embryos. Some 2,4,5-T preparations previously contained 2,3,7,8-tetrachloridilbenzo-p dioxine (TCDD) which also has a

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strongly teratogenic influence. The results of the investigations on damage to the embryo are difficult to interpret and are ambiguous. The influence of the phenoxy compounds on various mammal embryos differs. Mice are the most strongly influenced out of six mammals (mice, rats, gerbils, rabbits, sheep, and rhesus monkeys); toxic effects have been observed in 1971 with a dose of 100 mg/kg (NIEHS investiations, Giftnämnd 1971). Rats showed embryotoxic effects with 2,4-D and 2,4,5-T doses over 100-150 mg/kg/day (Khera and McKinley 1972). The observed effects included mild cases of defects in the bone structure and did not cause death. Wilson's (1971) investigations for the Swedish Giftnämnd on the embryonic toxicity of 2,4,5-T showed no defective development among the rhesus monkeys even with large quantities, 40 mg/kg/day.

Several chemicals are known to cause defective embryonic developments when given in large enough quantities [e.g. regular salt (NaCl), cafein, salicylates, and leucine amino acid are some of these (Giftnamnd 1971)]. It should be pointed out, too, that the most affected mammal in 2,4,5-T experiments (mice) can tolerate a dose (100 mg/kg) that would be lethal for a man even if taken only once. It is possible that the frequency of birth defects has been increased in Vietnam due to the chemical warfare. Such effects are not due to phenoxy compounds alone, since other chemicals with teratogenic side effects have been used in large quantities and since the phenoxy compounds that were used have contained contaminants such as dioxine. Swedish investigations in 1970 on two cases of alleged embryonic defects showed, according to the experts in teratology, no obvious connection with the minor allergic reactions and the defects (Giftnämnd 1971).

4.4.4. Mutagenic and carcinogenic effects

2,4-D and 2,4,5-T are mutagenic in plants. It is not certain yet whether this phenomenon is secondary in its influence in a way similar to that of the growth hormone or whether it is of primary mutagenic influence. Due to their chemical structure, the phenoxy

compounds cannot be expected to react with the hereditary material of the cell (DNA), whereas some other currently used chemical could (Myrkkyasian Neuvottelukunta 1972). Dioxines could also react with DNA. The mutagenic influence of the chemical can be caused by its interaction with the synthesis of the hereditary material in the splitting and doubling of the cell; in this case, however (unlike the previous case), the influence will show up only after the concentration has risen to a high enough degree. Even though the hereditary material of the animal and plant cells does not differ significantly, the metabolism of chemicals in plants and animals is so differnet that mutagenic effects in plants do not imply similar effects in animals. Dävring and Sunner (1971) observed, however, genetic defects in drosophila that were fed with 250 ppm of 2,4,5-T and Dävring (1971) observed traces of similar phenomenon in mice fed with 250 ppm of 2,4,5-T. The significance of these observations is not clear at the moment.

The occurrence of cancer cells also involves the mutation of the hereditary material. Consequently, if a chemical is mutagenic, it should be able to cause cancer cells--and further cancer, provided the cells will not be destroyed. 2,4-D and 2,4,5-T have never been observed in animal experiments to cause severe cancer. The evidence, however, cannot be considered conclusive enough to exclude the carcinogenic possibilities.

Summary

The acute toxic effects of 2,4-D and 2,4,5-T on various mammals and men is known. Chronically toxic effects have been investigated with animal experiments and based on this evidence it appears that the chronic and acute toxic effects are similar. It should be pointed out that most of the experiments have been of short duration (a few days or weeks) and only a few have been long term (months or years).

The damage to the embryo by 2,4-D and 2,4,5-T seems to be so negligible that, under usual circumstances, man is not likely to be exposed to a potentially dangerous dose. Dioxines are very

toxic and teratogenic compounds. They could increase the effects of the phenoxy compounds.

The mutagenic effects of 2,4-D and 2,4,5-T are mild, according to current knowledge. Carcinogenic effects cannot be shown, but there is not enough evidence to exclude the possibility with certainty. The mutagenic or carcinogenic effects of dioxines have not been investigated according to the literature available. So far there is no evidence that phenoxy compounds have caused embryonic defects, genetic damage or cancer in humans.

5. SURVEY OF THE LITERATURE CONCERNING THE HEALTH HAZARDS TO HUMANS THAT HAVE BEEN EXPOSED TO PHENOXY COMPOUNDS

5.1. Symptoms following exposure

Russian researchers have frequently reported that persons exposed to phenoxy compounds both in spraying (e.g. Fetisov 1966) and in industrial preparation (e.g. Basirov 1969) have experienced unusual fatigue, headaches, giddiness, loss of apetite and stomach aches as well as irritation in the respiratory organs and the skin.

5.2. Illness and poisonings

The irritation in respiratory organs (asthma) by defoliants containing phenoxy compounds has been studied by Forbes et al. (1966). In this case, however, the asthma was not considered to be caused by the defoliant.

In the late 1950's and early 1960's six cases of illness caused by 2,4-D were published, some of which developed into severely disabling polyneuropathy (Goldstein et al. 1959, Todd 1962, Foissac-Gegoun et al. 1962, Berkley and Magee 1963). In most of these cases the duration of exposure to 2,4-D was reported as being rather short, just a few days, and in one case it consisted of just a very brief splashing of the legs with the preparation; in this case, however, there was an intensive rash. In three cases the authors point out that an earlier contact had caused various symptoms in the patients, although it was only after the second contact that

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the neurologic illnesses showed up after a latent period of a few days. It is strange that after 1963 there have been no reports of similar symptoms in the literature. The cases indicate an illness based on allergic reactions or other idiosyncracies. It is also possible that 2,4-D was not the only cause of the illness, but that some impurity in the preparation might have been a factor.

Lorenzen and Lyngsøe (1957) have published a case history about two workers that became ill after having sprayed MCPA; the problem was in sugar metabolism (in one case a temporary problem and in the other case permanent diabetes). Animal experiments did not indicate diabetogenic effects. Other cases are not reported in the literature. Monarca and Divito (1961) have described one case of acute poisoning without permanent damage due to the spraying of 2,4-D.

Statistics on the health factors in the industrial processing of phenoxy compounds exist in Germany (Kimmig and Schulz 1957, Bauer et al. 1961, Kleu and Götz 1971), in the Soviet Union (Basirov 1969) and in the United States (Bleiberg et al. 1964, Poland et al. 1971, Johnson 1971).

Kimming and Schulz reported as early as 1957 that among the 31 workers of a factory producing 2,4,5-T there occurred chloric acne and it turned out that three of the workers had damaged livers. Ten persons of the group were investigated later on (Bauer et al. 1961) and again about 15 years after the first observations (Kleu and Göltz 1971). During these years it was observed that all ten showed symptoms of a psychopathologic state that corresponds to a known psychoorganic syndrome caused by damage in the brain. Kimming and Schulz observed in animal experiments that pure 2,4,5-T or its initial compound trichlorophenol did not cause chloric acne. The synthesis of trichlorophenol did not result in a pure product, but the authors did observe such impurities as tri- and tetrachlordibenzofuran and tetrachlordibenzodioxine. These compounds, in particular the last one, caused chloric acne and damage to the liver in rabbits even in small quantities. Schulz even experimented with his own skin and observed the above mentioned rash caused by tetrachlordibenzodioxine (TCDD).

Unfortunately these investigations by Kimming and Schulz were not noticed. It was only in the late 60's that it was rediscovered in the U. S. that commercial 2,4,5-T contained the dangerous TCDD and that further investigations on the properties and effects of the dioxines were initiated.

It is very likely that the illnesses reported among the workers producing 2,4,5-T in the U. S. (Bleiberg et al. 1964, Poland et al. 1971) are due to the impurities of the synthesis (e.g. dioxines) and/or the initial or intermediary compounds (tetrachlorobenzene, di- and trichlorophenol). In addition to the chloric acne, the workers were diagnosed as suffering from hyperpigmentation of the skin, hirsutism and tardive cutaneous prophyria, a disturbance in porphyria metabolims caused by the injured liver. On the other hand, Johnson (1971) could not observe anything abnormal in his investigation concerning 220 workers of a factory producing 2,4-D (where the estimated daily dose was 30-40 mg/day) and in another investigation on 64 workers dealing with the synthesis of 2,4,5-T (where the estimated daily dose was as low as 2-8 mg/day). Even though the investigation is not well described, it is reported to be based on extensive lab tests. Ten 2,4-D cases and 52 2,4,5-T cases were given karyotype test of lymphocytes with regular results.

Basirov (1969) reports that the workers of a factory producing 2,4-D compounds commonly had neurasthenic syndromes and symptoms of imbalances in the autonomic nervous system.

Several epidemiological investigations have been reported among farm labor spraying defoliants (Long et al. 1969) but the cases described have always been due to several different kinds of defoliants and the results are not important.

Summary

Use of phenoxy compounds causes several temporary symptoms such as fatigue, headaches, giddiness and irritation of the skin and of the respiratory system. The irritation can cause asthma if a patient has a disposition to it. In the early 60's there were

descriptions concerning injuries to the peripheral nervous system due to acute and mild or repeated exposure to the skin. No reports exist later on almost similar cases. It seems evident that the defoliants contained chemical impurities; for example, chlorinated phenols have caused similar injuries (Richert 1962).

The best evidence comes from the investigations concerning people who are employed in the industrial production of phenoxy compounds, since the exposures are usually strong and take place over years and, in the ideal case, the effects of other chemicals are insignificant or easy to evaluate. In the processing of 2,4,5-T the workers have been exposed to very damaging impurities, dioxines, and initial chemicals (tetrachlorobenzene), as well as to intermediary compounds (chlorinated phenols). It is very likely that the observed illnesses are due to some chemicals other than 2,4,5-T. The health hazards of 2,4-D and 2,4,5-T should be investigated epidemiologically in the future under conditions where the above confusing factors are removed. The possible mutagenic and carcinogenic effects should be investigated. The epidemiological investigations about the health hazards to workers spraying defoliants are inconclusive concerning the knowledge of the effects of the phenoxy compounds.

6. DEFOLIANTS USED IN FINLAND

6.1. Chemical structure

Currently, approval of a defoliant requires the filing of the complete chemical composition of the defoliant with the Institute of Plant Ecology. No exact knowledge about the chemical composition of the defoliants is available from the 50's and the 60's.

The active ingredients of the defoliants, the phenoxy compounds, form about one-half of the spraying liquid by weight; the rest consists of various additives and solvents. The spraying liquid usually contains additives that lower the surface tension (surfactants) to spread the liquid evenly on the applied surface. The amine salts of 2,4-D and 2,4,5-T dissolve in water. The defoliant used contains about 0.5-1.3% of the active ingredient.

The additives of the phenoxyacetic acid esters are, in addition to the surfactants, various emulsifiers that make the dissolution in water possible.

The solvent is usually gasoline. The esters are dissolved in water (concentrations as above for the salts), but the preparation for trees is dissolved in diesel oil or gasoline, and the concentration of the active ingredient is 2% (tree trunk spray) or 15% (tree stump spray).

6.2. Effective compounds

Currently the 2,4-D and 2,4,5-T compounds in use are either amine salts or macromolecular esters, e.g. isooctyle, butoxethyl and butoxyethanol esters. Previously small molecular esters were used, e.g. butyl ester, but these are easy to gasify, dangerous for the health of the user and difficult to use on warm days. The vapor pressures of isooctyl and butoxiethanol esters are so low under normal conditions [e.g. the vapor pressure of isooxtyl-2,4,5-T is 0.54×10^{-5} mmHg at 25°C (Jensen and Schall 1966), and isooctyl-2,4-D 0.2×10^{-5} mmHg, and butoxyethanol-2,4-D is 0.45×10^{-5} mmHg (Flint et al. 1968)] that they render no problems from gasification.

The amine salt, isooctyl ester, but butylester preparations of 2,4-D were not observed to cause systematic poisoning during daily application on the skin over a three-week period (Kay et al. 1965). The solutions were of two kind, 0.63% and 3.13%. The water solution caused mild or moderate irritation, rash, and even tissue necrosis.

6.3. Effect on health of various compound, additives, and solvents

There are no investigations available to clarify sufficiently the metabolism (absorption, transportation, secretion and dissolution) and the differences in effects of the salts and esters of phenoxyacetic acid. The polaric salts can be assumed to penetrate the skin and various membranes less easily than the esters. The penetration property of esters may be increased by various additives

that reduce the surface tension [e.g. anionic and cationic detergents which damage the skin very much (Schleuplein and Blank 1971)]. The effect of the oil based ester solutions is particularly damaging both locally for the skin and for the mucous membrane as well as internally for the organs.

Conclusion

In addition to the phenoxy compounds, the defoliants contain various additives (e.g. ones to reduce the surface tension, emulsifiers for esters, and solvents such as oil). The differences in their effects and their metabolism are not well known. The esters contain detergents that can significantly irritate the skin and the mucous membranes, and the irritation is even worse with oil based solutions.

7. IMPURITIES OF DEFOLIANTS

7.1. 2,3,7,8-Tetrachlorodibenzo-p-dioxine (TCDD) content in defoliants

TCDD can be developed in the synthesis of 2,4,5-T from chloro benzene using alcalic hydrolysis if the reaction temperature exceeds 160°C (Schulz 1968) (see Figure 1).

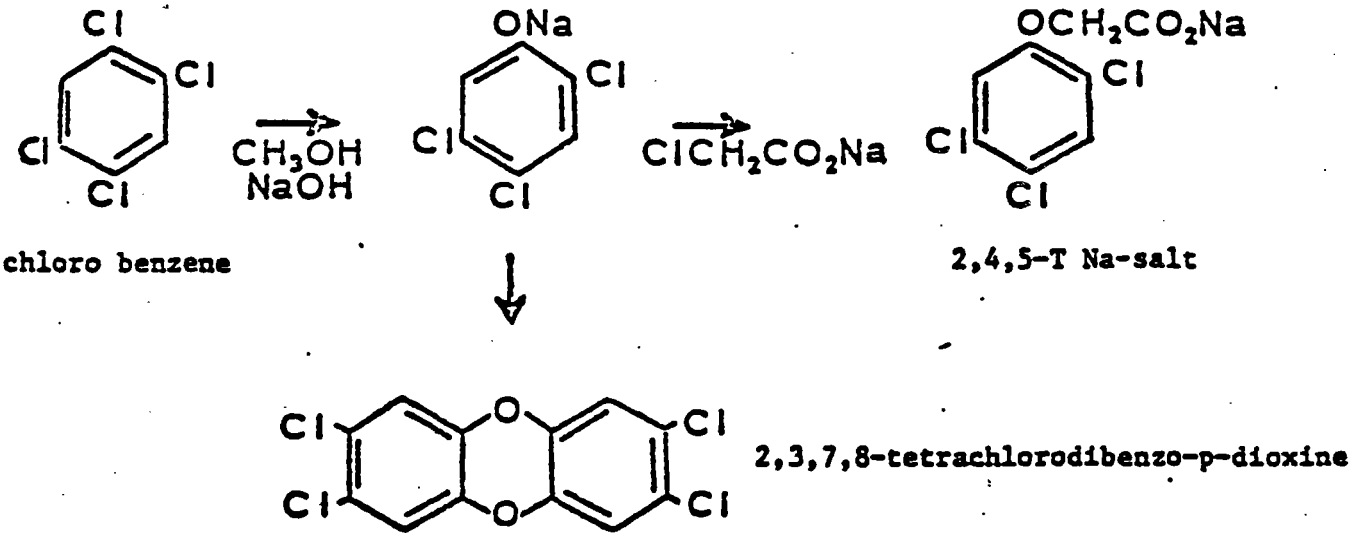


Figure 1. The by-product of 2,4,5-T synthesis (2,3,7,8-tetrachlorodibenzo-p-dioxine).

This way of synthesis has been widely used previously and in the U.S. it was abandoned in the late 60's. Woolson *et al.* (1972) have recently investigated the dioxine content of 17 defoliants containing chlorinated phenols and the results are in Table II. It is significant that TCDD was observed primarily in 2,4,5-T; in 22 samples the content was less than 0.5 ppm but in 20 samples it was greater than that. The high values all came from the same manufacturer during 1966-1970 and towards the end of this period there was a decrease in comparison to the previous values (20-30 ppm). This company later gave up the production of 2,4,5-T. At the time of the investigation the products never exceeded 0.5 ppm in the content of TCDD. One sample of 2,4,5-trichlorophenoxy-propioneacid contained TCDD over 0.5 ppm. No sample of 2,4-D contained TCDD, but one of them a small amount of hexa-CDD.

Hexa-, hepta-, and octachlorodibenzo-p-dioxine were observed in large quantities in some chlorinated phenols (e.g. pentachloro-phenols).

Table II. The content of dioxin in samples of defoliants (Woolson *et al.* 1972)

Pesticide	ppm of chlorodibenzo-p-dioxin: tetra <10	tetra <100	hexa <10	hexa <100	hexa <1000	hepta <10	hepta <100	hepta <1000	octa <10	octa <100	octa <1000	No. of samples contaminated	Total no. of samples tested
	Number found												
2,4,5-T	7[a]	13	3	1	0	ND[b]	...	...	ND	...	...	23	42
Silvex	1	0	ND	...	...	ND	...	...	ND	...	...	1	7
2,4-D (-DB,-DP)	ND	...	1	0	0	ND	...	...	ND	...	...	1	28
Dicamba	ND	...	ND	...	...	ND	...	...	ND	...	...	0	8
Chlorophenol													
tri-	ND	...	4	0	0	1	0	0	2	0	0	4	6
tetra-	ND	...	1	1	0	1	2	0	1	2	0	3	3
penta-	ND	...	0	7	0	0	4	6	0	4	6	10	11
Others	ND	...	1	0	1	3	0	1	3	1	0	7	24

[a] Any sample may contain one or more different dioxins. [b] ND = <0.5 ppm of any one chlorodioxin.

In Finland beginning on October 31, 1971, the Chamber of Agriculture and Forestry has prohibited (703/71) the sale and use of those defoliants which contain TCDD over 0.5 mg/kg computed from the

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content of 2,4,5-T acid. Currently, it is not likely that the defoliants containing 2,4,5-T have TCDD more than 0.5 ppm. The TCDD content of older defoliants has been determined in the State Institute for Agricultural Chemistry, contracted by the working group, and those results follow later on. The defoliants may contain small quantities of other contaminants. Huston (1972) observed 3 neutral contaminants in 2,4-D, one of which contained 30 ppm of them. The toxology of these compounds is not known.

7.2 The effects of TCDD

TCDD is one of the most poisonous compounds known today. It's LD_{50} value for the gerbil is 0.0006 (Table I). In other words, 0.6 micrograms per kg is lethal. TCDD is strongly teratogenic. Sparschan *et al.* (1971) observed 0.5 µg/kg daily to be teratogenic. It should be pointed out, too, that embryotoxic effects of 2,4-D and 2,4,5-T among rats occurred when the daily dose exceeded 100-150 mg/kg (Khera and McKinley 1972).

Above, the chloric acne and liver damages caused by TCDD were discussed. The illnesses among workers producing 2,4,5-T could be caused primarily by TCDD, but other (unknown) contaminants and chemicals that are used may be partially involved. The possible mutagenic and carcinogenic properties of TCDD are not known.

Several Cl atoms (6,7,8) containing dibenzo-p-dioxines may be contaminants in the defoliants, but they also appear in many other widely used chemicals such as pentachlorophenol. There is no exact knowledge of the properties of these compounds, but, evidently, they are toxic. Several dibenzoparadioxines have caused in hens the so-called "Chick-Edema" disease (Kimbourgh 1972).

TCDD is a very stable, insoluble, and lipophilic compound. It might accumulate in living organisms. Investigations of this are under way and the first observations (Kearney 1970) on radio-active TCDD indicate that the compound will be absorbed in the ground and that the plants will collect it only in small quantities.

8. THE INVESTIGATIONS OF THE INSTITUTE OF LABOR HEALTH

8.1 Questionnaires

8.1.1 The purpose of the investigation

After the health hazards of the defoliants gained publicity, the personnel in health care and occupational hygiene received several reports from individuals about alleged illnesses. Via the media, people were advised to file the reports with the Institute of Labor Health.

8.1.2 Method

The Institute sent questionnaires to these individuals and to some employers (see Appendix 8) in which such questions as the context and duration of the exposure, the symptoms of the alleged illness, and its duration were asked, as well as information about the possible time and place of hospitalization or of care by private doctor. This investigation was supposed to reach as many users of defoliants as have suffered from the use of defoliants. The data depended on the initiative of the individuals. The main points to be clarified by this method were:

- what are the most common symptoms in connection with the defoliants?
- are there cases of acute poisoning?
- are there cases that are similar to those reported in the literature or that could be connected with poisoning by defoliants?

8.1.3 Material

The Institute received 213 replies. The distribution of the persons according to age, sex, method of contact and time of exposure are described in Figures 1 and 2. 33 persons reported no symptoms during and after defoliation.

Figure 1. The statistics on persons who returned the questionnaire, according to age and sex

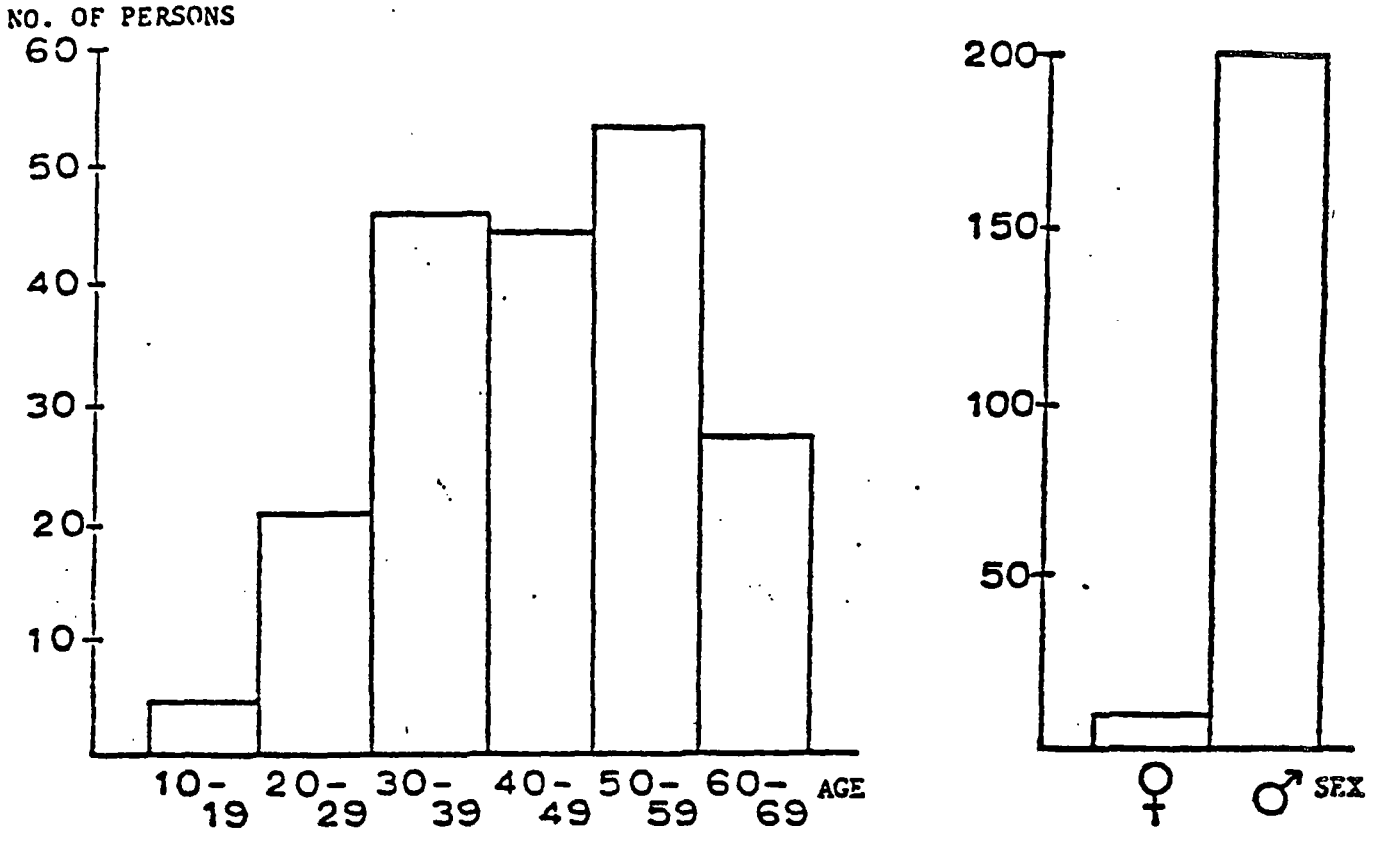
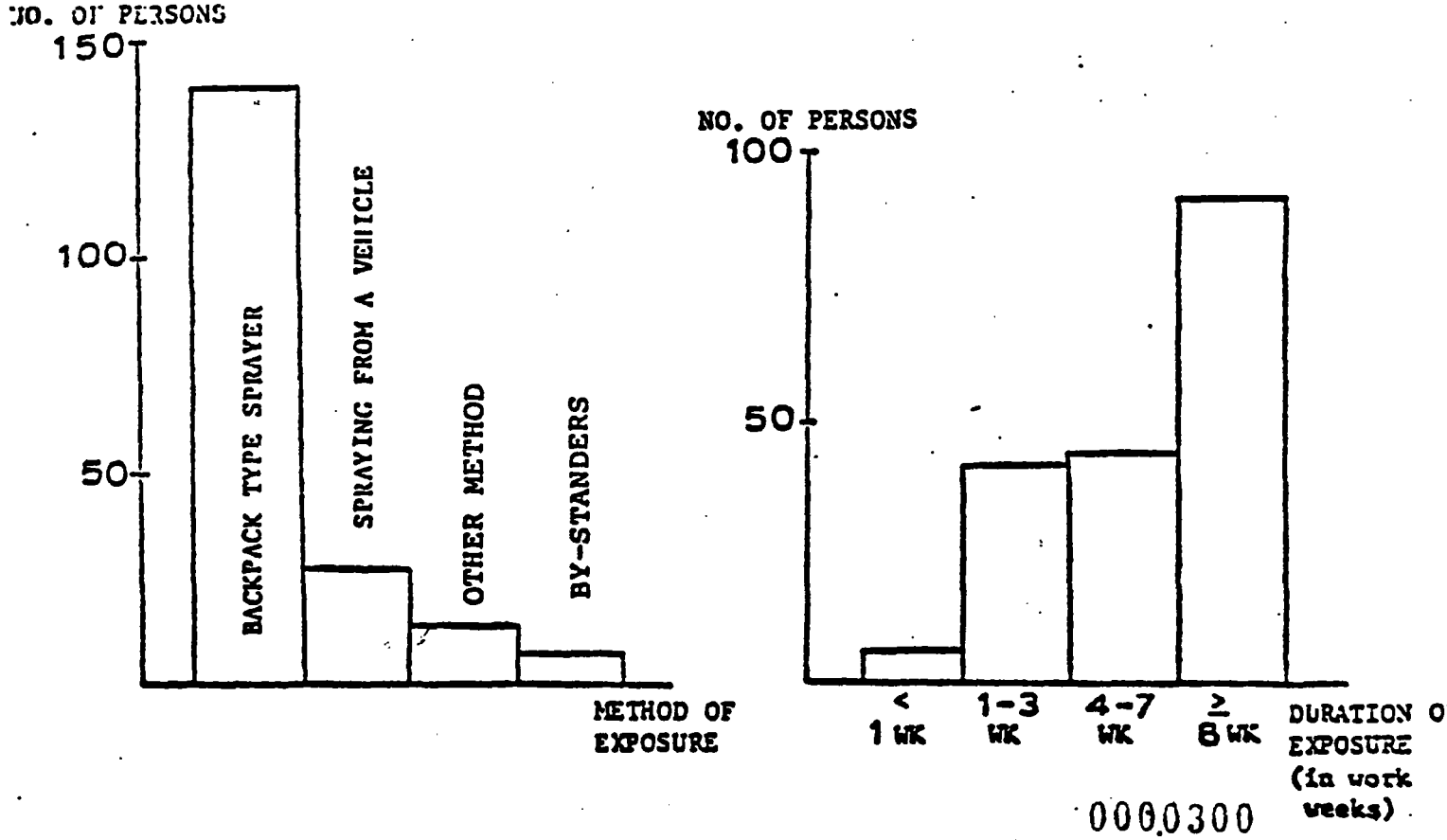


Figure 2. The statistics on persons who returned the questionnaire, according to the method and duration of the exposure



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The majority of replies comes from old people and the distribution of age differs significantly from that concerning the cause of death in the population (see p. 44, Table IX). In some cases the time elapsed since the last contact with herbicides was several years. The total population clearly contained longer exposure times (about half of them, more than 40 days or 8 weeks) in comparison to the population in the cause of death investigation (see Figure 7, p. 43). About three-fourths of those replying were exposed to defoliants when spraying them with the backpack type spray.

8.1.4 Reported symptoms and illnesses

The most common symptoms reportedly found in persons that were exposed to the defoliants.

Table III. The symptoms of the 180 persons participating in the inquiry*

	At the moment of exposure no. of persons	(% from total)	At the moment of investigation no. of persons	(% from total)
Headache	99	(55.0)	59	(32.8)
Giddiness	22	(12.2)	20	(11.2)
Fatique	18	(10.0)	7	(3.9)
Stomach ache	13	(7.2)	18	(10.0)
Irritation of respiratory organs	20	(11.2)		
Difficulties in breathing	14	(7.8)		
Irritation of the skin	20	(11.2)		
Rash	13	(7.2)		
Irritation of eyes	16	(8.9)		

*One person might have one or more symptoms.

Some of the infrequent symptoms and illnesses reported were (the number of cases are in parentheses): allergic reactions such as asthma, allergic cold or rash (5), insensitivity of legs (6), weakness in legs (4), decreased sense of smell or taste (2), impotence (1) and increased perspiration (2).

No acute poisoning cases showed up in the inquiry. One person, however, reported a headache, a stomache ache and an intensive rash so as to cause him to go to a hospital. No mention of disabling peripheral nervous illnesses was contained in the material returned. One person reportedly became diabetic some months after the defoliation, and another contracted a liver disease (nephrosis) during the work. Severe cases of cancer were also reported (acute leukemia and cancer of the bladder).

8.1.5 Summary and conclusions

Two hundred thirteen people responded to the inquiry of which 180 reported symptoms and illnesses connected with the defoliants and 33 reported no symptoms. The questionnaire was distributed mainly among persons who voluntarily reported to the officials in health care or occupational hygiene or at the Institute, but some were sent by the employers. The population was rather old, primarily male (only 10 women), and were exposed while spraying the defoliant with a backpack type spray. The duration of the exposure was rather long (compared to the death cases) and about half have had an exposure of more than 8 weeks.

In evaluating the significance of the symptoms, one must note that the inquiry was addressed to persons who had some symptoms. That is why no external reference could be found. It should be pointed out that about one-fourth of the people [40] reported the headache only during spraying. The headache might be caused by the defoliant but it could also be due to the strenuous physical work, the temperature, the unpleasantness of the spraying (the liquid stinks), or the constriction around the neck caused by the backpack. The exhaust gases of the engine could also cause a headache. The irritation of the respiratory system, the eyes and

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the skin was less frequent. These symptoms did not last long. The allergic reactions should also be considered, since in three cases the trouble resembled asthma. The symptoms reported in the inquiry matched well with the previous reports in the literature (e.g. Fetisov 1966) concerning the phenoxy compounds. On the other hand, there was no report of acute poisoning. There was no illness that resembled previous reports (e.g. Goldstein *et al.* 1959) on neurological illnesses.

8.2 Clinic research

8.2.1 Introduction

The clinical medical investigation on people exposed to the defoliants was conducted in two parts. The first one (8.2.2 Preliminary investigation) was intended to describe the health of people intensively exposed (spraying method, during long periods of work) to the defoliants regardless of the reported symptoms. The second one (8.2.3 Health investigation) was intended to find out about the background and other results pertaining to the illnesses, according to the reported symptoms. The mutual goals of both investigations were a) to find out traces of poisoning by looking at different organisms, b) to look for functional changes in the respiratory system, c) to investigate the possible diseases of the skin and the mucous membranes, and d) to identify a possible group of symptoms characteristic of the poisoning.

It should be pointed out that the investigation started only after one year had elapsed since these persons had been exposed to the defoliants (the summer of 1971). During the months after the exposure, the organism is expected, to a large extent, to recover from the alleged illness or malfunction. Some organs recover slowly and incompletely; this is the case with the nervous system. The clinical investigations have been emphasized to study the nervous system. The justification for this is that, in addition to a slow recovery, the nervous system is generally injured in poisonings (e.g. solvents, heavy metals). There have been

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descriptions about the injury to the nerves caused by 2,4-D (e.g. Goldstein *et al.* 1959) and the neurophysiology related to it [e.g. by Fullerton (P. M. Fullerton: Toxic chemicals and peripheral neuropathy, Proc. Ray. Soc. Med. 62 (1969), 201-204)]. A clinical investigation immediately during or after the spraying of defoliants would have been well justified, but that could not be included in the planning, since it was not known if the use of defoliants would be prohibited in 1972. Also, the initiation of the investigation was considered important.

Table IV The symptoms of the 30 persons participating in the preliminary investigation*

Symptom	No. of cases at the time of exposure	No. of cases still having the symptom	No. of cases having the symptom before exposure
Headache	10	7	3
Giddiness	4	3	0
Fatigue	4	2	1
Stomach ache	1	1	0
Irritation of respiratory organs	6		
Irritation of the skin	4		
Rash	6		
Irritation of the eye	6		

*One person might have one or more symptoms

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8.2.2 Preliminary investigation

Material

The population was chosen as 30 workers among the State Railway System, of which 25 were currently working and 5 were retired due to various illnesses. The persons were chosen from among those with the longest exposures to the defoliants according to the employee records. Out of the original group, 4 refused. According to their health records they had no symptoms. The statistics according to age and sex, as well as the method and duration of exposure are represented in Figures 3 and 4. Their most common symptoms are listed in Table 4.

Out of the group of 30, 13 were over 50. The exposure to the defoliants was over 8 weeks in 12 cases. The most common subjective symptoms that were reported were headaches and various irritations.

The method of investigation

The attending doctor made a detailed sickness and working history (see attached form 8b) and made the physical investigation (see attached form 8a), excluding neurology. The lab tests, X-ray's, and lungs were investigated according to Appendix 1. The clinic neurologist recorded subjective symptoms, the occurrence of any organic psycho-symptoms, exceptional findings from the autonomic, central and peripheral nervous systems, and the condition of the muscles. The neuro-physiological exam included the usual EEG, and the peripheral nervous system was investigated using a method called ENMG, developed in the Institute of Labor Health. For a comparison, a sample of neurologically unspecified industrial workers were investigated (50 with EEG, 100 with ENMG).

Results

The most important results are in Table 5. The deviations from

the normal were few and were mild. Only one person out of 30* was observed to respond positively to the sugar test indicating symptoms of latent diabetes. This was a new discovery for that one person. The performance of the lungs indicated two substandard cases. No asthma was recorded. According to the project, other than the lab investigations were given, e.g. the blood composition, the cholesterol content of the serum, EKG, and X-ray of the chest and throat. These were used to describe the general health condition (the usual health checking) and in diagnostic analysis. With the exception of the blood composition test, abnormalities were observed in the fat content of the serum and in the blood pressure among the old persons, as well as changes in the shape of the heart due to malfunctioning, all of which had no obvious connection with the use of defoliants.

The neurologic investigation indicated two cases of an organic psycho-syndrome, 8 cases of mild irregularities in the autonomic nervous system (increased perspiration, variations in the blood circulation in the limbs and cardiac arythmia), five cases of motoric disturbances, and 8 cases of malfunctioning of the peripheral nervous system. Except in one case the findings for the central nervous system could be ascribed to obvious causes (large intakes of alcohol or medicines, blood circulation problems). The symptoms of the autonomic nervous system were probably due to the situation (e.g., excitement, fatigue, etc.) In the case of the peripheral nervous system, the anomalies were confined to one or two nerve fibers which indicates a local injury, a compression of the nerve fiber or base.

In the neurophysiological investigations the EEG was irregular in 2 cases and the ENMG in 8 cases showed slowed conductivity in the nerves. The irregularities in the EEG were mild, except for

*Hernberg *et al.* (1971) found in a random group of industrial workers a frequency of 4.5% that deviated from the normal.

Figure 3. The distribution according to age and sex in the preliminary investigation

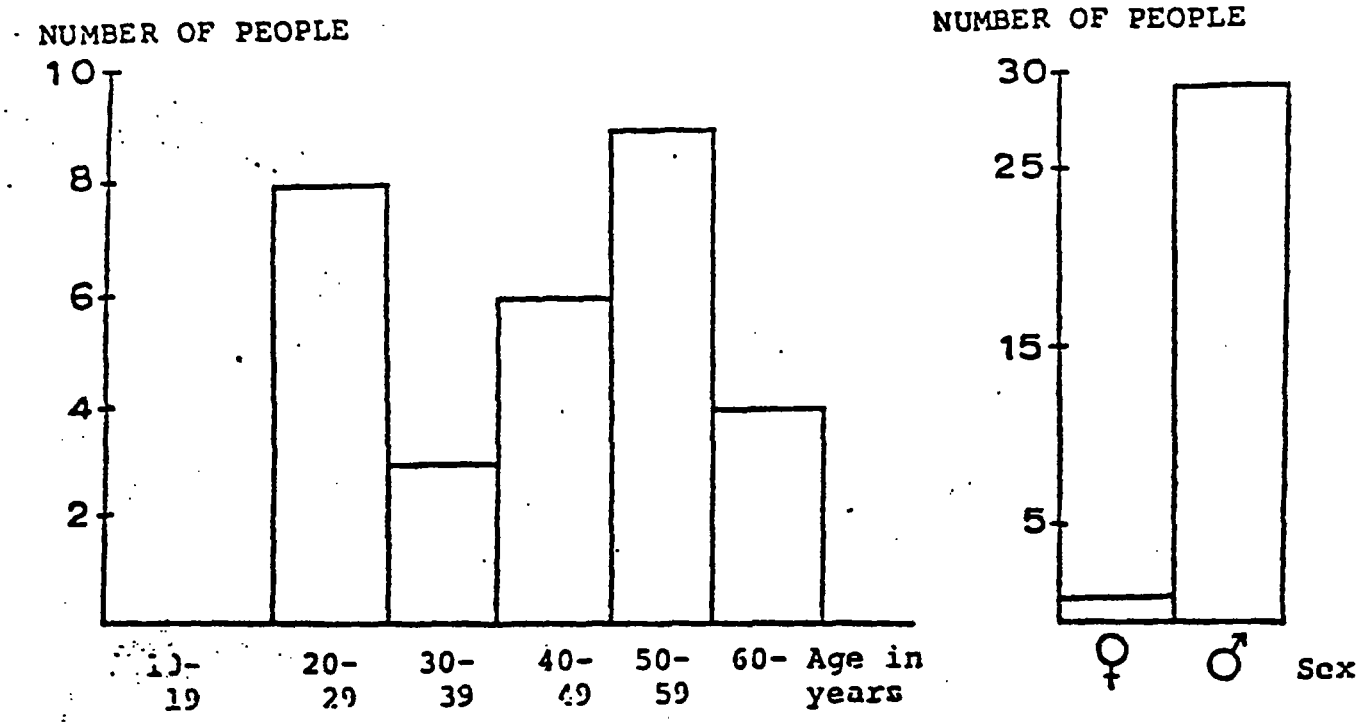
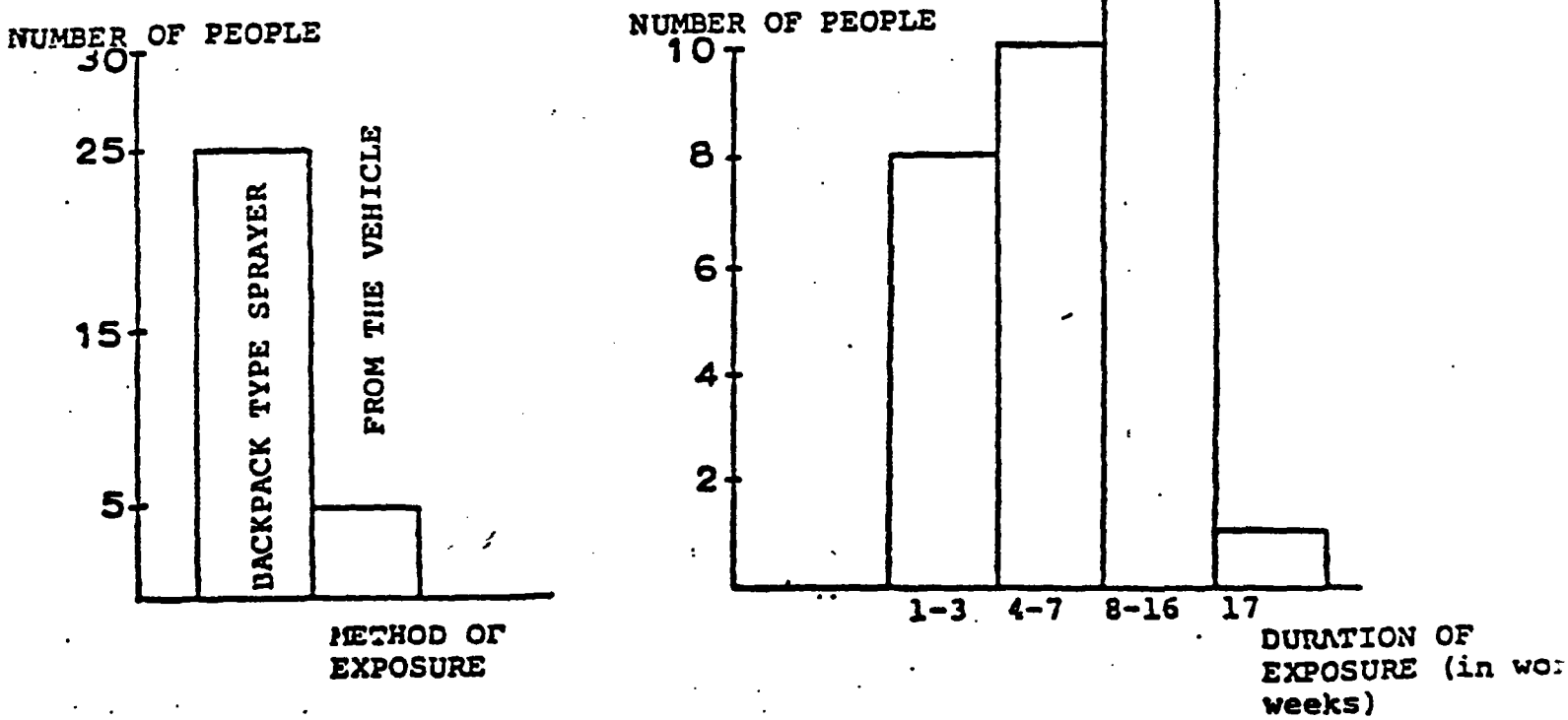


Figure 4. The distribution according to the method and duration of exposure



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one case, and could be normal. The irregularities in ENMG could be explained in 6 cases due to local injury, ischialgia, or use of vibrating power tools.

TABLE V. In the preliminary investigation observed malfunctioning in the lungs and Lab tests

Organ	What was looked at	Normal range	Irregularities found: Number of	Irregularities found: Quantity
Liver	Aspartateamino-transferase (GOT)	12-35 Wr units/ml	0	
	Alanineamino-transferase (GPT)	4-30 Wr units/ml	1	38
	Alcalic phos-phatase (S-AFOS)	0.8-2.9 B-L units	1	3.2
Kidney	Creatinine (S-Krea)	0.7-1.3 mg/100 ml	2	1.4; 1.4
	Urine glucose	-	0	
	Urine albumin	-	1	+ (weak)
Sugar Metabolism	Sugar test 1 hr.	210 mg/100 ml	1	223
Thyroid	Iodine bound in protein (S-PSJ)	4.0-8.0 µg/100 ml	0	
	Triiodothyronine binding test ($S-T_3$ test)	80-120%	0	
Respiratory System	Spirometry	VC, $FEV_{1.0}$ 80%	2	$FEV_{1.0}$ 71;80% Vc 108; 75%

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Conclusions

The investigation convinced us that the health of the sample of 30 persons was normal, corresponding to a similar random group of same age.

The neurologically exceptional cases were old persons, although mild irregularities in EEG were also observed among the young. In the case of the peripheral nervous system, the neurological results corresponded well to those of the ENMG. In particular, the neurophysiological tests are very sensitive, and similar irregularities can be obtained from a "normal population". In this case, the control group consisted of a sample of neurologically unspecified industrial workers. The frequency of exceptional EEG's among the 30 workers was about twice the frequency of the control sample. The difference is not significant ($p > 0.05$). ENMG did not show any difference between the two groups.

Five persons were referred for further investigation and care after this investigation.

8.2.3 The Health investigation

Material

The participants were chosen from the 180 who had reported symptoms. The criteria were a) duration of the exposure, b) occurrence of "typical" symptoms and c) occurrence of other interesting symptoms (reported in the literature). Since age is crucial in evaluating the results, no one over 50 was chosen. The statistics on age, sex, and the method of exposure, as well as its duration are shown in Figures 5 and 6. It should be pointed out that out of 18 persons 11 have been exposed to the defoliants 17 weeks or more and 14 persons have been using the most hazardous method, the backpack type sprayer.

Methods

The plan described in the preliminary investigations was

Figure 5. The distribution of age and sex in the health investigation

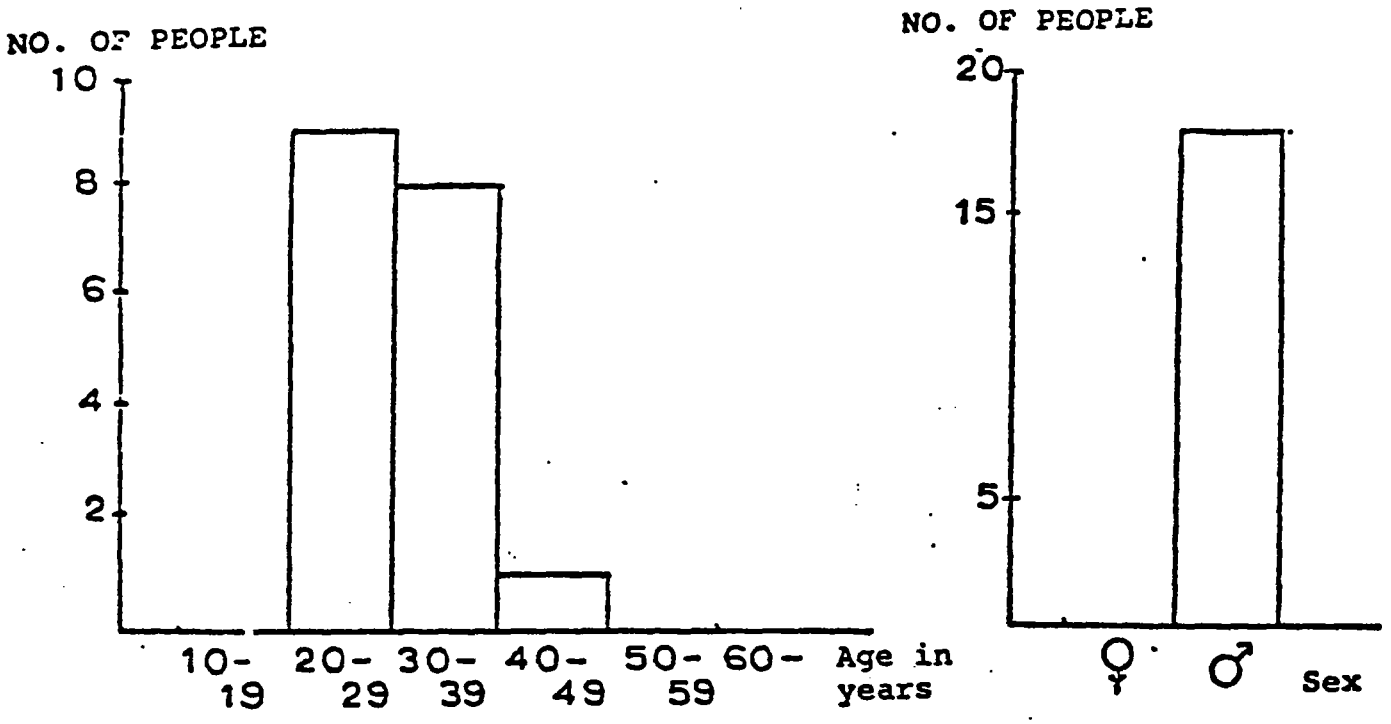
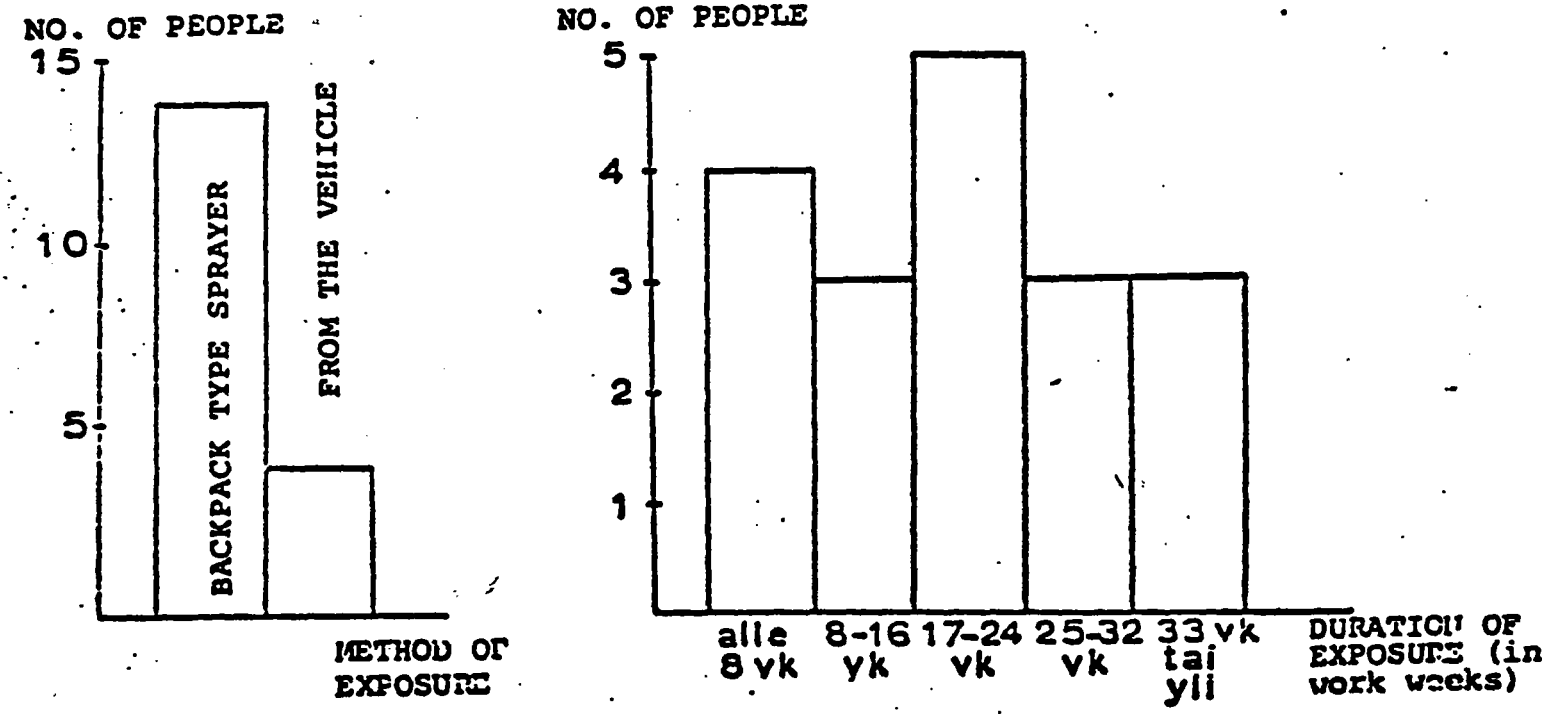


Figure 6. The distribution of age and sex in the health investigation



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followed. In lab tests, the ones concerning the malfunctioning of the liver and thyroid were removed, since the working group considered them insignificant. Instead, as a new program, all persons were submitted to a toxi-psychological test, given by Mr. Kari Lindstrom, MSc. That includes the following usual sequence of tests used in the Institute of Labor Health:

1. General Intelligence performance. This is tested using the Wechsler intelligence test for adults (WAIS): similarity test, number sequences, sign test, completion of pictures and the cube test.
2. Memory and learning test. This was tested using Wechler's memory tests (WMS), limited to the three areas of memory:
   - -mechanical-verbal memory
   - -logical-verbal memory
   - -visual memory
3. Sensorimotor response has been measured using three different tests of speed, which were combined to give one variable. The methods are:
   - -the Bourdon Wiersma test
   - -the Santa Ana test
   - -the sign test (WAIS)
4. Psychomotor disturbances have been estimated by the MIRA-test which is a test of motor performance without using vision as a control.
5. The characteristic reactions were estimated using the Rorschach projection test and another one specially designed to describe the personality of workers exposed to industrial poisonings. This measures primarily affectivity, amount of psychic energy and sense of reality.

The results are transformed to a standard scale to make it possible to compare with a standard sample of male

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weeks and 8 weeks and one year and more than one year.

The deaths were divided into similar groups according to the age of death.

From the above statistics the expected number of deaths were obtained using the mortality statistics of the whole country. The expected total mortality (mortality due to natural causes, to tumor, and to cancer of the respiratory systems) was evaluated for the total mortality, it has been possible to evaluate the expectancy among the working population. This has been done using the statistics on the probability of the cause of death in one year collected by the Department of Health and Social Affairs using their health insurance statistics.

8.3.2. Results

The mortality for various causes (Table III) was almost consistently lower than the expected values, and even in those cases where the values were high they were not statistically significant. There was no significant difference in mortality between the two groups from 1955-1965 and from 1966-1971. The duration of exposure (2-7 weeks vs. 8 or more weeks of defoliation) did not increase mortality (Table X).

8.3.3 Further Considerations

There were some difficulties in collecting the statistics due to the lack of a file containing job assignments. It seems that, in particular, the defoliation in the Department of Forestry is poorly documented, because their labor force is more transient than that among other employers. It seems that, due to the physical requirements of the work, only workers in good health were selected for the job. The workers were fairly young at the beginning of the defoliation, one-third being 24 or younger (see Table VII). The majority (61%) were exposed to the defoliants in 1966 or after. The defoliation reached its peak years in the late 60's. Since for the majority the exposure to defoliants took place during the

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past 5-6 years, one would not be able to detect illnesses that take theoretically a long time to show up. It is known that cancer can take 10 years to develop. It is justifiable that the investigation will be repeated in the future (e.g., after some 3-5 years). On the other hand, it should be pointed out that for three-fourths of the workers (See Figure 7), the time of exposure was brief (2-7 weeks) and the influence would, therefore, be small.

When the mortality rate of the group of workers is compared to the mortality rate in Finland, it should be kept in mind that the total population contains such cases as birth defects and the chronically ill who are not (completely) able to work. Hence, it is to be expected that the mortality rate of the working population is lower than the mortality rate of the total population. The insurance statistics used as an alternative reference are more representative of the mortality rate of the labor population, but since this system has been in existence for a short time (since 1962) it might contain "an over-representation of healthy persons". The results of this investigation show that, even though the mortality rate due to other illnesses is low, the mortality rate for tumor and lung cancer is almost as expected from the total population. It cannot be assumed that the general risk among this group (due to smoking) to contract cancer and lung cancer is less than that among the total population.

The cause of death investigation shows that among those exposed to defoliants the mortality rate due to natural causes or various groups of illnesses does not exceed the total mortality rate in Finland or that among labor. In other words, there is no evidence that the defoliants increase mortality.

An investigation in the Swedish State Railway System on epidemiologic mortality (Axelson, *et al.*, 1972) did not indicate any excess mortality due to natural causes or cancer or lung cancer among people exposed to the phenoxy compounds.

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Figure 7. The distribution of the time of exposure in defoliation

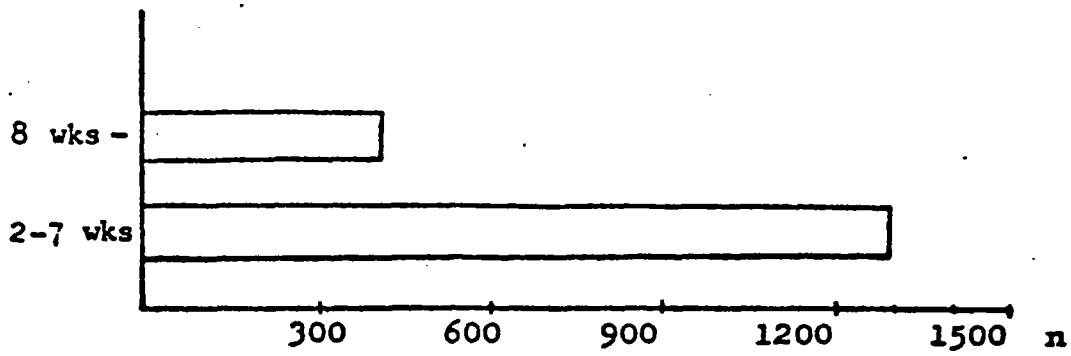


TABLE VII: Distribution of the cause of death

Employer	Defoliation started 1955-1965	%	1966-1971	%	Total	%
State Railway System	251	32.4	190	15.9	441	22.4
Dept. of Highway Construction	211	27.3	291	24.3	502	25.5
Dept. of Forestry	241	31.1	693	57.9	934	47.4
IMATRAN VOIMA CO.	71	9.2	23	1.9	94	4.8
Total	774	100	1197	100	1971	100

TABLE VIII: The age distribution in the cause of death

Age when starting the defoliation	Defoliation started 1955-1965	%	1966-1971	%	Total	%
15 - 24	189	24.4	408	34.1	597	30.3
25 - 34	201	26.0	238	19.9	439	22.3
35 - 44	191	24.7	279	23.3	470	23.8
45 - 54	146	18.9	199	16.6	345	17.5
55 - 60	47	6.1	73	6.1	120	6.1
Total	774	100	1197	100	1971	100

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TABLE IX. The cause of death distribution in various illnesses and their expectancy in the cause of death investigation

	Starting 1955-1965 Years				Starting 1966-1971 Years				Total			
	Deaths	Expec-tancy I	Expec[x] tancy II		Deaths	Expec-tancy I	Expec-tancy II		Deaths	Expec-tancy I	Expec-tancy II	x
Cancer in respiratory system	6	5.0	..	n.s.[xx]	3	3.7	..		9	6.6	..	n.s.
All severe cases of tumor	10	12.9	..		3	1.5	..	n.s.	13	16.4	..	
Irregularities in the blood circulation	11	24.2	..		6	8.3	..		17	33.5	..	
All natural causes	26	54.8	..		10	16.6	..		36	71.4	..	
Total of causes	30	66.1	37.1		15	21.9	11.5	n.s.	45	88.2	48.7	

x The expectancy is computed in different age groups using the mortality rate of:
I Total population in Finland
II Those belonging to the health insurance system.

xx n.s. = non-significant difference

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TABLE X. The mortality[x] distribution according to the duration of the defoliation and the expectancy

	Starting 1955-1965 years			Starting 1966-1971 years			Total		
2-7 weeks of defoliation	Deaths	Expectancy I	Expectancy II xx	Deaths	Expectancy I	Expectancy II xx	Deaths	Expectancy I	Expectancy II xx
Cancer in respiratory system	2	1.7	.. n.s.[xxx]	3	1.0	.. n.s.	5	2.7	.. n.s.
All severe cases of tumor	4	4.6	..	3	2.4	.. n.s.	7	7.0	..
Irregularities in the blood circulation	5	8.6	..	3	5.4	..	8	14.3	..
All natural causes	11	19.8	..	7	10.9	..	18	30.7	..
Total of causes	13	24.7	13.6	12	14.6	7.7	25	39.4	21.3
≥ 8 weeks of defoliation									
Cancer in respiratory system	1	1.4	..	-	0.4	..	1	1.8	..
All severe cases of tumor	2	3.6	..	-	0.9	..	2	4.4	..
Irregularities in the blood circulation	2	6.8	..	1	1.9	..	3	9.0	..
All natural causes	5	15.4	..	1	3.9	..	6	19.2	..
Total of causes	6	18.9	10.4	1	5.0	2.7	7	23.9	13.1

x Only includes cases where the duration of the defoliation is known

xx The expectancy is computed in different age groups using the mortality rate of:
I Total population in Finland
II Those belonging to the health insurance system.

xxx n.s. = non-significant difference

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9. INVESTIGATION IN SWEDEN

9.1 Swedish mortality investigation

9.1.1 Material

In 1972 the Clinic of Occupational Medicine, OREBRO, investigated the mortality among people using herbicides in the Swedish State Railway System (Axelson, *et al.* 1972). The workers had sprayed various herbicides such as compounds containing a mixture of amitrole and diuron, amitrole, a mixture of amitrole and monuron, and a mixture of atrasin and phenoxy. The total population was 324 workers, of which 194 has been exposed to the phenoxy compounds and 143 to amitrole or its mixtures.

9.1.2 Results and conclusions

The results are in Table VIII. The data is displayed in such a way that the effect of time can be seen after 3 and 5 years for those times when the duration of exposure exceeds 46 days or more. In case of amitrole and mixtures, 4 cases of cancer were observed with an expectancy of 1.07, and out of these, two were lung cancer, with an expectancy of 0.19. It is significant that no excess in mortality rate was observed among those exposed to the phenoxy compounds.

10. URINE TEST RESULTS CONCERNING ALLERGIC REACTIONS AMONG DEFOLIANT SPRAYING GROUPS (A case history from the Summer of 1972)

10.1. Method and Results

The working group thought it would be important to investigate, even if only superficially, the allergic reactions during spraying of the defoliants. Urine tests were performed on four persons working in various ways with spraying. From the urine test, the quantities of 2,4-D and 2,4,5-T were analyzed. The liquid which was sprayed is called VESAKONTUHO 500, containing

TABLE III. OBSERVED AND EXPECTED TOTAL AMOUNT OF DEATHS, THE NUMBER OF DEATHS IN TUMOR (WHO VOL II) AND LUNG WITH RESPECT TO THE LATENCY TIME AMONG THOSE WHO HAVE BEEN EXPOSED (46 or MORE). CANCER CASES (WHO A50) 1957-72 IN SWEDISH STATE RAILWAY SYSTEM (SJ) IN 1947-71. THE EXPECTANCY BAND ON MORTALITY REPRESENTING THE WHOLE COUNTRY (SWEDEN) DURING THE PERIOD.

PREPARATION	CAUSE OF DEATH	0 Year OBS. PERS. PER YEAR	0 Year EXP.	0 Year OBS.	3 Year OBSER. PERS/YR.	3 Year EXP.	3 Year OBS.	5 Year OBS. PERS/YR	5 Year EXP.	5 Year OBS.
TOTAL	TOTAL MORTALITY	2,802	19,33	17	1,341	14,41	15	1,289	10,96	13
	TUMOR		4,59	5		3,47	5		2,66	5
			0,78	2		0,60	2		0,47	2
PHENOXY ACID AND COMBINATIONS	TOTAL MORTALITY	1,621	11,58	9	1,046	8,55	7	712	6,36	6
	TUMOR		2,76	1		2,06	1		1,55	1
			0,47	0		0,36	0		0,27	0
AMITROLE AND COMBINATIONS	TOTAL MORTALITY	1,228	7,94	9	807	5,90	8	561	4,46	6
	TUMOR		1,87	4		1,41	4		1,07	4xx)
			0,32	2x)		0,24	2xx)		0,19	2xxx)
OTHER PREPARATIONS AND COMBINATIONS	TOTAL MORTALITY	293	1,63	4	209	1,34	4x)	156	1,09	4xx)
	TUMOR		0,39	3xxxx)		0,32	3xxxxx)		0,26	3xxxx
			0,06	1		0,05	1x)		0,04	1x)

x) $p < 0,05$ xx) $p < 0,03$ xxx) $p < 0,02$ xxxx) $p < 0,01$ xxxxx) $p < 0,005$

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2,4-D and 2,4,5-T in a ratio of 2:1. The samples were collected the next morning and evening after work and the second morning after the spraying. The jobs, working conditions and duration of the work was as follows:

1. Driver of a tractor spray, one door of the tractor open, no prevention. Three consecutive days.
2. Driver of a tractor spray, closed doors, a mask used for respiration. Five consecutive working days.
3. A man on board a truck. No mask used. Three consecutive working days.
4. Driver of a spraying truck, no mask used, windows open. Three consecutive working days.

TABLE XII. The content of 2,4-D and 2,4,5-T in urine among the defoliant sprays in State Department of Highway Construction. Determinations individually and using spectrophotometry. (Behm. E. and Schroder, I. Weed Res. 9 (1969) 43-7).

	1. First day after spraying morning	evening	2. Second day after spraying morning	3. Estimate of 2,4-D and 2,4,5-T (in mg) in the organism after the spraying xx
1.	19[x]	57	22	c.a. 98
2.	3	3	3	c.a. 8
3.	8	17	13	c.a. 35
4.	3	11	4	c.a. 18

x content of 2,4-D and 2,4,5-T in urine mg/ℓ

xx the samples are over a period of 40 hours after the spraying. Total amount of urine is assumed to be 2 ℓ, that is, 0.5 ℓ during the first night, 1.0 ℓ during the next day and 0.5 ℓ during the second night. The half life of 2.4-D and 2.4.5-T is assumed to be 1 day (24 hours).

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10.2 Discussion of the results

The amounts were measured only once and the accuracy of the results cannot be verified. The sampling, mailing, and storing may be sources of errors that cannot be considered here. It was intended to obtain only a rough estimate of the concentration of the phenoxy compounds in urine after spraying. The estimate of the amounts of 2,4-D and 2,4,5-T immediately after the spraying was only meant to give some indication. The largest amount was found in worker 1 (98 mg), which is the amount accumulated in the organism over 3 days if the daily dose is 50 mg. on the average. This is about 1/70 of an immediately dangerous amount (a lethal dose in man is about 50-100 mg/kg). Disregarding the adaptation that takes place in connection with chronic exposure, the amount accumulated over 3 working days would be about 1/35 of the lethal amount. Sprayer number 3 collected a dose in his body about 1/100 of a dangerous dose. The rest of the amounts were much smaller.

The results correspond to the expectations about exposure in various tasks of defoliation, and the small amounts in sprayer number 2 should be pointed out, which demonstrates that closing the windows and using a mask seems to help.

Even though one sprayer, according to the data, was significantly exposed to the defoliants during work, there is no method for interpreting the amount of exposure in relation to any alleged health hazards. In general, the influence of chronic exposure is poorly known.

11. THE CONTENT OF DIOXIN IN THE DEFOLIANTS USED IN FINLAND DURING 1960-1971*

11.1 Investigation contracted by the working group

The working group contracted an evaluation of the content of

*The working group would like to thank the following companies for financial aid during this investigation: Berner Co., Farmos Co., Kemira Co., Nykema Co., and Eero Helkavaara Co.

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dioxin in seven different samples of the commercial defoliants. The results were given on February 16, 1973 (see Appendix 2 and table below).

The method of evaluation is described in Elvidge, D. A. Analyst, October, 1971, 96, pp. 721-727. The content of dioxin (TCDD or 2,3,7,8-tetrachlorodibenzo-p-dioxin) is in a table below:

Defoliant (Brand Name)	Origin and Years of use	Active Ingredient		Dioxin content ppm (mg/kg)
Vesakontuho 500	VR 1967 - 70	2,4-D	2,4,5-T	0.31
Tributon DT	VR 1971	"	"	0.20
Bruspon	VR 1968	"	"	0.10
Spray 201 E/3	VR 1960 - 64	" DCA	" MCPA	0.31
KVK/Køge	KSL sample from year 1961			0.14
Hormoslyr 500 T	KSL " " 1965		2,4,5-T	0.21
Regulan	KSL " " 1962		"	0.51

VR = State Railway System

KSL = The Institute of Plant Ecology

11.2 Conclusions

The data in the above table shows that the most common defoliants from 1960-1971 did not exceed the upper limit of 0.5 ppm of dioxin (TCDD) set up by the Ministry of Agriculture and Forestry (decision 703/71). The only sample exceeding this limit is not statistically significant since the evaluation was inaccurate.

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12. CONCLUSION

12.1 General

12.1.1 The phenoxy compounds, which are usually the active ingredients in the defoliants, are the most commonly used in herbicides. That is why the hazards in connection with the use of the phenoxy compounds should be investigated.

12.1.2 The phenoxy compounds have been called "action hormones", because their influence resembles that of a natural growth hormone of plants called 3-indoleacetic acid. Previously, it was believed that since the defoliants are "action hormones" they are not poisonous. This is wrong. One should always consider all herbicides to be toxic. The phenoxy compounds in the defoliants are fairly poisonous based on evidence of acute poisonings.

12.2 Earlier reports on phenoxy compounds, a survey of the literature

12.2.1 Connected with the spraying and production of the phenoxy compounds, there had been descriptions of various acute symptoms such as fatigue, headaches, stomach troubles, and irritation of the skin and the respiratory system.

12.2.2 In spite of the common use of the phenoxy compounds there are few published observations about dangerous poisonings or illnesses among the sprayers.

12.2.3 In the industrial synthesis of one of the important ingredients of the defoliants, 2,4,5-T, the workers have been reported as suffering from skin disease, liver illness, and, probably, a nervous illness. The probable cause of these illnesses is not pure 2,4,5-T, but contaminants used during the industrial process (e.g. TCDD, as well as the initial and intermediary chemicals).

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12.2.4 The liquids containing 2,4,5-T might have a contaminant called TCDD (2,3,7,8 tetrachlorodibenzo-p-dioxin). The TCDD is very toxic and causes deformation in the embryos.

12.2.5 2,4-D and 2,4,5-T have been observed (in animal experiments), to damage the embryo slightly and interact with the hereditary material of the cell. No carcinogenic effects could be detected in animal experiments. No evidence exists that the phenoxy compounds have caused cancer, hereditary diseases, or deformation of the embryo.

12.3 Research of the working group

12.3.1 Sometimes the workers spraying defoliants have shown symptoms and, in general, acute effects such as headaches, vomiting, stomach trouble, giddiness, and irritation of the respiratory organs, the eyes, and the skin.

12.3.2 In some cases these irritations caused by the defoliants could lead to asthma or allergic rash in some particularly sensitive persons. Many years after the beginning of the illness, it is usually impossible to verify the cause in these persons.

12.3.3 In the investigation the effect of defoliants on the nervous system was tested using very sensitive methods. This was considered important, since previously it has been suspected that the phenoxy compounds cause damage to the nerves. On the other hand, the persons under investigation have shown nervous symptoms (e.g. headaches). One can expect to detect, at the moment of investigation, only those injuries for which recovery is slow or incomplete; the time since the last exposure to the defoliants was at least a year. The team from the Institute of Labor Health, with experience in the neurophysiology of various poisons (e.g., solvents and heavy metals) could

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not detect a single case of poisoning among the persons under investigation.

In delicate tests to describe the functioning of the nervous system, it was discovered that there was twice the normal frequency of disturbances in the electrical functioning of the brain as compared to a group of workers that had not been exposed to chemicals affecting the nerves. In statistical analysis, the difference was not significant ($0.5 < p < 0.10$), but, however, it was indicative.

The observed irregularities were generally mild and they do not necessarily indicate illness. In the severe cases, further investigations and follow-up studies were considered necessary.

12.3.4 The investigation of the cause of death showed that the mortality rate from all natural causes or various specific groups of illnesses among persons exposed to the defoliants did not exceed the figures of the total population in Finland or those of the working age population. This discovery applies also for the mortality rate from cancer. Consequently, the defoliants do not seem to increase the mortality rate. It should be pointed out that the number of people in the investigation, about 2,000, is rather small. It is possible, too, that chronic illness will show up in the future, and it is recommended that the investigation be repeated after 3-5 years.

12.4 Results on the content of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in defoliants used in Finland

In the State Institute of Agricultural Chemistry, seven samples from 1960-1971 were analyzed to contain TCDD less than 0.5 ppm in six cases, this amount has been recommended the upper limit by the Ministry of Agriculture

and Forestry (decision 703/71). One sample contained 0.51 ppm. Random samples of old defoliants did not reveal any dangerous quantities of the contaminant TCDD.

12.5 The TVL Case history of allergic reactions among defoliant spraying groups

The urine tests given to workers of TVL exposed to phenoxy compounds show that significant quantities of the poison can get into their bodies if adequate measures for protection are not used. No health hazards were found during these investigations.

13. RECOMMENDATIONS BY THE WORKING GROUP

13.1 Protection for the sprayers of defoliants from exposure should be improved. The practical recommendations for this have been given to the committee on safety regulations set up by the Ministry of Social and Health Affairs.

13.2 The working group has seen the recommendations by this committee on safety regulations and considers that they will protect the sprayer and any by-stander from health hazards, if followed carefully in using and dealing with defoliants. The effects of the proposed safety measures should be checked among the sprayers in the future (e.g., by measuring the individual exposure in various spraying methods).

13.3 The working group could not investigate the environmental impact of the use of defoliants including points other than human health. The impact of the defoliants on nature should be investigated separately.

13.4 The working group considers that workers, other than transient workers in defoliations, should be given a health check as required by the work safety law, § 44 (the decision

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637/71 by the State Council, subsection 21 in the list of cases).

The preliminary check on the workers should screen out persons who are exceptionally sensitive to the hazards of the above chemicals, (e.g., pregnant women or persons with illnesses or allergies or injuries of the respiratory passages, skin, nerves, liver or kidney). Consequently, the preliminary check should precede the beginning of any work of this kind.

At regular intervals, a check should be done immediately after the completion of the spraying season. This check should include, in particular, any symptoms of the respiratory passage, eye and skin, the neurological status and lab tests in order to evaluate the functioning of the liver and kidney (evaluation of the transaminase, albumin and glucose in the urine).

13.5 The working group considers it important to have reliable tests on the content of the poisonous contaminants, such as dioxins, to be done prior to granting sales permission. The chemical composition of these products should be checked repeatedly in the future.

13.6 The working group expects that world wide research on the phenoxy compounds will be followed. Special emphasis should be placed on the epidemiological investigations of the health hazards among people such as cancer, injuries to embryos, and hereditary diseases. The epidemiological mortality investigation by the working group is proposed to be followed by another one (e.g., after three years). At the same time, the possible chronic health hazards should be considered.

13.7 The follow-up check for chronic injuries requires that the employers keep detailed records on defoliation which indicate the workers, the methods, the duration of the work

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and the defoliants used.

13.8 If new discoveries on the health hazards of the defoliants show up in the future, the health hazards connected with these chemicals should be re-evaluated.

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Appendix 2

CERTIFICATE

No.: A 121-127/73

Ordered by: the working group on health hazards and working safety with defoliants

Date: January 5, 1973

Sample: 9 samples of defoliants

Task: 2,3,7,8-tetrachlorodibenzo-p-dioxin analysis

Results:

Brand	Maximum amount of dioxin (from the amount of 2,4,5-T) ppm (mg/kg)
A 121/73 Vesakontuho 500	0.31
A 122/73 Tributon DT	0.20
A 123/73 Bruspon	0.10
A 124/73 Spray 201 E/3	0.31
A 125/73 KVK-Køje	0.14
A 126/73 Hormoskyr 500 T	0.21
A 127/73 Regulan	0.51

Method of analysis: Elvidge, A. A., Analyst, October 1971, 96, pp. 721-727

Due to shortages of time two defoliants "Vesakonhauite F 500" and "Bruspon" (from 1957) could not be received by February 16, 1973. The result on "Regulan" A 127/73 is based on one measurement only.

Helsinki, February 16, 1973

Veijo Mattinen

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Appendix

CURRENT KNOWLEDGE ABOUT THE EFFECT OF DEFOLIANTS ON HUMAN HEALTH

In Finland the main defoliants are 2,4,5-T and 2,4-D as esters (which dissolve in oil) or salts (which dissolve in water). These phenoxy compounds are considered second class poisons based on LD_{50} values in animal investigations.

During the past two years the question has been raised whether these compounds - in particular 2,4,5-T - cause damage to the human embryo. In Vietnam, these phenoxy compounds have been extensively used to defoliate, and it has allegedly caused deformed embryos. The investigation results are not conclusive so far. However, it is known that 2,4,5-T compound might have contained large quantities (up to 30 ppm) dioxin, which is a known poison and causes deformation of embryos.

Recent investigations with mice indicated that pure 2,4,5-T could cause changes in the chromosomes. But the animal experiments did not indicate any carcinogenic effects for 2,4-D or 2,4,5-T [Myrkkyasian neuvottelukunta, March, 1972].

It has been alleged that the workers of defoliation in SJ (State Railway System) have become ill or died in Sweden in winter 1972. An investigation was started there. The medical investigation was done by Regional Hospital in Örebro (Department of Occupational Medicine, Dr. Lannart Sundell) and a committee of the Parliament was set up (including some experts). In Sweden the main ingredients in defoliants are other than phenoxy compounds, namely, amitrol and diuron.

In Finland the working group on defoliation has kept touch with the medical investigation group in Sweden.

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In literature there are reports of some twenty poisonings of human beings by phenoxy compounds of which some have been exposures during work, some have been suicides.

These cases have been described in literature as neurological cases with usually the similar kind of symptoms (disabling neuropathy). In some cases there have been disturbances in the performance of the liver and the kidney.

The closer details about the poisonings for people are described in Appendix 3 "The effect of defoliants on people". It seems possible that the illnesses in these cases were due to some allergic reactions, since 2,4-D had usually very little effect.

The working group considers that there is enough medical evidence and allegations about the health hazards of the defoliants that the most exposed workers should be brought into medical investigation.

Plan of investigation

Preliminary investigation

For the preliminary investigation, 30 workers of VR from Oulu area will be chosen according to the length of exposure to defoliants. This group will contain both currently working, retired, and persons in illness pension.

The medical check-up will be done in the Institute of Labor Health in Helsinki according to Appendix 2 and the investigation will include lab, X-ray, lung functioning, and neurophysiology as well as check-up by a specialist in occupational health and neurology. The program takes one day for each worker.

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The occurring symptoms and findings of illnesses will be processed statistically (possibly multivariable analysis) in computer. The final statistical analysis will be determined only after it is known how frequent abnormal findings are. In the first phase, no control group is intended to be used, but if more than expected pathological findings show up, one has to compare this with a normal control group (which is not included in the cost estimate).

The results will be reported individually to each worker.

The primary purpose of the preliminary investigation is to find facts about poisonings caused by the defoliants and the results are hoped to help in planning and guiding further investigations.

The cause of death investigation

Simultaneously with the preliminary investigation an extensive cause of death investigation is carried over among workers exposed to defoliants. Special emphasis will be exercised to get workers who sprayed defoliants in 1950, if reliable information of them is available.

At this moment, it seems that the most reliable data about workers exposed to the defoliants comes from VR (State Railway System). Preliminary estimates puts 1000 the number of people to be investigated, but the final figure may change since the data is not yet complete.

For comparison, another group of 1000 persons should be chosen as a control group. This group, not exposed to the defoliants, should closely resemble the group exposed to defoliants (as far as age, sex, geographic location, social status, similarity of the work is concerned).

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The cause of death is purely statistical file research. The purpose is to determine the cause of death among workers who were exposed to the defoliants and died in 1953-1971 based on the death certificates and reports of autopsies.

Special emphases will be exerted on finding out the possible overmortality in cancer, chronic lung diseases, cardiovascular diseases, and other chronic illnesses.

This kind of investigation is the only easy method to find out if the defoliants increase the rate of mortality as compared to the so-called usual illnesses.

Health investigation

The Institute of Labor Health will choose 50 workers among those who responded to the questionnaire as becoming ill due to the defoliants, and who have had the most common symptoms of the whole group.

The purpose of the health investigation is to identify the possible joint symptoms of poisonings and the connected permanent or chronic changes in the functioning of the organism. It should be pointed out that only permanent and chronic changes can come out of the investigation, since the workers have not been exposed to the defoliants at least for a year.

The final decision about the program for investigation is not yet done, but it should probably include neurological investigations, since that kind of injury is permanent.

The estimate of cost is just preliminary for one patient.

The principal investigators:

The responsible investigator
Sven Hernberg, assistant professor

Occupational medicine investigations
Vesa Riihimäki, M.D.
Juhani Hassi, M.D.

Neurophysiology (EEG and EMG)
Anna-Maria Seppäläinen, M.D., specialist

Neurology
Pauli Karli, M.D., specialist

Internal diseases
Simo Tarpila, assistant professor

Statistical analyses
Sisko Asp, MS.

In addition to the above, some staff is required for other jobs (labs, files, typing, mailing, etc.) and computer time.

Schedule

The preliminary investigation can be started right away, if funds are available. The investigation will take about 2 months excluding the statistical analysis. The cause of death investigation will be carried over simultaneously with the preliminary investigation. It will take more time depending on how quickly the material will be gathered. In the data collection the local civil servants of VR will do troublesome and tedious chores

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laria Seppäläinen, M.D., specialist

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Karli, M.D., specialist

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Appendix 8a

INSTITUTE OF LABOR HEALTH
Medical Department

HEALTH HAZARDS OF THE DEFOLIANTS

Name: _____ Social Security No.: _____

Profession/occupation: _____ Phone: _____

Address: _____ Date: _____

_____

Who has employed you to defoliate?

_____

When (how many years, how many days a year)?

_____

_____

_____

Which brand names of defoliants have you used?

_____

_____

How have you used defoliants?

_____

_____

_____

Have you used a mask? _____

Have you used a raincoat? _____

Other possible ways being exposed to defoliants:

_____

_____

_____

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THE TOXICITY OF POLYCHLORINATED POLYCYCLIC COMPOUNDS AND RELATED CHEMICALS

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INTRODUCTION

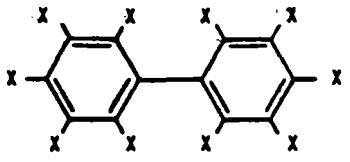
Only the small group of the polychlorinated polycyclic compounds given in Figures 1 and 2 will be reviewed in this article. Several years ago when this laboratory was the Atlanta Toxicology Branch of the Food and Drug Administration, concern was voiced over the possible long-term effects of polychlorinated biphenyls, and we began to study the toxicity of Aroclor 1254 and Aroclor 1260. About this time a follow-up study was performed on a group of workers in New Jersey who were engaged in the production of 2,4,5-T.[1] These workers had very severe chloracne and had previously been shown to have porphyria.[2] During that same time, a petition was filed with the Food and Drug Administration for a residue level of hexachlorophene on certain food crops. Hexachlorophene, as well as 2,4,5-T, is made from trichlorophenol, although the chemical reactions are quite different. When we checked the available literature on the toxicity of hexachlorophene we felt that adequate toxicity data by present standards were not available, particularly not if the compound was meant to be ingested. Furthermore, Larson[3] had pointed out that hexachlorophene was more toxic than commonly acknowledged.

Hexachlorophene and similar chemicals behave differently from such polycyclic polychlorinated compounds as the chlorinated biphenyls (PCBs). Hexachlorophene in particular is acutely much more toxic than the PCBs and is fairly rapidly eliminated from the body, while the PCBs have a very low acute toxicity, but are very poorly metabolized, particularly those with more than four chlorine atoms on the phenyl rings. The long-term effects are therefore more critical for compounds such as PCBs. The chlorinated dibenzodioxins and furans, some of which are extremely toxic on an acute basis, may also show persistence and their long-term effects may be equally as important. These observations show that it would be erroneous to make predictions on long-term effects from results obtained in acute toxicity studies.

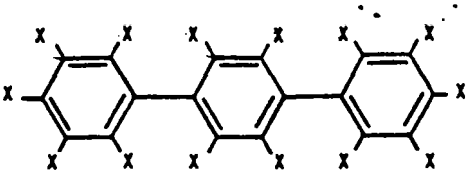
*Formerly with the Environmental Protection Agency, Chamblee Toxicology Laboratory, 4770 Buford Highway, Chamblee, Ga 30341.

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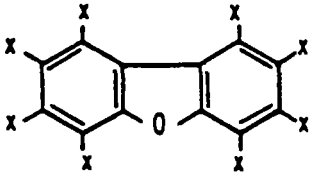
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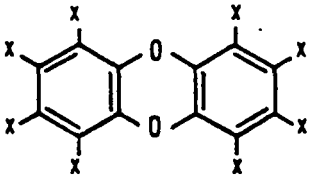
Polychlorinated Biphenyls (PCB's)



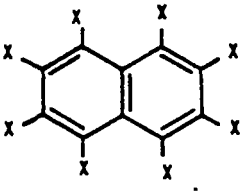
Polychlorinated Terphenyls



Chlorinated Dibenzofurans

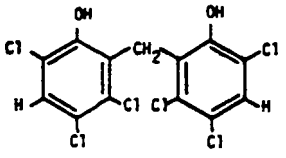


Chlorinated Dibenzodioxin

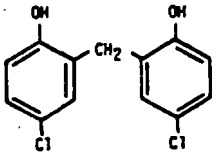


Polychlorinated Naphthalenes

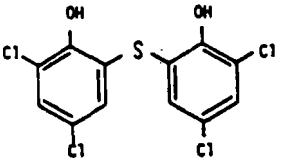
FIGURE 1. The materials shown in this figure are mixtures of compounds in which several but usually not all of the positions (-X) are chlorine and the rest are hydrogen. The properties of the materials depend on the degree of chlorination of the mixture. All are insoluble in water but soluble in varying degrees in organic solvents and in fats. The trade name for the U.S. product (Monsanto) of the chlorinated biphenyls and terphenyls is Aroclor. The trade name is followed by the number. For the biphenyls, the first 2 digits of this number are 12, and for the terphenyls, 54. In each instance the last 2 digits give the percentage of chlorine.



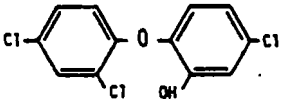
HEXACHLOROPHENE
2,2'-Methylene-bis(3,4,6-trichlorophenol)



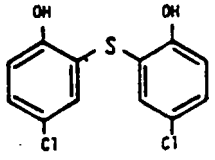
DICHLOROPHENE
2,2'-Methylene-bis(4-chlorophenol)



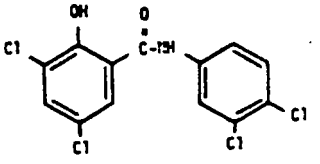
BITHIONOL
2,2'-Thio-bis(4,6-dichlorophenol)



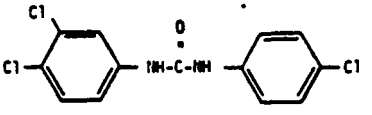
2,4,4' trichloro- 2'hydroxy-diphenyl ether
(Triclosan = Irgasan DP300)



FENTICLOR
2,2'-Thio-bis(4-chlorophenol)



3,3',4,5'-Tetrachlorosalicylanilide (TCC)



3,4,4'-Trichlorcarbanilide (TCB)

FIGURE 2. The compounds in this figure are white crystalline solids with clearly defined melting points above 160°C. They are practically insoluble in water and soluble in varying amounts in organic solvents and in fats. Those containing an -OH group, all the above excepting 3,4,4'-trichlorocarbanilide, are soluble in aqueous alkali.

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The present review is an attempt to correlate various toxic effects produced by the chemicals listed in Figures 1 and 2 in order to better understand and appreciate their behavior.

The subjects covered in this article have lately enjoyed great interest and a rapidly growing number of scientific reports are available. Since a number of the chemicals discussed produce similar or related effects, their toxicology is discussed together rather than listing each compound separately, which would have led to a great deal of repetition.

A detailed discussion of the various chemical reactions, analytical methods for the determination of the compounds, is not given. The review has been written with the toxic effects of the various compounds as the focal point and the literature cited serves to illustrate these points. No attempt has been made to present a complete review of the literature in this area.

DEVELOPMENT, USAGE, AND OCCURRENCE

A description of the synthesis of *chlorinated biphenyls* (PCBs) can be found in *Liebig's Annalen* as early as 1881.[4] Successful production of diphenyl in commercial quantities was not reported until 1930.[5] At that time the chlorination of technical biphenyl was also described.[6] Biphenyl itself is fungistatic and used as a preservative for citrus fruits.[7] Chlorinated biphenyls are very stable, and have been used for protective coatings, as plastic resins or gums for varnishes, and for waterproofing and flameproofing of wood.

The excellent dielectric properties of these compounds were noted as early as 1930. Until the advent of the PCBs, mineral oil was used as a cooling and dielectric liquid impregnant for high voltage electric apparatus such as transformers and capacitors. Since mineral oil is flammable and flammable gases could also be produced, restrictions for the use and installations of these transformers had to be set, particularly for indoor installation. When mineral oils were replaced by the Aroclors, the fire and explosion hazards were eliminated. This was recognized by the National Board of Fire Underwriters and the National Electric Code was modified.[8] Use of Aroclors also greatly reduced the physical size of the capacitors.[9]

A recent summary of the varied uses of PCBs has been provided by Broadhurst.[10] With a peak production of 42,527 short tons of PCBs in 1970, Monsanto is the sole producer of Aroclors (U.S. tradename for PCBs) in the U.S. Yearly PCB production figures for the period 1960-1971 were reported by Monsanto.[11] The production increased steadily until 1970 and dropped to 20,236 short tons in 1971 due to a voluntary restriction of the use of PCBs by the company. Trade names in other countries include Kanneclor and Santhaterm in Japan, Clophen, Flenclor, Phenoclor, and Sovol in Europe.

The polychlorinated capacitor liquids, commonly called askarels, are mixtures of chlorinated biphenyls and chlorinated benzenes. A variety of other industrial applications[10] have been found for PCBs. They have served as fluids for hydraulic, gas turbine, and vacuum pumps, as heat transfer fluids, as plasticizers, adhesives, textile coatings, surface coatings in paints and varnishes, as sealants, and as part of formulations to prepare pressure sensitive records and color copying papers. The extent of the current use of PCBs in printing processes is unknown. Other suggested uses over the years have included catalyst carrier for polymerization of olefins, conversion of water permeable soil to a nonpermeable state, and combined insecticide and bactericide formulations. Mixtures of chlorinated biphenyls and chlorinated naphthalenes have been employed to insulate electric wires and cables, particularly when they were used in the mining industry and on warships.[12]

Residues of polychlorinated biphenyls have been reported in the environment by a number of authors in different parts of the world. These reports were usually from areas where industrial pollution was responsible for the environmental contamination. Holden,[13] after finding PCBs in marine fish and mollusks from Scottish waters, noticed that they were generally highest in samples from the Firth of Clyde and he discovered that the PCB contamination probably originated from industrial sewage. The sewage sludge was transported from two Glasgow purification works by boats and dumped in deep water six miles south of Garroch Head. The disposal of sewage sludge on land could lead to the introduction of PCB residues into the terrestrial ecosystem by transfer through food chains. So far PCB residues have not been detected in fresh water fish in Scotland.

Polychlorinated biphenyls have also been found

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in wildlife samples collected along the Dutch coast and the Rhine River[14] and in fish and seal in different parts of the Baltic Sea along the Swedish coast.[15]

Risebrough et al.[16] compared the presence of PCBs with that of DDT and its metabolites in various birds. These authors found very low concentrations of PCBs in wildlife species in Baja, California, Mexico, and the Gulf of California, which are very remote areas with a high ratio of DDT to PCB of approximately 9 or 10. In sea birds from the Pacific, the ratio was between 5 and 10 and at that time, PCBs were not found in eggs of the Adelie penguin from Cape Crozier, Antarctica. Relatively high PCB concentrations were found in San Francisco Bay, Puget Sound, and San Diego Bay and the ratio of DDT to PCBs in wildlife samples from this area was between 1 and 2. This is again emphasized by the findings of Dustman et al.[17] Twelve Alaskan bald eagle eggs had median PCB residues of 1.65 ppm while 11 eggs collected in Maine, Michigan, Minnesota, and Florida showed a median concentration of 9.7 ppm.

Zitko[18] found low concentrations of PCBs in different types of fish taken from several lakes and the St. John River System, New Brunswick, Canada, and the Nova Scotia banks. Fish from the Milwaukee River and Lake Michigan in the midwest part of the U.S., where the river enters into the lake, contained varying amounts of PCBs[19] while fish taken from various small lakes in Wisconsin without industrial discharge failed to show PCBs. Large predators from Lake Michigan contained as much as 10 to 25 ppm PCBs on a fresh weight basis expressed as Aroclor 1254 and salmon eggs contained as much as 12 to 17 ppm PCBs on a fresh weight basis. Further studies by Veith[20] of the concentration of PCBs in different rivers emptying into Green Bay indicated a trend towards a decrease in PCB concentrations since the *voluntary* partial ban on PCB sales by Monsanto was implemented in September of 1970.

If PCB discharges are very high a fishkill may result such as the one in Escambia Bay, Florida in 1969.[21] In that particular episode the PCBs had entered a plant's effluent through accidental leakage of heat exchange fluid. The PCB found in this case resembled Aroclor 1254 most closely. The peak amount found in water of the Escambia River at the outfall amounted to 275 ppb and the peak concentration found in oysters from Escambia Bay was 3.0 ppm. Aroclor residues in sediment samples taken near the outfall reached 486 ppm. When the leakage from the plant was corrected, Aroclor levels gradually decreased in this area.

The wildlife portion of the U.S. National Monitoring Program analyzed starlings, mallard and black ducks, bald eagles, and fish of several species for PCBs. According to Stickel,[22] monitoring for terphenyls is also done but not for any of the other chlorinated compounds in this report. Research surveys that include fisheating birds, insectivorous birds, ospreys, woodcocks, and certain ducks also monitor only for PCBs and for pesticides. Oysters (*Crassostrea virginica*) are a good indicator of PCB and other chlorinated hydrocarbon concentrations in water and a national monitoring program in these mollusks in 15 coastal areas has been developed.[23] So far, mollusks have only very occasionally contained low levels of PCBs.

In summary, the PCB concentrations in water are low or nonexistent in remote lakes and fresh water streams and can be as high as 50 μg/l (5 ppb) or higher in polluted rivers such as the Hudson River.[24] The PCB concentration is usually higher in sediment from these polluted waters because PCBs are only very slightly soluble in water.[25] Since PCBs are stored in living matter, they are concentrated in the food chain and the usual "biomagnification" is encountered as we know it from other poorly metabolized and excreted substances. The concentration of PCBs in fish, particularly those in the upper end of the food chain from polluted rivers and lakes, is usually estimated in the ppm range and fisheating birds will have somewhat higher levels of PCBs than the fish from the same geographic location.[26] Young animals may contain less PCBs than their older counterparts of the same species in a particular location.

PCBs are also infrequently encountered in dairy products, meat, fish, and poultry in ready-to-eat human food.[27,28] Occasionally, human food and domestic animal feed have been more extensively contaminated with PCBs. In these instances, the contamination could usually be traced to local misuse of transformer fluid, leakage of PCBs from heat exchange fluid, or contamination of recycled paper with PCBs where the paper had been used for food packaging such as cereal boxes.[29] The U.S. Food and Drug Administration has recently set temporary tolerances for PCBs in certain food products (see Table 1).

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TABLE 1

Temporary Tolerances Expressed as Parts per Million (ppm) Established by U.S. Food and Drug Administration

(1) Milk (fat basis)	2.5
(2) Dairy products (fat basis)	2.5
(3) Poultry (fat basis)	5.0
(4) Eggs	0.5
(5) Complete and finished animal feeds	0.2
(6) Animal feed components	2.0
(7) Fish and shellfish (edible portion)	5.0
(8) Infant and junior food	0.2
(9) Paper food-packaging material	10.0

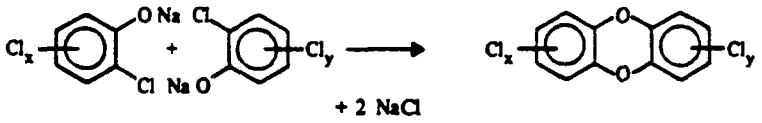
Note: The tolerance for paper food-packaging material shall not apply to such material separated from the packaged food by a functional barrier impermeable to PCB migration.
The Food and Drug Administration will provide upon request the analytical methods it will use for enforcing the temporary tolerances.

All of these aspects of PCBs in the environment have been the subject of a number of reports, reviews,[30] and conferences.[31-35]

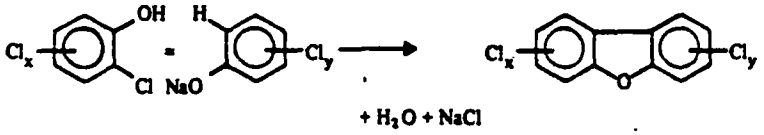
Terphenyls and chlorinated terphenyls are used as reactor coolants. The terphenyls consist of mixtures of ortho-, meta-, and paraterphenyls. Chlorinated terphenyls are produced by Monsanto and termed Aroclor, followed by numbers. The first 2 digits of these numbers are 54 and the last 2 indicate the percent of chlorine such as Aroclor 5460. Aroclor series 25 and 44 consist of mixtures of polychlorinated biphenyls and polychlorinated terphenyls.[36] According to Zitko et al.,[37] polychlorinated terphenyls have been found in environmental samples. Polychlorinated terphenyls do not elute from GLC columns under conditions used for PCBs,[38] which may explain why they have only been detected occasionally, but the way they are used probably also precludes their extensive spread in the environment.

Chlorinated dibenzodioxins and *chlorinated dibenzofurans* may occur as contaminants of cyclic chlorinated hydrocarbons.

In 1959 Tomita et al.[39] reported on the synthesis of polyhalodibenzo-p-dioxins. According to these authors, chlorophenols, when heated to about 200°C or more under alkaline conditions and pressure, will form chlorodibenzodioxins, as shown below.



Under similar conditions a chlorodibenzofuran may also be formed.



Chlorinated dibenzodioxins and chlorinated dibenzofurans are formed, or can be formed, as contaminants in the manufacture of a variety of products which use chlorophenols and chlorobenzenes as starting materials, such as 2,4,5-T, pentachlorophenol, nitrofen, hexa- and pentachlorobenzene, and tri- and tetrachlorophenol. Polychlorinated biphenyls may also be contaminated with chlorinated dibenzofurans,[40,41] and chlorinated dibenzofurans may result from polychlorinated biphenyls under UV irradiation.[42] The toxicity of these products varies depending on the position and number of chlorines attached to the phenyl rings. Products from different manufacturers may show a great deal of difference in the type and amount of contamination and different lots of the same product may vary in composition, making toxicological evaluations of these products very difficult. Rigorous production control and cleanup procedures need to be implemented so that the composition of different lots manufactured by the same company becomes more uniform.

Villanueva et al.[43] and Jensen and Renberg[44] reported the presence of chlorinated dibenzo-p-dioxin in pentachlorophenol. Hydroxynonachlorodiphenyl ether was also found and referred to as "predioxin" under the assumption that it represented a precursor of dioxin.

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According to Johnson et al.,[45] the predominant dioxin in a commercial pentachlorophenol was octachlorodibenzodioxin while trace amounts of hexachlorodibenzodioxin and no tetrachlorodibenzodioxin were found. Since the technical pentachlorophenol was in some respects more toxic in animal studies, than the pure product, the production process of pentachlorophenol was changed to minimize the dioxin concentration and a new product has now been registered under the Federal Insecticide Fungicide and Rodenticide Act with a lower dioxin content.

Technical 2,4,5-T has also been found to be contaminated with a dioxin. In this instance, unfortunately, the very toxic 2,3,7,8 tetrachlorodibenzodioxin,[46-48] was present. The contamination of the 2,4,5-T with 2,3,7,8-tetrachlorodibenzodioxin ranged from < 1 ppm to as much as 32 ppm over the years. In newly manufactured 2,4,5-T in the U.S. the content of tetrachlorodibenzo-p-dioxin has been substantially reduced and is usually kept below 0.1 ppm. Whether other dioxins or furans have ever been detected in 2,4,5-T in the U.S. has not been reported in the literature.

Chlorinated dioxins have also been found in so-called "toxic fat," which produces chick edema disease when fed to chickens. After several years of study by industry and the Food and Drug Administration, Flick et al.[49] were able to separate a purified crystalline product which produced chick edema disease. Two years later, Cantrell et al.[50] announced that 1,2,3,7,8,9-chlorodibenzo-p-dioxin was one of the toxic compounds that produced chick edema. The origin of the chlorinated dibenzodioxin in "toxic fat" was not clear. It was suspected that it arose from chlorophenols which were used to preserve hides for the manufacture of leather. Tallows and greases were obtained from these hides[51] and a more recent outbreak of chick edema disease was traced to the contamination with chlorophenols of soapstock used as feed fats. Higginbotham et al.[52] showed that chlorophenols and their salts, when heated, undergo condensation reactions and form chlorinated derivatives of dibenzo-p-dioxin; furthermore, chlorinated dioxins and predioxins were originally present in some of the chlorinated phenols. Generally no efforts have been made to determine whether *various chlorinated dioxins and furans* are present in the environment. However, Baughman and Meselson[53] recently reported the presence of dioxins in fish taken from four locations in Vietnam in Military Region III, which includes Saigon and several provinces to the north.[54] The concentrations ranged from 18 to 814 ppt.

Zitko[55] was not able to demonstrate chlorinated dibenzodioxins and dibenzofurans in aquatic animals from various locations in Canada.

Chlorinated naphthalenes may so far have remained unidentified since they interfere with other chlorinated compounds in any multiresidue analysis and are detected only when mass spectrometry is also performed.[56]

The various *germicides* given in Figure 2 have been used as antiseptics, deodorants, and particularly, as far as hexachlorophene is concerned, also as preservatives for various cosmetics, soaps, and lotions. A listing of different products that contained hexachlorophene prior to the new regulations was given by the American Pharmaceutical Association.[57] Registration of bithionol was cancelled in the U.S.[58] because it produced photosensitivity reactions in humans using soaps that contained this product. None of the polychlorinated polycyclic compounds used as germicides have been shown to accumulate in the environment. Hexachlorophene has additional uses as a fungicide on ornamental plants, on conveyor chains as a mildewstat, in laundry rinses as a mildewstat, in shoes and air filters and assorted industrial uses as a fungistat, and on leather, paper, and textiles.[59] It is also registered as a pesticide for use on cucumbers, peppers, and tomatoes,[60] and for cotton.

Dichlorophene is used as a mildew preventive on cotton and is recommended as a fungicide and bactericide for the protection of textiles and materials from molds.[61]

CHEMISTRY: METHODS FOR CHEMICAL DETERMINATION AND PHOTOLYSIS

Much of the chemistry of *polychlorinated biphenyls* has recently been reviewed.[33-34] Commercial chlorinated biphenyls are mixtures of biphenyl groups with different numbers of chlorine atoms (Figure 1). When hydrogen atoms are replaced by chlorine on the biphenyl ring, a large number of substitution combinations can arise since the number of chlorine atoms on the molecules can vary and the chlorines can also be

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attached to the molecule in different positions, resulting in a variety of isomers. Even if the chlorination may be the same, different commercial batches may vary in the relative concentration of isomers. The PCBs produced in the U.S. by Monsanto fall under the trade name Aroclor. The various Aroclor mixtures have a great number of constituents, and when the biphenyl is chlorinated, 210 different chemicals are theoretically possible. Aroclor 1254, for instance, yielded 69 constituents while the fractionation of Aroclor 1242 and 1260 yielded 45 and 78 components, respectively.[34]

The chlorinated biphenyls (PCBs) are chemically very inert, resistant to corrosive chemicals, insoluble in water, and have a low vapor pressure. Aroclor 1221, for instance, has a boiling point of 278°C. The boiling point increases with increasing chlorination. The chlorinated biphenyls are determined in biological and environmental samples by a combination of electron capture gas liquid chromatography and mass spectrometry. Nuclear magnetic resonance spectroscopy is used in addition to the above two procedures in some laboratories. PCBs were first identified in environmental samples by Jensen in 1966.[62] They were detected as interfering peaks on GLC analysis of environmental samples that were analyzed for chlorinated pesticide residues.[63]

The PCBs interfered with routine pesticide analyses for chlorinated hydrocarbons. Various authors have therefore published separation methods[64] and Reynolds[38] discussed this extensively in a review.

According to Safe and Hutzinger,[65] the use of mass spectrometry for structural studies of PCBs is limited because the chlorine atoms may be randomized between the phenyl groups when the PCBs are fragmented by electrons. The only exception to this is 2,2′-dichlorobiphenyl.

The quantitation of polychlorinated biphenyl in environmental samples has met with difficulty since the PCBs represent mixtures. Several workers have compared PCB components in field samples to Aroclor 1254 because the sample chromatograms were most similar to these mixtures.[66-68] Rote and Murphy[68] quantitated individual peaks of different Aroclors by means of a response curve. The semilogarithmic relationship of detector response (total peak area/16 ng) to average chlorine content was obtained for each Aroclor by the method of least squares. From this relationship the theoretical response of the detector to each chlorinated biphenyl was calculated. When this approach was used, the levels of PCBs were lower than when the PCB levels were compared to the Aroclor 1254 standard. Because of these difficulties in quantitation, the PCB levels reported from different laboratories may vary greatly. The various problems encountered in the analytical methodology of polychlorinated biphenyls and their quantitation are also described in a U.S. FDA report which can be obtained upon request from the U.S. Food and Drug Administration in Rockville, Md. The title of the report is *Analytical Methodology for Polychlorinated Biphenyls* (Feb. 1973).

Polychlorinated terphenyls (Figure 1) are not eluted from gas chromatography columns under conditions used for polychlorinated biphenyls.[38] By altering their gas chromatograph column, Zitko et al.[37] were able to detect polychlorinated terphenyls.

Chlorinated naphthalenes (Figure 1) elute at the same time and cause interference with the determination of chlorinated biphenyls and chlorinated hydrocarbon pesticides in general, unless mass spectrometry is performed to establish the identity of the various chemicals involved.[56]

The methods presently available for the determination of *trichlorocarbanilide* and *other germicides* (Figure 2) are not very well developed. Graber et al.[69] outlined a thin layer chromatography method for the determination of these chemicals in soap and very briefly mentioned other methods that have been employed in the past.

Several methods for the determination of *chlorinated dibenzodioxins* and *chlorinated dibenzofurans* have been described. The goal of these methods is two-fold. First of all, quite a number of technical polychlorinated organic compounds may contain one or several of these compounds[70-72] as contaminants and methods have been developed to determine them in various technical products. Other methods dealt primarily with toxic fats where many attempts were made to isolate and identify the compounds responsible for chick edema disease.[73] Some of these methods were described in the papers that will be cited in connection with the description of chick edema disease in this article.

When preparing the various chlorinated dibenzodioxins and dibenzofurans for toxicity studies in

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order to obtain pure products, methods also need to be available for their separation and identification.[74]

One of the problems with tetrachlorodibenzodioxin as well as tetrachlorodibenzofuran is their severe toxicity in the microgram range which will probably not result in an accumulation within the ppm in biological tissues, for instance, for residue analysis. Baughman and Meselson[53] have recently described a method for the determination of these materials, within the ppt range.

Hexachlorophene is made from trichlorophenol. A process for its production was patented by Gump.[75] Additional methods for the production and purification for hexachlorophene have been published and are cited in *Chemical Abstracts*.

Recently several methods[76-79] have been developed for the determination of small amounts of hexachlorophene in blood and biological tissues. Some methods were based on extraction of hexachlorophene with ether or ethanol or a mixture of ether and ethanol. An acetyl or methyl derivative was formed and the hexachlorophene was determined with electron capture gas chromatography.[79] In an earlier method described by Bachmann and Shetlar,[80] simple benzene extraction was used and no derivative of hexachlorophene was made, which led to very variable results. The various advantages and disadvantages of these methods were discussed by Ulsamer.[79] In earlier work my co-workers used ether for extraction and prepared a dimethyl ether derivative of hexachlorophene.[81] Recovery with this method was only about 75% and we have recently altered our method of extraction to obtain better recovery.[82] A method developed by Kabacoff et al.[78] for the determination of hexachlorophene is, according to the authors, also suitable for determination of dichlorophene and Gutenmann and Lisk[83] have described a method for the determination of hexachlorophene in several agricultural products.

The production of hexachlorophene from trichlorophenol proceeds at low temperature and under acidic conditions which makes the production of a chlorinated dibenzodioxin as an impurity highly unlikely.

Since chemicals that are found in the environment are exposed to sunlight, the effect of *UV light* on the compounds under discussion has been given considerable attention. Hustert and Korte[84] synthetized a few PCB isomers, namely, 2,4,6,2′,4′,6′-hexachlorobiphenyl, 2,4,5,2′,4′,6′-hexachlorobiphenyl, 2,4,2′,4′-tetrachlorobiphenyl, 2,5,2′,5′-tetrachlorobiphenyl, and irradiated the various isomers with UV light. A mercury vapor lamp with quartz filters was used as a light source. The UV spectrum of the synthetized chlorinated biphenyls only shows significant absorption below 300 nm. When these compounds were irradiated in a solvent such as hexane, acetone, methanol, or a mixture of methanol and water, dechlorination and polymerization of the compounds was observed. When the PCB isomers were irradiated in perfluorinated dimethylcyclohexane, an inert medium, isomerization, and chlorination dechlorination were observed. These experiments indicate that, under environmental conditions, photolysis products may result with a lower chlorine content but small amounts of higher chlorinated biphenyls may also occur in low concentrations. Safe and Hutzinger[85] reported that the irradiation of 2,4,6,2′,4′,6′-hexachlorobiphenyl at 310 nm resulted in compounds of lower chlorine content, such as di-, tri-, tetra-, and pentachlorobiphenyls. In these investigations hexane and methanol were used as solvents. It is possible that new compounds are formed by loss of chlorine. Rearrangement and condensation may lead to chlorobiphenyls in environmental samples that are not present in commercial PCB mixtures. The 24-hr irradiation of 3,3′,4,4′-tetrachlorobiphenyl, 2,2′,6,6′-tetrachlorobiphenyl, 2,2′,5,5′-tetrachlorobiphenyl dissolved in hexane resulted in about 70% loss of the starting material, while with 2,2′,4,4′,5,5′-hexachlorobiphenyl, only 3.8% remained and less than 1% of 2,2′,3,3′,4,4′,5,5′-octachlorobiphenyl remained. The irradiation of Aroclor 1254 in hexane resulted in a change of the composition of the Aroclor mixture. The gas chromatogram obtained from the irradiated Aroclor showed shorter retention times than the standard Aroclor 1254. The irradiation of Aroclor 1254 in a dioxane water mixture also containing sodium bicarbonate did not lead to the formation of chlorodibenzofurans or hydroxychlorobiphenyls. The compounds that were observed corresponded to PCB molecules to which water was added and a more polar "carboxylic" acid fraction. Irradiation of Aroclor as a thin film with a frequent addition of water led to newly formed hydroxylated compounds and a more polar "carboxylic" fraction. The results obtained from irradiating

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PCBs therefore depended greatly on the solvents used and the pH of the solution in addition to the light source used and may result in dechlorination, formation of polymers, hydroxylation, and carboxylic products.[86]

Exposure of various chlorinated dibenzodioxins to summer sunlight or fluorescent UV light with an intensity of roughly 100 $\mu w/cm^2$ in the presence of organic solvents such as methanol led to rapid photolysis of 2,3,7,8-tetrachloro- and 2,7-dichlorodibenzodioxin, while 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin produced a series of chlorinated dioxins with uniformly decreasing chlorine content. Some evidence was also found that reactions other than reductive dechlorination occurred.[87] When 2,3,7,8-tetrachlorodibenzodioxin was spread on a glass plate in a methanol solution and the methanol was allowed to dry, quantitative recovery of the dioxin was possible even after 14 days of UV irradiation. Similar results were obtained when 2,3,7,8-tetrachlorodibenzodioxin was dissolved in methanol, put on a glass plate, covered with soil, and irradiated.[87] These results demonstrated that under favorable environmental conditions, when organic hydrogen donors are present, photolysis will take place. Bare surfaces of soil, concrete, or water are not effective inducers of photolysis. Chlorinated dibenzodioxins could at times prove to be quite persistent. Partial dechlorination may also occur with chlorinated diphenyls or the higher chlorinated dibenzodioxins and may result in less chlorinated compounds.

The possible long lasting presence of the dioxins is also emphasized by Kearney et al.,[88] who recovered 56 and 63% of originally applied tetrachlorodibenzodioxin after one year in Hagerstown and Lakeland, Md. soils.

Tetrachlorosalicylanilide and similar compounds split off chlorine atoms when exposed to sunlight. This is briefly mentioned in connection with photosensitivity reactions of the skin. Shaffer et al.[89] irradiated *hexachlorophene* in absolute ethanol with UV light and obtained a number of dechlorinated bisphenols resulting from loss of chlorine in the ortho and para positions relative to the hydroxy group.

GENERAL TOXICITY AND EFFECT ON REPRODUCTION

Diphenyl itself, without any chlorine atoms, is a fungistatic agent which is used as a preservative for citrus fruits, and is usually impregnated into the wrapping paper. The protected citrus fruit contains a certain amount of biphenyl residue. In different countries the amount allowed as a residue on citrus fruit varies between 70 to 110 ppm of biphenyl. Booth et al.[90] found a reversible nephrotoxic effect of biphenyl on the rat kidney when rats were fed 0.5% or 1% (10,000 ppm biphenyl in the diet). None of these effects was observed on diets containing 0.1% (1000 ppm biphenyl). Deichmann et al.[91] showed that prolonged exposure tc air containing diphenyl in high concentrations of 5, 40, and 300 mg/m^3 produced liver and kidney injury as well as bronchopulmonary lesions in mice and rats. In general, diphenyl has always been considered to be relatively nontoxic. In 1969, in Finland, a man who had been intensively exposed to diphenyl for 11 years became ill and died.[92] Diphenyl impregnated wrapping paper had been manufactured in Finland for about 15 years in the plant where the man was employed. The equipment used in the impregnating process was cleaned 5 to 10 times a year with tri- or tetrachloroethylene. The cause of death of the worker in this particular report was not satisfactorily explained and neither were the complaints of 31 other workers engaged in the same factory. Since chlorinated ethylene compounds were used for cleaning, it should be established whether organic solvents containing halogens could lead to the production of toxic substances when they are brought in contact with diphenyls. If this proves to be the case, then care should be taken that this situation is avoided.

The *PCBs* are also not very toxic when given as a single or a few repeated doses to birds and mammals. However, the juvenile pink shrimp is very sensitive to PCBs in water, while trout and catfish can tolerate higher doses; insects are also not very sensitive to PCBs. Pin fish are not quite as sensitive to a single dose, but will die when exposed for 14 to 45 days to 5 ppb of Aroclor 1254.[93] PCBs are of a low order of toxicity to rats and rabbits when given as a single dose. The acute oral toxicity decreases in rats with increasing chlorine content, while this is not as obvious when the dermal toxicity is tested in rabbits.[33] This may be due to poorer absorption of the higher chlorinated compounds. In studies with adult Sherman strain rats, we found that the oral LD_{50} for Aroclor 1254 and 1260 was somewhere in the

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range of 4 to 10 g. These two compounds proved to be more toxic in weanling rats. The single oral LD_{50} for weanlings was 1295 mg/kg, and 1315 mg/kg for Aroclor 1254 and 1260, respectively, and the lowest lethal doses were 1000 mg/kg for Aroclor 1254, and 1200 mg/kg for Aroclor 1260. The intravenous LD_{50} for Aroclor 1254 in adult female rats was 358 mg/kg.[94]

A number of short-term studies have been reported where the PCBs were given to animals over a period of one or two weeks. When the PCBs were given in this fashion they were somewhat more toxic than when they were given in a single dose. These studies are reviewed in great detail by Nelson et al.[32] and will not be reported here since they do not give a great deal of additional information.

Vos and Koeman[95] studied the toxicity of three PCB preparations in chickens, Phenoclor DB6, a French product, Clophen A60, a German product, and Aroclor 1260, a product from Monsanto in the U.S. The birds were fed 400 ppm of PCBs in their diets for 60 days; the Phenoclor and Clophen were much more toxic than Aroclor. The birds fed the two European samples died between 12 and 60 days after onset of exposure. The findings at autopsy consisted of hydropericardium, ascites, subcutaneous edema, and liver necrosis. Only 3 of 20 birds fed Aroclor 1260 died during this time with hydropericardium. None of the Aroclor fed birds showed liver necrosis.

With the help of mass spectrometric and microcoulometric analyses, the authors[95] determined that the two European samples were contaminated with tetra- and pentachlorodibenzofurans. As an additional contaminant, chlorinated naphthalenes were found.

Dermal toxicity studies with the three compounds, Clophen, Phenoclor, and Aroclor 1260, in rabbits[96] after two days led to reddening of the skin where the materials were applied. The skin reaction was more pronounced after one week, desquamation of the external epidermal layers of the skin was observed, the hair grew at a reduced rate, the skin became thickened, and prominent transverse wrinkles developed. The test animals showed a gradual loss of weight, and one of the rabbits given Phenoclor and three of those given Clophen died. All but one rabbit showed fluorescence of the liver under UV light, and microscopic changes were also observed in the livers and the treated skin.

Vos and Notenboom-Ram[97] compared the dermal toxicity of the isomer 2,4,5,2′,4′,5′-hexachlorobiphenyl with that of a PCB mixture (Aroclor 1260) in rabbits. Their dermal application resulted in early microscopic skin lesions in the Aroclor group. The lesions in the 2,4,5,2′,4′,5′-hexachlorobiphenyl group appeared later and were less severe. Both experimental groups showed liver changes. The liver damage was more pronounced in the animals that had been exposed to hexachlorobiphenyl than in those that had been exposed to the Aroclor.

In inhalation studies with Aroclor 1242 and 1254, rats, mice, rabbits, guinea pigs, and one cat were exposed to PCB vapors five days a week for several weeks. The concentration of Aroclor 1242 vapor was 1.90 to 8.63 μg/l and no ill effects were observed. The exposure of animals to the vapor of Aroclor 1254 in the concentration of 5.4 or 1.5 μg/l produced enlarged livers in rats. An intercurrent respiratory infection in some control as well as experimental rats made it difficult to interpret the results.[98] Since it is now known that the chronic toxicity of PCBs is a more important parameter in establishing their effects than short-term exposures, further studies with lower levels over longer periods of time would give more meaningful information. Fortunately, most of the compounds under discussion have a low vapor pressure so that exposure by inhalation probably does not present a great problem.

A number of reproduction studies in various species have been conducted with PCBs; usually, embryo toxicity was observed but there were no malformations. Heath et al.[99] were unable to demonstrate an effect on *reproduction* with dietary levels of 25 and 50 ppm of Aroclor 1254 in mallards and bobwhites. An increase in eggshell cracking or reduction in shell thickness was also not observed while DDE, which was used as a positive control, induced significant shell thinning and cracking and a reduction in hatching successes in mallards but not in bobwhite.

In field studies with brown pelican shells, it was found that thinning could be best correlated to DDE residues rather than dieldrin or PCBs. This finding indicates, according to Blus et al.,[100] the PCBs are not responsible for eggshell thinning in most birds following environmental exposure.

Peakall[101] fed ringdoves 10 ppm Aroclor 1254

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for 6 months. He injected others with 160 mg/kg intraperitoneally for 4 days, and evaluated the ashed eggshell weights of these birds. He found no difference in eggshell thickness between the experimental and the control groups.

In a pheasant reproduction study with Aroclor 1254,[102,103] a significantly greater difference in the number of eggs that were pipped but not hatched was found in the group of hens that received 50 mg of PCBs weekly. Egg production and hatchability was lower in this group, the survival of chickens that did hatch at six weeks of age was reduced, and the ones that did survive were significantly lighter than the controls.

Aroclor 1242 at 10 ppm or 100 ppm and Aroclor 1254 at 100 ppm in the diets of chickens did reduce egg production and hatchability and caused thin eggshells, while Aroclor 1242 at 1 ppm, Aroclor 1254 at 10 ppm, and Aroclor 1260 at 100 ppm did not affect reproduction in chickens.[104] In a rat reproduction study, decreased survival of pups at a dietary level of 100 ppm of either Aroclor 1242 or 1254 was observed. The results obtained from the reproduction studies in birds particularly seem to indicate that the lower chlorinated biphenyls affect reproduction more than the higher chlorinated biphenyls, and it is very important to determine whether the lower chlorinated biphenyls that have been suggested as replacements for presently employed chlorinated biphenyls have an effect on reproduction.

In another rat reproduction study[94] the lowest dose of Aroclor 1254 that affected reproduction was 20 ppm (1.5 mg/kg/day) which manifested itself in a decreased number of litters and less pups per litter. Neither 1 ppm nor 5 ppm of Aroclor 1254 had an effect on reproduction in the Sherman strain rat; however, an exposure of the dams to the Aroclors at 5 ppm or higher increased the liver-to-body weight ratios in weanling rats of both sexes. At 1 ppm the increase in liver weight was observed only in F1a and F1b weanling *male* rats. The dietary levels of 100, 20, and 5 ppm of Aroclor 1260 did not affect reproduction; however, the liver-to-body weight ratio of 21-day-old pups was increased at all exposure levels.

Mink are highly susceptible to the toxic effects of PCBs. A daily dietary intake of 30 ppm resulted in death in about 6 months. Exposure to 5 ppm Aroclor 1254 in the daily diet severely affected reproduction.[105]

Unfortunately, it is often very difficult to compare the various dietary levels that affect different species, since the food consumption may vary decisively from one animal species to another and the daily food intake in g/kg body wt. is often not included in the scientific report. Some mice strains, for instance, will be exposed to as much of a given substance that is present in the diet at the concentration of 300 ppm as rats on a diet containing 600 ppm or more. With some species it is of course difficult or almost impossible to measure the food consumption, but a greater effort in establishing these very basic facts would be helpful for the interpretation of toxic effects in different species.

Not very much is known at present about the toxicity of *terphenyls* or *chlorinated terphenyls*. These materials have not been studied to any great extent. The few studies that are available only describe very specific effects such as microsomal enzyme induction in the liver and give no overall evaluation of their toxicity.

The most toxic of the various *chlorinated dibenzodioxins* and *dibenzofurans* is 2,3,7,8-tetrachlorodibenzodioxin which, in the German literature, is sometimes referred to as 2,3,6,7-tetrachlorodibenzodioxin. Single oral LD_{50} values are female rats: 44.7 μg/kg; male rats: 22.5 μg/kg; male guinea pigs: 0.72 μg/kg; rabbits: < 30 μg/kg; and dogs: between 30 and 300 μg/kg.[106] It is possible that young animals are more susceptible to the toxic effects of this compound than older animals and LD_{50} values should therefore also be determined in weanling animals. One of the characteristics of this compound was that it caused delayed death, sometimes as delayed as 40 days after a single dose. During the period of intoxication, the animals lost a great deal of weight.[106]

On the other hand, 2.0 g/kg of dichlorodibenzo-p-dioxin and 4.0 g/kg body weight octachlorodibenzo-p-dioxin were not lethal to rats. Hexa-, hepta-, and trichlorodibenzo-p-dioxin were more toxic.[106]

Schulz[107] in earlier experiments found that oral doses of 20 to 50 μg/kg/body weight of 2,3,7,8-tetrachlorodibenzodioxin resulted in fatal liver necrosis in rabbits. More recently Milnes[108] reported that 10 μg/kg was lethal to rabbits.

The 2,3,7,8-tetrachlorodibenzodioxin has a variety of specific toxic effects. Whether these effects vary in different species is presently not well defined. With the interest in this and the other related compounds, more information on

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their toxicity probably will soon become available. The problems inherent in studying these compounds are the unavailability of some of them, the fact that they may not be completely pure, particularly the hex- and hepta- preparations, and of course with respect to the tetrachlorodibenzodioxin, its extreme toxicity, which makes it difficult to handle.

In contrast to PCBs, teratogenic as well as fetotoxic effects have been reported for 2,3,7,8-tetrachlorodibenzodioxin. Sparschu et al.[109] gave 0, 0.03, 0.125, 0.5, 2.0, and 8.0 μg/kg/day of 2,3,7,8-tetrachlorodibenzo-p-dioxin to pregnant rats on days 6 to 15 of pregnancy. With the 0.03 μg/kg dosage level, an effect on the fetus was not observed, but doses from 0.125 μg/kg on up resulted in fetal mortality, early and late resorptions, and fetal intestinal hemorrhage. Khera and Ruddick[110] gave doses ranging from 16 to 0.125 μg/kg to Wistar rats on days 6 to 15 of pregnancy. These authors also observed pronounced fetotoxicity at all dosage levels except 0.125 μg/kg. The affected fetuses showed cerebral and intestinal hemorrhage as well as subcutaneous edema at autopsy. The offspring of mothers given 0.5 and 1 μg/kg of the tetrachlorodibenzo-p-dioxin gained less weight during their suckling period and their survival to weaning was reduced. This could have been caused by the intrauterine effect of the dioxin or by excretion of the dioxin in the milk of the dams given the material during pregnancy. Courtney and Moore[111] observed cleft palates in 3 strains of mice given 3 μg/kg TCDD subcutaneously from days 6 to 15 of pregnancy and an increased incidence of kidney anomalies. Hydronephrotic kidneys were also produced in mouse pups that were nursed by dams treated with TCDD during pregnancy or at time of parturition.[112]

The 2,3,7,8-tetrachlorodibenzo-p-dioxin is extremely toxic in the chick embryo assay.[52] In the rat, hexachlorodibenzodioxin caused fetotoxic effects at 1 and 10 μg/kg and teratogenic effects at doses of 100 μg/kg.[106] Harris et al.[113] gave female rats daily doses of 0.1, 1, or 10 μg/kg TCDD for 31 days. Rats given 10 μg/kg lost weight and most of them were moribund within 3 weeks. Weight gain was also less at the 1 μg/kg dietary level. The rats receiving 0.1 μg/kg were not affected. In experiments with guinea pigs, 9 of 10 animals died after a single dose of 3 μg/kg TCDD, while guinea pigs receiving a single dose of 1 μg/kg gained less weight. All guinea pigs receiving 1 μg/kg/week died within 24 to 32 days and a weekly dose of 0.2 μg/kg depressed their weight. Of the various animal species tested, the guinea pig seemed to be most susceptible to the toxic effects of TCDD.

Reduced thymus and spleen weights were observed in rats that received a single dose of 25 μg/kg TCDD. The rats that died at the various dosage levels showed massive hemorrhages into the heart, liver, brain, adrenal glands, and gastrointestinal tract. Necrosis and ulceration of the glandular part of the stomach were observed. Occasionally organized thrombi were noted and the liver revealed necrosis of parenchymal cells. Sublethal doses of TCDD produced microscopic changes in liver, kidneys, and thyroid. In the kidneys the collecting tubules were primarily affected. Doses of 0.1 μg/kg/day for 31 days, a single dose of 5 μg/kg, and multiple weekly doses of 1.0 and 0.2 μg/kg did not produce changes in rats discernible with the light microscope.

Toxicity studies were conducted in our laboratory with *dichlorophene*.[114] The oral LD_{50} for adult female rats when the material was given in peanut oil by stomach tube was 1,660 mg/kg and in males it was 1,500 mg/kg. The only signs of toxicity observed in these rats were diarrhea and some depression. When 200 mg/kg was applied daily in propylene glycol to the skin of a clipped area of the back of male adult rats as a 12.5% solution, local irritation of the skin with some ulceration resulted but there was no systemic toxicity. The acute intravenous LD_{50} in adult male rats was 16.8 mg/kg when it was given in a saline lecithin suspension. In a two generation rat reproduction study, a dietary level as high as 1,000 ppm (50 mg/kg/day) had no effect. Gross and microscopic examination of the various organs including the brains of the parents as well as the offspring did not reveal any morphological changes that could be related to dichlorophene exposure. These findings indicated that dichlorophene was not very toxic to rats. The great difference between the oral and the intravenous LD_{50} suggested either poor absorption or rapid breakdown of the material in the gastrointestinal tract. Martins[61] lists the acute LD_{50} for guinea pigs as 1,250 mg/kg and for dogs, 2,000 mg/kg. Rats fed for 90 days on a diet containing 2,000 ppm showed no evidence of toxicity.

The acute toxicity of *hexachlorophene* is much greater and varies in different animal species and is

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also dependent on the route of administration and the solvent in which it is given. Gump[115] lists the acute oral LD_{50} in mice as 168 mg/kg and cites LD_{50} values reported by other investigators ranging from 80 mg/kg to 215 mg/kg. Dogs and sheep were more susceptible to the toxic effects of hexachlorophene. The interest in the effect of hexachlorophene on sheep in particular stemmed from the fact that it is effective against liver flukes[116] and advocated for its anthelmintic properties. This even led to the treatment of children with orally administered hexachlorophene in China.[117] Intravenously administered hexachlorophene in rats resulted in an LD_{50} value of 9.1 mg/kg and in rabbits of 8.5 mg/kg.

More recently we found that the oral LD_{50} (single dose) for adult male rats was 66 mg/kg and for adult female rats 56 mg/kg; for weanlings it was 120 mg/kg when the material was given in peanut oil by stomach tube to Sherman strain rats. The acute intravenous LD_{50} was 7.5 mg/kg in adult male rats.[118] Nakaue et al.[119] reported similar oral LD_{50} values in Wistar rats. The single oral LD_{50} for sheep and cattle lies between 30 and 60 mg/kg.[120]

Single dermal LD_{50} values have not been reported in the literature. We found in rats[118] that single applications of 600 mg/kg hexachlorophene in 95% ethyl alcohol solution caused death in 1 of 10 rats. Dermal application of 24 and 48 mg/kg/day for 30 days in propylene glycol or in a detergent as a 3% solution caused ulceration of the skin and affected the white matter of the central nervous system in at least half of the rats. A dose of 12 mg/kg/day as a 1.5% solution caused only mild erythema and desquamation of the skin of rats. No effect on the central nervous system was observed. Whether a single dose given in either propylene glycol or a detergent would be more toxic than one given in ethyl alcohol was not established.

The signs of acute hexachlorophene poisoning following a single oral dose varied with the age of the rats.[118] Adult rats suffered from severe depression and diarrhea, but showed few signs of leg weakness. Weanlings, on the other hand, developed posterior leg weakness as well as depression. The leg weakness became more apparent in the adults when they were given repeated doses of hexachlorophene. Death was usually preceded by convulsions in rats dosed intravenously. In rats killed with hexachlorophene, rigor mortis developed very rapidly and was very pronounced. Body temperature elevation following hexachlorophene poisoning has also been recorded in rats.[119] Both changes, the pronounced rigor mortis and the temperature elevation, may be related to the fact that hexachlorophene uncouples oxidative phosphorylation. Additional symptoms of hexachlorophene poisoning particularly in other species are discussed under neurotoxicity.

Nakaue et al.[119] fed 400 ppm hexachlorophene (28.9 mg/kg/day) to Wistar rats. They developed leg weakness within 5 days, diarrhea, emaciation, and they died in 7 to 10 days. No deaths occurred in the rats that were fed 200 ppm (about 13 mg/kg/day) for 16 weeks.

In a two generation rat reproduction study which we conducted in our laboratory, we found a reduced survival in the offspring of the F_1 generation fed 100 ppm (11.8 to 5.5 mg/kg/day) in their diet for 54 and 166 days. This was statistically significant in the second breeding of the F_1 generation. In the second generation the litters were slightly smaller, but the survival to weaning was not significantly reduced.[118] A dietary level of 20 ppm (2.3 to 1.1 mg/kg) did not influence reproduction. Thorpe[121] reported that the oral administration of hexachlorophene to male rats as well as single doses of 25 and 50 mg/kg hexachlorophene to sheep produced degeneration of spermatogenic cells; multinucleated cells were observed within the tubules of the testes. The doses given by Thorpe were higher than the ones we used in the reproduction study, and microscopic examination of the testes of the rats used in our reproduction study did not reveal any abnormalities. Toxic doses of hexachlorophene given to the dam during pregnancy may result in malformations of the offspring. Thus far, two studies have been reported in the literature; both studies were conducted in rats.[122,123] It should be determined whether malformations can also be produced in other species and with lower doses before any conclusion can be drawn from these findings.

A number of *human* poisoning cases have been reported in the literature and some of these were fatal. Most of the poisoning cases in humans have either been caused by accidental ingestion, because a hexachlorophene detergent solution was mistaken for milk of magnesia, or by the application of hexachlorophene to large areas of burned or otherwise damaged skin.[115,124] Larson[3] was

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the first to recognize that burned patients developed convulsions and other toxic symptoms after repeated exposure to hexachlorophene which was applied to burned areas of the skin. The incidence of convulsions in burned patients decreased when the use of hexachlorophene was discontinued.[125]

Eleven cases have been reported[126,127] in which a single dose of 3% hexachlorophene detergent solution had been taken orally. Gastrointestinal symptoms including nausea and vomiting were observed in ten of these cases; the eleventh case, a child who drank about 250 mg/kg hexachlorophene, became comatose and died. One additional death occurred in this group, however, this was most likely due to other causes. A 17-day-old infant was accidentally given daily oral doses of 37 mg/kg hexachlorophene for 7 days. On the third day the child started to have loose stools. On the fourth day of hexachlorophene administration the child developed spasms of the extremities with twitching of the face, sucking movements of the lips, and lateral shaking movements of the head. It was also noted in the case report that the child refused to take the medicine, there was drooling and frequent vomiting, and it is not quite clear whether the child received 37 mg/kg/day. The child survived. A week after the hexachlorophene suspension was discontinued, persistent spastic flexion of the fingers, hands, and arms was still observed. After a month the hands were slightly flexed at the wrists but were not spastic. Horizontal nystagmus was observed but cleared later. The child was discharged when it was two months and three weeks old. At this point it had shown great improvement and when checked at six months the child was healthy looking.[128] Pilapil mentioned in his discussion a number of other unpublished cases, some of which were reported to the National Clearing House for Poison Control Centers. Among these was the case of an infant who by mistake got a spoonful of PhisoHex in each feeding bottle for approximately two weeks, when it suddenly died.

Chung et al.[117] treated a total of 105 patients 5 to 15 years old with hexachlorophene for chlonorchiasis sinensis infestation. Sixty of these patients received 1 dose of 20 mg/kg, 32 received 2 doses of 20 mg/kg/day, 8 patients received 3 daily doses of 20 mg/kg, and 5 patients received 20 mg/kg body weight on the first day and 10 mg/kg on the second day. The toxicity reactions observed in these patients were mainly confined to the central nervous system and to the gastrointestinal tract. The group that received 3 doses of 20 mg/kg/day had the most severe reaction. One patient in this group became comatose on the fourth day with loss of light reflex and positive pyramidal signs. Fundus examination of the eyes revealed mild papillary edema. A low grade fever was also observed. Complete recovery occurred within three days. One half to two thirds of the patients had diarrhea within 1 to 2 hr after medication and in some cases a marked depressive effect on the central nervous system was also observed. An additional case of poisoning after oral ingestion was reported by Korlof and Winsten[129] in Scandinavia and two fairly recent cases with fatal outcome occurred in the U.S.[130]

Herter[131] was probably the first to report a human poisoning case caused by the dermal absorption of hexachlorophene. Because of a language barrier, the mother of a newborn infant applied 3% hexachlorophene as a lotion after the infant's bath without rinsing the baby. After four days, excoriations appeared on the child's face and buttocks, the infant developed convulsions, his face twitched and his extremities jerked. A roving nystagmus was present. This child recovered within 16 days after exposure to hexachlorophene had been discontinued.

In addition to the two cases already mentioned in which poisoning occurred because of oral ingestion, Mullick[130] also reported four cases – children who died following the treatment for burns in two instances and the treatment for severe ichthiosis with a 3% hexachlorophene bath.

Larson[3] reported six burned patients who developed central nervous system symptoms following the dermal exposure to hexachlorophene. All six patients recovered. Korlof and Winsten[129] reported two cases of poisoning following the dermal application of hexachlorophene. Both patients were fairly extensively burned, and developed central and peripheral nerve damage as well as some kidney impairment.

Lockhart[132] mentioned six deaths that were reported to the FDA caused by the topical application of hexachlorophene on burned patients. In all of these cases the skin was damaged, probably enhancing absorption of hexachlorophene, and although skin does represent a barrier, it is not impermeable – a fact we tend to forget at times. Damaged or abnormal skin may be

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more permeable, and the skin of prematures, newborns, and children in general may have absorption characteristics other than those of adult human beings, another factor that is often neglected. Recently[133,134] 41 deaths due to hexachlorophene poisoning have been reported in France. The deaths occurred in infants who were treated with a powder to which 6% hexachlorophene had been added by mistake. This powder was mainly applied in the diaper area where the skin became inflamed, this as well as the diaper which served as an occlusive dressing, probably enhanced absorption. These cases will not be made public until litigation problems have been settled.

Little published information is available on the toxicity of the germicides in Figure 2 aside from hexachlorophene and dichlorophene. The acute oral LD_{50} for triclosan (TCC) in mice and rats is about 4,000 mg/kg.[135] The acute subcutaneous toxicity for rats was 14,700 mg/kg and the acute dermal toxicity was about 9.3 g in rabbits. Concentrations of 1 to 5% solutions caused eye irritation in rabbits. Daily oral doses of TCC up to 1,000 mg/kg/day for 4 weeks did not produce an effect in rats. When 2.5 and 5% suspensions in gum arabic were applied dermally to rats for 4 weeks, the animals did not gain as much weight. Unpublished material cited in the minutes of the OTC (Over the Counter Drugs) Panel listed a "no effect" level as 170 mg/kg/day in rats and for a 90-day feeding study in rabbits, 138 mg/kg/day. In baboons a daily oral dose of 100 mg/kg/day did not produce a toxic reaction. The minutes also mentioned some work where lower doses showed an effect on rats and dogs. Since some of these results are conflicting, further studies are indicated and should be performed with products used as ingredients in soaps; a chemical analysis of these products is necessary to determine whether impurities might be responsible for varying results. Chloroaniline as a result of heating during manufacture could be present as such an impurity. Of the anilines, according to Hamblin,[136] only p- and m-chloroaniline may cause methemoglobinemia if absorbed through the skin. The o- and m-chloroaniline may cause liver and kidney damage. Triclosan itself is also absorbed through the skin and absorption is greater when applied to damaged skin.

SPECIAL TOXIC EFFECTS

Skin Reactions

Chloracne

This occupational skin disease can be produced by a number of chemical compounds.[137] Polychlorinated biphenyls as well as the chlorinated naphthalenes, a few isomers of the chlorinated dibenzodioxins, and chlorinated dibenzofurans are all able to produce chloracne in a certain proportion of the human population. Oily skin and large pores seem to predispose to the disease while the opposite is true for smooth, tender skin and it is possible that young children might be less susceptible because of the nature of their skin.

Chloracne has also occurred in workers engaged in the production of 2,4,5-T[1]. During the industrial production of trichlorophenol a sudden rise in temperature within the reactor has occasionally resulted in accidents. In one instance this exothermic reaction caused an explosion.[108] When the rise in temperature occurred, 2,3,7,8-tetrachlorodibenzodioxin was formed and severe outbreaks of chloracne were observed among the workers.[137a]

The term chloracne was first used by Herxheimer in 1899,[138] who thought it was caused by free chlorine generated in certain factories. Wauer in 1918[139] and Teleky in 1927[140] suggested the term "Pernakrankheit." Another term sometimes used is halogenwaxacne.

As the word chloracne implies, part of the lesion of this occupational skin disease resembles adolescent acne; however, it is generally more severe and the distribution of the lesions is not consistent with adolescent acne, although it may be superimposed on adolescent acne. Chloracne is described in the literature as consisting of the formation of comedones with or without cysts and pustules. The follicular orifices are filled with sebaceous and keratinous material. Melanosis and a secondary inflammatory reaction may also exist. In addition to this description, skin changes occur that have been discussed less frequently in the more recent literature. These skin changes are usually referred to as cable rash or cable itch. Cable rash has been observed in workers who have insulated cables with various mixtures of chlorinated naphthalenes and chlorinated biphenyls and in workers handling these cables.[141] It has been observed also in factories that make condensers.[142,143] According to Braun,[142] a few of

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the cases with manifest chloracne started with changes in the skin of the face resembling photo sensitivity and the bearers of these lesions suffered severe puritus in the areas of the skin lesions. This lesion termed cable rash occurred predominantly on the cheeks but also on the skin of the arms. The outcome of cable rash varied; it could either disappear or it could develop into full-blown chloracne.

In addition to the pustules and comedones of typical chloracne, brownish keratinization of the skin was observed in some patients. Braun[142] pointed out that the workers, particularly those with poor hygiene, transmitted the disease to their spouses and children through direct contact.

Many of the occupational cases of chloracne that have been reported in the literature have been reviewed previously.[137,144] Jones and Alden[145] reported one of the first outbreaks of chloracne in a company that was engaged in the manufacture of chlorinated biphenyls. They described one typical case and mentioned 24 men working on the manufacture of chlorinated biphenyls; 23 had had an acneform eruption on the face and body. Particularly during the 1930s and 1940s large outbreaks of chloracne occurred in a number of factories.

Much discussion has been devoted in the literature to the problem of whether the chloracne was produced by external contact alone or whether it was caused by systemic absorption, particularly the inhalation of vapors. One question asked was whether internal administration could cause chloracne, and if so what part it played in any particular case. As we know now from outbreaks of poisoning due to the consumption of rice oil that was contaminated with polychlorinated biphenyls in Japan (see under "Yusho"), chloracne can be produced by the systemic absorption of chlorinated biphenyls. The experiments by Shelley and Kligman[146] and Plewig[147] showed that the skin lesions could also be produced by applying the chloracnegenic compound to the skin.

After occupational chloracne manifested itself, the patients also complained of systemic effects such as loss of appetite, nausea, edema of the face and hands, abdominal pain, vomiting, and burning and soreness of the eyes. Particularly with the chlorinated naphthalenes, hepatotoxic effects have also been observed. Application of chemicals to the skin can lead to their absorption and cause systemic toxicity. Whether a systemic toxic effect results will depend on the skin surface area covered, the amount of the toxic substance applied, and the ability of the skin to absorb it. If dermal absorption is poor, a single application will usually not be very toxic. However, if the compound is not easily metabolized and excreted, repeated dermal application may result in sufficient accumulation in the body to cause systemic toxicity.

Crowe[144] has pointed out that chloracne due to chlorinated naphthalenes has decreased because these compounds have been used less since the Second World War. However, Weber in 1969[148] reported an outbreak of chloracne in a company engaged in the production of electric coils that were coated with a mixture of chlorinated naphthalenes with the tradename "Nibrenwax."

Detailed examination by several German investigators indicated that the capacity of 2,4,5-trichlorophenol as well as 2,4,5-T (2,4,5-trichlorophenoxyacetic acid)[149,150] to produce chloracne was due to contamination with 2,3,7,8-tetrachlorodibenzofuran or 2,3,7,8-tetrachlorodibenzodioxin.

Schwartz et al.[151] indicated that the most potent chloracne producing agents were the chlorinated naphthalenes, chlorodiphenyls, and chlorodiphenyloxides. Persons who worked with chlorinated naphthalenes usually developed acne after a month or more of exposure.

Light microscopic observations made of the chloracne lesions of humans varied somewhat, depending primarily on the length of time the lesions had existed at the time of examination. The earliest change encountered consisted of acanthotic widening of the external root sheath of the hair follicle. Above a layer of basal cells 6 to 10 layers of large round cells were observed, followed by a layer of 2 to 3 cell rows of granular cells. In later skin biopsies, folding of the basal layer occurred and comedo formation was observed, resulting in dilatation of the follicle and atrophy of the epithelium surrounding the comedo. The contents of the follicles consisted predominantly of keratinous material rather than sebum. The sebaceous glands also underwent changes. Within the alveoli of the glands the basophil cells increased in number and basal cell hyperplasia was noted. The ducts of the sebaceous glands were filled with a solid mass of basophil epithelial cells. The sebaceous glands gradually

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disappeared and in later secretions were only recognized as solid protrusions of the comedo wall. Aside from atrophy of the sebaceous glands, hyperplasia has also been described and many large sebaceous glands forming huge cysts filled primarily with keratinous material have been observed. Hyperkeratosis and acanthosis of the surrounding epidermis has usually accompanied these lesions. Foreign body granulomata which are thought to result from rupture of the follicular cysts have been found in the skin.[147,151] In the Japanese poisoning incident (Yusho), the predominant skin lesion was marked hyperkeratosis of the epidermis, cystic dilatation of the hair follicles, and an increase in the melanin pigment in the basal cells of the epidermis. Foreign body granulomata were also observed. The contents of the cysts consisted partially of keratin. In a stillborn child the hyperkeratosis was even more pronounced, accompanied by atrophy of the epidermis; cystic dilation of hair follicles was observed, especially in the skin of the head. According to earlier reports[151] some differences exist in the microscopic changes observed in the skin following exposure to the various chlorinated cyclic compounds that can produce the lesion. It is, of course, possible that these histopathologic variations are in reality only differences that exist between an early, a well-developed, and a subsiding lesion.

Many theories have been advanced to explain the development of chloracne. None is satisfactory. The concept of mechanical plugging of the hair follicles on skin exposure to polychlorinated polycyclic compounds is not supported by the fact that chloracne develops following ingestion of these compounds. It is possible that the skin lesions are caused by a toxic effect on the epithelium of the skin and its appendages which is manifested in proliferation and loss of normal function of the cell. Since the chemicals are lipid soluble they could occur in a more concentrated form in sebum and therefore affect sebaceous glands more than sweat glands or salivary glands. The reports on the Yusho outbreak suggest that proliferation of the epithelium of the mucous membranes may also occur. The increased pigmentation of the skin and mucous membranes may indicate a functional disturbance resulting in increased melanin production. Hyperpigmentation is also observed when the adrenals are damaged, as in Addison's disease, and adrenal function should be investigated in chloracne patients who manifest hyperpigmentation.

In none of the human case reports on chloracne have the mucous membranes been studied to any great extent. Vaginal smears in women and sputa, corneal, and nasal scrapings may give helpful information in this respect. Since in animals exposed to polychlorinated polycyclic compounds low vitamin A levels have been observed in the liver, it is possible that the compounds may affect vitamin A absorption and storage, and adequate utilization in the liver and elsewhere in the body. If the lesion is produced by inadequate utilization of vitamin A, then administration of vitamin A per se may not necessarily alter the course of the disease. Protein deficiency may also mimic hypovitaminosis A in that it affects transport of vitamin A. The liver stores 90% of the available vitamin A and these stores may be affected in liver disease. The microscopic appearance of the skin lesions resembles in some respects observations made in skin of patients suffering from vitamin A deficiency. In vitamin A deficiency, hyperkeratosis of the hair follicles is observed. Multiple firm papules may develop as a result of the keratin plugs in the sebaceous glands. Dryness and scaliness as well as furunculosis of the skin may also be present. In contrast, night blindness has not been reported as a symptom in patients suffering from chloracne. Unfortunately, chloracne outbreaks are often not well investigated and, because of problems with litigation, accounts are often only published many years after the outbreak has occurred if they are published at all.

Chloracne is a very persistent skin disease and in the Japanese outbreak of poisoning with chlorodiphenyls, the skin changes were still present in a number of patients three years after exposure to the chlorodiphenyls had been discontinued. What needs to be investigated is whether this persistence of the skin disease is caused only by the fact that once the lesion has developed it regresses very slowly, or whether it may be sustained by the chloracne-producing chemicals which have been absorbed and stored in the body, particularly the adipose tissue, and are only very gradually excreted. In patients with chloracne, adipose tissue biopsies should be analyzed for the presence of the offending polycyclic polychlorinated compounds. If adipose tissue is unobtainable, the sebum of the ear canal or the content of the skin lesions may represent a good substitute.

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Apparently there is no known effective treatment for chloracne and the best control measure is prevention. The manufacture of chlorinated hydrocarbons and the coating of wires and condensors with insulating materials containing them should be done in totally enclosed areas so that the fumes of the insulating substances and of the solvents if any are used do not come in contact with the workers. The workers should be provided with clean overalls and underclothes daily. These should be laundered at the plant and cleaned in such a way that no chlorinated hydrocarbons remain on them. Shower baths after work should be compulsory and supervised and there should be sufficient shower facilities provided so that the workers are not unduly delayed after work. Special synthetic wetting agents[151] seem to remove the material from the skin more effectively than ordinary soaps.

The experimental induction of chloracne in men has also been accomplished with 2 applications of 10 μg 2,3,7,8-tetrachlorodibenzo-p-dioxin[107] and with Halowax 1014, pentachloronaphthalene, and hexachloronaphthalene.[147]

Experimental work in animals has shown that most species do not develop the same type of dermatitis. The rabbit's skin,[152] particularly the inner aspect of the ear lobe, provides a good test medium for the development of acneform dermatitis. The rabbits, in addition to the development of hyperkeratosis of the ear, will also show a hepatotoxic effect.[150] Microscopic examination of the changes in the rabbit ear were described by Jones and Krizek.[153] Inagami and Koga[154] were able to produce hyperkeratosis in hairless mice that were fed rice oil contaminated with Kaneclor 400. These mice also showed hepatomegaly. Microscopic examination of the skin revealed cystic dilation and hyperkeratosis of hair follicles and sweat glands. Hairless mice may be another suitable experimental animal for testing the effect of these chemicals on the skin.

X-disease in Animals

Chloracne-type lesions do not usually occur in other species except for the rabbit and the hairless mouse, already mentioned. However, in 1947 Olafson[155] described a disease in cattle which was designated as *X-disease* or hyperkeratosis. Symptomatology included excessive lacrimation, diarrhea, polyuria, marked salivation, and discharge from the nostrils. The animals developed a chronic cough, poor appetite, numerous red maculae in the buccal mucosa; in addition, hyperkeratosis of the skin developed. The skin was hard, and fissured particularly across the withers and sides of the neck. Many wart-like proliferations on the lips, tongue, and hard palate were also observed. The abomasum was greatly inflamed, swollen, and edematous with many superficial ulcers.[156] In the cows that had X-disease, a low vitamin A plasma level was also observed, which again indicates that vitamin A may play a role in the etiology of these various disease entities.

The disease was first thought to represent a virus infection and other factors were also suspected. After numerous investigations it was finally established that X-disease could be produced in cattle by the ingestion of highly chlorinated naphthalenes, and also by petroleum products such as crankcase oil. Bell[157] tested the ability of the various compounds of the chlorinated naphthalene group to produce X-disease. He demonstrated in his studies that dichlorinated and trichlorinated naphthalenes did not result in X-disease, while tetrachloronaphthalene had an effect and the higher chlorinated naphthalenes such as pentachloronaphthalene, hexachloronaphthalene, heptachloronaphthalene, and octachloronaphthalene caused severe disease. Octachloronaphthalene was less toxic than hexa- and heptachloronaphthalene. Wagener[158] reported the occurrence of hyperkeratosis in cattle in Germany between 1946 and 1948. The cause of this outbreak was traced to a wood preservative which was used in postwar Germany. The author proved experimentally that animals housed in cabins painted with the wood preservative developed typical X-disease. The poisonous ingredient in the wood preservative appeared only in certain lots of this product and later lots did not contain it. The poisonous ingredient was never identified by chemical analysis.

Animal experiments were conducted with the toxic ingredients of rice bran oil that produced Yusho in Japan. These toxic substances consisted of a mixture of chlorinated biphenyls containing 48% chlorine, with a trace of 0.01% of naphthalenes.[159] When mice were exposed to them for 3 to 4 months, they showed eczematous changes of the skin around the eyelids, erosion, ulceration and perforation of the ear laps, loss of hair, and erosion and ulceration of the skin around the neck, forelegs, and sides of the chest. The authors

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stated that, at the end of the experiment, some changes in the skin similar to those in the experimental mice were seen in some of the control mice also, which makes it difficult to interpret their findings.

In 1957 a disease that will be discussed in greater detail later in this article occurred in a large number of chickens. It was termed chick edema disease and was found to be caused by certain toxic fat. Allen and Carstens[160] fed this substance to monkeys and found that, among other changes which will be discussed later, the monkeys developed a generalized alopecia and subcutaneous edema one to two months before death. Schoettle et al.[161] produced hyperkeratosis of the skin in rats by feeding them hexachloronaphthalene. Young swine[162] and sheep[163] did not develop skin lesions as a result of exposure to chlorinated naphthalenes.

Photocontact Dermatitis and Irritation of the Skin

As was pointed out earlier, the hyperpigmentation observed in patients with chloracne as well as the reaction described as cable rash could also be the result of a photosensitive reaction. This photosensitive reaction is the predominant lesion in the skin produced by a number of germicides, namely, bithionol (Figure 2), tetrachlorosalicylanilide (TCSA), and a number of other halogenated phenolic compounds including fenticlor. Patients who develop photodermatitis complain initially of a burning sensation, and this is followed by itching. A rash develops which in the beginning is erythematous and associated with varying degrees of edema. As it subsides, pigmentation is observed.[164] Wilkinson[165] was the first one to describe cases where patients developed a photo allergy which was due to the incorporation of TCSA into popular soaps. He observed that the outbreak of dermatitis was confined to the areas that usually were exposed to light. Most of the cases reported from England were transient and as soon as exposure to the compound was discontinued the reaction subsided. Jillson and Baughman[166] and Jillson,[167] on the other hand, reported that their cases were more persistent.

Willis and Kligman[168] demonstrated that the photocontact allergy of the skin with these various compounds was simply a contact sensitization. The reason that allergic reactions of the skin developed only after exposure to the sun was due to the photodecomposition of the various products to which the skin had been exposed. If various brominated and chlorinated salicylanilides were irradiated in vitro in an appropriate solvent, the resulting breakdown products caused an allergic reaction on contact, without exposure to UV light. These studies were performed because of information provided in earlier studies by Coxon et al.,[169] who had irradiated brominated and chlorinated salicylanilides and found that these compounds gave off halogens. With 3,5 substituted salicylanilides, the halogen substituted on the 3 position was lost. Tetrachlorosalicylanilide photosensitized subjects reacted to 3′, 4′, 5-trichlorosalicylanilide and to 3′, 4′-dichlorosalicylanilide. A reaction to nonhalogenated salicylanilide was not observed. Halogens on the anilide ring were unaffected by UV radiation, while the halogens on the salicyl ring were replaced stepwise by hydrogen upon irradiation.

Among the patients who developed photosensitivity to tetrachlorosalicylanilide reported by Wilkinson,[165] a group of patients in a shop of a factory was also included. This same outbreak was reported by Jones,[170] who found that among 106 employees who used the same soap very frequently, 31% were affected by a dermatitis due to contact with tetrachlorosalicylanilide present in the soap.

Additional cases of photodermatitis due to the exposure to tetrachlorosalicylanilide were reported by Calnan et al.,[171] who pointed out that pronounced edema of the eyelids was also observed when involvement was severe. Some cases were persistent or developed acute relapses on further exposure to sunlight without further exposure to the offending chemical. Frenk[172] reported an additional nine cases of acute contact dermatitis of the hands due to tetrachlorosalicylanilide. Epstein[173] described a photoallergic reaction in two patients to tribromosalicylanilide (TBS). With patch tests these patients showed a cross reaction to TCSA. A similar cross reaction pattern to a number of halogenated salicylanilides was also observed by Osmundsen[174] in patients who had developed photodermatitis following exposure to tribromosalicylanilide.

Because of the photosensitivity reactions and the cross reactions that were also found with bithionol, which is similar in structure to tetrachlorosalicylanilide (Figure 2), the U.S. Food and Drug Administration, on October 24, 1967, withdrew all new drug applications of all drugs that

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contained bithionol. In the notice of withdrawal of approval in the Federal Register, the Commissioner of the Food and Drug Administration pointed out that bithionol may in some instances cause a very persistent photosensitization and severe dermatitis may occur with exposure to sunlight without further contact with the sensitizing articles, and that bithionol may produce cross photosensitization with other commonly used chemicals such as certain halogenated salicylanilides and hexachlorophene.

Hexachlorophene preparations may also cause irritation of the skin if these preparations are used frequently. A true allergic reaction to hexachlorophene is apparently rare.[175] The more severe reactions were usually confined to the skin of the scrotum and consisted of a primary irritant contact dermatitis.[176,177] The cases reported by Baker et al.[176] also included a 33-month-old Caucasian female who developed a rash around the buttocks, vulva, and upper thighs following the use of a hexachlorophene preparation in her bath water.

We observed severe skin irritation and ulceration in rats that were exposed dermally to a 3% hexachlorophene solution in propylene glycol or a detergent. The skin reaction was not as severe when the hexachlorophene concentration was reduced to 1.5%.[118] Boutwell et al.[178] reported considerable skin irritation in mice that had 5 mg/mouse applied twice weekly for 21 weeks. Harber et al.[179] were able to induce contact photosensitivity with TCSA or TBS. These authors also found that cross contact sensitivity and photosensitivity to hexachlorophene existed in guinea pigs with primary photosensitivity to TCSA and TBS. Photosensitivity to trichlorocarbanilide (TCC) was not produced in guinea pigs.

The guinea pig may represent a suitable animal species to study contact and photosensitivity of these and related compounds. After exposure to the photosensitizing agents has been discontinued, humans may still be sensitive to sunlight, a fact that needs further study. To a certain extent this could be due to a persistence of the chemical compound on or in the skin.

Dichlorophene used as an antimicrobial preservative may also cause contact dermatitis. Cross-sensitivity to hexachlorophene was not demonstrated.[180]

Chick Edema

Simpson et al.[181] and Sanger et al.[182] described a new highly fatal disease in chickens. The first outbreak occurred in 1957 in Georgia. Large numbers of birds from approximately three weeks of age to adult laying hens were affected. Clinical signs consisted of dyspnea, reduced body weight gain, stunting, subcutaneous edema, paleness, and sudden death. In young chickens, gasping was the first noticeable sign. This was followed by a waddling, unsteady gait. Gross inspection of the birds revealed a pale heart, fluid was noticed in the pericardial sac, and the livers were pale, mottled, and had an irregular granular surface. In advanced stages of the disease the chickens had large distended abdomens that were filled with fluid. Ecchymotic hemorrhages were present in the skin beneath the wings, on the legs, and over the Q bone in a few chickens. The kidneys were pale and swollen. Following the first outbreak, no additional cases were reported until late summer and early fall of 1957, when extensive losses of chickens and turkeys occurred throughout the southeastern part of the U.S. Suspicion soon developed that losses were caused by the consumption of rations containing certain lots of animal fat.[181] The animal fats contained tallows and greases obtained as by-products from hides preserved with chlorophenols prior to leather manufacture.[51]

This material was excluded from commercial poultry rations and no additional cases occurred where animal fats were responsible. Simpson et al.[181] came to the conclusion that the edema in the chickens was the result of vascular changes that seemed to be the primary lesion. The vascular lesion, or endotheliosis, consisted of proliferation and hypertrophy of the endothelial cells of arterioles and small arteries in most tissues. Pronounced proliferative changes in endothelium of the glomerular capillaries were observed, but no significant changes were noticed in the tubular epithelium of the kidney. Necrosis of the parenchymal cells of the liver was also noted.

In some reports it was pointed out that exposure to toxic fat resulted in a decrease of the total serum protein and a shift on the albumin to globulin ratio with a decrease in the albumin fraction. This could, of course, lead to edema formation, particularly when combined with malfunctioning glomerular capillaries. It has also been suggested that increased permeability of the cardiovascular bed contributed to the edema forma-

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tion.[160,183] In other studies the serum protein concentration did not differ from that of the controls[184,185] and increased permeability of the cardiovascular bed was advanced as the sole cause of the fluid accumulation. In addition, pulmonary edema was observed in diseased chickens and perivascular lymphocytic infiltration as well as edema of the cardiac muscle with interstitial lymphocytic infiltration has been noted.[186] Electron microscopic examination of the cardiac muscle revealed degeneration and loss of mitochondria.[184] It is possible that the heart may play a primary role in the development of chick edema, but a hepatorenal syndrome could be the underlying cause as well. Studies that investigate the development of the lesion more closely are needed to elucidate these problems. The type of diet also influenced the development of the disease. It could be brought on more rapidly with a semipurified diet than with a natural grain ration; sodium chloride in the diet was a prerequisite for the development of the syndrome.[187]

If low rations of the toxic fat were fed to chickens they did not develop chick edema but reproduction and hatchability were drastically reduced.

Toxic fat was not the only product that caused the chick edema syndrome. Chlorinated biphenyls caused the disease in chickens[188] and in Bengalese finches.[26] A mixture of pentachloronaphthalene and hexachloronaphthalene fed to chickens also resulted in chick edema disease.[189] Cantrell et al.[190] announced in 1967 that one of the toxic compounds capable of producing chick edema found in toxic fat was 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin. Then Higgenbotham et al.[52] showed that the chick edema factor was produced by two principal compounds isolated from toxic fats: 2,3,7-trichlorodibenzo-p-dioxin, and 2,3,7,8-tetrachlorodibenzo-p-dioxin. They also suggested fats and fatty acids that contain commercial chlorophenols as possible sources of contamination. When crude fats and tallows are heated to produce fatty acids, chlorophenol residues may be converted to a chick edema factor. Whether these last three compounds mentioned are the only ones that produce chick edema disease is questionable unless the chlorinated naphthalenes as well as chlorinated biphenyls that produced chick edema were also contaminated with them. Another outbreak of chick edema disease occurred in 1969.[51] In this incident millions of birds in North Carolina were involved. The outbreak was traced to a vegetable oil refinery where feed fat became contaminated with "chlorophenols." The company formulated the chlorophenols as antimicrobial water treatment products. An underground pipeline from the "pesticide" plant led to traps used to collect by-product fatty acids held as feed fats.

Flick et al.[185] have recently shown that pyrolytic products of 2,3,4,6-tetrachlorophenol were toxic to chicks and produced chick edema if 50 ppb were fed for 21 days. The product fed consisted of 53% hexa-, 45% hepta-, and 25% octachloro-p-dibenzodioxin. The pyrolytic product of pentachlorophenol consisted of a mixture of 5% hexa-, 62% hepta-, and 33% octachlorodibenzo-p-dioxin that produced mild edema and increased mortality when fed a diet containing 1 ppm (200 μg total ingestion) of the material for 21 days. When a mixture of tri- and tetrachlorodibenzo-p-dioxin was fed to the animals at a dietary concentration of 0.01 ppm (a calculated intake level of 1.9 μg), the birds developed edema and 83% of them died. Further studies with pure compounds are necessary to confirm these findings.

The effect of PCBs on the chicken was again demonstrated when PCBs that were used in the U.S. as heat transfer fluids in food processing plants contaminated large quantities of fish meal that was processed for animal feed in a plant in Wilmington, North Carolina.[191] From about April 1971 until July 1971, PCBs leaked into the fish meal and 16 thousand tons of contaminated fish meal were distributed to more than 60 companies in 10 states. One of the purchasers of the fish meal, The Holly Farms, the nation's largest poultry producer, had to slaughter 77,000 fowls after discovering contamination of the chickens with PCBs. Some of the levels found in the adipose tissue of the animals were as high as 40 ppm. The Holly Farms first discovered that the chicken meal was contaminated because the hatchability of their eggs was drastically reduced to 18% of normal.

In February and March of 1968 an outbreak of chick edema also occurred in Japan, where more than 400,000 chickens reportedly died. The disease was traced to the contamination of animal feed with Kanechlor 400, a Japanese PCB. The chick edema disease occurred simultaneously with an outbreak of polychlorinated biphenyl poisoning in people that was due to the contamination of

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rice bran oil, also contaminated with Kanechlor 400 and produced by the same company.[192]

Metcalfe[193] suggested that chick edema factor could be minimized if fleshings grease from hides preserved with technical pentachlorophenols containing chlorinated dibenzodioxins were no longer used for food products. It was also pointed out that the use of glue emulsions containing chlorophenols in rendering operations is another possible source of the toxic factor. Whether elimination of these sources from animal food products will prevent chick edema epidemics remains to be seen, since polychlorinated biphenyls that may be contaminated with chlorinated dibenzofurans have also produced chick edema disease.

Yusho

During the summer of 1968, 13 cases of chloracne were observed in the western part of Japan, centering around Fukuoka prefecture. The incidence of the disease seemed to be familial and it became apparent that all patients had used the same brand of cooking oil (canned Kanemi rice oil). These findings were picked up by the newspapers and a strong demand was voiced to elucidate the cause. Therefore, a Yusho study group was established by Kyushu University.[192] The term Yusho stands for rice oil disease. After extensive investigations it was found that several illnesses occurred in a number of households that had used common cans of rice bran oil.

When a total number of 89 males and 100 females that suffered from Yusho were examined, a variety of symptoms were found in these patients. The incidence of the different symptoms that varied a great deal is given in Table 2. The epidemic outbreak was finally, after very extensive investigation, pinpointed to Kanemi oil, manufactured on February 5, 1968. Chemical analysis of this oil established that it was contaminated with 2,000 ppm of Kanechlor 400, a chlorobiphenyl that contains 48% chlorine. It was also demonstrated that most of the components of the chlorobiphenyls were stored in adipose tissue of the patients for quite a long time and passed through the placenta into the fetus of a few women who happened to be pregnant.[194]

The amount of oil necessary to produce disease was found to be at least 2.5 l. The younger the patient, the higher the morbidity rate with a smaller total amount of ingested oil. The average amount of chlorobiphenyl consumed by individual patients was calculated to be a total of 2 g.[195] (Various reports written on this subject give different accounts of what actually was consumed and the amount can at best only be estimated.)

When the rice bran oil was heated under reduced pressure, Kanechlor leaked from the old pipes in which holes were subsequently discovered.

The Yusho outbreak spread not only over Fukuoka, Japan, but also over 20 other prefectures in the western part of Japan. A total of 1,057 poisoning cases resulted according to the latest tabulation (August 1971, Ministry of Welfare).[196] The most common initial symptom experienced by 136 patients with Yusho was increased eye discharge and swelling of the upper eyelids (38.3%) followed by acneform eruption, follicular accentuation (33.1%), and pigmentation of the skin (9.6%). Itching of the skin and "stiffening" of the soles of the feet and the palms of the hands were also observed. Pigmented mucus membranes were noticed in many of the patients; a small percentage of them developed jaundice, many complained of a feeling of weakness and headaches; occasionally vomiting, diarrhea, and fever also occurred. Various neurological symptoms manifested themselves such as transient visual disturbances, numbness and spasms in the limbs, and hearing difficulties. Most patients showed acnelike skin eruptions that resembled the chloracne observed after occupational exposure to chlorinated naphthalenes or chlorinated biphenyls. When the age and sex specific incidence rates were analyzed it was found that a significant sex difference was not present, but a lower risk for both males and females in the age groups over 60 years was noted. The proportion of severe cases among those age 13 to 29 was significantly larger than that of other age groups.

During the Yusho epidemic, 11 women with Yusho and 2 wives whose husbands had Yusho, but who themselves did not show the disease, delivered 10 liveborn and 2 stillborn babies. Nine of these babies had unusually greyish, dark brown stained skin, and dark pigmentation of the gingiva and nails was noted in five of them. Most infants also had heavy eye discharge. Histological examination of a stillborn fetus showed a marked hyperkeratosis and atrophy of the epidermis and cystic dilation of hair follicles, especially of those of the head. A marked increase of melanin pigment in the basal cells of the epidermis was also noted. Most fetuses that were born were smaller

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TABLE 2

Symptoms (complaints) of Patients (89 males, 100 Females, as of October 31, 1968)*

Symptoms	Males %	Females %
Blackening of nails	83.1	75.0
Black spots in all pores	64.0	56.0
Excessive sweating in palms	50.6	55.0
Acnelike skin eruptions	87.6	82.0
Red spots on limbs	20.2	16.0
Itching	42.7	52.0
Change in skin color	75.3	72.0
Swelling of hands and feet	20.2	41.0
Hardening of backs and hands	24.7	29.0
Pigmentation of mucous membranes	56.2	47.0
Sebum (gum secretion in eyes)	88.8	83.0
Hyperemia of mucous membranes in eyes	70.8	71.0
Temporary failing of eyesight	56.2	55.0
Jaundice	11.2	11.0
Swelling of upper eyelids	71.9	74.0
Sense of weakness	58.4	52.0
Numbness of hands and feet	32.6	39.0
Fever	16.9	19.0
Hearing difficulty	18.0	19.0
Spasms of hands and feet	7.9	8.0
Headaches	30.3	39.0
Vomiting	23.6	28.0
Diarrhea	19.1	17.0

*Reprinted with permission from Kuratsune et al.[193]

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than the national standards and four of them were small for gestational age babies. As they grew older their skin gradually became lighter over a period of three months.

Okumura and Katsuki[197] studied a group of 24 patients whose main complaint had been acneform skin eruptions and who had been diagnosed as definitely having Yusho. Eighteen of these were adults and six were children. Irrespective of the seriousness of the disease in all patients, the initial symptoms were eye discharge associated with edema of the eyelids, defects in vision, generalized fatigue, anorexia followed by comedo and acneform eruptions of the face, and pigmentation and flattening of the nails. The principal subjective symptoms were fatigue, numbness of the lower limbs, and intolerance for *sake*, which is a Japanese alcoholic beverage made from fermented rice. Three of twelve females had an irregular menstrual cycle. Seven of nine patients with serious disease showed a low grade fever. Most laboratory tests conducted on these patients were normal, including serum enzyme values. A slight elevation in alkaline phosphatase was observed in over half of the seriously ill groups. An increase in the α_2 globulin fraction of the serum protein was noted with a concomitant reduction of the albumin fraction, but the total serum proteins were within the normal range. These same 24 patients had abnormally elevated serum triglycerides ranging from 200 to 600 mg %. In 12 of 24 cases, while the total serum cholesterol remained unchanged, phospholipids tended to be somewhat lower.[198] Preliminary animal experiments in rabbits conducted by the same authors showed that lipemia was consistently induced within several days. The levels returned to their previously normal values several weeks after exposure had been discontinued.

The serum concentration of triglyceride is usually elevated in humans in glycogen storage disease (Von Gierke's disease). An increase in plasma triglyceride concentration is also observed in *essential hyperglyceridemia*, an apparent con-

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genital anomaly. Essential hyperglyceridemia has been observed in siblings of a number of families, but the mode of transmission is not known. The course of this disease is generally benign and is consistent with a normal life span.[199] Observation of the familial cases shows that the mere elevation of triglyceride does not in itself increase the risk of arteriosclerosis.

Another interesting fact in this poisoning epidemic was that out of 23 cases with obvious Yusho, 10 showed symptoms or signs of a sensory neuropathy that included numbness, pain and hypoesthesia, and, in addition, 1 case also showed areflexia.[200,201] This effect on the sensory nerves would not be immediately obvious in animal experiments and probably should be investigated further.

Samples of sputa and adipose tissue collected from patients who suffered from Yusho contained chlorinated biphenyls on chemical analysis.[194] The concentration in adipose tissue was much higher than in sputa. Recently Kuratsune et al.[196] reported on the present status of the patients who suffered from Yusho. Many of the patients were still suffering from the illness. When the findings made in 159 patients in 1970 were compared with those observed in 1969, it was found that half of the patients had improved clinically, while the remaining half showed no such improvement and more than 10% of the patients had become worse.

Immunosuppression

Several reports have indicated that PCBs and possibly some of the chlorinated dibenzodioxins and furans may alter the immune response in animals. Atrophy of the thymus and reduced total white count were observed in rabbits following the dermal exposure to PCB fractions containing tetra- and pentachlorodibenzofuran.[96] In fish, exposure to Aroclor 1254 for 14 to 45 days led to emaciation and funguslike skin lesions.[93] However, no attempt was made to isolate organisms from these lesions. When 10-day-old mallard ducklings were fed diets containing PCBs (Aroclor 1254) and were subsequently injected interperitoneally with a duck hepatitis virus, significantly more ducklings died than when they received the virus alone.[202] In guinea pigs, sublethal dosage levels of 2,3,7,8-tetrachlorodibenzodioxin produced severe atrophy of the thymus with destruction of lymphocytes, lymphoid depletion in spleen and lymph nodes, as well as extensive hemorrhages into various organs including the adrenals. Reduced thymus and spleen weights were also observed in rats that received a single dose of 25 μg/kg TCDD and in mice the cell mediated immunity was found to be suppressed.[203] The various findings listed here do not conclusively prove that the immune response is specifically altered by these compounds as we encounter it following radiation exposure or therapy with alkylating agents. In most studies relatively high doses of the chemical compounds were given, resulting in general toxicity accompanied by weight loss. Weight loss in itself may lead to atrophy of the spleen. In humans maintained on a low caloric intake with negative nitrogen balance, a general reduction of lymphoid tissue results which is not specifically related to the immune response and vitamin A deficiency is also accompanied by a reduction in lymphatic tissue. In this respect it is also of interest that when monkeys were fed "toxic fat" containing polycyclic polychlorinated hydrocarbons. The germinal centers in the lymph nodes and spleen as well as islands of hematopoietic cells in bone marrow were extremely sparse.[160] This is more extensively discussed in connection with chick edema. The fact that a combination of duck hepatitis virus and PCBs is more toxic to 10-day-old ducklings than the virus alone suggests a reduced immune response but could also merely represent an additive damaging effect on the liver, made more susceptible to infection by the toxic effects of PCBs. So far no convincing evidence of "wasting disease" as a result of exposure to chlorinated polycyclic compounds has been reported in rodents or any other species in the literature.

Atrophy of the thymus in newborn rodents reduces the capacity of these animals to produce serum antibodies, resulting in wasting disease, also referred to as runt disease or homologous disease. Such animals are then incapable of rejecting foreign skin grafts,[204] which probably represents the most convincing evidence for homologous disease. A shift in the immune response could influence the development and growth of cancer and alter the defense mechanism in infections. In order to prove that PCBs as well as chlorinated dibenzodioxins and furans do indeed influence the immune response, the effect of these substances on newborn animals of several species should be tested with adequate parameters such as skin

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grafting and the measuring of the development of antibodies. Whether the other chlorinated polycylic compounds in this article also affect the immune response has not been suggested or reported.

Biochemical Effects and Chemical Toxicology

Porphyria

Porphyria cutanea tarda in humans is an acquired defect in hepatic porphyrin metabolism, characterized by uroporphorinuria, photosensitivity as manifested by blisters, and mechanical fragility of the skin. The hepatic porphyria (Porphyria cutania tarda) which is responsible for the increase in porphyrins and the skin photosensitivity can be produced experimentally by a number of drugs; all of these have the ability to stimulate the activity of the initial enzyme in the heme synthesis δ-aminolevulinic acid synthetase (ALA). In addition to this acquired porphyria, an acute intermittent porphyria erthropoietica and an acute intermittent porphyria hepatica, which are both hereditary, exist together with a number of subgroups which will not be considered here.

In 1964, Bleiberg et al.[2] reported studies of 29 workers in a 2,4-D (2,4-dichlorophenoxyacetic acid) and a 2,4,5-T (trichlorophenoxyacetic acid) factory. Many of the workers had chloracne and 11 had abnormal excretion of urinary uroporphyrins. Many of the workers with uroporphyrinuria had hirsutism, hyperpigmentation, and increased skin fragility. Liver dysfunction was noted in two hospitalized patients. Poland et al.[1] reinvestigated the workers of the same plant studied by Bleiberg et al.[2] They studied a total of 73 male employees; chloracne was found in 80% of the workers. In this study six years after the first report, clinical porphyria was not observed and only one worker had persistent uroporphyrinuria. In the Japanese poisoning epidemic (Yusho), increased uroporphyrin excretion was not reported.

Apparently polychlorinated biphenyls, as well as tetrachlorodibenzodioxins, produce hepatic porphyria in animals. Experimental hepatic porphyria was observed in Sherman strain rats when the rats were exposed to Aroclor 1254 in the diet. The porphyria resembled hexachlorobenzene poisoning and human porphyria cutanea tarda.[205] When female rats were fed 100 ppm Aroclor 1254 in the diet (approximately 7.9 mg/kg/day) they became porphyric after a delay of about 2 to 4 months. At this time the urinary porphyrin excretion increased rapidly. Uroporphyrin excretion was elevated to a maximum of 550-fold, prophobilinogen 27-fold, and δ-aminolevulinic acid 18-fold. The major porphyrins found in urine from Aroclor treated rats were 8-carboxyporphyrin (73%) and 7-carboxyporphyrin (16%). Substantial increases in amounts of 5 and 6 carboxcylic porphyrins were also observed. A 10-fold increase in the excretion of coproporphyrins was observed, but the increase in uroporphyrin excretion was 1,400-fold. In control rat urines, coproporphyrin (81%) represented the major porphyrin fraction. A good correlation between urinary porphyrins and liver porphyrins was also observed. Only a trace of porphyrin was found in liver samples in control rats.

When feeding rats 100 ppm Aroclor 1254, δ-aminolevulinic acid synthetase was not increased in the first month of exposure. After this time δ-aminolevulinic acid synthetase was elevated 3- to 6-fold in porphyric rats but was normal in nonporphyric Aroclor treated rats. Cytochrome P-450 and microsomal heme were also increased throughout the study but the increase was maximal in one week despite the absence of an increase of the rate limiting enzyme in heme synthesis δ-aminolevulinic acid synthetase. When a large single dose of Aroclor 1254 (500 mg/kg) was given, a 4-fold induction of δ-aminolevulinic acid synthetase could be demonstrated in the liver 5 hr after dosing the animals. These results suggested that δ-aminolevulinic acid synthetase induction occurred when porphyria had developed if lower levels of Aroclor 1254 were fed over a period of time, but when high doses of Aroclor were given it was induced immediately.

Vos and Koeman[95] reported that chemical porphyria was produced by the two European PCB products, Phenoclor DP6 and Clophen A60, as well as the American product, Aroclor 1260. The porphyrogenic action was observed in chickens, quail, and rats. It has not been established so far whether only certain isomers of the polychlorinated biphenyls or contamination with a chlorinated dibenzofuran is responsible for the production of hepatic porphyria cutanea tarda.

Poland and Glover[206] used fertile chicken eggs at a developmental stage of 15 to 20 days and injected them with 25 μl of the *halogenated dibenzo-p-dioxins*. They found that 2,3,7,8-tetrachlorodibenzo-p-dioxin produced a dose-related

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increase in ALA synthetase activity. The lowest dose tested, 4.66×10^{-12} mol/egg (1.5 ng) doubled the enzyme activity. These results were statistically significant.

Studies with other halogenated dibenzo-p-dioxins gave the following results: the 2,3,7-trichloro and 2,3,6-tribromo isomers are potent inducers of ALA synthetase while 2,3-dichloro and 2,7-dichloro, 2,8-dichloro, 1,3,6,8-tetrachloro, and 1,2,3,4-tetrachloro isomers all failed to induce ALA synthetase at doses up to 2.5 mg/egg. Of the limited number of halogenated dibenzo-p-dioxins that have been tested for fetal toxicity and the ability to produce chloracne, those that are toxic at low doses also induce ALA synthetase. Whether some of the polyneurotic symptoms reported in Yusho and also in severe exposure to technical 2,4,5-T or technical trichlorophenol that were heavily contaminated with tetrachlorodibenzodioxin are caused by an effect on the porphyrin metabolism merits investigation since neurotoxic effects have been reported in acute intermittent porphyria.[207]

The dioxins that induce ALA synthetase in the liver of the chick embryo may also induce it in the mammalian system. Woods[208] was not able to induce δ-aminolevulinic acid synthetase (ALA synthetase) in the liver of male rats up to 30 days after oral doses of 2,3,7,8-tetrachlorodibenzodioxin. Guinea pigs and mice also did not show an increase of ALA synthetase following the administration of 2,3,7,8-tetrachlorodibenzodioxin. However, it was possible to demonstate an increase in ALA synthetase and uroporphyrins in the liver in C_{57} black male mice given 25 μg/kg/week for 4 weeks.[209] It is possible that ALA synthetase induction varies in time with age and sex and may show species variation. Induction in mammals may require longer dosing, since, for instance, porphyria and induction of ALA synthetase with moderate doses of Aroclor 1254 did not develop until 2 to 4 months after dosing was started.[205] Female animals and the embryo may be more susceptible than adult male animals.

Effect on Other Enzymes and on Vitamin A

A number of investigators have shown that the *polychlorinated biphenyls* induce liver microsomal enzymes.[210,211] Norback and Allen[211] correlated an increase of hepatic microsomal enzyme activity with smooth endoplasmic reticulum proliferation. Litterst et al.[212] studied the effect of four PCB mixtures, namely, Aroclor 1242, 1248, 1254, and 1260. The animals were fed the material for 4 weeks and dietary levels employed were 500, 50, 5 and 0.5 ppm. At higher dietary levels an increase in liver weights was observed. Elevation of triglycerides in the liver was seen at 500 ppm with a maximum effect occurring at approximately 50% chlorine content. An increase in cytochrome P-450 was observed at the 50 and 500 ppm dietary levels of all PCB mixtures. An induction of the pentobarbital hydroxylation and an increase in methylase activity were observed at the higher dietary levels. Induction of pentobarbital hydroxylation increased with increasing chlorine content of the PCBs. Nitroreductase activity was markedly stimulated with increasing doses of PCB and the induction was seen at levels as low as 0.5 ppm for all mixtures. It is difficult to evaluate the enzyme induction at these low dietary levels since the control animal food can be contaminated at times with PCBs.

Two types of inducers of drug-metabolizing enzymes of the liver have been reported; one group to which phenobarbital belongs resulted in increased P-450 content of the liver and induction of a variety of pathways including benzpyrene hydroxylase and demethylation of ethylmorphine. The polycylic hydrocarbons such as 3,4-benzpyrene belong to the second group, which resulted in the formation of an abnormal cytochrome, P-448, and increased benzpyrene hydroxylation, but not ethylmorphine demethylation. Alvares et al.[213] reported that PCB treatment produced an increase in cytochrome P-448, an increase in benzpyrene hydroxylation, and N-demethylation. Since the PCBs represent mixtures, it is possible that the type of induction may depend upon the isomers present. The various effects of PCBs on liver microsomal enzymes can be blocked by the prior administration of actinomycin D. When the effect of a number of PCBs on the beef heart mitochrondrial enzyme system was studied, it was found that they inhibited NADH-oxidase, succinoxidase, and cytochrome oxidase activity. It has not been established whether these same effects will occur in vivo. Benthe et al.[214] induced liver microsomal enzymes by intraperitoneal injection of a single dose of Aroclor 1248 or 1232.

Most of the microsomal enzymes have been studied in the rat, but they are affected in other species as well. Allen et al.[215] reported liver

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hypertrophy after feeding polychlorinated biphenyls (Aroclor 1248) or a polychlorinated triphenyl (Aroclor 5460) for three months to primates. The increase in liver size was attributed to the increase in smooth endoplasmic reticulum and induction of microsomal enzymes was observed. Proof of the induction of microsomal enzymes in birds is the finding made by Lincer and Peakall[216] that livers obtained from American kestrels exposed to either Aroclor 1254 or Aroclor 1262 showed an increase in the in vitro breakdown of oestradiol to a more polar metabolite. This effect on sex hormones is also emphasized by Platonow et al.[217] Aroclor 1254 given to Yorkshire boars reduced the urinary excretion of gonadal steroids.

Both the *polychlorinated biphenyls* as well as the *chlorinated naphthalenes* affect the vitamin A content of the liver. This was particularly studied with the chlorinated naphthalenes since hyperkeratosis and other abnormal keratin formation of the skin is often accompanied by vitamin A deficiency. Low vitamin A plasma levels were observed in cattle with X-disease and young swine.[155,162] A decrease in vitamin A storage in the liver of Japanese quail and rats that were fed Aroclor 1242 was also demonstrated.[218] Since vitamin A deficiency affects reproduction, this aspect should be further investigated to determine whether the effect of PCBs on reproduction is secondary to vitamin A deficiency.[219]

Ecobichon and Johnstone[220] studied the effect of various PCB isomers on the hepatic enzyme system. Biphenyl itself did not cause induction of hepatic drug metabolizing enzymes and neither did monochlorobiphenyl (4-Cl). Substitution of chloro groups at the three and four positions of the di- and tetrachlorobiphenyls caused induction of microsomal monooxygenases. Induction of microsomal monooxygenases occurred also when hexa- and octachlorobiphenyls were given to the rats. Nitroreductase and carboxylesterase activities were not affected while all compounds produced a marked induction of the sulfobromophthalein conjugating system. *Terphenyls* as well as chlorinated terphenyls increased the pentobarbital metabolism in the rat with corresponding increase in smooth endoplasmic reticulum which suggested microsomal enzyme induction.[221,222]

Lucier et al.[223] reported that a single oral dose of 5 or 25 μg/kg body weight of *tetrachlorodibenzodioxin* induced cytochrome P-450 significantly in male rats for a period of up to 28 days. Cytochrome b-5 content was also increased. Hydroxylation of aniline was induced and aminopyrine demethylation rates were decreased. Microsomal proline was increased by approximately 15%. The induction of UDP glucuronyltransferase was most striking. Oxidative phosphorylation rates in rat liver mitochondria using succinate as a substrate were unchanged. The enzyme induction was observed as early as one day – the peak induction was reached between three and ten days after treatment. The enzyme induction was accompanied by an increase in smooth endoplasmic reticulum and a mild increase in rough endoplasmic reticulum.[224] These changes were first observed three days after the injection of the tetrachlorodibenzodioxin. Thus, enlargement of the liver during exposure to various chlorinated compounds is accompanied by an increase in smooth endoplasmic reticulum.

Furthermore, if "inclusions" or "hyaline bodies" are seen within the cytoplasm with the light microscope, concentrically arranged membranes that often surround lipid vacuoles can be seen with the electron microscope (Figure 6).

Hexachlorophene, on the other hand, does not increase the size of the liver.[118] The livers show no ultrastructural changes and probably microsomal enzymes are not induced. Whether other compounds listed in Figure 2 affect liver microsomal enzymes is not known. Hexachlorophene has an effect on a variety of other enzyme systems than those mentioned thus far. Cammer and Moore[225] and Caldwell et al.[226] have shown that hexachlorophene uncoupled oxidative phorphorylation, increased adenosine triphosphatase activity, but did not affect the activities of electron transport enzymes when compared to controls. Gould et al.[227] demonstrated that hexachlorophene inhibited succinoxidase activity of rat heart, liver, and kidney preparations. According to unpublished material cited by Gluck,[228] hexachlorophene is conjugated to the glucuronide conjugate which is excreted in bile. The proportion of conjugated hexachlorophene to unconjugated hexachlorophene in blood may be more significant in determining the toxicity of hexachlorophene than the hexachlorophene blood levels as they are presently determined.

Methemoglobinemia

In the early 1960s outbreaks of methemoglob-

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inemias were reported from hospital nurseries.[229,230] One outbreak involved a total of 18 infants with a level of methemoglobin from 4.9 to 30% of the total hemoglobin. An epidemiological investigation traced the outbreak to *3,4,4'-trichlorocarbanilide (TCC)* that was used as a 2% solution in a rinse in the laundering process, after which the laundry was neutralized with an acid and then dried in an autoclave. Twelve of the eighteen cases in this report were prematures who were probably longer exposed to the offending chemical than term infants since their hospital stay is usually longer. It is also possible that chemicals penetrate more easily through the skin of prematures.

In another outbreak, methemoglobinemia occurred among a group of custodial patients in an institution. These patients were given soap suds enemas with boiled soap that contained 2% TCC.[231]

It is possible that by heating TCC a breakdown product occurred which produced the methemoglobinemia. P-chloroaniline and 3,4-dichloroaniline have been suggested as breakdown products. None of the other compounds discussed in this article have thus far been shown to produce methemoglobinemia.

Storage, Distribution, Metabolism, and Excretion

As we progress further in our study on the distribution, metabolism, excretion, and absorption of compounds such as chlorinated biphenyls, chlorinated terphenyls, the chlorinated dibenzodioxins, and chlorinated naphthalenes, we will probably find that since they are lipid soluble they are stored in quite the same way as the more studied persistent pesticides such as DDT and dieldrin. From the evidence presently available on the chlorinated biphenyls it can be assumed that the turnover of these compounds in the body, particularly of those that have a high chlorine content, may be slower than some of the persistent pesticides. The turnover rate will vary with the degree of chlorination. Hexachlorophene, on the other hand, is fairly rapidly excreted and presently no information is available on bithionol and related compounds that have a germicidal effect. All of these compounds are not very well absorbed by the gastrointestinal tract or the skin.

Analysis of various tissues of rats given a single dose of the PCB Aroclor 1254[232] showed the highest concentration of PCBs in adipose tissue and the least in plasma. Brain, liver, and kidney had levels of the same order of magnitude but were lower. If, for instance, 1,600 mg/kg body weight of Aroclor 1254 were given by stomach tube, 24 hr later the mean concentration in adipose tissue was 1,146 ppm. The concentrations observed in individual animals varied greatly. Similar levels were found in adipose tissue when 3,200 mg/kg of Aroclor 1260 were given by stomach tube to adult female rats. The levels of Aroclor 1254 found in the brain showed a mean of 138 ppm and the values observed for Aroclor 1260 were 145 ppm. All of the values were calculated on a wet weight basis. The same concentrations in ppm were found with both compounds even though only half of the amount of Aroclor 1254 was given. This is probably due to the fact that only a certain amount of Aroclor is absorbed at a given time.

When 100 ppm of Aroclor 1254 was fed to male weanling rats for 58 days the mean concentration of Aroclor derived materials found in the feces varied roughly between 11 and 35 ppm. After the animals had been removed from the experimental diet for a week, the excretion in the feces was 8 ppm and after 16 days' recovery an average of 2.5 ppm was found in the feces. PCBs at the concentration of about 2 ppm were still observed in the feces 24 days after the animals were removed from the experimental diets. During the entire study the amount of PCB derived material recovered from the urine remained below 1 ppm. While PCBs were fed to the rats, the gas chromatogram obtained from the feces was superimposable on the one obtained from the standard Aroclor 1254 that was fed to the rats. Once the animals were no longer fed Aroclor 1254 the number of peaks observed in the gas chromatograms that resulted from the analysis of the fecal material decreased. Fewer peaks were also observed in the gas chromatograms obtained for the tissues and from urine. During the 58-day feeding period, a gradual increase in the concentration of the PCB derivatives was observed in tissues. The highest concentration was found in adipose tissue; after 58 days it reached mean values of about 500 ppm. The concentration in the liver only amounted to a mean of 12 ppm at that time. Following a recovery period of 71 days when the animals were fed plain chow, the concentration of the PCB derived material in adipose tissue had not decreased appreciably and

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still showed values in the neighborhood of about 400 ppm and 4.7 ppm was found in the liver. The concentration in the brain never reached very high proportions; it ranged from about 2 to 4 ppm. Following a 71-day recovery period the amount in the brain was still about 2 ppm. When Aroclor 1254 was fed for 240 days at the dietary level of 100 ppm, the concentration of the PCB derived material observed in adipose tissue had reached values of about 1,000 ppm. If, on the other hand, 100 ppm of DDT is fed to male rats for 2 years, the concentration in adipose tissue is about 95 ppm.[233] In a separate study where 500 ppm of Aroclor 1254 was fed to male rats for 6 months and the exposure to PCB containing diets was subsequently discontinued for an additional 10 months, the concentration of the PCBs ranged from 924 to 1,688 ppm in adipose tissue with an arithmetic mean of 1,192 ppm. The concentrations in the liver ranged from 17.3 ppm to 26.24 ppm with an arithmetic mean of 22.65 ppm.[234]

Further studies are necessary to determine whether PCBs are stored in tissues to a certain point when a steady state is reached and the concentration does not increase further. The given concentration at which this equilibrium is reached in adipose tissue would vary with the amount fed to the animals and the type of compound. The effect of starvation or illness when the amount of adipose tissue in the mammalian body is suddenly reduced also needs to be investigated further. Would this lead to increased excretion of the material or to redistribution or both? Dahlgren et al.[235] who determined PCB tissue levels in pheasants, found that a concentration of 300 to 400 ppm of the PCB derived material was usually found in the brains of birds that had died, while live birds that were fed the same amount of PCBs had much lower concentrations in their brain tissue.

Studies in bobwhite quail showed that relative peak heights and chromatograms for the tissue extracts were similar to the Aroclor standard after partitioning with acetonitrile-hexane when the birds were given a single capsule of Aroclor and their carcasses were analyzed soon after exposure. This suggests absorption of all components of Aroclor. The gastrointestinal tract, liver, skin, wings, and feet had been removed prior to analysis. When bobwhite quail were fed Aroclor 1254 for 14 days and then allowed to recover, the gas chromatograms showed a gradual reduction in the number of peaks and after 42 days on a control diet only 4 major PCB components remain, which suggested selective excretion and/or metabolism of some of the components of the Aroclor tested.[236] In a reproduction study, Heath et al.[99] analyzed 2 second-year eggs of mallards fed 25 ppm Aroclor 1254 in their diet for 2 years and found 56 and 33 ppm PCB by wet weight. The gas chromatogram showed a pronounced diminution of the early peaks when it was compared to the standard, suggesting again a selective reduction of the components with lower chlorine concentrations. This is also evident in environmental samples. Lower organisms such as mussels and fish contained a higher concentration of PCBs with lower chlorination than birds.[15]

In mammals, excretion of PCBs in milk and transplacental passage is also observed. Feeding cows 200 mg/day of Aroclor 1254 for 60 days[28] resulted in higher PCB levels in milk fat than in body fat. At 30 days' exposure, 45.6 ppm was found in milk fat and 21.9 ppm in body fat; at 60 days' exposure, 66.7 ppm was found in milk fat and 44 ppm in body fat. However, once exposure to PCBs was discontinued, the concentration in milk fat sank below that of body fat.

Transplacental passage of PCBs also occurred.[237,238] Sherman strain rats were given Aroclor 1254 in peanut oil by stomach tube during days 7 to 15 of gestation. A mean of 20.60 ppm PCB derived material was found in their milk when 10 mg/kg/day was given to the dams during pregnancy, while those given 50 mg/kg/day excreted a mean of 66.34 ppm in their milk on a wet weight basis. Fetuses taken by Caesarean section on day 20 of pregnancy contained a mean of 0.63 ppm and 1.38 ppm of PCBs, respectively. With a fivefold difference in dosage, the concentration in fetuses showed a twofold difference and in milk a threefold difference.[238]

The hyperpigmented babies observed in the Yusho incidents in Japan represent additional circumstantial evidence of transplacental passage of PCBs.

Polychlorinated biphenyls have also been detected in human adipose tissue. Biros et al.[239] analyzed two human adipose tissue samples which contained 200 and 600 ppm PCBs ranging from pentachlorobiphenyl to decachlorobiphenyl and Price and Welch[240] found 100 ppm PCBs in another human adipose tissue sample. According

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to Price and Welch,[240] 41 to 45% of the general population in the U.S. contain 1 ppm or more PCBs in their adipose tissue. Among PCB sources they discussed the food chain and house dust which, from residences in southwestern Michigan, contained up to 180 ppm PCBs. Polychlorinated biphenyls were found in 31% of 637 samples of human adipose tissue that were collected from the general population of the U.S., as part of the Human Monitoring Survey. Of the samples analyzed, 5% contained more than 2 ppm.[241] PCBs have also been found in human adipose tissues of the general population of Scandinavia[242] and Japan,[244,245] and in human milk and human adipose tissue of the general population of Germany.[243] In general, low concentrations of PCBs were stored in adipose tissue in a certain proportion of the general population. This is probably more prevalent in the highly industrialized countries and almost nonexistent in underdeveloped countries unless most of the food was imported from polluted areas. These low levels of PCBs are harmless, as far as we know at the moment; however, the occasional levels found in the 100 to 1,000 ppm range give rise to concern. It would be of interest to establish whether average adipose tissue levels increase with age and whether those in certain professions such as painters, printers, textile workers, workers in plastic factories, and those engaged in the manufacture of transformers and capacitors had higher PCB levels than the general population.

Reports on the absorption, storage, and metabolism of chlorinated naphthalenes, terphenyls, dichlorophene, bithionols, fenticlor, triclosan, and tetrachlorosalicylanilide are almost nonexistent in the literature. Some unpublished results on some of the compounds used as *germicides* are mentioned in the summary minutes of the Over the Counter Drug Panel on antimicrobials. Copies can be obtained by writing to Dr. Mary Bruch, Bureau of Drugs, Rockville, Md.

Approximately 9% of *triclosan*, also called Irgasan DP300 and CH 3565 (2,4,4'-trichloro-2'-hydroxydiphenyl ether), is absorbed when it is applied dermally in a soap solution. The halflife of the chemical was 20 hr when applied dermally and 10 hr when given intravenously. Humans excrete 65% in urine and 20% in feces after intravenous injection. It is excreted either as a free compound or as a glucuronide.

In dogs nearly 100% of the injected material is recovered as the glucuronide or sulfate in urine and feces over a 5-day period. It has been shown that TCC is absorbed through the skin.

Tissue levels of the *chlorinated terphenyl* Aroclor 5460 were determined in codfish.[246] In this study it was found that Aroclor 5460 was apparently poorly absorbed from the gastrointestinal tract; it was stored in all tissues that were analyzed. The highest concentration was found in the liver and following a single dose of 1 g some of it was still present in the codfish after 70 days, an indication of slow excretion. The authors pointed out that with the method used to determine the chlorinated terphenyls, it would be difficult to distinguish chlorinated biphenyls from chlorinated terphenyls. A comparison of the chromatograms of the standard with those obtained from chlorinated terphenyls extracted from tissues revealed more of the earlier peaks in material obtained from tissues and fewer of the later peaks. A variation in the intensity of the peaks was also observed. There seems to be a change in the chlorinated terphenyls within the living organism; however, part of this could also have been introduced as an artefact by the analytical methods or by differential absorption from the gastrointestinal tract.

Calves born to cows with X-disease developed hyperkeratosis and it was found that highly *chlorinated naphthalenes* were excreted in milk.[156] This suggests that chlorinated naphthalenes may well follow similar storage and excretion patterns as PCBs.

Chlorinated dibenzodioxins and *furans* are probably also stored in tissues of animals and men who are exposed to them, but again not much information is presently available. Firestone et al.[247] fed the unsaponifiable fraction isolated from "toxic fat" or 3% "toxic fat" itself to young cockerels. The "toxic fat" contained chlorodibenzodioxins with 2 to 8 chlorine atoms. When the excreta were analyzed it was found that a certain percentage of the hexachlorodibenzodioxins had been absorbed from the gastrointestinal tract, while all of the octachlorodibenzodioxins remained in the gastrointestinal tract, and only very little of the heptachlorodibenzodioxin was absorbed. Because of the presence of interfering components, the chick tissues and excreta could not be examined for the lower chlorinated dioxins. In the organs, the highest concentration was found in the liver and an appreciable amount

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was also found in the bone and skin, while the other organs contained very few dioxins. Some evidence for a possible metabolism of hexa- and heptachlorodibenzodioxin by the chick was also observed. Zitko and Wildish[248] fed di-, tri-, tetra-, and octachlorodibenzofuran to fish and detected only octachlorodibenzofuran in muscle and gut of dead fish. One problem with metabolism, storage, and distribution studies of chlorinated dibenzodioxins and furans is that not all of the compounds are readily available and, if they are available, any long-term feeding studies in large animals are expensive. The very toxic dioxin compounds can be given only at such low levels that they escape detection easily. In our laboratory we detected a chlorinated dibenzofuran in the urine of rats fed Aroclor 1254. We found this same chlorinated dibenzofuran in the Aroclor itself[40] as a contaminant.

Williams et al.[249] fed rats a total of 22.7 and 120.7 μg of octachlorodibenzodioxin. They recovered a great deal of the compound in the feces. Very small amounts were recovered in the liver at both dosage levels and in adipose tissue at the higher dosage level. Further studies are needed to establish the absorption, excretion, and storage of these compounds, and their persistence. Studies with labeled material may eventually give us some information on their metabolism. Piper et al.[250] gave a single oral dose of C^{14} labeled 2,3,7,8-tetrachlorodibenzo-p-dioxin to rats and found that about half of the radioactive material was recovered within 21 days in the feces and only 13% in urine and 3% in expired air. Most of the remaining radioactive material was found in the liver.

In another study[251] 100 μg of Cl^{36}-octachlorodibenzodioxin was given daily to rats by stomach tube for 21 days. Less than 0.3% of the material was absorbed. Subsequently the rats received a control diet for 7 weeks and still showed 20% of the absorbed material found at 21 days in their tissues.

The in vivo metabolites of the various compounds under discussion have in most instances not been identified. Block and Cornish[252] studied the urinary excretion of biphenyl and 4-chlorobiphenyl in rabbits. Biphenylglucosiduronic acid and 4-hydroxybiphenyl were isolated from urine of rabbits fed biphenyls. The rabbits fed the 4-chlorobiphenyl excreted 4-(p-chlorophenyl)-phenol and 4-chlorobiphenyl glucosiduronide. Twice as much 4-chlorobiphenyl as biphenyl was excreted as the glucosiduronic acid derivative. It is possible that other low chlorinated biphenyls and naphthalenes are excreted in urine in a similar manner; whether they would be detected by present gas chromatographic methods would have to be checked by combining the identification of radio labeled material with gas chromatography.

Hutzinger et al.[253] found evidence that some chlorinated biphenyls are hydroxylated by some species. Apparently trout is not able to hydroxylate 4-chlorobiphenyl, 4,4'-dichlorobiphenyl, and 2,2',5,5'-tetrachlorobiphenyl, while the rat and the pigeon both formed monohydroxychlorobiphenyl from 4-chlorobiphenyl. In addition, the rat also produced dihydrochlorobiphenyl. Large quantities of unchanged material were excreted by the rat when 4,4'-dichlorobiphenyl and 2,2',5,5'-tetrachlorobiphenyl were given to them and only a monohydroxyderivative was identified in their urine. Pigeons followed a similar pattern but no dihydrochlorobiphenyl was observed in the bird excreta fed 4-chlorobiphenyl. No hydroxy metabolites could be identified in the excreta of the three species when they were given 2,2',4,4'5,5'-hexachlorobiphenyl. No evidence of reductive dechlorination was observed in any species.

Only very few studies have been conducted with radiolabeled *hexachlorophene* to study its absorption, distribution, and metabolism. Wit and Van Genderen[254] gave rats and rabbits hexachlorophene-C^{14} (2,2'-methylene-C^{14} bis(3,4,6-trichlorophenol). Complete recovery of radioactivity in excreta after a single dose of 50 or 15 mg/kg of radioactive hexachlorophene was observed in rabbits within 5 days. Roughly one third was excreted in urine and feces, respectively, as unchanged hexachlorophene and the remainder was excreted in the feces as unidentified metabolites. The metabolites could not be extracted from the feces with ethanol. The biological halflife calculated from the experimental results was found to be approximately 17.8 hr. In rats, about two thirds of the radioactivity was recovered in feces within seven days and only very little in urine. A complete recovery of radioactivity was not obtained in these studies within seven days. Five cows given a single dose of 15 mg/kg body weight of hexachlorophene excreted about half to two thirds in their feces within five days. Very little was excreted in urine and none in milk.

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Metabolites were not identified and tissue levels were not determined in this study. The authors cited a study by Kok,[255] who did determine hexachlorophene in various organs of rats.

Carroll et al.[256] studied the absorption of C-14 labeled hexachlorophene on the tail skin of the rat. Hexachlorophene in a detergent solution was absorbed by the intact skin of the tail at the average rate of 1.7 $\mu g/cm^2$ of exposed surface area/hr. Immediately after the infliction of second degree burns, the absorption rate increased 2.5 times that of the normal skin surface. After 24 hr the absorption rate dropped to normal or subnormal levels. Immediately after traumatizing the tail, the absorption rate of hexachlorophene was even greater than following the infliction of a burn wound.

Manowitz and Johnston[257] found that hexachlorophene was deposited on the skin when used in various formulations and could be extracted from the skin by soaking the exposed areas (hands and forearms) in alcohol. The amount recovered increased in proportion to the amount applied. These authors were unable to confirm earlier investigations which suggested that the quantity of hexachlorophene retained by the skin reached a plateau level after a number of washings and remained relatively constant thereafter. Arms were washed and rinsed every 20 min. When extractions with alcohol were made on different arms after different numbers of washings, it was found that the amount of hexachlorophene recovered increased with the number of washings and no plateau was reached. A greater amount of hexachlorophene was also deposited on the skin with increasing length of time of a single washing. Since we now know that hexachlorophene is also absorbed from the intact skin and some of it is probably also lost from the surface of the skin, the time interval between the washings plays a role in the amount of hexachlorophene found to accumulate on the skin. Also of interest is that bathing water containing 4 mg/l of hexachlorophene deposited approximately the same amount of the chemical on the skin as a single washing with a 2% hexachlorophene soap.

Since hexachlorophene was used very extensively and often unknowingly by the general population, we investigated whether it was present in the blood of a few random samples of the general population. We found that the arithmetic mean concentration of hexachlorophene in adults was 0.028 ppm ($\mu g/g$) of whole blood of a total of 14 samples. In infants who were washed with hexachlorophene in the nursery the amount of hexachlorophene found in whole blood after the infants had anywhere from 2 to 14 washes averaged 0.109 ppm ($\mu g/g$ whole blood). Blood obtained from the cord at the time of birth contained an arithmetic mean of 0.022 ppm hexachlorophene.[258,259]

In the rats that were used in a reproduction study and were fed 100 ppm hexachlorophene in the diet for 258 days, the hexachlorophene blood levels averaged 1.21 ppm, while the blood levels of rats fed 500 ppm of hexachlorophene for 55 days who had severe symptoms of poisoning amounted to 8.5 ppm.[259,260] At the time we reported these blood levels, our recovery rate was only about 75%. All blood levels reported at that time were determined in whole blood. (It is assumed at the moment that blood levels are equivalent to half the amount of that found when plasma is analyzed.) A study undertaken at the request of Sterling Laboratories[132] illustrated that newborn rhesus monkeys following total body bathing with a 3% detergent solution for 1 week had blood levels of about 1.5$\mu g/ml$ (ppm). In subsequent months a plateau effect was observed and at 90 days the blood level was equivalent to 1.1 $\mu g/ml$. These monkeys showed status spongiosus of the white matter of the brain. this indicates that at least in the monkey, blood levels of about 1.5 $\mu g/ml$ whole blood were consistent with fluid accumulation in the myelin sheath of the central nervous system.

Lockhart[132] cited unpublished studies by Gluck, who found that hexachlorophene blood levels in term infants after washing with a 3% hexachlorophene detergent solution were somewhat higher than those reported by us with a mean concentration of 0.345 ppm ($\mu g/g$ whole blood). This difference could have been due to a difference in washing procedure and a better recovery rate in the analytical method in Gluck's study, or both.

Premature infants apparently absorbed hexachlorophene more readily. In 15 premature infants that were washed with a 3% hexachlorophene detergent solution, the average blood concentration amounted to 0.475 ppm ($\mu g/g$).[261] Abbott et al.[262] using a different washing regimen found a mean hexachlorophene blood concentration 12 to 18 hr after the initial wash of 0.154 ppm ($\mu g/g$

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whole blood) and on the 7 to 9th day of life, 24 to 48 hr after the second or third application of hexachlorophene, the mean blood level amounted to 0.287 ppm (μg/g). The amount found in cord blood of 16 infants was 0.013 ppm (μg/g). When hexachlorophene was applied only on the day of delivery, the hexachlorophene blood levels gradually decreased from a mean of 0.145 ppm on the first day to a mean of 0.057 ppm on the ninth day, following an increase to 0.194 ppm on the fourth day. The authors suggested that premature infants may absorb more hexachlorophene through their skin since in one infant 29 weeks of gestation and weighing 1.42 kg the blood level was 1.17 ppm 24 hr after the initial wash and 0.655 ppm at 48 hours. Alder et al.[263] reported mean hexachlorophene blood levels of 0.18 ppm in infants who were dusted with a powder containing 0.33% hexachlorophene.

Adults using hexachlorophene preparations also absorbed it via the skin.[132] The blood levels obtained in adults that exposed their total body surface to a 3% hexachlorophene preparation twice daily for 60 days, 5 min each time, showed blood levels of approximately 0.68 ppm (μg/ml whole blood) with a range of 0.25 to 1.08 ppm (μg/ml whole blood). If only hand washing was employed for a period of 28 days, 4 times daily, a mean blood level of 0.07 ppm was reached and in a face hand washing study the mean blood level after 28 days was 0.196 μg/ml.

Blood levels obtained in a patient with burns who died between 24 and 48 hr after the last hexachlorophene application were 2.2 ppm (μg/g whole blood). The blood levels that have been reported all indicate that following dermal hexachlorophene exposure the chemical can be found in blood, and from information through animal studies as well as some human experience, levels of approximately 1.5 to 2 ppm (μg/g) in whole blood in humans are presently considered to represent toxic levels if the analysis for blood was performed with the methods mentioned elsewhere in this article.

Probably a better way of assessing a toxic dose would be to measure the amount of hexachlorophene in the brain, but for obvious reasons that is not possible. Female rats fed hexachlorophene at the rate of 500 ppm (25 mg/kg body weight/day) for 55 days with very definite lesions had about 4 ppm hexachlorophene in their brain and 3.91 ppm in the liver. Squirrel monkeys given 5 mg/kg body weight/day of hexachlorophene for 38 days showed a mean hexachlorophene concentration in the brain of 0.38 ppm and in adipose tissue the hexachlorophene concentration was 0.94 ppm.[264] The recovery rate in this study was only 75%. These squirrel monkeys had no overt clinical signs of toxicity, but showed mild status spongiosus of the white matter of the brain. Electroencephalographic changes had also been observed in these primates. Ulsamer et al.[265] found hexachlorophene brain levels of about 1 ppm in newborn rats with signs of central nervous system toxicity. In 1 suspected case of human hexachlorophene poisoning in a child, the hexachlorophene brain level was about 2.2 ppm.[261] Adult rats dying after the administration of a single dose of hexachlorophene showed concentrations of 6 to 9 ppm of hexachlorophene in the brain.[266] Some variation of the concentration of hexachlorophene in the brain which can be related to neurotoxic symptoms must be expected in different species and perhaps also with age and sex. Whether the fluid accumulation which may occur in the myelin sheaths after repeated exposure to hexachlorophene would alter hexachlorophene brain levels has thus far not been reported.

With present methods, we are probably only determining free hexachlorophene while the conjugated portion remains undetected. The toxicity of hexachlorophene will vary with the ability of the organism to conjugate, metabolize, and excrete this material.

We found in rats that hexachlorophene is excreted in milk of dams fed hexachlorophene at the rate of 20 and 100 ppm in the diet (2.3-1.1 mg/kg/day and 11.8-5.5 mg/kg/day, respectively, for 241 days). Electron capture, gas-liquid chromatographic determinations revealed levels of 0.07 and 0.33 ppm in the milk. Since hexachlorophene did not seem to accumulate to a great extent similar values would probably have been obtained, had the levels in milk in these animals been measured earlier. Although we now have some information on the absorption and distribution as well as excretion of hexachlorophene, the possible storage of the material as well as its metabolism needs further investigation. Since hexachlorophene was found in cord blood of infants, it can be assumed that transplacental passage also occurred.

Morphologic Effect on the Liver

Some of the chlorinated naphthalenes, chlori-

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nated dibenzodioxins, chlorinated dibenzofurans, the chlorinated biphenyls, as well as the chlorinated terphenyls have an effect on the liver in various species, including humans. Bennett et al.[267] compared the toxicity of differently chlorinated naphthalenes and a biphenyl that contained 65% chlorine. Tri-chloronaphthalene apparently was less hepatotoxic than the higher chlorinated compounds and the chlorinated diphenyl was the most toxic of all. The studies were done in rats and the exposure was by feeding as well as inhalation. The findings described in the liver consisted of liver necrosis, fat accumulation, and the presence of hyaline bodies in the cytoplasm of the liver cells. A marked deposit of finely divided granular yellow or yellow-brown pigment was observed in Kupffer cells, particularly in those animals that had been exposed to the chlorinated biphenyls. This pigment apparently did not stain characteristically for either hemosiderin or hemofuchsin. Of course, all livers were enlarged macroscopically; in addition, these authors described proliferative changes in occasional bile ducts, particularly after feeding mixtures of chlorinated naphthalenes and chlorinated biphenyls. The number of bile ducts was increased in certain areas and the epithelial cells lining the bile ducts showed mitotic figures.

In inhalation studies with low concentrations of PCBs which consisted of either a 16-hr daily exposure to average concentrations of 0.57 mg/m^3, or to 8 hr daily with an average concentration of 0.93 mg/m^3, neither group of rats appeared ill. The periods of exposure varied from 37 to 143 days. When the animals were sacrificed the livers were pale or slightly yellow and somewhat mottled. Microscopic examination of the livers revealed similar changes as had been observed after feeding the material, and an increased vacuolation of the liver cells. Microscopic evidence of recovery was not observed in the livers of rats that had been exposed to the chlorinated diphenyl for a total of 105 days and were removed from exposure for 2 months.

Similar findings were made by Miller[268] who studied the effect of a PCB which contained approximately 42% chlorine. This author observed varying degrees of liver damage after subcutaneous injection as well as oral ingestion of the toxic substance in guinea pigs, rats, and rabbits. The liver damage consisted of fatty degeneration and atrophy of the central lobular cells. In the rat, hyaline bodies within the cytoplasm of the liver cells were also noted. Most of the liver damage was found in the guinea pig, less in the rabbit, and least in the rat.

Nishizumi,[269] who studied the effects of a Japanese biphenyl Kaneclor 400 with 40% chlorine in monkeys and mice, described enlargement of liver, fatty changes, granular cytoplasm of the hepatocytes, increases in the size of Kupffer cells and hepatocytes, and a brown pigment in some of these cells. He also studied the livers under the electron microscope and found an increase in smooth endoplasmic reticulum, variation in the appearance of mitochondria, and an increase in the number of microbodies. In addition, "myelin figures" were observed in the cytoplasm of hepatocytes.

Vos and Koeman[95] observed liver necrosis in chickens that were dosed with European PCBs, while a Monsanto product with 60% chlorine did not produce this effect. Increased amounts of iron were demonstrated with Perl's iron stain in the livers of all chickens exposed to the European as well as the American PCB sample. Vos and Beems[96] studied the PCB-induced lesions in the liver of rabbits, following dermal exposure to Aroclor 1260, Phenoclor, and Clophen. The microscopic changes observed in the liver included fatty degeneration, focal necrosis, centrolobular liver cell atrophy, ceroid pigment in Kupffer cells, periportal fibrosis, and cytoplasmic hyaline degeneration.

The feeding of Aroclor 5460, a chlorinated terphenyl, Aroclor 1254, or a chlorinated dibenzo-p-dioxin, not further specified in the article, led to liver hypertrophy of Sprague-Dawley rats within eight days to three weeks. Ultrastructural alterations were similar in the three groups and consisted of numerous multilayered, concentric membrane arrays and proliferated smooth endoplasmic reticulum. In many instances these membrane arrays surrounded lipid droplets.[270] Rabbits given 300 mg of Aroclor 1242 and 1254 once a week for 14 weeks developed enlarged livers and the ones given Aroclor 1254 showed midzonal necrosis of the hepatic lobules. The livers of rabbits fed Aroclor 1221 in a similar fashion did not show any histologic changes.[271]

We conducted studies to compare the toxicity of Aroclor 1254 and Aroclor 1260 in Sherman strain rats. The acute effect of these two mixtures on the liver was negligible. If rats were given a

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FIGURE 3. Section of liver of a rat fed Aroclor 1260 for 90 days. This figure illustrates a mitotic figure and a hyperchromatic nucleus (arrows) H & E X 500.

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single dose of 10.000 mg/kg in peanut oil by stomach tube, and were sacrificed the next day, the liver was essentially normal microscopically. Doses of 1,000 ppm (72 mg/kg) of Aroclor 1260 for 3 months produced only an increase in mitotic figures (Figure 3) and enlarged hepatocytes. When groups of rats were fed dietary levels of 20, 100, 500, and 1,000 ppm of Aroclor 1260 and 20, 100, and 500 ppm Aroclor 1254 for 8 months, the liver changes were pronounced.[272] The hepatocytes were enlarged in many of the rats of the experimental groups and lipid accumulation, foamy cytoplasm, and a brown pigment which stained partially positive for hemosiderin were observed in the livers. The pigment was primarily observed in macrophages and Kupffer cells. Inclusions (Figure 4) that stained slightly more eosinophilic than the surrounding cytoplasm were present in the cells. These inclusions within the cytoplasm have been described for many compounds such as mirex[273] (dodecachloro-octahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalene), DDT[274], 1,1,1-(trichloro-2,2-bis(p-chlorophenyl) ethane dieldrin[275], 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a-5,6,7,7,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene, and piperonyl butoxide[274] (α-(2(-butoxyethoxy)ethoxy)-4,5-methylenedioxy-2-propyltoluene). They are identical to the so-called hyaline bodies that were described in the earlier literature by Bennett et al.[267] and Miller.[268]

Aside from these general changes, extensive, grayish-white, firm, glistening areas were noticed in the livers of a number of the experimental animals, particularly at the high dietary levels. Microscopic examination of these grayish-white areas showed that the hepatic parenchyma had been replaced by glandular pale staining epithilial cells that formed ducts and were surrounded by proliferating fibrous tissue (Figure 5). Larger lesions often had extensive fibrosis and also contained collagen and the ducts that were formed by epithilial pale staining cells were markedly dilated and contained necrotic debris or mucus. The lesion has been classified as adenofibrosis for the time being. It has been described in detail by Edwards and White[276] who observed it in rats that were fed butter yellow (p-dimethylamino-azobenzene). These lesions enlarge peripherally by the formation of new glandular structures. Hepatic

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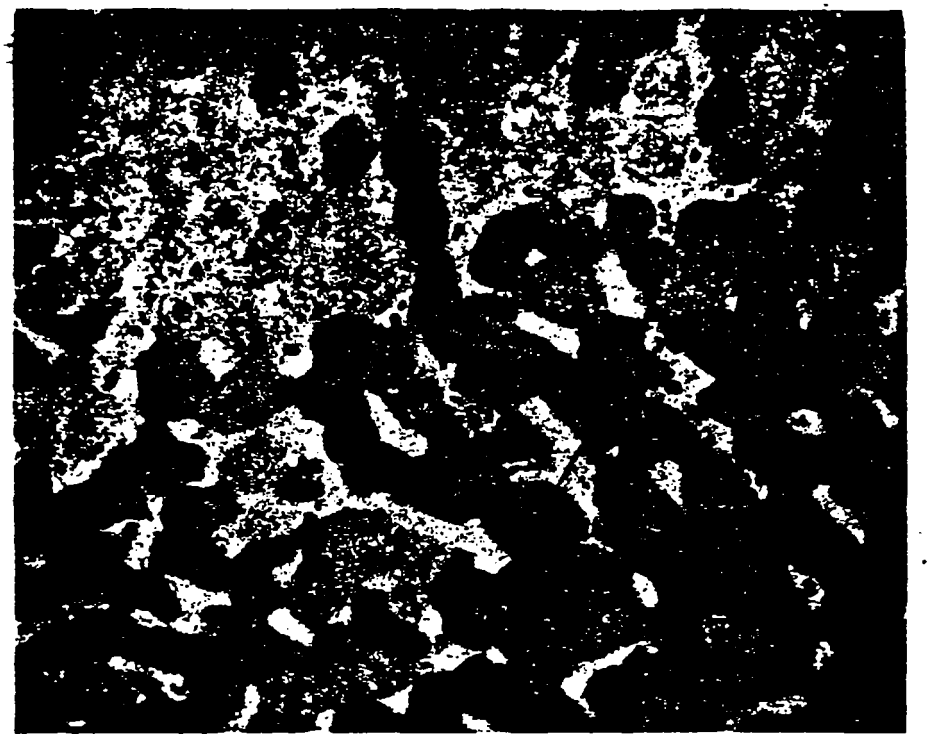


FIGURE 4. Section of liver from rat fed Aroclor 1254. A cluster of inclusions is present in the cytoplasm of one hepatocyte (arrow). Usually individual cells contain only one of these formations at a time. Toluidine blue stain of maraglass embedded material X 300.

tissue may be trapped between the glands of the peripheral area of adenofibrosis. The older central parts of the lesions sometimes contain pigmented avascular collagenized stroma with atrophic epithelial cells lining dilated ducts that appear atrophic and dead. The glands of actively growing adenofibrosis show atypical epithelium with large pleomorphic nuclei. The epithelium can occasionally become stratified. Parts of the lesion may be quite extensive, while in addition, in many areas of the same liver small foci of adenofibrosis exist simultaneously in close relationship to portal areas. It has therefore been assumed that these lesions arise from bile ducts, but they can also be primarily confined to the region of the hepatic veins. The absence of bile ducts around hepatic veins led to the conclusion[277] that a precursor, other than bile ducts, existed from which adenofibrosis developed. However some investigators of the lesions have assumed that it represented an atypical bile duct proliferation, also referred to as cholangiofibrosis.[278] In addition to this lesion we also observed small clusters of glandular cells within areas of adenofibrosis as well as adjacent to blood vessels that were surrounded by relatively normal appearing hepatic parenchyma with staining characteristics of salivary gland tissue. These clusters of cells very closely resembled pancreatic tissue.[279] The distinction between adenofibrosis and adenocarcinoma is difficult. The glands are quite atypical in adenofibrosis but it may be helpful to remember that in adenofibrosis, in contrast to adenocarcinoma, the irregularity observed is limited and duplicated with considerable exactness from lesion to lesion. Mucus is always present in adenofibrosis, but not always in adenocarcinoma. The stroma of adenofibrosis is concentrically arranged, young and cellular at the periphery and old and hyalinized towards the center of the lesion.[277] Of course a liver showing adenofibrosis may also contain carcinomatous lesions. So far conclusive evidence is lacking in the literature that adenofibrosis gives rise to carcinomas. However, it has been found to coexist with carcinoma of the liver in rats and some investigators feel that the lesion is in fact a precursor of cancer.[280] Choline deficient rats apparently, in addition to other changes in the liver, also develop

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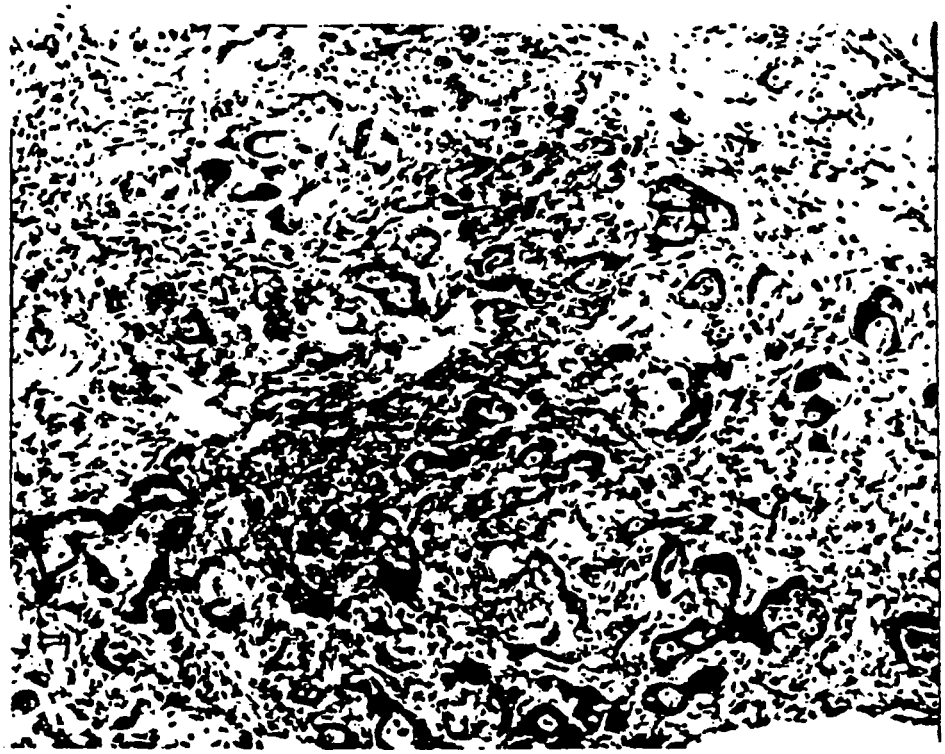


FIGURE 5. Section illustrates an area of adenofibrosis of the liver of a rat fed Aroclor 1254. Note the epithelial cells forming small ducts. The ducts contain cellular debris and are surrounded by fibrosis which can become quite pronounced, H & E X 125.

adenofibrosis.[281] However, these rats were fed peanut meal and soymeal and therefore the possibility exists that the experimental diet was contaminated with a mycotoxin. Bennet et al.[267] also described the lesion (adenofibrosis) in rats when a mixture of chlorinated naphthalenes and chlorodiphenyls was fed to them. A critical review of the histopathogenesis of this lesion was made by Stewart and Snell.[277]

Ultrastructural changes of the livers of animals exposed to the Aroclor consisted of an increase in smooth endoplasmic reticulum and atypical mitochondria. Many lipid vacuoles were observed, particularly at the higher dietary levels. They were at times surrounded by concentrically arranged membranes. The "inclusions" or "hyaline" bodies observed in the cytoplasm with the light microscope correspond to these formations when they are examined under the electron microscope. Their ultrastructural appearance has led to their being referred to as "finger prints" (Figure 6). The epithelial component of the adenofibrosis outlined consisted of cuboidal or columnar cells with the free surface lined by microvilli, granular cytoplasm with many ribosomes, and a few endoplasmic membranes. Some of the cells contained a great deal of mucus and resembled goblet cells. Tonofilaments and terminal bars were also observed. These various observations suggested that the epithelial component of adenofibrosis had all the morphological characteristics of bile duct epithelium.

Chickens with chick edema disease and cattle with the X-disease, already mentioned, usually showed changes in the liver which consisted predominantly of liver cell necrosis and fat accumulation. The hepatotoxic effect of chlorinated naphthalenes has also been described in rats.[267]

In a number of reports on the occupational occurrence of chloracne in workers that were exposed to chlorinated naphthalenes, chlorinated diphenyls, or 2,4,5-T, and technical trichlorophenol, reference is made to the hepatotoxic effect of these compounds. This hepatotoxic effect can manifest itself only in a decreased tolerance for alcohol; it may also develop into hepatic deficiency with acute yellow atrophy and jaundice. Apparently the effect on the liver was

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FIGURE 6. Electron micrograph of a portion of the cytoplasm of a hepatocyte. The section shows concentrically arranged arrays of membranes which surround vacuoles containing lipid. Lead citrate, uranyl acetate X 34,200.

particularly bad following exposure to halo-waxes, which are chlorinated naphthalenes.[282]

Cotter[283] reported seven cases of pentachlorinated naphthalene poisoning in workers who were engaged in manufacturing wire cable during World War II for the navy. Four of the workers developed jaundice and two died. Microscopic examination of the liver of the two workers that died showed complete loss of liver cells in some areas, the centrolobular areas were hemorrhagic, and prominent bile duct proliferation was seen in the periphery.

Another group of compounds that have at least some representatives that cause a hepatotoxic effect are the chlorinated dibenzodioxins and chlorinated dibenzofurans.

Single oral doses of 1 to 10 μg/kg *2,3,7,8-tetrachlorodibenzodioxin* given to rabbits produced a hepatotoxic effect. At the higher dosage level the animals died and the lower doses caused serious liver damage.[108] Schulz[107] had reported earlier that single oral doses of 20-50 μg/kg body weight resulted in fatal liver necrosis in rabbits.

Weanling rabbits were treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin by applying a dose of 1.67 μg daily to the inner aspect of both ears for three days. The total dose given was 7.05 μg/kg body weight. When these rabbits were sacrificed 18 days following the last application of the dioxin, their livers were significantly larger than those of the controls. Microscopic examination of the livers showed enlarged hepatocytes. Some of the hepatocytes were multinucleated. The cytoplasm was foamy or vacuolated. A light brown pigment in some liver cells as well as Kupffer cells was also observed. Inclusions were seen in the cytoplasm in some liver cells, slight interstitial fibrosis was present, and two of three rabbit livers examined showed foci of necrosis that were surrounded by fibrosis.[284]

Studies with "toxic fat," the cause of chick edema disease, showed that it had a necrotizing

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effect on the liver in chickens. As we now know, the toxic agents responsible for chick edema disease are chlorinated dibenzo-p-dioxins. Toxic fats also altered liver morphology in Macacca mulatta monkeys.[285] Hepatocytes were enlarged, multinucleated and focal necrosis in the centrolobular zone was observed. Many liver cells were vacuolated and stained positive for neutral fat.

In our studies of rats given *hexachlorophene*, we did not observe any liver pathology with the light microscope and no change with the electron microscope at the dosage levels that we employed.[118] Thorpe[121] on the other hand, observed periportal fatty changes in livers of sheep given three or four doses of 50 mg/kg hexachlorophene and histochemical changes were also observed in the hepatocytes. Pugh and Crowley[286] also observed hepatotoxicity in sheep after giving them hexachlorophene.

Of the other germicides discussed in this article, only triclosan (Irgasan DP-300) (2,4,4-trichloro-2'-hydroxydiphenyl ether) has been reported to have a toxic effect on the liver at a dose of 125 mg/kg and enlarged the liver of rats at a dose of 25 mg/kg, according to the summary minutes of the OTC Panel, U.S. Food and Drug Administration. It was not stated whether these were single or repeated doses. Oral administration of triclosan to beagle dogs produced jaundice and severe liver damage was observed. The effect that these compounds have on the liver will have to be studied further.

The accumulation of fat in the liver indicates that either fat transport or fat metabolism is impaired; if the insult to the liver is removed, the liver gradually recovers unless the lesion has become irreversible. The increase in smooth endoplasmic reticulum as well as the "inclusions" will also disappear after a certain amount of time if exposure to the material is discontinued.[287] The significance of the increase in smooth endoplasmic reticulum and the hyaline bodies with a concomitant rise in liver microsomal enzyme activity is presently not understood. These changes are considered adaptive and beneficial, but in addition to metabolizing unwanted chemicals at a higher rate, the stimulated liver also metabolizes some medications more rapidly,[288] as well as substances produced by the body such as steroids. Whether the inclusions within the cytoplasm represent the so-called inactive smooth endoplasmic reticulum which is deposited in the form of arrays of membranes within the cell is not known. The fact that smooth endoplasmic reticulum may become hypoactive was first postulated by Hutterer et al.[289]

Fibrosis of the liver as well as necrosis and the adenofibrosis observed after PCB exposure in rats should definitely be considered a very serious lesion. Usually adenofibrosis occurs concomitantly with hepatomas or hepatocarcinomas in rodent livers. A recent publication by Kimura and Baba[289a] showed that hepatomas can be induced in rats with Kanechlor 400. Chronic feeding studies may show that other PCBs will also induce hepatomas in rodents.

Neurotoxicity

In some of the reports on chloracne and also in the Yusho incident, peripheral neuropathy is mentioned as well as psychic alterations[1,290] as part of the symptomatology of poisoning, but in most reports not much emphasis was put on it. In the Yusho epidemic Murai and Kuroiwa[290] performed more detailed examinations in 21 cases admitted consecutively to Kuyushu University Hospital in the northern part of Japan. Seven of the patients complained of numbness or pain in the peripheral portion of their extremities and in five cases, hypoesthesia, hypalgesia, and thermohypesthesia were noted. Slowing as well as reduction of the sensory nerve conduction velocity in the radial and sural nerve was observed in a number of these patients. Headache and peripheral nervous system symptoms were also reported in a poisoning episode which occurred in a Finnish company where paper was impregnated with biphenyl.[92]

The only chemical of the group of polychlorinated, polycyclic compounds discussed in this report that has a predominant central neurotoxic effect in animals as well as humans is hexachlorophene. Adult female rats that were fed 500 ppm hexachlorophene in the diet for 14 weeks (about 30 to 18 mg/kg body weight/day) developed leg weakness after 12 to 19 days of exposure. This leg weakness which was only evident in the hindlegs progressed to paralysis within 3 to 5 weeks.[291] At autopsy the brains of the exposed rats were enlarged; they weighed an average of 2.6 g while the control brains weighed an average of 1.99 g. Microscopic examination of the brains revealed normal gray matter throughout the brain and the spinal cord in the few instances that the spinal

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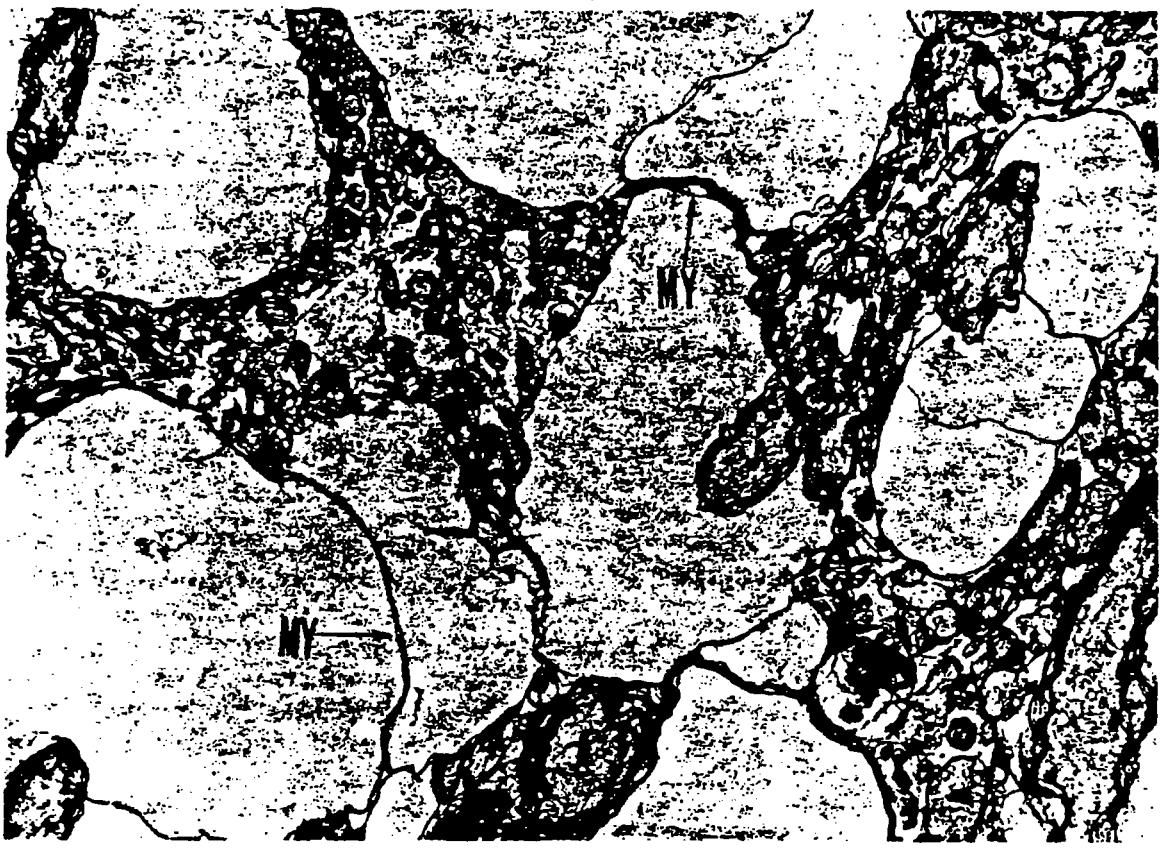


FIGURE 7. Electron micrograph from the white matter of the brain of a rat, illustrating status spongiosus. Note the large empty appearing vacuoles lined by myelin (MY = myelin, A = axon). Lead citrate, uranyl acetate X 34,200.

cord was examined. The white matter on the contrary seemed widened and showed many cystic spaces that appeared empty and were lined with strands of material that stained positive for myelin. The vacuoles were very numerous and varied greatly in size giving the tissue a spongy appearance. The brains in these studies were usually fixed in either Bouin's solution or buffered formalin for light microscopic study.

Brain tissue, particularly that of rodents, may normally show small vacuoles that are considered to represent an artefact.[292] These vacuoles are smaller, usually very uniform, and at times contain a homogeneous pink staining material. They are usually separated by a wider area of normal appearing white matter than the vacuoles in status spongiosus. Brains that show this artefact do not weigh significantly more than those that do not show it.

Electron microscopic examination of brains of rats given hexachlorophene showed that the vacuolation in the white matter of the brain revealed large vacuoles lined by myelin. The axons that were present appeared usually to be quite normal; occasionally strands of myelin had separated and traversed the vacuoles. The vacuoles were otherwise empty except for some electron dense granular material (Figure 7). No other alterations were observed in the white matter. This lesion is not unique for hexachlorophene; other chemicals that have produced status spongiosus as we have called the lesion include triethyltin,[293] isoniazid,[294] the halogenated benzanilide, 2-acetoxy-4-chloro-3,5-diiodobenzanilide,[295] and rafoxanide,[296] which is the 3,5-diiodo-3'-chloro-4'-(p-chloro-phenoxy) salicylanilide. Triethyltin seemed to produce the lesion at a much lower dietary level and more rapidly than the other chemicals mentioned, particularly hexachlorophene.

In an additional study, after feeding rats 500 ppm hexachlorophene in the diet for 10 weeks and then discontinuing the exposure to hexachlorophene, function began to return in the hindquarters of paralyzed rats after 2 weeks and within

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6 weeks the rats had almost completely recovered clinically. However, microscopic examination of the brains of these rats showed that a few cystic spaces were still present 12 weeks after the exposure to hexachlorophene had been discontinued. In weanling rats, but not in adult rats, the brain lesion and symptoms of paralysis could be produced with a single oral dose of 100 mg/kg of body weight given by stomach tube in peanut oil.[118] Part of the reason for being able to produce the brain lesion in the weanling rats with a single dose may be related to the fact that the weanling was able to survive a higher single dose than the adult rat.

Other species that have also shown paralysis and other neurological deficits after exposure to hexachlorophene are rabbits,[297] pigs,[298] and cats.[299] Sheep become blind according to Udall and Malone.[120] Hanig et al.[299] recorded the neurological deficits produced in cats in more detail. Early symptoms consisted of lassitude, weakness and ataxia of the hindlegs, impaired righting reflex, patellar hyperreflexia which later developed into hyporeflexia, urinary retention, and eventually complete flaccid paralysis. The cranial nerve functions remained intact except preterminally. In these cats an increase in cysternal cerebralspinal fluid pressure was observed.

In our first studies we continued exposure to hexachlorophene for a period of 14 weeks because we had designed the experiment to conduct an approximate 90-day feeding study. It is not necessary to feed rats for this length of time. As we determined later, microscopic changes can be observed in rats after a feeding period of two weeks when the animals were exposed to levels high enough to produce the lesion. Some vacuolation can already be observed 3 to 4 days after onset of exposure to hexachlorophene in some rats. When lower dietary levels were fed in a reproduction study,[118] the dietary level of 20 ppm (2.4–0.9 mg/kg body weight/day) had no effect on the brain. At the dietary level of 100 ppm (11.8–4.6 mg/kg body weight/day) focal areas of vacuolation of the white matter of the brain were observed in some of the rats, but they were never extensive. The rats in these lower dietary levels did not show any clinical-neurological symptoms.

The brain lesion observed in rats was also described in monkeys following the exposure to hexachlorophene. Newborn monkeys washed with 3% hexachlorophene detergent solution developed a diffuse status spongiosus of the white matter of the brain.[300] Santolucito[264,301] was able to produce the vacuolation of the white matter, the so-called status spongiosus, by injecting squirrel monkeys daily subcutaneously with 5 mg hexachlorophene/kg of body weight for 37 days. When monkeys were *fed* hexachlorophene they did not develop the brain lesion but showed electroencephalographic changes which need further investigation. The monkeys that showed the vacuolation also demonstrated electroencephalographic changes. Sheep have apparently also shown status spongiosus of the white matter according to Hale and Reid.[302] Lampert et al.[303] were able to produce the lesion in mice as well as rats. These authors reported that the morphological changes were also observed in frozen sections of brains quenched in liquid nitrogen. Trypan blue injected intraperitoneally did not stain the brain, an indication that the blood brain barrier was intact as far as trypan blue was concerned.

The brain changes consistent with status spongiosus due to hexachlorophene exposure have also been described in humans, particularly children. Mullick[130] examined the brains of six human cases that died from hexachlorophene poisoning. The two adults in this group did not show vacuolation of the white matter of the brain. They had died less than 48 hr after exposure to hexachlorophene. The four children (two with congenital ichthyosis, two with burns) had at least three or more days of exposure to hexachlorophene baths in which a 3% hexachlorophene preparation was used. The final concentration of hexachlorophene in the bath water was not known. All four children showed status spongiosus of the white matter. The brain weight given in one of the cases, a 12-day-old child, was 425 g. The normal brain weight for a child that age is 382 g. Shuman et al.[304] in a retrospective blind study were able to demonstrate that the incidence of status spongiosus in the brain stem reticular formation in infants who had died from a variety of causes could be correlated to hexachlorophene exposure. The authors found 21 cases in a series of 250 autopsies of children. Of these cases, 18 were premature infants weighing less than 1,400 g and were of less than 30 weeks' gestation. All but 2 of the cases had 3 or more total washes with 3% hexachlorophene. In the group of cases that were studied were a number of stillborns who did not

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show the lesion and of course had not had dermal hexachlorophene exposure. Shuman et al.[304] mentioned earlier cases of Letterer Siwe's disease in which they had observed status spongiosus of the white matter. Each of the children with Letterer Siwe's disease had had pronounced exposure to hexachlorophene because of the skin lesions which had been vigorously scrubbed with a detergent containing the chemical. The various observations show that, in patients with abnormal skin as well as the skin of the premature, hexachlorophene may be absorbed in sufficient amounts to cause status spongiosus. The fact that blood levels in premature infants washed with hexachlorophene tend to be higher than in mature infants also substantiates these findings.

Almost identical observations were made by Powell et al.[305] In premature infants weighing less than 1,400 g, 7 of 13 that had 4 or more exposures to hexachlorophene washes showed status spongiosus of the myelinated tracts of the brain stem. If this group was further subdivided according to the number of exposures, 6 of 9 infants that had 9 or more exposures to hexachlorophene washes showed the lesion, and 1 of 4 that was washed 4 to 8 times. Four stillborns and 13 infants washed 0 to 3 times with hexachlorophene and weighing less than 1,400 g did not show the spongy change. Electron microscopic examination showed that the spongy change observed in the premature infants was due to a split in the myelin sheaths.

In August of 1972 the press reported a number of deaths of infants in France that occurred because a baby powder to which hexachlorophene had accidentally been added at a concentration of 6% was used on the infants. The final death toll amounted to 41 infants and young children[306] (New York News, Dec. 13, 1972). Detailed accounts have not been published of this incident because of litigation problems. As we pointed out earlier, under acute toxicity, the higher the concentration of hexachlorophene is in a preparation applied to the skin, the more likely it is that skin damage will result. Skin damage also occurred in the French incident. In addition to this, the powder was probably used in many instances where some diaper rash already existed and was then well covered with a diaper, which may have contributed to increased absorption in this area. The brains and spinal cord of a few infants from the French poisoning outbreak that were examined microscopically showed status spongiosus of the white matter.[306]

These various accounts show that status spongiosus can also be produced in humans and probably represents a nonspecific reaction which can be elicited by a number of chemicals.

The lesion status spongiosus of the white matter as far as we now know seems to represent a specific or restricted type of brain edema where the fluid accumulation is confined to the inside of the myelin sheath and myelin is not actually damaged or at least breakdown products of myelin have not been observed. The term status spongiosus or spongy change has been used as a descriptive term and a spongy change of the gray matter may also occur in certain diseases. Status spongiosus together with other morphological changes can be observed in the brain in a variety of many etiologically different diseases and the reader is referred to Klatzo,[307] Ardonato and Lampert,[308] and a recent editorial in the British Medical Journal.[309] From clinically observed poisoning cases as well as animal experiments it is evident that the brain lesion is to a great extent reversible. However, in our own studies as well as those by Lampert et al.,[303] microscopic changes in rodents did not completely regress and the fact that sheep do not recover their eyesight after hexachlorophene poisoning[120] indicates that repair may not always be complete. No reasonable explanation exists at the moment to indicate why certain chemicals produce this very specific edema confined to certain structures of the central nervous system.

Gastrointestinal Lesions

Only two types of compounds under discussion have produced lesions in the gastrointestinal tract. We observed in our study with Sherman strain rats that high single oral doses of 3,000 mg/kg Aroclor 1254 and 1260 or more caused ulceration of the gastric and duodenal mucosa (Figure 8). Ulcers in the GI tract were not observed in the long-term feeding studies with 500 ppm or less. No other changes in the gastrointestinal tract were observed. Ringer et al.,[105] in their reproduction study with mink, observed that Aroclor 1254 produced hemorrhage in the gastrointestinal tract of the offspring.

Allen and Norback[310] observed hyperplasia and dysplasia of the gastric mucosa in rhesus monkeys. Six of these rhesus monkeys were fed

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FIGURE 8. Section of the glandular portion of the stomach of a rat given a single dose of 3,000 mg/kg of Aroclor 1254. This figure illustrates the focal loss of mucosa and the infiltration by inflammatory round cells. H & E X 50.

300 ppm Aroclor 1248 and six were given 5,000 ppm Aroclor 5460, a polychlorinated triphenyl. The glandular formations in this lesion were highly atypical; penetration of the muscularis mucosae and invasion of the submucosa by the mucosal epithelium were observed. Allen and Carstens[285] reported an effect of toxic fat on the gastric mucosa in 18 of 27 monkeys, *Macaca mulatta*, which consisted of hypertrophy of the gastric mucosa and small gastric ulcers within the mucosa. The toxic fat used in these studies, as we now know, contained chlorinated dibenzodioxins.

It has also been observed that hemorrhaging occurred into the gastrointestinal tract of rat fetuses who were exposed to 2,3,7,8-tetrachlorodibenzo-o-dioxin in utero. It was not stated in the reports whether the gastrointestinal hemorrhages were caused by ulceration of the mucosa.[110,111] Ulceration of the glandular portion of the adult rat stomach was also observed.[113]

Patients with chloracne frequently complained of loss of appetite, nausea, vomiting, and abdominal pain, and Goldmann[311] described a worker with a follicular dermatitis who died six months after exposure to 2,3,7,8-tetrachlorodibenzodioxin. At autopsy a ruptured duodenal ulcer and a gastric ulcer were found. Whether any relationship existed between the ulcers and the exposure to the poisonous chemical was not mentioned in the paper.

It should be established if these gastric lesions also occur at lower dietary levels and whether the contamination of the chemicals with various chlorinated dibenzodioxins and furans is responsible for them. Valuable information might be obtained from retrospective studies of workers following occupational exposure. Whether the ulcers are caused by a direct effect of the chemical on the mucosa of the gastrointestinal tract or whether stress plays a primary role in their etiology similar to ulcers that may occur in burned patients for instance (Curling's ulcer) is not clear.

COMMENTS

The acute toxicity of compounds such as the chlorinated biphenyls, the chlorinated naphthalenes, and the chlorinated terphenyls is very low.

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On the other hand, they are poorly broken down in the environment and the metabolism of the biphenyls with more than four chlorine atoms seems to be negligible. Because of these characteristics, the use, wherever possible, of chlorinated biphenyls that consist of mixtures of compounds with one to four chlorines has been proposed. Neither the chlorinated naphthalenes nor the chlorinated terphenyls have so far been shown to present a pollution problem.

Whether the various disease entities reviewed in this article are produced by all the different products mentioned in connection with them is not established with certainty. For instance, some of the compounds that produce chloracne or chick edema have been shown to be contaminated with various chlorinated dibenzodioxins and chlorinated dibenzofurans. The possibility remains that the compounds illustrated in Figure 1 are contaminated with these chemicals and the products themselves are not really responsible for the observed symptoms. On the other hand, a number of structurally unrelated compounds may produce the same disease entity.

Some of the chlorinated dibenzodioxins and the chlorinated dibenzofurans are highly toxic. The most toxic is 2,3,7,8-tetrachlorodibenzodioxins, while the dibenzodioxins without any chlorine and those with eight chlorines are much less toxic. The difference in toxicity of these compounds may be partly caused by the fact that they are very poorly dissolved and absorbed. Intravenous toxicity data are thus far not available on most of these compounds.

We also do not know at present if the porphyria which develops after prolonged exposure to a number of technical compounds such as hexachlorobenzene, chlorinated biphenyls, chlorinated dibenzodioxin, and 2,4,5-T is in many instances due to the contamination of these products with chlorinated dibenzodioxins; of course it may be possible that one or more chlorinated dibenzofurans also produce porphyria. It has also not been established whether all compounds that produce porphyria in animals will do so in humans as well.

In general, the exposure of the various population groups to chlorinated biphenyls and other cyclic chlorinated hydrocarbons is at too low a range to induce porphyria. Whether this can also be said for occupational exposure is not known. When production conditions and general hygiene of the workers are poor, porphyria cutanea tarda can apparently occur.[2] It is also not known if the combined effect of different porphyria inducing agents is cumulative. Whether the photosensitivity observed in humans with some of the germicides discussed in this paper is related to an effect on the porphyrin metabolism was not evident from the published literature.

So far the mechanism that causes chick edema, chloracne, X-disease, and liver disease has not been satisfactorily demonstrated. Chick edema disease has been thought to be caused by proliferation of the vascular endothelium. This might explain the fluid accumulation observed in diseased animals, but so far the sequence of events that led to this disease is not really clear and factors other than the embarrassment of the vascular system may play a role in its etiology such as a toxic myocarditis, decrease in serum proteins, and renal failure. More detailed study of the pathology of the various disease entities is needed for a better understanding of the development of the various lesions mentioned.

The chlorinated polycyclic hydrocarbons seem to alter the immune response of certain animal species and perhaps also of people. These very important findings need to be pursued further. Thus far it has not been established beyond doubt whether this is caused by a general debilitating effect on the animal of which atrophy of the lymphoendothelial system may be one manifestation, or whether this is a specific alteration, as we know it from the effects of radiation and exposure to certain alkylating agents. In this respect not enough attention has been paid to the function of the adrenal, and several of the observed symptoms in humans such as the hyperpigmentation suggest hypofunction of the adrenal cortex. Depending on the information obtained from these studies, a tolerance level of 5 ppm in some food products as set by the U.S. Food and Drug Administration may have to be revised particularly since no long-term low level feeding study has been reported in animals.

Many questions have recently been raised about the use of germicides in various toiletries, common soaps, etc. The objection to their use in the specific case of hexachlorophene is its neurotoxic effect, that can also occur in humans, particularly if the skin is defective in some way. Of course, limited localized exposure, such as handwashing or treatment of a small wound, will not produce neurotoxicity.

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In infant nurseries in hospitals, one of the greatest problems is the constant threat of a staphylococcus infection of epidemic proportions. In order to prevent outbreaks of staphyloccocus infections, a routine was developed in many hospitals in the U.S. and elsewhere in the early 1960's where infants were washed once daily with a 3% hexachlorophene detergent solution, and in addition the diaper area was washed several times a day. The detergent was subsequently rinsed off more or less vigorously, depending upon the philosophy of the different nurses. In some hospitals the detergent was directly applied to the infant, in others it was first diluted in water. Since the neurotoxic effect of hexachlorophene has become known, many hospitals have changed their policies on the use of hexachlorophene. Another drawback to this routine, in addition to a possible neurotoxic effect, is that hexachlorophene keeps not only the staphylococcus of certain phage types from growing on the skin, but reduces all Gram-positive bacteria,[312] which is highly undesirable and may lead to disease. Recently Evans et al.[313] have pointed out that a marked annual variation exists in the prevalence of several bacteria in the nares as well as the umbilical cord of newborns. A decline of staphylococcus aureus was observed in the fall; the prevalence of *Escherichia coli* and streptococcus also varied. Staphylococcus epidermis and Enterobacter organisms did not show this fluctuation. Any studies of efficacy undertaken in a clinical setting should take these normal fluctuations into account and it is possible that good general housekeeping practices are ultimately more effective in preventing bacterial disease in newborns than various germicides. Unfortunately, breaks in technic do occur which in many hospitals makes the use of germicides on infants desirable.

The information reviewed in this article shows that the toxic effects of the polycyclic polychlorinated compounds are complex. The mere knowledge of an LD_{50} value in an animal species does not give us a great deal of information about the toxicity of a compound. Toxicity or toxic effects are a relative concept and such household items as table salt, vitamins, pepper, and allyl propyl disulfide volatilizing from onions to name a few are also toxic. Nevertheless, we have to learn not to use chemicals indiscriminately, without knowing a great deal about them, particularly their long-range effects, when they are persistent and poorly metabolized. The indiscriminate use of these products and discharge into the environment invariably lead to their restriction or ban, which is accompanied by a great deal of emotionalism, distorted facts, and often great economic loss. Prudence, better judgment, and a critical approach to the usage of chemicals and drugs, particularly an evaluation of whether in specific situations they are necessary or not, may help us avoid these situations in the future. Particular attention should be paid to avoiding unnecessary exposure of children to chemicals that we know little about. Children may react differently from adults and as far as long-term exposure is concerned, they would be the most vulnerable.

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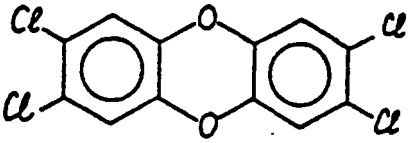
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TCDD

A Deadly Molecule

rdon W. Gribble
rtmouth College
nover, N.H. 03755

TCDD is incredibly toxic. The lethal dose is less than one millionth of a gram for guinea pigs. In man, a single exposure to a few micrograms of TCDD may cause a severe skin disease. TCDD has also been responsible for the death of millions of chicks. TCDD is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.



TCDD

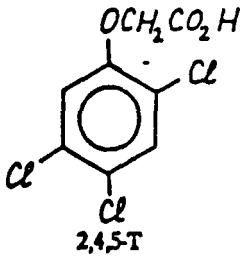
In 1899 a German physician, G. Herxheimer, described a severe skin disease contracted by workers involved in the electrolytic production of chlorine gas using carbon electrodes. He incorrectly attributed the disease to free chlorine. Subsequent reports of chloracne continued through 1964, when 60 Dow Chemical Co. workers developed the disease. Chloracne, a generalized dermatitis composed of comedones (blackheads) and papules with inflammation so severe it may cause permanent scarring, is morphologically similar to teenage acne. Although face, neck, and arms are the most common sites, areas covered by clothing also may be affected.

Chloracne is a hazard for industrial workers producing chlorinated hydrocarbons such as polychloronaphthalenes (Halowax), polychlorobiphenyls (Arochlor), and polychlorophenols. In 1957, TCDD was isolated from 2,4,5-trichlorophenol and found to be the cause of chloracne.

Teratogenicity of TCDD

In 1969, the teratogenicity (birth abnormality) of pesticides was studied at the Bionetics Research Laboratories under contract to the National Cancer Institute. Laboratory animals fed samples of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were observed to have given birth to offspring having fetal defects such as lack of heads

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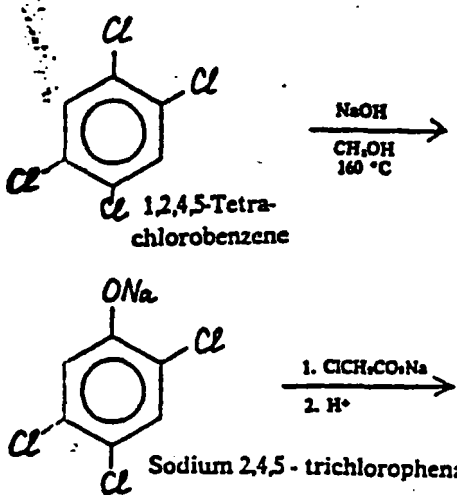
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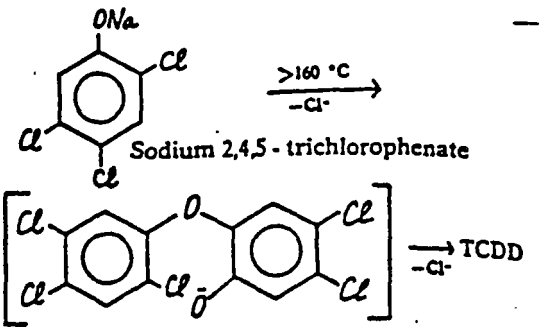
and eyes, cystic kidneys, cleft palates, abnormal livers, or other biological abnormalities. This study was widely quoted by the lay press and caused public concern since 2,4,5-T and a related herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), had been widely used domestically for weed control and in South Vietnam as a jungle defoliant.

Later it was learned that the study sample of 2,4,5-T was contaminated with 30 ppm of TCDD. Further, other studies of TCDD revealed it to be so powerful a teratogen that it could completely account for any birth malformations observed in the Bionetics study. Purified 2,4,5-T, containing less than 1 ppm TCDD, was found not teratogenic. Unfortunately, the lay press in discussing the herbicides 2,4,5-T and 2,4-D continues to overlook the fact that it is the TCDD impurity that is responsible for fetal abnormalities.

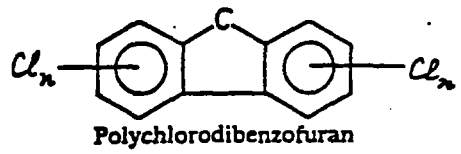
Preparation of TCDD

1,2,4,5-Tetrachlorobenzene and sodium hydroxide in methanol are heated under pressure; then the intermediate sodium 2,4,5-trichlorophenate is treated first with sodium chloroacetate and second with acid to yield 2,4,5-T. The temperature for the sodium hydroxide reaction is crucial. If it is not carefully maintained at 160 °C the intermediate can condense with itself to form TCDD.

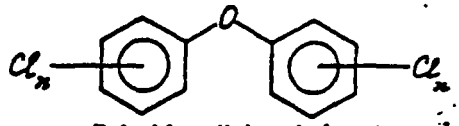




At temperatures above 160 °C polychlorodibenzofuran and polychlorodiphenyl ether, much less toxic than TCDD, may also be

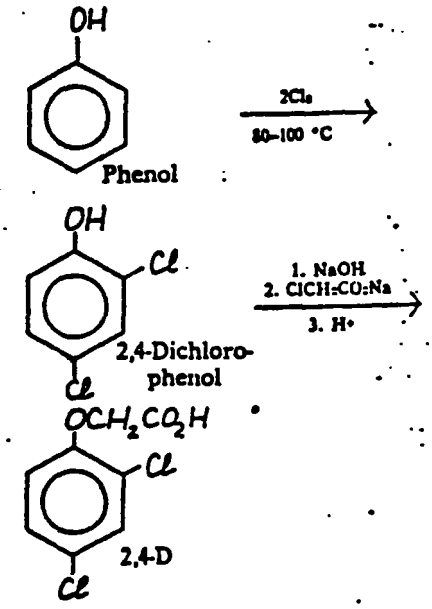


Polychlorodibenzofuran



Polychlorodiphenylether

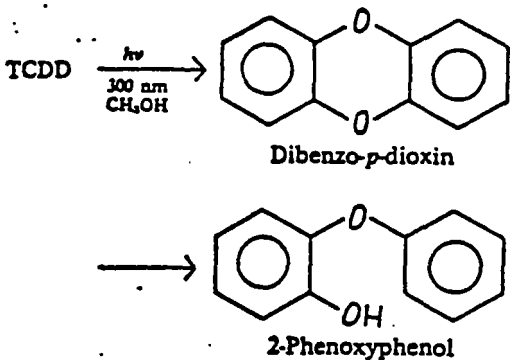
produced. Control experiments demonstrate that TCDD is formed when pure 2,4,5-trichlorophenate is heated 350–400 °C. The related herbicide, 2,4-D, is not usually contaminated with chlorodioxins since it is prepared at lower reaction temperatures by chlorination of phenol as shown in the following equation:



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Chemical Properties of TCDD

TCDD is a chemically stable water-insoluble, white solid having a melting point of about 300 °C. Thermal decomposition begins at 700 °C and is complete at 800 °C. TCDD does not react with strong acids and is inert to most reagents. It slowly photodechlorinates in methanol to give dibenzo-*p*-dioxin and, eventually, 2-phenoxy phenol.



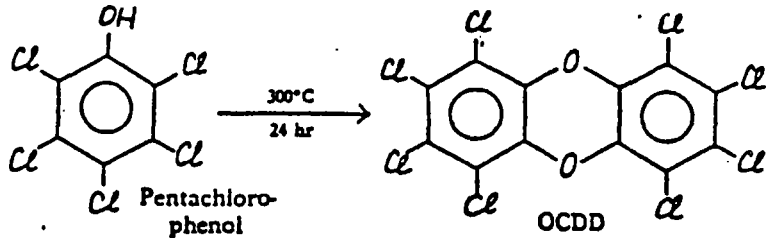
Other Chlorinated Dibenzo-*p*-dioxins

Other chlorinated dibenzo-*p*-dioxins can be formed in reactions involving chlorinated phenols. Isomeric tetrachlorophenols and pentachlorophenol are prepared at high temperatures by exhaustive chlorination of phenol or hydroxide displacement of chlorine from polychlorobenzenes. Consequently, production of chlorinated dibenzo-*p*-dioxins may be thermally promoted. For example, heating pentachlorophenol to 300 °C gives octachlorodibenzo-*p*-dioxin (OCDD) in 4% yield.

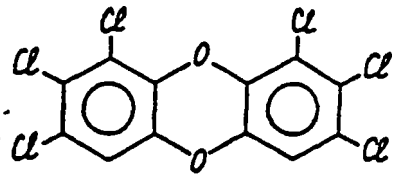
Table I. Relative Toxicity of TCDD

Compound	LD_{50} mg/kg*			
	Guinea pig	Rat	Mouse	Dog
DD[b]	0.0006	0.04	0.001	0.1
5-T[c]	470	375	370	100
D[c]	380	500	390	100
ychnine		5	0.5	
traodotoxin			0.008	
in (nerve gent)	0.05		0.2	
tenone		130		
CN			10	
F			23	20
rathion		10		

*The dose required to kill 50% of the animals. [b] Reference Reference *10*.



Another dioxin, 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (HCDD), has been identified as a toxic factor in chick edema disease.



This compound presumably arises from polychlorophenols used to preserve animal hides which are subjected to hydrolysis and heating during fatty acid manufacture. The fatty acid component of chicken feed becomes contaminated with toxic polychlorinated dibenzo-*p*-dioxins and leads to flock mortality as high as 90% with disastrous results to the chicken broiler industry.

Chick edema factor or disease periodically kills millions of young chicks in the U.S. It causes a buildup of fluid in heart sac and lungs. HCDD was identified in the toxic fat of feeds responsible for the disease

Toxicity of TCDD

TCDD is acnegenic, teratogenic, and deadly toxic. Guinea pigs are particularly vulnerable —the lethal dose for an average size guinea pig is about 5×10^{-7} gram! Table I compares the amazing toxicity of TCDD with other poisons.

The mechanism of the systemic toxic action of TCDD is unknown. It is known, however, that TCDD concentrates mainly in the liver and to a lesser extent in the brain and fatty tissues of laboratory animals. Oral administration of TCDD to laboratory animals has caused edema and hemorrhaging in their subcutaneous tissues, intestine, and brain ventricles, and general liver injury. Death occurs anytime from one to seven weeks after treatment with TCDD.

A solution of TCDD applied daily in microgram amounts to the inner surface of a rabbit's ear causes severe acne characterized by papules, comedones, and cysts. The chloracne response is judged by the formation of comedones. Microscopically, the skin shows marked plugging of hair follicles from an abnormal increase in skin keratin (hyperkeratosis), also the basic pathological defect in human chloracne.

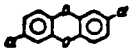
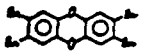
nm = nanometre = 10^{-9} metre

Structure-Activity Relationships

The extraordinary biological activity of TCDD is almost unique to its particular ar-

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Table II. Activity of TCDD and Related Compounds[a]

Compound	LD_{50} (mg/kg), rats	Chloracne response	Teratogenic response	Chick edema response
TCDD	0.04	Strongly positive	Strongly positive	Strongly positive
	>1000[b]	Negative	Negative	—
HCDD (mixture of two isomers)	>10[b]	Positive	Positive	Positive
OCDD	>1000[b]	Negative	Negative	Negative
	—	Strongly positive	—	—
	—	Negative	—	—

[a] Reference 3.
[b] Largest dose fed at which there were no deaths.

rangement of halogens. Other chlorinated dibenzo-*p*-dioxins are much less active or inactive (Table II). In addition, the 2,8-dichloro-, the 2,3-dichloro-, the 1,2,4-trichloro-, and the 1,2,3,4-tetrachlorodibenzo-*p*-dioxins are not acnegenic and probably not teratogenic.

Environmental Studies of TCDD

Although the use of herbicides 2,4,5-T and 2,4-D has been partially banned, mainly because of the Bionetics study, the major concern should be with TCDD and other dioxin contaminants. Fortunately, the supplier of most of the highly contaminated 2,4,5-T (≥30 ppm TCDD) no longer manufactures it.

The U. S. Department of Agriculture and other agencies have subjected TCDD to environmental scrutiny and have learned that in soil TCDD is not readily degraded, photochemically or otherwise; or biosynthesized or photosynthesized from 2,4,5-trichlorophenol; or absorbed into plants; or mobile in the soil. TCDD applied to leaves is not translocated within the plant. The most important of these facts as far as the environment is concerned is that the soil is an unreactive storage container for TCDD.



GORDON W. GRIBBLE, assistant professor of chemistry at Dartmouth since 1968, has research interests in natural product synthesis and nuclear magnetic resonance spectroscopy.

The USDA recently tested 129 samples of 17 pesticides prepared from polychlorophenols and also 20 samples of polychlorophenols for dioxin contamination. Most of the pesticide samples analyzed (76%) contained from no TCDD to less than 0.1 ppm, 15% contained 0.1 to 10 ppm TCDD, and 9% contained more than 10 ppm TCDD. 2,4,5-T samples were shown to contain most of the TCDD and only one other pesticide, silvex, contained more than 0.5 ppm TCDD. Analyses of chlorophenol samples showed more than 0.5 ppm TCDD. Higher chlorinated dioxins were present in many of the chlorophenol samples. For example, analysis of pentachlorophenol samples showed 30% contained more than 100 ppm OCDD. 2,4,5-T currently produced by Dow Chemical Co. contains fewer than 0.5 ppm TCDD.

Human Health and TCDD

A direct relationship between TCDD and chloracne is clear. TCDD's effect on laboratory animals has been established. But no relationship between 2,4,5-T and/or TCDD and human birth abnormalities has been found, despite studies of the 5 million acres of South Vietnam sprayed from 1962 to 1969 with 60,000 tons of 2,4,5-T.

Research is needed to determine the effects of 2,4,5-T TCDD, and related compounds on human health. As future studies unfold decisions can be made regarding the proper role of these chemicals in our society. Ω

Suggested Reading

(1) Aaronson, T., *Environment*, 1971, 13 (7), 21.
(2) Abstracts, Chlorodioxin Symposium, 162nd National American Chemical Society Meeting, Washington, D.C., Sept. 17, 1971, Pesticide Division.
(3) Collins, T. F. X., Williams, C. H., *Bull. Environ Contam. Toxicol.* 1971, 6, 559.
(4) Courtney, K. D., Gaylor, D. W., Hogan, M. D. Falk, H. J., Bates, R. R., Mitchell, I., *Science* 1970, 168, 864.
(5) Friedman, L., Firestone, D., Horwitz, W., Banes D., Anstead, M., Shue, G., *J. Ass. Offic. Anal. Chem.*, 1959, 42, 129.
(6) Kearney, P. C., presentation before the Council on Environmental Quality and President's Cabinet Committee on the Environment Working Group in Pesticides, Washington, D.C., Nov 5, 1970.
(7) Pohland, A. E., Yang, G. C., *J. Agr. Food Chem.* 1972, 20, 1093.
(8) Poland, A. P., Smith, D., Metter, G., Possick, P. *Arch. Environ. Health*, 1971, 22, 316.
(9) "Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health," 1969, pp. 665-674. U.S. Department of Health, Education, and Welfare, Washington, D.C.
(10) Rowe, V. K., Hymas, T. A., *Am. J. Vet. Res.* 1954, 15, 622.
(11) Sparschu, G. L., Dunn, F. L., Rowe, V. K., *Fd Cosmet. Toxicol.* 1971, 9, 405.
(12) Whiteside, T., "Defoliation," 1970, Ballantine New York, N.Y.
(13) Woolson, E. A., Thomas, R. F., Ensor, P. D. J. *J. Agr. Food Chem.*, 1972, 20, 351.

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ST. LOUIS ENCEPHALITIS – Continued

house sparrows. On June 25 intensified larvacide and adulticide of mosquitoes was begun in areas of Memphis known to be heavily infested. A total of 18,000 gallons of 95% malathion were sprayed over the next 2 months in an attempt to avert or lessen an epidemic. As soon as human cases were confirmed, ground spraying of 95% malathion was initiated in all urban areas of Memphis and Shelby County.

(Reported by Alan L. Bisno, M.D., Director, Infectious Diseases, Department of Medicine, University of Tennessee College of Medicine, Robert C. Rendtorff, M.D., Director, Division of Communicable Diseases, J. K. Musley, Director, Field Services Division, Harold Carver, Administrative Assistant, and Irene Duncan, R.N., Supervisor, Communicable Diseases, Memphis-Shelby County Health Department, Robert H. Hutcheson, Jr., M.D., M.P.H., State Epidemiologist, the Arbovirus Reference Branch, Vector-borne Diseases Division, and the Virology Division, Bureau of Laboratories, CDC; a Public Health Advisor; and an EIS Officer.)

Table 1
Hemagglutination Inhibition (HI) Titers to St. Louis Encephalitis Virus in 6 Patients, Memphis, Tennessee, August 1974

Case No.	Serum Specimen	
	Acute	Convalescent
1	1:20	1:160
2	1:40	1:160
3	1:20	1:160
4	1:10	1:80
5	1:10	1:80
6	1:10	1:40

Reference

1. Sudia WD, Fownkle E, Coleman RH: St. Louis Encephalitis in Memphis, Tennessee, 1964. J Med Entomol 4:77-89, 1967

ILLNESS ASSOCIATED WITH TCDD-CONTAMINATED SOIL – Missouri

In 1972 the Missouri Division of Health and CDC investigated a horse arena in eastern Missouri where 54 of 57 horses exposed to the arena had died of an illness characterized by skin lesions, severe weight loss, and hepatotoxicity. Birds, dogs, cats, insects, and rodents were also found dead in and around the arena, and one 6-year-old girl exposed developed hemorrhagic cystitis. Urine cultures for bacterial and viral pathogens were negative. Immediately prior to the onset of illness, the arena had been sprayed with salvage motor oil for dust control.

Similar horse illnesses and deaths occurred in 2 other horse arenas in the eastern Missouri area sprayed by the same salvage oil company. The 2 arenas had been sprayed within 1 month of each other. Subsequent to investigation, soil from all 3 arenas was excavated and disposed. No further problems have occurred since these excavations.

Recent results from laboratory analysis of soil samples taken from the initial arena implicate 2,4,5-trichlorophenol and 2,3,7,8-tetrachlorodibenzodioxin (TCDD) as the probable toxic substances. TCDD is a waste byproduct in the synthesis of trichlorophenol and the herbicide 2,4,5-trichlorophenoxyacetic acid. An investigation is underway to determine the source and extent of the TCDD disposal.

(Reported by H. Denny Donnell, Jr., M.D., State Epidemiologist, and Pat Phillips, D.V.M., Division of Health, Missouri Department of Health and Welfare, the Toxicology Branch, Clinical Chemistry Division, Bureau of Laboratories, and the Cancer and Birth Defects Division, Bureau of Epidemiology, CDC; and 2 EIS Officers.)

Editorial Note

TCDD is a very persistent chemical and a potent contact poison *(1)*. Its acute oral LD-50 for rabbits, mice, guinea pigs, and other species is in the microgram-per-kilogram range. TCDD may cause chloracne and liver damage in humans, chick-edema disease in chickens, and porphyria cutanea tarda in animals and perhaps man. It has been shown to be teratogenic in some mice strains and has also a general embryotoxic effect.

Reference

1. Kimbrough RD: Toxicity of chlorinated hydrocarbons and related compounds. Arch Environ Health 25:125-131, 1972

TULAREMIA MIMICKING PLAGUE – New Mexico

On May 19, 1974, a 39-year-old man from Coolidge, New Mexico, developed rhinorrhea, headache, generalized malaise, and a fever of 103°F. He was seen by a physician at a Gallup outpatient clinic and given long-acting penicillin intramuscularly. His symptoms improved until May 24, when he developed a tender swelling at the anterior axillary line. On June 6 physical examination revealed a rectal temperature of 100°F and 2 right para-axillary and 3 right epitrochlear tender lymph nodes.

An interview revealed that 1 day prior to the onset of his illness, the man had shot a prairie dog near Coolidge. He took the carcass to his wife, who skinned and dressed the animal. Both the man and his wife ate some of the meat after it had been cooked for 2 hours over a wood fire. Bubonic plague was suspected, and the patient was hospitalized.

A direct smear of lymph node aspirate stained by Gram's method and with *Yersinia pestis* fraction 1 sp. [illegible] fluorescent antibody did not reveal suspicious organisms. Cultures of blood, sputum, and lymph node aspirate were negative for *Y. pestis*. Table 2 gives the results of serologic tests performed on a serum specimen obtained on June 6.

The patient denied a history of recent insect bites, tick attachments, or carbuncles. He repeatedly denied having skinned or dressed rabbits or other small mammals. The

Table 2
Results of Serologic Tests for Plague and Tularemia, June 6, 1974

	Tube Agg. Titer	PHA* Titer
Plague	Negative	Negative
Tularemia	1:320	1:8192

*Passive hemagglutination

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THE NEW YORK TIMES, WEDNESDAY, AUGUST 28, 1974

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DEATH OF ANIMALS LAID TO CHEMICAL

Dioxin Identified as Cause of Fatalities in Missouri— 2 Children Became Ill

Special to The New York Times

JEFFERSON CITY, Mo., Aug. 27—Dioxin, the deadly chemical contained in a herbicide once used to defoliate forests in South Vietnam, has been identified as the agent that killed many horses and scores of other animals in Missouri.

Two young girls, one 6 years old and the other 10, became ill because of exposure to the substance. The younger suffered such severe kidney damage that she was hospitalized for four weeks at St. Louis Children's Hospital.

Identification by health scientists of the dioxin is one more step in a medical mystery that began in 1971. The exact source of the chemical, a contaminant in the herbicide 2,4,5-T is still unknown. More than 50 horses, 70 chickens, several dogs, a dozen cats and hundreds of wild birds were killed.

Dr. Renate Kimbrough, a toxicologist at the Federal Center for Disease Control at Atlanta, said that the Missouri incident, as far as she knew, was the first in which dioxin had been identified as the cause of animal deaths and injury to humans outside of plants where the herbicide was manufactured.

No Other Incidents

Dr. Barry Commoner, director of the Center for the Biology of Natural Systems at Washington University in St. Louis who is a renowned environmental scientist, said that he knew of no other incident in which dioxin had been identified in deaths outside the laboratory, although it had been suspected in some field cases.

A tea mat the laboratories in Atlanta identified the highly toxic substance—a danger even at a few parts a billion—after almost two years of analysis. The Atlanta center came into the case after the involvement of the two small girls.

"We didn't know what we were looking for," said Dr. Kimbrough. "That is why it took so long. We thought at first it might be some kind of a nerve poison and that sent us off on the wrong track. It was a needle in the hay stack."

The laboratory team found the needle, known by chemists as 2,3,7,8-tetrachlorodibenzo-para-dioxin, also known as dioxin or TCDD. That substance had been suspected by many scientists as a cause of birth defects in children born in defoliated areas of South Vietnam.

Use of Herbicide Halved

Laboratory tests with animals found that dioxin not only caused birth defects in guinea pigs but also killed the animals at levels as low as 0.0006 milligrams for each kilogram of body weight. Because of these findings the use of herbicide orange, which contains 2, 4, 5-T and which is contaminated by dioxin, was suspended in September, 1971. Its use in the United States as a herbicide had been placed under restrictions in 1970.

Dr. Patrick E. Phillips, a veterinarian in the Missouri Division of Health, said that the Missouri incident began in May and June of 1971. Four stable areas were sprayed with waste oil to control dust. It was this oil in which the dioxin eventually was identified.

Within two days after the spraying, sparrows and barn swallows began to die at one of the stables.

"There literally were bushel baskets full of those dead wild birds," recalled Dr. Phillips.

Horses began to get sick, their skin erupting in ulcers. They refused to eat. Fifty-four horses were affected. "As of this date, 35 of these horses have died," the state veterinarian said yesterday. "There were 12 abortions in mares of young colts."

The mares lost the colts in the summer of 1971. The horses began to die in 1972.

2 Girls Stricken

Then two young girls who lived at one of the stables became ill with what was then an unidentifiable kidney disease. Their mother and the co-owner of the stables complained of constant headaches.

Soil samples taken in the area were the ones in which the dioxin was identified early this month. Preliminary data indicated that the dioxin was in concentration of 60 parts waste oil contaminated by 2, 4, 5-T was sprayed has been removed.

"The man who sprayed the stable areas was not at fault," Phillips said. "He simply bought the stuff. We believe he is telling the truth. The oil with the dioxin should never have gotten into the hands of the waste oil carrier. This guy bought some oil under the idea that this was all it was."

Dr. Herbert R. Domke, director of the State Division of Health, called the incident an "episode of environmental contamination with a highly toxic chemical which should bring home the very real dangers of environmental pollution. It is remarkable that it it did not cause more human ailments."

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THE DOW CHEMICAL COMPANY

BENNETT BUILDING
2030 DOW CENTER
MIDLAND, MICHIGAN 48640

DOW 428192

MN056835

February 28, 1980

STATE DEPARTMENT OF AGRICULTURE

Gentlemen:

REVISED SPECIMEN LABEL FOR ESTERON* 245 HERBICIDE

Enclosed is a copy of the revised specimen label for Esteron 245 Herbicide. The revisions are noted on the bottom of the label. Please place this in your file in connection with our registration for said product. Discard previous specimen labels.

Very truly yours,

Conrad D. Woods

Conrad D. Woods
Regulations/Labeling Specialist
Quality Assurance Department

sre

enclosure

*A Trademark of The Dow Chemical Company

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PRODUCT NAME: ESTERON 245 (464-205)

LABEL/CONTAINER SIZE: 1, 5, 30, 55 gallon Litho

DATE CODE Old/New	EXPLANATION OF CHANGE (Initiator, Reason, Disposal Action)	INITL & DATE
P676 to Q1077	W.R. Mullison - Delete: "Do not apply where runoff is likely to occur". Use up old labels	10/12 [illegible]
Q1077 to R278	C.D. Woods - Add: Emergency response phone number and "Do Not Cut or Weld Container" (on 5, 30, 55). Use up old labels	Cow 4/27/
R278 to S979	C.D. Woods: EPA suspended use of 2,4,5-T Deleted (Note: 1 gallon not not corrected at this time. Do not use). Do not use old labels	Cow 11/21
S979 to T180	C.D. Woods: Use on industrial sites and fencerows reinstated by EPA Do not use old labels. 30 gallon size discontinued	Cow 2/20/8

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DOW 6973

DOW 2756626

U.S. Scientist Disputes EPA on Toxic Herbicide

By Morton Mintz
Washington Post Staff Writer

The Environmental Protection Agency and one of its top scientists have split over continued use on rice fields, pastures and rights of way of a herbicide contaminated by the most toxic chemical known to man.

"I think it should not be used," Dr. Diane Courtney, chief of the EPA's toxic effects branch, told a Senate Commerce environment subcommittee hearing Aug. 9. "We are using a chemical that we can barely handle."

EPA officials agreed with Dr. Courtney that the contaminant, dioxin, which is invariably present in the herbicide, 2,4,5-T, causes birth defects in laboratory animals in concentrations "so low we cannot measure them."

But EPA officials told the subcommittee Aug. 16 that they not only favor continued use of the herbicide but see no substantial question of safety which would require a public hearing.

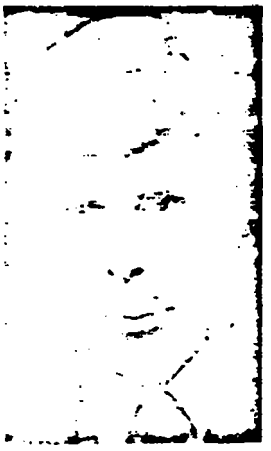
They said the lack of an adequate method to detect dioxin in parts-per-billion ratios leaves the EPA with no data to show that residues get into the food chain. Moreover, they testified, the probability of exposure appears to be minimal because dioxin apparently decomposes swiftly in the presence of sunlight.

Sen. John V. Tunney (D-Calif.), who presided over the hearings, strongly disagreed.

It is a "crazy kind of logic" to let a toxic substance continue in use "because we don't have adequate monitoring data to determine how toxic it is," he said.

"Why shouldn't you have a public hearing and just let people express their viewpoints?" Tunney asked. "I just can't understand why you are so frightened of having a public hearing."

"Well, I guess our basic disagreement is over the level of



SEN. JOHN V. TUNNEY
... fears dioxin use

doubt necessary before you get rid of the product," EPA Deputy General Counsel Robert Zener said.

"I don't think we can properly assess the risk in a public hearing" which would not be "based on any data," said Edwin L. Johnson, an agency pesticides official.

Tunney said he feared the agency will let use of 2,4,5-T continue "until there is a substantial adverse impact upon the health and welfare of the population." The EPA said it is stressing the need for "continued and intensive" efforts to resolve questions about the use of 2,4,5-T and related compounds.

The senator, told by Dr. Courtney that dioxin might be in a steak bought at a supermarket, said, "It makes me very angry. . . . because I did not know that I was being exposed to dioxin, and I don't like the fact that I am being exposed without knowing about it."

In South Vietnam, military pilots defoliated nearly 5 million acres with Agent Orange of which 2,4,5-T—90 million pounds of it—was an ingredient. The herbicide, independently of dioxin, was reported in 1970 to cause birth defects when injected into mice.

Last year, Harvard scientists reported that dioxin in significant amounts had entered the food chain in South Vietnam. Conclusive studies have not been done on whether defoliation has caused birth deformities in humans. Dioxin is so lethal that a teaspoonful is believed sufficient to kill 1,200 persons.

After the 1970 report on 2,4,5-T, made by Department of Health, Education, and Welfare panel, the Agriculture Department ordered a ban on the use of 2,4,5-T for certain uses—around homes, in gardens and recreational areas, or where it could contaminate water supplies—but allowed it to go on being used in forests and on rights-of-way, rangeland and, later, rice fields.

Does dioxin get into the food chain in this country? Tunney asked researcher Courtney?

No one can say for certain because "the food chain is not monitored for dioxin," she replied.

In July, 1973, EPA announced that it would hold a public hearing on whether these uses of 2,4,5-T should continue. At such a hearing, which was to have been held this month, the sole manufacturer, Dow Chemical Co., would be obligated to demonstrate with clear and convincing evidence that—in the words of EPA Deputy Administrator John R. Quarles Jr.—2,4,5-T and dioxin "do not present an unacceptable risk of adverse environmental effects."

At the time of the announcement, the EPA expected that a sensitive new method for detecting dioxin parts per trillion would be able to pick up dioxin traces, if present, in human fat and milk, and in beef, rice and wildlife.

The new method, which was developed by the Harvard scientists, chemist Robert Baughman and geneticist Matthew Meselson, worked in Vietnam. But it turned out not to work under the much different conditions encountered in the United States.

Finally, Quarles convened a meeting with Dow Chemical, the Agriculture Department, and the Environmental Defense Fund Inc., which long had been battling to halt all usage of 2,4,5-T.-Dr. Courtney, although an expert in birth deformities and a pharmacologist, was not asked to attend—a point that irked Tunney.

Then, on June 24, Quarles announced there would be no public hearing, because without a "break-through" in dioxin detection such as had been counted upon "a hearing would not be productive."

But he acknowledged in a letter to William Butler of the Environmental Defense Fund, "The decision to refrain from proceeding at this time should, . . . permit no inference that the doubt at to the safety of 2,4,5-T and related herbicides has been resolved."

At the Senate hearings, Dr. Courtney said that cattle which has grazed on pastures sprayed with 2,4,5-T were found to have dioxin in their liver and fat, and that it consequently could be in beef and dairy products. Tunney cited evidence that dioxin also had been found in goats and sheep which had grazed on land treated with 2,4,5-T.

Tunney told the EPA officials that he "cannot square" Dr. Courtney's view that 2,4,5-T "should not be used" with the agency decision that there was no "substantial" question of safety. He was told that a much broader scientific investigation that Dr. Courtney's had been made by the EPA's criterion and evaluation division.

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Herbicide Killed Missouri Animals

New York Times Service

Jefferson City, Mo.—A lethal concentration of Dioxin; the deadly chemical contained in a herbicide once used to defoliate forests in South Vietnam, has been identified as the agent that killed many horses and scores of other animals in Missouri.

Two young girls, one six years old and the other 10, became ill because of exposure to the substance. The younger suffered such severe kidney damage that she was hospitalized for four weeks at St. Louis Children's Hospital.

The exact source of the chemical, a contaminant in the herbicide 2,4,5-T is still unknown. More than 50 horses, 70 chickens, several dogs, a dozen cats and hundreds of wild birds were killed in Missouri.

THE HERBICIDE 2,4,5,-T is planned for use in Michigan U.S. Forest Service to kill 84 acres of aspen and hardwood trees in Ottawa National Forest, allowing more room and sunlight for more commercially valuable spruce and red pine trees.

The spraying has been delayed until at least next year by federal court suit brought by Michigan Attorney General Frank Kelley. The Wisconsin attorney general has also filed suit against the Forest Service, halting spraying of the herbicide in northern Wisconsin forests.

Dr. Renate Kimbrough, a toxicologist at the Federal Center for disease control at Atlanta, said that the Missouri incident was the first in which dioxin had been identified as the cause of animal deaths and injuries to humans outside plants where the herbicide was manufactured.

She said that if the concenthation of dioxin were kept to one-tenth of part per million parts of 2,4,5-T it "would not be hazardous" to wildlife when used as a defoliant.

"The case in Missouri is an isolated one where the dioxin was much more concentrated than in the 2,4,5-T used for spraying," she said. The 2,4,5-T in Missouri was estimated to have a dioxin concentration of 60 parts per million.

"We are not sure why," said Dr. Kimbrough. Herbicide manufacturers can control the amount of dioxin in 2,4,5-T, she said.

DIOXIN had been suspected by many scientists as a cause of birth defects in children born in defoliated areas of South Vietnam.

Laboratory tests with animals/ found that dioxin not only caused birth defects in guinea pigs but also killed the animals at levels as low as 0.0006 milligrams for each kilogram of body weight. Because of these findings the use of the herbicide agent Orange, which contains, 2,4,5-T and which is contamined by dioxin, was suspneded in September 1971. Its use in the United States as a herbicide had been placed under restrictions in 1970.

The Missouri incident began in May and June of 1971, after four stable areas were sprayed with waste oil containing dioxin, to control the rust.

Within two days after the spraying, sparrows and barn swallows began to die at one of the stables. "There literally were bushel baskets full of those dead wild birds," recalled Dr. Patrick G. Phillips, a state veterinarian.

Horses began to get sick, their skin erupting in ulcers. They refused to eat. Fifty-four horses were affected. Thirty-five died and 12 mares aborted.

Then two young girls who lived at one of the stables became ill with what was then an unidentifiable kidney disease. Their mother and the co-owner of the stables complained of constant headaches.

Thursday, Aug. 29, '74 DETROIT FREE PRESS

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THE DOW CHEMICAL COMPANY

BIOCHEMICAL RESEARCH LAB. MIDLAND, MICHIGAN 48640

TOX. NO. K 65681

FILE NO.

REQUEST FOR TOXICOLOGICAL STUDIES

1803 BLDG.

NAME OF MATERIAL TO BE TESTED: 2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD)

SUBMITTED BY (Signature): Sylvia J. Orm (Date) (Building) (Phone) 1710 Bldg. 4/9/74

SUPERVISOR (Signature) (Date and Building) PROJECT SAFETY COORDINATOR Date:

ESTIMATED COST: $ 85,000 for reproduction studies

ACCOUNT NUMBER

LEDGER	LOCATION GROUP / FUNCTION	SECTION / SUB. FUNC	SUFFIX

PROBLEM NO. TO BE USED BY TOXICOLOGY

PROBLEM INDEX NO. LAB. PROBLEM

DO NOT WRITE BELOW THIS LINE — COMPLETE ALL INFORMATION ON REVERSE

ROUTE OF ADMINISTRATION: dietary feeding studies

LENGTH OF TREATMENT: 2 years

SPECIES:

RAT	☒M ☒F	RABBIT	☐M ☐F	GUINEA PIG	☐M ☐F
DOG	☐M ☐F	MONKEY	☐M ☐F	HAMSTER	☐M ☐F

OTHER

DOSES: 0, 0.1, 0.01 and 0.001 mg/kg/day

CHECK POINT	DATE
PLE RECEIVED TOXICOLOGY	9-28-70
ESTIMATED STARTING TIME	May 74
ESTIMATED REPORTED DATE	
REVISED ESTIMATE REPORTED	
REPORT DRAFTED	
REPORT ISSUED	

FATE OF SAMPLE	DATE
☐ RETURNED	
☐ DESTROYED	
☐ REFERENCE SAMPLE STORED	
☐ REFERENCE SAMPLE TO K STORAGE	

NOTES

Sample extremely toxic + Chloroacnegenic

DATE PROJECT COMPLETED

NOTES, CHANGES, ADVERSE EFFECTS NOTED

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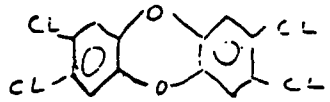
SAMPLE INFORMATION

NAME
2,3,7,8-Tetrachlorodibenzo-p-dioxin

MOLECULAR FORMULA	MOLECULAR WEIGHT	SAMPLE REFERENCE, SOURCE AND OR IDENTIFICATION
		340-2-54B

ACRONYMS
TCDD

STRUCTURAL FORMULA OR COMPOSITION



PHYSICAL AND CHEMICAL PROPERTIES

ESTIMATED PURITY OF SAMPLE	SAMPLE ANALYSIS	RESULTS
in excess of 90%	☑ YES ☐ NO	AL-23-483 Sept 28, GW

BOILING POINT	EXPLOSIVE LIMITS	FLASH POINT	IGNITION TEMP.	MELTING POINT	VAPOR PRESSURE
°C mmHG	(% BY VOLUME IN AIR)	°F	°C	°C	mm 25°

CORROSIVENESS (to common metals)	PHYSICAL STATE	COLOR

CHEMICAL REACTIVITY	ODOR (include concentration in air)

STABILITY (to pH change, heat, light)

Why are toxicological studies needed? Include stage of development, proposed use, method of handling, and amount handled. Do you know of any adverse effects from human exposure?

Material is highly toxic and chloracnegenic, and must be handled with extreme caution.

This study is to generate data regarding possible adverse effects associated with chronic ingestion of low levels of TCDD in the diet. BJ Kouba 4/5

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	DATE		DATE		DATE
☑ PROTOCOL COMPLETED	4-9-74	☐ STATISTICS DONE		☐ SLIDES TO BE READY	
☐ ANIMALS ORDERED		☐ CLINICAL TESTS REPORTED		☐ SLIDES READ	
☐ ANIMALS STARTED ON TEST		☐ HEMATOLOGY TESTS REPORTED		☐ HISTOPATH REPORTED	6980
☐ NECROPSY DONE		☐ SLIDES STARTED		☐ WORK ALL DONE, READY TO WRITE	

B. A. Schwetz - 1803
E. E. Kenaga - 9008
H. L. Gordon - 2030
J. G. Gleeson - 2030
J. Kilian - Freeport
INDEX

Ag-Organics Dept.
7/3/75

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Tsitologiya i Genetika 9, No. 3, 202-206 (1974)

CYTOGENETIC EFFECT OF THE HERBICIDE 2,4-D ON HUMAN AND ANIMAL CHROMOSOMES

M. A. Pilinskaya

202

Introduction. Certain pesticides can make a definite contribution to the induced mutation process in humans. Consequently, the problem of ascertaining potential mutagens among the pesticides assumes special urgency, the accumulation of which mutagens in various objects of the external atmosphere can have genetic danger not only for the individual but also for the population as a whole.

A study of the mutagenic activity of the widely used herbicides of the 2,4-D group (derivatives of chlorophenoxy compounds) is highly practical on this score.

The cytogenetic action of the chloro derivatives of the phenoxy acids has been studied mainly on various plants /1-5/. In recent years some members of the chlorophenoxy compounds have been studied on insects and mammals /6-8/.

In the present paper we made a study of the cytogenetic action of one of the compounds of the given group, and specifically 2,4-dichlorophenoxyacetic acid (2,4-D), on the somatic cells of humans in vitro and of mice in vivo. This herbicide was selected from a fairly extensive group of chloro derivatives of the phenoxy acids in view of the fact that the residues of any of the compounds of this group on plants mainly represent 2,4-D /9/.

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202 Material and operational procedure. In the in vitro experiments we used the blood of two clinically healthy donors who had a normal karyotype and had not been exposed to any factor capable of affecting the frequency of the aberrations. The lymphocytes were cultured and the chromosome preprations were prepared as described in /10/. The herbicide 2,4-D was added to the culture 3 hr after the start of incubation and allowed to remain until the end of culturing.

203 For the in vivo experiments we used white male mice that weighed 18 - 20 g. The chromosome preparations from the bone marrow cells were prepared by the procedure used in our laboratory /11/. The herbicide 2,4-D was given orally as a single dose with the milk. The animals were killed by decapitation 20 hr after giving the compound.

The chromosome analysis was run as described in /10/.

The uniformity of the experiments on the frequency of the aberrant cells was verified by the x^2 method. Substantial differences were not observed ($P > 0.1$) between the duplicated experiments and consequently the experimental results were added. The statistical reliability of the differences between experiment and the control and between the different doses was determined by the t method /12/.

Study results and their discussion. The cytogenetic activity of 2,4-D in the culture of human lymphocytes was studied at concentrations that had a cytotoxic effect (50 and 20 μg/ml), and in amounts that failed to exert a toxic effect on the cells (2.0, 0.2, 0.02, and 0.002 μg/ml). The data, obtained in the chromosome

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20 analysis of the cultures that had been treated with different doses of 2,4-D, are given in Tables 1 and 2.

From Table 1 it can be seen that the number of metaphases with aberrations at all of the 2,4-D concentrations, except the minimum, differed reliably from the spontaneous level ($P < 0.001$; $P < 0.05$). Increasing the 2,4-D concentration in the culture above 0.2 μg/ml was not accompanied by a reliable increase in the number of aberrant metaphases ($P > 0.05$), although a tendency toward a maximum cytogenetic effect was observed at a dose of 2.0 μg/ml. Evidently, high doses of the compound, which exerted a cytotoxic action on the culture, led to a destruction of part of the aberrant cells or else they retarded their entry into mytosis, and as a result the total frequency of the aberrations remained at the same level. A decrease in the herbicide concentration in the culture, beginning with 0.2 μg/ml, caused a decrease in the number of damaged cells. The obtained data do not permit judging the character of the dose relation in the verified dose range.

The aberrations caused by the action of 2,4-D were of the chromatid and chromosome type in a 4 : 1 ratio (Table 1). Single acentric fragments predominated among the chromatid rearrangements. Symmetrical chromatid interchanges were observed in three metaphases at the minimum concentration of the compound (Fig. 1, see first insert at end of number), i. e. this concentration of 2,4-D is not "noncperative", as it would seem if the spectrum of the disturbances is neglected. The chromosome type of aberrations are mainly represented by paired acentric fragments.

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From Table 2 it can be seen that the identified cleavages are distributed at random among the chromosome groups ($P > 0.05$). However, a nonuniform distribution of the damages was observed along the shoulders of the chromosomes, and here 81.4% of the cleavages was localized in the long shoulders. The maximum of the disturbances was found on the telomeric third of the long and short shoulders (70.6 and 74.3%).

The cytogenetic activity of 2,4-D on mice was studied at doses of 300 mg/kg (LD_{50}), 100 mg/kg (1/3 LD_{50}), 50 mg/kg (1/6 LD_{50}), and 10 mg/kg (1/30 LD_{50}). The data, obtained in the chromosome analysis of the bone marrow cells of mice, are given in Table 3, from which it can be seen that, at dose levels of the compound of 10 and 50 mg/kg, which failed to cause visible signs of intoxication, the frequency of the aberrant cells did not differ from that of the control. After administering 2,4-D at doses of 100 and 300 mg/kg, where expressed signs of intoxication were observed, the number of metaphases with aberrations reliably differed from the spontaneous level ($P < 0.05$; $P < 0.001$). An increase in the frequency of the aberrations was accompanied by an increase in the average number of disturbances, both in a single studied and in a single aberrant metaphase, mainly due to the appearance of cells with multiple aberrations (Fig. 2, see first insert). Single acentric fragments predominated among the induced injuries.

Consequently, as a result of the performed study it was found that the herbicide 2,4-D is capable of damaging the chromosomes of the somatic cells of animals and humans. However, the cytogenetic

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effect in the bone marrow cells of mice arose only by the action of toxic doses of the herbicide, on which basis 2,4-D can obviously be regarded as being a weak mutagen for mice. In human lymphocytes the compound induced chromosome aberrations at concentrations that exerted and did not exert a toxic action on the cells.

Consequently, human chromosomes in an in vitro culture are more sensitive to the damaging action of 2,4-D than the chromosomes of the bone marrow cells of mice in vivo. These data again emphasize the importance of the specificity of the object when studying induced chemical mutagenesis.

From the literature data on the distribution of 2,4-D in the organism of mammals it is known that the compound does not metabolize, is excreted in unchanged form, penetrates into the gonads and can pass through the placental barrier /13, 14/, which is probably the reason for its teratogenic effect /13 - 15/.

It is possible that the studied herbicide is distributed in a similar manner in the human organism. This postulation is supported by the data that were obtained on volunteers who took the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), a compound that differs from 2,4-D by having an extra chlorine atom /16/. After a single oral administration of 2,4,5-T the herbicide was determined in the blood and for three days was excreted unchanged in the urine. If the fate of 2,4-D in the human organism is the same, then the given compound represents a genetic hazard as a cytogenetically active substance, in view of which it should not be permitted to enter the human organism from various objects of the external atmosphere.

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Table 1

Frequency and Types of Chromosome Aberrations in Culture of Human Lymphocytes at Various 2,4-D Concentrations

1) Концентрация 2,4-Д: мкг/мл	Mole моль	2) Количество исследованных метафаз	3) Частота метафаз с аберрациями, %	4) Количество аберраций: Total всего	5) на 1 исследованную метафазу	6) на 1 аберрантную метафазу
50,000	$2,5\times10^{-4}$	500	8,00±1,213	43	0,086	1,075
20,000	10^{-4}	500	8,40±1,241	44	0,088	1,048
2,000	10^{-5}	500	9,40±1,343	58	0,116	1,234
0,200	10^{-6}	500	8,60±1,253	48	0,096	1,116
0,020	10^{-7}	500	4,20±0,907	22	0,044	1,048
0,002	10^{-8}	500	2,80±0,735	14	0,028	1,000
Контроль Control		800	1,75±0,469	14	0,017	1,000

7) Аберраций на 100 клеток								
8) хроматидного типа				9) хромосомного типа				
10) одиночных фрагментов	11) асимметричных обменов	12) симметричных обменов	13) всего	14) парных фрагментов	15) дицентриков	12) симметричных обменов	16) кольцевых хромосом	13) всего
6,2	0,2	0,2	6,6	1,4	0,4	0	0,2	2,0
7,2	0	0	7,2	1,4	0,2	0	0	1,6
9,8	0	0	9,8	1,6	0,2	0	0	1,8
6,8	0	0	6,8	2,6	0,2	0	0	2,8
3,6	0	0	3,6	0,6	0	0,2	0	0,8
2,0	0	0,6	2,6	0,2	0	0	0	0,2
1,1	0	0	1,1	0,5	0	0	0	0,5

1) Concentration of 2,4-D
2) Number of investigated metaphases
3) Frequency of metaphases with aberrations, %
4) Number of aberrations
5) Per investigated metaphase
6) Per aberrant metaphase
7) Aberrations/100 cells
8) Chromatid type
9) Chromosome type
10) Single fragments
11) Asymmetric exchanges
12) Symmetric exchanges
13) Total
14) Paired fragments
15) Dicentrics
16) Ring chromosomes

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Table 2

Distribution of Number of Cleavages, Caused by 2,4-D, By Chromosome Groups

1) Концентрация 2,4-Д, мкг/мл	2) Группы хромосом									3) Всего
	A-1	A-2	A-3	B	C	D	E	F	G+Y	
50,000	3	1	1	8	21	2	2	1	2	41
20,000	3	3	1	6	19	2	3	1	0	38
2,000	5	8	2	8	15	1	0	1	1	41
0,200	4	5	2	3	7	3	3	1	4	32
0,020	0	1	1	4	5	2	5	2	0	20
0,002	2	0	0	2	9	1	1	1	0	16
4) Всего (наблюдаемое число разрывов)	17	18	7	31	76	11	14	7	7	188
5) Ожидаемое число разрывов (на основании длины хромосом)	16,19	15,15	12,71	22,64	71,99	18,76	10,07	8,46	6,04	188
χ^2	0,04	0,53	2,56	3,09	0,22	3,21	0,26	0,25	0,15	10,31

6) Примечание. $\chi^2=10,31$; число степеней свободы равно 8; $P>0,05$.

1) Concentration of 2,4-D, μg/ml

2) Chromosome groups

3) Total

4) Total (observed number of cleavages)

5) Expected number of cleavages (on basis of chromosome length)

6) Remark. $\chi^2 = 10.31$; number of degrees of freedom = 8; $P > 0.05$.

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20)

Table 3

Frequency and Types of Aberrations for Mice That Had Been Poisoned by Various Doses of 2,4-D

1) Доза 2,4-Д, мг/кг	2) Количество животных	3) Количество исследованных метафаз	4) Частота метафаз с аберрациями, %	Частота клеток со множественными повреждениями хромосом		8) Число аберраций			12) Типы аберраций на 100 клеток		
				6) % к числу исследованных клеток	7) % к числу аберрантных клеток	9) всего	10) на 1 исследованную метафазу	11) на 1 аберрантную метафазу	13) одиночные фрагменты	14) парные фрагменты	15) транслокации
10	6	1200	0,333±0,165	0	0	4	0,0033	1,00	0,250	0,083	0
50	6	1200	0,833±0,264	0,08	10,0	14	0,0117	1,40	1,000	0,083	0,083
100	6	1200	1,750±0,378	0,16	9,5	25	0,0208	1,19	1,833	0,250	0
300	6	1200	3,083±0,499	1,28	43,3	114	0,0950	3,08	8,583	0,75,	0,167
16) Контроль	10	1000	0,700±0,264	0	0	7	0,0070	1,00	0,700	0	0

1) Dose of 2,4-D, mg/kg
2) Number of animals
3) Number of studied metaphases
4) Frequency of metaphases with aberrations, %
5) Frequency of cells with multiple damages of chromosomes
6) In % to number of studied cells
7) In % to number of aberrant cells
8) Number of aberrations
9) Total
10) Per investigated metaphase
11) Per aberrant metaphase
12) Types of aberrations/100 cells
13) Single fragments
14) Paired fragments
15) Translocations

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Conclusions. A study was made of the cytogenetic action of the herbicide 2,4-D in both in vitro experiments (on a culture of the lymphocytes of peripheral human blood) and in vivo experiments (on bone marrow cells of white male mice). It was shown that the human chromosomes in the culture are more sensitive to the damaging action of the compound. The obtained results make it possible to classify the herbicide 2,4-D with the compounds that are potentially dangerous in a genetic sense.

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John Livak
June 26, 1975

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8 (3), 202-6 (1974)
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УДК 615—099—036.7

ЦИТОГЕНЕТИЧЕСКИЙ ЭФФЕКТ ГЕРБИЦИДА 2,4-Д НА ХРОМОСОМЫ ЧЕЛОВЕКА И ЖИВОТНЫХ

М. А. ПИЛИНСКАЯ

Введение. В индуцированный мутационный процесс у человека определенный вклад могут вносить некоторые пестициды. Поэтому особую актуальность приобретает задача выявления среди пестицидов потенциальных мутагенов, накопление которых в различных объектах внешней среды может представлять генетическую опасность не только для отдельных индивидуумов, но и для популяции в целом.

С этих позиций весьма целесообразным является исследование мутагенной активности широко применяемых гербицидов группы 2,4-Д (производных хлорфеноксисоединений).

Цитогенетическое действие хлорпроизводных феноксикислот в основном исследовалось на различных растительных объектах [1—5]. В последние годы некоторые представители хлорфеноксисоединений изучались на насекомых и млекопитающих [6—8].

В задачу настоящей работы входило исследование цитогенетического действия одного из препаратов данной группы — 2,4-дихлорфеноксиуксусной кислоты (2,4-Д) — на соматические клетки человека in vitro и мышей in vivo. Этот гербицид был выбран из довольно обширной группы хлорпроизводных феноксикислот в связи с тем, что остатки любых препаратов этой группы на растениях в основном представляют собой 2,4-Д [9].

Материал и методика. В опытах in vitro использовали кровь двух клинически здоровых имеющих нормальный кариотип доноров, которые не подвергались действию какого-либо фактора, способного повлиять на частоту аберраций. Культивирование лимфоцитов и приготовление препаратов хромосом проводили по методике [10]. Гербицид 2,4-Д вносили в культуру через 3 ч после начала инкубации и оставляли его до конца культивирования.

Частота и типы хромосомных аберраций в культуре

Концентрация 2,4-Д		Количество исследованных метафаз	Частота метафаз с аберрациями, %	Количество аберраций		
мкг/мл	моль			всего	на 1 исследованную метафазу	на 1 аберрантную метафазу
50,000	$2{,}5\times10^{-4}$	500	8,00±1,213	43	0,086	1,075
20,000	10^{-4}	500	8,40±1,241	44	0,088	1,048
2,000	10^{-5}	500	9,40±1,313	58	0,116	1,231
0,200	10^{-6}	500	8,60±1,253	48	0,096	1,116
0,020	10^{-7}	500	4,20±0,907	22	0,011	1,019
0,002	10^{-8}	500	2,80±0,735	14	0,028	1,000
Контроль		800	1,75±0,469	14	0,017	1,000

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Для опытов in vivo использовали беспородных белых мышей-самцов весом 18—20 г. Препараты хромосом из клеток костного мозга готовили по методу, принятому в нашей лаборатории [11]. Гербицид 2,4-Д вводили однократно внутрижелудочно с молоком. Животных забивали декапитацией через 20 ч после введения препарата.

Хромосомный анализ проводили так же, как в сообщении [10].

Однородность опытов по частоте аберрантных клеток проверяли по методу χ^2. Между повторностями экспериментов не наблюдали существенных различий ($P>0,1$), поэтому результаты повторностей суммировали. Статистическую достоверность отличия опыта от контроля и различных доз между собой определяли по методу φ [12].

Результаты исследований и их обсуждение. Цитогенетическую активность 2,4-Д в культуре лимфоцитов человека исследовали в концентрациях, обладающих цитотоксическим эффектом (50 и 20 *мкг/мл*), и в количествах, не оказывающих токсического действия на клетки (2,0; 0,2; 0,02; 0,002 *мкг/мл*). Данные, полученные при хромосомном анализе культур, обработанных различными дозами 2,4-Д, приведены в табл. 1 и 2.

Из табл. 1 видно, что число метафаз с аберрациями при всех концентрациях 2,4-Д, кроме минимальной, достоверно отличалось от спонтанного уровня ($P<0,001$; $P<0,05$). Увеличение концентрации 2,4-Д в культуре более 0,2 *мкг/мл* не сопровождалось достоверным повышением количества аберрантных метафаз ($P>0,05$), хотя наблюдалась тенденция к максимальному цитогенетическому эффекту при дозе 2,0 *мкг/мл*. Очевидно, высокие дозы препарата, оказывающие цитотоксическое действие на культуру, приводили к гибели части аберрантных клеток или задержке вступления их в митоз, благодаря чему общая частота аберраций сохранялась на одном и том же уровне. Снижение концентрации гербицида в культуре, начиная от 0,2 *мкг/мл*, вызывало уменьшение количества поврежденных клеток. Полученные данные не позволяют судить о характере дозовой зависимости в проверенном интервале доз.

При действии 2,4-Д возникали аберрации как хроматидного, так и хромосомного типа в соотношении 4 : 1 (табл. 1). Среди хроматидных перестроек преобладали одиночные ацентрические фрагменты. При минимальной концентрации препарата в трех метафазах наблюдали симметричные хроматидные обмены (рис. 1, см. I вклейку в конце номера), т. е. эта концентрация 2,4-Д не является «недействующей», как казалось, без учета спектра нарушений. Аберрации хромосомного типа в основном были представлены парными ацентрическими фрагментами.

Из табл. 2 видно, что идентифицированные разрывы распределялись случайно по группам хромосом ($P>0,05$). Однако отмечалось неравномерное распределение повреждений по плечам хромосом — 81,4% раз-

Таблица 1

лимфоцитов человека при различных концентрациях 2,4-Д

Аберраций на 100 клеток								
хроматидного типа				хромосомного типа				
одиночных фрагментов	асимметричных обменов	симметричных обменов	всего	парных фрагментов	дицентриков	симметричных обменов	кольцевых хромосом	всего
6,2	0,2	0,2	6,6	1,4	0,4	0	0,2	2,0
7,2	0	0	7,2	1,4	0,2	0	0	1,6
9,8	0	0	9,8	1,6	0,2	0	0	1,8
6,8	0	0	6,8	2,6	0,2	0	0	2,8
3,6	0	0	3,6	0,6	0	0,2	0	0,8
2,0	0	0,6	2,6	0,2	0	0	0	0,2
1,1	0	0	1,1	0,5	0	0	0	0,5

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Таблица 2

Распределение числа разрывов, вызванных 2,4-Д, по группам хромосом

Концентрация 2,4-Д, мг/кг	Группы хромосом									
	A-1	A-2	A-3	B	C	D	E	F	G+Y	Всего
50,000	3	1	1	8	21	2	2	1	2	41
20,000	3	3	1	6	19	2	3	1	0	38
2,000	5	8	2	8	15	1	0	1	1	41
0,200	4	5	2	3	7	3	3	1	4	32
0,020	0	1	1	4	5	2	5	2	0	20
0,002	2	0	0	2	9	1	1	1	0	16
Всего (наблюдаемое число разрывов)	17	18	7	31	76	11	14	7	7	188
Ожидаемое число разрывов (на основании длины хромосом)	16,19	15,15	12,71	22,64	71,99	18,76	16,07	8,46	6,04	188
χ^2	0,04	0,53	2,56	3,09	0,22	3,21	0,26	0,25	0,15	10,31

Примечание. $\chi^2 = 10{,}31$; число степеней свободы равно 8; $P > 0{,}05$.

рывов локализовалось в длинных плечах. Максимум нарушений приходилось на теломерную треть длинных и коротких плеч (70,6 и 74,3%).

Цитогенетическую активность 2,4-Д на мышах исследовали в дозах 300 *мг/кг* ($ЛД_{50}$), 100 *мг/кг* ($^1/_3$ $ЛД_{50}$), 50 *мг/кг* ($^1/_6$ $ЛД_{50}$) и 10 *мг/кг* ($^1/_{30}$ $ЛД_{50}$). Данные, полученные при хромосомном анализе клеток костного мозга мышей, представлены в табл. 3, откуда видно, что при действии препарата в дозах 10 и 50 *мг/кг*, не вызывающих видимых признаков интоксикации, частота аберрантных клеток не отличалась от таковой в контроле. После введения 2,4-Д в дозах 100 и 300 *мг/кг*, при которых наблюдались выраженные признаки интоксикации, число метафаз с аберрациями достоверно отличалось от спонтанного уровня ($P < 0{,}05$; $P < 0{,}001$). Повышение частоты аберраций сопровождалось увеличением среднего числа нарушений как в одной исследуемой, так и в одной аберрантной метафазе в основном за счет появления клеток со множественными аберрациями (рис. 2, см. I вклейку). Среди индуцированных повреждений преобладали одиночные ацентрические фрагменты.

Таким образом, в результате проведенных исследований обнаружено, что гербицид 2,4-Д способен повреждать хромосомы соматических клеток животных и человека. Однако цитогенетический эффект в клетках костного мозга мышей возникал только при действии токсических доз гербицида, на основании чего 2,4-Д, очевидно, можно считать слабым мутагеном для мышей. В лимфоцитах же человека препарат индуцировал хромосомные аберрации в концентрациях, оказывающих и не оказывающих токсического действия на клетки.

Следовательно, хромосомы человека в культуре in vitro более чувствительны к повреждающему действию 2,4-Д, чем хромосомы клеток костного мозга мышей in vivo. Эти данные еще раз подчеркивают значение специфичности объекта при изучении индуцированного химического мутагенеза.

Из литературных данных о распределении 2,4-Д в организме млекопитающих известно, что препарат не метаболизирует, выделяется в не-

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Таблица 3

Частота и типы хромосомных аберраций у мышей, затравленных различными дозами 2,4-Д

Доза 2,4-Д, мг/кг	Количество животных	Количество исследованных метафаз	Частота метафаз с аберрациями, %	Частота клеток со множественными повреждениями хромосом		Число аберраций			Типы аберраций на 100 клеток		
				% к числу исследованных клеток	% к числу аберрантных клеток	всего	на 1 исследованную метафазу	на 1 аберрантную метафазу	одиночные фрагменты	парные фрагменты	транслокации
10	6	1200	0,333±0,165	0	0	4	0,0033	1,00	0,250	0,083	0
50	6	1200	0,833±0,264	0,08	10,0	14	0,0117	1,40	1,000	0,083	0,083
100	6	1200	1,750±0,378	0,16	9,5	25	0,0208	1,19	1,833	0,250	0
300	6	1200	3,083±0,499	1,28	43,3	114	0,0950	3,08	8,583	0,75,	0,167
Контроль	10	1000	0,700±0,264	0	0	7	0,0070	1,00	0,700	0	0

изменном состоянии, проникает в гонады и может проходить через плацентарный барьер [13, 14], с чем, вероятно, и связан его тератогенный эффект [13—15].

Возможно, что в организме человека исследуемый гербицид распределяется подобным же образом. В пользу этого предположения говорят данные, полученные на добровольцах, принимавших гербицид 2,4,5-трихлоруксусную кислоту (2, 4, 5-Т) — препарат, отличающийся от 2,4-Д лишним атомом хлора [16]. После однократного перорального приема 2,4,5-Т гербицид определялся в крови и в течение трех суток выделялся в неизменном виде с мочой. Если судьба 2,4-Д в организме человека такова же, то данный препарат как цитогенетически активное вещество представляет генетическую опасность, в связи с чем необходимо исключать попадание его в организм человека из различных объектов внешней среды.

Выводы. Исследовано цитогенетическое действие гербицида 2,4-Д в опытах in vitro (на культуре лимфоцитов периферической крови человека) и in vivo (на клетках костного мозга беспородных белых мышей). Показано, что хромосомы человека в культуре более чувствительны к повреждающему действию препарата. Полученные результаты позволяют отнести гербицид 2,4-Д к веществам, потенциально опасным в генетическом отношении.

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CYTOGENETIC EFFECT OF THE HERBICIDE 2,4-D ON HUMAN AND ANIMAL CHROMOSOMES

M. A. PILINSKAYA

Summary

The frequency of chromosome aberrations was studied in the culture of human peripheral lymphocytes in vitro and in the mouse bone marrow cells in vivo treated with the herbicide 2,4-D at different doses. The human lymphocyte chromosomes in culture are more sensitive to the injuring effect of the preparation. These results emphasized potential genetical hazard of 2,4-D, that should be taken into account in hygienic reglamentation of the herbicide application.

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The Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Guppies (*Poecilia reticulatus* Peters)

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a contaminant of the commercially produced herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). TCDD is immobile and only slowly degraded in soils, not readily taken up by plants, subject to photodecomposition, and low in water solubility (0.2 ppb)[1/] (ISENSEE and JONES 1971, KEARNEY et al. 1971, 1972). It is highly toxic and teratogenic in mammals (COURTNEY et al. 1970, COURTNEY and MOORE 1971). The effects of TCDD on aquatic organisms have not been previously reported.

2,4,5-T is an extremely important tool for reforestation of brush-dominated forest sites. During 1969, 358,800 pounds of 2,4,5-T were applied to forest and rangelands managed by U.S. Department of Agriculture and U.S. Department of Interior (MacLEAD 1971). 2,4,5-T probably presents minimum hazard to organisms in the forest environment, but the effect of TCDD must also be considered (NORRIS 1971). Small quantities of 2,4,5-T are found for short periods in streams after aerial application on nearby forest land. TCDD is presumably also present because most of the herbicide in the stream results from drift or direct application of spray formulation to the stream surface (NORRIS 1967). We are determining the toxic characteristics of TCDD in aquatic organisms and report here some of these characteristics in guppies, *Poecilia reticulatus* (Peters).

MATERIALS AND METHODS

The TCDD used in these tests was part of lot 851:142-22 from the Dow Chemical Company, Midland, Michigan.[2/] Based on mass spectral analysis, the chemical was 98 percent 2,3,7,8-tetrachlorodibenzo-p-dioxin and the balance was 1.3 percent trichloro- and 0.7 percent pentachlorodibenzo-p-dioxin.

Stock solutions (166.6 ppm) were made in chloroform and subsequent dilutions in acetone.

Guppies of various sizes (9 to 40 mm) were placed in 1-gallon glass jars (20 to 22 fish per jar) and received one of four treatments (three replications). The fish were exposed to 0, 0.1,

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1/ ppb - parts per billion.

2/ Mention of a company does not imply endorsement by the U.S. Department of Agriculture.

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1.0, or 10.0 ppb TCDD in 3 liters of well water. WILSON (1968) reported the composition of the well water. All jars received 1.2 ml acetone and were maintained at 20 C for the duration of the experiment. After 120 hours exposure, fish were transferred to clean jars with well water containing no dioxin. Water was changed at 14-day intervals. Dead fish were removed each day and their length recorded (DOUDOROFF et al. 1951). Fish were fed live tubifex worms *ad lib* daily except during the exposure period.

RESULTS

The effect of 120-hour exposure to 0.1, 1.0, or 10.0 ppb TCDD on guppies was irreversible, and all guppies eventually died. Some of the treated guppies died during the 5-day exposure period, but most fish died after they were placed in clean water. All treated fish were dead 37 days after the beginning of the exposure period, and in general, the smaller fish died first. There was no mortality among control fish (Table 1).

TABLE 1

CUMULATIVE GUPPY DEATH AFTER 120-HOUR EXPOSURE TO TCDD

Days since start of exposure period	Mean cumulative death (percent)[1/] TCDD (ppb)[2/]			
	0	0.1	1.0	10.0
5	0	8	11	18
10	0	24	38	32
15	0	34	65	42
20	0	45	80	56
25	0	61	96	83
30	0	72	100[3/]	97
35	0	97		100
37	0	100		
Days to 50 percent mortality		21.7	11.6	18.2

[1/] Mean of three replications.

[2/] Parts per billion.

[3/] Last fish died in this treatment on 28th day.

The data were expressed as survival time in days and body length in mm for each fish and subjected to analysis of covariance. Survival time was significantly different at the 5 percent probability level for fish exposed to different concentrations of TCDD ($F_{2,5} = 11.818$). The analysis showed the effect of body length on survival of fish exposed to TCDD was significant at the 1 percent

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level ($F_{1,5}$ = 65.896) (Figure 1). Using the regression equation in Figure 1, we adjusted the mean survival time of fish exposed to each concentration of TCDD for the effect of body length on survival (Table 2).

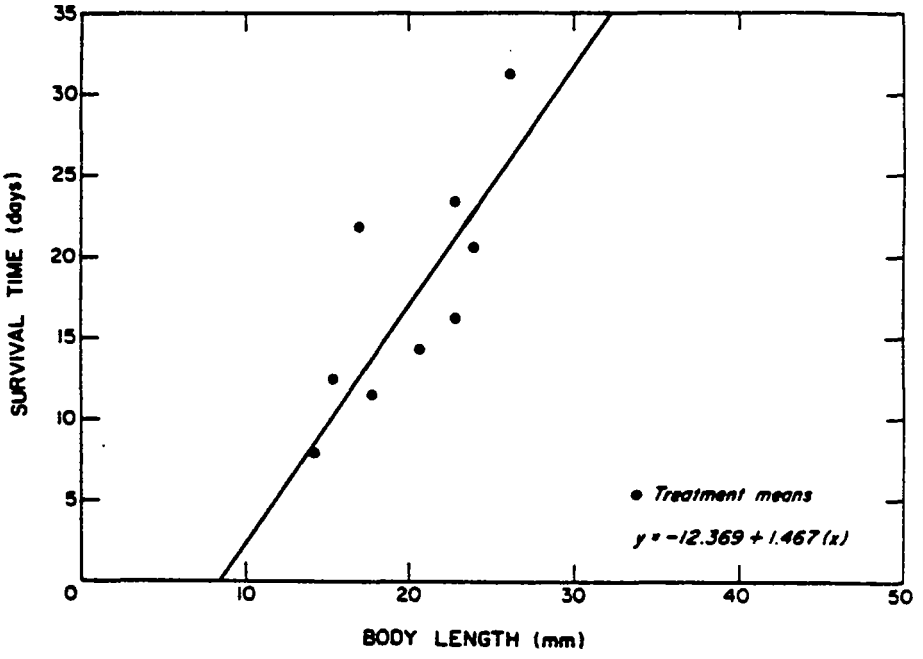


Figure 1. Effect of body length on survival time of guppies exposed to 0.1, 1.0, or 10.0 ppb dioxin in water for 120 hours.

TABLE 2

GUPPY SURVIVAL TIME AFTER 120-HOUR EXPOSURE TO TCDD

Mean[1/]	TCDD (ppb)[2/]		
	0.1	1.0	10.0
Fish length (mm)	20.1	20.7	20.6
Survival time (days)	21.3	14.1	17.2
Survival time adjusted for fish length (days)	21.9	13.7	17.0

[1/] Mean of 3 replications.

[2/] Parts per billion.

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DISCUSSION

Our results show smaller fish are considerably more sensitive to TCDD than larger fish. Similar results have been reported for several other toxicants. Many organisms increase their tolerance to environmental stresses with increasing age and body mass. Toxicant uptake, storage, and detoxication patterns probably change with fish age as lipid levels increase and the ratio of gill surface area to body mass decreases (COPE 1971, MURPHY and MURPHY 1971).

We are uncertain about the reason for the apparent decrease in toxicity between 1.0 and 10.0 ppb TCDD, but suggest it may be the result of working beyond the normal limits of TCDD solubility. Fish in the 1.0-ppb treatment may have been exposed to supersaturated levels of TCDD, possibly mediated by interaction with fish skin mucus in the water. The solubility of DDT in water is enhanced in the presence of some large organic molecules (WERSHAW et al. 1969).

Approximately 1 week after initial exposure to TCDD, guppies exhibited a declining interest in feeding and swimming. All treated fish surviving more than 10 days showed fin necrosis which became more pronounced with time. Necrosis of the maxillary cartilage appeared in two large females surviving more than 25 days after exposure to 0.1 and 10.0 ppb TCDD. None of these behavioral or pathological signs of distress were observed in control fish. The site of action of TCDD in guppies is not known; however, experiments with other animals suggest the liver may be involved (BUU-HOI et al. 1972a, 1972b, CUNNINGHAM and WILLIAMS 1972, PIPER and ROSE 1971). The delayed mortality we observed with guppies is consistent with the hypothesis that TCDD induces liver dysfunction.

Static water bioassays are not particularly satisfactory for determining chemical toxicity in terms of field exposures (JUNTUNEN and NORRIS 1972). They can, however, provide information on the toxic characteristics of a particular chemical and on the relative toxicity among chemicals. Most static water tests are terminated after 96 hours of exposure. The degree of TCDD toxicity in guppies was hardly apparent in our tests even after 120 hours of exposure. We suggest all tests of chemical toxicity to aquatic organisms provide for observation of delayed mortality.

The concentrations of TCDD used in our tests were much higher than those likely to be encountered in forest streams. Concentrations of 2,4,5-T in excess of 0.1 ppm have not been found in Northwest forest streams (NORRIS 1967). Current production grade 2,4,5-T contains less than 0.1 ppm TCDD: therefore, streamwater containing 0.1 ppm 2,4,5-T should contain no more than 0.01 part per trillion TCDD. Experiments are now in progress to determine the threshold-response level of salmon fingerlings and guppies to TCDD in water.

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SUMMARY

Exposure of guppies to 0.1, 1.0, or 10.0 ppb TCDD for 120 hours caused complete mortality in the next 32, 21, and 30 days, respectively. Duration of survival was significantly and positively correlated with body length. The concentrations of TCDD used in these tests were considerably greater than expected to occur in forest streams after aerial application of 2,4,5-T. The threshold-response level for TCDD in fish has not been reported.

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"A Report to the Consumer"

DIRTY TRICKS

Vol. V No.113/114 (Nov '75)

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This special 8-page newsletter deals with two dastardly and possibly-illegal deeds. 1) Deliberate falsification of facts by the L.A.County Chief Administrative Officer (CAO) on a vital health issue, aided and abetted by the Los Angeles County Health Services, the Forester and Fire Warden and the Agricultural Commissioner! 2) Violation of Federal Law, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) by the United States Department of Agriculture Forest Service, with the approval and blessings of the California Agricultural Commissioners!

#1. Ever since 1945, charitable donations have been doled out to the National Forest Service by the L.A. County Board of Supervisors under "Aid to Other Governments and Non-Profit Organizations" to maintain "recreational" services for persons living in the County. The amount ranged from $20,000 to the current $90,000. The Sept.2, 1975 agenda item (for another hand-out) was challenged by this Consumer Activist, charging that "the continued use of herbicide/defoliants (2,4-D, 2,4,5-T, 2,4-DP,etc.) by the Forest Service throughout the U.S. is a hazard to the entire community, especially to pregnant women and women of child-bearing age, due to the teratogen DIOXIN that is present in all phenoxy herbicides - the very reason that the U.S.Surgeon General in 1970 ordered them banned from military use...Since the market for herbicides was lost when the war ended in Vietnam, the chemical companies have accelerated the big push to unload these deadly defoliants on US."

The $3½ million government "Bionetics Study" (commissioned by National Cancer Institute) revealed that these herbicides caused cancer, birth deformities and genetic damage. They were developed by the military at Fort Detrick, Md. to be used as Chemical Biological War Weapons, and only recently our Senate renounced their use as weapons of war. It is immoral that their use continues, especially since evidence has shown that they accumulate in the food-chain, presenting a long term danger to all living things. The herbicides are "genetic atomic bombs timed to explode in the future with an unpredictable outcome" said Senator Gaylord Nelson and he justifiably insisted that funds sought by the National Forests to spray 2,4,5-T be deleted from the budget of the U.S.Forest Service.

The National Cancer Institute (NCI) statistics for the first 7-months of '75 showed that CANCER MORTALITY has increased 5.2% this year, higher than it's been since they began gathering mortality data in 1933! The recent Library of Congress report "Effects of Chronic Exposure to Low-Level Pollutants in the Environment" documented the inadequacies of our current research and monitoring activities, and reported that environmental problems might account for as much as 70 to 90% of causes of cancer, and were responsible also for heart diseases. It is no surprise that the only pollutants named were asbestos, chlorines, fluorides, nickel and mercury.

2,4 ichlorophenoxyacetic acid caused cancer in 22 laboratory animals, focal pneumonitis in 11 animals and metamorphosis of the liver. 2,4-DP caused cancer in 7 laboratory animals and focal pneumonitis in 8 animals. 2 4,5-T caused cancer in 9 animals, folicular hyperplasia of the spleen, lymphoid hyperplasia of the lungs, focal pneumonitis, lymphoid nodules, pulmonary edema, lymphoid infiltration in the lungs, and monstrous birth deformities. They are all toxic chlorinated hydrocarbons in addition to being hormones. There are no known antidotes for them and they should have been outlawed

The item ($90,000 to Forest Service) appeared on the agenda for 6 consecutive weeks and was finally heard on Oct. 30th., just prior to Halloween, and as usual, the CAOs office was ready with their bag of tricks. In his letter to the Board of Supervisors, CAO Harry Hufford conveyed the following misinformation: "Herbicides which produce toxic dioxins have not been used by the Forest Service in 1973, 1974, or to date in 1975. This is confirmed by use permits to the Forest Service and the County monitoring program which has discovered no trace of dioxins (emphasis added) in any of the 250 samples of soil, water and air examined in the County pesticide laboratory over the past 12 months." THIS STATEMENT IS A BLATANT FALSEHOOD! The County does not have facilities or equipment to test for dioxin - THEY DID NO TESTING FOR DIOXIN!



In addition to testing "soil, water and air," the CAO stated that they had tested horses, rabbits, coyotes, squirrels, deer, fish and 2 lions for residues of phenoxy herbicides and dioxin. Since the issue raised was contamination within the forest service, one would assume that the animals tested had come from within the Angeles National Forest. Under questioning they admitted that one of the rabbits came from Downey, the other from the Long Beach Naval Base - one of the lions came from "Lions, Inc." owned by John Marshall Farms, nobody knew where the other lion came from, or whether lions still roam in our forests! One coyote came from Malibu, another from Antelope Valley - a deer from William S. Hart Park - a horse from La Canada, another from Altadena, and the fish from Rio Hondo! If one has integrity... in order to determine residues of toxic chemicals in wild animals that live in the national forests, one would go directly to the scene of the crime (the forest). It is highly irregular and downright dishonest, but very convenient for the agencies named, to secure specimens from Tim-Buck-Two.

October 30th. Acting Board Chairman, Baxter Ward, instructed the CAOs office to get an immediate response to the questions I had raised, and a meeting was held with Dr. John Affeldt (County Health Services), Paul Engler (Agricultural Commissioner), Dick Baird and Jack Michaels (CAOs office) and your Consumer Activist. After one hour of dickering with Affeldt, Engler and Baird, who finally admitted that they knew little, if anything, about dioxin, we awaited the arrival of their expert, Dr. Naresh Jain (Toxicologist, Rancho Los Amigos Hospital/Downey) who was unaware that 2,4,5-T is not the only phenoxy herbicide that contains Dioxin. I pointed out that scientists Edwin A.Wilson, Ronald F. Thomas and Peter D.J.Enser with the USDA Plant Research Division at Beltsville, Md. on 8-19-71 had proven that hexachlorodibenzo-p-dioxin was present in 2,4-D up to 1 part per million - that 13 dioxin isomers had been isolated in the phenoxy herbicides, and that there were reams of government publications showing 2,4-D, 2,4-DP, 2,4,5-T, 2,4,5-TP,etc. contain dioxin!

The County "experts" treated the information with disdain and even though they had been told to answer my questions, there was no way of penetrating their skulls. Hours of valuable time were wasted and since Baird had trouble formulating my questions, I wrote them for him, but neither the questions nor the answers were submitted to the Board. I charged the CAOs office with deliberately lying to the Board (Hufford should have verified the facts before submitting such misinformation to the Board). Dr. Affeld defended the CAO saying "It was an honest mistake - they weren't able to test for dioxin, and so they didn't find any." Dr. Affeldt holds one of the most responsible and highest paid jobs in our County - Our health rests on his "educated judgement." We finally agreed that it was imperative that there be routine testing for dioxin, but since the equipment would cost around $150,000. the suggestion was made that the Environmental Protection Agency (who now has the equipment) be contacted to see whether they could do the testing for the County. The recommendation was never made to the Board.

The following was submitted to the Board of Supervisors: "The controversy

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is that on one hand Ms. Honorof feels these herbicides (2,4-D, 2,4-DP) form highly toxic dioxins, while our Health Services and the Agricultural Commissioner do not agree based on their analysis (THERE WAS NO ANALYSIS FOR DIOXIN) In addition, the herbicides presently being used by the Forest Service have been approved by theEnvironmental Protection Agency. While we will be happy to work with Ms. Honorof further on the matter, we feel the Forest Service's use of herbicides is proper, and recommend the Board sign the policy budget contract with the Forest Service." The Board approved the recommendation!

The three Supervisors (Ward, Edelman and Schabarum) admitted that they knew nothing about the herbicides, or about dioxin, at the same time that they refused to listen so they could learn. The question we must raise is HOW MANY TIMES HAS THE CAO's OFFICE SUBMITTED TOTALLY FALSE AND MISLEADING INFORMATION WHICH WAS APPROVED BY THE BOARD OF SUPERVISORS? If these people had been under oath, any statement they submitted would be under penalty of perjury, they would be mighty careful with their recommendations. The issue on hand isn't just one of total waste of taxpayer's money - it is a serious health problem. Now the Board of Supervisors will be held liable for perpetuating a program that is detrimental to health and life.

In a personal letter to Supervisor Baxter Ward I pointed out that I had correspondence from Drs. Granville Knight (Santa Monica), Terry Lash (Environmental Defense Fund/Palo Alto) and E.W. Pfeiffer (Univ/Montana- Missoula)with the warning that PREGNANT WOMEN AND WOMEN OF CHILD BEARING AGE SHOULD NOT BE PERMITTED TO ENTER ANY AREAS WHERE PHENOXY HERBICIDES HAVE BEEN SPRAYED, due to the birth deforming contaminant that is present in all of the phenoxy herbicides. Four years ago statistics showed that "8% of all live babies are born with some birth defect in the U.S." This figure is nothing compared with what will happen in the future. How will our scientists be able to pinpoint the cause of increased birth deformities? Babies born with gross deformities will not have "finger-prints" or a label attached saying: "THIS DEFORMITY WAS CAUSED BY 2,4-D," or some other phenoxy herbicide.

Dr. Gerhard Grummer (Rostock Univ/Germany) toured both South and North Vietnam and returned with documented evidence proving that these herbicides should NEVER AGAIN BE USED, either during periods of war or periods of peace. 25% of all mothers that came in contact with "Agent Orange" (50%-2,4-D and 2,4,5-T) gave birth to deformed babies suffering from a most serious hereditary disease-"Down's Syndrome". (They had 47 chromosomes instead of the normal 46). Children with this surplus chromosome will at best attain the physical constitution of a 10-year old when they are adult. They remain small in stature, their limbs are short and stunted and their bodies are clumsily structured. The neurocranium is not infrequently smaller than normal. At best they will acquire the mental status of a normal 7-year-old when fully grown. The harm done to their minds is even graver than the damage done to their bodies. MUST WE WAIT FOR THIS SAME TRAGEDY TO OCCUR IN THE U.S. BEFORE WE WAKE UP AND STOP THE USE OF THESE DEFOLIANTS?

Birth deformities are on the increase in the U.S., but there isn't a single government agency that has an on-going monitoring system to determine what caused the increase. I spoke to one of the people with Department of Health in Sacramento (who asked to remain nameless) who stated: "The data we have isn't worth a damn - there is no standardization among doctors. Sometime ago they used to put down congenital birth defects on the certificate, but the doctors are lax because it is not demanded of them...We don't have the hard data to collect - WHY MASSAGE IT?" Another call to Berkeley netted: "There is no on-going monitoring program, simply because many times doctors don't know what to look for, and often the birth deformity will show up after the doctor has signed the birth certificate, so it isn't included on the certificate." "The information is sketchy," said Mitch Tashiro, a statistician with the Health Department in Sacramento, "fragmented - trying to monitor off of birth certificates would be enormous - birth certificates are poorly kept -

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There is no program geared to identification and reporting birth deformities."

The Pesticide Chemical News of 9-10-75 revealed the information that had been suppressed for over 6 months, that "TCDD (tetrachlorodibenzo-p-dioxin) had been found in cattle tissues in levels up to 100 parts per trillion (ppt) p[illegible]f that dioxin is present in our food chain. The Department of Agriculture and the chemical companies were aware that the phenoxy herbicides remained and bio-magnified in our food chain in the early 70s (Sen.Hart Hearings). Samples are now being taken by the EPA from small mammals who were exposed to TCDD, and samples will be taken from mothers milk as well, to determine residues of TCDD, and the Hearings that were postponed on June 24,1974, "to permit the EPA to set up a monitoring program to determine if TCDD is bioaccumulating in man and the human food chain," will resume soon. The EPA must also include monitoring for everyone of the phenoxys and their metabolites - DIOXIN IS AN INTEGRAL PART OF THE PHENOXY HERBICIDES.

The Board of Supervisors have allowed themselves to be lied to - by the CAOs office - the Health Services white-washing and covering up (which makes them equally guilty) - with the inadequate monitoring and non-existing "testing" for dioxin. I suggested a public hearing in L.A.County to expose the insanity of herbicide use - working towards the State of California enacting a total ban. The herbicides are too toxic to be used by the military - how can they justify their safety on our forests, rights of way, schools, agricultural use, etc. I also suggested that legislation be enacted whereby heavy fines and jail sentences would be imposed on government officials, public health service personnel and scientists, who deliberately make false statements concerning data not borne out by proper analysis and evaluation. Jail sentences would be the only way of curing the problem, especially since these deadly compounds jeopardize the health, welfare and life of the entire community. We pray that our public servants will rise to the occasion.

I[illegible]he Environmental Impact Report (EIR) that was prepared by L.A.County Agricultural Commissioner for their "Alligatorweed Eradication Project in '73, AND WHICH NONE OF THE COUNTY SUPERVISORS HAD BOTHERED TO READ, the facts presented proved that "2,4-D and 2,4,5-T have mutagenic potential as demonstrated in tests with several plant systems (Mrak,1969), Unrau and Larter (1952), Unrau (1953,1954) found 'highly significant' abnormalities of chromosome behavior in rapidly dividing cells of wheat and barley sprayed with 2,4-D ethyl ester. Muhling et al (1960) also found chromosomal effects in peas treated with 2,4-D. 2,4-D isoctyl ester, 2,4-D butyl ester as well as 2,4-D isopropyl ester produced statistically significantly higher incidences of congenital malformation in mice or rats. Innes, et al (1969) reports 2,4-D isopropyl ester and 2,4,5-TP yielded an increased carcinogenic tumor incidence in comparison to negative controls. Mrak (1969) suggests these compounds need more testing but the priority for testing is not high in comparison with some other pesticides." The Agi. Comm. should review this EIR!

TCDD(dioxin) is so lethal that only 6 parts of dioxin per 10 billion parts (bodyweight) was lethal in laboratory tests. It is the world's most lethal synthetic agent. The Library of Congress lists its acute oral toxicity for humans as 42 micrograms. One drop can kill 1200 persons- 30 gallons could kill every living thing on this earth! Records show that millions of chickens died in the U.S. in 1957 from pericardial edema, which was traced to the inclusion of fat in the diet, which contained hexachlorodibenzo-p-dioxin (Cantrell, Webb & Mabis,1969). Structurally similar compounds, tetra and penta chlorodibenzofurans are contaminants of polychlorinated biphenyls that cause hy[illegible]opericardium in chicks and hyperkeratosis of the rabbits ear (Vos & Beems, 19[illegible]; Vos & Koeman, 1970; Vos Koeman, van der Maas ten Noever de Brauw & deVos, 1970). Other studies have shown that dioxin is a potent teratogen and foeticidal agent for rats and mice (Courtney & Moore,1971; Neubert & Dillmann,1972; Sparschu, Dunn & Rowe,1971). Since the 1930s, chloracne has been recognized as an occupational hazard associated with the production or use of

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chlorinated aromatic compounds. Chloracne is not an insignificant disease, on the contrary, it is a very serious one, accompanied by loss of vigor and mental ability, as well as many physical manifestations. It was this same contaminant that forced the closing of Dow's Midland Michigan plant in the late 60s, until they could overcome the problem. Since 1950 Dow had been keenly aware of the possibility of a highly toxic impurity formed in 2,4,5-T. Workers developed chloracne and their bioassay program showed that chloracne potential of the waste-oil from 2,4,5-T process was building up to a danger point - Neither the FDA nor the USDA were notified in 1965, because no-effect levels were ever reached even at the lowest dose tested. The Diamond Alkali plant stopped manufacturing 2,4,5-T. (Julius Johnson at Hart Hearings,1970)

Despite the fact that the L.A. County expert Naresh Jain, PhD, stated that the only toxic dioxin he was aware of was tetrachlorodibenzo-p-dioxin in 2,4,5-T. Page 29 of the L.A.County EIR reads: "Dioxin content of phenoxy herbicides is important. Dichlorodibenzo-p-dioxin would be the major species of dioxin in 2,4-D and tetrachlorodibenzo-p-dioxin would be the major species of dioxin in both 2,4,5-T and 2,4,5-TP." Page 107: "The highest tissue levels of 2,4-D and 2,4,5-T were found in liver, kidney, lung and spleen, the levels sometimes exceeding the plasma level...In blood cells, 10-20% of the plasma level was found. Penetration of 2,4-D into adipose tissue and into the central nervous system was restricted, whereas a ready placental transfer was demonstrated in swine. (K.Erne,1966)."

There are additional facts that the County experts omitted. The fact that many plants have accumulated lethal quantities of nitrates after being sprayed with 2,4-D. The County EIR p-143 states: "The accumulation of nitrates in the leaves of treated sugar beets is well known (Savage 1949). Increased levels of nitrate in the leaves of this crop as a result of herbicide applicated have been reported by Willard (1960), Stahler & Whitehead (1950), & Whitehead et al (1956). Isolated incidents have been reported of nitrate poisoning of cattle in America as a result of feeding on sugar beets that had previously been sprayed...N.Dakota, the nitrate content of sugar beet leaves after spraying was found to vary from 1.81 to 8.77% of the dry weight, as against 0.22% for untreated plants and a toxic level of 1.5%. (Stahler and Whitehead (1950)." p-144:"Studies on forage crops (Berg & McElray, 1953)and on a range of weed species (Frank & Grigsby,1957) have shown which of these may contain high levels of nitrates after auxin application...Nitrate poisoning in stock does occur from time to time and it is possible for the hazard to be increased by application of auxin herbicides to nitrate-accumulating plants. P-145: "2,4-D and MCPA were toxic to bees, not only from visiting the flowers but also as a result of drinking contaminated water trapped on treated plants. (Wahlin,1950) 2,4-D might have some effect on nectar which made it toxic to bees. (Palmer-Jones,1964; Antoine,1966).

It is a pity that the Board of Supervisors did not read the Alligator Weed EIR. They would not have approved the project. In the recommendations made by the Agri. Comm. the following appears: "Herbicide monitoring of the Angeles Forest area could be increased. The Agri.Comm- Health Services herbicide monitoring program has thus far been concentrated in more highly populated areas and comparatively few samples have been taken from the Angeles Natl. Forest. It should be noted that testing samples is a laborious costly process as is the collection of samples. To gather a greater number of samples from relatively inaccessible areas in the forest would add appreciably to the time demands upon the monitoring employee." This recommendation will be closely followed, inasmuch as I have a solemn promise that I will accompany whoever goes out to the Forest Service to collect specimens for analysis.

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#2. Some months ago I requested information from the Angeles Forest Service pertaining to what herbicides had been used by the Forest Service during the years 1973, 1974, 1975; and what do they have planned for 1976, etc. etc.

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The cheerful reply I received from Robert Reese, (Watershed & Resource Management Officer) was: "...The amassing of the data you have requested exceeds the time that I have to allocate - considering my other heavy duties in the management of the Angeles National Forest." He invited me to call for an appointment - he would provide the work space - the charge was "0.10/page for large amounts of zerox copy work."

I had also communicated with the San Francisco office and received a report from John A.Vance, (Chairman Pesticide Use Coordination Committee) and T.W.Costella (Deputy Regional Forester in San Francisco). This report listed those herbicides that had been used in the California National Forests in 1975. Based on their information and the information shown on the Monthly Pesticide Use Report received from L.A.County Agricultural Commissioners office, I learned that the California Forest Service as well as the Agricultural Commissioners (who approve pesticide use) had violated the Federal Insecticide, Fungicide and Rodenticide Act, inasmuch as the herbicides had been used contrary to the instructions on the label.

Some of you will recall the expose in RTC #60- Sept.'73, and the near fatal incident where a L.A.City Park employee, Harry Fales, had been applying pesticides without ever being advised of their toxicity or their identity. The herbicide PARAQUAT had been transferred from the original container to an unmarked one. Protective attire was not provided - and other serious violations (FIFRA - - - then there was the death of a student at El Dorado High School, Jerry Maes, after drinking what he thought was fruit juice in the principal's office. Jerry Maes died from drinking PARAQUAT, despite the fact that ALL Paraquat was to have been removed from schools, universities, playgrounds, golf-courses, etc.,etc. It was being used in violation of the label precaution. (See RTC #81) The label specifically reads "KEEP PESTICIDE IN ORIGINAL CONTAINER - DO NOT PUT CONCENTRATE OR DILUTE INTO FOOD OR DRINK CONTAINERS, KEEP OUT OF REACH OF CHILDREN, DO NOT USE AROUND HOME, GARDENS, OR OTHER AREAS LIKELY TO BE CONTACTED BY CHILDREN OR PETS."

On Feb. 2, 1974, in an unprecedented action, the EPA formally charged three government agencies (Los Angeles County, Los Angeles City, and Thousand Oaks School District) with violation of FIFRA, and warned them that future violations would be punishable with fines up to $25,000. and prison terms up to 1 year for violators. PARAQUAT HAD BEEN IN USE FOR SOME 20 YEARS, BUT NOBODY, NO AGENCY, HAD EVER TAKEN THE TROUBLE TO READ THE LABEL WARNINGS, UNTIL R.T.C. PUBLISHED IT IN 1973.

In a Certified (return receipt requested) letter to Russell E. Train, Administrator of the Environmental Protection Agency, I informed him that there has been a violation of FIFRA "on the part of (1) the USDA Forest Service on a State (California) level; The individual Forest Services at Angeles, Los Padros, Cleveland, Mendocino and Sierra. (There may also be others throughout the U.S., but I only have the report for California) By the Los Angeles County Agricultural Commissioner as well as the Agricultural Commissioners of San Diego County, Santa Barbara (or Ventura) County, etc.,etc. and other agencies who have been responsible for concealing the violation. The above named Forest Services each used AMCHEM's Weedone-170 (butoxy ethanol ester of 2,4-D and 2,4-DP - 31.8% of 2,4-Dichlorophenoxyacetic acid, butoxyethanol ester and 31.1% of 2,4-Dichlorophenoxypropionic acid butoxyethanol ester) in addition to using Dow's Esteron-99. The issue of violation is with the use of Amchem's Weedone, prohibited from being used in recreational areas. (Many of these pesticides are used in direct violation of the label, where they are

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restricted if the pesticide gets into water supplies,etc., but we were concerned with a stark violation that even a child can understand: "DO NOT USE AROUND THE HOME OR I RECREATIONAL AREAS." The Forest Service are a multi-[illegible] use natural resource. Despite the fact that there has been a ban on the use of 2,4,5-T in "recreational" areas, and hundreds of people enter OUR forests for recreational purposes, the Forest Service harbored the thought that they were above the law - immune from regulations.

The Amchem Weedone-170 label reads: "CAUTION, Harmful if swallowed. Avoid contact with skin, eyes or clothing. Avoid spray drift to susceptible plants such as cotton, beans, tomatoes, tobacco and ornamentals as this product may cause injury...Under very high temperature of use, vapors from the product may injure susceptible plants in the immediate vicinity. This product is toxic to fish. Keep out of lakes, streams or ponds. Do not apply where runoff is likely to occur. Do not apply when weather conditions favor drift from treated areas. Do not contaminate any body of water by washing or spray equipment or disposal of waste...Do not contaminate water used for irrigation or domestic purposes. Do not use in a greenhouse. Do not use around the home or in recreational areas."

Our National Forests ARE recreational areas! It is inconsistent and outrageous that government agencies do not conform to laws. There are 43 National Forests in our country (17 of them are in California) and millions of persons are exposed to these toxic defoliants.

According to the 1975 report I received from the Regional Forest Service in San Francisco, Angeles National Forest, in addition to using 2,4-D Ester, 2084 lbs. a.e. on 521 acres, also used 1076 lbs a.e. of Amchem's Weedone-170 on 269 acres. Cleveland National Forest used 3344 lbs a.e.(on 836 acres) of Amchem's Weedone-170. Los Padres National Forest used 2528 lbs a.e. on 632 acres of Amchem's Weedone-170. Mendocino National Forest used 5400 lbs. a.e. on 1600 acres of Amchem's Weedone-170. Sierra National Forest used 1200 lbs. a.e. on 300 acres of Amchem's Weedone-170.

The Amchem Weedone-170 carries the "EPA Reg. No. 264-222 AA, EPA Est 264-PA-1 Form No.3757-30, M-10/74-MP." The date stamped is May 1975. The label is specific in cautioning that the product (propionic acid mixed with phenoxyacetic acid) is not to be used in recreational areas. The Pesticide Use Report (dated June 1975) for Angeles shows that 226 gallons were used in 1975, and the July 1974 report shows that 175 gallons were used in 1974. The response from Robert Reese was a violation of Freedom of Information Act (18-U.S. Code Sect. 1001). Under this Act I am entitled to the information on request, but I imagine Mr. Reese is still smarting from L.A.City Council's historic motion of September 20, 1972, censoring the USDA Forest Service for using 2,4-D, 2,4-DP and 2,4,5-T. At that time he stated "Passage of the motion need have no effect on the operations of the Angeles National Forest - the effect of such passage however would tend to place an undeserved stigma on the use of these sophisticated tools of land management...such stigma is undeserved." And Mr. Reese and all of the other Resource Management Officers continue to rain down these teratogenic, mutagenic and carcinogenic hazards on the populace.

Senator Gaylord Nelson recently stated "We are closer now to the truth on 2,4,5-T than we were when we so blindly dumped one hundred million pounds of herbicides on South Vietnam. We are closer to the truth than we ever were when the Grant County rangeland spraying occurred two years ago. But all the facts are not in. Knowing what we do, however, it seems to me the height of folly to have the U.S. Forest Service shutting its eyes to the EPA pre-trial brief while they routinely spray in the U.S. National Forests."

Senator Nelson was referring to the EPA's statement indicating that they were having technical difficulties "in the use of its method for detecting dioxin (July 1974) and noting new uncertainties about the persistence of 2,4,5-T."

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The EPA brief summarized the potential dangers of 2,4,5-T. "Hazards of birth defects from 2,4,5-T and TCDD - TCDD was mutagenic - There was carcinogenic potential - a pernicious, little understood toxicant, capable in minute quantities of inducing a variety of chronic illnesses and, perhaps of causing c th as a delayed response to exposure - TCDD poisoning may be cumulative - because the effects of long-term exposure to low levels of TCDD remain undetermined, an acceptable level for man cannot be set. If TCDD exposure causes delayed lethality, or if continuous impingement of TCDD on human organisms otherwise causes cumulative effects or if TCDD concentrates in human tissue, a level of exposure which would be safe for the general population may not exist. Even residues below the current level of detection may be unsafe... The capacity of TCDD to penetrate, persist, to move and to bio-concentrate in the acquatic and terrestrial environment, given the incomparable toxicity of this small molecular compound, and given the practical nonexistence of facts about its ecological effects..there cannot be a reliable conclusion that TCDD is not causing serious environmental injury.

"The polychlorophenal in 2,4,5-T may decompose into dioxin when exposed to high temperatures such as might occur with incineration or even cooking of food. TCDD can be generated by the thermal stress of 2,4,5-T and some of its metabolites, raising the potential for the generation of additional dioxin under environmental conditions...People may be exposed to a latent destructive force - accidental or unknown triggering of the thermal release mechanism by which 'harmless' amounts of 2,4,5-T, its esters or salts, convert to letnal tetra-dioxin...dioxin and 2,4,5-T could build up to dangerous levels within man and animals - possibly in the food chain as well.(This was proven 9-10-75)

"Model ecosystem studies suggest that TCDD bioconcentrates more than DDT... acquatic ecosystem showed catfish accumulated tetra-dioxin in only 3 days by a factor of 14,000...Analysis of residues in Vietnamese shrimp and crusta- c ns detected significant levels of tetra-dioxin following defoliation in regions draining into the area from which the shrimp were collected...Residues have not declined between 1970 and 1973, although defoliation ceased in 1969. Wildlife in the vicinity of"Agent Orange"application at Eglin Air Force Base (Florida) retained measurable levels of TCDD several years after use of the herbicide (2,4-D and 2,4,5-T) was stopped...25% of the dietary intake of tetradioxin may be stored in body tissues...withdrawal of cattle from a diet contaminated with dioxin for as long as one week may have little effect in decreasing TCDD residues...Current label provisions requiring 'feed off' periods on dioxin free food to assure absence of dioxin residues in the meat are not likely to be effective in reducing tetra-dioxin residues...Monitoring wildlife collected along rights-of-way in the U.S. demonstrates as does the Vietnamese aquatic residue data, that 2,4,5-T related TCDD can enter the food chain from 'non-food' uses." The above holds true for all of the phenoxy herbicides. These facts will be brought out during the upcoming Hearings.

PS: Russell Train was also advised that after talking with back-packers, we have been advised that the Angeles Forest Service has violated a contract they had with L.A.City Attorney's Office to post areas in Angeles prior to treatment with any herbicide, warning the public that such treatment will take place, by either stationing of guards or posting of signs. THERE IS NO ALIBI FOR VIOLATIONS OF LAW! THE ONLY ALTERNATIVE IS A TOTAL BAN!

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Removal of 2,4-Dichlorophenoxyacetic Acid (2,4-D) Formulations from Noncombustible Pesticide Containers

by T. E. ARCHER

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Retained pesticides in discarded used containers can be a serious environmental pollution problem. If 1, 5, 30 and 55 gal[1] metal pesticide drums are defined as noncombustible packaging materials, then the large number of these containers that accumulate yearly entail special disposal considerations.

Published reports have discussed the decontamination of organophosphorus insecticides on and around aircraft and spray equipment (WOLVERTON et al. 1970); edaphic aspects of the disposal of unused pesticides, pesticide wastes, and pesticide containers (STOJANOVIC et al. 1972); chemical and thermal aspects of pesticide disposal (KENNEDY et al. 1972); engineering aspects of the disposal of unused pesticides, pesticides wastes, and pesticide containers (SHUMAN et al. 1972); and decontamination of noncombustible agricultural pesticide containers (HSIEH et al. 1972 and ARCHER and HSIEH 1973).

The previous investigations, particularly in regard to detoxication of metal drums, were directed toward organophosphorus insecticides. The present work was undertaken to investigate procedures for the removal of retained amounts of formulations of 2,4-dichlorophenoxyacetic acid in a water miscible (non-volatile weed killer) and a paraffinic (low-volatile weed killer) solvent base from 5, 30, and 55 gal metal drums so that the detoxified metal containers could be disposed by conventional methods or reclaimed for non-food usage.

Materials and Methods

Pesticide formulations. (1) Dow Esteron^R 99^R Concentrate Low-Volatile Weed Killer containing propylene glycol butyl ether esters of 2,4-D; acid equivalent: 4 pounds per gallon; active ingredient: 2,4-dichlorophenoxyacetic acid, propylene glycol butyl ether esters, 72.8%, 2,4-dichlorophenoxyacetic acid equivalent 44.9%; inert ingredients, 27.2%. (2) Dow Formula 40 Non-Volatile Weed Killer containing alkanolamine salts of 2,4-D; acid equivalent: 4 pounds per gallon; active ingredients: alkanolamine salts (of the ethanol and isopropanol series) of 2,4-dichlorophenoxyacetic acid, 59.7%,

[1] Throughout this paper 1 gal = 3.8 ℓ.

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2,4-dichlorophenoxyacetic acid equivalent 38.6%; inert ingredients, 40.3%.

Containers. The containers were flat-head steel drum types commonly used for agricultural pesticides and represented 5, 30, and 55 gal sizes.

Washing solvents. The aqueous solvent was deionized water, and the ethanol was freshly distilled absolute ethyl alcohol.

Diazomethane preparation. Diazomethane was prepared by the method of ZWEIG et al. 1964, and the etheral solution was adjusted to 25 ml volume. One ml of this solution was added to 1 ml of the drum wash extracts and allowed to stand at room temperature for 15 min. The excess reagent and solvent were evaporated by a warm air stream to dryness, the volume adjusted and an aliquot injected into the gas-liquid chromatograph (GLC) for analytical measurement of the 2,4-dichlorophenoxyacetic acid equivalent.

Container retention volume. Excess spray formulation was added to empty, dry, weighed containers. The containers were closed, shaken, and swirled so that the inner surfaces were wetted with the formulation. The containers were opened, inverted, and the excess formulation allowed to drain into a graduated cylinder until dripping stopped (at least 5 min), and the containers were reweighed. The formulation retention in the container was expressed as net retention volume or weight of active ingredient. The retention volumes were determined in triplicate for each container size and the mean retention volumes were added to the containers for the washing experiments.

Container washing. Adequate volumes of washing solvents, determined by visual observations, were added to each size container to result in efficient washing of the inner surfaces. These volumes were 250 ml for the 5 gal, 750 ml for the 30 gal, and 1000 ml for the 55 gal containers. The washing solvent was added to the container, the container was closed, and it was shaken, swirled, and tumbled repeatedly. The container was opened, the wash mixture was drained into a graduated cylinder until dripping stopped (at least 5 min), and the solution was measured and stored for extraction and analysis. Each container was washed repeatedly with each wash solvent, and the data are shown in Tables II through V.

Washing mixture extraction. Ten ml aliquots of the aqueous wash mixture containing the propylene glycol butyl ether esters of 2,4-D were acidified with 5 ml of 5N HCl and extracted four times in a separatory funnel with 25 ml diethyl ether. The ether extracts were filtered through a Whatman #1 filter paper, combined and adjusted to appropriate volumes. To 1 ml of the ether extracts was added 1 ml of the etheral diazomethane for methylating the 2,4-D present, and the excess reagent and solvents were evaporated to dryness after the solutions remained at room temperature for 15 min. Dilutions were made to approximate 1 ng methylated 2,4-D per µl hexane

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and the samples were analyzed by GLC. The aqueous wash mixture containing the alkanolamine salts of 2,4-D were extracted as described above. Each total ethanol wash was concentrated in vacuo at 50-60°C to approximately 1 ml after the pH was adjusted to less than 2 with 10 ml of 5N HCl. The solution was adjusted to an appropriate volume with diethyl ether and 1 ml of this solution was added to 1 to 3 ml of the etheral diazomethane (until the color remained yellow) and allowed to stand at room temperature for 15 min. The excess reagents and solvent were evaporated by a warm air stream and the methylated 2,4-D was adjusted with hexane to approximate 1 ng/µl for analysis.

Detection and determination of the methylated 2,4-D. Gas-liquid chromatography (GLC) procedures were employed routinely. All chemicals used, with the exception of the pesticide, were reagent grade and the solvents were redistilled shortly before use. The gas chromatograph was a Varian Aerograph (Model 1200) equipped with an electron-capture detector and a Leeds and Northrup Speedomax W 1-mv recorder with a chart speed of 1/2-inch per min. Areas under the peaks were measured with a polar planimeter. The chromatographic column, made with a 6-foot x 1/8-inch glass tube packed with 60-80 mesh silylated Chromosorb W, acid washed, was coated with 5% SE-30 silicone gum rubber and 5% Dow 710 silicone fluid. Nitrogen carrier gas (50 p.s.i., 30 ml per min) and a column temperature of 190°C gave the best results and were used in these experiments.

Results

The retention volumes of emptied metal drums of various sizes are shown in Table I. Considerable amounts of 2,4-dichlorophenoxyacetic acid (2,4-D) equivalent in the formulation in terms of ml retention volumes were found actually retained in the drums when they were drained as completely as possible. However, these retention volumes represented approximately 0.12% of the 5 gal container volume and ranged from 0.05% to 0.07% for the 30 and 55 gal containers.

Tables II to IV and V to VI contain data on removal from the emptied containers by water and ethanol-acid or ethanol washing of the 2,4-D acid equivalent in the formulations containing the alkanolamine salts of 2,4-D and the propylene glycol butyl ether esters. The initial high removal efficiency for water washing is probably due to the presence of emulsifiers or other adjuvants in the formulations which were diluted rapidly during the repeated container washings. The remaining 2,4-D was removed by the ethanol-acid or ethanol washings in which the 2,4-D in the respective formulations is soluble. Washing the various sized containers with eight water washings followed by three ethanol-acid or ethanol washings (Tables II to V) removed 95 to 99% of the retained formulations in the supposedly empty containers. When the 55 gal drum containing 83.6 g of the 2,4-D acid equivalent of the propylene glycol butyl ether esters formulation of 2,4-D (Table VI) was washed with four water washings followed by three ethanol washings, approximately 96% of the 2,4-D contaminants were removed from the container.

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TABLE I

Retention Volume of 2,4-D Formulations in Various Sized Metal Containers

Drum Size (gal)	Retention Volume* (ml)	Grams Actual 2,4-D	% of Container Total Volume
	Alkanolamine Salts of 2,4-D		
5	24	11.5	0.12
30	83	39.8	0.07
55	146	69.9	0.07
	Propylene Glycol Butyl Ether Esters of 2,4-D		
30	72	55.5	0.06
55	109	83.6	0.05

*Rentention volumes were determined in triplicate for each sized container and mean values are reported.

TABLE II

Five Gallon Metal Drum Washed with 0.250 ℓ Solvent
2,4-D Removed Per Wash (g)*

Wash	Removed	Present Before Washed**	% Removed in Wash	Total Removed	% Total Recovered
Water					
1	10.200	11.500	88.6	10.200	88.6
2	0.624	1.300	48.0	10.824	94.1
3	0.060	0.676	8.8	10.884	94.6
4	0.025	0.616	4.0	10.909	94.8
5	0.008	0.591	1.3	10.917	94.9
6	0.007	0.583	1.2	10.924	94.9
7	0.008	0.576	1.3	10.932	95.0
8	0.007	0.568	1.2	10.939	95.1
Ethanol-Acid					
1	0.031	0.561	5.5	10.970	95.3
2	0.004	0.530	0.7	10.974	95.4
3	<0.001	0.530	<0.1	10.974	95.4

*2,4-D acid equivalent in alkanolamine salts of 2,4-D.

**Twenty-four ml (11.5 g 2,4-D) added to drum before washing.

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TABLE III

Thirty Gallon Metal Drum Washed with 0.750 ℓ Solvent
2,4-D Removed Per Wash (g)*

Wash	Removed	Present Before Wash**	% Removed in Wash	Total Removed	% Total Recovered
Water					
1	34.900	39.760	87.7	34.900	87.7
2	3.200	4.860	65.8	38.100	95.8
3	0.682	1.660	41.0	38.782	97.5
4	0.175	0.978	17.8	38.957	97.9
5	0.089	0.802	11.0	39.046	98.2
6	0.067	0.714	9.3	39.113	98.3
7	0.039	0.647	6.0	39.152	98.4
8	0.028	0.608	4.6	39.180	98.5
Ethanol-Acid					
1	0.088	0.580	15.1	39.268	98.7
2	0.032	0.492	6.5	39.300	98.8
3	0.019	0.460	4.1	39.319	98.8

*2,4-D acid equivalent in alkanolamine salts of 2,4-D.

**Eighty-three ml (39.8 g 2,4-D) added to drum before washing.

TABLE IV

Fifty-Five Gallon Metal Drum Washed with 1 ℓ Solvent
2,4-D Removed Per Wash (g)*

Wash	Removed	Present Before Wash**	% Removed in Wash	Total Removed	% Total Recovered
Water					
1	59.900	69.940	85.6	59.900	85.6
2	8.520	10.040	84.8	68.420	97.7
3	1.070	1.520	70.3	69.490	99.3
4	0.150	0.450	33.3	69.640	99.5
5	0.041	0.300	13.6	69.681	99.6
6	0.021	0.259	8.4	69.702	99.6
7	0.012	0.238	5.0	69.714	99.6
8	0.009	0.226	3.9	69.723	99.6
Ethanol-Acid					
1	0.009	0.217	4.1	69.732	99.7
2	0.007	0.208	3.3	69.739	99.7
3	0.005	0.201	2.4	69.744	99.7

*2,4-D acid equivalent in alkanolamine salts of 2,4-D.

**One hundred forty-six ml (69.9 g 2,4-D) added to drum before washing.

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TABLE V

Thirty Gallon Metal Drum Washed with 0.750 ℓ Solvent
2,4-D Removed Per Wash (g)*

Wash	Removed	Present Before Wash**	% Removed in Wash	Total Removed	% Total Recovered
Water					
1	30.500	55.500	54.9	30.500	54.9
2	9.800	25.000	39.2	40.300	72.6
3	5.540	15.200	36.4	45.840	82.5
4	3.540	9.660	36.6	49.380	88.9
5	1.500	6.120	24.5	50.880	91.6
6	1.040	4.620	22.5	51.920	93.5
7	0.390	3.580	10.8	52.310	94.2
8	0.380	3.190	11.9	52.690	94.9
Ethanol					
1	1.340	2.810	47.6	54.030	97.3
2	0.550	1.470	37.4	54.580	98.3
3	0.390	0.920	42.3	54.970	99.0

*2,4-D acid equivalent in the propylene glycol butyl ether esters of 2,4-D.

**Seventy-two ml (55.5 g 2,4-D) added to drum before washing.

TABLE VI

Fifty-Five Gallon Metal Drum Washed with 1 ℓ Solvent
2,4-D Removed Per Wash (g)*

Wash	Removed	Present Before Wash**	% Removed in Wash	Total Removed	% Total Recovered
Water					
1	58.850	83.600	70.3	58.850	70.3
2	12.200	24.750	49.2	71.050	84.9
3	4.110	12.550	32.7	75.160	89.9
4	1.650	8.440	19.5	76.810	91.8
Ethanol					
1	2.060	6.790	30.3	78.870	94.3
2	0.860	4.730	18.1	79.730	94.1
3	0.160	3.870	4.1	79.890	95.5

*2,4-D acid equivalent in the propylene glycol butyl ether esters of 2,4-D.

**One hundred nine ml (83.6 g 2,4-D) added to drum before washing.

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Generally the 2,4-D in these formulations was more persistent against removal by the aqueous and organic solvent washing treatments than as previously discussed for emulsifiable concentrate formulations of parathion (ARCHER and HSIEH 1973). However, 95 to 99% of the retained formulations were apparently removed from the containers, and considering the low mammalian toxicity of 2,4-D, this is probably an adequate efficiency.

Discussion

Supposedly empty noncombustible metal containers retained as much as 24 to 146 ml of formulation for 5 and 55 gal sizes, respectively, after the containers were drained as completely as possible. Although 2,4-D is harmless to mammals at phytotoxic concentrations, it has been widely used as a herbicide toward most broad-leaved plants since it is relatively nontoxic to monocotyledonous plants. It has also been used for the prevention of preharvest fruit drop, the production of seedless fruit, and the regulation of the growth of plants.

These effects are useful when 2,4-D is applied to plants intentionally. However, what results would occur if 2,4-D were to appear in and pollute the environment by unintentional application such as contamination of surface and ground waters as well as soil? The improper disposal of noncombustible used metal containers which previously contained formulations of 2,4-D could contribute to such environmental pollution.

It has been shown by previous studies (HSIEH et al. 1972 and ARCHER and HSIEH 1973) that the efficiency of parathion removal from noncombustible used pesticide containers by solvent washing is independent of the chemical properties of the solvents, and this also appears to be true for 2,4-D formulations. The important factors to be considered are solubility of the pesticide in the solvent, or partition between the inner surface of the container and the solvent, and the presence of adjuvants or emulsifiers. The latter is particularly important when water is used. Formulations of 2,4-D can most efficiently be removed from a used noncombustible container, first by four water washes, followed by three washes with a water miscible organic solvent such as ethanol or ethanol-acid depending upon the formulation in the container. The aqueous and alcoholic washes can be added to the spray tank and disposed with the spray mixture provided that this does not adversely affect the properties of the spray liquid or constitute a flammability hazard. The decontaminated drums could then be disposed in a conventional manner or reclaimed for pesticide use if their condition warrants it.

This procedure is a convenient and economical method for removal of potentially hazardous pesticides remaining in emptied noncombustible metal containers, and is a practical solution for the disposal of the containers as well as for the prevention of environmental pollution with unwanted chemicals.

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E. L. Delvaux, J. Verstraete and A. Hautfenne, in collaboration with F. DeSart and G. Goffin*: Polychlorodibenzo-*p*-dioxins [Les Polychloro Dibenzo-*p*-Dioxines].

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POLYCHLORODIBENZO-*p*-DIOXINS

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Summary

Polychlorodibenzo-*p*-dioxins (chlorodioxins) are produced most frequently when chlorophenols are heated in an alkaline solution, particularly in the preparation of chlorophenol derivatives. Consequently chlorodioxins may contaminate a great number of products which are widely used, such as certain pesticides for example. Occasionally, chlorodioxins are also synthesized from chlorophenols or their derivatives *in situ*, outside of industrial processes, when all the required conditions are accidentally met.

It has been shown that chlorodioxins are the cause of chloracne as well as of chick edema which killed thousands of chickens in American farms. Furthermore, chlorodioxins are suspected to cause congenital malformations, possibly even in humans. It is thus undeniable that the toxic effects of chlorodioxins must be studied, and the importance of analytical detection methods becomes understandable.

In this first part of our study we survey the research work which has led to the discovery of chlorodioxins and their properties.

In the future, we shall publish a review of analytical methods and the results of our experiments.

Introduction

The designation and numbering of the atoms of substances presently called polychlorodibenzo-*p*-dioxins (or possibly chlorodioxins, an abbreviated and unspecified designation) have changed in the course of time.

The paper by Gilman and Dietrich [1] seems to represent the starting point of our present designations, at least as far as the numbering of the atoms is concerned. "Chemical Abstracts" uses

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this numbering system. Some authors, generally predating Gilman and Dietrich, use other names for chlorodioxins or other numbering systems. In such cases, we kept the earlier designation and put it in quotation marks. To the degree that the indications of the authors permit an identification, we give the present designation in parentheses. In a table included as an appendix we list the formulas and the current designations for all the polychlorodibenzo-*p*-dioxins cited in this paper.

Chlorodioxins had already been discovered a century ago as the products of the pyrolysis of phenochlorinated compounds or as secondary products which form during the synthesis of such chlorinated derivatives. Workers who handled chlorodioxins were afflicted with chloracne even if they had handled only traces of the substance and they rapidly identified the responsive agent. Later the extremely acute toxicity of the chlorodioxins was again discussed when several thousand chickens died in American farms. The substances acquired the name "chick edema factor". Their importance has not decreased since. At the present time, chlorodioxins receive priority in toxicological research as a result of the harmful effect they seem to have on human heredity factors and on the enzymatic system (in this respect chlorodioxins can be compared to carcinogenic substances such as benzpyrene. The importance of the problem is increased by the fact that many products of the phytopharmaceutic industry (pesticides and defoliants of the chlorinated *para*-phenoxyacetic acid type) contain chlorodioxins. Furthermore, there is the undeniable possibility that chlorodioxins can form and concentrate by pure chance. It seemed of interest to us to conduct, parallely to our laboratory work, a bibliographic research to determine the present level of scientific knowledge with respect to polychlorodibenzo-*p*-dioxins.

This publication is the first part of our work and it consists of a bibliographic survey of chemical and toxicological studies on chlorodioxins.

In the future we shall publish a survey of the analytical methods and the results of our experimental work.

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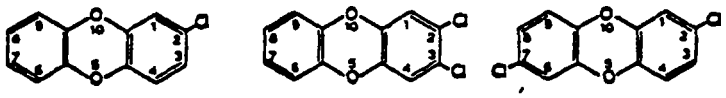
We certainly realize that our paper is a modest contribution to the study of polluting agents, an enormous, world-wide project. Nevertheless, the work contains a great number of salient traits which marked the discovery of the insidious and often violent effects of the chemical substances which we use. May this contribution increase the interest in toxicological research and in identification methods.

Chemical aspects

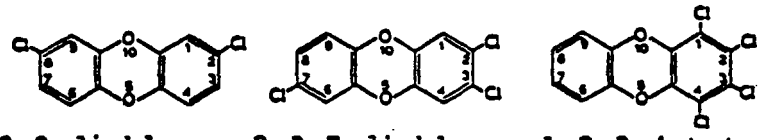
First studies

The first studies dealing with polychlorodibenzo-*p*-dioxins were published in the last century. Denivelle [2] has reviewed these publications.

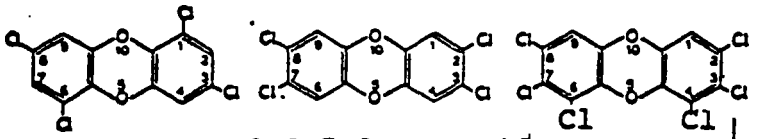
At that time octachlorodibenzo-*p*-dioxin called "perchlorodioxydiphénylène" in French and "Perchlorphenylenoxyd" in German was formed as a reaction product from chlorocyclohexadienones or similar products heated to temperatures between 210° and 300°.



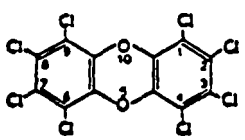
2-chloro 2,3-dichloro 2,7-dichloro



2,8-dichloro 2,3,7-dichloro 1,2,3,4-tetrachloro



1,3,6,8-tetrachloro 2,3,7,8-tetrachloro 2,3,4,6,7,8-hexachloro



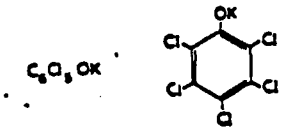
1,2,3,4,6,7,8,9-octachloro

Principal formulas for syntehsized polychlorodibenzo-*p*-dioxins.

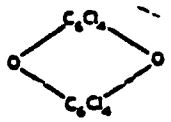
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In 1872 Merz and Weith [3] announced that they heated "Kalium-Perchlorphenylate"

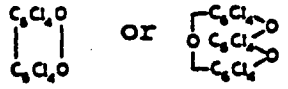


and observed the formation of "Perchlorophenyloxyd", a substance which was sparingly soluble in most conventional solvents (ethyl alcohol, ethyl ether, chloroform) and whose white needle-shaped crystals melted at 320°. The found this compound's reactivity to be low and, although unable to determine its molecular weight, they attributed a formula to it:

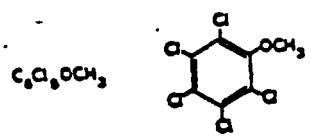


The substance was octachlorodibenzo-*p*-dioxin.

Several years later, in 1889, Hugounenq [4] heated "hexachlorphénol" to produce "perchlorodioxydiphenylene" whose physical and chemical properties led him to believe that the formula should be a multiple of C_6Cl_4O. He proposed the following formulas:



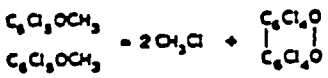
One year later he [5] produced the same compound by sulfuric acid action on pentachloranisole



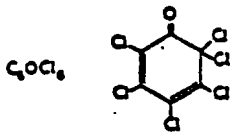
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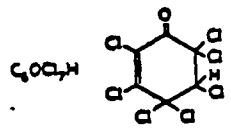
according to the reactions:



In 1894 Zincke and Schaum [6] succeeded in producing a $C_{12}Cl_8O_2$ compound by heating "hexachloroketodihydrobenzol"



or "heptachlorketotetrahydrobenzol"



In the first case the reaction path was:

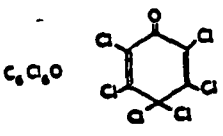
$$2\,C_6OCl_6 \xrightarrow{200-210^\circ} (C_6OCl_4)_2 + 2\,Cl_2;$$

in the second case:

$$2\,C_6OHCl_7 \xrightarrow{220^\circ} (C_6OCl_4)_2 + 2\,HCl + 2\,Cl_2$$

In 1904, the first reaction was confirmed by Biltz [7] who produced what he called "Perchlorphenylenoxyd".

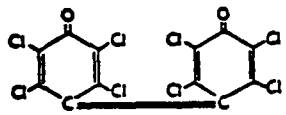
In 1895 Barral [8] clarified the structure of "hexachlorophénol a"



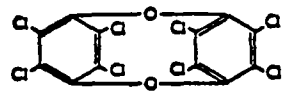
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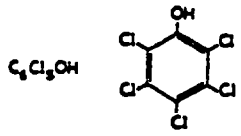
using the known fact that heating of this molecule would produce "perchlorodioxydiphenylene" whose formula, according to him, could be:



or

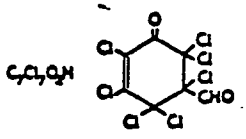


Furthermore, in 1900, Barral and Johnson [9] discovered during the preparation of "pentachlorophenol"



by chlorination of phenol the formation of a significant amount of "perchlorodioxydiphenylene" in the presence of excess of chlorinating substances, especially $SbCl_5$ and $SbCl_3$. This was the result of the conversion of the "pentachlorophenol" formed. In some cases the conversion reached 50%.

Finally, in 1908, Zincke and Broeg [10] discovered the formation of a compound with the empirical formula $C_{12}Cl_8O_2$ by heating to 240° "aldehydoheptachlorketotetrahydrobenzol":



This compound was identical to the one Zincke and Schaum [6] had produced earlier by a method described above.

In these first studies octachlorodibenzo-*p*-dioxin has usually been considered a reaction by-product or even an impurity.

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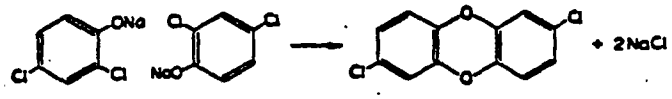
First syntheses

It was not until the work done by Ueo [11] in 1941 that chlorodioxins became the deliberate target of organic syntheses.

Meanwhile, Ullman and Stein [12] as well as the chemists of Bayer [13] had succeeded in 1910 in synthesizing "Diphenylendioxyd", the unchlorinated dibenzo-*p*-dioxin nucleus.

Ueo [11] succeeded in producing "2,6-dichlorodiphenylenedioxyde" (2,7-dichlorodibenzo-*p*-dioxin) via catalytic reduction of "2,6-dinitrodiphenylene dioxide" followed by a denitration with CuCl.

In this manner he obtained a light yellow compound with a fusion point of 207°C and a dipolar moment of 0.62. The paper by Julia [14], in 1953, mentions the formation of the same "dichloro-2,6-dibenzo-*p*-dioxine" (2,7-dichlorodibenzo-*p*-dioxin) by two different paths. In the first reaction path the formation takes place during certain condensations between *p*-bromoacetophenone and 2,4-dichlorophenol, but it also occurs in the absence of *p*-bromoacetophenone which led Julia to conclude that the dichlorophenol was the responsible substance. The second possible reaction occurs during the heating of sodium dichlorophenoxylate in the presence of copper powder. The path is as follows:



sodium 2,4-dichlorophenoxylate

"2,6-dichloro dibenzo-*p*-dioxine" (2,7-dichlorodibenzo-*p*-dioxin)

These works by Julia also make mention of a preparation of dibenzo-*p*-dioxin from *o*-chlorophenol. They are a sign of a new emphasis on studying the biological effects of chlorodioxins. In tests on weevils and Drosophil a "dibenzo-*p*-dioxine, 2,6-dichloro" (2,7-dichlorodibenzo-*p*-dioxin) had no effects.

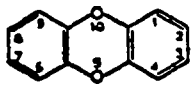
Gilman and Dietrich's 1956 study [1] is strictly chemical, but it is nevertheless of great use. It is devoted to the proportion of halogenated derivatives of dibenzo-*p*-dioxins and describes

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the synthesis procedures for 2-chlorodibenzo-*p*-dioxin and 2,7-dichlorodibenzo-*p*-dioxin.

In addition, this study presents the numbering system for the dibenzo-*p*-dioxin nucleus mentioned in the introduction of this paper. The principle is diagramed below



It has been used by "Chemical Abstracts" since Volume 74 (1971) in the "Subject Index", p. 1137S.

In 1957 Sandermann et al. [15] published the results of their search for pentachlorophenol derivatives which could be used as wood protection agents. They showed that the pyrolysis at 300° of pentachlorophenol or potassium pentachlorophenate resulted in the formation of "Oktachlor-Diphenylendioxyd" (octachlorodibenzo-*p*-dioxin). This compound was of no interest to them and this particular synthesis was already known since it was at the origin of the discovery of chlorodioxins. The chlorination of dibenzo-*p*-dioxin lead to the formation of "Tetrachlorodiphenylendioxyd" (tetrachlorodibenzo-*p*-dioxin) which was an effective agent against substances which damage wood. But, after noticing that even traces of this compound produced "mild chloracne", the authors decided that it should be researched further.

Toxicological Aspects

Chloracne

This fortuitous medical observation by Sanderman and his conclusion to study which dealt basically with chemical issues had served for years as the starting point of medical contributions to the study of chlorodioxins.

As soon as 1899, Herxheimer [16] had described the symptoms of chloroacne (appearance of pustules, comedones and lesions of the parenchyma of the liver) without knowing exactly the nature of the responsible agent. The symptomatology of the disease and the

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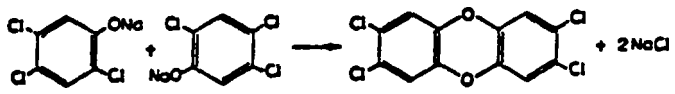
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evolution of its etiology are presented in a work written by Bauer et al. [17] in 1961 after a study of a hundred cases of severe poisoning in workers who had been making or handling chlorophenols.

As the number of chloracne cases increased in industrialized countries all over the world it became certain that neither the chlorine nor the chlorinated hydrocarbons alone were the responsible agents.

Kimmig and Schultz's papers [19, 20], published in 1957, represent the last step toward the final recognition of the role which the chlorodioxins played. The starting point of their investigations was an observation of chloracne patients who had worked in plants where 2,4,5-trichlorophenol was manufactured by alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene at high pressures in the so-called "Druck-Phenolprozess" [21, 22]. The chloracne was not produced by the main reaction products in pure form, but by secondary products. Since the authors were at first unable to identify these products they conducted chloracnogenic tests on rabbit ears and even on one of themselves (Schultz) using synthetic products which could be produced in possible secondary reactions of the "Druck-Phenolprozess". The active substances tested were: "trichlordibenzofuran", "tetrachlordibenzofuran" and "2,3,6,7-tetrachlor-dibenzodioxin" (2,3,7,8-tetrachlorodibenzo-*p*-dioxin).

It was not proven that the first two compounds are actually formed during the "Druck-Phenolprozess". 2,3,7,8-tetrachlorodibenzo-*p*-dioxin however corresponds to the reaction discovered by Julia [14]: the decomposition of two molecules of sodium trichlorophenoxylate molecules according to the path:



This chlorodioxin which turned out to be the most active of the tested compounds was detected in the unrefined products of the "Druck-Phenolprozess". Chemists who had been working at the synthesis of the compound were afflicted with severe chloracne.

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Kimmig and Schultz [20] suggested also that other chlorodioxins could form in reactions similar to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, for instance octachlorodibenzo-*p*-dioxin during the heating of sodium pentachlorophenoxylate.

Thus a link between medical and chemical research was established.

The toxicological potency of chlorodioxins did not fail to impress. Indeed, three drops of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin diluted in polyglycol with concentrations from 0.01 to 0.005% were sufficient to cause chloracne when applied to a rabbit's ear. Oral doses ranging from 0.05 to 0.1 mg/kg weight of body caused the death of test rabbits within one to two weeks as a result of necrosis of the liver [17].

In 1962 Jones and Krizek [23] suggested that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin be used as a standard by which to measure various substances' power to cause chloracne. These authors used the preparation method of Tomita [24]; it consists in heating sodium 2,4,5-trichlorophenate.

Most of the information concerning the pathological effects on humans have been collected on patients who worked in plants where chlorophenolated products were made. 9 out of 31 workers who had contracted chloracne were still being treated five years after the outbreak of the disease for acne, permanent weakness of the leg muscles, for vasovegetative fragility and especially for psychopathological trouble [17].

Recent bibliographical surveys on chloracne have been gathered by Poland and Smith [25], in 1971, and by Kimbrough [26], in 1972.

The chick edema factor

As mentioned above 1957 was a year in which chemical and medical researchers had joined their efforts to confirm the role chlorodioxins played in the "chloracnogenesis". In the same year an avian disease killed several million farm chickens in the United States. The autopsies of the chickens revealed in most cases an

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accumulation of fluids in the pericardium and in the peritoneal cavity (for this reason the responsible agent was called chick edema factor) as well as swollen livers and damaged kidneys [27, 28].

The course of the studies which permitted the identification of the factor has been discussed in Nutrition Reviews in 1968 [29], by Brion in 1969 [30] and by Parsons in 1970 [31].

These studies, including the most recent ones will be surveyed again below. However, the emphasis will lie on the discovery of the chlorodioxins rather than on the biological implications of their effects.

The first authors [27, 28, 32] to mention the disease concluded that the noninfectious affliction was caused by an extremely toxic chemical agent which was heat-resistant and remained stable over an extended period of time. This agent, it was concluded, must be contained in the fatty substances included in the feed ration of the chickens.

Friedman et al. [33] isolated the factor from the nonsaponifiable content of tar by-products of the preparation of oleic and stearic acids from different manufacturers.

Wootton and Alexander [34] isolated the factor from animal fat, and although their analyses were based on products of doubtful purity, they correctly assumed that the toxic factor had an aromatic structure and that it had a high molecular weight.

Brew et al. [35] separated the factor from the particularly high nonsaponifiable content of toxic animal fat. They found that the molecular weight was between 366 and 360. Separation chromatography and a study of the infrared and ultraviolet spectra led them to attribute a steroid structure to the factor.

In 1960 Harman et al. [36] submitted the factor which they had isolated from the nonsaponifiable part of tallow to silane paper chromatography and to an analysis of its IR and UV spectra. But due to the small isolated quantities, they could not draw any conclusions.

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Ames and Swanson [37] found the toxic agent in samples of oleic acid and in some commercial glyceric acids. However, these were cases of accidental contamination.

In 1961 Firestone et al. [38] published the results of their reserach for the chick edema factor in several fatty substances. After having analyzed a great number of samples they concluded that the toxic factor must have a cyclic structure. It is astounding that they found the factor in distillates and distillation residues which had been produced during the preparation of commercial fatty acids, in hydrogenated and nonhydrogenated inedible tallow, in cotton oil, in acid oils, in saponified palm oil which had been recovered from the production of tin plates and in numerous samples of commercial oleic acid and stearic acid.

Yartzoff et al. [39] isolated the factor from triolein which has toxic effects on monkeys and which causes chick edema. They obtained a crystalline substance which was quite similar to that discovered by Harman [36]. The spectra were reminiscent of highly substituted naphthalenes and the presence of chlorine - a new and important factor - led to the assumption that the chick edema factor was a chlorinated naphthalene. Chlorinated naphthalenes are in fact known toxic agents, but they do not cause chick edema. Microcoulometric gas chromatography showed that the retention time of the isolated substances was 37 to 38 min as opposed to approximately 10 min for the chlorinated naphthalenes. Yartzoff et al. [39] formulated the hypothesis that the molecular weight of the chick edema factor should be higher than that of the chloronaphthalenes or that it would contain an additional substituent other than chlorine. For this reason the solubility and the polarity of the chick edema factor would be different from those of chloronaphthalene.

In 1962, Wootton et al. [40] isolated three active compounds, two of which in their pure form, from the nonsaponifiable content of commercial fat which had caused chick edema. They succeeded in crystallizing the two pure substances. The fusion temperatures were: 313.5°-314.5° and 246°-246.4°. The molecular weight was 391 in both cases. The two compounds had 6 chlorine atoms per molecule

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in addition to an aromatic nucleus. Two years later Wootton and Courchene [41] concluded that the substances were 2 isomers with the empirical formula $C_{14}H_{10}Cl_6$ and they felt justified to assume that they were hexachlorohexahydropheanthrenes.

Meanwhile, in 1962, McCune et al. [42] had discovered by chance that chlorinated biphenyls are toxic for chickens and that they cause symptoms which are quite similar to those caused by the chick edema factor. Flick et al. confirmed this in their paper [43].

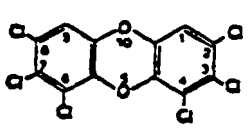
In 1963 Firestone et al. [44] designed a method for detecting the chick edema factor in fatty substances and in fatty acids by microcoulometric gas chromatography.

The use of gas chromatography with an electron-capture detector became later on a helpful tool in the research on the edema factor [45].

In 1965, this method made it possible for Flick et al. [46] to separate 8 compounds from a crystalline substance which had been extracted by the Yartzoff method from the nonsaponifiable content of a toxic fat [39]. One of these compounds corresponded to one of the active substances isolated by Wootton and Courchene [41].

In 1966, Campbell and Friedman [47] detected 9 compounds by applying this chromatography method after a purification by thin-layer chromatography. The detection limit was 0.2 ppb.

The publications by the crystallographers Cantrell, Mabis and Webb added the final conclusion to the clarification of the nature of the chick edema factor. The results of their studies had been announced since 1967, but they were not published in *extenso* until 1969 [49]. According to this study the chick edema factor is "1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin" (2,3,4,6,7,8-hexachlorodibenzodioxin) as shown by an analysis of x-ray diffraction patterns obtained with a monocrystal of one of the toxic factors isolated by Wootton and Courchene. The molecule is almost flat and has the formula:



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The authors also report that once this chlorodioxin was synthesized it was submitted to toxicity tests by Wootton. It indeed caused chick edema.

In 1972, Kimbrough [26] published a bibliographic survey of the work which had been done on the chick edema factor.

Questions of present interest

The origin of the chlorodioxins

The question of the origin of the chlorodioxins remained unanswered. In 1969 it gained renewed interest in the U.S. following another outbreak of chick edema. This time soapstocks, by-products from alkaline refining of a vegetable oil, were suspected to be the source of the toxic factor.

Campbell and Firestone [50] summed up the situation at the International Symposion on the Identification and Measuring of Pollutants held in Ottawa in 1971. Their lecture was based on the most recent studies such as those by Higginbotham et al. [51] published in 1968 and those by Metcalfe [52] which were published in 1972. It dealt with the presence of chlorodioxins in fatty substances, in pesticides which seem to be one of the most important vectors and in the atmosphere in general.

The formation of chlorodioxins seems indeed to occur in high-temperature alkaline reactions of chlorophenol according to a reaction path which has already been mentioned repeatedly.

This formation occurs particularly in the preparation of chlorophenols or during their conversion into certain derivatives which are often pesticides or defoliants. Chlorodioxins which are preformed in this way contaminate the derivative and accompany it.

But *in situ* reactions of this type occur in cases where all the prerequisites are met by accident, for example during the alkaline refining of vegetable oils which have been contaminated with chlorophenols or closely related compounds.

In addition, Firestone et al. [53] have discovered that all chlorodioxins, with the exception of octachlorodibenzo-*p*-dioxin, remain and concentrate in the chicken tissues into which they have

DOW 117069

been absorbed. This may possibly trigger a dangerous cumulative process in the food chain. However, Zitko [54] reported in 1972 that he failed to detect any chlorodioxins in marine animals, but his studies dealt with the population of a very small geographic area. In the case of chick edema it has been established that the toxic tallow which was responsible for the chick edema came from tanneries in which chlorophenols were used to conserve the skins. As far as the soapstocks are concerned, they had been contaminated by chlorodioxins contained in pesticides produced nearby.

Campbell and Firestone [50] showed in their lecture that chlorodioxins are formed and accumulate during the manufacturing processes of certain pesticides which are used all over the world. In addition, these compounds can be produced accidentally in the food industry and they can contaminate animals which are consumed by humans. Their discussion was a message of warning recommending prudence and reflection. While the spectacular effects of the chick edema factor received the most attention, chlorodioxins had been identified as the responsible factor for chloracne. In spite of this the chemical operations which involve the use of chlorophenols or its derivatives had not been discontinued. In fact the use of the toxic agent had increased significantly and several severe cases of poisoning had occurred in chemical industry workers of the chemical industry who had been exposed to chlorophenolated products. In Vietnam, where defoliants have been used intensively since 1964, an increase in congenital malformations has been observed.

The works published until today can in some respect be divided into two categories. The first category contains works which deal only with the chemistry of dioxins and the second, which accounts for most of the papers written, covers research work which has been prompted by the extraordinary toxicity of the chlorodioxins. These works deal often with observations and studies of the many effects chlorodioxins can have, in addition to and sometimes to the exclusion of chemical studies. As a result the distribution between chemical and toxicological studies becomes more and more tenuous.

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The links which unite the two disciplines became increasingly numerous and it is not always an easy task to classify the publications in one category or the other.

The classificatory system we adopted to group papers published after the discovery of the role chlorodioxins play in the development of chloracne does not account for the above-mentioned research work on the chick edema factor and it attempts to circumvent the difficulty by collecting the publications under three headings: 1) new chemical properties of chlorodioxins, 2) the toxicological importance of chlorodioxins, 3) chlorodioxins in the environment, using based on the general purport of the text.

New chemical properties of chlorodioxins

In 1959 Tomita et al. [24] published preparation methods for several polyhalogenated dibenzo-*p*-dioxins such as 2,7-dichloro-, 2,3,7,8-tetrachloro- and octachlorodibenzo-*p*-dioxin.

In 1960, Denivelle [2] described preparation methods for octachloro- and octabromodibenzo-*p*-dioxin.

In 1961, Kulka [55] studied the conversion of pentahalophenols into octohalogenodibenzo-*p*-dioxin using halogens or halogenated compounds. He suggested that a pentahalophenoxy radical forms during the reaction and acts as a reactive intermediate. By this method he had succeeded in synthesizing octachlorodibenzo-*p*-dioxin.

More recently, in 1970, Darsow and Schnell [56] of the Bayer A.G. developed the synthesis of 2,3-dichloro- and 1,2,3,4-tetrachlorodibenzo-*p*-dioxin to improve the fire-resistance of polymers and polyesters. Their invention was patented.

In 1971, Buu-Hoï et al. [57] published a preparation method for 2,3,7,8 tetrachlorodibenzo-*p*-dioxin in its pure form. This distinguishes their work from that of Tomita et al. [24]. They also list the physical properties of this chlorodioxin and the results of mass spectrography of tetrachlorodibenzo-*p*-dioxins. These last mentioned results were full of mistakes due to catalytic decompositions in the spectrometer. They were corrected shortly after [58].

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In 1972, Pohland and Yang [59] published a systematic study of the preparation and characterization of chlorodibenzo-*p*-dioxins which contain from 1 to 8 chlorine atoms. They review the various preparation techniques, discussing the yield and the ease of the reactions as well as the degree of product purity obtained. They list chemical and physical properties and mention the stability, the coloring reactions, the IR, UV, NMR and phosphorescence spectra. They define a relation between the wavelength of the phosphorescence and the life of the triplet on the one hand, and on the other the number of chlorine atoms and their position on the nucleus of the dibenzo-*p*-dioxin. Boer et al. [60-62] published in 1972 the results of their preparations and studies of crystals and molecules of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,7-dichlorodibenzo-*p*-dioxin. According to them 2,3,7,8-tetrachlorodibenzo-*p*-dioxin can be prepared in a reaction of chlorine and a heated chloroform solution of dibenzo-*p*-dioxin or 2,7-dichlordibenzo-*p*-dioxin in the presence of a catalytic amount of ferric iodide and iodine. It crystallizes in the triclinic system and the molecule is almost planar [60, 61]. The 2,7-dichlorodibenzo-*p*-dioxin is prepared by heating sodium 2,4-dichlorophenate in the presence of copper as catalyst for several hours at temperatures from 180° to 200°C. The crystal belongs to the triclinic system and the molecule can be considered planar [60, 62].

Neuman et al. [63] continued this research showing that the octachlorodibenzo-*p*-dioxin crystal belongs to the monoclinic system. With the exception of slight deviations the molecule is planar.

Finally, in 1972, Boer et al. [64] published a study dealing with 2,8-dichlorodibenzo-*p*-dioxin which crystallizes in the orthorhombic system. It is prepared by pyrolysis of the potassium salt of 5-chloro-2-(2,4-dichlorophenoxy) phenol. The molecule is not entirely planar.

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The toxicological aspects of chlorodioxins

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The publication of works focusing on a better understanding of the toxic properties of chlorodioxins goes back to 1969-1970 when congenital malformations were observed in animals which had been exposed to the herbicides 2,4,5 T and its esters and to Silvex-2® [65-68]. It is very likely that many of these research projects were triggered by the controversial and alarming reports observers brought back from Vietnam where they had witnessed the damages caused by massive use of defoliants for strategical purposes [65, 66, 69, 70, 71]. At that time, the American Government proceeded to cutbacks in the use of 2,4,5T [65, 66, 68, 72].

The program of the first research steps has been retraced by various authors in publications which report on the danger of the contaminants.

The work by Golberg [73] generally speaking deals with food contamination. Clegg [72] looks at the question of toxicity to the embryo, especially the toxic effects of 2,4,5T. Somers and Smith [74] deal with environmental contaminants focusing particularly on chlorodioxins.

It was in fact a chlorodioxin, specifically 2,3,7,8-tetrachlorodibenzo-*p*-dioxin which had caused malformations in new-born animals who had come into contact with 2,4,5 T and Silvex-2®.

The toxins in these herbicides which are derivatives of phenochlorinated products were impurities which had probably formed during the preparation according to a mechanism which has already been mentioned.

It did not take long before this chlorodioxin was suspected to be responsible for the anomalies. Dow, the main producer of 2,4,5 T had thought so from the beginning on [66, 68]. Nevertheless, the very first works dealt with the action of the herbicides as manufactured, partially since no pure chlorodioxins were available, and the hypothesis of a combined action was not excluded [67, 68, 75, 76].

Sparschu et al. [77] studied the effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the rat. Courtney and Moore [76] also studied

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the effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on mice and rats.

A work by Collins and Williams [78] dealt with the teratogenic effect of 2,4,5 T and 2,4 D (2,4-dichlorophenoxy acetic acid) on the hamster.

Diether and Imke [79] studied the embryotoxic effect of 2,4, 5 T and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on mice.

The import of the conclusions depends on the type of test animal, on the ingested doses and on whether the experiments have been conducted with pure or technical 2,4,5 T or with pure 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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The research work is still in progress. In 1971, Firestone et al. [53] studied the distribution of hexa-, hepta- and octa-chlorodioxins in the tissue of chickens.

In 1972, Cunningham and Williams [80] published a study dealing with the effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the growth rate and the synthesis of lipids and proteins in rats.

Williams et al. [81] studied the distribution and excretion of octachlorodibenzo-*p*-dioxin in the rat.

The effect of chlorodioxins on human embryos is less well known, but the congenital malformations observed on Vietnamese who lived in zones which had been the target of massive defoliant raids cannot be ignored [65, 69, 71]. Several authors report these observations [65, 66, 71, 72].

The danger of chlorodioxin contaminations produced a 1971 publication which dealt with health protection problems of workers in the 2,4 D and 2,4,5 T manufacturing industry [25] and in a 1972 article which reviewed the toxic effects of chlorinated hydrocarbons and their derivatives, including chlorodibenzo-*p*-dioxins and chlorodibenzofurans [26].

The dangerous biological effects cited above, the chloracne and related diseases, the chick edema, the embryotoxic, feticide and teratogenic effects (possibly affecting humans) together with the cumulative effect contribute to make of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on of the most dangerous toxins known. This has

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been pointed out by Buu-Hoi et al. [82]. According to these authors, the extreme toxicity is due to the fact that this chlorodioxin causes malfunctioning of the enzymatic system affecting the liver, the thymus, the heart and the lungs [82-84].

They made the same observation in experiments on rats and mice. This led them to attribute carcinomimetric properties to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin similar to those of benzo[α]pyrene and hepatocarcinogenic substances.

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Chlorodioxins in the environment

The extraordinary toxicity of certain chlorodioxins has given rise to a trend in research which studies their identification, their photochemical reactions and their stability in the atmosphere.

In this respect, the article by Buu-Hoi et al. [57] is a very valuable contribution. The authors describe ways of detecting 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the pyrolysis products of defoliants (2,4,5 T and butyl 2,4,5T the base of the defoliant "Grange") or in a substance which is impregnated with these defoliants. According to the same authors the pyrolysis of such defoliants at approximately 500° results in a significant production of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The yields are reported to reach 5 to 15%.

Very recently, in 1972, Saint-Ruf [85] has shown that the pyrolysis of sodium 2,4,5T at 500° during 5 hours also resulted in the formation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. For this reason he condemned the use of these products.

It is to be noted that the experimental conditions of the pyrolyses mentioned above occur during the combustion of plants. The humidity facilitates the hydrolysis and the alkalinity of the ashes facilitates the transformation of chlorophenolated products into chlorodioxins. Thus populations whose domestic combustibles are vegetal material and wood are in serious danger if the burnt material was exposed to defoliants such as 2,4,5 T. This applies to the population of Vietnam (cf. the alarming reports of the first observers) and also to the populations of most developing countries

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where the use of organic chlorinated products is becoming more and more widespread for fighting parasites and for increasing the agricultural output. Finally, this shows that there is a real risk that chlorodioxins may appear at every point of the globe, since chlorophenolated products play a role in the production of paper and paint as well as in the production of pesticides [68].

In 1971, Crosby et al. [86] studied the reactions of 2,7-dichlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachloro- and octachlorodibenzo-*p*-dioxin under the action of light. An irradiation with sunlight or with a lamp which puts out an equivalent radiation produces identical results. The photolysis observed in alcohol solutions depends on the number of chlorine atoms. It is weak in the case of octachlorodibenzo-*p*-dioxin and faster in the case of 2,7-dichlorodibenzo-*p*-dioxin. No photolysis occurs in an aqueous medium or if the chlorodioxins impregnate a sandy soil directly without solvents. The influence of a hydrogen donor organic solvent seems decisive. Since such donors are part of the leaf surface of plants, the photolysis of chlorodioxins could take place there, while it is unlikely that it could occur on the open ground, in water or on stone.

According to Plimmer and Klingebiel [87] there is a possibility that chlorodibenzo-*p*-dioxins form in water by photoxidation of 2,4-dichlorophenol due to the influence of riboflavin. The fact that they have discovered dimers under such conditions, the most frequent dimer was 4,6-dichloro-2-(2,4-dichlorophenoxy)-phenyl, and the fact that chlorodioxins can be formed by the closing of a ring of a 2-phenoxyphenol derivative speak in favor of their hypothesis. However, the authors were not able to verify their theory in their experiments, but according to them the photolysis of chlorodioxins which contain few chlorine atoms is so rapid that their presence could not have been detected.

Another field of investigation opened by the study of the toxic effects of chlorodioxins is the study of their stability in the soil and their absorbtion into plants.

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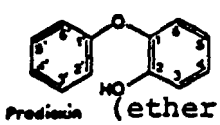
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Experiments carried out by Isensee and Jones [88] on oats and soybeans show that these plants accumulate neither 2,7-dichloro- nor 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The chlorodioxins introduced were either dissolved in the food, or the soil of the culture was impregnated with them, or they were applied onto the leaves, or dissolved in an aqueous solution with a surfactant, Tween 80. No migration of the chlorodioxins which had been applied to the leaves toward the root system was observed.

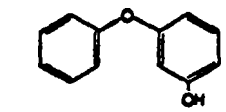
Helling [89] studied the mobility of 2,7-dichloro- and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in soil. Tests conducted by thin-layer chromatography on clay plates revealed no mobility. Most likely this is due to the insolubility of chlorodioxins in water. However, it is possible that chlorodioxins are carried by the wind.

The studies by Kearney et al. [90] showed, in 1972, that the stability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is such that one year after its application 60% of it is still present. According to these authors it seems that no microorganisms can transform 2,4-dichlorophenol or 2,4,5-trichlorophenol into 2,7-dichloro- or into 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

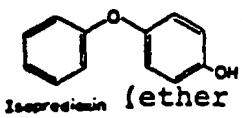
Research on chlorodioxins is obviously a subject of current interest. In 1972, Jensen and Reuberg [91] found chlorodioxins, especially octachlorodibenzo-*p*-dioxin, in pentachlorophenols used as fungicides for the preservation of wood. They called the ethers of 2-hydroxydiphenyl "predioxins" and the ethers of 3-hydroxy- or 4-hydroxydiphenyls "isopredioxins" and detected some "octachloroisopredioxin".



(ether of 2-hydroxydiphenyl)



(ether of 3-hydroxydiphenyl)



(ether of 4-hydroxydiphenyl)

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According to these authors the late discovery of predioxins was a result of the compounds' instability.

In 1972, Woolson et al. [92] published the results of their analyses of chlorophenol derived pesticides. They detected various chlorodioxins at different concentration loads.

Finally, in 1973, Edmunds et al. [93] published the results of their analyses of 2,4,5 T and the alkyl esters of this acid. They were trying to detect 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The highest concentration found was 28.3 ppm.

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P. J. Gehring	411-0000110	2/9/76	K-002372

THE FATE OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) FOLLOWING ORAL ADMINISTRATION TO MAN

M.W. Sauerhoff, W.H. Braun, G.E. Blau and P.J. Gehring

REPORTED BY G.E. Blau and P.J. Gehring CHECKED BY: J.E. LeBeau

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The pharmacokinetic profile of 2,4-D is defined in man. Five male human volunteers ingested a single dose of 5 mg/kg 2,4-D without detectable clinical effects. Concentrations of 2,4-D were determined in plasma in three of five subjects and in urine in all subjects at intervals after ingestion. The elimination of 2,4-D from plasma in all subjects occurred by an apparent first order rate process with an average half-life ($t_{1/2}$) of 11.6 hr. All subjects excreted 2,4-D from the body in the urine with an average $t_{1/2}$ of 17.7 hr. 2,4-D was excreted mainly as 2,4-D (82.3%) with smaller amounts excreted as a 2,4-D conjugate (12.8%). Essentially all of the 2,4-D is absorbed from the gastrointestinal tract in man. There is no evidence that clearance of 2,4-D from the plasma or excretion from the body involve non-linear kinetics.

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INTRODUCTION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a chlorophenoxy acid herbicide. Following oral administration to rats, pigs, calves and chickens, 2,4-D is readily absorbed, distributed and eliminated in the urine (Erne, 1966). The half-life values for the apparent first order clearance of 2,4-D from plasma were reported to be 3 hr for rats, 8 hr for calves and chickens, and 12 hr for pigs. The fate of 2,4-D following ingestion by man has not been reported. Human male volunteers ingested 5 mg/kg 2,4-D. Samples of blood and urine collected at intervals after dosing were analyzed for 2,4-D. This report presents results of the fate of 2,4-D following oral administration to five human male volunteers.

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MATERIALS AND METHODS

Subject. Five male human volunteers age 29 to 40, weighing 70 to 90 kg, participated in the study. The medical history of each individual was reviewed and a physical examination conducted prior to the study. Included in the examination were blood pressure, pulse rate, pulmonary function, electrocardiogram, chest x-ray, hemoglobin, packed cell volume, red and white blood cell counts including differential, and sedimentation rate, serum concentration of total calcium, cholesterol, triglycerides, glucose, inorganic phosphate, albumin, total protein, uric acid, bilirubin, glutamic-oxaloacetic transaminase, lactic dehydrogenase, alkaline phosphatase activities, as well as urinary protein and sugar.

Dosage and Sample Collection for Analysis. Five subjects ingested 5 mg/kg analytical grade 2,4-D (sample AGR 30653C, Dow Chemical Co.). Two subjects ingested the free acid of 2,4-D as a slurry in milk and three ingested it directly from a weighing paper followed by a few swallows of water. All subjects ingested 2,4-D between 8:00 and 8:05 AM. Subjects were not fasted prior to ingestion of the dose.

Blood samples, 10 ml, were collected from three subjects in tubes containing 4 mg of potassium oxalate at 1, 4, 8, 12,

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24, 36, 48, 72, 96, 120 and 144 hr after ingestion. Following collection, the whole blood samples were centrifuged, the plasma separated and placed in tared vials and frozen for subsequent analysis of 2,4-D.

All the urine voided by each subject during successive 12-hr intervals post-dosing was collected and the volume recorded. The urine collected during each 12-hr interval was thoroughly mixed and aliquots were frozen until subsequently analyzed for 2,4-D.

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Samples of plasma and urine were analyzed by gas chromatography-mass spectrometry. Urine and plasma samples (5 ml) were acidified with 1 ml of 1.0N HCl and extracted twice with 5 ml of diethyl ether. To determine whether hydrolyzable conjugates of 2,4-D may be present, additional samples of urine (5 ml) from the 0-12, 12-24 and 24-36 hr collection intervals were acidified with 1 ml of concentrated hydrochloric acid and heated at 80°C for 1 hour. Subsequently these samples were extracted twice with diethyl ether and analyzed for 2,4-D together with non-hydrolyzed urine samples. 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 long x 2.0 mm (I.D.), packed with 10% OV-1 on Chromosorb W 80/100 mesh. The m/e 234, 236, and 238 peaks were monitored for sample quantitation. Recorded peaks were symmetrical; therefore, peak heights, not peak areas, were used for sample quantitation.

Standards of 2,4-D prepared in plasma and urine were stored with the experimental samples. No decay of 2,4-D was observed in any of the samples or standards during the storage period.

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RESULTS

No untoward effects associated with ingestion of 5 mg/kg 2,4-D were detected in any of the subjects.

The concentration of 2,4-D in plasma, µg/g, is shown in Figure 1 as a function of time following administration. The concentrations found in the plasma of each subject are presented. The data suggest that subjects 2 and 3 can be characterized by a one-compartment pharmacokinetic model while subject 1 may be characterized by a one or two-compartment model. Pharmacokinetic parameters for the three subjects were calculated using a non-linear parameter estimation program. Parameters for a two-compartment model were calculated for subject 1. They are reported in Table 1 with their linearized standard deviations. Comparing the parameter estimates reveals the importance of characterizing each subject individually. For example, the absorption constant (k_a) for subjects 2 and 3 are significantly different at the 95% confidence level as are the elimination rate constants K_e.

A lack of fit test was performed for all compartmental models based on the known analytical error. Each model was shown to be inadequate to describe the data at the 95% confidence level.

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The urinary excretion of 2,4-D expressed as a percent of the dose excreted during each collection interval is presented in Table 2 and represented graphically in Figure 2. Subjects were modeled individually using a one-compartment linear pharmacokinetic model. The pharmacokinetic parameters determined using a non-linear parameter estimation program are presented in Table 3. The half-life of elimination ranged from 10.2 hr to 28.5 hr for subjects 2 and 4, respectively. Recovery of 2,4-D ranged from 87.6% to 106.3% of the administered dose.

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DISCUSSION

These data demonstrate that orally administered 2,4-D is well absorbed in man and excreted in urine as 2,4-D. A fraction of administered 2,4-D is also excreted in urine as a conjugate. The small fraction of 2,4-D not recovered from some individuals may represent lack of absorption, excretion of 2,4-D and/or conjugation in feces, formation of a metabolite which was not detected or a combination thereof.

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After ingestion of 5 mg/kg 2,4-D plasma concentrations increased and reached a peak of approximately 25 µg/ml at 4 hr. Clearance of 2,4-D from the plasma of subjects 2 and 3, as well as the urine from all 5 subjects, was monophasic. The plasma data for subject 1 exhibited a biphasic clearance phenomenon. The explanation for this is unclear. It is interesting to note that this biphasic clearance in subject 1 is accompanied by the delayed peak in the urine excretion data. The slow clearance phase parameter for the plasma data of subject 1 is only slightly slower than the monophasic clearance for subjects 2 and 3. This suggests that the overall clearance characteristics of subject 1, 2 and 3 will not be markedly different.

Statistical tests performed to determine the suitability of the compartmental models to characterize the 2,4-D plasma

data based on a combined analytical and sampling error of 10.2%, indicates that there was a significant lack of fit between the models and the experimental data. A significant lack of fit implies either that a more complex model (i.e., one having more parameters such as a two-compartment model) is required or that the data are sufficiently precise to reveal deviations from the linear approximations inherent to compartment models. In this case, the latter situation is occurring. If, for example, the experimental error had been 15% rather that 10.2%, no significant lack of fit would have been detected. This means that the differences between data points and the line drawn through the plasma data in Figure 1 are real differences and not random scatter. Moreover, these differences represent real departures from the assumed first-order rate processes.

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The volume of distribution was calculated for all subjects (refer to Table 1). The V_d for subjects 2 and 3 were 238 and 294 ml/kg, respectively. The V_d for subject 1, V_1 and V_2 were 83 and 218 ml/kg. This indicates 2,4-D is not extensively distributed to tissues.

Excretion data are presented for five subject ingesting 5 mg/kg 2,4-D. It is not reasonable to pool the individual subject data because of the difference in the pattern of

excretion. One of the five subjects did not form the conjugate of 2,4-D while other subjects excreted between 4.8 to 27.1% of the administered dose as 2,4-D conjugate. Modeling of the data was based on the excretion of 2,4-D and not the conjugate.

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The data presented in Table 2 and Figure 2 show significant between subject variation in the excretion of 2,4-D from the body. The slower excretion of 2,4-D in subjects 4 and 5 may be attributable to factors including increased protein binding, decreased capability of the kidney to excrete 2,4-D or differences in reabsorption from the tubular filtrate. Data presented in this report indicate first order excretion of 2,4-D.

The pooled data half-life values for clearance of 2,4-D from plasma is 11.6 hr. It may be useful to compare this data with pooled data from human 2,4,5-T pharmacokinetic studies. Gehring et al., 1973 have shown the plasma clearance $t_{1/2}$ of 2,4,5-T was 23.1 hr. Decreasing the number of chlorines from 3 to 2 on the phenoxy ring apparently increases the rate of clearance of these chlorophenoxy acids from the body.

In conclusion, 2,4-D is well absorbed following oral administration to man and eliminated as unchanged 2,4-D (82.3%) and

2,4-D conjugate (12.8%) in urine. Clearance of 2,4-D from plasma and elimination from the body occur by apparent first order rate processes. No evidence of non-linear kinetics were observed following the 5 mg/kg oral dose of 2,4-D.

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TABLE 1

Rate Constants and Half-Life Values for Absorption and Clearance of 2,4-D From Plasma in Three Male Subjects Given a Single Oral Dose of 5 mg/kg 2,4-D

		Absorption		Elimination		
		Rate Constant	Half-Life	Rate Constant	Half-Life	Distribution
Subject	Model	k_a (hr^{-1})	$t_{1/2}$ (hr)	k_e (hr^{-1})	$t_{1/2}$ (hr)	V_d (ml/kg)
1	Two Compartment	0.165±0.055	4.20±1.40	k_{12}[a]=0.013 hr^{-1}±0.009		V_1[d]=83.6±28.4
				k_{21}[a]=0.048 hr^{-1}±0.007		V_2[e]= 218±15.7
				k_e[b]=0.145 hr^{-1}±0.049		
				α[c] =0.163 hr^{-1}±0.048	4.25±1.25	
				β[c] =0.043 hr^{-1}±0.012	16.2±4.31	
2	One Compartment	0.202±0.047	3.43±0.80	0.062±0.002	11.2±0.36	283±42
3	One Compartment	0.415±0.082	1.67±0.33	0.095±0.003	7.29±0.23	294±39

[a] k_{12}, k_{12} First order distribution constants between the plasma and slow exchange compartments

[b] k_e First order rate constant for overall elimination from the central compartment

[c] α and β hybrid rate constants for α and β portions of plasma clearance curve

[d] V_1 Volume of central compartment

[e] V_2 Volume of slow exchange compartment.

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TABLE 2

Percent of 2,4-D (Free and Conjugate) Excreted in Urine
During Successive Time Intervals Following a Single
Oral Dose of 5 mg/kg 2,4-D

SUBJECT	1		2		3		4		5	
	Free	Conjugate	Free	Conjugate	Free	Conjugate	Free	Conjugate	Free	Conjugate
0-12	14.3	2.50	29.9	-	40.6	10.5	21.0	-	24.7	5.70
12-24	13.9	8.80	33.9	-	17.3	11.8	17.1	3.10	13.8	3.00
24-36	31.6	10.8	9.18	-	8.64	4.76	14.1	1.70	10.7	1.10
36-48	15.9		8.78		2.33		11.0		10.0	
48-60	5.07		3.91		0.68		8.98		8.99	
60-72	1.10		1.10		0.31		7.15		7.90	
72-84	0.86		0.49		0.16		4.04		6.03	
84-96	0.93		0.22		0.12		2.97		1.25	
96-108	0.27		0.06		0.02					
108-120	0.11		0.05		0.04					
120-132	0.08		0.03		0.01					
132-144	0.07		0.01		0.01					
TOTAL	84.2	22.1	87.6	0.00	70.1	27.1	86.3	4.8	83.4	9.8

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TABLE 3

Rate Constants and Half-Life Values for the Elimination of 2,4-D in the Urine of Individuals Following a Single Oral Dose of 5 mg/kg

Subject	Estimated Fraction of Dose Eliminated (%)	Absorption Constant Kt(hr^{-1})	t 1/2 (hr) Absorption	Elimination Constant Ke(hr^{-1})	t 1/2 (hr) Elimination
1	71.4±18.9	0.108±0.052	6.42±3.09	0.0566±0.0060	12.2±1.3
2	84.3±14.5	0.192±0.098	3.61±1.84	0.0678±0.0023	10.2±0.44
3	47.8±15.5	-	- [1]	0.0638±0.0029	10.9±0.50
4	96.5±4.67	0.522±4.67	1.33±1.44	0.0244±0.0020	28.4±2.38
5	88.4±18.5	-	- [1]	0.0290±0.0065	23.9±5.42

[1]Meaningful estimate of absorption constant could not be obtained from these two subjects.

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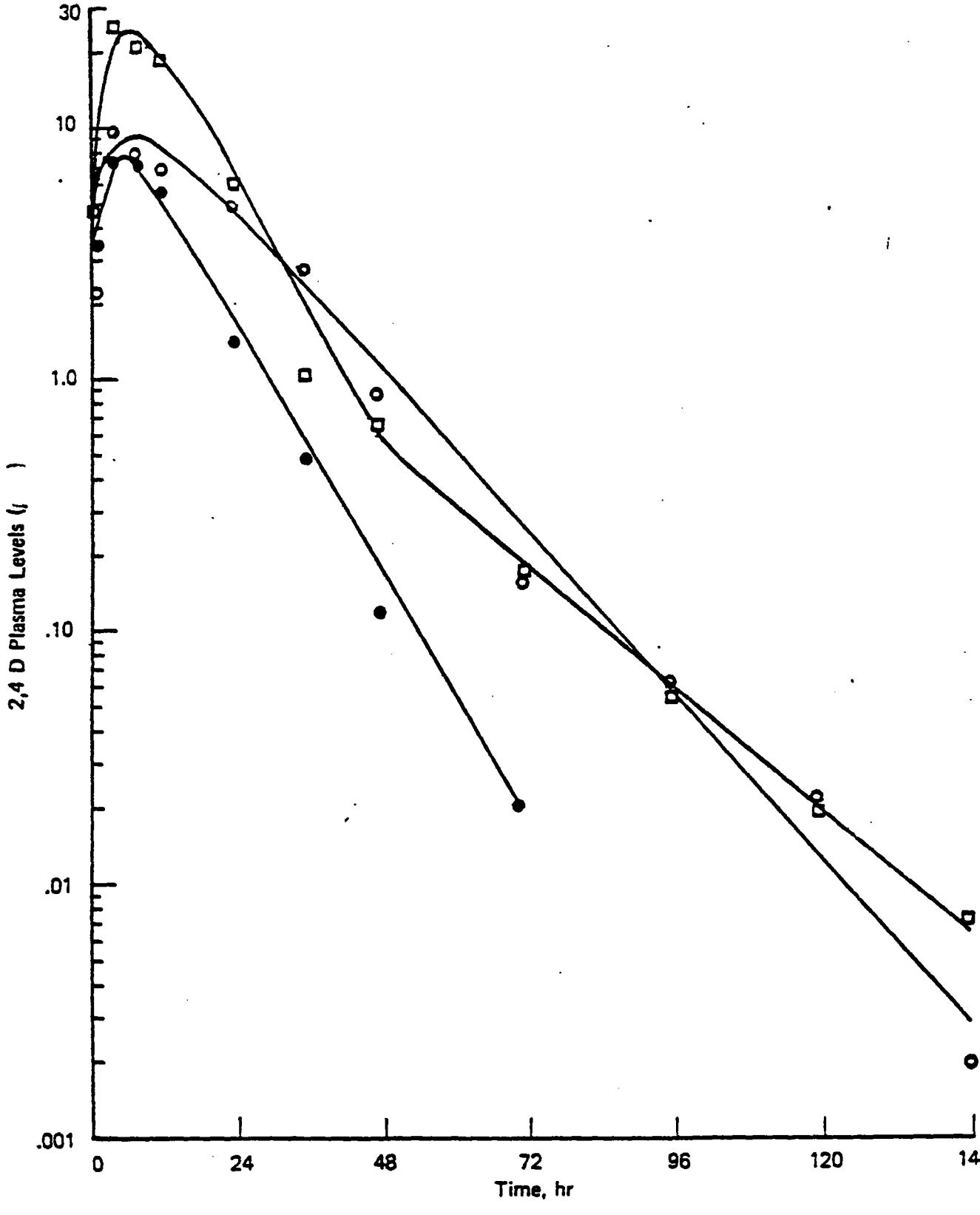
LEGEND

Figure 1 — Plasma levels of 2,4-D in man following a single oral dose of 5 mg/kg 2,4-D.

Figure 2 — Excretion of 2,4-D in urine expressed as percent of dose excreted during successive time intervals following a single oral dose of 5 mg/kg 2,4-D.

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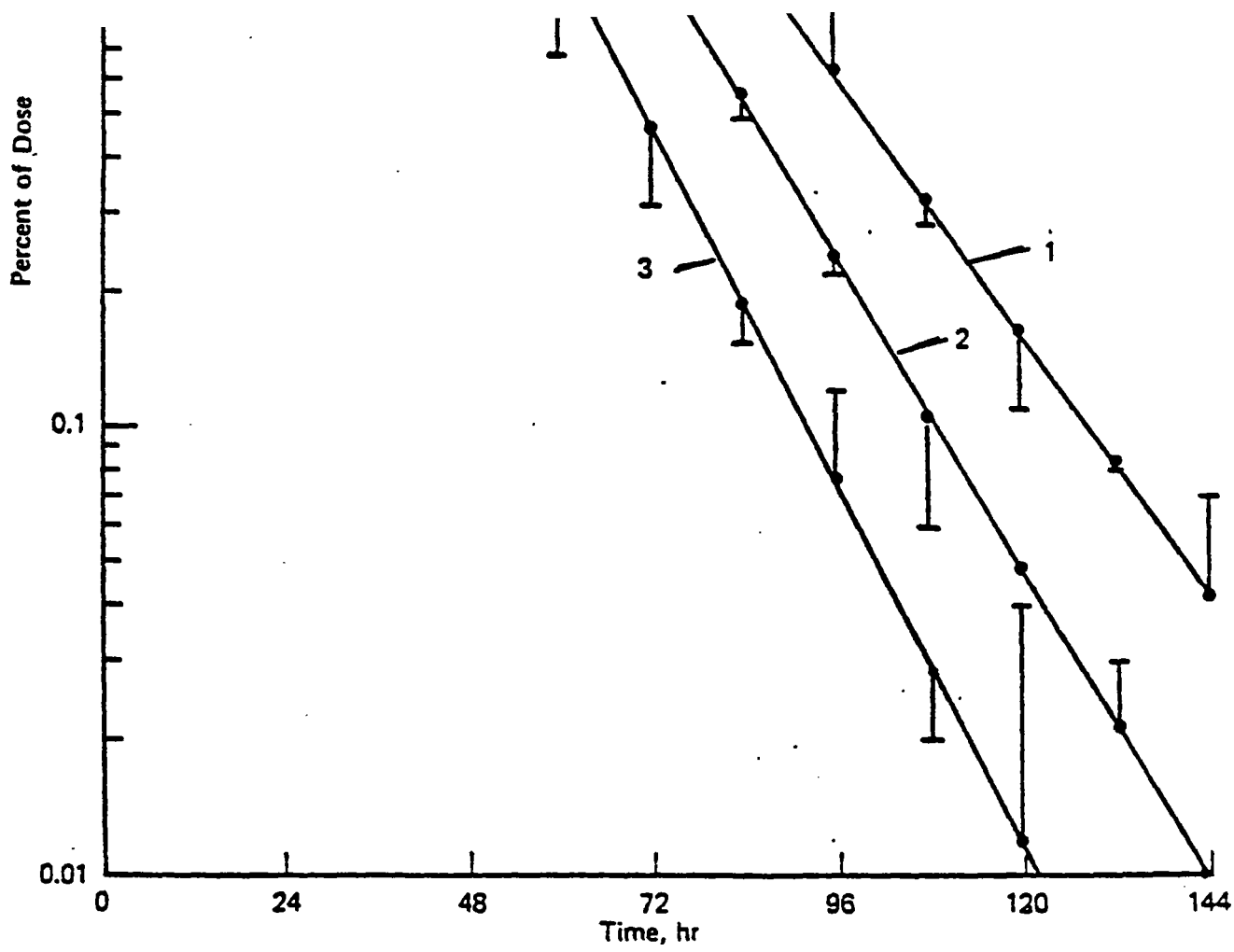
FIGURE 1



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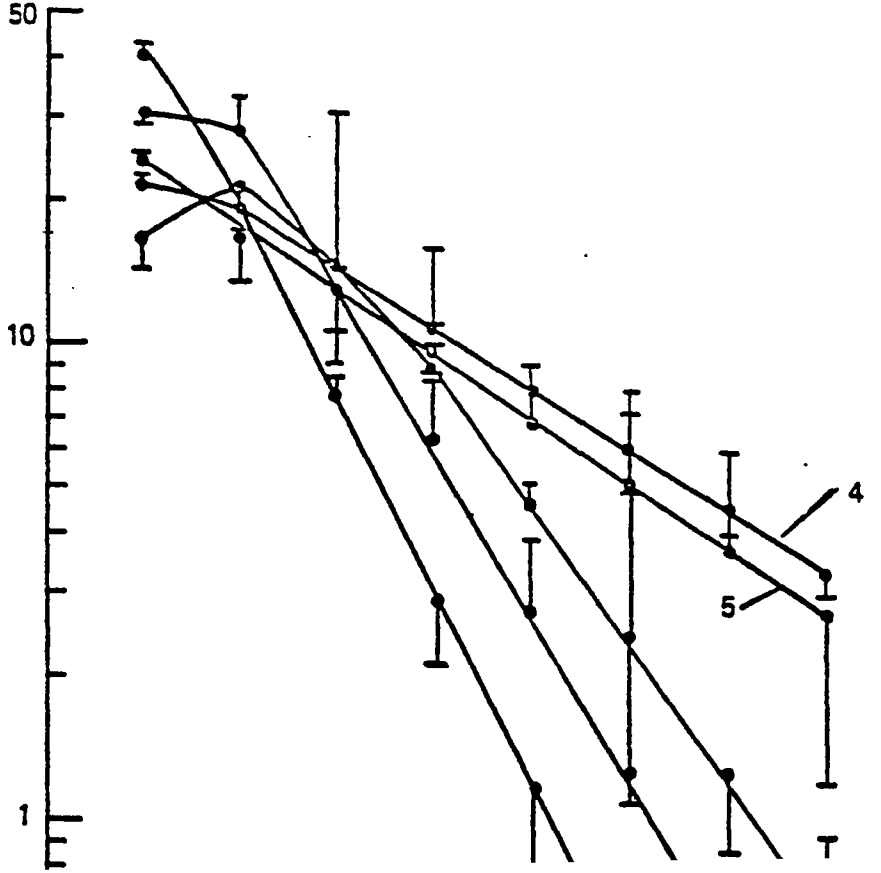
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FIGURE 2



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To / RLO

JEFFERSON SERVICE COMPANY'S PESTICIDE SALES IN N.C. HALTED BY STATE BOARD

Jefferson Service Company, Baltimore, Md., has been barred from selling pesticides for an indefinite period in North Carolina. The State's Pesticide Board and the firm have agreed that:

-- Pesticide sales by the company are prohibited in the State "until such time as the Board may reconsider application for registration.

-- The firm will refund dissatisfied farmers' money. Total refund not to exceed $5,000.

-- Farmers are to initiate refund requests.

-- The company will take back unopened containers of pesticides from farmers who have not paid their invoices and close the accounts.

-- Unopened pesticides can be returned to the company freight collect.

The agreement, the Board noted, was in lieu of a scheduled hearing for the firm.

William B. Buffaloe, Secretary, North Carolina Pesticide Board, in a letter sent last week to the President of Jefferson Service Company, advised "that should evidence constituting a breach of this agreement, or a failure on the company's part to abide by all of the terms and conditions agreed to, the Board will, upon reapplication by the company for registration, consider all allegations of violations, and evidence pertaining thereto, which occurred prior to the date of this agreement" (See Feb. 18, Page 5 and April 21, Page 13).

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POSSIBLE TOXIC EFFECTS OF TCDD IN HUMANS LISTED BY DIOXIN GROUP

A summary of the January 21 meeting of the Toxicology Working Group of the Environmental Protection Agency's dioxin project (See Dec. 24, Page 14) included a list of 13 possible toxic effects of TCDD in humans agreed to by the group:

"Chloracne, porphyria, lethargy, anorexia, liver alterations, diarrhea, headache, alopecia, thrombocytopenia, loss of libido, neuropathy, lowered hemoglobin, and hyperpigmentation."

According the the summary, "chloracne and increased excretion of porphyrins are probably the most common findings associated with TCDD overexposure ... The group agreed that the other items may be associated with TCDD but that the relationship between cause and effect is ill-defined."

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EPA last week decided that future meetings of the dioxin project and its working group will be open to the press and public, an agency official stated. EPA officials also decided that as soon as research data and results came into the project manager, they

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will be given to project participants and to the EPA's Office of General Counsel, the official said.

At its January 21 meeting, the group discussed certain animal study results which appeared to indicate few major adverse effects of dioxin in the test animals.

The Director of the National Cancer Center for Toxicological Research (NCTR), Dr. Cranmer, indicated last December that in his opinion, the teratological risk to humans of 2,4,5-T with less than 0.1 p.p.m. dioxin was nil.

The meeting summary included studies relevant to exposure to low levels of TCDD.

The summary noted that the NCTR "has studied the teratogenic potencies of both technical (< 0.05 p.p.m. dioxin) and purified (< 0.005 p.p.m. dioxin) 2,4,5-T in various strains of mice. There were no statistically significant differences for cleft palate incidence, embryolethality and fetal weight. The absence of differences in potencies between the two 2,4,5-T preparations suggests that dioxin contaminants are not the teratogenic agents in such preparations or that the increased adverse effects due to dioxins are insignificant in relation to effects produced by 2,4,5-T at doses studied."

Dr. B. A. Schwetz, Dow Chemical Company, reviewed teratology studies on TCDD in rats and mice, the summary, dated May 3, noted.

It stated that the "consensus of the studies (reviewed by Dr. Schwetz) is that TCDD is fetotoxic but not teratogenic in rats." The summary continued:

> "A study was undertaken to determine a non-teratogenic dose level of TCDD in mice. The non-teratogenic dose level of TCDD given to CF-1 mice by gavage on days 6-15 of gestation was found to be 0.1 ug TCDD/kg/day ... Results to date indicate an interference with the reproductive capacity at a dose level of 0.1 ug/kg/day."

Dr. R. J. Kociba, Dow Chemical, USA, the summary noted, presented results of a 13 week oral toxicity study in rats.

The summary said that from this study "'the data indicate that no discernible ill-effects occurred in rats given 0.01 or 0.001 ug TCDD/kg 5 days/week for 13 weeks.' He also discussed a presently ongoing 2 year chronic toxicity study of TCDD in rats ... As of January 1976, (the 16th month of the study) a dose of 0.1 ug/kg/day of TCDD affected body weight, mortality, urinary porphyrins and hematological parameters but no effects were noted at a dose of 0.01 ug/kg/day."

Dr. Renate Kimbrough, Communicable Disease Center, according to the meeting summary noted that "TCDD has been involved in a large horse poisoning episode in Missouri. This incident was a case of gross accidental overexposure to TCDD and is not indicative of the effect of TCDD as it may exist elsewhere in the environment."

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The group heard a report on results of a rat study, the summary said, which showed that "single oral doses of 0,1,3,10 and 30 ug/kg TCDD did not enhance porphyrin excretion, indicating porphyria occurs only after chronic dosing whereas 16 weeks after single doses of 3, 10 and 30 ug/kg, enzyme activity was significantly increased."

Results of another rat study discussed in the summary indicated "that TCDD approaches steady state levels in the body within 13 weeks. The rate constant defining the approach to steady state levels is independent of the dose level of TCDD over the dose range of 0.01 to 1.0 ug TCDD/kg/day. This means that the results found in the 90 days feeding study may be indicative of the results of the 2 year feeding study."

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The summary noted that the group rejected advised teratological studies of TCDD in primates.

There may be additional follow up on industrial workers exposed to TCDD, the summary indicated.

It stated that "Dr. Cranmer at NCTR is requesting the data for the last 25-30 years on cleft palate and cleft lip in the State of Arkansas. This will be compared with the use histories of 2,4,5-T to see if there is any correlation between cleft palate and high 2,4,5-T usage."

Dr. John Spaulding, Agriculture Department, presented a brief report to the group on "The Impact of Dioxin Residues to Agriculture." The summary said "if and when EPA decides to set an action level, the methodology for testing and economic cost of enforcement must be considered." Dr. Spaulding said:

> "The increased production expenses and any changes in the prices at the market place will be the responsibility of the agency establishing the tolerances or advising on action levels. In those instances where the Department believes the projected benefits do not outweigh the proven risks, they will utilize the administrative and legal options available under the law to assure the American public is fully informed concerning the basis of the decision and the potential consequences."

His report stated that the USDA "will not encourage the use of any toxic material in a manner that is known to create an unacceptable level of a toxicant in the food supply at the time of marketing."

Dr. Cranmer, NCTR Director, gave his opinion of the relative risks to man and the environment of 2,4,5-T with less than 0.1 p.p.m. dioxin in a Dec. 17, 1975 memorandum to EPA's dioxin project manager.

"I personally feel that at judicious use levels, the teratological risk is nil," his memorandum asserted.

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He added that "2,4,5-T is teratogenic and must be dealt with as such. NCTR decided that research on TCDD was important, but not in the context of 2,4,5-T and, therefore, we junked plans for such." His memorandum said:

> "I believe that dioxins in the environment are important, but I feel that pesticides will contribute little, if control over the quality of production is maintained. The problem lies not with the pesticide, but with industrial chemicals escaping into the environment."

Dr. Cranmer stated that "At NCTR we proved, I believe, that the currently available 2 4,5-T is teratogenic in several dose-response studies and that the effect is not due to a generalized non-specific effect on the maternal animal, and that TCDD plays no discernible role."

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PBB CONTAMINATION OF LIVESTOCK BRINGS PRESSURE FOR TOXIC SUBSTANCES BILL

Toxic Substances Control legislation was called for by Bobby D. Crim, Speaker of the Michigan House of Representatives in testimony last week before the House Agriculture Committee's Subcommittee on Conservation and Credit (See April 21, Page 15). The Subcommittee is reviewing the current situation regarding the toxic contamination of livestock. Most discussion centered on the problems resulting from the mixing of polybrominated biphenyls (PBB) in animal feed in 1973 in Michigan and the need for some form of financial relief for farmers.

Crim told the Congressmen that "in order to know what we are dealing with, we needed rigorous pre-market testing of toxic substances before the contamination occurred. Information on the effects of toxic substances is our most powerful weapon in combating them." He called for thorough testing of the effects on humans and animals of all toxic substances being marketed where such information is now lacking.

Also needed, he said, are minimum standards forlabeling and packaging of toxic substances. as well as precise records of where and when each container of toxic material was distributed. Stringent requirements for the labeling of toxic substances are needed throughout the country, Crim asserted.

Many toxic substances are potentially lethal to humans and animals and they should be manufactured, packaged, distributed and marketed with appropriate knowledge and caution, Crim told the Congressmen. Little or no legislation now exists which takes these into account, he added. He warned the Congressmen that similar incidents could take place in other states and that is why it is vital for Congress to approve legislation to deal with situations such as the Michigan PBBs before they happen.

The National Milk Producers Federation called on the House Subcommittee to include a provision, similar to one already in the Senate-passed version. to provide for loans to processors whose products are destroyed as the result of such toxic chemicals.

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3,7,8-Tetrachlorodibenzo-*p*-dioxin: environmental contaminant and molecular probe[1,2]

cc- Rowlison Hudson Blair K-file

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ABSTRACT

The chlorinated dibenzo-*p*-dioxins and dibenzofurans are formed as trace contaminants during the synthesis of a number of commercially important chemicals. The prototype compound of this group, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is one of the most potent low molecular weight toxins and teratogens known, and its inadvertent dispersion in the environment has caused concern about the potential hazard to human health. In studying the biochemical effects of TCDD, it was found to be extraordinarily potent as an inducer of two hepatic enzymes: *1*) δ-aminolevulinic acid synthetase, the initial and rate-limiting enzyme in heme synthesis, and *2*) aryl hydrocarbon hydroxylase, a cytochrome P-450–mediated microsomal monooxygenase. Among a series of halogenated dibenzo-*p*-dioxins there is an excellent correlation between their toxic potency and their potency as inducers of these two enzymes. The administration of polycyclic aromatic hydrocarbons (e.g., 3-methylcholanthrene (MC)) to certain inbred strains of mice induces aryl hydrocarbon hydroxylase, while other inbred strains fail to respond; and the trait of aryl hydrocarbon responsiveness is inherited as an autosomal dominant. TCDD, about 30,000 times as potent as MC, induces all strains whether responsive or nonresponsive to MC; however, the responsive strains are more sensitive ($ED_{50} \sim 1 \times 10^{-9}$ mole/kg) to TCDD than are the nonresponsive strains ($ED_{50} \geq 1 \times 10^{-6}$ mole/kg). The results suggest that the mutation in the nonresponsive strains results in a ligand binding site (an induction receptor) that has a diminished affinity for MC and TCDD. The correlation among the halogenated dibenzo-*p*-dioxins, between their potency as toxins and their potency as inducers of aryl hydrocarbon hydroxylase, is discussed in relationship to various proposed mechanisms of toxicity. —Poland, A., and A. Kende. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: environmental contaminant and molecular probe. *Federation Proc.* 35: 2401–2411, 1976.

The chlorinated dibenzo-*p*-dioxins and dibenzofurans are among the most potent toxins and teratogens known. The present concern about these compounds and the potential human health hazard they pose is a result of a growing awareness of their extraordinary toxicity and their inadvertent dispersion in the environment as trace contaminants of important commercial chemicals. Over the past two decades, hundreds of chemists, biologists, and clinicians have made important contributions to our understanding of this problem. Several excellent symposia (3, 38), reviews (26), and government-sponsored reports (13, 52, 53) have appeared on the subject. The present paper makes no attempt at a comprehensive review; rather it presents the work done in our laboratory, prefaced by a brief review of pertinent investigations of others.

FORMATION AND OCCURRENCE OF THE CHLORINATED DIBENZO-*P*-DIOXINS AND DIBENZOFURANS

The chlorinated dibenzo-*p*-dioxins are formed from the condensation of orthochlorophenates. The particular dibenzo-*p*-dioxin formed depends on the chlorophenols present. In the commercial synthesis of the herbicide 2,4,5-T, the first step is the conversion of 1,2,4,5-tetrachlorobenzene to 2,4,5-trichlorophenate (Fig. 1). At high temperatures an unwanted contaminant, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is formed by the condensation of two trichlorophenate molecules (31).

In the commercial synthesis of pentachlorophenol, a widely used wood preservative and bacteriostat, octa-, hepta- and hexachlorodibenzo-*p*-dioxin are formed (23, 43). Chlorinated dibenzofurans are formed as unwanted contaminants in the production of the polychlorinated biphenyls (PCBs) that are

[1] From the Symposium on *Mass Health Disasters Related to Environmental Chemicals* presented by the American Society for Pharmacology and Experimental Therapeutics at the 59th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 17, 1975.

[2] Supported by National Institute of Environmental Health Sciences Grant 1-R01-ES-00965-02.

[3] Recipient of Career Development Award, 1 K04-ES00017-01.

Abbreviations: 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; MC, 3-methylcholanthrene; ALA synthetase, δ-aminolevulinic acid synthetase; LD_{50}, mean lethal dose—the dose of a drug that when administered kills 50% of the animals; ED_{50}, mean effect dose—the dose of a drug that when administered elicits half the maximal response that that drug can produce; and *Ah*, aromatic hydrocarbon responsiveness.

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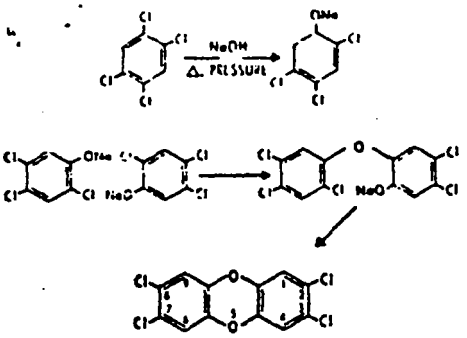


Figure 1. Origin of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the synthesis of 2,4,5-trichlorophenate. In the commercial synthesis of the herbicide 2,4,5-T, the first step is the conversion of 1,2,4,5-tetrachlorobenzene to 2,4,5-trichlorophenate. At elevated temperatures two trichlorophenate molecules can condense to yield TCDD (shown at bottom) in trace amounts.

widely used as plasticizers and heat exchange fluid (54, 62).

Historical background

Our current understanding of the chlorinated dibenzo-*p*-dioxins and dibenzofurans arose from several seemingly unrelated lines of investigation, each stimulated by a poisoning epidemic or concern about the pot al for poisoning.

Chick edema factor

In the southeastern and central United States in 1957 there were several outbreaks in domestic poultry of a disease characterized by hydropericardium and gross kidney and liver damage which produced substantial economic losses (37). The etiologic agent was traced o a toxic impurity sporadically present in the nonsaponifiable fraction of fats added to commercial eed. Intensive investigations of this "chick edema factor" eventually led o the isolation and identification of ,2,3,7,8,9 - hexachlorodibenzo - *p* - lioxin (7, 12, 66). The probable origin of this compound was as a contaminant formed in the synthesis of hlorophenols that were used as reservatives for hide curing operaions (14). The contaminated fat ripped from the hides was then old as a by-product and used in hicken feed.

Chloracne

n Germany in 1951 there was an utbreak of severe acne among the employees of a chemical plant manufacturing 2,4,5-T. In a pioneering investigation, Kimmig and Schultz and co-workers found that acne could be produced in rabbits by direct dermal application of the technical grade trichlorophenol which was contaminated with a number of chlorinated dibenzo-*p*-dioxins and dibenzofurans (27, 58). The most potent acnegenic compound was TCDD.

In the late 1950's and early 1960's several outbreaks of chloracne were reported among workers in 2,4,5-T factories in Germany, France, and the United States; manufacturing conditions were altered to minimize the formation of TCDD and the contaminant concentrations were monitored during production and in the final product.

The most recent poisoning attributed to TCDD occurred in 1971 in eastern Missouri, where waste oil contaminated with TCDD was sprayed for dust control in riding areas (8). This mishap was responsible for one human illness and for the death of 48 horses and numerous small animals.

Vietnam war and NCI study

A mixture of the *n*-butyl esters of 2,4-D and 2,4,5-T (Agent Orange) was used extensively in the Vietnam war from 1962 to 1969. Besides the ecological effect of this defoliation, which has been the subject of several reports (13, 52, 53), application of Agent Orange contaminated the environment with large amounts of TCDD, which was present as a trace impurity.

A final stimulus was a National Cancer Institute study on the carcinogenicity and teratogenicity produced by a number of insecticides and herbicides in which a 2,4,5-T sample containing a significant amount of TCDD was shown to be teratogenic (40). Subsequent work has shown both the herbicide and TCDD to be teratogenic and TCDD to be one of the most potent teratogens known (9, 60).

Chemical and biological properties

1) Chemistry. The prototype compound of the dibenzo-*p*-dioxins is 2,3,7,8 - tetrachlorodibenzo - *p* - dioxin (TCDD). TCDD is a nearly planar tricyclic aromatic molecule with twofold symmetry. It is remarkable for its lack of reactive functional groups and its chemical stability. TCDD is an extremely lipophilic molecule, notably insoluble in water (6×10^{-10} M) and only sparingly soluble in most organic solvents.[a]

TABLE 1. Comparative toxicity of selected poisons

Substance	Molecular weight	Minimum lethal dose, moles/kg
Botulinus toxin A	9×10^5	3.3×10^{-12}
Tetanus toxin	1×10^{-6}	1×10^{-12}
Diphtheria toxin	7.2×10^{-4}	4.2×10^{-12}
TCDD[a]	322	3.1×10^{-9}
Saxitoxin	372	2.4×10^{-8}
Tetrodotoxin	319	2.5×10^{-8}
Bufotoxin[b]	757	5.2×10^{-7}
Curare	696	7.2×10^{-7}
Strychnine	334	1.5×10^{-6}
Muscarin[b]	210	5.2×10^{-6}
Diisopropylfluorophosphate	184	1.6×10^{-5}
Sodium cyanide	49	2.0×10^{-4}

These data were compiled by Mosher et al. (39) and the values indicate only relative toxicity. It should be noted that one is comparing different species, routes of administration, survival times, and in one case the mean lethal dose rather than the minimum lethal dose. Except where noted, administration was by the intraperitoneal route in mice. [a] LD_{50}; oral administration in the guinea pig. [b] Intravenous injection in the cat.

2) The most striking fact about TCDD is its potency: lethal potency, and potency as a teratogen and as an acnegen. In guinea pigs the oral LD_{50} is approximately 1 μg/kg (3×10^{-9} mole/kg) (59). TCDD is perhaps the most potent toxin of low molecular weight known (Table 1). Almost all the other poisons listed in Table 1 act rapidly and kill by impairing the physiologic function of the nervous system. TCDD, in contrast, is a "cellular poison." In the rat, death appears to result from hepatic necrosis, and ensues weeks after a single dose.

3) There are large differences in species susceptibility to TCDD (Table 2). The dog is almost 1,000 times less sensitive than the guinea pig. The cause of death is uncertain in all cases. In the rat, rabbit, and mouse, hepatic necrosis seems to be the cause of death. However, in the

[a] Dow Chemical Company.

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TABLE 2. Mean lethal dose of TCDD in various species

Species	Sex	LD_{50} μg/kg
Rat	Male	22
	Female	44
Mouse	Male	>64
	Female	>130
Guinea pig	Mixed	0.5–2.0
Rabbit	Mixed	~30
Dog	Mixed	1,000*
Chick embryo	Mixed	<1

The table was compiled largely from data obtained by Schwetz et al. (59). TCDD was administered orally in all cases except the chick embryo (19), in which case it was administered parenterally into the yolk sac. * Estimated.

guinea pig, one of the most sensitive species, liver damage is minimal.

4) TCDD produces a number of specific toxic actions in different species: teratogenesis occurs in several species, edema in the neonatal chick, and dermal contact produces acne in rabbits and man. Among the chlorinated dibenzo-*p*-dioxins studied, those that produce [illegible] of these responses seem to evoke all of them; and changes in the number or position of the chlorine atoms produce large changes in potency (59).

5) TCDD is a remarkably stable compound in biological systems. The whole body half-life of TCDD in rats is about 24 days (42, 56), and despite a rather thorough search, no metabolites have been found over a 20-day period following in vivo administration, nor after incubation with liver microsomes in vitro (61). This suggests that if TCDD is metabolized, the rate is very slow. In contrast, the parent ring, dibenzo-*p*-dioxin, is rapidly converted to polar metabolites by hepatic microsomes (15, 61). TCDD is also quite stable in soil, and microbial degradation is limited (25).

2,4-D and 2,4,5-T factory study

The starting point of our work was a study of Bleiberg et al. (4) in 1961 who noted that of 29 workers in a 2,4,5-T factory, all of whom had industrially-acquired acne, 11 had porphyrinuria and several had overt clinical porphyria cutanea tarda. TCDD was known to be a contaminant in the 2,4,5-T and was known to be the causative agent of the acne; however, the cause of the porphyria was unclear. Porphyria cutanea tarda is an acquired defect of hepatic porphyrin metabolism characterized by an overproduction of porphyrins by the liver, increased urinary excretion of porphyrins, mechanical fragility and photosensitivity of the skin (blistering in areas exposed to sun light), hyperpigmentation, and hirsutism (35).

We restudied the same factory 5 years later and found the acne was reduced in severity and there was no evidence of porphyria among the employees (50). The fact that this syndrome abated following measures to reduce the formation of TCDD and minimize exposure of the employees to this contaminant suggested that TCDD might have been the causative agent of the porphyria outbreak originally reported by Bleiberg et al. (4).

Laboratory investigations

Porphyria

A variety of xenobiotics produce hepatic porphyria in animals, and most if not all have in common the ability to induce the initial and rate-limiting enzyme in the heme biosynthesis pathway, δ-aminolevulinic acid synthetase (ALA synthetase). To test whether TCDD was porphyrigenic, we administered the compound dissolved in *p*-dioxane to chick embryos (17-day gestation); 48 hours later the embryos were killed and ALA synthetase activity was assayed in their livers (44). As seen in Fig. 2*A*, TCDD produced a dose-related increase in enzyme activity. As little as 4.66×10^{-12} mole/egg (1.5 ng) produced a doubling of enzyme activity and at the highest level tested, 1.55×10^{-9} mole/egg, there was a 35-fold induction. TCDD is more potent, by at least three orders of magnitude, than any other inducer of ALA synthetase yet reported, and unlike many other porphyrigenic chemicals, the duration of induction is prolonged (most likely a result of the long biological half-life of TCDD).

We next screened a series of 15 halogenated dibenzo-*p*-dioxins for their ability to induce ALA synthetase (45). As seen in Figure 2*B*, the five isomers that were inducers had two common properties: *1*) halogen atoms occupy at least three of the four lateral ring positions (2, 3, 7, and 8), and 2) there is at least one free, nonhalogenated ring position. The inactive compounds were tested at 200 to 400 times the molar concentration of TCDD that produced a significant response.

Woods (65) reported that administration of TCDD had no effect on ALA synthetase in rats, mice, and guinea pigs. Goldstein et al. (17) found that while TCDD produced only a modest induction (twofold) of ALA synthetase in C57BL/6J mice, a gross accumulation of hepatic porphyrins occurred (30). Thus, it seems likely that TCDD was the causative agent of the porphyria cutanea tarda reported in workers in a factory manufacturing 2,4,5-T.

Aryl hydrocarbon hydroxylase

Numerous investigators have noted that many, but not all, compounds that induce ALA synthetase also induce microsomal mixed function oxygenase activity in the liver (also called "drug metabolizing enzymes"). (For a more extensive discussion of the relationship between heme synthesis and drug metabolism, see refs 11 and 18). Hepatic microsomal oxygenase activity is produced by a membrane-bound enzyme complex requiring NADPH and molecular oxygen, which is responsible for the oxidative metabolism of many foreign compounds.

Reconstitution experiments of microsomal oxygenase activities in vitro have shown that the enzyme complex consists of three components: a phospholipid, a flavoprotein (NADPH–cytochrome P-450 reductase), and cytochrome P-450 (32). The cytochrome is the enzyme active site and determines the reactivity of the microsomal oxygenase towards any drug. There is a large body of evidence to suggest there are at least two types of cytochrome P-450, and recent evidence reveals four or five different bands of the cytochrome on gel electrophoresis (1, 64). The administration of polycyclic hydrocarbons selectively increases one type of cytochrome P-450, termed P_1-450, which is spectrally distinct (31). Associated with the increase of this cyto-

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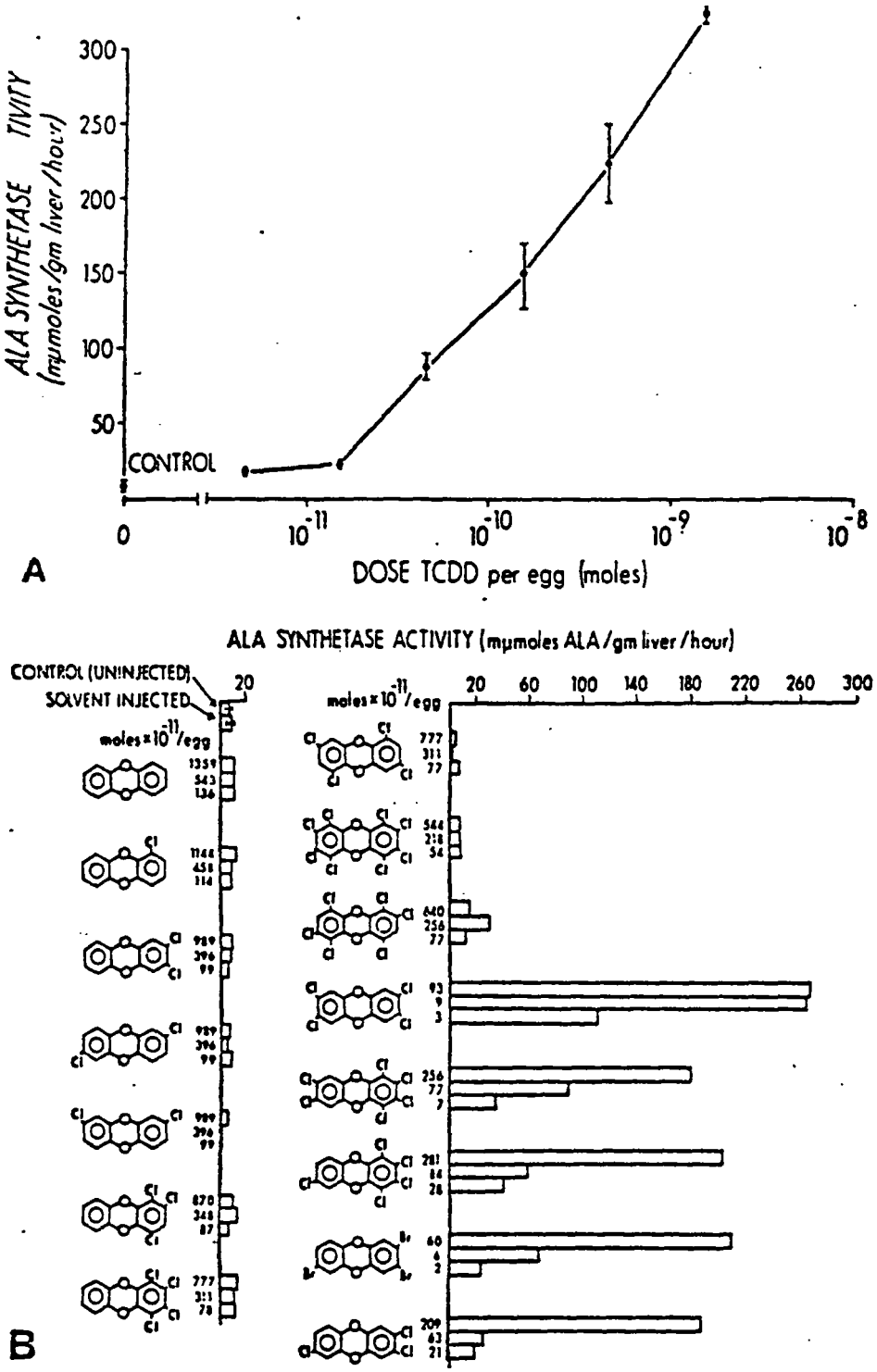


Figure 2. Induction of ALA-synthetase by halogenated dibenzo-*p*-dioxins. Chicken eggs of 17 days' gestation were injected with 25 μl of solvent (control) or solvent containing various concentrations of TCDD or other dibenzo-*p*-dioxins. The embryos were killed 48 hours later and assayed for hepatic ALA-synthetase activity (44). *A*) log dose–response relationship of TCDD; each point represents the mean ± standard error of three or four groups of pooled livers. *B*) structure–activity relationship; each bar represents a single group of 3 to 5 pooled livers except the uninjected controls (no. = 9) and the solvent-injected controls (no. = 12).

chrome P_1-450 are a limited number of microsomal oxygenase activities, the most studied being aryl hydrocarbon hydroxylase (41). Because TCDD resembles the polycyclic hydrocarbons, we chose to study its effect on aryl hydrocarbon hydroxylase activity.

As seen in Fig. 3*A*, TCDD produces a dose-related induction of aryl hydrocarbon hydroxylase in chick embryo liver (15). At the lowest dose tested (1.55×10^{-12} mole/egg (0.5 ng)) there is a nearly twofold increase in enzyme activity, and maximum induction is produced by 1.55×10^{-10} mole/egg (50 ng). We screened 15 halogenated dibenzo-*p*-dioxins for their ability to induce aryl hydrocarbon hydroxylase activity at three dose levels, 4.7, 47 and 470×10^{-11} mole/egg. The structure–activity relationship is the same as that seen with induction of ALA synthetase (Fig. 3*B*): *1*) compounds that are potent inducers (induction of aryl hydrocarbon hydroxylase at 4.7 or 47×10^{-11} mole/egg) have halogen atoms at at least three of the four lateral ring positions and *2*) they have at least one nonhalogenated ring position.

The potency of the halogenated dibenzo-*p*-dioxins congeners to induce ALA synthetase and aryl hydrocarbon hydroxylase corresponds precisely with their lethal, teratogenic, and acnegenic potency, to the extent that toxicologic data are available (59). To date we have tested over 40 dibenzo-*p*-dioxins and 20 dibenzofurans; the basic requirement for halogens in the lateral ring positions holds in all cases. The striking correspondence between the potency of these compounds as inducers of ALA synthetase and aryl hydrocarbon hydroxylase and their whole animal toxicity, and the sensitivity of the biochemical response, prompted the suggestion that enzyme induction be used as a screening bioassay for the presence of these compounds in environmental samples (45). Bradlaw and colleagues at the Food and Drug Administration are currently exploring this possibility, measuring the induction of aryl hydrocarbon hydroxylase in cell culture (5).

The most commonly used inducer of aryl hydrocarbon hydroxylase activity is 3-methylcholanthrene (MC). A comparison of MC and TCDD on the induction of hepatic hydroxylase activity in the rat showed that these drugs evoked the same maximal response; simultaneous administration of maximally inducing doses of both drugs evoked no greater response than that produced by either drug alone; and the slopes of the log-dose response curves were parallel (Fig. 4) (46). TCDD, however, is 30,000 times as potent as MC. These results suggest that the drugs share a common mechanism for the induction

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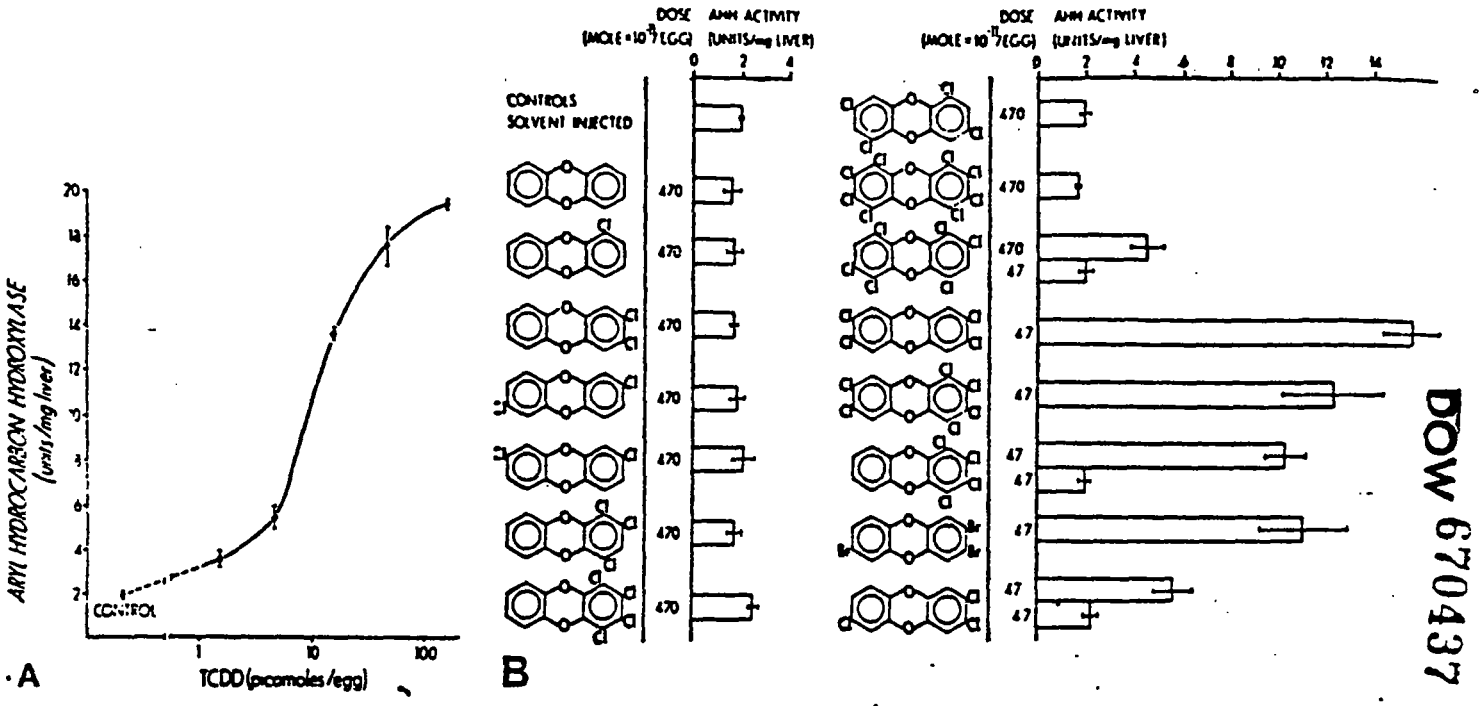


Figure 3. Induction of aryl hydrocarbon hydroxylase by halogenated dibenzo-*p*-dioxins. Chicken eggs of 18 days' gestation were injected with 25 μl of *p*-dioxane or the test compound dissolved in *p*-dioxane. The embryos were killed 24 hours later and assayed for hepatic aryl hydrocarbon hydroxylase activity. *A*) log dose–response relationship of TCDD; each point is the mean ± standard error of four groups of pooled livers. *B*) structure–activity relationship; each point is the mean ± standard error of four groups of pooled livers except the solvent-injected controls and TCDD, where no. = 12.

...ryl hydrocarbon hydroxylase and ...haps act at the same receptor.

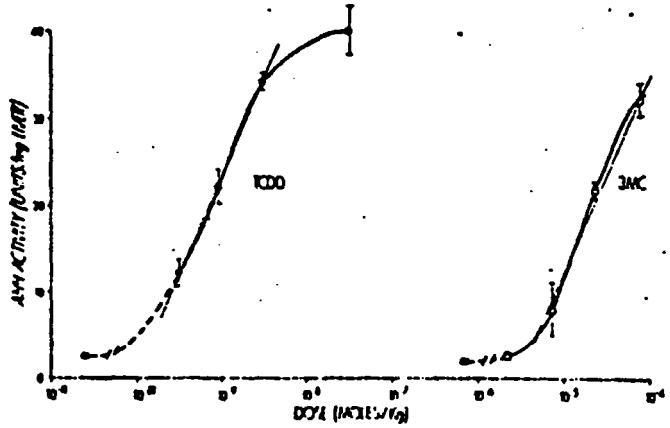
The administration of TCDD induces hepatic aryl hydrocarbon hydroxylase activity in the chick embryo, rat, and mouse, and the dose of TCDD that elicits half-maximal induction is very similar: $ED_{50} = 4 \times 10^{-10}$ mole/kg in chick embryo; 8.5×10^{-10} mole/kg in the rat, and 4.0 to 12.0×10^{-10} mole/kg in various strains of inbred mice (46).

Genetic expression of aromatic hydrocarbon hydroxylase

When 3-methylcholanthrene is administered to wild type and certain inbred strains of mice, there is induction of aryl hydrocarbon hydroxylase activity and the formation of cytochrome P_1-450 in a number of tissues (16). In contrast, other strains of inbred mice do not respond to administration of MC. In crosses between the prototype strains C57BL/6 (responsive) and DBA/2 (nonresponsive), the phenotypic characteristic of aromatic hydrocarbon responsiveness is inherited in a simple autosomal dominant mode. Extensive studies of crosses among 12 different inbred strains have shown that aromatic hydrocarbon responsiveness is controlled by multiple alleles at at least two, and possibly more loci (55). The important point is that in nonresponsive mice, MC fails to induce hepatic aryl hydrocarbon hydroxylase activity.

Because of the greater potency of TCDD, we administered it to inbred responsive and nonresponsive mice. As shown in Fig. 5, TCDD induced hepatic aryl hydrocarbon hydroxylase activity in all strains regardless of whether or not they responded to MC (49). The aryl hydrocarbon hydroxylase activity and cytochrome P_1-450 level, characterized by difference spectra, electron paramagnetic resonance, and in vitro enzyme inhibition studies, were qualitatively the same in all induced mice, whether obtained from responsive mice treated with MC or TCDD, or from nonresponsive mice treated with TCDD. This implies that the prod-

Figure 4. Log dose–response curves for the induction of aryl hydrocarbon hydroxylase in rats by TCDD and 3-MC. Young Sprague-Dawley male rats were injected intraperitoneally with *p*-dioxane (0.3 ml/kg), TCDD in *p*-dioxane, corn oil (2 ml/kg), or MC in corn oil, and killed 24 hr later. The livers were removed and assayed for aryl hydrocarbon hydroxylase activity. The initial point on each curve is the enzyme activity for control animals treated only with the vehicle (■, *p*-dioxane; □, corn oil). Each value represents the mean ± standard error of four or five rats.



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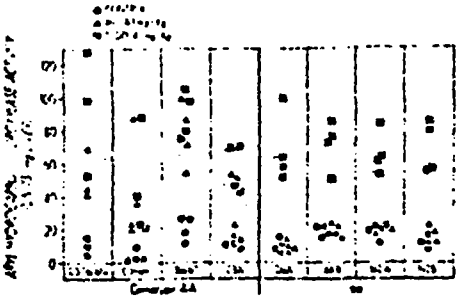


Figure 5. Hepatic aryl hydrocarbon hydroxylase activity in "responsive" and "nonresponsive" inbred strains of mice. Mice were administered an intraperitoneal dose of *p*-dioxane (0.4 ml/kg, control ●), MC in corn oil (80 mg/kg, ▲), or TCDD in *p*-dioxane (10 μg/kg, ■). Forty-eight hours later, the animals were killed and their livers were assayed for enzyme activity.

uct(s) of gene expression are the same for MC and TCDD.

What is the defect in nonresponsive mice? Clearly they do respond to a potent stimulus, i.e., TCDD. Therefore, nonresponsive mice do possess the necessary regulatory and structural genes for the expression of aryl hydrocarbon hydroxylase activity.

We postulated that the mutation in nonresponsive mice that resulted in a failure to respond to MC was a failure recognize MC. In biochemical ...ns, we suggested that the mutation resulted in a recognition site for MC (an induction receptor) with a diminished affinity for the ligand. Why then can TCDD induce aryl hydrocarbon hydroxylase and cytochrome P_1-450 in these nonresponsive mice? Two hypotheses come to mind: *1*) TCDD acts as a receptor site different from that of MC, a site not affected by the mutation in nonresponsive mice, or *2*) TCDD acts at the same receptor site as MC, but because of its greater potency (and presumably greater receptor affinity), it is able to saturate this site and initiate the process that results in the induction of aryl hydrocarbon hydroxylase. One can design experiments to differentiate between these two hypotheses: *1*) if TCDD acts at a site different from that of MC, a site unaffected by the mutation in nonresponsive mice, then responsive and nonresponsive mice should not differ in their sensitivity to TCDD, and *2*) if, however, TCDD acts at the same induction rec... as MC, then the diminished affinity of this receptor in nonresponsive mice should be diminished for MC and TCDD. Thus, the nonresponsive mice should be uniformly less sensitive to TCDD than the responsive mice.

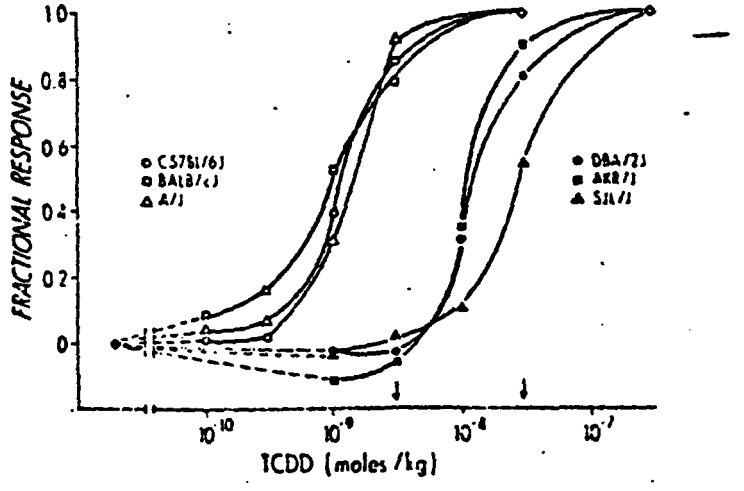
As shown in Fig. 6, the dose of TCDD that produces half-maximal induction (ED_{50}) in three responsive inbred strains of mice is about 1×10^{-9} mole/kg, and the ED_{50} in the three nonresponsive strains is equal to or greater than 1×10^{-8} mole/kg (47). As one might anticipate, hybrid mice of responsive and nonresponsive parents—(C57BL/6J × DBA/2)F_1—show a sensitivity intermediate to that of the parent strains.

All the data are consistent with the hypothesis that: *1*) the *Ah* locus is a regulatory gene whose product is the receptor that binds TCDD and MC and initiates the events that ultimately result in the expression of aryl hydrocarbon hydroxylase, and *2*) the mutation in the *Ah* locus in nonresponsive mice results in a receptor with a diminished affinity for these inducing drugs.

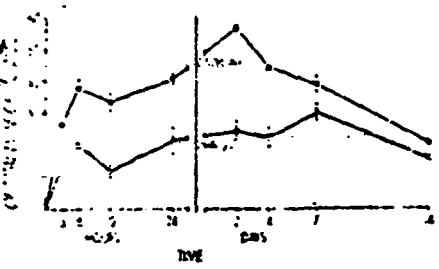
This hypothesis proved useful in suggesting further experiments on the nature of the induction receptor. We reasoned that after administration of low doses of radiolabeled TCDD, insufficient to saturate the receptor, hepatic uptake of TCDD might be determined at least in part by the affinity of the receptor: one should see greater hepatic uptake in responsive mice and a lesser concentration in nonresponsive mice. There is an implicit assumption that TCDD, an extremely lipophilic molecule, is handled by passive diffusion. Following intraperitoneal administration of ^{14}C-labeled TCDD at 6×10^{-9} mole/kg, the C57BL/6J (responsive) mice concentrate more TCDD in their livers than do DBA/2J mice (nonresponsive) for all time periods examined (3 hr to 2 wk; Fig. 7). The hybrid mice, (C57BL/6J × DBA/2J)F_1, show hepatic uptake intermediate to the two parental strains.

Our recent research has been directed toward identification and characterization of the induction receptor in vitro: that is, the isolation of a macromolecular fraction from liver that binds TCDD and that has the binding properties in vitro would be predicted from the in vivo biology. We have identified and begun to characterize a liver protein fraction that has the following properties: *1*) TCDD is bound with a high affinity, and the binding is saturable and reversible; *2*) congeners of TCDD that do not induce aryl hydrocarbon hydroxylase do not bind, and the binding affinity of active congeners of TCDD corresponds to their in vivo potency; and *3*) the protein fraction from the liver of responsive mice has a greater affinity for TCDD than the comparable fraction from nonresponsive mice.

Figure 6. Log dose–response curves for the induction of aryl hydrocarbon hydroxylase activity by TCDD in six inbred strains of mice. Mice were injected intraperitoneally with *p*-dioxane (0.4 ml/kg) or varying doses of TCDD dissolved in the vehicle. Twenty-four hours later the animals were killed and hepatic aryl hydrocarbon hydroxylase activity was assayed. The absolute enzyme activities have been converted to fractional responses (control = 0%, maximally induced = 100%) for ease of comparison (For raw data see ref 47). Each point is the mean of 4 or 5 animals. The responsive strains are those with open symbols; the nonresponsive strains are shown with black symbols.



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igure 7. Hepatic uptake of ^{14}C-labeled TCDD C57BL/6J and DBA/2J mice. C57BL/6J responsive) and DBA/2J (nonresponsive) mice ere administered a single intraperitoneal se of ^{14}C-labeled TCDD (6×10^{-9} mole/kg; ecific activity, 114 mCi/mmole). At the indiated times after administration, the animals ere killed, the livers were removed, weighed, omogenized, an aliquot solubilized with Protool™ and counted by liquid scintillation ectroscopy. The results represent the perntage of the administered dose concentrated the liver. Each point represents the mean standard error of 5 or 6 animals.

DISCUSSION

s apparent from the data presented at TCDD is a powerful probe in ploring the mechanism of gene exession of aromatic hydrocarbon droxylase. A model to explore a ulatory gene in eukaryotic cells of c siderable interest to basic carc But one might justly ask, how does this help us with our ginal intent to explain the mechan by which TCDD acts as a poison, eratogen, and an acnegen? The etic expression of aryl hydroon hydroxylase and the toxicity chlorinated dibenzo-*p*-dioxins n to be related: induction of this me is one of the earliest, most itive, and most universal (in diffnt tissues and different species) of e ents produced by TCDD, and structure–activity relationship ng the halogenated dibenzo-*p*-ins for induction of hydroxylase isely matches their potency as s. How then is hydroxylase inon related to toxicity?

ries of toxic action

nechanism by which TCDD exts toxic action is unknown. A er of proposed mechanisms bservations that suggest mechaof toxicity are summarized berst, posals that relate toxicity luction of microsomal oxyactivities, followed by a more geneous group).

Overstimulation of microsomal oxygenase activities

Since one of the most sensitive and prolonged responses to TCDD is induction of aryl hydrocarbon hydroxylase (and other cytochrome P_1-450–mediated microsomal oxygenase activities), it has been suggested that this prolonged enzyme stimulation might result in excessive inactivation of endogenous substrates (e.g., steroids) or produce an excess of harmful metabolites of endogenous compounds (20). It should be noted that in addition to induction of the microsomal monooxygenases, TCDD is also a potent stimulator of UDP-glucuronyl transferase (33) and ligandin (glutathione-*S*-transferase B) (29).

Reactive metabolite formation

We suggested that perhaps TCDD is metabolized to some reactive intermediate(s) and that this metabolite binds covalently to an essential macromolecule (48), analogous to the mechanism of hepatotoxicity proposed for bromobenzene (6) and phenacetin (24, 36, 37, 51). If the rate-limiting step is the formation of the arene oxide of TCDD, then those congeners that induce aryl hydrocarbon hydroxylase would stimulate their own metabolism and flood the system with an excess of toxic metabolites. (For a review of the proposed mechanism of arene oxide toxicity, see refs 10, 22.) To date, there is little evidence supporting metabolism or covalent binding to TCDD.

Tissue localization as a result of the induction receptor

Our data on the hepatic uptake of radiolabeled TCDD in responsive and nonresponsive strains of mice suggest that hepatic uptake and retention of TCDD are in part determined by the genetic constitution of the animal, in particular by the affinity and perhaps the concentration of the induction receptor. One might speculate that the halogenated dibenzo-*p*-dioxins that induce and thus bind to the induction receptor are thus concentrated in the liver (or other parenchymal organs containing substantial receptor concentrations) whereas dibenzo-*p*-dioxins that do not induce hydroxylase activity and do not bind to the induction receptor have more uptake in less critical organs (e.g., fat). Although this speculation does not propose a mechanism of toxicity, it does suggest a reason for the correlation of toxic potency of dibenzo-*p*-dioxins and the potency as inducers of aryl hydrocarbon hydroxylase.

Intercalation

TCDD is a planar tricyclic aromatic compound similar chemically to many polycyclic hydrocarbon carcinogens and tricyclic compounds that bind to DNA, directly or through the formation of a metabolite, and produce mutation and consequent teratogenesis or carcinogenesis (2). Using specific mutant strains of *Salmonella typhimurium* Ames was unable to demonstrate reversion of mutants by TCDD (personal communication, B. N. Ames and E. Yamasaki). At present, the available evidence suggests that TCDD is not a mutagen.

Delayed hypersensitivity

Vos and Moore (63) found that TCDD produces atrophy of the cortical cells of the thymus, a reduction in the general lymphoid mass, and an accompanying depression of cellular immunity. They suggested that this may be related to toxicity.

Inhibition of mitosis

Jackson reported that TCDD inhibited mitosis of the dividing endosperm of the African blood lily (21). No similar effect has been reported in animal cells.

We have not yet exhausted the list of theories proposed for the toxicity of chlorinated dibenzo-*p*-dioxins. At present our knowledge is inadequate to tentatively accept or reject any one of these. There are two sources of encouragement on this search for a mechanism of toxicity. First, there is an ever-increasing body of data concerning the dibenzo-*p*-dioxins and dibenzofurans, and any proposed theory must account for *a*) differences in species sensitivity, *b*) different histological patterns in different species, *c*) the structure–activity relationship, and *d*) the correlation of the various types of toxicity. Second, TCDD is among the most potent small molecule toxins known. If one reexamines Table 1, virtually every toxin of comparable potency has a well-established mechanism of action, and

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most have become useful tools in further biologic research. 57

We would like to express our appreciation to Drs. James Wade and Mark DeCamp for their skill and effort in synthesizing the many congeners of TCDD, and to Mr. Edward Glover for his meticulous efforts in carrying out most of the biological experiments. In addition we gratefully acknowledge the collaboration of Dr. Daniel Nebert and his colleagues at the National Institute of Child Health and Human Development in many of the genetic experiments, and Drs. David Firestone, Albert Pohland, and Irwin Pomerantz, of the Food and Drug Administration, for their generous gifts of congeners of TCDD and for their continued interest.

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Mouse Teratology Studies with Chlorodibenzo-P-Dioxins

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Recently, much attention has been focused on the polychlorinated dibenzo-p-dioxin class of compounds. The extremely high toxicity of the 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) isomer warranted an examination of additional isomers and members of this class of compounds.

TCDD was produced as a contaminant or by-product in the manufacture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), a herbicide which was widely used in this country. Awareness of the toxicity of TCDD occurred as the result of an industrial accident that produced chloracne in the workers (BLEIBERG et al., 1964) as well as porphyria cutanea tarda (POLAND et al., 1971). In 1968, chlorodibenzo-p-dioxins were found to be the causative agent of chick edema disease first observed in 1957 (HIGGINBOTHAM et al., 1968). More recently TCDD was found to be teratogenic and fetotoxic in mice (COURTNEY and MOORE, 1971; NEUBERT and DILLMAN, 1972) and rats (SPARSCHU, et al., 1971). The dosage levels for these effects are in the range of microgram per kilogram of body weight.

Since TCDD affected fetal development so markedly and at such low dosage levels, this compound ranks as one of the most biologically active chemicals studied in mammalian fetuses. The uniquely high degree of toxicity of TCDD has generated interest in other tetrachloro-isomers as well as other members of this class of compounds containing more or less chlorines. Two current reviews of the wide range of toxic and pathological effects has been presented by Kimbrough (1972, 1974). Possible mechanisms of actions of TCDD were suggested at a symposium on dioxins (MOORE, 1973). It was not known how many of this class of compounds shared the extremely toxic and teratogenic properties of TCDD in the mouse. Thus, this study was undertaken to evaluate the teratogenic potential in mice of other members of the class of chlorinated dibenzo-p-dioxin compounds.

MATERIALS AND METHODS

Female CD-1 mice and pregnant CD-1 mice with known insemination dates were procured from Charles River Laboratories, Wilmington, Massachusetts. Detection of a vaginal plug indicated day 1 of pregnancy. The mice were randomly selected and assigned to control or experimental groups. The various chlorinated dibenzo-p-dioxins were prepared and supplied by Dr. A. Pohland of the Food and Drug Administration (POHLAND and YANG, 1972). The abbreviation TCDD is reserved for the 2,3,7,8-tetrachlorodibenzo-p-dioxin compound.

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Compounds were administered orally or subcutaneously. Oral administration was by means of gastric intubation using a volume of 0.1 ml/mouse/day. The following solutions were used for oral administration. Dibenzo-p-dioxin was dissolved in corn oil. The octachlorodibenzo-p-dioxin was dissolved in 15% anisole in corn oil. The remaining compounds were dissolved in 5% anisole in corn oil. Compounds administered subcutaneously were dissolved in DMSO employing 0.1 mg/mouse/day.

In order to select dose levels for the teratology studies, the various chlorinated dioxins were administered by gastric intubation daily to female CD-1 mice for 14 days. They were observed for an additional 7 days.

For the teratology studies, compounds were administered from the 7th through the 16th day of gestation. These mice were sacrificed on day 18 except those receiving octachlorodioxin and their respective controls. They were sacrificed on day 17 of gestation. Upon sacrifice, the fetuses were weighed, examined and stored in Bouin's solution until necropsied. The following conventions were observed in compiling data. If a fetus was either dead or resorbed, it was regarded as a dead fetus. Only live fetuses were examined for physical abnormalities. A fetus was classified normal if it was alive and had at least one type of anomaly, regardless of type. A fetus was said to have abnormal kidneys if at least one of its kidneys were affected. In calculating the ratios of liver to body weight in the mother, maternal body weight was defined as the difference between the weight of the mother on the day it was killed and the gravid uterus weight. Maternal weight gain was defined as the difference in the corrected maternal weight on the day it was killed and its weight on day 6 of pregnancy. Averages were calculated for each litter, then across litters. Statistical analyses were performed using the Student "t" test.

RESULTS AND DISCUSSION

The results from treating female CD-1 mice with various doses of the different dibenzo-p-dioxins are presented in Table 1. Administration of the compounds for 14 days at the doses indicated produced no lethal dose values. The dosage for the dibenzo-p-dioxin, dichlorodibenzo-p-dioxin, and octachlorodibenzo-p-dioxin are expressed as milligrams per kilogram of body weight per day while the others are expressed as micrograms per kilogram of body weight per day. The change in body weight reflects the difference in body weight on the first day of the study to the last, which was seven days after the last dose. The only unusual value was from those mice which received 50 mg/kg of dibenzo-p-dioxin. Further study is needed to determine if this increase of almost seven grams was real or not. Limitations of the supply of these compounds precluded further exploration of dose studies at this time. In general, none of the dibenzo-p-dioxins studied

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Table 1. Oral Administration of Dioxins to Female CD-1 Mice for 14 Days

Compound	No. of Mice	Dose/ Day	No. Dead/ Doses	Change Body Wt. (gms)
Dibenzo-p-dioxin	6	1 mg/kg	0	+0.6
	6	10	0	-0.3
	6	50	1/14	+6.8
2,7-Dichlorodibenzo-p-dioxin	6	0.5 mg/kg	0	-0.1
	6	1.0	1/8	+1.5
Combination of:				
40% 2,7-Dichlorodibenzo-p-dioxin	6	10 µg/kg	1/9	+1.2
60% 2,3,7-Trichlorodibenzo-p-dioxin	6	50	0	+0.8
	6	100	1/13	+1.6
1,2,3,4-Tetrachlorodibenzo-p-dioxin	6	5 µg/kg	0	0
	6	10	0	-0.2
	6	50	0	+0.8
	6	100	0	-0.9
2,3,7,8-Tetrachlorodibenzo-p-dioxin	10	10 µg/kg	0	+0.1
Octachlorodibenzo-p-dioxin	6	0.5 mg/kg	1/11	+0.1
	6	0.75	0	+0.7
	6	1.0	0	-0.4

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Table 2. Toxicologic Evaluation of Chlorinated Dibenzo-p-dioxin Compounds in Pregnant CD-1 Mice
(Compounds Administered from Day 7 to 16 of Gestation)

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Table 2. Toxicologic Evaluation of Chlorinated Dibenzo-p-dioxin Compounds in Pregnant CD-1 Mice (Compounds Administered from Day 7 to 16 of Gestation)

Dibenzo-p-dioxin Compound	Route	Dose/kg/day	No. of Litters	Av. % Fetal Mortality/ Litter	Fetal Weight (gms)		Maternal Weight Gain (g)		Maternal Liver/Body Weight x 100	
					$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
5% anisole:corn oil	oral	0.1 ml/mouse	15	6	1.02	0.16	3.5	1.93	7.8	0.59
2/3 mixture[a]	oral	100 µg	6	3	1.36	0.13***	4.6	1.10	8.0	0.84
	oral	200 µg	5	5	1.25	0.19**	4.4	1.59	7.5	0.46
1,2,3,4-tetrachloro	oral	100 µg	4	10	1.09	0.09	4.8	0.51*	7.8	0.47
	oral	250 µg	4	20	1.10	0.12	4.8	1.44	7.7	0.37
	oral	500 µg	5	5	1.08	0.08	4.5	1.56	7.8	0.43
	oral	1000 µg	5	10	1.03	0.09	3.7	1.31	8.3	0.58
	subc	500 µg	5	7	1.24	0.07	3.4	0.82	7.6	0.40
	subc	1000 µg	6	2	1.31	0.07	2.3	0.57	8.1	0.44
DMSO[b]	subc	0.1 ml/mouse	6	14	1.19	0.17	3.1	1.96	7.5	0.94
2,3,7,8-tetrachloro	oral	25 µg	7	6	1.13	0.13	3.4	2.20	8.6	0.68**
	oral	50 µg	7	13	1.01	0.13	3.4	1.07	9.4	0.44***
	oral	100 µg	6	14	0.95	0.12	2.1	3.57	7.8	1.00
	oral	200 µg	6	87	d		d		d	
	oral	400 µg	5	97	d		d		d	
	subc	25 µg	5	36	1.25	0.07	3.0	0.86	9.7	0.42***
	subc	50 µg	6	56	1.20	0.15	2.3	1.17	9.0	1.09*
	subc	100 µg	6	72	d		d		d	
	subc	200 µg	6	76	d		d		d	
15% anisole:corn oil[c]	oral	0.1 ml/mouse	5	8	0.61	0.05	0.8	1.47	6.2	0.22
octachloro[c]	oral	5 mg	6	14	0.60	0.06	0.3	1.40	6.6	0.22
	oral	20 mg	6	12	0.53	0.05	-0.1	2.31	7.2	0.60*

a mixture = 40% 2,7 dichlorodibenzo-p-dioxin and 60% 2,3,7 trichlorodibenzo-p-dioxin
b DMSO = dimethylsulfoxide
c sacrificed day 17 of gestation: all others sacrificed day 18
d marked edema
p values: * = 0.05; ** = 0.01; *** = 0.001

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were as toxic as TCDD, the 2,3,7,8 tetrachlorodibenzo-p-dioxin isomer, and some of the compounds could be considered relatively non-toxic.

Toxicologic results of administering the various dibenzo-p-dioxins to pregnant CD-1 mice are presented in Table 2. The diluent control solutions of 5% anisole in corn oil, DMSO or 15% anisole in corn oil did not adversely affect fetal mortality or fetal weight.

The mixture of the dichloro- and trichlorodibenzo-p-dioxin administered orally at doses of 100 and 200 mg/kg/day had no adverse effects on the fetal and maternal parameters. The fetal weights of these two groups are higher than those of their respective controls. They are also higher than values which might be anticipated for a gestational day 18 mouse fetus. It was assumed that these mice were very near term because the weights reflect a gestational day 19 mouse and one member of the experimental groups littered (omitted from these data). The maternal weight gains of the experimental groups were slightly higher than the values of the control groups, but these differences were not statistically significant. This slight increase in maternal weight gain was also seen in mice treated with the 1,2,3,4 tetrachlorodibenzo-p-dixoin isomer administered orally at doses ranging from 100 to 1000 mg/kg/day. Whether or not this increased weight gain was a true increase in weight or a manifestation of edema needs to be explored since studies with TCDD produced maternal edema. In contrast, the subcutaneous administration of the 1,2,3,4-tetrachloro-isomer produced a slight decrease in maternal weight gain compared to controls. The difference in these values was not statistically significant.

In marked contrast, TCDD adversely affected fetal weight and survival and maternal weight gain at a dose of 100 mg/kg/day. Dose levels of 200 and 400 µg/kg/day produced generalized edema in the mothers and vaginal bleeding after the sixth dose with some mice aborting shortly thereafter.

The increase in the ratio of liver to body weight seen in the mice receiving the lowest dose either orally or subcutaneously was primarily due to an increase in the liver weight. The dose of 50 µg/kg/day produced a decrease in maternal weight gain, and at the two highest doses the obvious edema made it difficult to interpret maternal weight changes. The subcutaneous administration of TCDD produced more fetal mortality at lower doses than oral administration. This suggests that the bioavailability of a subcutaneous dose is greater than that of an orally administered dose.

Treatment of pregnant mice with octachlorodibenzo-p-dioxin at 5 mg/kg/day had no adverse effects on fetal or maternal parameters. At a dose of 20 mg/kg/day, there was a slight

Table 1. Teratogenic Evaluation of Chlorinated Dibenzo-p-dioxin Compounds in CD-1 Mice

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Table 3. Teratogenic Evaluation of Chlorinated Dibenzo-p-dioxin Compounds in CD-1 Mice

Dibenzo-p-dioxin Compound	Route	Dose/kg/day	No. of Litters	Av. No. Live Fetuses/ Litter	Av. No. Abnormal Fetuses/ Litter	% Anomalies/Total Fetuses		
						Cleft Palate	Kidney	Clubfoot
5% anisole:corn oil	oral	0.1 ml/mouse	15	11.0	0.8	0	1	4
2/3 mixture[a]	oral	100 µg	6	12.3	3.2	0	10[d]	9
	oral	200 µg	5	12.8	3.8	0	1	22
1,2,3,4 tetrachloro-	oral	100 µg	4	11.8	0.8	2	0	2
	oral	250 µg	4	11.5	0.5	0	1	3
	oral	500 µg	5	11.6	0.2	0	0	0
	oral	1000 µg	5	11.8	1.0	0	0	0
	subc	500 µg	5	11.6	5.4	0	0	2
	subc	1000 µg	6	12.2	1.0	1	4	3
DMSO[b]	subc	0.1 ml/mouse	6	11.3	0.2	0	0	1
2,3,7,8 tetrachloro-	oral	25 µg	7	10.9	4.6	3	34	3
	oral	50 µg	7	11.0	8.1	19	72	7
	oral	100 µg	6	9.7	8.3	66	71	13
	oral	200 µg	6	1.5	1.5	100	100	14
	oral	400 µg	5	0.4	0.4	100	50	50
	subc	25 µg	5	7.7	6.7	82	53	11
	subc	50 µg	6	5.3	5.0	79	58	17
	subc	100 µg	6	3.5	3.5	85	95	0
	subc	200 µg	6	3.3	3.1	100	38	18
15% anisole:corn oil[c]	oral	0.1 ml/mouse	5	11.2	0	0	0	0
octachloro-[c]	oral	5 mg	6	11.2	0.2	1	0	0
	oral	20 mg	6	11.6	0	0	0	0

a mixture = 40% 2,7 dichlorodibenzo-p-dioxin and 60% 2,3,7 trichlorodibenzo-p-dioxin
b DMSO = dimethylsulfoxide
c sacrificed day 17 of gestation: all others sacrificed day 18
d 9/10 from one litter

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reduction of fetal weight which was not statistically significant. Also, the reduction in maternal weight gain was not statistically significant due to a very large standard deviation. The increase in the ratio of liver to body weight was primarily due to a loss in body weight. Fetal mortality was not affected at either dose.

All of the live fetuses were examined for malformations. These results are shown in Table 3.

The mixture of dichloro- and trichlorodibenzo-p-dioxin produced a slight increase in the number of abnormal fetuses. At the lower dose this was partly due to an increase in kidney malformations which were a mild form of hydronephrosis. Since most of these fetuses (9/10) were from one litter and kidney malformations were not observed at the higher dose, it is doubtful that this malformation was produced by the compound under study. At both dose levels there was an increase in the incidence of clubfoot. This may reflect both a natural incidence of the malformation and uterine crowding, since these fetuses weighted slightly heavier and the litters were slightly larger than the controls. However, this does not negate a possible compound effect.

The 1,2,3,4 tetrachloro-isomer did not increase the incidence of malformation at any dose level by either oral or subcutaneous administration. Since this strain of mouse has a tendency to display clubfoot, the 8% incidence of this anomaly observed at the 1000 mg/kg/day dose level needs further substantiation before being accepted as a compound effect.

In contrast TCDD produced many abnormal fetuses at all doses studied and by both routes of administration. The majority of the malformations were cleft palates and hydronephrotic kidneys, both unilateral and bilaterial. A few other anomalies such as hydrocephalus and open eye were occasionally seen. TCDD administered subcutaneously produced a greater teratogenic response at a lower dose than administration by the oral route. Administration by the subcutaneous route at the lowest dose produced about 87% abnormal fetuses per litter. This made it difficult to demonstrate a dose related response since this was close to being a maximum response. At the higher dose with both routes of administration many fetuses were observed with marked edema and petechiae.

The oral administration of 5 or 20 mg/kg/day of octachlorodibenzo-p-dioxin to pregnant CD-1 mice did not affect fetal development morphologically. The only malformation detected in this group of fetuses was a single cleft palate at the low dose.

In conclusion, TCDD, the 2,3,7,8 tetrachlorodibenzo-p-dioxin member of this class of compounds was the most fetotoxic and teratogenic of the compounds studied. The related compounds were relatively non-toxic and were not teratogenic at the doses studied.

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INDEX
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W. R. Mullison

FROM: J. H. Davidson
1/24/77

и мутилирующими язвами по всему телу. Разнообразная клиническая картина документирована рядом фотографий. Двенадцать пострадавших имело поражения печени с признаками porphyria cutanea tarda, а у некоторых пациентов были обнаружены нервные и психические расстройства.

Оказалось, что кожные и общие повреждения были вызваны 2, 3, 6, 7 — тетрахлордибензодиоксином, который возникает при алкалическом гидролизе тетрахлорбензола в трихлорфенолят солистый. Реакция производилась неподходящей технологией при температуре 190 °C и давлении 45 атм. На основе работ Шульце и Кичмира известно, что это максимально токсическое вещество не образуется, если же вышеуказанная реакция проводится при температуре ниже чем 153 °C. Поражеными были не только работающие при непосредственном производстве, но и рабочие по ремонту, а также научно-исследовательские работники и рабочие — землекопы, копающие канализационный канал.

У 6 из них началось заболевание острым солярным дерматитом, у остальных — медленным рассеянием комедонов и их переходом в кисты. Воспаления и язвы наблюдались в наиболее тяжелых случаях. Локализация и клинические признаки соответствовали тем, которые известны из современных литературных источников. У 17 больных, однако, acne chlorina началась необыкновенным способом — папулопустулами на конечностях. У 20 больных авторам удалось обнаружить локализацию в азилах, этот факт не был отмечен в современной литературе. Заболевание не проявляется на тех местах, где растут волосы, а у одного больного совершенно исчезло в тех местах лица и шеи, где выросли усы и борода, причем в остальных местах заболевание продолжалось. Больные с нарушениями метаболизма порфирина имели типичную гиперпигментацию и гипертрихоз, причем один из них имел bullosis actinica et mechanica. Однако авторы обнаружили гипертрихоз и гиперпигментацию и у 19 пациентов, имеющих acne без лабораторных признаков порфирии.

В период перед заболеванием у больных не был найден предопределяющий момент. Латенция была различной. В некоторых случаях и после весьма короткой экспозиции наблюдались тяжелые повреждения как кожные, так и внутренние. В других случаях и длительная и массивная экспозиция вызвала только незначительные повреждения. Несмотря на это авторы уверены, что интенсивность заболевания в большей степени зависит от экспозиции, чем от предрасположения. У больных наблюдались субъективные ощущения в соматической, душевной и сексуальной областях, а у некоторых и потеря веса. Два умерли от общей интоксикации, другие два от рака легких, взаимоотношение которого к профессиональной экспозиции, затем еще невозможно выразить. Раковое действие хлорированных углеводородов, на основе описанного производства затем еще нельзя доказать. В других работах авторы дают подробное описание внутренних и неврологических признаков своих больных.

Все больные находятся под постоянным наблюдением терапевта и невролога. После достижения максимума — обычно между 1-ым и 2-ым годом — заболевание постепенно уменьшается. У некоторых больных кожные заболевания и симптомы общей интоксикации продолжаются напротяжении 8 лет. Все применяемые методы оказались недостаточными. Улучшение наблюдалось при регулярной экспрессии комедонов при сауне. Все пострадавшие проходили лечение и им были возмещены убытки согласно с существующим законом. Км.

Cs. Derm., 48, 1973, č. 5, s. 306—317.

Jirásek L., Kalenský J., Kubec K.: Acne Chlorina and Porphyria Cutanea Tarda during the Manufacture of Herbicides

The authors submit after an evaluation of data reported in the literature results of the long-term follow up of 78 patients with an occupational disease caused by manufacture of herbicides — pentachlorphenol and derivatives of 2,4,5-trichlorophenoxydacetic acid. Of this number 76 subjects suffered from acne chlorina manifested in mild cases only by comedones and in severe cases by large cysts, abscesses and mutilating scars on the whole body. The varied clinical picture is demonstrated on photographs. Twelve of the patients had a hepatic lesion with symptoms of porphyria cutanea tarda and some patients had nervous and mental disorders.

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It was revealed that the skin and general affections were caused by 2,3,6,7-tetrachlorodibenzodioxin which is formed during alkaline hydrolysis of tetrachlorobenzene into sodium trichlorophenolate. The reaction was performed by an unsuitable procedure at a temperature of 190 °C and pressure of 45 atm. From the work of Schulz and Kimmig it is known that this highly toxic substances is not formed when the above reaction is carried out at a lower temperature of 153 °C. Not only workers engaged in production of herbicides were affected but also maintenance workers, research workers and workers digging the sewage channel.

In six the disease began as acute solar dermatitis, in all the remainder it started by a slow eruption of comedones and their development into cysts. Inflammations and scarring were found only in the most severe and extensive affections. The site and clinical manifestations corresponded to data reported in the literature. In 17 patients, however, acne chlorina started in a unusual way by papulopustulous eruptions on the extremities. In 20 patients the authors observed localization in the axillae which was not reported previously. The disease does not affect the hairy part of the skull and in one patient it disappeared even from those sites in the face and on the neck where he grew a beard, while the condition persisted at other sites. Patients with an impaired porphyrin metabolism sufered from typical hyperpigmentation and hypertrichosis and only one of them from bullosis actinica et mechanica. Hypertrichosis and hyperpigmentation was observed also in 19 subjects with acne without laboratory signs of porphyria.

In the period prior to the disease no significant predisposing factors were noted. The latency differed. In some instances severe dermatological and internal damage developed after short exposure. In other instances apparently long and massive exposure caused only mild symptoms. Despite this the authors are convinced that the intensity of the disease depends more on exposure than predisposition. The patients had various complaints-somatic, mental and sexual and some of them hac considerable weight losses. Two died from general intoxication, two from lung cancer but so far it is not possible to state reliably whether it was related to the occupational exposure. The cancerogenic effect of chlorinated hydrocarbons produced in the particular plant was not proved so far. The medical and neurological symptoms will be discussed by the authors in subsequent work.

All patients are being followed up in collaboration with specialists in internal medicine and a neurologist. After the peak has been reached — usually after 1—2 years duration — the disease recedes very slowly. In some patients, however, the manifestations and symptoms of general intoxication persist already for 8 years. All therapeutic methods proved little effective. A certain improvement was obtained by regular expression of comedones and sauna. In all patients secondary prevention was ensured, treatment and damages in accordance to legal provisions. O.

Cs. Derm., 48, 1973, č. 5, s. 306—317.

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was caused by some lead compound that formed on the chamber's lead walls as a result of a reaction with chlorine. Renon [35] also connected chloracne with lung tuberculosis in one of his patients and ascribed both diseases to gaseous chlorine.

However, Hirsch [20] concluded in that same year that the cause must be a solid substance which formed on the anodes during electrolysis because exposure to chlorine gas alone did not cause the disease. This finding was supported by Bettmann [2] who observed 21 patients who were engaged in the production of hydrochloric acid and who were in contact with chlorinated tar compounds but not with chlorine gas.

Lehmann [26], in 1903, concluded that the actual causes of th disease were chlorinated products such as hexachlorobenzene, pentachlorobenzoic acid and hexachloroethane which formed on the carbon anode during electrolysis. Because similar cases of acne chlorina were also observed in the production and processing of *p*-nitrochlorobenzene, W. Lehmann [27] concluded that acne chlorina was caused primarily by chlorobenzenes. As soon as carbon anodes were replaced by magnetite anodes, the incidence of acne during electrolysis ceased.

Before World War I, Wahle [46] observed acne chlorina in two chemists which was probably caused by a chlorine compound - phenyl dioxide - not identified or known in more detail at that time.

The next wave of the disease's incidence occurred at the end of World War I during the production of chlorinated naphthalenes or perchloronaphthalenes. In this period the disease was given an additional new name - Pernakrankheit (Wauer [42]). Chlorinated naphthalenes and diphenyls with a waxy consistency, also known as halowaxes, have some very valuable properties such as waterproofness, flame resistance and acid resistance. These properties carmarked these materials for the production of protective gas masks and other military equipment.

A new increase in the incidence of the disease occurred in the nineteen-twenties during the use of chlorinated naphthalenes

as an insulating material in the mining industry (Teleky [42]). Acne chlorina also appeared in the electrolytic production of chlorine when insulating coatings of tar were used. This fact brought about numerous disagreements and questions as to whether the disease in those production processes was not actually acne picea. On the other hand, Teleky supported the contention that the tar acne could be caused by chlorinated hydrocarbons contaminating the tar.

The excellent dielectric properties of the chlorinated naphthalenes ("haftax", "halowax", "nibrene") led to their wide utilization in the production of cables and condensers and anothe: mass occurrence of the disease was produced in the nineteen-thirt: when the U.S.A. and other countries recorded hundreds of stricken individuals. Holtzmann [22] also noted the occurrence of acne among the family members of workers employed in the production of condensers. These family members handled and washed work clothes contaminated with the chlorinated naphthalenes. Based on these observations, Holtzmann corrected his previous original conclusio: and stated that the chloracne was actually tar acne and judged the effect of halowaxes to be that of an external acnegenic cause.

Ormsby [31] observed acne chlorina with the use of a fungicic used in the impregnation of lumber (sodium tetrachloro-*o*-phenylphenolate). Jones and Alden [24], Schwartz [39] and later many others also observed total disorders in their patients and Flinn and Jarvik [8] noted a death due to yellow liver atrophy. In 193: Mayers and his coworkers [28] were unsuccessful in clarifying the mechanism of occurrence of dermatitides and acne chlorina in thei: patients with the aid of epicutaneous tests - the tests were negative.

During World War II, chlorinated naphthalenes were used for the impregnation of ships' hulls when it was discovered that thes substances could protect ships from damage of magnetic mines. Th use of halowaxes increased significantly, especially in the elect: cal industry. American authors gave acne chlorina another name,

"cable rash", because of the occupation (cable production) of the patients (Good and coworkers [14], Schwartz [40], Morris [29]). At that time, massive outbreaks of acne and several deaths were recorded. After World War II, the incidence of acne did not subside because the undesirable experiences with the chlorinated naphthalenes did not prevent their use in the electrical industry for some time. An interesting observation was recorded by Herzber [19] in 1947. He observed seven cases of acne chlorina in patient who had ingested chlorinated paraffins in the form of a food shortening. This prompted Herzberg to conclude that acne chlorina must occur after the internal ingestion of chlorinated hydrocarbons. Similarly, Fuchs [9] also noted the affliction of three children and their mothers after they had eaten potatoes fried in the "paraffin". Winkler [48] recorded 10 cases of dermatitis and 13 cases of acne chlorina among 23 workers in a condenser factory that used nibrene in the production sequence. From these cases Winkler concluded that "chlorinated hydrocarbons cause the formation of reagents that in turn cause an increased secretion from lubricating glands". In 1949 Gavrilova [10] warned of the photosensitization effect of polychloronaphthalenes. In recent years, plastics have replaced chlorinated naphthalenes in industrial use.

Even today the production of sodium hydroxide may be a source of acne chlorina. We have observed more cases between 1950 and 1965. The main causes of acne chlorina in these cases have been the combination of chlorine gas with the hydrocarbons in the tar gaskets and fittings on the electrolytic containers and tubs and with the mineral oils which were used to protect the electrode contacts. The authors noted several cases of acne chlorina among laboratory workers after the use of nibrene in the electrical industry in the 1950's.

Furthermore, the acnegenic effects of the mineral oil based coolant mixtures used in metal working increase when chlorinated hydrocarbons are added. Therefore, some cases of acne oleosa were observed which exhibited a partial acne chlorina character (more

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distinct follicular hyperkeratosis, less inflammation, cyst formation and atypical localization).

The last wave of the disease's occurrence was recorded in the 1950's. This outbreak was noted among persons engaged in the production of chlorinated phenol type insecticides and herbicides. From 1950 through 1951, Baader and Bauer [1] and Brinkmann [7] published information on the occurrence of acne chlorina which involved the complete internal, nervous and psychic symptoms among 17 workers engaged in the production of pentachlorophenol. Several years later, Hergt [17], Oettel [30] and Hoffmann [21] observed a total of 80 afflicted workers who were employed in the production of 2,4,5-trichlorophenol by the alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene. From 1954 through 1956, Kimmig and Schultz [25] recorded 31 cases of acne chlorina in one Hamburg (Germany) plant that produced 2,4,5-trichlorophenoxyacetic acid and its esters. By very detailed research and tests, these authors have shown that acne chlorina and total intoxication with damage to the internal organs, mainly to the liver, with psychovegetative disorders were not caused by the basic chlorinated hydrocarbons, i.e. tetrachlorobenzene, trichlorophenol and trichlorophenoxyacetic acid, but rather were caused by a ballast substance concentrated in these compounds in small quantities, namely, 2,3,6,7-tetrachlorodibenzodioxine. These authors contributed significantly with their other experimental works to the question of what substances cause acne chlorina and how these substances act.

In 1964, Bleiberg and coworkers [3] observed 29 cases of acne chlorina among persons engaged in the production of 2,4,5-trichlorophenol and 2,4-dichlorophenol. In eleven of these cases they also diagnosed symptoms of porphyria cutanea tarda. In 1957 Bowen and coworkers [4] observed acne chlorina among workers employed in the production of DDT from trichlorobenzene and hexachlorobenzene. The fates of 53 patients who in 1953 had worked in the alkaline hydrolysis of tetrachlorobenzene to trichlorophenol were described only last year by Goldmann [11]. In addition to acne, Goldmann also diagnosed in his patients bronchitides and

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disorders of the liver, kidney, myocardium and nervous system. The author described the death of a patient from necrosis of the pancreas as a special case. Furthermore, he also observed dermatitis in a nurse who had had contact only with experimental animals. Finally, he diagnosed acne chlorina in the fourteen year old son of an employee whose disease was contracted only from the household environment - by wearing his father's contaminated scarf and by using his father's towel.

Actual observations

The process called PCP (pentachlorophenol) which caused mass damage in Czechoslovakia was, as far as production is concerned, identical to the process from which Kimmig and Schulz drew their patients for observation. The final products of this process were sodium pentachlorophenolate, 2,4,5-tricholorphenoxyacetic acid and the latter's sodium salt and butyl ester. The primary raw material was technical grade trichlorobenzene which was produced in a neighboring building for the production of hexachlorocyclohexane (HCH) and lindane. The table shows the entire production scheme (Table I). The chlorination of trichlorobenzene produced tetrachlorobenzene and hexachlorobenzene. Sodium pentachlorophenolate was formed by the alkaline hydrolysis of hexachlorobenzene. Sodium trichlorophenolate was produced by the alkaline hydrolysis of tetrachlorobenzene. The hydrolysis of tetrachlorobenzene with sodium hydroxide in the presence of methanol took place in an autoclave at 190°C and at 45 atm of pressure for one hour. After cooling, the methanol was distilled from the hydrolysate, the hydrolysate was diluted with water to a 25% concentration and it was then syphoned into a storage tank. The condensation of sodium trichlorophenolate with monochloroaceti acid produced sodium trichlorophenoxyacetate. Following cooling, centrifugation and flushing with water, the dried sodium trichlorc phenoxyacetate was picked out by hand and placed in small barrels. Some of this product was used to produce the butyl ester of

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trichlorophenoxyacetic acid. This production took place in another building.

The production of both the primary products took place in a four story building with grated floors (WEMA grates) which was one reason why the entire building was contaminated with the high risk substances. The building was not sufficiently airtight, the local exhausts were in most cases improperly installed and hermetization and mechanization were insufficient. Initially, th sodium tetrachlorophenolate [sic] was escaping from the centrifuge into the work space in the form of a mist. A series of tasks such as the removal of solid substances and the pumping of liquids were performed by hand. The workers were dressed in linen work clothes. The thorough cleaning and frequent changing of the work clothing was impossible to guarantee over the entire interval of production. Rubber gloves and respirators were used at some work sites. The conditions surrounding the production of the butyl ester of trichlorophenoxyacetic acid were the same as those described above.

Kimmig and Schulz [25] found that the main source of acnegenic hydrocarbons was that segment of the production process in which sodium trichlorophenolate is formed by the alkaline hydrolysis of tetrachlorobenzene in the presence of methanol. A coworker of these authors, Doctor (of Chemistry) Sorge, had deduced on theoretical grounds that if this reaction were conducted at 190°C and 45 atm of pressure, chlorinated hydrocarbon by-products such as polychlorodibenzo-p-dioxines, polychlorodibenzofurans, polychlorodiphenyl ethers and polychlorodiphenyl oxides may be formed in small amounts (Schulz [36]). Sorge also prepared some of these substances by his synthetic process.

Animal tests carried out by the authors (Schulz and Kimmig) with tetrachlorodibenzofuran and 2,3,6,7-tetrachlorodibenzodioxine have proved an extraordinarily high toxicity for these substances. A simple smear of a 0.1% solution of tri- and tetrachlorodibenzofuran on a small area of a rabbit's auricle caused heavy liver

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damage and death of the test animal within 2 to 3 weeks (Schulz [36]). 2,3,6,7-Tetrachlorodibenzodioxine, which also has very strong acnegenic effects, was proved to be far more toxic. Only then was Dr. Sorge successful in the identification of this enormously toxic substance in the production process and in the final product. He proved that this toxic substance is formed only at certain temperatures and pressures. If during the alkaline hydrolysis of tetrachlorobenzene to sodium trichlorophenolate the temperature is reduced below 153°C, the toxic substances do not form. However, at this low temperature, the reaction time is extended from one to five hours and the productivity is obviously lower. Furthermore, it was proved that the condensation of two molecules of trichlorophenolate to give tetrachlorodibenzodioxine is an exothermic reaction. Several explosions of autoclaves with injuries and the lethal intoxication of workers were reported to be the result of this reaction in the German Democratic Republic (East Germany) (Schulz [38], Goldmann [11,12]). Tetrachlorodibenzodioxine was also identified in the Czechoslovakian production process and in the products as well.

The first two cases of acne chlorina were noted in 1965 in two technicians who operated the production machinery. It was assumed at that time that these two cases were caused by careless work and an undeveloped production technology under semiproductio: conditions. After one year without a reported case and during which production was temporarily halted, a mass outbreak of 78 cases was recorded within the following three years. Finally, after a detailed investigation and study of the entire problem, the process was terminated and production ceased in 1968. Worker: from all stages of production were stricken. At that time, it was impossible to determine which production stage was the most risky because most of the workers were involved in several stages at various locations in the plant within a relatively short time. Workers directly involved in production as well as maintenance personnel and workers digging sewage ditches were stricken. Later, a research scientist working to isolate the acnegenic ballast

substances from the products of the individual production phases was even stricken. His research work was carried out outside the production plant in a research institute's laboratory. In addition to the 78 patients with dangerous symptoms of acne chlorina or porphyria, other workers in this plant had symptoms of acne which were, however, symptoms of juvenile acne or of a similar character and could not therefore be counted as an occupational disease.

With the exception of two female laboratory technicians, the patients consisted of males from 18 to 57 years of age (4 persons below 20, 46 persons from 21 to 30, 11 persons from 31 to 40, 10 persons from 41-50 and 7 persons from 51 to 57). Therefore, the acne chlorina patients were mostly young people. On the other hand, more than half the patients with porphyria cutanea tarda were older than 40 years.

Generally, the disease followed a normal course as described by other authors. In only six patients did the disease begin suddenly as acute solar dermatitis, i.e., by erythrema and edema in regions exposed to solar radiation and continued by acne symptoms (Photographs 1 and 2), as observed by Goldmann [12], Grimmer [15], Braun [6] and before these authors, also by Touraine and coworkers [45]. Therefore, chlorinated phenols in some cases have similar photodynamic effects as the chloronaphthalenes described by Gavrilova [10]. Erythema and edema in these patients were obvious mainly on their auricles. Sometimes, small blisters in thick groupings were observed there.

In all the other patients the acne developed slowly. Among the less severe cases the disease was limited to single or densely grouped comedones, conditoned by follicular hyperkeratosis, which were so small and sometimes so numerous that they appeared to be a slate grayish-brown color, like dirty spots on the skin. These symptoms were noted mainly on the face, with maximum occurrence above the cheek bones (Photograph 3). In the more severe cases, the comedones gradually developed into small white cysts similar

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to milia, although with black centers, and into large cysts ranging from the size of a pea to that of a nut (Photographs 4, 5 and 6). As opposed to other acne forming dermatoses, the described symptoms and manifestations mainly lacked signs of an infection. Only in the more severe cases and rather in the vicinity of large cysts were some reactive inflammations, infections and abscesses noted. The emptied cysts and abscesses left behind large atrophied scars with raised edges and with bridges of intact skin under which a probe could be inserted (Photograph 7). This basic clinical picture was supplemented by diffused hyperpigmentation in the face and by papulopustules on the torso and extremities. Patients who were found to suffer from a disorder in porphyrin metabolism had symptoms of porphyria cutanea tarda in regions exposed to the sun (hyperpigmentation and hypertrichosis, blisters, excoriation and small scars). However, in this group there were two patients with prophyria but almost without any acne at all.

Histological investigation is not much help in diagnosis. The symptoms begin with hyperkeratosis at the mouth of the follicl After one to three weeks, the follicle changes into a cystic formation filled with a horny substance [keratin?] (Photograph 11) and to a small extent with sebum. However, the epidermal changes are small. After the rupture of the full follicle, the contents of the cyst penetrate into the surrounding tissue and a granuloma of foreign bodies forms around the area (Photograph 12).

The disease normally starts on the face above the cheek bones However, in 17 patients the disease started in an unconventional manner by papulopustular eruptions on the extremities, primarily on the lower extremities. This characteristic, resembling more acne oleosa, remained in some of the patients for the entire duration of their illness.

The most common localizations of acne chlorina were the cheek temples and to a lesser extent, the entire face. Eyebrows, eyelids, the perioral region and the scalp and hair area remained fre

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Other predilected areas were the auricles, the back of the neck, the back and the chest in a seborrheic localization and the genitals. The auricles and genitals and especially the scrotum were stricken by the formation of large cysts (Photographs 8 and 9 Among the five most seriously ill patients, the disease spread all over the body and had a discouraging effect that was heightene by the disease's usual persistence, the long term healing and by medical helplessness (Photographs 10 and 7). The contents of the cysts produced a repulsive and rancid odor. For some of the patients the disease disrupted their social and family relations and caused severe depressions.

Some authors, such as Braun [6], emphasize itching as the initial symptom, but we noted this symptom in only three cases.

In 20 patients with extensive symptoms, the occurrence of pustules, cysts and abscesses was also noted in axillae although this location was not mentioned by other authors. Because of the great number of follicles in the axillae and the similarity of the axillary skin to that of the genitals and for several other reasons, this finding appears to be just as valid as localization on the genitals and the genital area. On the other hand, it is surprising that acne chlorina does not strike at the hairline or scalp. In the case of one patient, the acne symptoms disappeared in the facial areas when he grew a full beard while in other areas the symptoms continued without a reduction in intensity. So far there is no explanation for this phenomenon.

Hypertrichosis or hyperpigmentation or both were determined in the faces of 19 patients without a laboratory diagnosis of a deficiency in porphyrin metabolism. Bleiberg [3] made similar observations. These symptoms receded simultaneously with the healing of acne chlorina.

~~More younger persons suffered from acne chlorina than older persons~~.* This study group contained 50 persons (85%) below 30

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*~~Translator's note: Beginning of this sentence illegible.~~

*Many authors believe that younger persons have a greater tendency to develop acne chlorina than older individuals. (Correction by NIH Translation Un

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years of age. However, this fact cannot be used as a hard and fast rule or to support the findings because the production plant under study employed mostly young workers. The preillness medical history among the patients did not indicate any significant predisposing factors and no incidence of serious illnesses, intoxication or ~~etiolation~~*. Furthermore, no relation to hair color, eye or complexion color, to the degree of body hair, to the perspiration of skin oils or to hereditary follicular hyperkeratosis and other skin diseases was noted. Only one patient reported a more severe prior case of acne juvenilis, 12 patients reported mil cases during puberty and 14 patients reported only insignificant acne juvenilis symptoms. 18 patients also had some infrequent contact with mineral oils either during the appearance of the symptom or at some time in the past. These patients were mostly involved in maintenance work as mechanics. However none of them had ever had an oil acne in the past. Their illnesses following contact with tetrachlorodibenzodioxine were of the same character as the illnesses of the other patients. Their diseases were not similar to acne oleosa either in appearance or in localization.

The latent period from the initial contact with the risk substance to the appearnace of symptoms was quite varied. Some workers became ill after exposure of from several months to severa years and the disease in their cases was rather mild although the indications pointed to a massive exposure. In other cases, severe damage occurred after a very short time of several weeks and after an apparently small exposure. For example, one of the most seriou ill patients worked under the semiproduction conditions for only two and a half weeks before the outbreak of the disease (acne and porphyria cutanea tarda). After that he had no contact with the risk materials.

It is not clear whether individual predisposition or the leve of personal and work hygiene is more responsible for the intensity

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~~*Editor's note: This may be a dubious choice. The Czech "etylism" transliterates to "etilism".~~

*alcoholism (Correction by NIH Translation Unit)

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of damage or latency of the disease. Most authors discount individual predispositon. However it is believed that the deciding factor must be the degree of actual exposure which is undoubtedly dependent on a series of other factors such as work discipline, personal discipline, adherence to preventive measures, frequency of changing work clothes and undergarments, the degree and regularity of hygienic decontamination, work site order and cleanliness, dust in the environment, etc.

These patients also had a series of subjective difficulties: tiredness, weakness in the lower extremities, muscle pains, sleepiness and insomnia, increased perspiration, lack of apetite, headaches and other disorders in the mental and sexual spheres. These complaints were more recurrent and more intense in patients with the more extensive skin symptoms. In more serious acne cases, a significant weight loss was also noted. It is our opinion that al these difficulties and symptoms are manifestations of a rather overall intoxication than of only the acne chlorina.

All the patients were admitted to a dispensary. Regular examinations of their dermatological, internal and neurological conditons were arranged. The patients who were still working were transferred to prevent further contact with any acnegenic factor. All contact with chlorinated hydrocarbons of any type was prevente even with those substances whose acengenic effects have not been thoroughly proved. Furthermore, all contact with mineral oils and tar and its products were prevented. The application of mineral based ointments was discounted for external therapy. The plant's medical doctors were cautioned against prescription of any interna medication with an acnegenic effect (iodine and bromium preparatio corticoids and others). The patients were given detailed medical directions concerning their daily regimen, personal and work hygiene and secondary disease prevention and treatment.

All available medications for acne were utilized in the treatment. The expressions of comedones and the pressure relief of the contents of cysts and abscesses necessitated many periods of

hospitalization and special ambulatory treatment hours attended by a specially trained nurse. No special results were observed as a result of repeated hospitalization. The best results were noted after the expression of comedones by the use of a sauna constructed for the patients by the factory on the author's recommendations. It was not possible to test the effects of vitamin A acid (retinoic acid) which has been praised by some authors in the medical literature. However, in agreement with the findings of other authors, it was determined that no local or internal medication exists which has an effective and permanent healing effect on acne chlorina. Some of the patients have been under observation for eight years and their disease still persists. Dr. Schulz's [38] patients have manifested continuous symptoms for more than ten years. Goldmann [11] has now been treating one patient with comedones and cysts on his penis and scrotum for 18 years! In most of the patients discussed by these authors, the symptoms and manifestations of the disease progressed and reached a maximum after about one year, even after transfer of the patient from the high risk work area. A regression takes place after two or three years at the earliest. In some isolated cases, the progress of a mild course of the disease was observed even after five years. However, the regression of the disease in all the patients was very slow and, it may be said, even lasts a number of years.

Of the 78 patients treated by the authors, 76 suffered from acne chlorina. Of these, 11 simultaneously had hepatic lesions with a deficiency in porphyrin metabolism. Two patients did not have acne chlorina. One of these two had porphyria and the second one died in 1966 from acute intoxication. Pentachlorophenol was considered to be the cause of death in that instance. When the toxicological analysis was carried out at that time, the investigators were unaware of tetrachlorodibenzodioxine and, therefore, it is our contention now that in that case, this highly toxic substance was primarily responsible for the acute intoxication.

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Gombos [13] mentioned one patient with acne chlorina and porphyria in connection with this production process.

The authors analyzed 55 patients internally and neurologically in detail. About half of these patients had a deficiency in lipid metabolism and in more than one-third, some minor biochemical deviations and mild hepatic lesions were noted in the first stages of the disease. In 17 patients, the authors found symptoms of central nervous system disorder, the majority with lesions of the peripheral neurons of the lower extremities (verified by EMG examination). In most of the patients, physical disorders, primarily an acute neurasthenic syndrome, were diagnosed. In addition to the death due to acute intoxication (pentachlorophenol? tetrachlorodibenzodioxine?), three more patients died. In one patient, the porphyria developed unusually rapidly into an arteriosclerosis of the brain with an atypical morphological diagnosis and demention. Two patients died from bronchogenic carcinom All these patients with their complete symptoms are discussed in other papers [32,23].

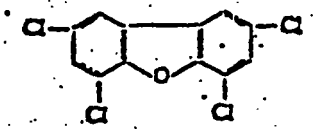
Discussion

In agreement with Kimmig and Schulz, we cite the tetrachlorodibenzodioxine which is formed during the production process as the main reason for the skin symptoms as well as for all the other deficiencies mentioned and diagnosed in the authors' patients. This toxic compound was confirmed in considerable quantities in the final product (Arboricide E50), in the butyl trichlorophenoxyacetate and also in the mortar and wall paint of the factory building. Even adjacent rooms such as worker dressing and locker rooms were contaminated with this compound. Test rabbits placed into these rooms soon died.

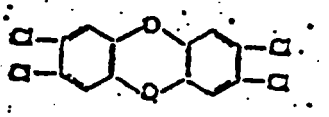
In his monograph, Braun [5] cited the following chlorinated hydrocarbons as the then known causes of acne chlorina: chlorobenzenes with various degrees of chlorination, chlorinated phenols (sodium tetrachlorophenolate, pentachlorophenol), *para*-nitrochloro benzene, sodium tetrachloro-*o*-phenylphenolate, chlorinated

naphthalenes, various chlorinated diphenyls, trichloroxydiphenyl, chlorinated diphenyl oxides, sodium o-(2-chlorophenyl)phenolate and others. However, Kimmit and Schulz [25] ascertained that pure pentachlorophenol and pure 2,4,5-trichlorophenoxyacetic acid do not have any acnegenic effects. Therefore, they checked the presumed effects of the other substances that were blamed for causing acne and found that neither pure nor chlorinated benzenes (from 1 to 8 chlorinated positions) nor chlorinated diphenyl-ethers have any acnegenic effects. A definite acnegenic effect was proved only for the following substances: chlorinated naphthalenes (5 and 6 chlorinated positions) in agreement with Shelley and Klingman [41], higher chlorinated dibenzofurans (diphenyl oxide and 2,3,6,7-tetrachlorodibenzodioxine.

Therefore, it is possible and even probable that some chlorinated hydrocarbons that have been previously considered to have an acnegenic effect do not have this effect and the actual causes were other ballast chlorinated hydrocarbons contained in those hydrocarbons.



Tetrachlorodiphenylene oxide (tetrachlorodibenzofuran)



2,3,6,7-tetrachlorodibenzo-dioxine (tetrachlorodiphen dioxide)

In an animal experiment, Kimmig and Schulz found an exceptionally high toxicity for tetrachlorodibenzodioxine. In a local application on rabbits' auricles (keratosis test), this compound produced a strong reaction even at a concentration of 0.001%. Also, a skin application of this substance at the same concentration produced acne chlorina (Schulz [38]). Tetrachlorodibenzofura was proved to be 10 to 20 times weaker. Chlorinated naphthalenes had a thousand times weaker effect. Generally, acne chlorina is caused by the direct contact of tetrachlorobenzodioxine with the skin. Absorption through the skin is not ruled out and an effect

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through the ingestive or respiration tracts is also conceded. However, in a pure ingestive experiment with a test animal, acne chlorina did not occur. However, the cases of acne chlorina contracted after the ingestion of chlorinated naphthalenes as food fat substitutes should not be forgotten [19,9].

Tetrachlorodibenzodioxine used internally has a strong hepatotoxic effect. A dose of 20 to 50 mg per kg of rabbit's body weight causes death in all the test animals by yellow liver atroph

Because of this enormous toxicity, all the preventive measure taken at the high risk processes in East Germany and Czechoslovakia were proved to be completely ineffective. The only dependable solution to that problem was the reduction of the reaction temperature and pressure during the alkaline hydrolysis of tetrachlorophenolate to sodium trichlorophenolate. In East Germany, the decontamination cost of the high risk areas and buildings was extremely high. One chemical plant attempted to use for other purposes a contaminated building which had not been decontaminated after the building had remained unused for two years, but new cases of acne chlorina were shortly reported. With normal decontamination procedures new outbreaks of the diseas occurred and therefore, decontamination had to be carried out usin special pressurized chemical protective suits with pressurized breathing apparatus because gas masks were not sufficient. Doors, windows, the wooden trim and other wooden building components had to be burned; the steel construction materials in the building had to be sandblasted; the plaster, facade and floors had to be torn out and cast into cement blocks together with the tubing, plumbing and other equipment. These blocks were then transported to the Atlantic Ocean and sunk. The disposition of those nonflammable materials by burying would have run the danger of contaminating the subterranean water supply. Only the most expensive components, autoclaves and some valves, were successfully decontaminated by repeated washing with special detergents.

The Czechoslovakian plants are still closed and their fate is still undecided. The patients are still under a continuous observation.

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[13] Gombos, et al., Porphyris cutanea tarda and acne chlorina occurring during the production of cyclic chlorinated hydrocarbons.

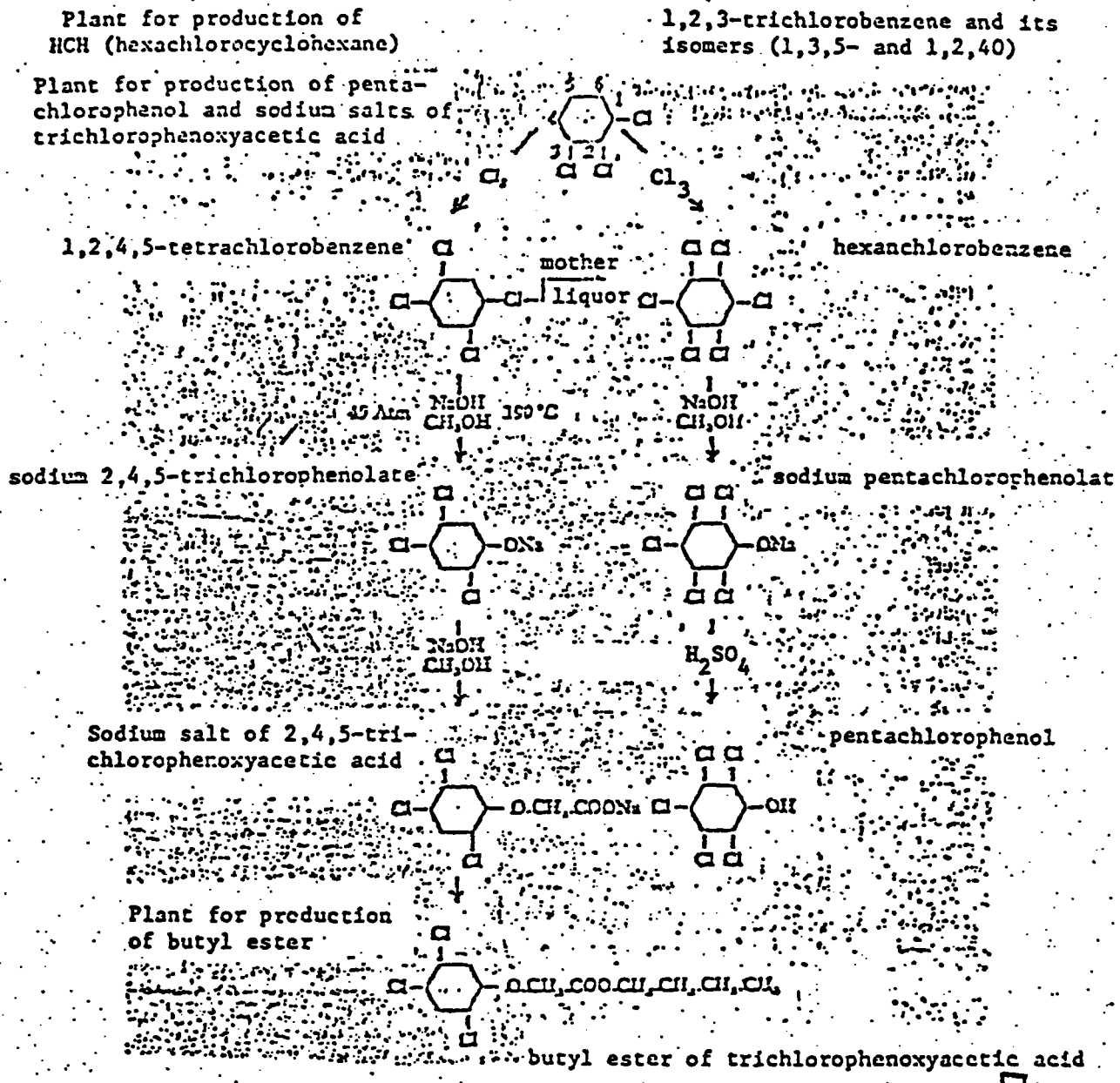
[23] Symptoms of total intoxication in acne chlorina during the production of herbicides - in final preparation for printing.

[31] Discussion on paper by Jones [24]

[32] Health hazards to workers in production of sodium 2,4,5-trichlorophenoxyacetate.

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TABLE I. Production Scheme



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Dioxin: Position Document

Dioxin Working Group
U.S. Environmental Protection Agency

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Dioxin: Position Document

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 DRAFT 26 APR 1977

I. Background

A. Chemical and Physical Properties of TCDD

1. Synthesis

TCDD is not found naturally in the environment, its source comes as a by-product in the manufacturing process of several chemical products.

Although there are several synthetic routes for the formation of TCDD, the more common reaction occurs under conditions which are used to prepare 2,4,5-trichlorophenol, tetrachlorobenzene being the precursor compound. Under extreme saponification reactions, 2,4,5-trichlorophenol is formed. This reaction was first established by Schwetz, et al. (1). This means that TCDD or other chlorodibenzo-p-dioxins will always be present in trichlorophenol products and/or their derivatives, and that any use of these products will result in environmental contamination by dioxins.

2. Environmental Characteristics

Basically there are three ways in which TCDD may be introduced into the environment: (1) it may be present as a contaminant in chlorophenols or their derivatives, (2) it may be formed from chlorophenols or their monomeric chlorophenoxy derivatives under conditions of use or storage, or (3) it may be formed from phenoxyphenols or other polymeric derivatives under similar conditions. In those cases where TCDD residues have been found in environmental samples, it is not known at present which of these routes is responsible. Kearney's, et al. (2) study of the fate of TCDD in the environment, however, has resulted in the following observations:

1). TCDD does not leach vertically in soils;

2). Significant amounts of TCDD are not taken up by plants and none could be harvested in grain or soybeans;

3). TCDD disappears slowly from soils and about half is lost after one year. It is less persistent than most chlorinated hydrocarbon insecticides, but more persistent than 2,4,5-T;

4). TCDD is not translocated from the point of application on the leaf surface to other parts of the plant. Some of it is washed off with rain water;

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5). TCDD destruction may be caused by sunlight in water, but not on soil surfaces;

6). TCDD is not made from breakdown products of 2,4,5-T in soils or in sunlight;

7). Although there are some residues in the liver, large amounts of TCDD fed in an animal's diet can be eliminated in the urine and feces;

8). TCDD was accumulated from water by fish in laboratory studies.

Further information on the persistence and mobility of TCDD in the environment will be available in late 1977 as a result of an interagency research agreement between EPA and USDA. The research being conducted at the USDA-ARS Degradation Laboratory is designed to provide data on the fate of TCDD when Silvex is applied in simulated home and recreational turf plots. Dr. Kearney is the Laboratory leader for the investigation.

Since TCDD is likely to be formed in the preparation of 2,4,5-trichlorophenol and since this compound is common to the manufacturing process of the pesticides 2,4,5-T, Silvex, Erbon, Ronnel and 2,4,5-trichlorophenol, all four compounds were included in the dioxin monitoring program as possible sources of TCDD contamination.

B. 2,4,5-T Litigation History

On October 29, 1969, the President's Science Advisor announced that a series of coordinated actions was being taken by several governmental agencies to restrict the use of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). This was precipitated by the release of a screening study conducted by the Bionetics Research Laboratories in which it was found that mice and rats treated during early pregnancy with large doses of 2,4,5-T gave birth to defective offspring.

The announcement, together with reports of an increased occurrence of birth defects by ~~South~~ Vietnamese newspapers (June and July 1969), elicited immediate reactions from governmental agencies, segments of the scientific community, various lay groups concerned with environmental problems, and from public communications media. Government-sponsored panels of experts, special commissions set up by scientific organizations, hearings before subcommittees of the U.S. Senate, and conferences attended by representatives from industry, government, and academia examined available data and heard expert opinions. None of these groups, however, were able to conclusively resolve the central question of whether 2,4,5-T constituted a risk for human fetuses during pregnancy as currently produced and used. At least one reason for failure to reach a satisfactory resolution of the issue was the paucity of reliable, scientific evidence.

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Additional animal experiments performed early in 1970 confirmed that the purest available sample of 2,4,5-T given in large doses to pregnant mice, did indeed result in the birth of malformed offspring (3,4). It was later reported (5,6) that the 2,4,5-T formulation employed in these studies contained 30ug/g of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD), one of the most toxic and potent teratogenic substances known (1,7). On April 14, 1970, precautionary actions were taken by the Secretary of Health Education and Welfare, who advised the Secretary of Agriculture that: "In spite of the uncertainties of the experimental data related to 2,4,5-T, the Surgeon General feels that a prudent course of action must be based on the decision that exposure to this herbicide may present an imminent hazard to women of child bearing age." Accordingly, on the following day the Secetaries of Agriculture, of Health, Education, and Welfare and of the Interior jointly announced the suspension of the registrations of 2,4,5-T for: "I. In all uses in lakes, ponds or on ditch banks. II. Liquid formulations for use around the home, recreation areas and similar sites" (USDA-PRD PR 70-1, 20 April, 1970). A notice for cancellation of registration was issued on May 1, 1970 for: "I. All granular 2,4,5-T formulations for use around the home, recreation areas and similar sites. II. All 2,4,5-T uses on crops intended for human consumption." (USDA-PRD PR 70-13, May 1, 1970) All registrants were advised of these actions, and two of the registrants, Dow Chemical and Hercules Incorporated, exercised their right under Section 4.e of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) [7 U.S.C. 135 et seq.] to petition for referral of the matter (for the cancellation of rice only) to an Advisory Committee.

As provided by that statute (8), the National Academy of Sciences supplied a list from which was selected a nine-member Advisory Committee of scientists with appropriate qualifications from universities and research institutes throughout the county. The charge given to the Committee was to: (a) consider all relevant facts, (b) submit a report and recommendations regarding registration for certain uses of 2,4,5-T, and (c) state the reasons or bases for these recommendations. The Report from the Committee was submitted to the Administrator of the Environmental Protection Agency on May 7, 1971 (9). The report recommended that 2,4,5-T may be permitted under certain conditions for uses in forestry, range and rights-of-way providing:

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1. That the limit of 0.1 ppm of contamination with TCDD be set for all future production of 2,4,5-T.

2. That 2,4,5-T be applied no more than once a year at any one site.

3. That 2,4,5-T be applied with proper caution so that it will not contaminate other areas where it may come into human contact. The Committee also recommended that this action be reviewed again when the existing deficiencies in information relative to possible magnification in the food chain of TCDD have been rectified by specific research directed toward that end.

In July 1972, Dow Chemical obtained an injunction against EPA enjoining further administrative action against 2,4,5-T. In 1973, the United States Court of Appeals for the Eighth Circuit overturned the injunction and administrative proceedings were allowed to go forward (10). Accordingly, a notice of intent to hold Public Hearings on all uses of 2,4,5-T brush-weed killer was filed with the EPA Hearing Clerk on July 20, 1973, under Section 6(b)(2) of FIFRA. All federally-approved uses of the controversial brush-and-weed killer, 2,4,5-T, alleged to have caused adverse effects on human and animal health, were to be explored in a public hearing scheduled for April 1974, following completion of an intensive monitoring program for detecting dioxin in the parts per trillion (ppt) range (38 FR 19869, July 24, 1973). The hearing was to afford ~~to~~ all concerned parties--manufacturers and formulators, the U.S. Department of Agriculture (USDA), the Environmental Defense Fund (EDF), and other interested groups as well as the EPA--the opportunity to present pertinent scientific, economic, and other relevant data needed by EPA to weigh the benefits of the herbicide against the risks involved.

The basic question to be resolved by EPA through the hearing process was whether the remaining Federal registrations of 2,4,5-T should be cancelled or the classification changed.

During the preparation of the hearings, it was evident that the pesticide 2,4,5-T, per-se, posed no special cause for concern, when used as directed.*

* Note: 2,4,5-T, 2,4,5-TCP, Silvex, Erbon, and Ronnel are now under review in the Office of Special Pesticide Reviews (OSPR), EPA, as candidates for Rebuttable Presumption Against Reregistration (RPAR). The toxicity of these pesticides and/or the associated dioxin contaminant are considered in this review process.

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Residue monitoring analyses for 2,4,5-T in environmental samples, including food, have for the most part led to negative findings. In those cases where positive results were obtained, levels were low (normally less than 0.1 ppm). This conclusion was based primarily on the results of an EPA forced feeding study of the levels of 2,4,5-T in cows milk and calf tissue, following exposure. These observations, coupled to the low order of teratogenicity for 2,4,5-T when tested at the highest purity available, lead to the conclusion that human exposure to 2,4,5-T from normal usage posed no discernible problem.

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On May 10, 1974, the information hearing was expanded to include all insecticides and herbicides having in their manufacturing process 2,4,5-trichlorophenol (TCP). These include the pesticides Silvex, Erbon, Ronnel and 2,4,5-TCP all of which have the potential of containing TCDD.

On July 24, 1974, the Agency withdrew cancellation and information gathering proceedings initiated against the herbicide 2,4,5-T and related compounds. The proceeding was withdrawn because of the inability of the Agency to monitor food for residues of 2,4,5-T's highly toxic contaminant TCDD with the necessary analytical precision. The lack of evidence that 2,4,5-T use results in exposure of man to TCDD made a final determination on the "unreasonable adverse effects" caused by the use of 2,4,5-T extremely difficult, if not impossible. Accordingly, while the 2,4,5-T notice of hearing was withdrawn, the Agency stated that it:

> "will continue its TCDD residue monitoring program and will take such further action as it deems appropriate once the results of the monitoring project are available." 39 FR 24050 June 28, 1974.

On July 25-26, 1974 the Agency held a Dioxin Planning Conference in Washington, D.C. The public meeting was held primarily for those parties having an interest in the withdrawn 2,4,5-T/dioxin hearings. The nature of the meeting was to address data analysis and retreival (in the areas of analytical methodology, toxicology and monitoring) with emphasis on analytical methodology for TCDD at the parts per trillion level (ppt). As a result, the Agency promulgated the Dioxin Implementation Plan which is designed to develop the required analytical methods for the determination of dioxin residues in environmental samples.

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II. Dioxin Implementation Plan

A. Introduction

In September 1974, the Agency sent out a Preliminary Dioxin Implementation Plan (DIP) to all attendees of the Planning Conference requesting that it be reviewed and that they provide input on what changes or additions would be advisable in the overall plan. After these responses were received, a final plan was prepared in February 1975. It consists of two parts, a short term monitoring program and a broad research plan which would take four to five years to complete. The EDF, USDA and Dow Chemical agreed to participate in a monitoring program established by EPA, which would utilize improved analytical methodology capable of TCDD detection at the ppt level.

The short term beef fat monitoring plan was a joint plan (representatives from EPA, USDA, EDF and Dow Chemical) in which beef fat and liver samples were to be collected at six month intervals to permit timely reevaluation of any available evidence for TCDD residues. Such reevaluation could lead to (1) modifications in plans for future research and monitoring and/or (2) reconsideration of the desirability of initiating some form of regulatory action on 2,4,5-T or other pesticides contaminated by TCDD. At each point all of the information then available should be considered even though the specific experiment producing it might not be completed. The guiding principles for the sampling plan were:

a. The samples should be representative of beef actually being consumed by some segment of the population.

b. The sample should represent cattle likely to be marketed for human consumption and grazed on lands treated with 2,4,5-T and thus likely to maximize the probability of containing 2,4,5-T (or TCDD).

Between February and March, 1975, the first 85 beef fat and 43 liver samples were collected. At the outset it was decided that emphasis would be placed on analytical methodology. Due to the complexity of the analytical technique to determine TCDD at the ppt level, analysis for these first 128 samples were delayed and not completed until May 1976. Approximately twenty-five percent of these samples was taken from non-treated areas, i.e., where 2,4,5-T or TCDD is not likely to be found. One laboratory prepared all sample extracts, and identical aliquots were sent to all participating analytical laboratories. The purpose of having one laboratory perform all cleanup of samples was to minimize possible errors in the procedure.

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B. Analytical

1. Phase I Beef Fat and Beef Liver Anaylyses

The primary analytical achievements of 1976 have been the; 1) completion of phase I beef fat and liver analyses using combined gas chromatographic (GC) and using high resolution, mass spectrometric (MS) techniques; 2) analysis of technical grade pesticides for TCDD residues; 3) further refinement of cleanup methodology for environmental samples; and 4) the development of a preliminary method for the extraction analysis of human tissues and milk for TCDD residues.

The progress made in the area of TCDD analytical methodology is noteworthy. By far the most significant data out of the above is the phase I beef and liver analyses. Based on the repeated ability of more than 1 laboratory to detect TCDD in identical sample aliquots, it is clear that dioxin can be detected at the ppt level. The data as reported by the collaborators at the June 15, 1976 meeting in Washington (see section V Appendix part A) are suggestive of TCDD residues in beef fat ranging from 20-60 ppt.

Phase I of the program was primarily intended to identify acceptable methods for extraction and analysis of TCDD in environmental samples. This phase of the program is essentially complete. A short term study (90 days) will be conducted, prior to initiation of phase II, to determine the optimal combination of extraction and analytical methods. Once this combination has been determined, the Agency will immediately proceed with the second phase of the program. Phase II will proceed under an experimental design intended to insure the accuracy and legal sufficiency of the analytical data being developed (see section III Future Analytical Studies).

2. Survey of Trichlorophenol Pesticides for TCDD Residues.

Samples of trichlorophenol materials were received from the basic manufacturers of pesticides for analysis of TCDD content by EPA. The limit of detection in the analysis was 0.1 ppm, corresponding to the 0.1 ppm limit for TCDD contamination of 2,4,5-T, as set by the 2,4,5-T Advisory Committee (NAS) in 1970. Seventy-three out of the 75 samples collected did not contain TCDD at the above limit of detection. The remaining 2 samples, Fonnel and Na-2,4,5-TCphenate are believed to contain 0.107 and 0.312 ppm of TCDD respectively. These samples will be reanalyzed for confirmation. If the residue level continues to exceed 0.1 ppm, the Agency will take regulatory action to insure that TCDD is reduced to an acceptable level.

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only in 3.5% of the 85 samples of beef fat (all from one area all with known exposure to 2,4,5-T

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(0.01 ppm)

A second study of the TCDD residues in technical pesticides will begin in mid-1977. The limit of detection in this study will be 10ppb. Samples for this study will be taken by representatives from Pesticide Toxic Substances Enforcement Division, EPA. The data will provide EPA with a more comprenhensive analysis of the intrinsic level of TCDD residues in the pesticides under consideration, prior to use.

C. Toxicological Effect Level

The TCDD toxicology data is well documented in the EPA conference report of January, 1976 and in the May, 1976 addendum and therefore does not need to be reviewed extensively herein. Additional data however have been generated which expand our knowledge of the risks associated with human and nontarget exposure to TCDD. Dow Chemical Company has recently completed a two-year feeding study with 2,3,7,8-TCDD in male and female rats. Preliminary data indicate that at a level of 0.001 ug/kg/day of TCDD (which approximates 20 ppt TCDD in the rat's daily diet) the effect was similar to controls in all parameters measured. At the dose of 0.01 ug/kg/day however increased urinary excretion of porphyrin and increased liver weights were observed (personal communication, Dr. Koziba, Dow Chemical Co.). Toxicologists from the Criteria and Evaluation Division (CED), OPP, EPA have reviewed this data and established a range of 80 to 200 ppt as the effect level (EL) for human exposure to beef fat containing TCDD. To determine the EL it was assumed that the amount of TCDD in the beef was constant and that the amount of beef fat ingested is from 1 to 2.5% of a humans total diet.

The data generated thus far for the 85 beef fat and 43 liver samples analyzed in phase I of the monitoring program do not indicate that the EL for TCDD exposure through beef fat ingestion has been exceeded or reached. It is emphasized however that exposure to TCDD through ingestion of beef fat is only one possible source of exposure. The environmental samples scheduled for analysis in phase II of the Dioxin Implementation Plan range from soil to human types. The diversity of these samples is intended to provide EPA with a better understanding of the potential for TCDD to bioaccumulate once it is released into the environment and thus present multiple sources of exposure to humans or other non-target organisms.

as to rats soil?

III. Future Analytical Studies

Phase II of the Dioxin Implementation Plan is designed to provide the Agency with precise analytical data on the residues of TCDD in environmental samples.

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The purpose of the design is to establish a practicable expermental procedure that (a) will permit development of a "best fit" recovery curve (or curves) for quantification of 35 Cℓ-TCDD spiked in beef fat and liver at levels ranging from 0 to 100 ppt, and (b) will allow statistically reliable measurements of the following parameters:

1. The accuracy and precision with which ppt levels of 35 Cℓ-TCDD added to beef fat and liver can be extracted at OPP's Pesticide Monitoring Laboratory and quantified at the collaborative analytical laboratories;

2. The accuracy and precision with which these analytical laboratories can quantify ppt levels of 35 Cℓ-TCDD provided as standards;

3. The relationships between measurements of TCDD in standards and measurements of TCDD extracted from beef fat and liver spiked at the same levels as the standards;

4. The consistency of laboratory performance throughout the experiments, as measured by the relative magnitude of statistical interactions;

5. The precision of PML's extraction procedure relative to the precision of the GC-MS quantitation.

Based on the ability of the collaborator to detect TCDD in spiked samples, an evaluation can be made of the validity of the TCDD levels reported in blind environmental samples. It is anticipated that the above procedure can be applied to all environmental samples scheduled for analysis on phase II. Highest priority however will be given to the analysis of phase II beef (fat and liver) and human (fat, liver, and milk) samples.

The Agency presently has a total of 167 human samples (milk, liver, adipose) in storage at our Pesticide Monitoring Facility, Bay St. Louis, Miss. These samples were collected in Mississippi and Arkansas from persons who might have been exposed to TCDD through the use of 2,4,5-T on rice.

Additionally, EPA is establishing a voluntary biopsy and mother's milk monitoring program in Oregon, to further investigate the significance of direct and indirect human exposure to TCDD through the use of 2,4,5-T. This program is intended to provide EPA with information on the significance of indirect and direct human exposure to TCDD through the comparison of samples taken from persons living in urban versus forested sites, respectively.

For specific details on the phase II analytical procedures refer to Section V, Appendix, part B.

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IV. Summary

EPA is investigating the frequency and level of TCDD residues in the environment as basis for determining the toxicological risk associated with human and other non-target exposure to TCDD.

A. Phase I of the Dioxin Implementation Plan was primarily designed to identify acceptable research approaches for extraction and analysis of TCDD residues in environmental samples. Existing data indicate that the methods are satisfactory for analysis of beef samples (fat and liver) and that TCDD is present in a small percentage (3.5%) of the beef fat samples taken from cattle with a known exposure to 2,4,5-T. All of the beef liver samples analyzed were negative. As a part of phase I, the Agency is conducting further research to determine the acceptability of these methods for analysis of other sample types (humans, small mammals, birds, soil, etc.) and the optimal combination of extraction and analytical methods. The latter study will be short term (90 days) in order that the Agency might rapidly proceed with phase II analysis.

B. The Agency has analyzed 75 samples of pesticides which, due to their manufacturing process, could contain TCDD. Seventy three of the 75 samples analyzed, did not contain TCDD at or above the 0.1 ppm tolerance level set by the Science Advisory Committee in 1970. The 2 remaining samples ronnel, and Na-2,4,5-T phenate are believed to contain .107 ppm and .312 ppm of TCDD respectively. These samples will be reanalyzed for confirmation. If confirmed EPA will take regulatory action to insure that the level of TCDD in the affected pesticides is reduced to the 0.1 ppm level.

An additional analytical study for TCDD residues in technical grade pesticides will be conducted in 1977. This study will analyze a greater number of samples at a lower limit of detection (10 ppb) than the preceeding analyses. EPA is presently selecting an analytical laboratory for this research.

C. Phase II of the Dioxin Implementation Plan is intended to provide the Agency with increased information on the range and possible tendency of TCDD to bioaccumulate in the environment.

All analyses in phase II will be based on a statistical approach designed to improve the precision of the quantification of TCDD residues reported.

Analyses of the human (fat, liver and milk) and beef (fat and liver) samples now in storage will receive the highest priority when the Agency proceeds with phase II of the plan.

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D. Existing beef fat and liver analytical data from phase I of the Dioxin Implementation Plan do not indicate that the toxicological effect level for exposure to TCDD through ingestion of contaminated beef, has been exceeded. This is only one source of exposure however. Additional analytical data are necessary to assess the overall toxicological risk associated with accumulative exposure from different sources of TCDD.

In addition to the human samples now in storage, EPA is establishing an elective biopsy and mothers milk program in Oregon to further assess the potential for human exposure to TCDD through the use of 2,4,5-T.

V. Appendix

A. Interpretation of Phase I Data by Analytical Collaborators

A meeting of the analytical collaborators (Dow Chemical, Harvard University, and Wright State University) was held on June 15, 1976, to discuss the results obtained to date. As a result of this meeting the following statement was issued by the analytical collaborators.

1. Of the beef fat famples (85) analyzed, one shows a positive TCDD level at 60 ppt; two samples appear to have TCDD levels at 20 ppt; five may have TCDD levels which range from 5-10 ppt. While several laboratories detected levels (5-10 ppt) in this range, the values reported were very near the sample limits of detection. There exist a great deal of uncertainty of the procedure below 10 ppt.

2. The analytical method is not valid below 10 ppt O'Keefe, Harvard.

3. An introduction to a neutral extraction technique shows promise of the capability of detecting levels below 10 ppt. This was demonstrated by comparative data at the lower parts per trillion range (5-10 ppt). However, this method has been demonstrated by only one laboratory at this time and has not been validated below 10 ppt by another competent analytical facility.

4. The samples analyzed were peritoneal fat and kidney fat taken from cattle which had grazed on rangelands of known treatment with 2,4,5,-T. Controls were the same sample type taken from cattle from non-treated areas within the same state.

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5. Of the liver samples (43) analyzed, only one sample suggest any TCDD residue, but the residue observed was too close to the sensitivity of the sample detection limits for quantitation. The fat sample analyzed from the same animal showed no TCDD residue. Three liver samples (for which fat samples were analyzed and showed positive data) showed no TCDD residues.

6. None of the collaborators reported TCDD in samples of beef fat taken from cattle in non-treated areas (at the sensitivity of the analytical method). Three of the laboratories receiving liver samples from cattle in non-treated areas observed no TCDD in the samples.

Due to the improvement and agreement among the analytical collaborators with the analytical data, the above data supercedes the EPA beef fat monitoring data released in memoranda dated August 5, 1975 and December 19, 1975.

B. Phase II Experimental Design

The design phase II calls for preparation of two "pools" of control beef fat (say, Pool F and Pool G) and two pools of control beef liver (Pool L and Pool M) from which all spiked samples are to be constructed. The fat pools are constructed from equal amounts of fat from each control animal selected, using a separate set of animals for each pool. Liver pools are constructed in a similar manner. (Necessary pool sizes are to be determined by Dr. Aubry Dupuy after study of the design).

Eleven samples each are prepared from fat pool F and liver pool L. The samples from each pool are spiked individually at levels between 0 and 100 ppt of 35 Cl-TCDD. Samples are then extracted, and the extract is divided into three equal aliquots for shipment to the analytical labs, (the spiking levels are systematically spaced). The spiking system allows close spacing at lower levls and moderate increases in spacing at higher levels).

Next, five samples each are prepared from fat pool G and liver pool M. The samples from each of these pools are spiked individually at levels between 0 and 100 ppt of TCDD. These samples are extracted and divided into three equal aliquots for shipment as above.

It is necessary that each laboratory receive enough extract from sets G and M and from the comparably-spiked samples of sets F and L to allow duplicate measurements of each sample. For other samples, labs need receive only enough extract for a single measurement.

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A single series of standards, prepared as set S and set T, will serve for comparison both with fat and liver samples. Set S will comprise eleven aliquots spiked at levels, comparable to fat set F and liver set L.

Standard set T will contain 5 aliquots spiked at levels comparable to fat set G and liver set M. Again labs must receive enough standard from set T and corresponding levels of set S to permit duplicate measurement of each standard.

Thus, a total of 48 samples -- 16 standards, 16 fat and 16 liver -- are needed; these will require a total of 78 measurements by each laboratory, counting duplicate measurements of specified samples. A diagram of the design is attached.

All samples -- fat, liver and standards -- are to be prepared and shipped in random order, except that aliquots from a given sample wil be shipped to the three labs simultaneously. Laboratories are to analyze the samples in the order in which they are received. Labs are to perform blind analyses, i.e., they are not to know either the origin of the material or the level of TCDD in any sample. A sample numbering system should be used that provides no clue to sample identity.

All data will be used to develop recovery curves. Data from duplicate analyses of replicated samples will be used to measure extraction and GC-MS precision. Data evaluation will be by analysis of variance and regression methods.

Detection limit defined as 2.5× background noise
Quantitation limit = 10× background
Must meet validation for recovery at that level

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TCDD Level (ppt)	Standard Measurements			Beef Fat Measurements				Beef Liver Measurements			
	Pool Code	Lab. A	Lab. B	Lab. C	Pool Code	Lab. A	Lab. B	Lab. C	Pool Code	Lab. A	Lab. B	Lab. C
A	S	2	2	2	F	2	2	2	L	2	2	2
A	T	2	2	2	G	2	2	2	M	2	2	2
B	S	1	1	1	F	1	1	1	L	1	1	1
C	S	1	1	1	F	1	1	1	L	1	1	1
D	S	1	1	1	F	1	1	1	L	1	1	1
E	S	2	2	2	F	2	2	2	L	2	2	2
E	T	2	2	2	G	2	2	2	M	2	2	2
F	S	1	1	1	F	1	1	1	L	1	1	1
G	S	2	2	2	F	2	2	2	L	2	2	2
G	T	2	2	2	G	2	2	2	M	2	2	2
H	S	1	1	1	F	1	1	1	L	1	1	1
I	S	2	2	2	F	2	2	2	L	2	2	2
I	T	2	2	2	G	2	2	2	M	2	2	2
J	S	1	1	1	F	1	1	1	L	1	1	1
K	S	2	2	2	F	2	2	2	L	2	2	2
K	T	2	2	2	G	2	2	2	M	2	2	2
Tot. Pools		16				16				16		
Tot.		26	26	26		26	26	26		26	26	26

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24 Nov 1977

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8. FIFRA, Section 6(d), as amended in 1972.

9. Report on the Advisory Committee on 2,4,5-T to the Administrator of the Environmental Protection Agency, May 7, 1971.

10. Dow Chemical Company vs. Ruckelshaus, 477 F. 2nd 1317 (8CRI 1973).

11. 6 (b) 2 FIFRA as amended in 1972, information gathering procedure to determine whether or not a pesticide should be cancelled or its registration changed. Changes in the Act in 1972 added 6(b) (2) to supplement 6(b) (1), cancellation of a pesticide or to change its classification.

12. Toxicology Meeting Summary, February 21, 1976. Office of Pesticide Program, U.S. Environmental Protection Agency, Washington, D.C.

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Letters to the Editor

EFFECTS OF DIOXIN EXPOSURE

SIR,—Dr Laporte (May 14, p. 1049) continues the interminable argument—often largely political—in which the alleged long-term effects of 2,4,5-T/2,4-D herbicide spraying in Vietnam are compared with industrial accidents over the 28 years or so during which factory workers have been exposed to tetrachlordibenzo-P-dioxin (dioxin, T.C.D.D.). Dr Laporte cites extensively from Prof. Ton That Tung and similar Vietnamese sources; having several times visited Italy to assist the Italian Government with the Seveso problem, I feel I must try to put some of these assertions into perspective.

I shall examine one quotation in detail; the others may be interpreted with similar caution. The journal *Sapere*, the source of almost all Dr Laporte's data, states that from 1961 to 1969, 500 kg of dioxin were sprayed over Vietnam. During these 9 years, the total U.S. production of 2,4,5-T for every purpose was 97·5 million lb of 2,4,5-T acid. This contained an average of 1·91 part per million of 2,3,7,8-T.C.D.D. (i.e., 2 p.p.m. approximately). Thus the total T.C.D.D. content of the entire U.S. output for this period was only 195 lb (82·5 kg)[1], and only part of this ever reached Vietnam.

Dr Laporte, by referring only to the explosions, also attempts to suggest that the numerous factory workers exposed to T.C.D.D. over a period of nearly 30 years, have not been chronically as well as acutely exposed. However, the explosions, dramatic though they have been, have poisoned far less workers than has poor plant hygiene with its chronic toxicity. The important Czech experience,[2] for example, is essentially one of chronic poisoning yet Dr Laporte does not mention it, and when we compare the relatively enormous concentrations of T.C.D.D. in such industrial exposures with an average concentration of T.C.D.D. in the Vietnam defoliant of only 2·0 mg/kg we can readily appreciate that those industrially exposed should by now be manifesting a high rate of liver cancer, chromosome damage, and so on if Prof. Ton That Tung's assertions are correct. So far there is no published evidence to confirm this among those who have been poisoned in industry in years gone by.

2,4,5-T and 2,4-D are not "both synthesised from trichlorophenol". 2,4-D is made from 2,4-dichlorophenol which contains no toxic chlorinated dioxin at all and made up 50% of the agent-orange herbicide used in Vietnam.

The statement that chloracne is a consequence of hepatic damage reveals a profound ignorance of the subject.

There is no foundation for the statement that T.C.D.D. is being disseminated either superficially or deep in the soil at Seveso.[4]

Finally, the spraying of a 37 hectare (ha) plot of lakeland soil in Florida from 1962 to 1964 with the Vietnam defoliant (50% 2,4,5-T and 2,4-D), at a rate of 4950 kg/ha, is informative. The T.C.D.D. contamination of the 2,4,5-T averaged 2·0 p.p.m. (i.e., 0·5 kg/km²). Since the total 2,4,5-T produced in the U.S. between 1961 and 1969 contained only 82·5 kg T.C.D.D. it follows that had the above concentration of herbicide been sprayed on to Vietnamese soil the total area covered each year would have been approximately 18 km², which we know to be absurdly low. Therefore the Vietnam concentration per km² must have been less than the Florida concentration. The significance of the Florida experiment lies in the fact that post-mortem and ultramicroscopic examination of beach mice, fish, and reptiles in the sprayed area revealed no significant alteration from controls some years later, although tissue levels of T.C.D.D. were still appreciable.[5]

All such facts, should be kept in the forefront of one's mind when evaluating such data about Vietnam and Italy as are cited by Dr Laporte. Only by so doing will the truth finally emerge and Seveso cease to be both a medical and political battleground.

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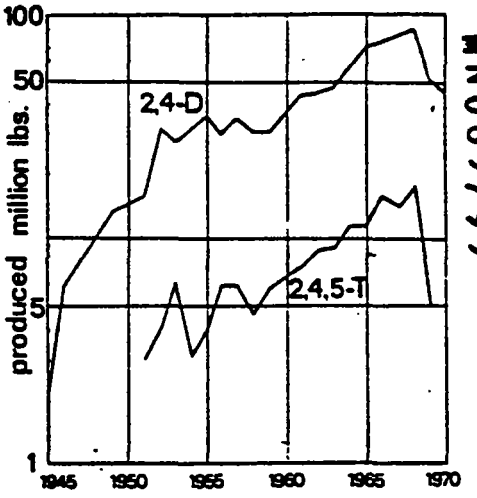
K. D. CROW

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**This letter has been shown to Dr Laporte whose reply follows.—ED. L.

SIR,—I thank Dr Crow for his interesting letter on dioxin and for his corrections to some of my statements.

As the accompanying figure shows, the total U.S. production of 2,4,5-T between 1961 and 1969 was 97·5 million lb. In 1969 2,4,5-T production dropped back to 5 million lb, so it may be concluded that about 52 million lb was used in Vietnam (i.e., about 44 kg of T.C.D.D. and not 500 kg, the figure I cited[1]). However, in Vietnam spraying may not have been the only source of dioxin. 2,4,5-T heated to 500–600°C will yield 50 000 p.p.m. T.C.D.D.,[2] and such temperatures may have been achieved on the battlegrounds of Vietnam.



U.S. production of 2,4-D and 2,4,5-T.
Source: Margnelli and Tibaldi.[16]

The commission of the American Association for the Advancement of Sciences, which went to Vietnam in 1972, took samples of edible fish from Vietnamese rivers. These were analysed at Harvard University.[3] A specially designed spectrophotometric method revealed mean dioxin concentrations of 548 µg/kg, the L.D.$_{50}$ for guineapig being 600 µg T.C.D.D./kg bodyweight.[3] In 1974 the U.S. National Academy of Sciences Committee on the Effects of Herbicides in Vietnam was not able to collect animals with a view to investigating the Vietnamese food chain more fully, but the Committee did say of dioxin that "it is a proven teratogen in mice and rats" and it strongly recommended "repeated sampling of the Vietnamese food chain, where low levels of dioxin have already been found".[4]

The Czech experience mentioned by Dr Crow could be one of chronic poisoning, though very different from the situation in Vietnam or Seveso. A high rate of liver cancer would be

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WORLD HEALTH ORGANIZATION

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IARC MONOGRAPHS

ON THE

EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO MAN

Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals

VOLUME 15

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INTERNATIONAL AGENCY FOR RESEARCH ON CANCER 7135

LYON

AUGUST 1977

DOW 1375306

1. Chemical and Physical Data

1.1 Synonyms and trade names

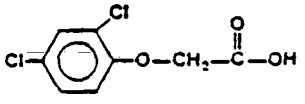
Chem. Abstr. Services Reg. No.: 94-75-7

Chem. Abstr. Name: (2,4-Dichlorophenoxy)acetic acid

Dichlorophenoxyacetic acid; 2,4-dichlorophenoxyacetic acid

For a representative list of other synonyms and trade names of products containing 2,4-D, its salts or esters, either as the sole active ingredient or as mixtures with other compounds, see Appendix A.

1.2 Chemical formula and molecular weight



$C_8H_6Cl_2O_3$ Mol. wt: 221.0

1.3 Chemical and physical properties of the pure substance

From Weed Science Society of America (1974), unless otherwise specified

(a) Description: Odourless white crystals

(b) Boiling-point: 160°C at 0.4 mm

(c) Melting-point: 140-141°C

(d) Spectroscopy data: Infra-red and ultra-violet spectra are given by Gore *et al.* (1971).

(e) Solubility: Soluble in 95% ethanol and in acetone, dioxane and isopropyl alcohol

(f) Stability: Stable up to and including its melting-point

(g) Reactivity: Forms salts that are soluble in water

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1.4 Technical products and impurities

Technical grade 2,4-D is available in the US as the free acid (98% purity), as salts (dimethylamine, mixed ethanolamine and isopropanolamine, lithium and sodium) and as esters of the following alcohols: isopropyl, *n*-butyl, sec-butyl, iso-octyl, 2-butoxyethyl and butoxypolypropylene glycol (US International Trade Commission, 1976a).

Of 28 samples of 2,4-D tested for content of chlorodibenzo-*para*-dioxins, one was found to contain <10 mg/kg hexachlorodibenzo-*para*-dioxin (Woolson *et al.*, 1972). Bis(2,4-dichlorophenoxy)methane has been identified as the major contaminant of 2,4-D, and bis(2,6-dichlorophenoxy)methane and 2,2',4,6'-tetrachlorodiphenoxymethane as minor contaminants (Huston, 1972).

N-Nitrosodimethylamine[1] has been detected at a level of 300 ug/l in dimethylamine salt of 2,4-D which was stored in metal containers the interiors of which had been presprayed with sodium nitrite as an antioxidant (Fine *et al.*, 1977).

Technical 2,4-D produced in Japan is more than 99% pure.

2. Production, Use, Occurrence and Analysis

For background information on this section, see preamble, p. 17.

2.1 Production and use

(a) Production

2,4-D was prepared in 1941 by the interaction of 2,4-dichlorophenol, monochloracetic acid and sodium hydroxide (Pokorny, 1941), and a similar process is believed to be used in its commercial production.

Production was first reported in the US in 1944 (US Tariff Commission, 1946). The quantity of 2,4-D produced increased steadily between 1963 and 1968, when it reached a maximum of 36 million kg; production decreased to about 20 million kg in 1970 (US Department of Agriculture, 1973) and

[1] See IARC, 1972

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gradually increased again to an estimated 27 million kg in 1974. In 1975, three US companies reported production of 2,4-D acid; three others reported the production of esters or salts of 2,4-D, presumably from purchased acid. Separate production data for 1975 are available only for the dimethylamine salt of 2,4-D, 11.6 million kg of which were produced, and for the iso-octyl ester, 4.5 million kg of which were produced (US International Trade Commission, 1976a). In 1973, 115 thousand kg of 2,4-D acid and 57 thousand kg of mixed butyl esters were imported through the principal US customs districts (US Tariff Commission, 1974). In 1974, 365 kg of the mixed butyl esters were imported (US International Trade Commission, 1976b). Combined US exports of 2,4-D and 2,4,5-T amounted to 5.7 million kg in 1975 (US Department of Commerce, 1975).

The Federal Republic of Germany and the UK are the major producing countries in western Europe, where annual production is estimated to be 3-30 million kg; in eastern Europe it is estimated to be less than 10 million kg.

2,4-D was first produced commercially in Japan before 1945 by a process similar to that used in the US. Production in 1975 by two producers amounted to 511 thousand kg, and 176 thousand kg were exported.

About 90,000 kg were imported into Australia in 1975-76.

(b) Use

2,4-D is a systemic herbicide widely used for control of broadleaf weeds in cereal crops and sugar cane and on turf, pastures and non-cropland (Weed Science Society of America, 1974). It is also used to control the ripening of bananas and citrus fruits, to delay preharvest dropping of some fruits and in some countries as a fungicide for the control of *Alternaria* rots when lemons are to be held for storage (WHO, 1975).

An estimated 27 million kg of 2,4-D acid equivalent, largely in the form of esters and salts, were used in the US in 1975, as follows: wheat and other small grains, 31%; corn and grain sorghum, 26%; pasture and rangeland, 25%; industrial and commercial uses, 9%; lawns and turf, 5%; aquatic weed control, 3%; rice and fruit, 1%.

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2,4-D was used to defoliate jungle areas in south Vietnam, where it was a component of 'Agent Orange' (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 TCDD) (Davis, 1974). About 40 million litres of 'Agent Orange' were sprayed in south Vietnam between 1965-1971 for defoliation or crop destruction (Committee on the Effects of Herbicides in Vietnam, 1974).

National tolerances are in effect in several countries. Examples were reported to the Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues in 1975. The previously established acceptable daily intake for man of 0-0.3 mg/kg bw was considered and confirmed at this meeting (WHO, 1977).

The US Occupational Safety and Health Administration health standards for exposure to air contaminants require that an employee's exposure to 2,4-D does not exceed an eight-hour time-weighted average of 10 mg/m^3 in the working atmosphere during any eight-hour work shift of a forty-hour work week. The corresponding standard in the Federal Republic of Germany is also 10 mg/m^3, and the acceptable ceiling concentration in the USSR is 1 mg/m^3 (Winell, 1975).

2.2 Occurrence

2,4-D is not known to occur as a natural product.

It is broken down by soil microorganisms, and there is reportedly no accumulation in the soil as a result of normal agricultural use (Weed Science Society of America, 1974).

In a continuing programme involving the monitoring of pesticide residues in food, the US Department of Health, Education and Welfare found a decreasing level of 2,4-D in food samples collected at retail outlets during the period 1965-1973. Between June 1964 and April 1965, 2,4-D was found in 10 of 216 composite food samples examined, including leafy vegetables, oils, fat and shortening, sugar and adjuvants (Duggan *et al.*, 1966). In 1973, an insignificant amount of 2,4-D was found during this programme: a trace was found in one of 360 composite potato samples (US Bureau of Foods, 1975).

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Air samples were collected near wheat-growing areas around Pullman and Kennewick Highlands, Washington, USA between April and August 1964 after applications of 2,4-D. Although data were given for several products containing 2,4-D, those for the isopropyl ester were the highest: an average concentration of 0.116 µg/m³ and a maximum concentration of 1.96 µg/m³ of an aerosol form of the ester and an average concentration of 0.007 µg/m³ and a maximum concentration of 0.69 µg/m³ of the vaporized ester were found in 24-hour samples near Pullman (Finkelstein, 1969).

Residues of 2,4-D in pond waters declined from maximums of 0.345 and 0.692 mg/l in Florida and Georgia, respectively, to less than 0.005 mg/l 28 days after treatment and from 0.630 mg/l in Missouri pond waters to less than 0.005 mg/l 56 days after treatment. Residues in mud from the Florida and Georgia ponds never exceeded 0.05 mg/kg and had declined to trace or nondetectable levels 56 days after treatment. The highest residue found was 0.170 mg/kg in samples of mud taken on the first and third days in the most heavily treated Missouri pond. In mud from one Missouri pond, residues were detected as late as 28 days after treatment; no residues occurred in any ponds after that time (Schultz & Harman, 1974).

In September 1971, soil samples were obtained from an area in Thailand that had been used for calibrating aerial herbicide spray equipment and that had received about 940 kg/ha 2,4-D and large amounts of other herbicides in 1964-65. Two of 6 samples contained 0.18 and 0.21 kg/ha 2,4-D (Committee on the Effects of Herbicides in Vietnam, 1974).

2.3 Analysis

The Association of Official Analytical Chemists (AOAC) has published three Official Final Actions for the determination of 2,4-D in formulations: (1) titration, for formulations of the free acid; (2) determination of total chlorine after calorimetric destruction for formulations of 2,4-D compounds; and (3) infra-red spectroscopy for 3,6-dichloro-2-methoxybenzoic acid (Dicamba)/2,4-D formulations. Official First Actions have been published for determination of the free acid in formulations of 2,4-D esters by titration to pH 7, and for determination in formulations with 4-amino-3,5,6-trichloropicolonic acid (picloram) by high-pressure liquid

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chromatography (Beroza, 1976; Horwitz, 1975). Results of collaborative studies of several of these methods have been published (Hammond, 1973; Malina, 1971; Skelly *et al.*, 1976).

Another method for determining 2,4-D in formulations involves gas chromatography of its trimethylsilyl derivative (Zweig, 1972). With a similar method, recoveries of 100.5 ± 1% compare favourably with those obtained using the AOAC infra-red spectroscopy method (Collier & Grimes, 1974).

Methods of analysis for 2,4-D residues in various commodities using gas chromatography have been summarized and tabulated in a recent review (WHO, 1975). In a more recent publication (WHO, 1977) attention was drawn to the low recoveries frequently encountered due to conjugation of 2,4-D with plant constituents. The Pesticide Analytical Manual (US Food & Drug Administration, 1975a,b) summarizes several methods for determining 2,4-D residues, using gas, thin-layer and paper chromatography; a general method for extraction and clean-up of chlorophenoxy acids in a variety of foods provides more than 80% recovery. Limits of detection for gas chromatography are in the order of 10 µg/kg when microcoulometric detection is used and 0.2 µg/kg with electron capture.

A gas chromatographic procedure has been used to separate mixtures of herbicidal acids as their pentafluorobenzyl derivatives, which generally provide better separation and better responses in the electron capture detector than do the methyl or silyl derivatives; the method has a sensitivity of 0.4 µg/kg for 2,4-D (Chau & Terry, 1976). An automated, gel-permeation chromatographic procedure permits clean-up of pesticide residues in lipid-containing plant and animal extracts prior to their determination by gas chromatography with electron-capture detection, and provides recovery of more than 80% 2,4-D esters (Johnson *et al.*, 1976).

A low-temperature extraction and clean-up method is used to separate multiple residues of 2,4-D and other pesticides for eventual gas chromatographic analysis with electron capture and flame photometric detectors; 82-108% of added 4 mg/kg 2,4-D could be recovered (McLeod & Wales, 1972).

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Gas chromatography combined with electron-capture detection has been used to determine 0.5 mg/kg 2,4-D residues in oysters (Duffy & Shelfoon, 1967) and 0.05 mg/kg in soil, wheat and barley (Khan, 1975).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Man

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Groups of 18 male and 18 female (C57BL/6xC3H/Anf)F_1 mice and 18 male and 18 female (C57BL/6xAKR)F_1 mice received commercial 2,4-D (90%, m.p. 136-140°C) according to the following dose schedule: 46.4 mg/kg bw in 0.5% gelatine by stomach tube at 7 days of age and the same amount (not adjusted for increasing body weight) daily up to 28 days of age; subsequently, the mice were given 149 mg/kg of diet. A further group of 18 male and 18 female (C57BL/6xAKR)F_1 mice were given oral doses of 100 mg/kg bw/day from 7-28 days of age and subsequently fed 323 mg/kg of diet. The experiment was terminated when the mice were about 78 weeks of age, at which time 15, 16, 16 and 16 given the lower dose level and 11 and 13 given the higher dose level were still alive in the respective groups. Tumour incidences were compared with those observed among groups of 79, 87, 90 and 82 control mice, which had either been untreated or had received gelatine only: the incidences were not significantly greater ($P>0.05$) when any group or combination of groups was considered. Similar results were obtained in groups of mice given 2,4-D isopropyl, butyl or isooctyl esters (99%, 99% and 97% pure) at doses of 46.4 mg/kg bw from 7-28 days of age and, subsequently, 111, 149 and 130 mg/kg of diet, respectively, up to 78 weeks of age (Innes *et al.*, 1969; NTIS, 1968a).

Rat: Groups of 25 male and 25 female 3-week old Osborne-Mendel rats were fed for two years on diets containing 0, 5, 25, 125, 625 or 1250 mg/kg of diet 2,4-D. The 2,4-D was 96.7% pure and contained no detectable levels of 2,7-dichloro- or 2,3,7,8-tetrachlorodibenzo-*para*-dioxin; the limit of sensitivity of the method of analysis was 1 mg/kg . The numbers of male and female rats with malignant tumours were 6 in controls and 8, 7, 7, 8

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and 14 in the treated groups, respectively. Tumours were randomly distributed and were also found in ageing rats of this strain. According to the authors, a statistical increase ($P<0.05$) in the number of treated rats with malignant tumours over controls was found only in males receiving the highest dose level (Hansen *et al.*, 1971).

Groups of 120 male and 45 female random-bred rats, weighing 80-100 g at the start of the test, were given 2,4-D as the amine salt (amount not specified) mixed in the food at a concentration which corresponded to a daily intake of one-tenth of the LD_{50} (not specified, but see Table 1, section 3.2). Two treated rats developed tumours (a mammary fibroadenoma and a haemangioma of the mesenterium) after 23 months, and one untreated rat had a mammary fibroadenoma after 27 months (Arkhipov & Koslova, 1974).

(b) Subcutaneous and/or intramuscular administration

Mouse: Groups of 18 male and 18 female (C57BL/6xC3H/Anf)F_1 mice and 18 male and 18 female (C57BL/6xAKR)F_1 mice were given single s.c. injections of 215 mg/kg bw 2,4-D (90% pure, m.p. 136-140°C) in dimethyl sulphoxide on the 28th day of life and observed up to 78 weeks of age, at which time 16, 17, 18 and 18 mice in the four groups, respectively, were still alive. Tumour incidences were compared with those in groups of 141, 154, 161 and 157 controls that were either untreated or were injected with dimethyl sulphoxide, 0.5% aqueous gelatin or corn oil. The tumour incidence in any group or combination of groups was not significantly different from that in controls ($P>0.05$). No increase in the incidence of tumours was observed in similar groups of mice treated with single s.c. injections of 21.5 mg/kg bw butyl or 100 mg/kg bw isopropyl esters of 2,4-D (both 99% pure). Of mice treated with 21.5 mg/kg bw isooctyl ester of 2,4-D (97% pure), 5/17 females of the second strain developed reticulum-cell sarcomas ($P=0.01$) (NTIS, 1968a).

3.2 Other relevant biological data

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(a) Experimental systems

The toxicity of 2,4-D was reviewed by Dalgaard-Mikkelsen & Poulsen (1962).

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Acute and short-term toxicity

There are species differences in the acute oral toxicity of 2,4-D and of its derivatives (Table 1). The symptoms of acute toxicity in mice, rabbits, guinea-pigs and rats are essentially similar (Bucher, 1946; Hill & Carlisle, 1947). Some animals died suddenly, apparently from ventricular fibrillation; those that did not die immediately developed stiffness of the extremities, incoordination, lethargy, stupor and coma prior to death (Hill & Carlisle, 1947). Symptoms of myotonia were evident in mice after the parenteral administration of 150-200 mg/kg bw; and in mice acutely intoxicated with 2,4-D, dilatation of the blood vessels of lungs, liver and kidneys was observed (Bucher, 1946). Rats and guinea-pigs administered lethal doses of 2,4-D exhibited congestion of the viscera and enlarged, swollen kidneys; microscopically, there was massive cloudy swelling of the proximal convoluted tubules with cast formation (Hill & Carlisle, 1947). S.c. injections of 100 mg/kg bw 2,4-D resulted in a decrease of both thyroid and body weights in male rats (Florsheim & Velcoff, 1962).

Oral administration of 625 mg/kg bw to rats resulted in changes in the levels of several serum enzymes and globulins (Szöcs *et al.*, 1970). 2,4-D was found to be a potent uncoupling agent for oxidative phosphorylation and to stimulate the respiration of rat liver mitochondria in a phosphate-deficient medium (Brody, 1952).

In dogs, toxic symptoms were often not present until 6 hours after oral administration of a lethal dose of 2,4-D; the animals became ataxic, with progressive increase in spasm. Death appeared to be due in most cases to hepatic congestion or to pneumonia. Pathological changes, limited to the gastrointestinal tract, lung and liver, followed the development of anorexia, weight loss and myotonia (Drill & Hiratzka, 1953). Dogs exhibited evidence of liver damage more frequently than other animals studied (Bucher, 1946; Drill & Hiratzka, 1953).

No toxic symptoms were noted in monkeys given 214 mg/kg bw orally, or 428 mg/kg bw intraperitoneally; however, oral plus i.p. injection of a total of 500 mg/kg bw 2,4-D caused nausea, vomiting, lethargy, muscular incoordination and head drop (Hill & Carlisle, 1947). Acute toxic doses

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TABLE 1

Acute oral toxicity of 2,4-D and esters

Compound of 2,4-D	Species	Sex	LD_{50} (mg/kg bw)	Reference
Acid	Mice	M	375	Hill & Carlisle (1947)
	Mice	M	368	Rowe & Hymas (1954)
	Rats	M	375	" " "
	Rats		666	Hill & Carlisle (1947)
	Guinea-pigs	M&F	469	Rowe & Hymas (1954)
	Guinea-pigs		1000	Hill & Carlisle (1947)
	Rabbits		800	" " "
	Dogs		100	Drill & Hiratzka (1953)
	Chicks	M&F	541	Rowe & Hymas (1954)
Butyl ester	Mice		380	Konstantinova (1970)
	Rats		1500	Schillinger (1960)
	Rats		920	Konstantinova (1970)
	Cats		820	" "
Esters of mono-, di- and tripropylene glycol butyl ethers	Rats	F	570	Rowe & Hymas (1954)
Isopropyl ester	Mice	M	541	Rowe & Hymas (1954)
	Rats	M&F	700	" " "
	Guinea-pigs	M	550	" " "
	Chicks	M&F	1420	" " "
Mixed butyl esters	Mice	F	713	Rowe & Hymas (1954)
	Rats	F	620	" " "
	Guinea-pigs	F	848	" " "
	Rabbits	M&F	1420	" " "
	Chicks	M&F	2000	" " "
Sodium salt	Mice		375	Rowe & Hymas (1954)
	Rats	F	805	" " "
	Rats		2000	Schillinger (1960)
	Guinea-pigs	M	551	Rowe & Hymas (1954)
	Rabbits		800	" " "
Alkanolamine salt	Chicks		380-765	Rowe & Hymas (1954)

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of 2,4-D (765 mg/kg bw) produce fatty degeneration of the liver, spleen, kidneys and heart and haemorrhagic gastroenteritis in chickens (Bjorn & Northen, 1948), and acute toxic doses have similar effects in sheep and cattle (Palmer & Radeleff, 1964).

Subacute and chronic toxicity

2,4-D administered subcutaneously to mice at doses of 50-90 mg/kg bw once or twice daily for 3 weeks to 3 months produced no clear-cut chronic symptoms. Levels of 70 mg/kg bw and more retarded growth, probably by reducing food intake (Bucher, 1946).

Rats fed 1000 mg/kg of diet 2,4-D for one month showed no signs of toxic effects (Hill & Carlisle, 1947). No adverse effects were noted in young female rats fed 100 and 300 mg/kg of diet 2,4-D (purity unspecified) for periods of up to 113 days, while those given 1000 mg/kg of diet over the same period had increased mortality, depressed growth rate, slightly increased liver weight and slight cloudy swelling of the liver. Animals fed 3000 or 10,000 mg/kg of diet were sacrificed after 12 days because of food refusal and rapid weight loss. Increased liver and kidney weights and unstated slight pathological changes were noted in these organs (Rowe & Hymas, 1954).

No significant adverse effects were noted when 15 ml of each of 3 commercially available formulations of 2,4-D (the dimethylamine salt and the isooctyl and butyl esters) were administered 5 times weekly for 3 weeks to the intact and abraded skin of rabbits at two concentrations, 0.626% and 3.13% (the dimethylamine salt was diluted in water and the ester in either oil or water) (Kay *et al.*, 1965).

Groups of 3 male and 3 female beagle dogs were fed 10, 50, 100 or 500 mg/kg of diet 2,4-D (96.7% pure, with no detectable chlorodibenzo-*para*-dioxin content) for 2 years, starting at 6-8 months of age. Twenty-eight dogs that survived the 2-year period were clinically normal. No adverse effects related to 2,4-D administration were observed (Hansen *et al.*, 1971).

Dogs of both sexes were given 2, 5 or 10 mg/kg bw commercial 2,4-D (98.5% pure) orally by capsule for 5 days per week for 13 weeks; no signs

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of toxicity were observed. Dogs given 20 mg/kg bw survived for periods ranging from 18-49 days, with loss of weight occurring after 7-12 days and with ataxia, increased muscle tonus and a terminal fall in lymphocyte count prior to death (Drill & Hiratzka, 1953).

Young pigs treated at varying intervals up to 103 days with 50, 100 or 300 mg/kg bw of the commercial triethanolamine salt or butyl ester of 2,4-D exhibited symptoms of intoxication and pathology analagous to those seen in laboratory animals. Clinical signs of anorexia and retarded growth were found in one animal given 51 doses of 50 mg/kg bw triethanolamine salt over 103 days. Pigs fed 500 mg/kg of diet triethanolamine salt of 2,4-D for up to 12 months developed locomotor disturbances of increasing severity after about one month. Animals sacrificed after 2-12 months had normal organ weights and no gross pathological changes. Clinico-chemical observations included lowered haemoglobin and haematocrit values, elevation of glutamic-oxaloacetic transaminase and reduced albumin and albumin: globulin ratios in the treated animals (Björklund & Erne, 1966).

Absorption, distribution and excretion

In rats, pigs, calves and chickens, 2,4-D administered in doses of 50-100 mg/kg bw orally as salts was readily absorbed and eliminated, mainly in the urine, with plasma half-lives varying from 3-12 hours (Erne, 1966a,b). The rate of 2,4-D elimination in rats was dosage dependent. Following administration of ^{14}C-2,4-D, radioactivity was found in all organs and tissues examined (Khanna & Fang, 1966).

2,4-D esters are hydrolysed in animals. The phenoxy acids are excreted predominantly as such in the urine of rats after their oral administration, although a minor portion is conjugated with the amino acids glycine and taurine and with glucuronic acid (Grunow & Böhme, 1974). No 2,4-dichlorophenol was, however, detected in the urine of C57BL/6 mice treated subcutaneously with 2,4-D or its butyl or isooctyl esters. The rates of disappearance from the plasma of 2,4-D and its butyl and isooctyl esters following single s.c. injections of 100 mg/kg bw of the compounds to female C57BL/6 mice were: butyl ester>isooctyl ester>2,4-D (Zielinski & Fishbein, 1967).

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After oral administration of 0.05 mg/kg bw 2,4-D to rats, traces were detected in the milk of lactating animals for 6 days. Within 24 hours after administration of 2,4-D to pregnant rats, 16.8% of the dose was detected in the uterus, placenta, foetus and amniotic fluid (Fedorova & Belova, 1974). 2,4-D was also found to pass the placental barrier in pigs (Björklund & Erne, 1966).

Embryotoxicity and teratogenicity

Results of teratological studies with 2,4-D were variable; teratogenic effects are observed with doses close to those which cause maternal toxicity.

Administration of 2,4-D or its isopropyl, butyl or isooctyl esters orally or subcutaneously during days 6-14 of pregnancy increased the incidence of foetal anomalies among HL6, AKR and/or C3H strains of mice but not among B6AK and A/Ha strains. The purity of the compounds was: 2,4-D, 90%, m.p. 136-140°C; isopropyl and butyl esters, 99%; isooctyl ester, 97% (NTIS, 1968a,b).

Sprague-Dawley rats were given 1000 mg/l 2,4-D in the drinking-water during pregnancy and for a further 10 months, and 2,4-D was administered to the second generation for up to 2 years. Pregnancy and parturition were normal; litter size was not significantly reduced, and no malformations were noted in the young. Except for retarded growth and increased mortality in the second generation, no unequivocal clinical or morphological changes were seen. When 2,4-D was administered at a concentration of 500 mg/kg of diet during the entire pregnancy of a sow, anorexia was noted; the newborn piglets were underdeveloped and apathetic, and 10/15 died within 24 hours. Continued feeding of 500 mg/kg of diet to the survivors until they were 8 months of age caused marked growth depression, persistent anaemia and moderate degenerative changes of liver and kidneys (Björklund & Erne, 1966).

Female rats (10 per group) were fed 2,4-D (purity unspecified) at levels of 0, 1000 and 2000 mg/kg of diet for 95 days and then mated with untreated males and continued on their respective diets through gestation and lactation. Pups born to females fed the highest level were small at

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birth; 94% died before weaning; some deaths also occurred in pups of rats fed the lower level (T.B. Gaines & R.D. Kimbrough, cited by Hansen *et al.*, 1971).

The maximum tolerated oral dose of 2,4-D (98.7% purity, no chlorinated dibenzo-*para*-dioxins found with the limit of detection of 0.2 mg/kg) or an equimolar dose of 2,4-D propylene glycol butyl ether ester or of the iso-octyl ester of 2,4-D had embryolethal and growth retarding effects but no teratogenicity when given to pregnant Sprague-Dawley (Spartan strain) rats on days 6-15 of gestation. Other signs of foetotoxicity were s.c. oedema, delayed ossification and wavy ribs. 2,4-D did not affect fertility, gestation, lactation or viability of the newborn; the propylene glycol butyl ether and isooctyl esters decreased viability of the newborn and lactation indices[1] (Schwetz *et al.*, 1971). Similar effects were observed in Wistar rats given single daily oral doses of 100-150 mg/kg bw 2,4-D or the butyl, isooctyl, butoxyethanol dimethylamine derivatives of 2,4-D on days 6-15 of pregnancy (Khera & McKinley, 1972).

No consistent embryotoxic effects were noted when 2,4-D was administered orally to hamsters at doses up to 100 mg/kg on days 6-10 of gestation (Collins & Williams, 1971).

Lutz-Ostertag & Lutz (1970) tried to simulate field conditions for spraying with 2,4-D and to evaluate the effect on pheasant eggs and development of chicks. A high mortality rate and morphological alterations were observed in the embryos and chicks, the toxicity apparently being higher than for mammalian species. Total or partial paralysis was observed in most of the surviving embryos, and 50% of the surviving chicks were sterile.

In a 3-generation reproduction study, Osborne-Mendel rats were fed 100 or 500 mg/kg of diet 2,4-D that was 96.7% pure, with no detectable (<1 mg/kg) 2,7-dichloro- or 2,3,7,8-tetrachlorodibenzo-*para*-dioxin. No adverse effects were observed. Diets containing 1500 mg/kg 2,4-D, while

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[1] lactation index: (pups weaned/pups alive on day 4) x 100

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apparently affecting neither the fertility of either sex nor litter size, sharply reduced the percentage of pups surviving to weaning and the weights of the weanlings (Hansen *et al.*, 1971).

Mutagenicity

2,4-D was not mutagenic in *Escherichia coli* WP2 *hcr*$^+$ or *hcr*$^-$ or in *Salmonella typhimurium* strains TA1535, TA1536, TA1537 or TA1538 (Andersen *et al.*, 1972; Nagy *et al.*, 1975; Shirasu, 1975; Shirasu *et al.*, 1976; Zetterberg *et al.*, 1977).

In the rec assay, which is believed to give an indication of reparable DNA damage, 2,4-D was not more toxic to *Bacillus subtilis* M45 (*rec*$^-$) than to H17 (*rec*$^+$), suggesting that this compound does not damage DNA (Shirasu, 1975).

In *Saccharomyces cerevisiae* D4, gene conversion was increased by concentrations of 2,4-D above 400 µg/ml. Mitotic recombination in *S. cerevisiae* D5 was also increased by 2,4-D (300 µg/ml) (Siebert & Lemperle, 1974; Zetterberg *et al.*, 1977). *S. cerevisiae* RAD 18 (a histidine-dependent haploid strain) was reverted to histidine independence by 250 µg/ml 2,4-D. Toxic and mutagenic effects in *S. cerevisiae* were dependent on low pH (4.3) (Zetterberg, 1977) [Metabolic activation systems were not included in any of these tests].

In host-mediated assays with *S. typhimurium* strains TA1530 or TA1531, or with *S. cerevisiae* D4, no mutagenic effects were observed when adult male mice were given 6 mg 2,4-D (200 mg/kg) by gavage (Zetterberg *et al.*, 1977). Serum from orally dosed rats was not mutagenic to *S. typhimurium* (Styles, 1973).

The effect of 2,4-D on chromosome aberrations in cultured plant tissues is complex because 2,4-D is required as a plant growth regulator in tissue culture. Singh & Harvey (1975a,b) found an inverse correlation between 2,4-D concentration and chromosome aberrations in *Vicia hajastana* and *Haplopappus gracilis*. However, with a strain of *Nicotiana* that does not require 2,4-D it increased chromosome breakage (Ronchi *et al.*, 1976,.

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2,4-D induced chromosome aberrations in a number of cultivated plants and weeds. The cytological abnormalities included chromosome bridges, fragments, lagging chromosomes, C-mitoses and chromatin bodies (Mohandas & Grant, 1972).

No increase in the number of recessive lethals was observed when 2-day-old adult male *Drosophila melanogaster* flies were fed 4.5 or 9.0 mM 2,4-D in sucrose (Vogel & Chandler, 1974).

In vitro exposure of embryonic bovine kidney cells and of bovine peripheral blood cells to concentrations of 1-1000 µg/ml 2,4-D for 6-96 hours resulted in stimulation of mitosis. Chromosomal aberrations were not detected in the peripheral blood cells, but nucleolar irregularities and polyploid mitotic stages were observed in the kidney cells (Bongso & Basrur, 1973).

Treatment of cultured human lymphocytes with 2.5×10^{-7} M (0.02 µg/ml) 2,4-D increased the number of chromatid aberrations (single acentric fragments) and, to a lesser extent, of chromosomal aberrations (paired acentric fragments). In mice, toxic concentrations (100-300 mg/kg bw) of 2,4-D administered as a single oral dose significantly increased the frequency of aberrant metaphases (2-4-fold); single fragments were the primary aberration (Pilinskaya, 1974). 2,4-D had no effect on cultured cells nor on bone marrow after its oral administration to rats (Styles, 1973).

There was no detectable increase of micronuclei in the erythrocytes of mouse bone marrow after i.p. administration of 100 mg/kg bw 2,4-D (Jenssen & Renberg, 1976).

2,4-D did not increase dominant lethal mutations in mice when given as a single i.p. injection of 125 mg/kg bw, or when given orally on 5 successive days for a total dose of 75 mg/kg bw (Epstein *et al.*, 1972).

(b) Man

In a case of suicide of a 23-year old farming student, the total amount of 2,4-D in the body was estimated to be no less than 6 g, corresponding to a dose of about 80 mg/kg bw. All organs showed marked acute congestion. Severe, degenerative changes of the ganglion cells were found

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in the central nervous system (Nielsen *et al.*, 1965).

A man who accidentally ingested about 30 ml of concentrated weedkiller containing 50% of a thiolcarbamate and 36% 2,4-D isooctyl ester in aqueous solution exhibited fibrillary twitching and paralysis of the intercostal muscles. There was evidence of generalized skeletal muscle damage, as indicated by marked elevation of the levels of several muscle enzymes, as well as haemoglobinuria and myoglobinuria. Recovery was complete after several months (Berwick, 1970).

No adverse effects were reported in a man who took 500 mg 2,4-D orally daily for 3 weeks (approximately 8 mg/kg bw/day) (E.J. Krauss, cited in Mitchell *et al.*, 1946).

When 2,4-D was used as a treatment for a patient in the terminal stages of disseminated coccidioidomycosis, no side-effects were observed following 18 i.v. doses over 33 days: in the 7th to the 17th injection the dose of 2,4-D was 800-960 mg (about 15 mg/kg bw), and the 18th dose was 2000 mg (about 37 mg/kg bw). A 19th and final dose of 3600 mg (67 mg/kg bw) produced symptoms of toxicity comprising a semi-stuporous status, fibrillar movements, hyporeflexia and urinary incontinence, which persisted for 24 hours. Seventeen days after the last administration, the patient died (Seabury, 1963).

Three cases of peripheral neuropathy have been reported following spraying of 2,4-D. Initial symptoms were nausea, vomiting, muscular weakness, diarrhoea and swelling or aching of the feet and legs, with malaise and headache, which persisted for 10-20 days. In one case, paresthesia in the extremities and pain in the legs appeared within 4-5 days, followed by twitching of the muscles in the calves and arms; fasciculations became generalized, without neurological or electromyographical changes. In the second case, one week after a second exposure to 2,4-D, numbness and aching of the fingers and toes occurred, followed 6 weeks later by a well-developed neuropathy. In the third case, after a second exposure, severe pains occurred in the legs, with swelling of the metacarpal joints of both hands; 5 months later flaccid paraparesis was seen (Goldstein *et al.*, 1959).

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Similar case reports of poisoning in agricultural workers have been reported following spraying of 2,4-D (Monarca & Di Vito, 1961; Paggiaro *et al.*, 1974; Todd, 1962). The main initial symptoms were muscular weakness, vomiting, diarrhoea, fever, hyperthermia and tachycardia. In two cases, neurological symptoms occurred, which continued for 40 days to 2 years after exposure and included loss of deep-tendon reflexes and paralysis of thigh and leg muscles.

Assouly (1951) reported that workers employed in the fabrication of 2,4-D developed symptoms of somnolence, anorexia and gastralgia, increased salivation, a sweet taste in the mouth, a sensation of drunkenness, heaviness of the legs and hyperacusia.

Subjective clinical symptoms reported among workers using various esters and salts of 2,4-D included rapid fatigue, headache, loss of appetite and pains in the region of the liver and stomach. Sensitivity to taste and smell was lowered (Fetisov, 1966).

Bashirov (1969) examined 292 persons (248 men and 44 women) engaged in the manufacture of the amine salt and butyl ester of 2,4-D, with exposure ranging from under 5 years to 6-10 years (for 194 and 98 persons, respectively); 63% of these workers complained frequently of weakness, rapid fatigue, headache or vertigo. About 20% had disturbances of the cardiovascular system (mainly hypotension and bradycardia) and of the digestive organs (dyspeptic symptoms and gastritis). The various liver dysfunctions that were found were more pronounced in workers with longer exposures to the herbicides.

Changes in metabolic processes were observed in workers engaged in the production of 2,4-D, in particular, increases in blood cholesterol content, with no change in the lecithin:cholesterol ratio. Decreases in serum albumins, increases in globulins, decreases in blood sugar levels and altered responses to sugar loads were also noted (Lukoshkina *et al.*, 1970).

In a report on 220 workers exposed in a manufacturing plant to 30-40 mg/day 2,4-D for periods ranging from 0.5 to 22 years, Johnson (1971) stated [without providing any supporting evidence] that no 'meaningful'

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differences were observed in [unspecified] clinical assessment, in comparison to a control group of 4600 men, and that no chromosomal effects were observed in 10 workers whose chromosomes were karyotyped.

Feldmann & Maibach (1974) studied the absorption through the skin of 4 µg/cm² ^{14}C-labelled 2,4-D dissolved in a small amount of acetone. ^{14}C activity was measured in urine over a 5-day period and compared with that in urine after i.v. administration of the compound: urinary excretion of 2,4-D after i.v. administration was 100% of the dose in 120 hours, while excretion after topical administration was 5.8% of the dose.

Kohli *et al.* (1974) administered 5 mg/kg bw pure 2,4-D in a gelatin capsule with water to 6 healthy male volunteers, aged 22-30 years. None of the subjects complained of any ill-effects; no changes in blood pressure, pulse rate, haemoglobin content or total or differential white cell counts were observed. 2,4-D was absorbed fairly rapidly; the highest concentration in blood was reached in 7-24 hours. In urine, 2,4-D was present as early as 2 hours after ingestion, and more than 75% was excreted in 96 hours without undergoing transformation in the body.

In 5 male volunteers given a single oral dose of 5 mg/kg bw 2,4-D, the half-life in the plasma was 11.7 hours, and elimination in the urine occurred with a half-life of 17.7 hours. About 82% was excreted as such and 12.8% as a conjugate (Sauerhoff *et al.*, 1976).

For details of adverse toxicological effects resulting from the spraying of 'Agent Orange' (a 50:50 mixture of the *n*-butyl esters of 2,4-D and 2,4,5-T, contaminated with up to 30 mg/kg or more TCDD) and other herbicides in Vietnam, see monograph on chlorinated dibenzodioxins, p. .

3.3 Case reports and epidemiological studies

Axelson & Sundell (1974) reported that in a cohort study of Swedish railway workers exposed to a variety of herbicides a significant, two-fold excess of all cancers was observed in exposed workers, as compared with the national average. The situation was difficult to evaluate because of the combined exposure of many workers to more than one herbicide. Most of the excess, however, seemed to be due to exposure to 3-amino-1,2,4-triazole

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(amitrole); within the subgroups that had been exposed to phenoxyacids (2,4-D and/or 2,4,5-T), only a small difference was detected: 5 cancers at all sites observed *versus* 2.8 expected. The authors stated, however, that use of 2,4-D and 2,4,5-T had probably been higher than that they could trace.

See also monograph on chlorinated dibenzodioxins, p. 41.

4. Comments on Data Reported and Evaluation

4.1 Animal data

2,4-D and several of its esters were tested in rats and mice by oral administration and in mice by subcutaneous administration. All of these studies had limitations, due either to inadequate reporting or to the small number of animals used. Therefore, although increased incidences of tumours were observed in one study in which rats received 2,4-D orally and in another in which mice received its isooctyl ester by subcutaneous injection, no evaluation of the carcinogenicity of this compound could be made.

4.2 Human data

The results of the single cohort study of a small number of workers exposed to various herbicides, including 2,4-D, 2,4,5-T and 3-amino-1,2,4-triazole (amitrole)[1], are not sufficient to evaluate the carcinogenicity of 2,4-D to man [Because 2,4-D may be used with 2,4,5-T, which is contaminated with 2,3,7,8-tetrachlorodibenzo-*para*-dioxin, see also monograph on chlorinated dibenzodioxins, p. 41].

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[1]See also IARC, 1974.

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5. References

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MINUTES*

Fifth Meeting of the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens
September 26, 1977
Building 31 Conference Room 10
NIH, Bethesda, MD

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* These minutes have not yet been accepted by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens.

the occurrence of rare tumors. Dr. Griesemer noted that, whenever significant, historical control data are included and analyzed in bioassay reports.

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Review of Bioassay Reports*

1) *Picloram*. Dr. Brown served as the primary reviewer of the report describing the bioassay of Picloram. In summarizing the findings, Dr. Brown said that hepatic neoplastic nodules were found in both male and female treated rats. In the females the incidence was significant in the test for a positive dose-related trend and the incidence in the high-dose group was statistically significant when compared to pooled controls. The bioassay reports concluded, he said, that "the findings are suggestive of the ability of the compound to induce benign tumors in the livers of female Osborne-Mendel rats."

Dr. Brown was particularly critical of the small number of matched control animals used, that the ten percent impurities in the Picloram were not analyzed, and that the diagnosis of "neoplastic nodules" had not been confirmed by additional pathologists. He felt that the conclusion drawn in the report on the carcinogenicity of Picloram was inordinately conservative. He thought that the findings lent enough support to the hypothesis that Picloram may be a carcinogen. He added that consideration should be given to repeating the bioassay using a more appropriate design.

A lengthy discussion ensued on the biological nature and significance of the neoplastic liver lesions found in the treated rats. Dr. Brown recommended that the lesions be reviewed by additional pathologists. Mr. Samuels said that Picloram should be regarded as a carcinogen since the liver lesions, if not malignant, are at least a step in the pathogenesis of cancer. In regard to the ten percent impurities in the tested material, Dr. Griesemer noted that the Picloram was the technical grade product to which humans are exposed. Dr. Brown moved that the Subgroup accept his critique of the bioassay of Picloram. Dr. Weisburger seconded the motion and it was accepted unanimously.

2) *Chloramben*. Dr. Wolfe served as the primary reviewer for the report describing the bioassay of Chloramben.

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* Single copies of the reports may be requested from the Office of Cancer Communications, National Cancer Institute, Building 31, Room 10A21, National Institutes of Health, Bethesda, Maryland 20014.

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4369. [illegible] USA., G. A. WICKS, and C. R. [illegible] picloram, and 2,3,6-TBA [illegible] acid (dicamba), 4-amino-3,5-dichloro-2-picolinic acid (picloram), and 2,3,6-trichlorobenzoic acid (2,3,6-TBA) [illegible] tions across Nebraska. Herein, the persistence was determined in the field by assays of field beans (Phaseolus vulgaris L.) and soybeans (Glycine max (L.) Merr.). Picloram and dicamba showed the most persistence, 2,3,6-TBA was intermediate, and dicamba showed the least persistence across Nebraska. The residual phytotoxicity of picloram in soil was not necessarily dependent upon rainfall but was greatest in the fine textured soil with higher organic matter content.

4370. SCIFRES, C. J., Dep. Range Sci., Tex. Agric. Exp. Stn., Texas A&M Univ., College Station, Tex., USA., R. R. HAHN, and M. G. MERKLE. Dissipation of picloram from vegetation of semiarid rangelands. WEED SCI. 19(3): 323-332. illus. 1971.--Sprays of 25 ppm 4-amino-3,5,6-trichloropicolinic acid (picloram) usually were detected on grasses primarily buffalograss (Buchloe dactyloides (Nutt.) Engelm.) and blue grama (Bouteloua gracilis Willd. ex HBK Lag. ex Griffiths) immediately after application of 0.26 kg/ha picloram + 0.26 kg/ha 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in northwest Texas. Less than 1 ppm of picloram usually was detected in grass tissue 30 to 60 days after treatment. At 1 location, detectable picloram increased in grass tissue from 32 to 60 days after application. Increases of picloram in aerial grass tissue were attributed to root uptake during a flush of vegetative growth. Picloram dissipation from grasses was not affected by irrigation to runoff at 10, 20, or 30 days after application. Detectable picloram was reduced by 93% in herbaceous, broadleaf species in 30 days after application. Treated sand shinnery oak (Quercus havardii Prohl.) leaves at the soil surface caused a slight increase of picloram in surface litter

4371. SCIFRES, C. J., R. R. HAHN, J. DIAZ COLON, and M. G. MERKLE. (Tex. A and M. Univ., College Station, Tex. 77843 USA. Picloram persistence in semiarid rangeland soils and water. WEED SCI. 19(4): 381-384. illus. 1971.--Residues in soil following application of 0.25 lb A of 4-amino-3,5,6-trichloropicolinic acid picloram to semi-arid rangelands, usually were restricted to the top 12 in. for 60 days. Five ppb or less picloram were detected below 12 in. at 120 to 180 days after application. Wet picloram was detected 5 in. after application at 6 to 18 in. deep at the lower ends of plots with 3% slopes than in plots with 0, 1, or 2% slopes. Runoff water from plots irrigated 10 days after treatment contained 17 ppb picloram. Irrigation or rainfall at 20, 30, or 45 days after picloram application resulted in less than 1 ppb picloram in runoff water. No more than 1 or 2 ppb picloram were detected after dilution of runoff water in large ponds.

4372. RUBIN, EMANUEL, and CHARLES S. LIEBER. Mt. Sinai Sch. Med., City Univ., New York, N. Y., 10029, USA. Alc. Exp. Alcohol. and Drug. SCIENCE WASHINGTON 172 3984: 1132-1135. illus. 1971.--The extent of drug metabolism in the chronic consumption of ethanol may explain, in part, the accentuated tolerance of alcoholics, whereas, for drugs such as barbiturates. The inhibition of drug metabolism by acute administration [illegible] effects of ethanol in the CNS system, plasma [illegible] and sensitivity of inebriated persons to drugs. Ethanol can be [illegible] on the list of drugs that are metabolized by hepatic microsomes. Chronic administration of ethanol and pentobarbital both produce the following: increased SER (smooth endoplasmic reticulum), increased activities of drug-metabolizing enzymes in the hepatic microsomes, increased proliferation of its own metabolism, increased rate of metabolism of other drugs, and increase in cholesterol biosynthesis. Acute administration of ethanol or other compounds leads to inhibition of the metabolism of other drugs by hepatic microsomes and other components in vivo and in vitro. Both ethanol and other drugs [illegible] microsomes (MEO) stimulate an activity of certain drugs and ethanol by hepatic microsomes leads to mutual potentiation of their metabolism.

4373. [illegible]

4375 [illegible]

4376 [illegible]

4377 [illegible]

4378 [illegible]

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HEALTH AND ENVIRONMENTAL RESEARCH
DOW CHEMICAL U.S.A.

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DATE REPORT ISSUED: September 28, 1977

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RESULTS OF A TWO YEAR CHRONIC TOXICITY AND ONCOGENICITY STUDY OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) IN RATS

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September 28, 1977

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SUMMARY AND CONCLUSIONS

Male and female rats were maintained continuously on diets supplying 0, 0.1, 0.01 or 0.001 µg TCDD/kg body weight/day for 2 years. These dose levels were equivalent to dietary levels of ~2,200, 210 and 22 parts per trillion (ppt) of TCDD. Ingestion of the high dose level of 0.1 µg TCDD/kg/day 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, gamma-glutamyl transferase, glutamic pyruvic transaminase and morphologic changes primarily of the hepatic, lymphoid, respiratory, oral and vascular tissues of the body. These morphologic changes included an increase in the incidence of hepatocellular carcinomas of the liver (females only) and squamous cell carcinomas of the lung, hard palate/nasal turbinates or tongue. Also seen at the high dose level was a significant decrease in the incidence of numerous age-related lesions, including a decrease in tumor incidence of the pituitary, uterus, mammary gland, pancreas and adrenal gland. The incidence and severity of chronic renal disease was also decreased. Terminal content of TCDD in female rats given this high dose level averaged 24,000 ppt in the liver and 8,100 ppt in the fat. Ingestion of the intermediate dose level of 0.01 µg TCDD/kg/day caused lesser degrees of toxicity, including primarily an increased urinary excretion of porphyrins (females only), liver alterations, including an increased incidence of hepatocellular nodules

and lung alterations in the form of an increased incidence of focal alveolar hyperplasia. Females given this dose level had a decrease in the incidence of tumors of the uterus. Terminal liver and fat content of TCDD averaged 5,100 and 1,700 ppt, respectively.

Ingestion of 0.001 µg/kg/day caused no discernible effects in male rats and a statistical increase above the background incidence of swollen hepatocytes in female rats that was judged to be reversible in nature and likely associated with the hepatic detoxification process for TCDD. At this dose level, both liver and fat of female rats contained approximately 540 ppt TCDD.

Evaluation of the data collected in this study indicates that doses of TCDD sufficient to induce severe manifestations of toxicity increase the incidence of some types of tumors in rats, while reducing the incidence of other types. No increase in tumors occurred in rats receiving sufficient TCDD during the two-year study to induce mild manifestations of exposure.

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TCDD ACCIDENT AT A CHEMICAL FACTORY IN THE NETHERLANDS

At the Philips-Duphar 2,4,5-T factory, sodium trichlorophenate was synthetized by the hydrolysis of 1,2,4,5-tetrachlorobenzene using sodium-hydroxide and methanol at a temperature of approx. 170°C and a pressure of approx. 22 atm. Temperature and pressure within the autoclave (capacity 900 litres) were registered automatically, but the machinery was hand-operated.

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Description of events

On March 6, 1963, during the start of the synthesis, the reaction became uncontrollable. Because of the "explosion" the safety-valve blew as well as the lid of the autoclave, and the contents of the vessel contaminated the inside of the 4,000 m^3 building, that became covered with soot. Nobody was injured by the explosion and there was no fire. It was estimated that the final temperature in the autoclave was 400-450°C and the pressure > 80 atm.

Concerning the cause of the accident, no mechanical failure could be found, but a mistake of the operators could not be excluded.

Immediately after the accident, the building was closed. Animal experiments were started since the management knew of problems encountered with 2,4,5-T production in the U.S.A. and in the Federal Republic of Germany.

Animal experiments

On March 7, 1963, the first animal experiment was started. Three groups of 10 rats were used. The first group was placed in cages inside the building (exposure by inhalation). The second and third group was treated respectively by the oral and dermal route with 1 g of soot per kg body weight. Five animals

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of the first group were killed after 3 days. At macroscopic examination, no lesions were observed. Microscopically, lipid accumulation was present in the liver. Of the remaining animals of this group, one died after 11 days. Of the dermally exposed rats, 2 out of 10 died within 11 days. All remaining animals were killed after 11 days. Microscopically, there was lipid accumulation in the liver. In a second dermal toxicity study, started on March 18, 10 rats were exposed to 1 g of soot/kg body weight. Four rats died after 6, 8, 13 and 14 days. Again at microscopic examination, there was lipid accumulation in the liver.

At the end of March the efficacy of the cleaning of the building was studied; 75 rats were placed inside the building (exposure by inhalation). Groups of 25 rats were killed after 1, 2 and 3 weeks. There was no mortality; microscopically there was lipid accumulation in the liver. In addition, 6 rabbits were placed in the building. After 3 weeks they were removed from the building. One animal died one week later. From these results it was concluded that the building was still contaminated despite thorough cleaning.

In June, another experiment was done. At that time, the activities in the building included removal of paint from machinery, walls etc. A total of 48 rats were kept in open cages. In this manner, dust, peeled-off paint etc. contaminated the inside of the cages. Six of these animals died. At pathological examination, thymus atrophy, enlarged testes and lipid accumulation in the liver was observed.

At the end of 1963, after the reconstruction activities, a final toxicity study was carried out in the building, using rats and rabbits. From the results it appeared that the factory-building was still contaminated. Therefore, the final decision was reached to close the building.

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Analyses

In March 1963, the compound TCDD was unknown to the plant management. At spectrophotometric analysis, a peak occurred in the toxic material that was present in the building. Using this peak, the effect of the different cleaning-phases was studied. From more recent data it is known that this peak did not represent TCDD, but possibly a phenolic compound.

In 1966, the GLC method for TCDD analysis, as developed by Dow Chemical Co., was introduced. TCDD levels in the soot that was formed during the explosion were 1,000 ppm ± 20% (samples obtained from the autoclave as well as from the building). Based on these levels it is estimated that 200-500 g TCDD were released in the building. Samples obtained after the cleaning and reconstruction of the building were also analysed. TCDD levels in samples of floor scrapings ranged from 0 to 3 ppm; in paint from columns 6 to 25 ppm, and in paint from machinery 10 to 30 ppm. On the basis of these data it is estimated that between 20 and 200 g TCDD was still present in the building after the thorough cleaning and reconstruction activities.

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Cleaning and reconstruction activities

As the results of the first toxicity study were not alarming, dust and soot in the building was removed and the building was cleaned by Philips-Duphar employees, with water and a surface-active compound. Since animal experiments and analyses (measurements at 290 nm) showed that the building was still contaminated, it was tried again to clean the building. During the months April and May, factory employees sprayed the building (for a total of 11 times) with 1,000 - 1,500 litres of 2% sodium hydroxide, followed by spraying with water and a surface-active compound. As a result of this, analyses at 290 nm showed that the building was practically clean. Next, the building was ventilated with fans (capacity of 40,000 m^3/hr).

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During the months May until July, reconstruction activities began. Paint was removed from machinery, walls etc., as was insulating material. Next, the building was painted and damaged machinery was replaced. During this period, also non-factory personnel was hired: a clean-up crew for removal of paint (this group made use of organic solvents) as well as personnel for plumbing, painting and insulating.

Protective clothing for all workers in the building included a mask (depending on the nature of the activities; ranging from a dust mask till a fresh-air helmet), overall, boots and gloves. In particular the hygienic measures (shower, changing of clothes) were very strict, but difficult to enforce for the non-factory employees.

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Demolition of the building

At the end of 1963, the building was closed, as decontamination was impossible. In 1973 the factory-building was dismantled, embedded in concrete and dumped in the Atlantic Ocean. Working with fully protective clothing (air-supplied suit with integral helmet) proved a suitable method to prevent TCDD exposure. Details of the safety measures are published by Dalderup (T.soc.Geneesk. 52 (1974) 582 and 616).

Medical data

A total of 106 men are likely exposed to TCDD as they were in the building during the period March-July 1963. These workers can be divided into three groups:

A. Factory employees (exposed during all phases of the cleaning and reconstruction activities).

B. Clean-up crew (non-factory personnel; exposed during the months May-July).

C. Personnel for plumbing, painting and insulating (non-factory workers; exposed during the months May-July).

During the explosion, 2 operators were present in the building. With the exception of these men, who were likely exposed by the inhalatory route, all other workers are probably exposed by dermal contact, because of the use of masks. This is possibly the reason that chloracne was the prominent feature of TCDD exposure. Liver function tests (thymol turbidity, serum glutamic-pyruvic and glutamic-oxaloacetic transaminases) 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 to 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.

Group A. A total of 44 factory employees had worked in the building. All were medically examined (during a period until March 1964; and further on at least once a year); 26 had chloracne. In 1977, of the 27 men who were still employed, 20 still had acne. Of the remaining 17 men, 3 died and 14 men went elsewhere or are retired. One man died in 1964, at the age of 54, from pancreas carcinoma with extensive metastases in the liver. He died 14 months after slight exposure (cleaning of slightly contaminated room, next to the autoclave room). Already before the accident he complained of abdominal pain; he had no chloracne. One died in 1977, at the age of 69, from a second myocardial infarction and one man died in 1972 from a traffic accident, at the age of 76.

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Group B. Of a total of 18 men of the clean-up crew, 16 were examined in 1963. Ten of these 16 had chloracne. In 1977, 13 out of 17 men were alive, but no medical data are available (one man could not be traced). Four died: two of them in 1966 (sudden death, probably from myocardial infarction, at ages of 41 and 53 years); one died in 1967 at the age of 44 (progressive shortness of breath, not known whether of pulmonary or cardiac origin); one died in 1976 at the age of 65 (sudden death, probably myocardial infarction).

Group C. Of a total of 44 men in this group of non-factory workers, 16 were examined in 1963, eight had chloracne. In 1977, 31 out of 32 were alive; one died in 1969 (sudden death, probably myocardial infarction, at the age of 50).

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From a total number of 93 men (of the remaining 13, no data are available), 7 died a natural death, in the period March 1963 till October 1977, between the age of 40 and 69 years. This does not seem to indicate an excess mortality, in comparison with mortality data of men of similar age group in the community of Amsterdam although this is of course not a matched control group.

However, taking group B (clean-up crew) separate, of 17 persons, 4 died which possibly indicates excess mortality. Moreover, three of these four persons died a cardio-vascular death. Deaths from malignancies were not observed, and morbidity data are not available. The causes of death of the group as a whole is unexpectedly drawn towards cardio-vascular death. In the male Amsterdam population, deaths in the same age groups occur more from malignancies than from cardio-vascular origin. The relative high incidence of chloracne and mortality in group B versus groups C and A, is

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remarkable. Especially, since group B was in the building at a time that the building was certainly less contaminated then early after the accident when factory employees removed contaminated dust and soot. Possibly, group B had increased exposition to TCDD because of the use of organic solvents to remove paint. According to their profession, they had in any case multiple exposures to more or less toxic substances both before and after the exposure to TCDD.

Proposed future medical studies

During 1978, the exposed factory employees (of whom 27 are still employed), will be medically examined. Laboratory tests will include haematological examination, liver function tests (serum glutamic-pyruvic and glutamic-oxaloacetic transaminases, lactic dehydrogenase, alkaline phosphatase), serum cholesterol levels, serum immunoglobulin levels, and possibly the in vitro responsiveness of lymphocytes to mitogens, and levels of porphyrins or porphyrin precursors in the urine. It will be tried to compare these data with a matched control group (non-exposed factory employees of similar age). Of all men traced, mortality data will be collected in future years. It will also be tried to obtain morbidity data, but medical examination of the non-factory employees is not possible.

The present report presents preliminary data, detailed information

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as well as the results of the proposed study will be reported in the near future.

November, 1977

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FORMATION OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS DURING COMBUSTION OF A 2,4,5-T FORMULATION

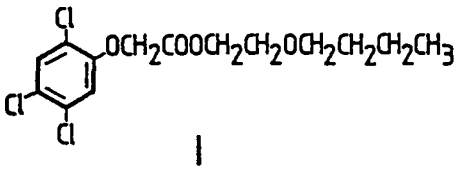
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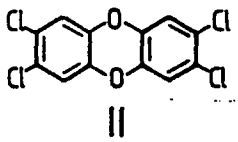
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The use in forestry and agriculture of products derived from 2,4,5-trichlorophenoxy acids (2,4,5-T), e.g. I, has given rise to much concern during a number of years. The major cause of this concern rose from the findings of the highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (II) as a contaminant in many of these preparations.[1,2] Toxicity tests with II have given LD_{50} values of 2 µg/kg in guinea pigs, the most sensitive species known, and around 80µg/kg in monkeys.[3] The compound also causes irreversible liver damage and is a potent inducer of enzyme systems. In man exposed to II one symptom is chloracne, a severe skin disease.[4,5]



I



II

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The presence of II in the chlorophenoxy acids is due to its formation as a byproduct in the preparation of 2,4,5-trichlorophenol used as a starting material for the manufacture of 2,4,5-T products. [5,6] It might also be formed during the coupling reaction between the sodium salt of 2,4,5-trichlorophenol and the appropriate α-chloroacid ester.

Although the content of II in 2,4,5-T preparations is nowaday under strict regulations and control, there is still some uncertainty about the possible formation of II from 2,4,5-T products during fires or by photochemical transformations. Laboratory experiments have shown that large amounts of II are formed when the sodium salt of 2,4,5-trichlorophenoxyacetic acid or of the 2-(2,4,5-trichlorophenoxy)propionic acid is heated in a melt at 500-600°. [7,8] The present investigation was performed in order to establish whether any formation of II might occur during burning of 2,4,5-T products, i.e. under simulated environmental conditions. These experiments were carried out in a combustion installation for pilot scale tests with possibilities for variation of conditions such as temperature, transit time and available oxygen. [9]

In this study account was also taken of the possible formation of other polychlorinated dibenzo-p-dioxins (PCDD) than II and also formation of polychlorinated dibenzofurans (PCDF). Although II seems to be the most toxic isomer of the chlorinated dibenzo-p-dioxins, many pentachloro-(5CDD) and hexachlorodibenzo-p-dioxins (6CDD) show similarly low LD_{50} values. [3] It was recently shown that some preparations of 2,4,5-T in addition to II also contain pentachlorodibenzo-p-dioxins and tetrachloro- (4CDF) and pentachlorodibenzofurans (5CDF). [10] Chlorinated dibenzofurans have been reported to show almost the same high degree of toxicity as the PCDD. [11]

EXPERIMENTAL

Analysis of the herbicide formulation. The 2,4,5-T product used was specified by the manufacturer to contain 500 g/l of 2-butoxyethyl 2,4,5-trichlorophenoxyacetate (I) in an inert hydrocarbon solvent. The concentration was determined by GLC using a synthetic standard. Free 2,4,5-trichlorophenoxyacetic acid in the formulation was determined by GLC as the methyl ester after appropriate dilution of the product with hexane-diethyl ether (1:1) followed by treatment with diazomethane. 2,4,5-Trichloro- and pentachlorophenol were determined as their methyl ethers after extraction from a dichloromethane solution of the formulation (0.1 g in 3 ml CH_2Cl_2) with 1 M potassium hydroxide solution (3 x 3 ml). After acidification and re-extraction with diethyl ether (3 x 3 ml) the extract was treated with diazomethane and

after appropriate dilution used for quantitation by GLC.

Analyses for chlorinated dibenzo-p-dioxins and dibenzofurans were performed by GC-MS after clean-up of the 2,4,5-T preparation by the use of a charcoal-polyurethane adsorbent according to Huckins et al.[10] with the exception that toluene (100 ml) was used to elute PCDD and PCDF.

Burning experiments. The pilot scale plant has been described earlier.[9] In a first series of nine experiments the possibility to destruct I was studied. The temperature was varied between 300° ("open fire") and 950° with transit times of 2-3 seconds. The formulation was, in all experiments except under "open fire" conditions, dissolved in isopropanol and injected through an atomizing nozzle into the oven, charged with sawmill wood chips. In the open fire experiment the formulation was mixed with sawdust and wood chips (30 kg) and introduced into the oven.

In a second series of experiments the formation of chlorinated dibenzo-p-dioxins and chlorinated dibenzofurans from I was studied. Three experiments (1-3 in Table 2) were carried out with a mixture of 30-75 kg sawmill wood chips and 1 kg 2,4,5-T formulation which was burned at temperatures between 500° and 850° with transit times of about 0.7 seconds in the burning zone. In experiments 4-6 a mixture of 50-100 kg wood chips, 8-10 kg dry leaves and 1 kg of the formulation was burned. One experiment (7) was carried out under pyrolytic conditions, i.e. in an oxygen-deficient atmosphere and one (8) as a simulation of an open fire. In the latter experiment sawmill wood chips (30 kg) and leaves (8 kg) together with 1 kg of the formulation were allowed to burn without any attempts to raise the temperature.

Sampling and clean-up of PCDD and PCDF. Equipment made entirely of glass were used for isokinetic sampling of emissions from the oven.[9] Particulate matter was collected on filters of glass wool which were eluted with toluene for analysis. Gaseous components were collected on concentration columns filled with Chromosorb W coated with 30% Apiezon M.[12] The columns were eluted with hexane and toluene.

From each experiment the three extracts were combined and the solvent evaporated. The residue was dissolved in 1 ml hexane and chromatographed on 1g basic alumina (Aluminiumoxid 90, aktiv, basisch, Merck, activated at 250° for 2 h). The column was eluted with hexane (10 ml, fraction A) to remove non-polar co-extractives followed by ethyl acetate (10 ml, fraction B) to elute PCDD and PCDF. Recovery experiments showed that PCDD added to the extracts were recovered to about 70% in fraction B.

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Analyses of PCDD and PCDF. The dioxins and furans were analysed by mass fragmentography on a HP 5930 A GC-MS with a HP 5933 A computer system. In each run five chlorination degrees of PCDD or PCDF with two ions each and one internal standard were analysed. As internal standard was used a 2,3,7,8-tetrachlorodibenzo-p-dioxin with four ^{37}Cl and four deuterium atoms (M=332).[13]

The reference compounds available for the quantitations were II, a mixture of two 6CDD (unknown isomers), 8CDD, 4CDF (2,3,7,8-) and 8CDF. Since these do not cover all the chlorination degrees determined, interpolations had to be made. Thus the area measured for one m/e value was corrected to a total area which correspond to all m/e values in the chlorine isotope cluster of that ion. The amount injected divided by this total area give fairly constant response factors for the references available.

RESULTS AND DISCUSSION

Impurities in the herbicide formulation. The results of the analyses of the formulation are given in table 1. A notably high concentration of the free 2,4,5-trichlorophenoxyacetic acid was found. In addition to the listed results, the specified content of the active substance (I) (500 g per l formulation) was verified.

Table 1. Impurities in the herbicide formulation used for combustion experiments.

Compound [1]		g per g formulation
2,4,5-Trichlorophenoxyacetic acid		$4.7 \cdot 10^{-2}$
2,4,5-Trichlorophenol		$2.3 \cdot 10^{-4}$
Pentachlorophenol		$1.2 \cdot 10^{-5}$
Dioxins	4CDD	$1.8 \cdot 10^{-8}$
	5CDD	$7 \cdot 10^{-9}$
	6CDD	$<5 \cdot 10^{-9}$
	7CDD	$<5 \cdot 10^{-9}$
	8CDD	$5 \cdot 10^{-9}$
Furans	4CDF	$1.3 \cdot 10^{-7}$
	5CDF	$8 \cdot 10^{-9}$
	6CDF	$<5 \cdot 10^{-9}$
	7CDF	$<5 \cdot 10^{-9}$
	8CDF	$<5 \cdot 10^{-9}$

1) 4CDD, 5CDD etc. comprise all isomers containing 4, 5 etc. chlorine atoms, respectively.

The content of 4CDD is well below concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin accepted by the Swedish authorities.

The level of 4CDF is much higher than that of 5CDF and also that of 4CDD. In an earlier investigation [10] the same dimers were found, but no quantitative data were given.

Destruction of 2-butoxyethyl 2,4,5-trichlorophenoxyacetate. The experiments performed to study the destruction of I showed that, with one exception, 99,995% of I is destructed at 400-500°C and a transit time of 2 sec. However, when wood chips with a very high water content was burned under "open fire" conditions, the temperature in the flue gas duct decreased temporarily to around 100°C and only 92% of I was destructed.

Emission of PCDD. The conditions in the burning experiments and the emitted amounts of PCDD and PCDF with 4 to 8 chlorine atoms are given in table 2.

Table 2. Emitted amounts of PCDD and PCDF from combustion of a herbicide formulation containing 2-butoxyethyl 2,4,5-trichlorophenoxyacetate.

Experiment no		1	2	3	4	5	6	7	8
Temperature (°C)		500	675	850	500	625	850	750	100
Transit time (s)		0.7	0.6	0.7	0.8	0.8	0.9	3.7	1.0
O_2 in flue gas (%)		15.0	12.0	10.5	13.0	12.0	7.5	0.0	17.5
CO_2 in flue gas (%)		2.6	4.0	6.1	4.1	7.2	7.7	9.0	0.9
CO in flue gas (%)		0.0	0.0	0.0	0.0	0.0	0.0	5.5	0.0
Emitted amounts (mg/kg formulation)									
Dioxins	4CDD	3.0	2.3	≤0.1	1.3	0.4	0.2	1.4	2.5
	5CDD	≤0.4	≤0.5	≤0.2	≤0.6	≤0.3	≤0.3	1.2	≤0.5
	6CDD	2.0	≤0.5	≤0.2	≤0.6	≤0.3	≤0.3	0.8	≤0.5
	7CDD	≤0.4	≤0.5	≤0.2	≤0.6	≤0.3	≤0.3	0.3	≤0.5
	8CDD	≤0.4	≤0.5	≤0.2	≤0.6	≤0.3	1.4	0.05	≤0.5
Furans	4CDF	≤0.2	8.0	1.3	0.6	0.2	2.9	7.6	1.0
	5CDF	1.8	7.1	1.9	1.3	≤0.3	4.2	5.7	0.5
	6CDF	1.3	2.8	1.8	≤0.6	≤0.3	4.9	1.7	≤0.4
	7CDF	≤0.7	≤0.7	1.4	≤0.6	≤0.3	3.4	≤0.2	2.3
	8CDF	≤0.7	≤0.7	≤0.6	≤0.6	≤0.3	3.9	≤0.1	≤0.4

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Background values were obtained after combustions without the herbicide. About 0.03 mg 4CDD were emitted during half an hour, which corresponds to the period required to burn 1 kg of the formulation in the experiments. Higher chlorinated dioxins could not be detected.

The results show that, with a few exceptions, only 4CDD was detected. This emission decreases with increased temperature and transit time. The very low temperature under "open fire" conditions (experiment 8) does not give extremely large 4CDD amounts. The explanation would be that the formation of dioxins is inefficient at this low temperature or that the formed dioxins are effectively destructed during the relatively long transit time. The formation of higher homologues of PCDD in some cases, expecially under the oxygen-deficient conditions in experiment 7, can not be explained at present.

The content of PCDD in the formulation can not explain the amounts of these compounds present in the flue gases. Furthermore, combustion experiments with chlorophenol formulations carried out by us [14] indicate that the content of chlorophenols in the 2,4,5-T formulation can not give the PCDD amounts found in the present study. The 2,4,5-trichlorophenoxyacetic acid in the formulation could, however, possibly be the precursor if extrapolations are made to earlier studies which showed that II was formed from the free chlorophenoxy acid in about 1% yield when heated as a melt to 500-600°C. [7,8]

Emission of PCDF. The results of the PCDF analyses are presented in table 2. The background levels of PCDF were unexpectedly high and emitted amounts up to 0.5 mg during a normal experiment run time have been detected. The results given in table 2 are not corrected for these background values and at least the low values must be regarded as unreliable.

In most experiments the emissions of PCDF are higher than that of the PCDD. These amounts of furans can not be explained by the content in the formulation. It is unlikely that the chlorophenols present in the formulation have any significance for these emissions. [14]

Under the experimental conditions used the emission of PCDF can not be correlated to either temperature or transit time.

CONCLUSIONS

Under normal combustion temperatures (>500°C) the investigated 2,4,5-T ester was destructed to 99.995%.

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It is evident that under certain conditions, the combustion of a 2,4,5-T formulation may lead to a formation of small amounts of PCDD and PCDF. The role of the free 2,4,5-trichlorophenoxyacetic acid in this context is not clear but should be further investigated.

The emission of PCDD decreases with increased temperature and transit time. The highest emitted amount was 3 mg 4CDD per kg formulation at a temperature of 500°C.

For the PCDF emissions up to 8 mg per kg formulation have been measured (4CDF at 675°C) but no correlation to either temperature or transit time can be found.

The concentration of the 2,4,5-T ester in the burnt material in the present study (about 10^{-2} g/g wood chips) is higher than the concentrations found after normal use in the environment (10^{-4} -10^{-3} g/g dry leaves [15,16]). A high concentration will favour the formation of dimers and smaller amounts are to be expected from e.g. a forest fire. The largest formation of 4CDD found in this work should correspond to a formation of about 1 µg 4CDD per m^2 in a forest fire directly after application of the investigated herbicide formulation.

Even if the formation in the environment is probably smaller than this amount, extreme caution is necessary not to disseminate these very toxic xenobiotics.

ACKNOWLEDGEMENT

We are indebted to Dr. S.Jensen for valuable discussions and critical advise. The skilful technical assistance by Miss L.Fahlberg, Mr L.Johansson, Mr A.Lindström, Mrs K.Nylund and Miss K.Wiberger is greatfully acknowledged.

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Deadly poison — dioxin — is showing up in mothers' milk

By the Washington Post

WASHINGTON — Harvard University researchers report that four samples of mothers' milk tested in two states contain traces of a super-toxic industrial chemical — a poison the Environmental Protection Agency describes as "perhaps the most toxic small molecule known to man."

The chemical is called dioxin. It's a manufacturing impurity that often contaminates certain industrial products, including the widely used herbicide 2,4,5-T. The herbicide has been used in this country since 1948 on pastures and in national forests, along highways and power line rights-of-way, and on rice crops.

Scientists have worried about the potential threat of dioxin since the early 1970's, when laboratory studies showed a single dose of the chemical kills animals in the several hundred parts per trillion range, lower than any other chemical ever tested.

Dr. Matthew Meselson, chairman of the department of biochemistry and molecular biology at Harvard, said that if confirmed by further testing, the new findings would provide the first concrete evidence that dioxin from 2,4,5-T and possibly other sources may be accumulating in human tissues. If researchers repeat Harvard's findings in a larger sample of women across the country, Dr. Meselson said "that would be cause for concern."

Dr. Meselson and other Harvard researchers tested the breast milk from 18 women living in Missouri, Texas and Oregon, near areas where 2,4,5-T is routinely sprayed.

According to a new chemical analytic technique developed at Harvard, the breast milk from four women in Texas and Oregon appeared to contain dioxin at levels from 0.6-1.6 parts per trillion (ppt). The levels translate to 10-40 ppt of dioxin in the milkfat, the researchers said.

Three of the breast milk samples, from Texas, were collected by La Leche League, a national group that advocates breast feeding. The Oregon sample was provided by a member of Citizens Against Toxic Sprays. It is one of numerous environmental groups across the nation that have sued the U.S. Forest Service in an effort to halt 2,4,5-T spraying in national forests.

Five more women were tested in Boston, where the herbicide is not widely used. They did not have dioxin in their breast milk. Dr. Meselson said the Harvard findings have not been confirmed by other laboratories — researchers used all the milk samples — and he characterized them as "preliminary."

But the Harvard findings may add weight to previous research suggesting that dioxin from 2,4,5-T may contaminate part of the nation's food supply.

Researchers at Harvard and Dow Chemical Co. of Midland, the major manufacturer of 2,4,5-T, reported last year they detected dioxin at levels up to 69 ppt in beef fat sliced from cattle which had grazed on pastures sprayed with 2,4,5-T.

"If there were levels of dioxin of even 1 ppt in the human diet, and if the material were highly accumulative, as monkey tests indicate," Dr. Meselson said in a recent telephone interview, "then that would put us over a period of years in the region of having a body burden that would be lethal for a guinea pig."

"We do not know whether 10 or 40 ppt of dioxin in mothers' milk fat will be injurious to human babies," said Dr. James R. Allen, a researcher at the University of Wisconsin Medical School, who is studying the effects of dioxin on monkeys. But he said the data has "some very serious implications."

Thomas Hollaway, an official in the Environmental Protection Agency's Office of Pesticides, says the agency will "reserve opinion" on Harvard's findings until they have been "confirmed by an outside laboratory."

William B. Seward, a spokesman for Dow, said, "We don't believe Harvard has a reliable method (for detecting dioxin) when dealing with levels below 10 ppt. We've analyzed mothers' milk, and we've never found any."

The Harvard mothers' milk findings open the latest round in the eight-year battle between environmentalists and the federal government over the herbicide 2,4,5-T. The herbicide first became the target of widespread public protests when U.S. warplanes sprayed it in massive quantities to defoliate Vietnam forests during the Indochina war.

When a government-sponsored study indicated in 1969 that 2,4,5-T caused birth defects in laboratory mice, the Pentagon bowed to public pressure and banned the herbicide from its Vietnam arsenal.

Later studies showed the chief culprit in causing the birth deformities and other toxic effects was the dioxin in 2,4,5-T. Soon after EPA was formed in 1971, the agency announced it would hold hearings to consider banning 2,4,5-T from the market.

But those hearings were canceled and have never been held. EPA officials contend they cannot ban the herbicide until, and unless, they gather conclusive evidence that dioxin actually threatens the public health by "bioaccumulating in man and the human food chain." For the past four years, researchers at the Environmental Protection Agency (EPA), Harvard, Dow and other research institutions have conducted a special "dioxin monitoring program" to find whether dioxin contaminates food and human tissues.

But their search has bogged down in a complex and frustrating technological battle. For even as researchers look for dioxin in the environment, they are still trying to perfect the analytic techniques capable of measuring dioxin in the parts per trillion range — a "pioneering effort," according to EPA documents.

Since 1974, researchers have reportedly detected dioxin residues in birds and fish in Oregon's Siuslaw National Forest, in beef fat and now mothers' milk. But scientists at EPA, Dow and Harvard have consistently disagreed just how many parts per trillion of dioxin they're finding in just how many samples. Hollaway said the data "certainly indicate from a qualitative standpoint that there is dioxin" in beef fat. And he says "I think Dr. Meselson's studies are indicative of dioxin residues in humans." But until Harvard's methods are "recognized by scientists in the field as being a positive and valid method," the EPA won't know the precise levels of dioxin, Hollaway said.

Without that information, the EPA won't be able to determine for sure whether dioxin in the environment

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threatens the public's health, he said. Hollaway said from the data he's seen so far, "we don't feel there is a significant risk of exposure to humans" from dioxin.

Some scientists and environmentalists reject EPA's view. For one thing, as Environmental Defense Fund general counsel William Butler says, "No scientist has ever been able to define a 'no-effect' level for a chemical which causes birth defects."

Scientists such as Dr. Meselson warn that studies at the University of Wisconsin suggest primates "are rather surprisingly sensitive (to dioxin) and that it is highly accumulative in their bodies."

While the dioxin debate has focused on the herbicide 2,4,5-T, an EPA report warns that dioxin may contaminate other herbicides and numerous chemicals used in the paper and pulp, leather tanning, textile and other industries.

Last summer, hundreds of people in Seveso, Italy, were evacuated — and much of the town was sealed off by barbed wire — after a nearby factory that makes chemicals for herbicides, medicines and cosmetics leaked a cloud of dioxin into the sky.

Thousands of animals died, pregnant women were urged to have abortions, and the once-lush croplands were contaminated, possibly for years.

Researchers see cause for concern from new tests in areas spraying that herbicide

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THE INFLUENCE OF pH ON THE EFFECTS OF 2,4-D (2,4-DICHLOROPHENOXYACETIC ACID, Na SALT) ON *SACCHAROMYCES CEREVISIAE* AND *SALMONELLA TYPHIMURIUM*

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Summary

The genetic effects of 2,4-D (2,4-dichlorophenoxyacetic acid, Na salt) have been investigated in cells of the yeast *Saccharomyces cerevisiae* and of the bacterium *Salmonella typhimurium* in experiments in vitro and in vivo. Experiments in vitro showed that the killing of both yeast and bacteria is dependent on the pH in the treatment solution of 2,4-D. A dose-dependent increase of the frequency of mitotic gene conversion and mitotic recombination in yeast was observed at pH 4.50 and 4.30. In experiments in vitro with two strains of Salmonella no significant increase of the number of revertants to prototrophy was obtained. The positive correlation between survival of cells and dissociation of 2,4-D in the pH region 2.8—5.0 indicates that the cells are unable to take up dissociated 2,4-D. Therefore the survival is high at a high pH when most 2,4-D is in dissociated form, and the survival is low at a relatively low pH when more of the 2,4-D is in its undissociated form. No genetic effects were induced by oral administration of tolerable doses of 2,4-D in host-mediated assays using mice as hosts and yeast or Salmonella as indicator cells.

Introduction

The intensive use of chlorinated phenoxyalkanoic acids as herbicides in agriculture and forestry and also as defoliants during the war in Vietnam has initiated a large number of investigators into their acute toxicity in mammals

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; MCPA, 2-methyl-4-chlorophenoxyacetic acid; Mecoprop, 2-(2-methyl-4-chlorophenoxy)-propionic acid; EMS, ethyl methanesulfonate.

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including man. The genetic effects seem to be much less investigated.

With mammals as test subjects the results are contradictory. Buselmaier et al. [3] observed no increase of dominant lethals in mice treated with 2,4,5-T. Cytogenetic tests on rat cells treated in vivo and in vitro with 2,4,5-T and 2,4-D gave negative results [16]. In mice [5] and Mongolian gerbils [11] chromosome aberrations have been induced with high doses of 2,4,5-T.

In higher plants many investigators have found cytological effects of chlorinated phenoxyalkanoic acids but their relevance is considered questionable because in plants these compound have a hormonal effect. However, Dark [4] and Kabiesch [8] have induced mutants in the offspring of tomatoes treated with 2,4-D and of potatoes treated with Mecoprop, respectively.

In experiments with Drosophila low concentrations of 2,4,5-T had effects on oogenesis and fertility [6,7], and high concentrations increased the number of X-bound recessive lethals [12].

Results from experiments with microorganisms are at variance. Andersen et al. [2] found no increase of the frequency of revertants in Salmonella after treatment with nine different chlorinated phenoxyalkanoic acids. Two of them were also tested in experiments with bacteriophage T4 and were found negative. Buselmaier et al. [3] reported negative results with 2,4,5-T, 2,4,5-T-*n*-butyl ester and MCPA tested in Salmonella (in vitro and in the host-mediated assay with mice) and Serratia (in vitro). Negative results were also reported by Styles [16] from treatments of Salmonella in serum of rats given 2,4-D and 2,4,5-T. The latter compound was also without effect on mitotic gene conversion in *Saccharomyces cerevisiae* [15]. Positive effects have also been reported: mutations having effects on sporulation and production of antibiotics have been induced with 2,4-D in Streptomyces [9]; mitotic gene conversion in Saccharomyces has been induced with Mecoprop [13] and 2,4-D [15].

We report in this paper the results from treatments in vitro and in vivo with 2,4-D on *Saccharomyces cerevisiae* and *Salmonella typhimurium*. For the treatments in vivo we used mice in the host-mediated assay.

Materials and methods

Test agents

2,4-Dichlorophenoxyacetic acid, Na salt, a preparation from Monsanto Chemical Co., St. Louis, Mo., U.S.A., was a gift from Dr. A. Aamisepp, Department of Plant Cultivation, Royal Agricultural College, Uppsala, Sweden. Freshly prepared solutions were used in all experiments.

Ethyl methanesulfonate, used as a positive control, was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.

Biological test material

Saccharomyces cerevisiae, strain D4, used for detection of mitotic gene conversion in two loci, *trp5* and *ade2*, and strain D5, used for detection of mitotic recombination, were obtained from Dr. F.K. Zimmerman, Technische Hochschule, Darmstadt, BRD.

Salmonella typhimurium, strains TA 1530, TA 1535 (his$^-$, reverting to prototrophy by base-pair substitutions), TA 1531 and TA 1538 (his$^-$, reverting

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to prototrophy by frame-shift mutation) were obtained from the Wallenberg Laboratory, Division of Genetics, Stockholm, Sweden.

Mice, used in the host-mediated assay, were males weighing about 30 g, of strain CBA, bought from the Institute of Genetics, University of Stockholm, Sweden.

1. Experiments in vitro

Mitotic gene conversion in Saccharomyces cerevisiae, strain D4

The method described by Zimmermann [17] was followed in general. Cells were grown in yeast-extract-peptone medium, with 2% glycerol as carbon source to eliminate respiration-deficient cells that occur in high proportion when this strain is grown in medium with glucose as carbon source. The cultures were incubated on a shaker at 18—20°C till late logarithmic phase, tested for spontaneous convertants, and cultures with low spontaneous frequency of convertants were kept at 4°C till they were used in the test (max. 3 weeks). Before the treatment the cells were washed and the density of the cell suspension was standardized to $E_{620} = 0.25$ (about 1×10^7 cells/ml) with a Linson spectrophotometer. The treatments were made in the dark at 37°C with fresh solutions of 2,4-D in buffers and stopped by repeated washing in phosphate buffer (pH 7.0). When the effects of low pH were studied 2,4-D was dissolved in McIlvaine's citrate-phosphate buffer. Cells of strain D4 formed red colonies on yeast extract-peptone agar. However, respiration-deficient colonies were white on this medium. The frequencies of respiration-deficient colonies (petites) were recorded in some of the experiments. The colonies were counted after 5-days' incubation at 30°C.

Mitotic recombination in Saccharomyces cerevisiae, strain D5

The method described by Zimmermann [17] was followed. The cells were grown overnight on a shaker at 30°C in yeast-extract-peptone medium with 2% glucose. The treatments with 2,4-D were made as described above. Colonies were counted after 7 days' incubation at 30°C.

Back-mutation test in Salmonella typhimurium

The methods described by Ames et al. [1] were followed in general. Cells were grown overnight on a shaker at 37°C in Difco Penassay Broth. The treatments were made as in the experiments with yeast (see above). The killing effect of 2,4-D was determined by plating the cells in Oxoid Nutrient Agar on which colonies were counted after 2 days' incubation at 37°C. The number of revertants was determined on minimal medium where colonies were counted after 3 days' incubation.

Experiments in vivo

The methods used in the host-mediated assay have been described by Legator and Malling [10] and were followed in general. Drinking water and food were removed from the mice 3 h before the start of the treatments. From a suspension of the indicator organisms (cells of yeast or Salmonella) 1.0 ml was injected into the peritoneum of each mouse. The yeast cells were sus-

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pended in yeast-extract-peptone medium, the bacteria in phosphate buffer (pH 6.8). Immediately after the injection of the indicator cells, 0.25 ml of a solution of 2,4-D (24 mg per ml buffer, = i.e. 6 mg per mouse) was administered orally with the aid of a ball-tipped syringe inserted about 20 mm into the esophagus of the mouse. After 3 h the mice were killed by cervical dislocation, and the indicator cells were recovered and washed twice. Plating, incubation of plates and counting colonies were done as described above. The dose of 2,4-D used was close to the maximal tolerable dose for these mice. During the period of treatment a single mouse died and was therefore eliminated from the protocol. As a negative control the mice were given 0.25 ml buffer orally but in other respects were treated identically.

Results

Experiments in vitro

Mitotic gene conversion in Saccharomyces cerevisiae, strain D4

Siebert and Lemperle [15] reported that 2,4-D gave an increased frequency of mitotic gene conversion in the strain of Saccharomyces used in our experiments. Siebert and Lemperle used a commercial preparation, U46 D-Fluid, and made the treatments at a low pH, 4.6. They did not give any reason for treating the cells under these conditions. With 1.0 mg 2,4-D/ml they got 22% survival after 16 h treatment at 25°C. In a series of experiments we have investigated the pH dependence for induction with 2,4-D of killing, mitotic gene conversion and petite character in strain D4 of *Saccharomyces cerevisiae*.

Fig. 1 shows that the killing effect is strongly dependent on the pH during the treatment. Owing to difficulties in determining and maintaining the pH at a desired value with high precision (to the second decimal) the results may be expected to vary from one experiment to another. This is also true for the results shown in Table I, a summary of the results from experiments where the pH was varied but the concentration of 2,4-D (0.6 mg/ml) and duration of treatment (3 h) were kept constant. Under these conditions there was a critical pH for survival around 4.5.

Table II shows the results from a series of experiments where the treatments were made at pH 4.50. There was a dose-dependent effect on the killing and on the frequency of convertants and petites. Also, when the duration of the treatment was varied a dose-dependence for these effects was obtained (Table III). The increase of the frequency of gene conversion after 3 h treatment with 0.6 mg 2,4-D/ml in buffer at pH 4.50 (Tables I—III) was tested statistically by the t test. The increase was significant on the 5% level (t 12/0.05 = 1.78) for the *ade2* locus (t = 1.96) but not quite significant for the *trp5* locus (t = 1.36). The increase of the frequency of petites was highly significant (t = 3.00).

The physiological condition of the cells was important for the sensitivity to 2,4-D. Fig. 2 shows that cells in the logarithmic phase of growth are more sensitive than cells in the stationary phase. Cells grown in medium with glucose as carbon source were more resistant than cells grown on glycerol as carbon source.

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TABLE I

THE EFFECTS ON THE FREQUENCY OF MITOTIC GENE CONVERSION, SURVIVAL AND FREQUENCY OF PETITE MUTATION IN STRAIN D4 OF *SACCHAROMYCES CEREVISIAE* AFTER TREATMENT FOR 3 h AT 37°C WITH 0.6 mg 2,4-D PER ml BUFFER AT DIFFERENT pHs

Numbers within parentheses are the totals of convertants scored. The results are from 4 independent experiments.

pH of buffer		4.0	4.2	4.3	4.4	4.5	4.6	4.7	5.0	5.1	5.5	6.0
0.6 mg 2,4-D per ml buffer	Convertants X 10^{-5} *trp5*					1.72 (32)	1.28 (338)	0.71 (291)	0.95 (241)	0.53 (285)	0.95 (35)	0.93 (40)
	ade2					1.27 (24)	0.70 (209)	0.67 (257)	0.59 (147)	0.44 (253)	1.08 (40)	0.98 (42)
	Survival (%)	<0.1	10^{-3}	0.11	7.3	26	35	61	98	98	90	105
	Petites (%)		12	51	83	46	40	11	5.5	2.8	5.6	6.8
Negative control	Convertants X 10^{-5} *trp5*	1.29 (44)	0.54 (237)	0.85 (175)					0.89 (190)	0.39 (241)		0.90 (37)
	ade2	1.23 (42)	0.34 (153)	0.72 (148)					0.50 (107)	0.30 (187)		0.78 (32)
	Survival (%)	98	94	64					100	100		100
	Petites (%)	7.0	2.5	4.4					2.8	3.4		2.4

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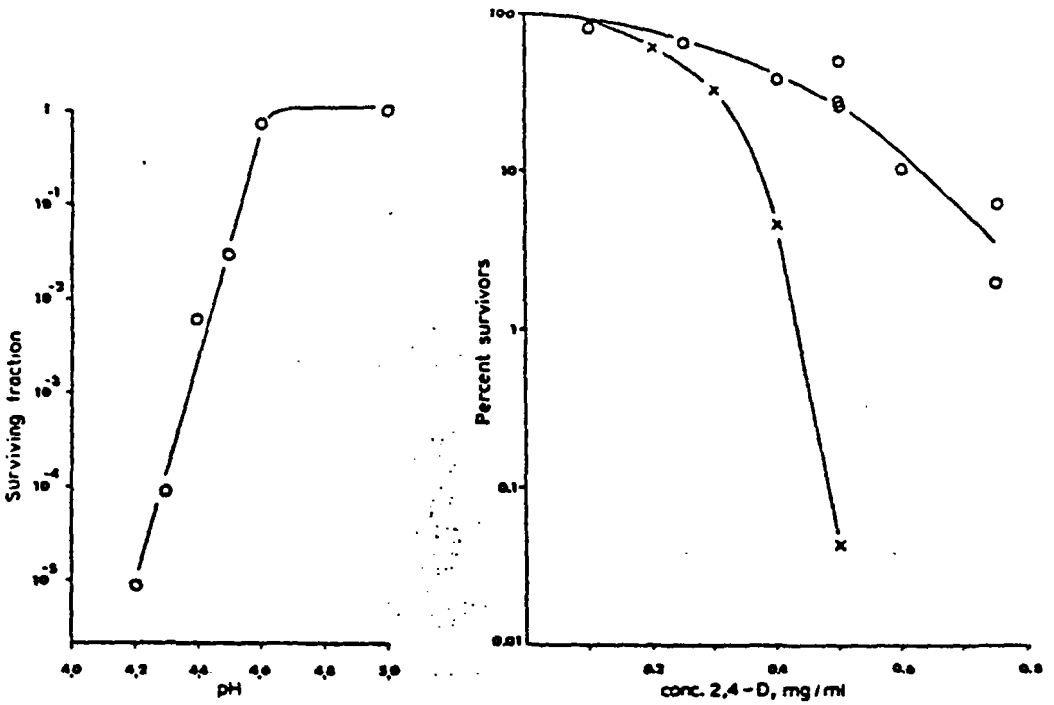


Fig. 1. The killing effect of 3 h treatment at 37°C in buffer of different pH containing 0.6 mg 2,4-D/ml. Strain D4 of *Saccharomyces cerevisiae*.

Fig. 2. The killing effect of 3 h treatment at 37°C with different concentrations of 2,4-D in buffer at pH 4.5 on stationary phase cells (○) and cells in logarithmic phase (×). Strain D4 of *Saccharomyces cerevisiae*.

TABLE II

THE EFFECTS ON THE FREQUENCY OF MITOTIC GENE CONVERSION, SURVIVAL AND FREQUENCY OF PETITES IN STRAIN D4 OF *SACCHAROMYCES CEREVISIAE* AFTER TREATMENT FOR 3 h AT 37°C WITH DIFFERENT CONCENTRATIONS OF 2,4-D (SODIUM SALT) IN BUFFER AT pH 4.5

The total number of convertants scored are within parentheses. The results are from 7 independent experiments.

2,4-D (mg/ml)	Duration of treatment (h)	Survivors (%)	Petites (%)	Convertants X 10^{-5} survivors	
				trp5	*ade2*
0	0	100	1.5	0.56 (616)	0.25 (263)
0.10	3	78	1.3	0.66 (154)	0.28 (66)
0.25	3	64	1.7	0.53 (94)	0.32 (57)
0.40	3	37	7.2	0.96 (173)	1.06 (192)
0.50	3	33	12	1.26 (368)	0.66 (194)
0.60	3	23	61	1.52 (248)	0.92 (160)
0.75	3	4.0	95	1.42 (36)	0.35 (10)
0	3	95	2.7	0.63 (1049)	0.42 (673)
Positive control EMS (1%)	3	88		36 (8000)	46 (10000)

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TABLE III

THE EFFECTS ON THE FREQUENCY OF MITOTIC GENE CONVERSION, SURVIVAL AND FREQUENCY OF PETITES IN STRAIN D4 OF *SACCHAROMYCES CEREVISIAE* AFTER TREATMENT AT 37°C WITH 0.6 mg 2,4-D PER ml BUFFER AT pH 4.5

The total number of convertants scored are within parentheses. The results are from 2 independent experiments.

2,4-D (mg/ml)		Duration of treatment (h)			
		0.5	1.0	2.0	3.0
0.6	Convertants $\times 10^{-5}$ *trp5*	1.67 (845)	1.92 (620)	2.21 (921)	3.48 (885)
	ade2	0.61 (293)	0.72 (233)	1.46 (606)	2.03 (520)
	Survival (%)	112	72	41	28
	Petites (%)	14	13	16	17
0	Convertants $\times 10^{-5}$ *trp5*	1.96 (1037)	2.07 (1093)	1.88 (1009)	2.12 (1004)
	ade2	0.68 (313)	0.69 (329)	0.69 (341)	0.82 (382)
	Survival (%)	100	100	100	100
	Petites (%)	7.0	4.0	5.7	5.4

Reconstruction experiments in which the sensitivity of convertants and their parent cells was compared showed no difference in sensitivity for the killing effect between the parent cells, *ade*$^-$ *trp*$^-$, on the one hand and convertants in the *ade-2* locus, *ade*$^+$ *trp*$^-$, on the other. Convertants in the *trp5* locus, *ade*$^-$ *trp*$^+$, were somewhat more sensitive than *ade*$^-$ *trp*$^-$ cells. Also petites were more sensitive than grandes for the killing effect of 2,4-D, which may be the reason for the smaller effect on petite induction obtained with the highest doses of 2,4-D. The reconstruction experiments showed that the increase of the frequency of gene conversion as well as the frequency of petites was a result of induction by 2,4-D and not dependent on selection of convertants or petites existing in the suspensions before the treatments.

Experiments in which the treatment was made at pH 4.30 gave a higher effect on survival and gene conversion than that at pH 4.50. Fig. 3 shows the dose dependence at concentrations up to 0.3 mg/ml. Fig. 4 shows the dose dependence when the duration of the treatment was varied.

EMS was used as a positive control. EMS is relatively non-toxic at high concentration (1%) and gave a strong increase of gene conversion (Table II). Fig. 5 shows that when the effects on survival and gene conversion of 2,4-D and EMS were compared on a molar basis there was a significant effect on both with 2,4-D at a molarity where EMS was almost without effect.

The effect of the low pH was reversible. Table IV shows how the killing during 1.5 h with 0.3 mg 3,4-D/ml buffer at pH 4.30 could be interrupted if the cells were moved to a medium containing the same concentration of 2,4-D but at pH 7.00. The effect on gene conversion was not so evident in this experiment owing to the low frequency obtained after 3 h at pH 4.30.

The effects of longer treatments with 2,4-D at pH 4.30 were also investi-

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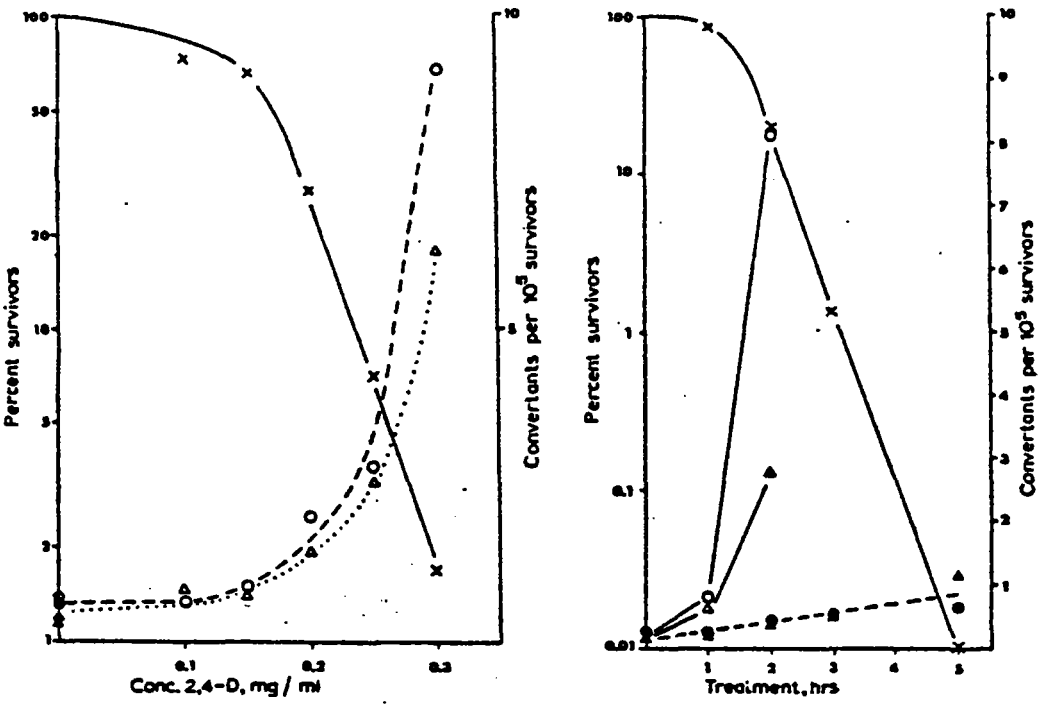


Fig. 3. The effects of 3 h treatment at 37°C with different concentrations of 2,4-D in buffer at pH 4.30 on survival (X) and the frequency of mitotic gene conversion in *trp5* (O) and *ade2* (Δ) loci of strain D4 of *Saccharomyces cerevisiae*. Filled symbols indicate the frequency of conversion in the control (buffer at pH 4.30).

Fig. 4. The effects of different lengths of treatment at 37°C with 0.30 mg 2,4-D/ml buffer at pH 4.30 on survival (X), and the frequency of mitotic gene conversion in *trp5* (O) and *ade2* (Δ) locus of strain D4 of *Saccharomyces cerevisiae*. Filled symbols indicate the frequency of conversion in the control (buffer at pH 4.30).

gated, but under these conditions the cells in the negative controls showed a low survival: therefore no data are reported from these experiments.

Mitotic recombination in Saccharomyces cerevisiae, strain D5

Table V shows the results from three different experiments made under the following conditions: pH 4.30, temp. 37°C, 3 h treatment, 0.20 and 0.30 mg 2,4-D per ml. After treatment with 0.30 mg 2,4-D/ml the percentage of aberrant colonies was about the same in all three experiments and clearly above the control value. Evidently 2,4-D increases the frequency of mitotic recombination under these conditions.

Back-mutation test in Salmonella typhimurium

A strong dependence on the pH in the treatment solution of 2,4-D was obobtained when the killing effect on Salmonella was studied (Fig. 6). The concentration of 2,4-D used in the experiment in Fig. 6 (0.5 mg/ml) was completely without effect when the treatment was made at pH 6.8 (Fig. 7). Treatments with 2,4-D at pH 4.30 did not increase the number of prototrophs in

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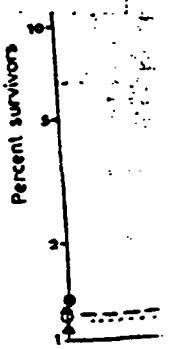


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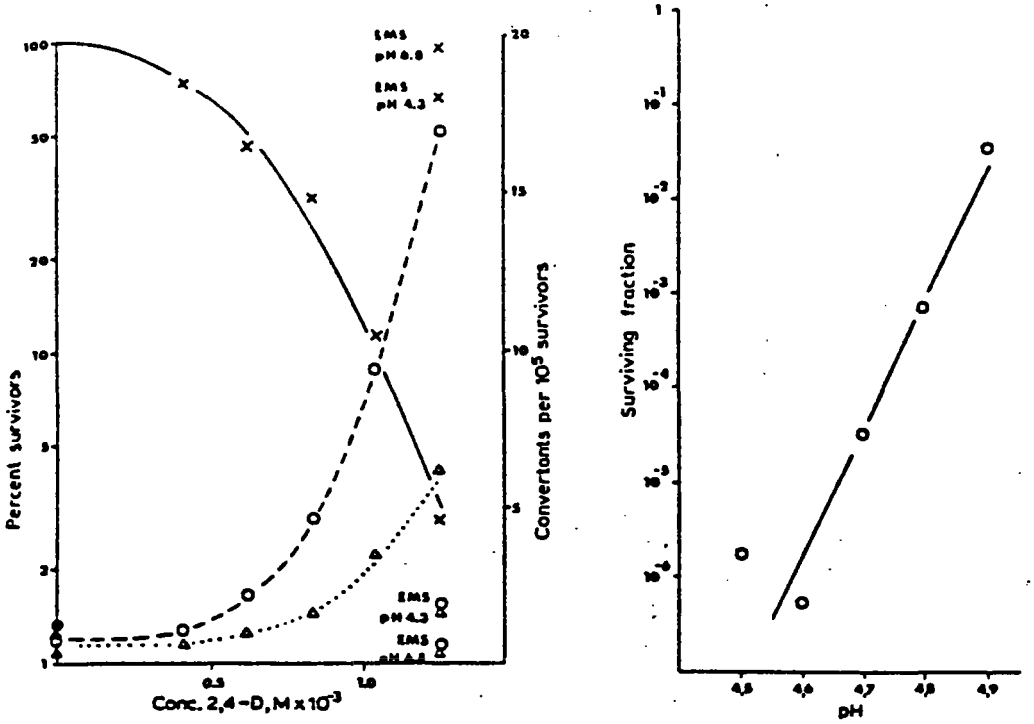


Fig. 5. The effects of 3 h treatment at 37°C with different concentrations of 2,4-D in buffer at pH 4.30 and of 1.24×10^{-3} M EMS at pH 6.80 and 4.30 on survival (X) and the frequency of mitotic gene conversion in *trp5* (○) and *ade2* (△) loci of strain D4 of *Saccharomyces cerevisiae*. Filled symbols indicate the frequency of conversion in the control (buffer at pH 4.30).

Fig. 6. The killing effect of 3 h treatment at 37°C with 0.5 mg 2,4-D/ml buffer at different pH. Strain TA 1530 of *Salmonella typhimurium*.

TABLE IV

THE EFFECTS ON THE FREQUENCY OF MITOTIC GENE CONVERSION AND SURVIVAL IN STRAIN D4 OF *SACCHAROMYCES CEREVISIAE* AFTER TREATMENT IN BUFFER AT pH 4.30 AND/OR pH 7.00 WITH 0.3 mg 2,4-D/ml.

Numbers within parentheses are the totals of convertants scored.

2,4-D (mg/ml)	H treatment at		Survivors (%)	Convertants X 10^{-5} survivors	
	pH 4.30	pH 7.00		*trp5*	*ade2*
0	0	0	100	0.54 (106)	0.50 (98)
0	3	0	100	0.74 (233)	1.06 (334)
0	0	3	100	0.33 (124)	0.37 (140)
0.6	1.5	0	70	3.60 (494)	2.26 (310)
0.6	1.5 +	1.5	80	3.46 (539)	2.03 (316)
0.6	3	0	11	2.02 (43)	2.49 (53)
0.6	0	3	100	0.33 (132)	0.28 (113)

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TABLE V

THE EFFECTS ON SURVIVAL AND THE FREQUENCY OF ABERRANT COLONIES IN *SACCHAROMYCES CEREVISIAE* STRAIN D5 AFTER TREATMENT FOR 3 h AT 37°C WITH DIFFERENT CONCENTRATIONS OF 2,4-D IN BUFFER AT pH 4.30

The results are from three independent experiments.

2,4-D (mg/ml)	Survival (%)	Number of colonies scored	Red/ pink	Pink/ white	Red/ white	Pink	Red	Hair-line	Total aberrant	Percent aberrant
0.20	53	510					1		1	0.2
0.30	11	1630	13	1	3	11	6	4	38	2.3
0	100	4557		1	1	1	1	2	6	0.1

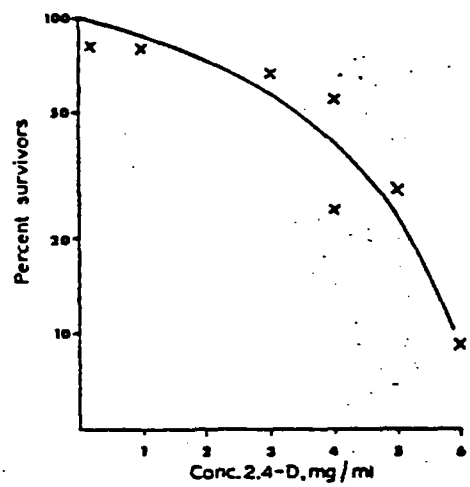


Fig. 7. The killing effect of 3 h treatment at 37°C with different concentrations of 2,4-D in buffer at pH 6.8. Strain TA 1530 of *Salmonella typhimurium*.

TABLE VI

THE EFFECTS ON SURVIVAL AND THE TOTAL NUMBER OF his^+ REVERTANTS AFTER 3 h TREATMENT IN VITRO AT 37°C WITH DIFFERENT CONCENTRATIONS OF 2,4-D OR EMS IN BUFFER AT pH 4.30

Strain TA 1535 of *Salmonella typhimurium*. The results are from two independent experiments.

Treatment with	pH	Survival (%)	Total number of revertants scored
2,4-D (0.08 mg/ml)	4.30	30	72
2,4-D (0.07 mg/ml)	4.30	57	51
2,4-D (0.05 mg/ml)	4.30	67	75
2,4-D (0.03 mg/ml)	4.30	108	63
Buffer only	4.30	65	92
Buffer only	7.00	100	70
EMS, 0.5%	4.30	10	1400
EMS, 0.1%	4.30	59	730

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TABLE VII

THE EFFECTS ON SURVIVAL AND THE TOTAL NUMBER OF his$^+$ REVERTANTS AFTER 3 h TREATMENT IN VITRO AT 37°C WITH DIFFERENT CONCENTRATIONS OF 2,4-D IN BUFFER AT pH 4.30

Strain TA1538 of *Salmonella typhimurium*. The results are from 4 independent experiments.

2,4-D (mg/ml)	pH	Survival (%)	Total number of revertants scored
0.08	4.30	27	122
0.07	4.30	42	179
0.05	4.30	73	227
0.03	4.30	93	194
0	4.30	72	157
0	7.00	100	154

TABLE VIII

INDUCTION IN VIVO (HOST-MEDIATED ASSAY) OF MITOTIC GENE CONVERSION IN *trp5* AND *ade2* LOCUS OF STRAIN D4 OF *SACCHAROMYCES CEREVISIAE* BY ORAL ADMINISTRATION OF 2,4-D, 6 mg PER MOUSE.

Duration of treatment, 3 h. Numbers within parentheses are the totals of convertants scored.

Expt. No.	Mouse No.	Number of cells tested		Convertants per 10^5	
		$\times 10^6$	% of contr.	*ade2*	*trp5*
1	1	5.0	199	2.24 (112)	3.90 (195)
	2	7.6		1.59 (121)	3.82 (291)
	3	4.1		2.05 (84)	4.35 (179)
	Contr.				
	4	2.8	100	1.47 (41)	2.24 (69)
2	1	2.8	47	2.73 (77)	2.98 (84)
	2	4.0		2.90 (117)	3.66 (148)
	3	1.2		3.62 (42)	2.59 (30)
	4	5.3		2.18 (115)	3.15 (166)
	Contr.				
	5	5.8		1.96 (114)	2.62 (153)
	6	6.7	100	2.70 (181)	3.34 (224)
	7	8.9		1.38 (123)	2.39 (213)
3	1	40.0	134	0.35 (143)	0.52 (208)
	2	38.1		0.37 (140)	0.72 (273)
	3	25.9		0.48 (124)	0.72 (186)
	4	33.8		0.30 (81)	0.57 (192)
	Contr.				
	5	26.9		0.48 (130)	0.78 (211)
	6	31.3	100	0.41 (128)	0.59 (184)
	7	19.0		0.51 (96)	0.75 (142)
4	1	28.0	118	0.46 (130)	1.15 (321)
	2	15.6		0.58 (91)	0.77 (120)
	3	3.4		0.71 (24)	0.97 (33)
	4	41.4		0.44 (180)	0.72 (296)
	Contr.				
	5	24.1		0.69 (166)	0.92 (222)
	6	23.4	100	0.68 (159)	0.86 (202)
	7	8.2		0.56 (46)	0.81 (66)

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two experiments with strain TA 1535 (Table VI) or in any of four experiments with strain TA 1538 (Table VII).

Host-mediated assay

Indicator organism: Saccharomyces cerevisiae, strain D4

Table VIII shows the results from four different experiments where we

TABLE IX

INDUCTION IN VIVO (HOST-MEDIATED ASSAY) OF his$^+$ REVERTANTS IN *SALMONELLA TYPHIMURIUM* STRAIN TA1530 WITH 2,4-D

Oral administration, 6 mg per mouse; duration of treatment, 3 h.

Expt. No.	Mouse No.	Number of cells tested		Total number of his$^+$ scored	his$^+$ × 10^{-8}
		×10^8	% of contr.		
	1	4.1	142	10	2.47
	2	6.4		19	2.96
	3	6.0		17	2.82
	4	6.8		16	2.35
1	Control				
	5	3.9	100	9	2.33
	6	3.6		8	2.49
	7	4.1		8	1.97
	8	4.8		14	2.92
	1	6.6	104	12	1.82
	2	10.4		21	2.03
	3	6.2		16	2.59
	4	6.1		14	1.85
2	Control				
	5	6.5	100	13	1.60
	6	2.7		4	1.46
	7	10.4		12	1.16
	8	8.2		12	1.46
	1	0.9	66	0	0.1
	2	8.3		1	0.1
	3	8.4		1	0.1
	4	11.0		2	0.2
3	Control				
	5	9.6	100	0	2.1
	6	6.3		13	0.3
	7	16.6		5	0.7
	8	11.4		8	
	1	4.8	338	90	18.7
	2	7.6		81	10.6
	3	4.3		91	21.2
	4	3.6		83	23.1
4	Control				
	5	1.2	100	76	62.8
	6	1.1		87	79.8
	7	1.7		85	50.9
	8	2.0		91	44.8

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TABLE X

INDUCTION ON VIVO (HOST-MEDIATED ASSAY) OF his^+ REVERTANTS IN *SALMONELLA TYPHIMURIUM* STRAIN TA1531 WITH 2,4-D

Oral administration 6 mg per mouse; duration of treatment, 3 h.

Expt. No.	Mouse No.	Number of cells tested		Total number of his^+ scored	$his^+ \times 10^{-8}$
		$\times 10^8$	% of contr.		
	1	1.3	219	0	
	2	2.9		1	0.3
	3	2.7		0	
	4	3.4		13	3.8
1	Control				
	5	0.9	100	0	
	6	1.1		5	4.6
	7	1.0		3	3.0
	8	1.7		6	3.6
	1	4.5	290	1	0.2
	2	2.9		7	2.5
	3	2.5		9	3.5
2	Control				
	4	0.6	100	7	12
	5	0.8		14	19
	6	0.6		0	
	7	2.5		10	4.1

tested whether oral administration of 2,4-D (6 mg/mouse) to mice could increase the frequency of mitotic gene conversion in cells of strain D4 during 3 h exposure in the peritoneum. Compared with the control values there was no significant increase.

Indicator organisms: Salmonella typhimurium, strains TA 1530 and TA 1531

In four different experiments with strain TA 1530 (Table IX) and two different experiments with strain TA 1531 (Table X) no increase in the number of revertants to prototrophy was obtained. The treatments were made under the same conditions as those with yeast (see above).

Discussion

Of the results presented above the observation that the effects of 2,4-D were pH dependent is the most interesting. We propose that the reason why 2,4-D was more active at a low pH is because more 2,4-D was in its undissociated form and that cells can only take up the undissociated form. Fig. 8 shows how the survival of yeast cells was correlated with the dissociation of 2,4-D.

Like the killing effect, the increase of mitotic gene conversion in yeast was dependent on the pH and the dose of 2,4-D. The increase was small compared with that obtained with EMS. However, it is worth noting that EMS was almost without effect at the low molarity where 2,4-D induced a clear increase of gene conversion. The two compounds behaved very differently as to dose

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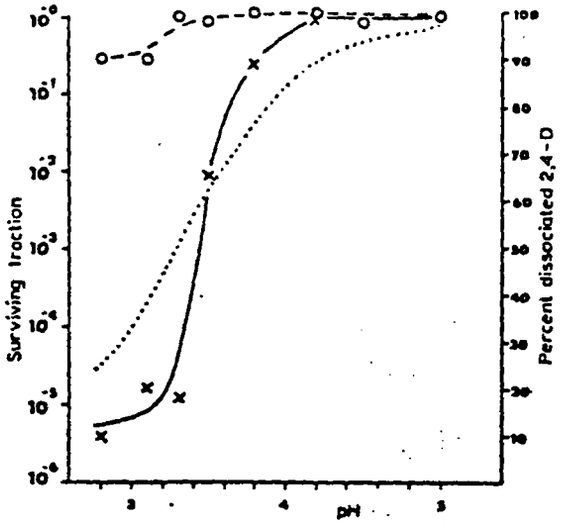


Fig. 8. The survival of cells of *Saccharomyces cerevisiae* strain D5 after 3 h treatment at 37°C with 0.05 mg 2,4-D per ml buffer at different pH (X). The negative control (O) was buffer. The dotted curve represents the dissociation of 2,4-D at different pH.

response: increased dose of 2,4-D gave mainly an increase of the toxic effect, whereas EMS with increasing dose became only slightly more toxic but very mutagenic.

At low pH, cells of Salmonella were killed by a much lower concentration of 2,4-D than were yeast cells. It may be that is Salmonella no mutagenic changes can occur at tolerable doses of 2,4-D.

The results from the host-mediated assays were negative for both Salmonella and yeast. It is possible that the conditions were such that the compound did not reach the indicator cells in the peritoneum at a concentration high enough to cause an effect [14]. Furthermore, the pH in the peritoneum is circumneutral, i.e. far from the optimal value for the effects of 2,4-D.

In situations where 2,4-D occurs at a low pH there are possibilities for toxic and mutagenic effects to occur. This indicates that eating food contaminated with 2,4-D would be a risk for animals with low pH in their alimentary canal, e.g. man. The high toxicity of 2,4-D on microorganisms may be of importance also for ruminant animals that are dependent on microbial processes in their rumen for the decomposition of food. The observation that the effects of 2,4-D were stopped by increasing the pH indicates that the compound when administered orally would have small chance to cause effects on cells such as gametes. The exposure of the gametes would have to be preceded by distribution in the blood plasma where the pH is about 7.4, i.e. much above the pH at which 2,4-D can enter the cells.

Acknowledgement

Part of the investigation was supported by Grant B-2468 from the Swedish Natural Science Research Council.

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The Problem of Mutagen Effects on the 2nd Generation after Exposure to herbicides

Ton-That-Tung
Ton-Duc-Lang
Do-Duc-Van

The aberrations of chromosomes, in both their structure and their number have been pointed out in 1970 by Ton-That-Tung and his collaborators[1] on vietnamese patients who have been exposed to the herbicide sprays, especially the later orange agent. This work has been confirmed by Fujita, Fujita and Funazaki[2] Yefimenko[3] and by Hew[4] following Seveso. The mutagenesis of the orange agent has b been affirmed for the Salmonella Bacteria by Hussein[5], with the flies Drosophila by Majumdar and Hall[6] with Rodents. In 1977, Murray and his collaborators[8] think that the TCDD can act like a dominant lethal agent, whereas Seiler[9] proves reduction of DNA production by experiment with testis rats with TCDD(1977).

Through the newspapers, one knows that in man, the former American veterans of Vietnam back in the U.S.A. complain about birth defects in their descent and about the abortion frequency among their wives.
At the time of a visit in the U.S., consequent to a private interview with American Veterans Administration in Washington in May 1979, it has been suggested to us to study the remote effects of herbicides on the 2nd generation among the former vietnamesesoldiers who fight in the South and who come back in the North to found a family, that is to say in the same conditions of the American veterans.
Here are the first results of our investigations:

Methodology

Our investigations first proceed on statistics in obstetrics services. Unfortunately in most of the cases, the father's military service has not been mentioned in the observations (besides, this has always been considered as a secret). By chance, we ran across the town of Yen-Bai; being coupled with a Southern town, it receives a certain number of Southern veterans (and usually native from the North). Yen-Bai belongs to the province of Hoang-lien-Son, whose sanitary service boss, Doctor Mai became interested in our work.
The town of Yen-Bai counts 10,000 inhabitants and about 700 former soldiers coming from the South. The former soldiers gather in agricultural and handicraft cooperatives in the town and its vicinity.

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After Yen-Bai, we appealed to former southern soldiers groups in 5 big provinces of the Delta of the Red River: Hanoi, Hunghoa, Namdinh, Haiphong, and Thai Binh. There, we sent teams in order to personally interview each individual.
Localities in which our investigations have been taking place, have never received herbicide sprays.

RESULTS

A) Hospital investigations

a)Maternity of Yen-Bai

Material : 3058 births from 1975 to 1978 (4 years)

Number of birth defects:	30
Number of birth defects depending on the couple: father, southern veteran, mother, native from the North, not having left the country:	15

Out of these defects there were 22 due date deliveries, 8 non due date deliveries.

Weight of these anomalous children:

Above 2.5 Kg (about 5.5 pounds)	20
Between 2,5 and 2 (between 5.5 and 4.4)	5
2 kg (4.4 pounds)	4
Non registered	1

I. Birth defects coming from father, former southern soldier, and mother, living in Yen-Bai:

Pure Anencephaly	2
Anencephaly with absence of nose and left ear hare lip short neck elevation of navel against sternum	1
Anencephaly with protuberant eyes short neck shoulder retraction facial cleft	1
Anencephaly with batrachian abdomen shortening of limbs (upper limbs 8 centimeters lower limbs 11 centimeters)	1
Anencephaly with absence of nose and eyes	1

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Hydrocephaly with angulation of upper limbs endowed of 3 fingers on the right hand, and four fingers on the left hand. absence of anus	1
Absence of nose with auricles jointed to the ear feet jointed to the legs hands jointed to the arms bilateral syndactyly	1
Abnormal location of the eyes with auricles jointed to the ear varus equine	1
Batrachian abdomen with agenesis of the upper lip	1
Absence of forearm	1
Polydactyly	1
Syndactyly of fingers and toes	1
Hare lip and cleft palate	1
Absence of the upper abdominal wall	1
II. Birth defects observed on the civil population:	15
Hare lip	2
Cleft palate and hare lip	1
Club-foot	1
Batrachian abdomen	4
Batrachian abdomen + Fallot	1
Anal imperforation	1
Batrachian abdomen + hermaphroditism	1
Hydrocephaly + batrachian abdomen	1
Hydrocephaly + batrachian abdomen + shortening of the limbs	1
Hydrocephaly + absence of arms and forearms, replaced by 3 fingers on each side	1
Hydrocephaly	1
	15

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On the whole, the number of anencephalies obviously predominates on the group of children born of southern fighters (6/15) relative to the civil population (0/15).

b) Maternity of Quy mong' district

Material : 233 births for a civil population of 4500 inhabitants with 30 families whose father has been a southern fighter during the years 1976, 1977, 1978 (3 years).

Number of birth defects	9
Number of birth defects among the civil population	0/9
Number of birth defects coming from father, southern veteran, mother living in Yen-Bai	9/9

These birth defects are dependent on only 6 couples:

Anencephaly with absence of upper maxilla shortening of limbs and batrachian abdomen	1
Pure anencephaly	1
Anencephaly with cleft palate batrachian abdomen absence of penis shortening of limbs (These 3 anencephalies come from the same couple)	1
Cleft palate, deceased on the 6th day (cardiac disease?)	1
Hydrocephaly	1
Hare lip , atrophy of uvula Deceased on the 3th day by cyanosis (cardiac disease?)	1
Hare lip, shortening of limbs Deceased on the 6th day	1
One single lower limb set up in a mass of shapeless flesh	1
Shortening of limbs, big ears , batrachian abdomen	1
	9

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In this little group predominate :

The anencephaly (3/9)

The hare lip with or without cleft palate (3/9)

The possibility of cardiac diseases (3/9)

The shortening of limbs (5/9)

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B) Investigations in the cities of southern veterans, in the delta of the Red River :

Our teams went to:
I. Gia lam (neighborhood of Hanoi)
II. Tu ky (Hai hung)
III. Hung ha (Thai binh)
V.Chuong My (Ha dong)
VI: Haiphong
VII. Ba dinh (Hanoi)
VIII. Nam dinh
IX. Ninh binh
X. Phu ly
XI. Thanh hoa

On the whole our teams have questionned 1102 veterans, of whom 836 coming back from the southern sprayed zones(Group A), and 266 not having been in the South (Group B).
Out of 1102 soldiers from group A, 670 couples are composed of a southern veteran husband and a wife having always lived in the North.
The group witness is composed of 116 couples.

The results are registered on the board I

BOARD I

Individual investigation on 1102 former soldiers (836 coming back from the southern sprayed zones) in the communities of North Vietnam.

	Birth Defects		Abortions and premature deliveries		Sterility	
	Number	%	Number	%	Number	%
Group A with 670 couples (former soldiers from the South married to a northern woman	43 (out of 1187 births on due time	3.6%	214 (out of 1401 pregnancies)	15.3%	23 (out of 670 couples)	3.3%
Group B (266) with 116 couples(former soldiers not having lived in the South,married to a northern woman	0 (out of 309 births on due time)	0%	36 (out of345 pregnancies)	10.4%	2 (out of 116 couples)	1.91%
	p 0.001		p<0,03		p<0,05	

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Here the nature of defects is:

Anencephaly
Microcephalia dementia
Anophtalmy
Hydrocephaly
Genu valgum
Absence of left forearm
Congenital bilateral cataract
Hare lip and cleft palate
Polydactyly
Retraction of the right upper limb in flexion
Hermaphroditism
Absence of the right auricular auricle
Congenital diseases of the heart
Paralysis of the external ocular motor (eye)
mongolism

In this group predominate:
1. Cardiac diseases 15/43
2. Defects of the neural tube 9/43

Dicussion

I. The clinical relationships

These first results seem to justify the complaints of the American VN veterans who have resided in the sprayed regions (Tay ninh, Da nang, etc...) and to suggest the spread through the father to the second generation.

We have been able to study 70 of these files whose rate of the birth defects and abortions on 70 couples is as follows:

	Birth defects	Abortions
70 couples (VN veterans + women in the U.S)	7/70	8/70

Here is the nature of these defects:

2 cleft palates
1 spina bifida
1 absence of anus with spina bifida
1 absence of genito-urinary system
2 congenital cardiac diseases

7

The former vietnamese soldiers coming back from the southern sprayed zones present suggestive signs of a mutagen ac transmitted by the father.

There is a significative difference for the defects between the group of vietnamese soldiers fighting in the South (group A), and the others (group B). In the first group, the congenital anomalies occur 43 times out of the 1187 births, i.e 3.6% (the normal number of defects seems to be about 1.5 to 2 %), whereas on the witness group , they are not any out of 309 births ($p < 0.001$). Abortions and premature deliveries represent 15.3% in the group A and 10.4% in the group B ($p < 001$)

About the characteristic of these defects, one must insist on the excessively high frequency of brain defects (anencephaly, microcephaly, anophtalmy) in Vietnam on the second generatic

We should normally have:

- One anencephaly out of 2777 births, whereas we have 1 anencephaly out of 197,8 births among the southern veterans.

- The microcephalic dementia exists once out of 25,000 birth and it represents among the southern soldiers once out of 1187 births.

- Anophtalmie should normally be 1 out of 10,000 births, whereas it is here 1 out of 1187 births.

On the other hand , the important place of the cardiac defects has to be emphasized: 34.8% of the defects.i.e. 15 cases out of 43 defects.

The elective harm of the neural tube on the second generatic seems to be in accordance with Barbara Field's works, who proves that in Australia, a linear relationship exists between the increasing rate of spina bifida on the newborns of the first generation and the rate of 2,4,5T used every year.

The number of molar pregancies is abnormally high in Hanoi: many of them stem from women whose husband has been a soldier in South Vietnam. For instance, at the time we are writing this, one can count, in the obstetrical Service of the Mother and the Child Institute,,19 hospitalized cases by mole or by chorioepithelioma: out of this number 19, there are 9 cases coming from southern veterans ' wives and 10 belonging to the civil population.*

II. Experimental tests

Jackson[11] was the first one to note that the dioxin inhibi mitoses of Haemanthus katharinae, Baker(1972), Davring and Summer

* This will be the object of another work

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reported that the commercial form of 2,4,5T with less than o.1ppm of dioxin, affects theoogenesis and causes sterility on the fly Drosophila melanogaster: for the first time, sterility with a genetic origin of the 2,4,5T is then evoked. Many authors, after a dioxin treatment, have observed multinucleate cells (Greig and his collaborators[13], Buu Hoi and his collaborators[14], Kimbroug and his collaborators[15]) on experimental animals.

Green Moreland and Sheu[16], in 1975, have been able to demonstrate that the dioxin could increase the number of the chromosomal aberrations in the rats' bone marrow in 1977, after Green and Moreland's failure[17] 2 years before. Czeizel and Kiraly[18] in 1976, i.e., 6 years after the work of Ton-That-Tung and his collaborators[1], studying Hungarian workers' blood working in a Budapest plant stained with dioxin, have found a high percentage of chromosomal aberrations. Fujita and his collaborators[2] have also observed chromosomal anc lies among the Japanese workers in contact with the 2,4,5T as well as Yefimenko[3] among rats in the cells of the bone marrow. After Seveso, a Hew seminary has allowed to report on the italian population, from 2 to 25 years old, a high number of chromosomal anomalies (gaps, breaks.....).

The mutagenesis of the dioxin has been the first assertion by Hussain and his collaborators[5] with ESCHERICHIA COLI Sd-4 Salmonella typhirmurium TA-1532. With Hussain, dioxin seems to be mutagenic by intercalation with DNA. Kondorosi and his collaborators[19] have found that the dioxin is a powerful mutager of Salmonella typhimurium TA-1532: according to them, the dioxin seems to cause an intercalation with DNA rather than to chemically react with the nucleic acid.

Khera and Ruddick's works have shown that on the Wistar rats, the (epididymises/ male rats), treated by the dioxin, present some granulomatous forms similar to the auto-immunologic reactions observed after a bacterial infection or a flow of foreign bodies. Van Miller and Allen[21] have proved that the Sprague Dawley male rats, after a treatment by oral route, by daily doses of 0.05, 0.5, and 1,0 ppm of TCDD present on the fourth week, a decrease of the spermatogenesis, whereas Seiler[9], points out that 0.4mg/kg of dioxin given by intraperitoneal route to the male mouse, provokes a 50% reduction of testicular DNA. Besides, one knows that dioxin causes adverse effects in the teste of mice, chickens, guinea-pigs, and monkeys. The double assertion that, first the dioxin reaches the testes, and secondly, provokes anomalies, implies the idea that the chromosomal dioxin can act as a dominant lethal agent, like Murray and his collaborators' wo seem to suggest[8], covering three generations of Sprague-Dawley rats.

It seems that the immediate problem is the cytologic and biologic paternal sperm in animals as well as in man, and moreover the paternal sterility can already appear like a problem according to the animal experimentation.

Furthermore, the study of herbicides and of dioxin seems to bring an unitarian proof to the actual conception according to which the transformations caused to DNA by environmental mutagens can be the main cause of cancer, birth defects, cardiac diseases and senility.

Work done by the surgical clinic of the University of Medecine in Hanoi.

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MORPHOLOGICAL CHANGES IN MONKEYS CONSUMING A DIET CONTAINING LOW LEVELS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN

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Abstract—Female rhesus monkeys given a diet containing 500 ppt (t = 10^{12}) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for 9 months became anaemic within 6 months and pancytopenic after 9 months of exposure. The marked thrombocytopenia was associated with widespread haemorrhage. Death occurred in five of the eight animals between months 7 and 12 of the experiment at total exposure levels of 2–3 μg TCDD/kg body weight. At autopsy, in addition to the 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.

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is produced in small amounts during the production of trichlorophenols. Uncontrolled chemical reactions in the presence of heat have led to the development of considerable amounts of TCDD in the commercial preparations. Explosions resulting from overheated chemical reactions have been responsible for human exposure to TCDD in Europe and the United States. One of the serious complications of this exposure has been persistent chloracne.

The health implications of TCDD, other than those related to industrial exposure, were brought to the attention of the general public during the Vietnam conflict. Defoliants widely used in the combat zones contained varying concentrations of TCDD, which has been held responsible for physical abnormalities that have developed in the human population (Rose & Rose, 1972). In addition to chloracne, exposed individuals have experienced fatigue, peripheral numbness (Rose & Rose, 1972), increased abortions and infant deaths, and liver cancer (Tung, 1973). More recently, serious physical problems, which included severe weight loss, acne, alopecia, haematurea and abortions, have appeared in horses that inhabited arenas sprayed with residues of trichlorophenol production containing TCDD (Carter, Kimbrough, Liddle, Cline, Zack & Barthel, 1975). The detection of minute amounts of dioxins in human milk (M. Meselson, personal communication 1977) and beef fat (R. T. Ross, personal communication 1976) have caused concern about the exposure of man and animals to TCDD. In the study now reported, emphasis is placed on determining the pathological alterations that occur in primates after low-level, long-term exposure to TCDD in food.

EXPERIMENTAL

Eight young healthy female rhesus monkeys weighing approximately 5·6 kg were placed on a diet of pelleted monkey food to which 500 ppt‡ TCDD (Dow Chemical Company, Midland, Mich.) had been added. The experimental diet was prepared in 100-lb lots by suspending TCDD (22·7 μg) in approximately 1 ml of acetone and adding this to 250 ml of corn oil; the corn oil was pre-mixed in 25 lb of finely ground monkey food (Purina Monkey Chow, Ralston-Purina Co., St. Louis, Mo.), and this mixture was then added to an additional 75 lb of food and mixed thoroughly for 10 min. The food was then pelleted and stored at −5°C until used. The monkeys remained on the experimental diet for 9 months. General health was monitored by daily observation, and in addition complete blood counts were made and serum protein, serum lipid, serum glutamic-pyruvic transaminase (SGPT) and blood-urea nitrogen were determined on a monthly basis. The food intake for each animal was monitored daily throughout the experiment.

A complete autopsy was performed on the monkeys that died. Sections of the tissues were fixed in 10%, neutral buffered formalin, dehydrated, embedded in paraffin and sectioned at 5 μm. The sections were stained with haematoxylin and eosin and examined using a light microscope.

RESULTS

Clinical observations

Following 3 months of TCDD feeding (5·8 ± 0·9 μg total intake per animal) the monkeys developed periorbital oedema, loss of facial hair and eyelashes.

‡t = 10^{12} throughout this paper.

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Table 1. *Haemograms of monkeys fed 500 ppt TCDD for 9 months**

Animal no.	White blood cells ($\times 10^3$ mm^3)		Blood platelets ($\times 10^3$ mm^3)		Haemoglobin (gm%)		Haematocrit (%)	
	Initial	Terminal	Initial	Terminal	Initial	Terminal	Initial	Terminal
7†	11·0	2·3	240	23	13·5	4·0	41·0	12·0
9†	7·9	2·4	360	44	14·1	6·9	43·0	21·5
23	10·3	3·8	380	28	14·6	12·6	45·5	40·0
31	10·6	10·4	400	480	14·1	10·7	45·0	35·5
32‡	8·5	4·1	300	50	14·1	6·0	43·0	19·5
38†	9·5	3·8	330	34	13·6	6·6	43·0	22·0
41	7·8	8·0	350	340	13·1	11·5	40·0	44·5
49§	8·6	8·7	260	54	13·7	8·5	43·0	29·5

*Values obtained 3 months after the end of treatment unless otherwise stated.
†Died during wk 44 of experiment.
‡Died during wk 28 of experiment.
§Died during wk 36 of experiment.

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accentuated hair follicles and dry scaly skin. These changes became more prominent in six of the eight animals by month 6 of exposure (11·3 ± 1·7 μg total intake per animal). The majority of the animals showed a decrease in haemoglobin and haematocrit by month 6. The haematological changes became more accentuated in six of the seven animals that survived 9 months of TCDD exposure (Table 1). However, blood urea nitrogen, total serum lipid, serum cholesterol, SGPT, total serum protein and albumin globulin ratios were not altered appreciably during the experiment except in terminal animals where there was a slight decrease in serum albumin and an increase in SGPT. The monkeys also lost weight throughout the experiment although their food intake was unaltered (Table 2).

Animal no. 32 became severely anaemic, thrombocytopenic and leucopenic in month 7 of exposure after a total intake of 11·2 μg (Table 1). Prior to death the peripheral blood smears were practically devoid of immature red and white blood cells. During the subsequent 4 months four additional animals (nos 7, 9, 38 and 49) showed similar clinical changes and died. Their peripheral leucocytes and platelets decreased to levels as low as 2300 and 24,000 mm^3 from normal values of 7–10,000 and 200–400,000 mm^3, respectively. The total TCDD intake of these animals at the time of death was 18·6 ± 1·8 μg/animal. Although all animals were removed from the TCDD diet after 9 months, the three animals (nos 23, 31 and 41) that survived for 12 months experienced a continuing loss of hair and periorbital oedema. One of the surviving monkeys (no. 23) developed a severe leucopenia and thrombocytopenia by month 12 (Table 1).

Table 2. *Body weights of monkeys fed 500 ppt TCDD for 9 months*

Animal no.	Body weight (kg)	
	Initial	Terminal*
7	6·19	5·86
9	6·74	5·74
23	6·62	5·03
31	6·02	5·35
32	5·01	4·22
38	5·63	5·22
41	5·73	5·44
49	5·39	4·46

*Weight at autopsy or after 12 months on trial.

Gross observations at necropsy

The animals that died experienced hair loss from all parts of the body. The skin was dry and flakey, while the hair follicles about the face became accentuated. Loss of eyelashes and swelling of the upper eyelids along with facial oedema and petechiae were present in all animals. There were also large haemorrhagic areas around the nares, on the gums and on the surfaces of the buccal cavity (Fig. 1). In addition, there were petechial haemorrhages over the entire surface of the body. Subcutaneous oedema was particularly prominent in the lower abdominal region and inner surfaces of the thighs. Marked irregularities in the growth of the toenails and fingernails (Fig. 2) and gangrenous necrosis of the distal phalanges were also recorded.

Ascites was prominent in all of the animals. There was marked distension of the intrahepatic biliary system, with oedema and dilatation (up to 1 cm in diameter) of the common bile duct (Fig. 3). Patency of bile ducts was demonstrated by the flow of bile through the ampulla of Vater and by careful dissection of the intrahepatic ducts. The lymph nodes throughout the abdominal cavity had undergone atrophy. In addition to the pale appearance of all abdominal organs, there were foci of haemorrhage in the adrenals, pancreas, liver, endometrium, serosal and mucosal surfaces of the gastro-intestinal tract and the urinary bladder.

In the thoracic cavity, the lungs exhibited focal haemorrhage in all lobes (Fig. 4). There was bilateral ventricular dilatation, cardiac enlargement and oedema. Focal areas of haemorrhage were present in the epicardium, myocardium and endocardium. The skeletal musculature, particularly in the extremities, was oedematous, pale and discoloured by haemorrhagic foci. Extensive haemorrhage was found in the meninges, and there were isolated haemorrhagic areas in the brain parenchyma. The bone marrow (from the femur, vertebrae and sternum) had the appearance

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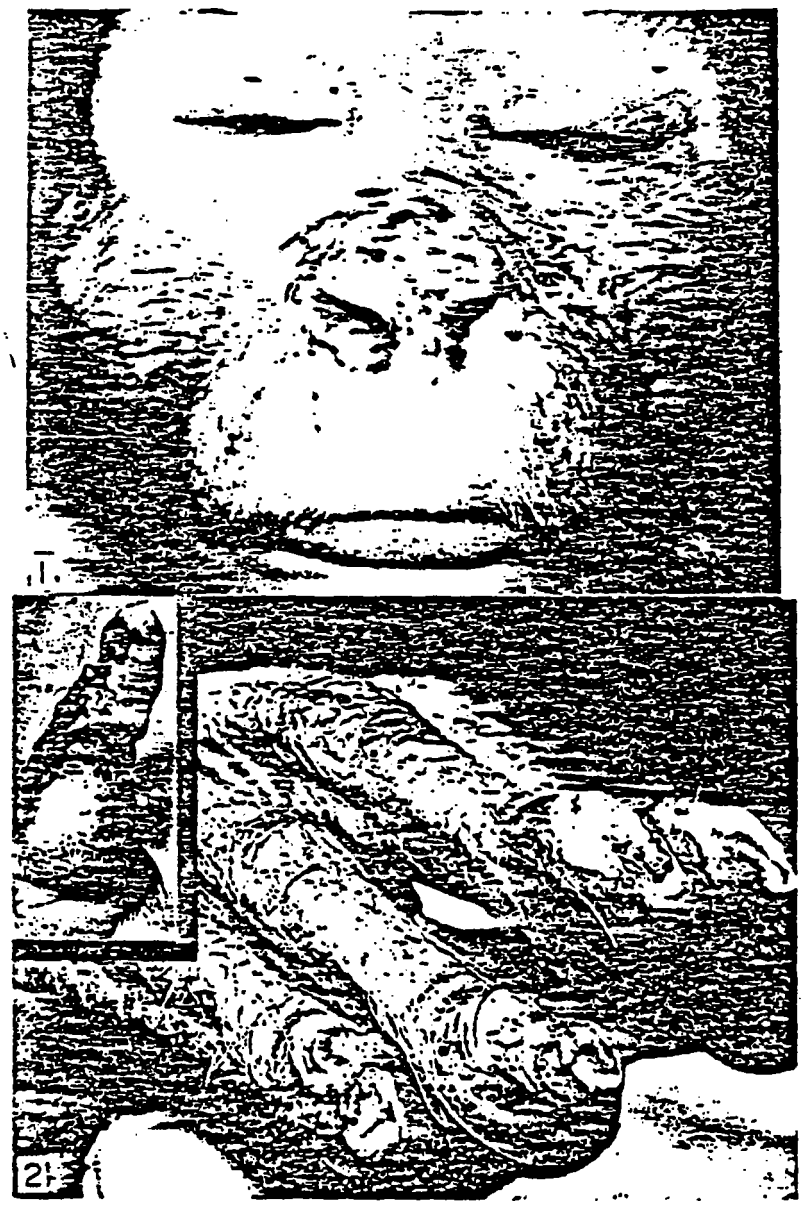


Fig. 1. Facial view of animal no. 38 which died after 9 months on a diet containing 500 ppt TCDD, showing petechial haemorrhages about the nose, absence of eyelashes and oedema of the upper eyelids and lips.

Fig. 2. Toes of animal no. 7, which died after 9 months on a diet containing 500 ppt TCDD, showing marked thickening and irregular growth of the toenails and swelling of the terminal phalanges. In the more severely affected animals, there was gangrenous necrosis of the terminal phalanges (see inset).

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Fig. 3. In animal no. 9, which died after receiving TCDD over 9 months, a probe (arrowed) was introduced through the ampulla of Vater to demonstrate oedema and dilatation in the common bile ducts.

Fig. 4. Lungs and heart of animal no. 49, which died after 35 wk on a diet containing 500 ppt TCDD, showing multiple haematomas in the lobes of the lung adjacent to apparently normal parenchyma, and an enlarged heart of globoid appearance.

Fig. 5. Sample of femoral bone marrow from animal no. 49, which died 1 wk after receiving a diet containing 500 ppt TCDD for 9 months, showing the fatty appearance of the marrow and focal haemorrhages in the darker areas.

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Fig. 6. Bone marrow from animal no. 32, which died with severe pancytopenia after ingesting 11·2 μg TCDD, showing small foci of darker cells dispersed among the lucent fat cells and consisting (inset) of small lymphoid-like cells with round basophilic nuclei and scant cytoplasm. Haematoxylin and eosin × 12 (inset × 104).

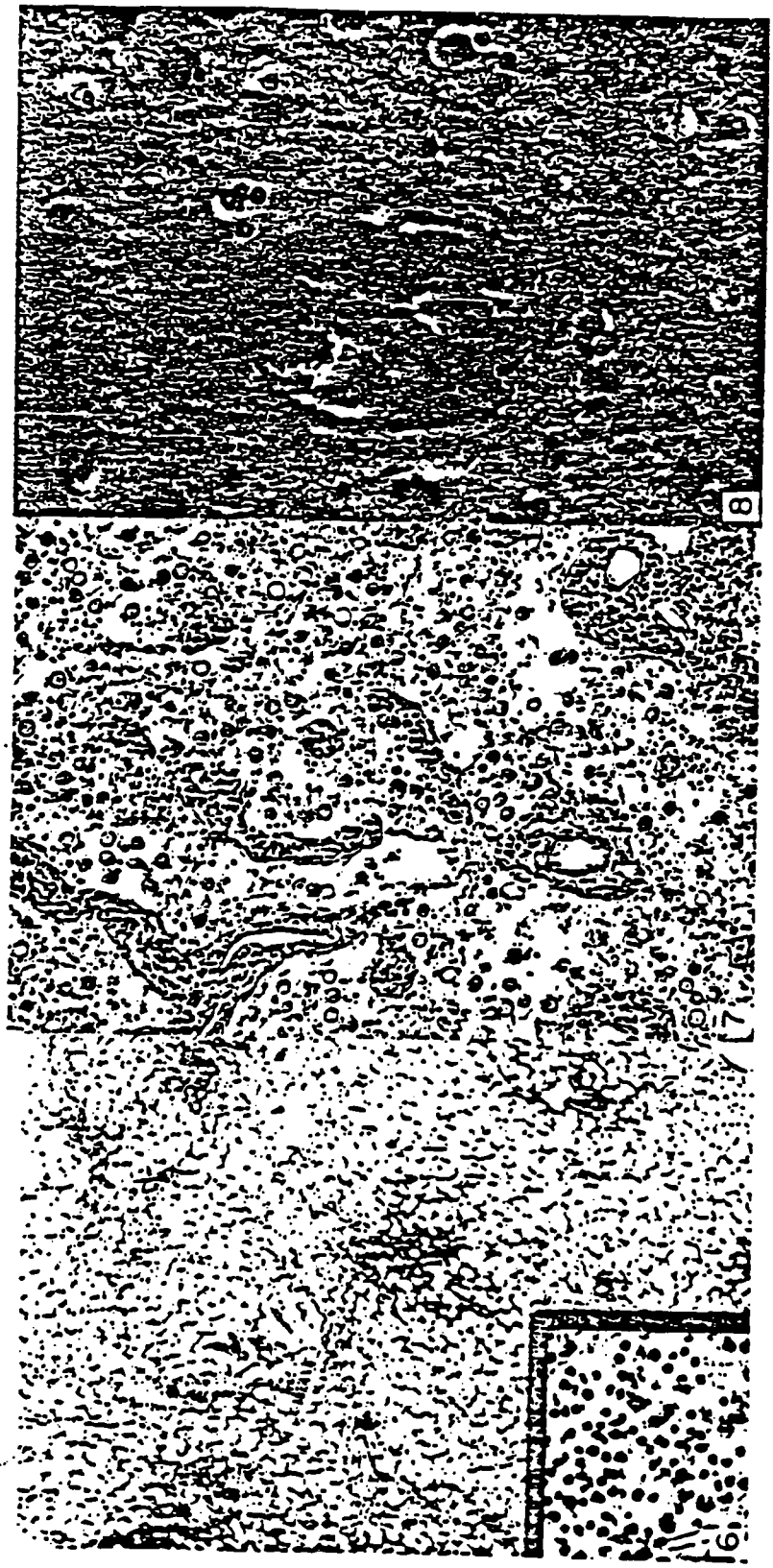
Fig. 7. Section of a mesenteric lymph node from animal no. 49, which died after receiving a diet containing 500 ppt TCDD for 36 wk, showing the hypocellularity of the medullary cords and, in the sinuses, widely dispersed cells which are mainly small lymphocytes and large vacuolated cells with eccentrically located nuclei. Haematoxylin and eosin × 104.

Fig. 8. Section of cerebrum from animal no. 38 which died after 9 months on diet containing 500 ppt TCDD, showing in the cortex extensive haemorrhage limited to the Virchow-Robin space around the blood vessels. Haematoxylin and eosin × 106.

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Fig. 9. Mucus-secreting cells (arrowed) in the ductal epithelium of the salivary glands from animal no. 9. Haematoxylin and eosin × 104.

Fig. 10. Hypertrophy, hyperplasia and metaplasia of the mucus-secreting cells in the gastric mucosa of animal no. 32, typical of all monkeys exposed to TCDD, with penetration of the muscularis mucosae by the epithelium (arrowed) and the development of large areas of mucus-secreting glands in the submucosa. Haematoxylin and eosin × 12.

Fig. 11. Keratinization in the Meibomian glands of the eyelids (K) occurring as an aftermath of TCDD exposure in the monkeys, with squamous metaplasia in the epithelial cells surviving around the periphery of the gland. Haematoxylin and eosin × 12.

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of fatty tissue in which there were focal areas of haemorrhage (Fig. 5).

Microscopic observations

The major microscopic changes may be separated into three categories: degeneration or atrophy of the bone marrow and lymphopoietic tissues, haemorrhage, and changes related to cellular hypertrophy, hyperplasia and metaplasia.

The bone marrow displayed a decided hypocellularity. The haematopoietic cells of the marrow were replaced primarily by fat cells with focal areas in which cells that were lymphoid in appearance predominated (Fig. 6). There was also a conspicuous reduction of discernible erythroid or myeloid stem cells as well as of megakaryocytes in the marrow. The lymph nodes throughout the body were hypocellular. In addition to the absence of any distinct lymphoid germinal centres, the cortical lymphocytes were sparse and the medullary cords were narrow or inconspicuous. Cellular debris and large vacuolated cells predominated in the sinuses (Fig. 7). The spleen, also, was devoid of any distinct lymphoid germinal centres and the small lymphocytes were widely dispersed.

The second major category of microscopic changes was related to the haemorrhage that occurred in tissues throughout the body. Well-circumscribed focal areas of haemorrhage in the heart, lungs, liver, adrenals, pancreas and skeletal muscle consisted of collections of intact red blood cells that partially disrupted the architectural pattern of the affected tissue. The acute nature of the haemorrhage was shown by the lack of tissue necrosis and an absence of reactive cells. Petechial areas were prominent in the dermis of the skin, submucosa of the urinary bladder and epithelium of the alimentary tract. Haemorrhages of the endometrium and meninges were more diffuse in their distribution. Those in the brain were limited to the Virchow-Robin spaces surrounding the blood vessels (Fig. 8). The haemorrhage in the bone marrow varied considerably, being diffuse in some areas and focal in others.

Considerable modification in the morphological features of epithelium occurred in the TCDD-treated animals. Metaplastic changes characterized by numerous mucus-secreting cells were present in the ductal epithelium of the salivary glands (Fig. 9), bile ducts and pancreatic ducts. Similar changes also occurred in the bronchial epithelium and in the palpebral conjunctivae. In addition to the metaplasia, the epithelium of the bile ducts and palpebral conjunctivae developed considerable hypertrophy and hyperplasia. Squamous metaplasia of the sebaceous glands occurred more extensively in the skin of the face and to a lesser extent in other areas.

Hypertrophy, hyperplasia and metaplasia were observed in the gastric epithelium where the parietal and zymogenic cells were replaced by those that secreted mucus. In addition to the thickened mucosal lining, many of the epithelial cells invaded the submucosa through interruptions in the muscularis mucosa (Fig. 10). Such ectopic epithelium assumed many different patterns. Some isolated cells encompassed large mucinous cysts, while others were arranged in sheets or formed acinar structures. Considerable oedematous fluid usually surrounded the epithelium. Inflammatory cells with a predominance of neutrophils were seen in close proximity to ruptured submucosal mucinous cysts. Rupture of such cysts produced the ulcers that were found in the gastric mucosa. Hypertrophy and hyperplasia were also apparent in the transitional epithelium throughout the urinary tract.

In addition to squamous metaplasia in the epithelium of the sebaceous glands, there was hyperkeratosis of the skin with keratinization of the hair follicles and the adjacent sebaceous glands. The latter alterations were particularly prominent in the Meibomian glands of the eyelids (Fig. 11). The pronounced thickening of the fingernails and toenails was considered to be secondary to excessive keratin production.

In the heart, petechial haemorrhages were prominent in the atria and in the tips of the papillary muscles. Additionally, foci of subendothelial and pericardial haemorrhage were frequently found in the ventricles. In two of the five hearts the erythrocytes were haemolysed, so that haem pigments and haemosiderin could be identified by Perls' stain in the intracellular spaces. Intra- and intercellular oedema led to separation of the muscle fibres and myofibrils.

DISCUSSION

The data presented in this report indicate that profound cellular alterations are induced in many tissues following the ingestion of minute concentrations of TCDD by primates for 9 months. Most notable are the effects of TCDD on the haematopoietic system. As exposure time lengthens, the severity of the cellular deterioration in bone marrow and lymphoid tissue becomes widespread. Terminally the animals develop severe pancytopenia. The haematological data presented here agree quite well with the previous studies conducted in this laboratory, where mixtures of dioxins, including TCDD, were fed to nonhuman primates (Allen & Carstens, 1967). In the previous investigation the monkeys also developed anaemia and leucopenia prior to death. Similar haematological changes have been recorded in rats given a single dose of 50 μg TCDD kg body weight (Van Miller & Allen,

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1977). In addition to the depletion of circulating lymphocytes, atrophy of the thymus, spleen and lymph nodes has been observed in several species (Vos, Moore & Zinkl, 1973). These changes may be related to the immune suppression which has been recorded in TCDD-exposed animals. Sublethal doses of TCDD have been shown to suppress cell-mediated and humoral immunity in guinea-pigs (Vos *et al.* 1973).

There are other complications that arise as a result of the decrease in haematopoiesis. The widespread haemorrhage that was observed in the monkeys prior to death corresponded to the decrease in blood platelets. It is logical to assume that the haemorrhage was associated at least in part with the altered clotting capability of the blood. The ventricular dilatation and myocardial hypertrophy in the TCDD-exposed monkeys may be related in part to the reduction of circulating erythrocytes and subsequent increase in the cardiac workload.

In this experiment it was shown that ingestion of 2–3 μg TCDD kg body weight over a 9-month period was capable of producing in several organs cellular destruction or alteration sufficiently great to cause death in over 50% of the non-human primates. It is of interest that the LD_{50} of monkeys exposed to a single oral dose of TCDD is 50–70 μg/kg body weight (McConnell, Moore & Dalgard, 1977) with death occurring within 45 days. There are some major differences in the changes that occur in monkeys acutely and chronically exposed to TCDD. While death was associated with severe pancytopenia in the chronically exposed animals, haematological changes were not a constant feature of those acutely exposed. In addition, widespread haemorrhage occurred only in the chronically exposed animals. Changes that followed both acute and chronic exposure were loss of hair and nails, keratinization of the Meibomian glands and hair follicles, hyperplasia of the transitional epithelium in the renal pelvis and of the mucosal epithelium of the stomach and bile duct, a decrease in serum albumin and an increase in SGPT.

The morphological changes that have been recorded in monkeys indicate that TCDD is capable of suppressing haematopoiesis and inducing cellular alterations in epidermal appendages and mucosal epithelium of the biliary, pancreatic, intestinal and urinary tract. After exposure to TCDD, the cell population in the bone marrow and lymphoid elements underwent degeneration and necrosis. Other less specialized cells such as the bile-duct epithelium and mucous cells of the stomach were injured less severely. However, as a result of injury the latter cells showed a tendency to compensate by undergoing hypertrophy and/or hyperplasia. Some of the injured cells also reverted to more primitive forms that were capable of surviving in a less desirable environment. This was seen in the sebaceous glands where the secretory and ductal epithelium reverted to squamous cells.

The observations made on the monkeys used in this experiment indicate that there are areas that need special attention in animals, including man, exposed to TCDD. Perhaps of foremost importance are the alterations that occur in the haematopoietic tissue. Anaemia, thrombocytopenia, and leucopenia were the most debilitating changes in the primates. The altered lymphopoiesis could be associated with immune suppression. The possibility of reproductive abnormalities also exists. Altered levels of serum progesterone and oestradiol associated with difficulties in conception and early abortions have been observed in female monkeys exposed to low levels of TCDD (Allen, Barsotti & Van Miller, 1977), and testicular atrophy has developed in male monkeys given small amounts of dioxins (Allen & Carstens, 1967). In addition, the widespread hypertrophy, hyperplasia and metaplasia that occurred in the epithelium of monkeys exposed to TCDD, and a greater frequency of tumours in rats (Van Miller & Allen, 1977) suggests that the compound has carcinogenic potential.

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Molecular orbital calculations were performed to analyze the electron distribution in both the unchlorinated dibenzo-*p* dioxin and TCDD, particularly their first unoccupied orbitals. It was hoped that this would allow a demonstration of the theory that neutral TCDD can be viewed as an electron acceptor, rationalize the necessity of halogenation for toxicity, and explain the chlorination pattern required for toxicity. The calculations made indication that halogenation of dibenzo-*p* dioxins at certain positions offers considerable structural stabilization to added charge. Greater chlorination at these positions yields stronger charge- transfer complexes. Chlorination at other positions provides poorer acceptors, precisely the observed chlorination requirement for dioxin toxicity. These calculations may help to explain the unusually firm binding of TCDD to soil and protein, both of which tend to be electron-rich or to contain electron-donors. TCDD may be solubilized by the presence of suitable electron- donors, displaced from its complexes by stronger electron- acceptors, or bound preferentially by a specific electronegative protein in target organs such as the liver.

PESTICIDES ABSTRACTS

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ACUTE AND CHRONIC TOXICITY OF 75 PESTICIDES TO VARIOUS ANIMAL SPECIES

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INTRODUCTION

Enactment of the Toxic Substances Control Act (TSCA) PL 94-469 on October 11, 1976 provided the U.S. Environmental Protection Agency (EPA) with the authority to require premanufacture testing of chemical substances for indications of human health and environmental effects. Successful implementation of TSCA may depend on the use of an effective toxicity screening process.

Considering the number of chemical compounds which may need quick environmental evaluation, the costs involved, and the shortage of toxicity testing facilities, the important question which remains to be answered is: "*What are the best and minimum number of indicator test methods and species for assessing toxicity to a broad spectrum of animal organisms (vertebrate and invertebrate, terrestrial and aquatic)?*"

There is a need for making a critical selection of good toxicity indicator organisms for evaluating hazards of chemicals. Data from a large base of acute toxicity for ten species of animal organisms and 75 chemicals was assembled from the literature for use for correlative purposes. These data are tabulated here and serve as the basis for another paper (8). The table has not been published previously due to space limitations. Because the tabulated data are so difficult to obtain in any one place and includes so many compounds, it appears valuable to make this data base available.

Indicator organisms and test methods are needed for both acute and chronic toxicity studies. Extensive data for chronic toxicity is not available on many species other than fish and rats. Chronic toxicity data on fish is discussed in a separate paper by the author (7). The present paper includes data on the chronic toxicity of 72 compounds to rats and a summary of the acute toxicity data the author has given previously (8).

TEST ORGANISMS AND METHODS

1. Acute Toxicity

Certain organisms have been used sufficiently in acute toxicity tests to support a common data base for 75 different pesticides. These organisms, listed in Table 1, include the rat (*Rattus norvegicus*) and mallard (*Anas platyrhynchos*) for acute oral LD50 studies; the bobwhite (*Colinus virginianus*) or Japanese quail (*Coturnix c. japonica*) for dietary LC50 feeding studies; the rainbow trout (*Salmo gairdneri*), bluegill (*Lepomis machrochirus*), fathead minnow (*Pimephales promelas*), the striped mullet (*Mugil cephalus*) or spot (*Leiostomus xanthurus*), *Daphnia magna* (or *D. pulex*), and shrimp (any of the three *Penaeus* species: *P. aztecus*, *P. duorarum*, *P. setiferus*) for aquatic LC50 studies; and the honey bee (*Apis mellifera*), a terrestrial arthropod for contact LD50 studies. A number of these species were chosen because they were under preliminary consideration for screening use by the TSCA Interagency Advisory Committee, and all are presently used for pesticide registration purposes by EPA (24). These species represent organisms which it is known from experience can be reared readily and tested in the laboratory and which are sensitive representatives of their family or class of organisms.

Most of the acute toxicity test methods used for data bases are those of the U.S. Department of Interior Fish and Wildlife Service for birds (5, 18), fish, shrimp and daphnids (2, 4, 10-14, 16-23, 26). The rat test methods and those for wildlife species are essentially those described in the EPA proposed Guidelines for Registration of Pesticides (24). Honey bee contact test methods are described by Atkins *et al* (1). The original references may be examined for details for variables in test methods. For rough toxicity screening purposes, such variables are considered here to result in variations to within about one order of magnitude for one compound on one species. If precisely standardized test methods were required for every test result here, little comparative data would be available for this paper. Thus, such a data requirement is not a practical solution for comparison purposes at the present time.

2. Chronic Toxicity

There is no published large data base for correlation of chronic toxicity tests with numerous chemicals other than that assembled by the FAO/WHO expert committees on pesticide residues in food (3). The only species on which acute and chronic (2 year) oral toxicity data are consistently available for comparison is the rat. Thus, the rat is presently the only choice of species available for correlation of acute and chronic toxicity. Seventy-two chemicals are thus compared.

3. Basis of Classification of Chemicals by Groups

The diversity of the structures of the 75 chemicals studied makes detailed chemical groupings of a useful correlative size unmanageable. Insecticides can, however, be grouped into four groups, chlorinated hydrocarbons, phosphorus containing chemicals, carbamates and miscellaneous. The herbicides

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[illegible] treatments. This classification system was followed in Table 1. The common names of pesticides are used in accordance with those shown in references 9 and 28.

4. Statistical Methods

The acute toxicity data for each species and chemical were intercorrelated in logarithmic form to prevent exaggeration of the correlations when many orders of magnitude are covered. Average values were used where a range was shown. The correlations were calculated using a multiple regression computer program. The validity of the correlations are presented in terms of the [illegible] number of orders of [illegible] values of one species from known toxicity values of another species where reasonably good correlations exist.

The correlation coefficients and 95% confidence limits are the principle indicators of the degree of success for predicting values from equations.

RESULTS

1. Acute Toxicity

Acute toxicity data for 10 species and 75 compounds are given in Table 1. This is the basis for the correlations but [illegible] species. Examples of the [illegible] correlations of the tenfold test species are summarized in Table 2. The data show that correlations between the results of terrestrial and aquatic organisms are not good. The best toxicity correlations with various species involve those concerning birds with mammals, various species of fish with each other, shrimp with fish. Honey bee data shows that bees are not very sensitive indicators over a large range

TABLE II. Comparative Acute Values with Birds and Fish, Chronic Responses and Bioconcentration Factors.

A. INSECTICIDES

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Common or other Name for Pesticide[a]	Chronic Toxicity Data: Rat No-effect mg/kg/day in diet	Acute TOXICITY Data: Rat LD50 mg/kg	Mallard LD50 mg/kg	Bobwhite LC50 ppm	Honey Bee LD50 µg/bee	Bluegill LC50 ppm	Rainbow Trout LC50 ppm	Fathead Minnow LC50 ppm	Salt Water Fish LC50 ppm	*Daphnia* LC50 ppm	Shrimp LC50 ppm
a. *Chlorinated Hydrocarbon Insecticides*											
aldrin	0.025	39-60	520	47	0.38	0.0052	0.0022	0.0082	0.00286 M; 0.0055 S;	0.029	0.0006
chlordane	1.0	283-590	1200	441	5.23	0.04	0.0078	0.069	0.0055 M;	0.029 *D. p.*	0.0044
DDT	0.05	87-500	2240	611	5.56	0.005	0.0016	0.019	0.0004 M; 0.002 S;	0.0014	0.001
dieldrin	0.025	40-100	381	37	0.14	0.0028	0.0014	0.0041	0.0071 M; 0.0055 S;	0.33	0.0055
endosulfan	2.5	18-110	33	805	7.81		0.0012		0.0006 M,S;	0.24	0.0004
endrin	0.05	3-45	5.6	14	2.0	0.0004	0.0086	0.0011	0.0026 M; 0.0006 S;	0.45	0.0001
heptachlor	0.25	40-100	2000	92	0.53	0.019	0.008	0.056	0.018 M 0.026 S;	0.058	0.0004
lindane	1.25	76-200	2000	882	0.56	0.068	0.027	0.087	0.16 M	0.46 *D. p.*	0.0004
methoxychlor	10	5000-7000	2000	5000	23.6	0.078	0.04	0.089	0.155 M 0.03 S	0.0096	0.0006
mirex	0.25	235-705	2400	2511	7.15		5.80		2 M,S		1.2 P
pentachlorophenol	8.0	125-210	980	3400	48	0.11	0.052	0.205		0.18	
Perthion	25	6680 M 70 S	10000 BW 455	5000	1.47		0.005			0.0042	
toxaphene	1.2	80-284	41	525	60.4			0.014	0.0005 M 0.00015 S	0.015	0.0014

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TABLE I Continued

A. INSECTICIDES

Common or other Name for Pesticide*	Chronic Toxicity Data: Rat No-effect mg/kg/day in diet	Acute TOXICITY Data: Rat LD50 mg/kg	Mallard LD50 mg/kg	Bobwhite LC50 ppm	Honey Bee LD50 µg/bee	Bluegill LC50 ppm	Rainbow Trout LC50 ppm	Fathead Minnow LC50 ppm	Salt Water Fish LC50 ppm	*Daphnia* LC50 ppm	Shrimp LC50 ppm
d. *Miscellaneous Insecticides*											
allethrin K-8077	25	680-1000	—	2000	0.31		0.019		—	0.021(D.p.)	—
cyhexatin K-53701	3.0	180-820	—	195	38.2	0.008	0.0012		—	0.0029	0.090P
pyrethrins K-15490	10.0	200-2600	10,000	5000(JQ)	0.63	0.070	0.054		—	0.025(D.p.)	—
rotenone K-14773	1.25	60-1500	2000	1882(JQ)	2.42	0.022	0.026		0.057(M)	0.1(D.p.)	—
B. HERBICIDES											
amitrole K-16556	0.025	24,600	2000	5000(JQ)	12		50(BG)		—	23	—
atrazine K-28261	5	3080	2000	5000	96	26	12.6		1.0(S)	3.6	1.0(B
2,4-D, BE ester K-17722	31 (acid) 62.5	666 acid	1000 acid	5000	18	0.39	2.1(BG)	5.0(K)	5.6	1.0(P)	
bensulide	25	770	—	10(JQ)	24	0.81	0.72		0.32(S)	—	1.0(B
bromoxynil K-34901	50	260-430	200	4000(RNP)	14		0.05		—	0.011	—
butylate	32	4659-5431	—	40,000	26	5.5	5.2		1.0(S)	—	1.0(B)
chloramben K-5222	500	3500	706.8(W)	—	2.0		4.2		1.0(C)	—	1.0(B)
dalapon, sodium salt K-[illegible]	15	7570-9330	5600 (chicken)	5000(JQ)	24		87		50(K)	1505	1(B)
dichlobenil K-30676	1	3160	2000	5000(JQ)	120	10		18.0	—	10.0	—
dichlone	—	1300	2000	5000(JQ)	7.25	0.12	0.34		—	0.025	—
dinoseb K-1774	5.0	58	9-13	400(JQ)	36		0.07-0.3		—	0.68	1.9(eP)
diquat (Br_2) K-24750	0.36(dog)	230	564	2932	—		10		1.0(K)	7.1	1(W)
diuron K-14276	—	3400	2000	1730	145	5.9	3-60 (no RT)		6.3(M)	47	1(B)
EPTC	16	1652-3160	—	20,000	12	27	19	20(M,K)	—	0.63(W)	
molinate	0.4	720	—	13,000 (Ma)	29	0.475	1.3		1(S)	0.60	1.0(B)
monuron	12.5	3500-3700	—	5000	110		76		16.3(M)	106	1(W)
picloram K-25323	150	8200	2000	5000	14	21	24		—	34.4	1.0(B)
propachlor K-27612	133	710	512	5000	311		0.16		—	7.0	—
silvex, PGBE ester K-4931	10.0 (acid)	300	—	12,979	36		0.65		0.36(S)	5.5	0.24(B)
simazine K-28205	5	5000	—	5000	96	100	25		—	1.0	—
2,4,5-T [illegible]	21.5	300	2000	2776	24		12		50.0(K)	53	71.0(B)
TCA, sodium salt K-3482	—	5000	4280 (chicken)	10000	48		100		1.0(M)	1428	1(B)
triclopyr K-44485	—	713	1698	2935	60	148	117-140		—	133	—
trifluralin K-51061	100	10,000	2000	—	24	0.047	0.01-0.09	0.093	—	0.56	—

Substitute Species

B brown shrimp; BG bluegill; BW bobwhite; C sheepshead minnow; D.p. *Daphnia pulex*; F fathead minnow; JQ Japanese quail; K killifish; M mullet; Ma mallard; P pink shrimp; RNP ring necked pheasant; RT rainbow trout; S spot; W white shrimp

Numbers in parentheses in the headings are assumed as the reference source of data unless a different reference number is cited in parentheses next to the individual data. The common names used in the table are in accordance with [illegible]

TABLE I Continued

V. INSECTICIDES

Common or other Name for Pesticide*	Chronic Toxicity Data: Rat No-effect mg/kg/day in diet	Acute TOXICITY Data: Rat LD50 mg/kg	Mallard LD50 mg/kg	Bobwhite LC50 ppm	Honey Bee LD50 µg/bee	Bluegill LC50 ppm	Rainbow Trout LC50 ppm	Fathead Minnow LC50 ppm	Salt Water Fish LC50 ppm	*Daphnia* LC50 ppm	Shrimp LC50 ppm
b. *Phosphorus Containing Insecticides*											
azinphosmethyl	0.125	13-16	1.96	488	0.42	0.052	0.014	0.245	0.0055(M);	0.0032(D.p.)	0.0044(B)
chlorpyrifos	0.03	97-276	70-80	2000(Q)	0.114	0.0033	0.003		0.07(S) 0.0036(K)	0.0017	0.0002(B) 0.0024(P)
chlorpyrifos-methyl	0.1	941-2140	2000	5000	0.383		0.014	1.22	0.15(S)	0.001	0.0007(B) 0.0007(P)
coumaphos	0.25	11-64	29.8	120	—	0.0062	0.082		0.26(C)	0.001	0.0036(P)
demeton	0.1	2-12	7.2	596	2.60		0.081(BG)		0.55(S)	—	0.063(P)
diamidafos	5	140-200	—	539	60	306	1		1.0(S)	10.0	1.0(B)
diazinon	0.1	66-600	3.8	245	0.37	0.082	0.09		0.25(M)	0.0043	0.044(B)
dichlorvos	0.033	25-170	7.8	298	0.52	0.48			0.56(S)	0.0000066(D.p.)	0.044(P)
dicrotophos	0.075	22-75	4.2	32	0.1		6.8		10(K)	0.6(D.p.)	0.25(B)
dimethoate	0.2mam	250-500	42	346(Q)	0.19	6.0	8.5		10(K)	2.5	10(B)
disulfoton	0.05	3-12	6.8	715	5.14		0.048(BG)		0.74(C)	—	0.025(B)
ethion	0.3	27-119	—	5000	20.6		0.23		0.069(C)	0.00001	0.036(P)
fenitrothion	0.25	250-670	1190	157	0.383		0.70		10(C)	—	0.0025(B)
fensulfothion	0.025	210	0.7	35	0.35		--		0.055(K)	—	0.01(B)
fenthion	0.15	256-298	5.9	30	0.308	1.88	0.93	2.44	1.59(M) 1.22(S)	0.0008 (D.p.)	0.00006 (P)
malathion	5.0	885-2800	1485	1397	0.71	0.103	0.17	8.65	0.57(M) 0.55(S)	0.0009	0.5(P)
methyl parathion	0.25 0.1mam	9-42	10	90	0.29	5.72	2.75	8.9	10(C)	0.0048	0.0055(B)
mevinphos	0.02	3.7	4.6	286.1(Q)	0.36	0.021	0.012		0.83(C)	0.00016(D.p.)	0.25(B)
monocrotophos	0.025	21	4.8	2.4	0.15	4.0	4.9		10(K)	—	0.069(B)
naled	0.37	430	52	2117	0.48	0.33	0.08 (19 hrs)	3.3	0.56(M) 0.44(S)	0.00015(D.p.)	0.0055(P)
parathion	0.05mam	3-30	2.1	194	0.18	0.047	2	1.9	0.065(C)	0.008	0.001(B)
phorate	--	1-8	0.6	373	10.1		0.089		0.00044(K)	—	0.00017(B)
pirimor	2.0	147-299	18.30	501	--		--		0.055(M,K)	—	0.0045(B)
phosphamidon	0.1	15-43	3.1	24	1.46		8.0		10(M,S)	0.0125	0.44(P)
ronnel	0.5	900-3025	4000 chronic	2600	5.74	1.0	0.74		0.32(S)	0.0057	0.0052(B)
stirofos	—	4000-5000	2000	5000	1.37		—		1.6(S)	—	0.28(P)
temephos	0.1	1000-8000	80-160	92	1.55		1.0		1.0(S)	—	0.0028(B)
trichlorfon	2.5	450-560	500-1000	720	59.8	0.26	1.4	51	10(C)	0.00012	0.39(P)
trichloronat	0.15	16-35	12	--	2.33		0.24		0.32(S)	-	0.0005(P)
c. *Carbamate Insecticides*											
aminocarb	10	30	23	2000	1.16		—		10(C)		0.55(B)
carbaryl	10.0	307-850	2000	5000	1.34	11.2	4.34	14.6	1.7(M)	0.006(D.p.)	0.0025(B)
methiocarb	5	100-135	—	1427(Q)	0.38	0.110	0.64		0.56(K)		0.055(P)
methomyl	5-10	15-64	3-8	500(Q)	0.91	16.7	15	17.0	10(C)	0.01(D.p.)	0.0026(B)
propoxur	12.5	95-104	12	2000	1.45	8.2		25	10(C)		0.006(P)

toxicity values. From these data certain organisms are more sensitive and show the greatest diversity of toxicity from various chemicals. From analysis of these data the key indicator organisms of toxicity to man and other animal organisms in the environment are the rat, a species of fish and daphnia.

2. Chronic Toxicity

The chronic no-effect data on rats shown in Table I are those from dietary feeding studies over a two-year period of time. The no-effect level is never lethality as it is in the acute toxicity LD50 and LC50 tests. The principal use for the chronic no-effect data here is for comparison with acute oral LD50 data using the rat.

3. Comparison of Chronic Toxicity No-Effect Levels with Acute Oral LD50 Data in Rats

By use of the statistical methods stated previously the chronic no-effect levels on rats were correlated with the LD50 on rats and with all of the other acute toxicity tests. The best statistical correlations from these pairings are shown in Table 3.

Correlation coefficients for some specific chemical groups are better than those from all combined chemical groups as shown in Table 3. While the correlations coefficients given are significant at the 1% level, the 95% confidence limits from calculated values are quite great for very accurate predictions.

A measure of chronicity for each chemical is the ratio of the rat dietary no effect (NE) level with the rat acute oral (AO) dosage. A NE:AO toxicity ratio was calculated for each compound from Table I. Due to lack of space, these individual ratios are not given here. For a data base, the extreme ranges in toxicity ratio values were taken from each chemical and summarized by chemical groups in Table 4. The non-uniformity of the NE:AO toxicity ratio values for each chemical group is shown.

According to widespread current opinion and scientific information, the "persistent chlorinated hydrocarbon insecticides" should have a high chronicity value, and indeed many do. In toxicity tests with chlorinated hydrocarbon insecticides, not many specific effects precede lethal effects. However, there is still wide variation in the NE:AO ratios which illustrates the wide variation in speed and degree of metabolism of chlorinated hydrocarbon insecticides. The speed of metabolism is one of the important indicators of chronic toxicity for all chemicals since organisms which rapidly metabolize compounds at the equivalent of several times the acute oral LD50 in their diet daily for a number of days before causing equivalent mortality (6). According to widespread current opinion phosphorus containing insecticides, carbamate insecticides, botanical insecticides, and herbicides [illegible] Contrary to this, some phosphorus containing insecticides and some herbicides appear to have high chronicity values as illustrated by use of the NE:AO ratio.

Most herbicides are not very toxic, either chronically or acutely, but the NE:AO ratios for some herbicides are large, indicating chronicity. Highly water soluble herbicides do not accumulate much in animal tissues such as fat and muscle above concentrations in the surrounding water or the animals diet and are often eliminated rapidly in the urine. However, if herbicides are not rapidly metabolized by animal organisms or are constantly present in the water or food of the organism they may exhibit chronic toxicity effects.

Organo-phosphorus containing insecticides may have a large NE:AO ratio not only because of great differences in metabolism but also because comparison of the NE:AO ratio involves cholinesterase NE dietary concentrations in treated rats. Cholinesterase measurements may be sensitive to 2-3 orders of magnitude lower concentrations than those necessary for lethality, the criteria used for AO tests. The five carbamate insecticides have small NE:AO ratios, probably because of the rapid metabolism known to occur with these chemicals in animal organisms. One herbicide compound known to

TABLE II — Statistical Correlations of Acute Toxicities between Species and Test Methods Shown in Table I

Comparative Organisms and Test Methods	Correlation Coefficient (r)	n
Rat acute oral LD50 vs Mallard LD50	0.77	74
vs Bobwhite LC50	0.59	71
Mallard LD50 vs Bobwhite LC50	0.67	60
Rainbow trout LC50 vs Saltwater fish species LC50	0.83	55
vs Bluegill LC50	0.96	41
vs Fathead Minnow LC50	0.91	20
vs Shrimp	0.63	57
Bluegill LC50 vs Fathead Minnow LC50	0.86	21
Saltwater fish species LC50 vs shrimp LC50	0.67	58

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[illegible]

Chemical Groups, Test Organisms, and Test Methods being Correlated[a]	Correlation Coefficient (r)	95% C.L. for Toxicity Value Predicted to Within (X) Orders of Magnitude X = ± value below
I. Chlorinated Hydrocarbon Insecticides		
Rat NE: dietary with:		
1. Rat LD50	0.71	1.59
2. Bobwhite LC50	0.82	1.29
II. Phosphorus Containing Insecticides		
Rat NE: dietary with		
1. Mallard LD50	0.78	0.98
2. Honey bee LC50	0.62	1.24
III. All Chemical Groups		
Rat NE: dietary with:		
1. Bobwhite dietary LC50	0.61	1.84
2. Rat LD50[b]	0.57	1.91
3. Shrimp LC50	0.55	1.93
4. Honey bee LD50	0.53	1.99

[a] Data taken from Table I

[b] An equation derived for converting rat LD50 to rat NE levels is as follows: Log rat NE [illegible] Log rat LD50

[c] All of these correlations are significant at the 1% level (r = .500)

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TABLE 3 Comparison of Rat Chronic No Effect Level (NE) with Acute Oral (LD50) Toxicity Levels

Chemical Group	No. of Compounds	Range of Rat NE:LD Ratios from Table I
Chlorinated hydrocarbon insecticides	13	0.139-0.0001
Phosphorus containing insecticides	28	0.039-0.00003
Carbamate insecticides	5	0.33-0.0325
Miscellaneous insecticides	4	0.05-0.0008
Herbicides	19[a]	0.19-0.0003[b]

[a] Does not include amitrole which has a ratio of 0.00001

have a large NE:LD ratio is amitrole where the NE level is based on goitrogenic responses.

When mutagenic, carcinogenic, teratogenic and other reproductive and enzymatic effects from all compounds are taken into account, the NE level may be considerably lower and thus the NE:LD ratios may change considerably. Thus, it is obvious that no dependable values can be gained from acute oral toxicity data alone for use in predicting NE levels on the same organism for a large group of chemicals. Predictabilities for NE levels in rats or other organisms can be developed from knowledge of physical and chemical properties for persistence and mobility of the various key chemicals in organisms and in the environment. This knowledge plus a knowledge of the most sensitive no effect measurement and mode of action of various chemicals on various organisms will be useful as predictive guides to chronic toxicity through comparison of these properties with those of [illegible]

CONCLUSIONS

By making tabulated toxicity data available, better decisions on the predictive relationships of different chemicals, test methods and test organisms can be made.

Based on data presented [illegible] organisms and [illegible] chemicals the following correlations were found statistically significant and useful for [illegible] toxicity screening tests for [illegible] chemical:

1. Mallard [illegible] oral LD50 [illegible] predict [illegible] LD50

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2. Salt water fish species LC50's can be predicted from rainbow trout LC50's.
3. Bluegill, rainbow trout, and fathead minnow LC50's can be predicted from each others LC50's.
4. Correlations between the above organisms are improved when derived from chemical groups closely related by structure. Other correlations may be useful depending on the sensitivity needed for decision making.
5. The most useful predictive indicator organisms and methods for acute toxicity from among the ten organisms tested would appear to be the rat LD50, one species of fish (rainbow trout) LC50, and one species of aquatic arthropod (daphnia) LC50.

Correlation of *chronic* and *acute* oral LD50 toxicity data for rats indicates that the predictive usefulness of equations are limited in sensitivity to generalizations with a few exceptions for results from related chemical using the identical test methods. This limitation is due principally to the use of many non-lethal, no-effect indicators for chronic toxicity from chemicals having different modes of toxicity compared to use of lethal effects as an indicator of acute toxicity, and to differences in the rate of metabolism of the various chemicals effecting chronicity.

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THE PHARMACOLOGY OF EXPERIMENTAL MYOPATHIES

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A large group of disorders affect striated muscle in man. The direct approach to the study of these diseases presents major difficulties. Biopsies can be useful in diagnosing a muscle disease and in confirming its progress. However, at a time when muscle weakness and other neurologic abnormalities become obvious, the myopathy is usually well advanced. It is also impossible to provide adequate controls to follow the development of the myopathy in man and its reversibility with experimental techniques. Consequently, a wide variety of animal models of human muscle diseases have been developed. These models include myopathies occurring spontaneously in highly inbred strains of mice, hamsters, goats, and other mammals. However, a new and rapidly expanding area of inquiry involves the pharmacologically induced myopathies developed with a wide variety of drugs. This review is concerned with this second area of research, that of pharmacologically induced experimental myopathies.

A survey of the literature of the past ten years reveals many attempts to reproduce pharmacologically human myopathies in animals. Three areas have been particularly active. These include experimental myotonia, experimental Duchenne's muscular dystrophy, and general neuromuscular disorders.

PHARMACOLOGICALLY INDUCED MYOTONIA

Of all the pharmacologic myopathies that have been attempted, experimental myotonia bears the closest resemblance to its human counterpart. When rats are injected with 2,4-dichlorophenoxyacetic acid or fed a diet containing 20,25-diazocholesterol, muscle fibers develop abnormal membrane parameters closely resembling those in patients with myotonia. A similar abnormality is observed in the myotonic goat.

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[2]The survey of literature for this review was completed June 1, 1976.

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Similarities Between Human and Drug-Induced Forms of Myotonia

In man, myotonia is characterized by sustained contraction of muscle produced either by a voluntary contraction, percussion with an instrument, or electrical stimulation of the muscle (1, 2). The irritability of the muscle is markedly increased after insertion of a needle electrode. Electromyography reveals the typical "dive-bomber" bursting of muscle action potentials, but the myotonic effect is reduced by repeated contractions ("warm up period"). Myotonia is particularly evident in myotonia congenita which is an autosomal inherited disease (2).

Myotonic effects are produced experimentally by a wide variety of compounds including the monocarboxylic acids (3), cholesterol derivatives (4), veratrum alkaloids (5), acridine (6), several amines (7), and indoleacetic acid (8). Closer examination at the membrane level reveals that only a few of these agents, monocarboxylic acids and cholesterol derivatives, produce effects approximating those seen in myotonia congenita (3).

The effects of two drugs in particular have been examined in detail. These are 2,4-dichlorophenoxyacetic acid (2,4-D) and 20,25-diazocholesterol (20,25-D).

The Myotonic Effects of 2,4-Dichlorophenoxyacetic Acid

2,4-D is a monocarboxylic aromatic acid which was also a widely used weed killer (9). Accidental ingestion of this drug by animals and man has led to symptoms of myotonia (10). When given to rats (200 mg/kg i.p.), 2,4-D produces a myotonic electromyogram (EMG) pattern within 30 min (11). Similar effects are observed when normal diaphragm muscles are perfused in vitro with 2.5 mM 2,4-D (4, 12). These studies have revealed several major changes in muscle contraction produced by 2,4-D: (*a*) Within the first few minutes of exposure to the drug, the peak amplitude of a single contraction is increased severalfold. Shortly thereafter there is a ten to twenty fold increase in the duration of contraction. (*b*) These effects are augmented when a tetanic burst of stimuli, instead of a single pulse, is given to the muscle. (*c*) A conditioning or "warm up" stimulus reduces the myotonic effect of a subsequent stimulus. (*d*) The prolonged contraction is associated with a prolonged series of spontaneous repetitive muscle action potentials (12). These major observations produced in vitro by 2,4-D closely parallel the abnormal muscle contractions observed in human myotonia (2) and in the goat (13).

The Myotonic Effects of 20,25-Diazocholesterol

The second drug that induces a myotonic-like condition in rats is 20,25-diazocholesterol (20,25-D). This drug inhibits the enzyme desmosterol reductase (14), resulting in decreased plasma cholesterol and increased plasma desmosterol, a precursor of cholesterol (15). The myotonia-inducing side effects of this drug were first observed in patients being treated for hypercholesterolemia (16, 17). Muscle cramping and spasm, together with EMG patterns similar to myotonia, were reported. In the experimental animal model, the drug is given in the diet or through an esophageal tube for 5 to 8 weeks (4, 18). Several other cholesterol analogues including 25-azocholesterol can be injected s.c. for a similar period to produce the same effect (19–21). Like 2,4-dichlorophenoxyacetic acid, the 20,25-D animal model closely

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parallels human myotonia with respect to abnormal contractile properties, as well as changes in electromyography.

Membrane Abnormalities in Experimental Myotonia

The underlying membrane abnormalities in both inherited and experimental myotonia are similar. Myotonic fibers of both man and the goat have an increased membrane resistance (3). In addition, the mean resting chloride conductance is significantly less than in normal fibers, while the potassium conductance is increased. However, muscle resting membrane potential was unaffected (3, 22). The myotonia produced experimentally in the rat with 2,4-D or 20,25-D displays similar changes in membrane cable properties (4, 23). There is an increase in specific membrane resistance and a fall in chloride conductance. Like inherited myotonia, muscle fiber resting membrane potential is normal or somewhat higher than normal (3, 4, 23–26). However, unlike human and goat myotonia, the myotonia produced by 20,25-D does not display increased potassium conductance (23). We have found no report on the effect of 2,4-D on potassium conductance. The effect on potassium conductance may depend on the type of myotonia-inducing drug employed since several monocarboxylic aromatic acids other than 2,4-D increase potassium conductance (3).

An abnormally low resting chloride conductance by itself could account for both increased membrane resistance and the spontaneous firing of action potentials (27, 28). When untreated rat diaphragm muscles were exposed to low chloride solutions, the muscles fibers became myotonic within 5 min (23). Membrane resistance increased with no change in potassium conductance. Spontaneous firing and the warm-up phenomenon (reduced myotonia with exercise) occurred during the first half hour. A short burst of stimulating pulses produced an augmentation and a prolongation of tension. Unlike the resting membrane potential in nerves, a major component of membrane conductance in skeletal muscle is chloride conductance. In frog muscles that do not display myotonia (3), chloride provides approximately 70% of the resting membrane conductance (29). Chloride is an even greater factor in mammalian muscle where it accounts for up to 85% of resting membrane conductance (30, 31).

Sites of Action of Myotonia-Inducing Drugs

The foregoing evidence indicates that the primary defect in inherited myotonia is an abnormally low membrane conductance to chloride ions, and that many of the properties of myotonic fibers can be reproduced in vitro with low chloride solutions or with various drugs. It appears that a wide variety of chemical agents with little structural similarity are all capable of producing a very specific effect, namely the reduction of chloride conductance. What is needed is a careful examination of the altered cable properties produced by each drug and a comparison of these effects with abnormal membrane characteristics in inherited myotonia. As previously mentioned, the monocarboxylic acids in general and 2,4-D in particular are capable of reproducing the myotonia-like condition in animals (3). On the basis of potency in blocking chloride conductance, Bryant & Morales-Aguilera (3) have determined the chemical requirements for agents that block the chloride channel and have sug-

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gested a steric block of the channel. Much less is known about the binding potency of cholesterol derivatives other than that 20,25-diazocholesterol also produces a reduced chloride conductance and increased membrane resistance (23). Most studies of membrane biophysics during experimental myotonia have been devoted to only 2,4-D and other monocarboxylic aromatic acids. On the other hand, biochemical studies have considered almost exclusively the inhibition of cholesterol biosynthesis, primarily 20,25-D. Consequently, it is difficult to make meaningful comparisons of their respective sites of action on muscle membranes. While the simplicity of a common mechanism of action for both groups of drugs is appealing, it appears that 2,4-D and 20,25-D may act at different membrane sites.

In sarcolemmal membranes isolated from 20,25-diazocholesterol–treated skeletal and cardiac muscle, the amount of cholesterol is reduced and desmosterol is increased (32). The myotonic effect is highly dependent on membrane cholesterol, since including cholesterol in the diets of 20,25-D–treated rats reverses the myotonia (19). Membrane cholesterol levels have not been reported after 2,4-D or other monocarboxylic acids.

Most reports show that Na^+-, K^+-stimulated ATPase activity is increased in 20,25-D-treated rats (15, 18, 32, 33), with one exception which demonstrates a decrease in activity (33). Sarcolemmal Ca-stimulated ATPase activity was also increased after 20,25-D treatment (18, 32). In contrast to the action of 20,25-D, 2,4-dichlorophenoxyacetic acid had no effect on Na^+, K^+-stimulated ATPase but increased the activity of basic *p*-nitrophenylphosphatase (*p*-NPPase) activity (34). There is evidence that this sarcolemmal enzyme may regulate the gate for passive flux of K ions (34). Increased activity of *p*-NPPase increases K^+ efflux from leukocytes (35). This may explain the increased K conductance observed after some monocarboxylic acids (3). The activity of *p*-NPPase in human or myotonic goat muscle has not been described, nor has the effect of 20,25-diazocholesterol been evaluated with respect to *p*-NPPase activity. Until this area is further explored, no unifying hypothesis can be put forward. Clarification of this point will be essential in determining the importance of altered potassium conductance.

A logical step in analyzing the mechanism of myotonia is to look for morphological abnormalities. Unlike human myotonic dystrophy, myotonia congenita is not associated with any generalized structural abnormalities. In the goat one minor change was an increased density of T-tubules (37). Similarly, rats fed 20,25-diazocholesterol did not show abnormal morphological correlates of altered function (38). However, a nonspecific toxic side effect of 20,25-D was observed, possibly due to the drug's effect on muscle sterols.

The Relationship of the Nerve to Drug-Induced Myotonia

The myotonic response does not involve the neuromuscular junction (39). Myotonia can still be induced pharmacologically in the presence of curare (11, 40). Rats treated with 25-azacholesterol demonstrate a tendency toward neuromuscular failure which may contribute to muscle weakness, but this is separate from the typical myotonic effect seen clinically and in vitro (21). However, an intact nerve supply may be required for the development of experimental myotonia (41–43).

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Once established, the myotonia produced by 2,4-D was not affected by subsequent nerve section. However, section of the sciatic nerve 7 days prior to administration of 2,4-D prevented the typical repetitive discharges and the waxing and waning of activity (41). Caccia et al (43) have observed a similar dependence on innervation for the myotonia produced by 20,25-D. However, when treatment with 20,25-D was extended for several weeks after denervation, EMG and muscle contractile behavior closely approximated myotonia (44). A difference is that typical myotonic potentials were rare.

The alteration in membrane cable properties following denervation is highly complex. Muscle membrane depolarization begins within a few hours after nerve section, followed after 3 to 4 days by increases in membrane resistance, capacitance, and time constant (45, 46). The increase in membrane resistance has been attributed to a reduction in potassium conductance (47–49). It is during this period of increased membrane resistance that 2,4-D no longer produces myotonia in diaphragm muscles (42). An explanation for this may lie in the observation that untreated muscles in chloride-free media display typical myotonia activity (27, 23). This spontaneous activity is abolished in high K^+ solutions where K conductance would be lower (28). Thus the relative insensitivity of denervated muscles to 2,4-D may be due to decreased K^+ conductance. However, much more work is required with both 2,4-D and 20,25-D treated animals before the subtleties of neural regulation of myotonia can be defined.

The pharmacologic model of myotonia congenita closely parallels its human inheritable form. While a wide variety of unrelated drugs produce myotonia symptoms in rats, those few drugs that have been closely studied show a common action on muscle membrane involving altered chloride conductance and possibly potassium as well. A more thorough examination of membrane transport proteins may also reveal a common biochemical change in the sarcolemma.

Myotonic changes in man precede the histologic changes in dystrophia myotonica (2). The slight histopathologic changes which have been reported in myotonia congenita are very similar to early changes observed in dystrophia myotonica (36). Recent studies show that chronic treatment with a myotonic drug, 2,4-D, began to reproduce some of the histopathologic abnormalities of dystrophia myotonica (50). The significance of the myotonia model may lie in the long-term effects of these drugs and the possibility of providing an animal model for the more severe disease, dystrophia myotonica.

PHARMACOLOGIC MODELS OF DUCHENNE'S MUSCULAR DYSTROPHY

The development of experimental models for progressive muscular dystrophies, of which the most debilitating is Duchenne's, has been much more difficult to achieve than the model for myotonia congenita. Myotonia is primarily a membrane-related dysfunction with minimal histopathologic abnormalities. Duchenne's muscular dystrophy is far more complex in both its etiology and its pathology. At the present time no animal model, either genetic or pharmacologic, accurately reproduces all

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aspects of Duchenne's muscular dystrophy. However, a first approximation to the disease has been achieved with several drug-induced myopathies.

For an animal model to reproduce accurately the pathology of Duchenne's dystrophy, several minimal criteria must be met: (*a*) Initial histopathologic changes in biopsies of Duchenne's patients include grouped fiber necrosis surrounded by normal muscle fibers (51) (*b*) As the disease progresses there is a wide variation in fiber diameter and an infiltration of endomysial connective tissue and fat (51, 53). (*c*) There is a fluctuating increase in serum levels of many enzymes including creatine phosphokinase (CPK), glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and lactic dehydrogenase (LDH) (52). (*d*) The proximal muscles of the pelvis and lower extremities are the first affected (53).

Some of these criteria are met by the genetically developed dystrophies in a wide variety of animals, of which the mouse and chicken have been studied most extensively (54) An alternative approach to simulating Duchenne's dystrophy in a genetic model has been the recent development of the pharmacologically induced experimental myopathies. Two general approaches have been followed. The first was based upon the initial studies of Hathaway et al (55), which involved circulatory obstruction with or without treatment with various monoamines. A second approach has been strictly pharmacologic. Either pharmacologic model meets several of the above listed criteria for Duchenne's dystrophy. In addition, they offer greater control in analyzing the basic mechanisms that produce the myopathy.

The Vascular Model of Duchenne's Dystrophy

A unique and diagnostic characteristic of a muscle biopsy from a patient in an early stage of Duchenne's dystrophy is the characteristic grouped fiber necrosis (51). This has led to the suggestion that the circumscribed area of necrotic fibers surrounded by normal muscle may represent the field of perfusion by a dysfunctioning terminal blood vessel (55). This vascular hypothesis is supported by histopathologic abnormalities in blood vessels of some patients in the early stage of the disease (55, 56), but others have not been able to confirm this (57).

Complete ligation of the femoral artery and vein yields no histologic abnormalities in the muscles of the lower extremity of rabbits (55). However, the relative ischemia produced by injection of 20μ–80μ dextran particles has led to a nearly complete reproduction of the histopathology of Duchenne's muscular dystrophy (55). Two weeks after an injection of the particles, early changes such as grouped fiber necrosis and phagocytosis occurred, and areas of regeneration were surrounded by fibers of normal appearance. The muscles of animals sacrificed 3 months after the initial treatment showed characteristic middle- and end-stage lesions such as proliferation of endomysial connective tissue, fatty infiltration, and a wide range of muscle fiber diameters. Occasional thickened walls of arterioles and some occluded vessels were also noted. These observations suggested that the histopathologic picture of Duchenne's muscular dystrophy could be reproduced experimentally by producing a relative ischemic condition in muscle.

A similar development of necrosis and phagocytosis at the light microscopic level was reproduced by aortic ligation by itself in rats (58). However, a thorough exami-

nation of the myopathy at electron microscopic level revealed significant differences from the ultrastructural changes seen in human Duchenne's dystrophy. The authors also noted that aortic ligation was selectively destructive to soleus muscle when compared to gastrocnemius muscle. This selectivity was presumably due to the higher oxidative metabolic demand of soleus muscles, since increasing the work load by contralateral denervation or tenotomy exacerbated the myopathy. The experimental methods of producing the partial ischemia differ greatly between the two studies. Consequently, until the ultrastructure of muscles treated with the microsphere approach is examined, the "relative ischemia" hypothesis cannot be fully accepted.

Ligation Plus Serotonin Model

Because very few abnormalities are seen in arteriolar walls of Duchenne's muscle biopsies, a new approach was developed combining aortic ligation plus the vasoactive agent serotonin (5-HT) (59). The rationale to using this combined approach has been given added significance in view of the observation that in Duchenne's patients, the platelet uptake of serotonin is greatly diminished (60). Serotonin itself has been suggested as a causative agent in carcinoid myopathy (61). Contrary to earlier observations (59), rats given 5-HT alone display some histopathologic abnormalities (62). In the rat, normal circulating levels of serotonin are too low to contract vascular smooth muscle (63). However, when the isolated rat hind limb was perfused with 2 to 50 mg/liter serotonin (5-HT creatinine sulfate) a decrease in blood flow in the femoral artery occurred (59). The combination of aortic ligation plus 20–75 mg/kg 5-HT, was given to rats for 5 days to 3 months either acutely or chronically. Either method alone produced no muscle necrosis, but in combination, a histopatholic picture reminiscent of Duchenne's dystrophy was produced (59). No structural abnormality was observed in blood vessel walls, leading the authors to suggest that a functional inadequacy rather than a structural lesion is responsible for the relative ischemia. Aortic ligation in the rat previously has been reported to render skeletal muscle more susceptible to vasoactive agents (64). The same characteristic pattern of muscle lesions has been reproduced with aortic ligation and another vasoactive agent, 3 mg/kg norepinephrine (59, 65).

Additional evidence for the "functional ischemic" model of Duchenne's dystrophy was obtained by examining plasma enzyme levels in rats given serotonin after aortic ligation (65). Aortic ligation itself produced some increase in plasma enzymes which returned to control levels within 72 hr. Administration of serotonin produced a marked increase in creatine phosphokinase (CPK), glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and lactic dehydrogenase (LDH). Enzyme levels returned to normal within 48–72 hr, but repeated injections produced a return to elevated enzyme levels (65). These data suggested that a second requirement for the animal model for Duchenne's dystrophy was achieved, that of increased levels of muscle enzymes. Damaged liver as a source for these enzymes was ruled out because there was no increase in alkaline phosphatase. Ligation plus norepinephrine was also effective in increasing enzyme levels (66). Pretreatment with phenoxybenzamine and chlorpromazine, both α-adrenergic blockers, pre-

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vented the elevated enzyme levels. Pretreatment with imipramine which blocks the preterminal amine uptake pump (67) had a paradoxical effect. Low doses (1–2 mg/kg) maintained low plasma enzyme levels whether noradrenaline or serotonin was studied. However, high doses of imipramine (20 mg/kg) actually increased the plasma enzyme levels elevated by norepinephrine (66). This interaction between imipramine and norepinephrine must be explored adequately before firm conclusions can be made with respect to overstimulation of α-receptors. In apparent conflict with these results, Melmed & Karpati (68) observed that phenoxybenzamine plus aortic ligation significantly increased the ischemic myopathy. It is quite clear that further studies are required to explain the fundamental difference between the myopathic effects of aortic ligation between the two groups. It is likely a difference in ligating techniques, but the question must be resolved before detailed pharmacology is explored. In addition, it will also be necessary to provide a more complete pharmacologic analysis in the prevention or exacerbation of histopathologic changes. Depletion of amines with reserpine, blockage of synthesis with *p*-chlorophenylalanine, and destruction of aminergic nerve terminals with 6-hydroxydopamine all must be thoroughly tested.

It has been emphasized recently that increased serum enzyme levels actually precede significant muscle necrosis in Duchenne's dystrophy (69). Significant abnormalities have been observed in dystrophic muscle and erythrocyte membrane composition and transport. It is conceivable that an expressed genetic error in membranes may be the earliest stage in the pathogenesis of Duchenne's dystrophy (69, 82). It should be noted that peak increases in enzyme levels occurred 12 hr after 5-HT, that is, well before the first lesions were apparent (66). Much work needs to be done to clarify early abnormalities in membrane transport and composition during the initial hours after drug treatment and before the development of lesions.

Vasoactive Amines and the Development of Myopathies

Two more specific pharmacologic approaches have been attempted. The first combined imipramine and serotonin. The second studied the myopathic effects of the monoamine oxidase (MAO) inhibitor, pargyline. Imipramine is a tricyclic antidepressant which blocks the uptake of primarily serotonin but also other amines into nerve terminals (67). Parker & Mendell (70) have succeeded in reproducing the characteristic histopathology of Duchenne's dystrophy by pretreating rats for three days with imipramine (10 mg/kg). On the fourth day serotonin was given (100 mg/kg) This 4-day procedure was repeated each week for eleven weeks. The combination of drugs closely reproduced the early and midstage lesions of Duchenne's dystrophy with the addition that proximal muscles were selectively affected. This procedure also raises plasma enzyme levels of CPK (71). When untreated muscle was perfused in vitro with imipramine and 5-HT, ^{14}C-labeled serotonin was taken up 3 times faster than control. However, oxygen uptake and the amino acid analogue γ-aminoisobutyric acid were both significantly reduced (71).

When rats were injected with the MAO inhibitor pargyline, soleus muscles contained a flurorescent material generally characterized by the Falck-Hillarp technique as catecholamines (72). Duchenne's dystrophy is the only human myopathy

known to contain catecholamine-fluorescent fibers (73). It is not known, however, whether the ligation-plus-5-HT or imipramine-plus-5-HT myopathies contain fluorescence. The pargyline myopathy displayed grouped fiber necrosis and, with seven-day treatment, significant connective tissue formation in the endomysium (72). It is very much unlike Duchenne's dystrophy in that only the soleus is affected with no lesions produced in the gastrocnemius (72), or diaphragm, or quadriceps muscles (M. B. Laskowski and W. D. Dettbarn, unpublished observations). Denervation reduced the myopathy, which was assumed to be "neurogenic." This approach is questionable since both motor, sensory, and sympathetic innervation are nonselectively removed and, of course, muscle activity and metabolic requirements are significantly reduced. In none of the above-mentioned experimental myopathies has any significant ultrastructural examination of the muscle, blood vessels, or nerve been reported. It is clear that before the 5-HT model can be accepted as a replica of Duchenne's dystrophy a thorough point-by-point comparison must be made with biopsied dystrophic muscle at the fine structural level.

Recent reports have suggested that an abnormal axoplasmic flow in motor nerves may be responsible for several of the myopathies produced by vasoactive amines (74, 75). Two MAO inhibitors, pargyline and phenelzine, were reported to increase the rate of fast axoplasmic transport (75). The increased flow rate was partially prevented by pretreatment with α-methylparatyrosine. Aortic ligation with or without 5-HT produced a similar increase in axoplasmic transport (74). However, Komiya & Austin (76) found that ligation of the iliac artery plus 5-HT did not alter flow rate although the myopathy was fully developed in the gastrocnemius muscle. It should be noted that in the reports of increased fast transport mentioned above, confirmatory histologic examination of muscle lesions was not described. Resolution of the controversy will add valuable information as to whether or not a trophic material from the motor nerve might be augmenting or reducing the myopathy.

Recently a "neurovascular hypothesis" has been proposed to explain the mechanism of the myopathies produced by vasoactive amines (77). A single genetic deficit was proposed, that of reduced effectiveness of sympathetic vasoconstriction. According to this hypothesis, the normal reflex vasoconstriction during exercise is impaired, resulting in an overperfusion of unused muscles (78). Reduced blood flow to exercising muscle in Duchenne's patients has been observed (79, 80). Such patients also display reduced oxygen tension in exercising muscles (81). While the hypothesis is simple and attractive, much more work is required at the pharmacologic and ultrastructural levels before it can be accepted. Sympathetic innervation of biopsies of Duchenne's patients must be examined at the electron microscopic level. In addition, more thorough pharmacologic analyses must be made of the "functional ischemia" experimental myopathies. What is required at this point is very basic and thorough work to help clarify the origin of ischemic lesions and the way these are enhanced with vasoactive amines. Such studies will provide pharmacologic models that will be an even closer approximation to Duchenne's dystrophy. This approach will certainly provide a potentially productive area for drug development and testing.

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GENERAL NEUROMUSCULAR DISEASES

Investigation of the involvement of the neuromuscular junction in muscle disease had been active before the development of electron microscopic techniques. Based upon preliminary work at the light microscopic level, ultrastructural investigations have revealed a frequent involvement of the motor end-plate in a wide variety of muscle diseases (82). Zacks (83) has written a thorough review of this topic. In a majority of muscle diseases, motor end-plate degeneration is secondary either to a primary myopathy or to motor nerve degeneration. Myasthenia gravis is a prominent exception (83). Less frequently occurring diseases that specifically affect the neuromuscular junction are carcinomatous neuromyopathy (84–86), amyotonia congenita syndrome (87), Isaacs' syndrome (88), Coxsackie B virus (89), and a variety of neurotoxins (83).

Experimental myasthenia gravis is being pursued in several laboratories. Such studies involve the myasthenic dog (90, 91) and rats made chronically autoimmune to ACh receptors (92). In recent years we have been actively pursuing studies of end-plate-mediated myopathies using a variety of cholinesterase inhibitors and cholinomimetic drugs (93–97). Rather than developing a model for a specific disease entity, this approach permits an examination of the delicate nerve-muscle relationship and explains how altering this relationship can initiate muscle lesions.

Neural Control of Muscle Properties

The now classic work of Buller, Eccles & Eccles (98, 99) revealed the significant influence that motor nerves have over the structural, functional, and biochemical properties of muscles they innervate. The trophic function of nerves was originally defined as a function that is not mediated by nerve impulses (100). However, only a few of these trophic actions are independent of nerve impulse transmission and muscle contraction (101, 102). The level of muscular activity influences contractile properties, content of specific enzymes, its sensitivity to ACh, and its ability to accept further innervation. The structural and functional integrity of skeletal muscle depends on the presence and normal function of neuromuscular transmission. Problems may arise from pharmacologic manipulation of neuromuscular transmission and may lead to changes in the development, maintenance, and integrity of the end-plate and muscle fiber. The physiology of neuromuscular transmission can be affected in several ways. The amount of the transmitter acetylcholine which is released may be increased or reduced by a wide variety of drugs (103–108). Postsynaptic sensitivity may be changed by altering the number of ACh receptors. Pharmacologic manipulation of the input resistance of the muscle fiber membrane will alter the threshold for generation of muscle action potentials. Variation in one of these factors or in combination with one another can be contributing causes to neuromuscular degeneration and can be altered experimentally (109–113).

Some of the changes that are seen after denervation may be due to disuse rather than loss of the trophic function of nerve. Other experimental procedures will interrupt various aspects of nerve-muscle interaction, while leaving the neuromuscular junction and its nerve structurally intact. Some of these approaches such as the

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use of vinblastine and colchicine block axoplasmic transport without interfering with the synthesis and release of ACh or the propagation and transmission of impulses (114). The effects of these compounds are almost similar to those of denervation. Total disuse of the neuromuscular junction can be induced by chemical application of local anesthetics (102) inducing block of conduction and transmission without changing structure and function of the neuromuscular junction. Such treatment also induces phenomena seen after denervation.

Cholinesterase Inhibition and Transmitter Release

Altered acetylcholine release or a reduction of its hydrolysis by loss in cholinesterase activity is observed as early pathophysiologic changes in murine dystrophy (115, 116), chicken dystrophy (117), atrophy (118), some experimental myopathies (93), and some forms of human muscle disease (119, 120). There has been considerable debate as to whether the nerve terminal or the postsynaptic region of muscle is the primary site of action of anti-ChE drugs (121), and whether either or both of these sites are affected by neuromuscular disease processes, such as muscular dystrophy and myasthenia gravis (112)

Few reports have been published on the effects of anticholinesterases, especially organophosphorus agents, on miniature end-plate potential (MEPP) frequency and end-plate potential (EPP) quantum content. Neostigmine increased MEPP frequency over a narrow concentration range but at higher doses decreased frequency (122, 123). Edrophonium was found to have similar effects on MEPP frequency (123, 124). These drugs, however, contain a quaternary ammonium ion which by itself is capable of increasing MEPP frequency (125). Indirect estimates of quantum content during neostigmine, edrophonium, and ambenonium, drugs that facilitate neuromuscular transmission, indicate that quantum content is elevated (123). Recent work has shown, however, that the method of paralyzing the muscle for intracellular recording affects the estimate of quantum content in edrophonium (124, 126).

Paraoxon, an irreversible organophosphorus inhibitor of ChE activity when injected into rats, causes (*a*) an augmentation of spontaneous transmitter release, (*b*) a reduction in quantum content, and (*c*) spontaneous and evoked antidromic nerve action potentials (94). Similar effects were observed when paraoxon was applied in vitro (94, 127).

A critical question is whether paraoxon produces these effects directly or acts indirectly through its inhibition of cholinesterase. The strongest evidence indicating that paraoxon acts through ChE inhibition comes from the experiments with pyridine-2-aldoxime methiodide (2-PAM). Reactivation of inhibited ChE with 2-PAM reduces the paraoxon-augmented MEPP frequency and eliminates antidromic nerve activity. Another organophosphorus compound diisopropylfluorophosphate (DFP) was without effect on MEPP frequency (128). However, these experiments were conducted while basal MEPP frequency was artificially accelerated with 10 mM K ion. Paraoxon depolarized muscle membranes by approximately 15 mV (127). This depolarizing effect was partly reversed with 10^{-6} M tetrodotoxin (TTX) and totally abolished in combination with α-bungarotoxin. The depolarizing effects of pa-

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raoxon are due to the combined depolarization of ACh receptors and extrajunctional receptors controlling Na-conductance channels. Paraoxon has no effect on the K-conductance channels (127).

Tetrodotoxin reduced the paraoxon-accelerated MEPP frequency but did not alter spontaneous release in control preparations. It has been reported previously that TTX blocks nerve and muscle action potentials while leaving the depolarization-release mechanism intact (129). Consequently, an intact action potential generating system must be present for paraoxon to yield its full effect. Whether the faster MEPP frequency is produced by reinvasion of nerve terminals with spontaneous nerve impulses or some other mechanism remains to be determined.

Acute Cholinesterase Inhibition and Muscle Fiber Degeneration

Irreversible inhibitors of the ChE activity such as paraoxon, DFP, tabun, sarin, soman, and parathion, as well as reversible inhibitors such as physostigmine and neostigmine, inhibit AChE activity at the neuromuscular junction. This loss of enzyme activity blocks the hydrolysis of ACh and leads to increased activity and stimulation of the skeletal muscle fiber. Reports from several laboratories have shown that all of the above-mentioned ChE inhibitors are myopathic (130–135).

The earliest evidence relating ChE inhibitors to necrotic lesions of skeletal muscle was in a report by Carey (130). Since then this observation has been repeated by several investigators; however, not all of them were aware of previous investigations (93, 131-134). Injection of paraoxon produces a progressive myopathy in the rat diaphragm, soleus, gastrocnemius, and quadriceps muscles. The diaphragm is the most severely affected of these muscles (133, 135), followed by the soleus and the gastrocnemius muscles. The earliest lesions noted were focal areas of abnormality close to the surface of the muscle fiber (93, 133). On H-E stain this area appeared to be more basophilic. The trichrome stain demonstrated an area of red-staining, and the normal basic pattern of mitochrondria, usually identified with LDH and NADH reactions, was disrupted by clumping of highly reactive material. These focal changes progressed to a generalized breakdown of fiber architecture, characterized by a loss of staining quality followed finally by phagocytosis. Longitudinal sections indicated that the early changes in a focal necrosis affected only a small segment of fiber lengths. The later stages affected progressively greater lengths of muscle fibers (95).

Following i.p. application of soman (methylpinacolyloxyphosphoryl fluoride), local necrotic lesions are seen scattered throughout the diaphragm muscle. Some fibers displayed severe lesions characterized by loss of cytoarchitecture and phagocytosis (131)

Within 2 hr of an injection of DFP or tabun the earliest light miscroscopic changes are seen characterized by localized eosinophilia, swelling of the sarcoplasm, and loss of striations in several muscle fibers. Approximately 12 hr after the i.v. injection a complete but localized necrosis has developed in the affected fibers (133). A delayed neuropathy beginning at the nerve terminals develops within three weeks after exposure to DFP (136, 137). At this time muscle contractile strength is returning toward normal.

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Ultrastructural Changes Produced by Cholinesterase Inhibitors

Motor nerve terminals showed varying degrees of changes within 30 min to 2 hr after injection of soman and paraoxon (131, 138). Some nerve terminals appear relatively normal with the exception of slightly swollen mitochondria. These nerve terminal abnormalities are more severe after soman than after paraoxon. More obvious changes are seen in the subsynaptic area and the surrounding muscle fiber. More severely affected nerve terminals display myelin figures, membrane enclosures, and an increase in the number of large coated vesicles. Soman, paraoxon, and neostigmine initiate the formation of vesicular structure in the primary and secondary subsynaptic cleft (95, 131, 139). Occasionally some of these are seen in the sarcoplasm. Many of the cleft vesicles are similar in density and size to synaptic vesicles but with considerable variations in diameter. The severity of lesions in the subsynaptic folds varies even within the same muscle. Normal subsynaptic clefts with few cleft vesicles are seen side by side with subsynaptic clefts with many cleft vesicles and a widening of the cleft itself (95, 131).

The ChE inhibitors, in addition to the changes seen in the region of the end-plate, cause changes in the muscle fiber itself. Muscle surrounding the motor end-plate shows a disruption of cytoarchitectural organization. Initially the first changes are in the mitochrondria which show swelling leading to lysis of the central cristae. Myelin figures beneath the end-plate are frequently observed while the region more distal to the end-plate is less affected. The nucleoli of the muscle cell nucleus are enlarged and move to the periphery of the nucleus. There is an increase in the sarcoplasmic ribosomes, the sarcoplasmic reticulum becomes dilated followed by a loss of striation of the myofibrils, and later total destruction of the myofilaments and fragmentation of Z bands occurs (95, 131).

Muscle Lesions Developing During Chronic Cholinesterase Inhibition

Treatment of rats with paraoxon over a period of seven consecutive days produces a progressive myopathy (93, 95, 135). In the diaphragm a maximal effect is seen after 3 days of treatment. All stages of the myopathic process were maximal at this point and 6.5% of all fibers were found to be affected. By day 7 the muscle appeared to be relatively normal. In soleus muscle the maximum number of lesions was seen at day 3, but only 0.7% of the total fiber population was affected. The gastrocnemius muscle, unlike the diaphragm and the soleus, exhibited maximal lesions on days 5 to 7. The total number of fibers involved at this time was 0.8%. In all muscles studied, maximal loss of ChE activity occurred during the first 30 min after the initial paraoxon treatment. Within 24 hr of each injection of paraoxon, the enzyme activity in diaphragm and soleus muscle had recovered to about 50% of control activity, while in gastrocnemius muscle the ChE activity had returned to 67% of control.

There is a marked difference in the susceptibility of muscle type to the paraoxon action. The muscles tested are distinct from each other as to predominant fiber type, rate of firing, speed of contraction, and inherent metabolism. The myopathy is much more severe in the diaphragm, which is predominantly a slow contracting, tonic or

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antidromic firing and the MEPP frequency. It also completely prevents the myopathy, unless given after a critical time, usually between 60–90 min (133–135). Hemicholinium has been shown to reduce the amount of ACh in the quantum released and reduces significantly the severity of the myopathy (93).

By inhibiting ChE in vitro, cholinergic as well as adrenergic stimulation is achieved. All preganglionic nerve fibers, as well as motor nerves, are cholinergic and the preganglionic transmitter is ACh. Under conditions when ChE is inhibited, simultaneous stimulation of postganglionic cholinergic and adrenergic and motor nerves will occur. This results in an increased release of ACh or norepinephrine from postganglionic fibers. Under these conditions not enough blood may be supplied to the most active muscle fibers and thus relative ischemia might result. This could lead to ischemic changes, leakage of muscle enzymes, and eventual necrosis. The effects of prolonged cholinergic vasodilation in combination with reduced adrenergic vasoconstrictor tone during the periods of increased ChE inhibitor-induced muscle activity could lead to functional ischemia in certain hyperactive muscle fibers and not in others. Less active muscle may have normal oxygen requirements, but may receive the same blood supply. The functional ischemia could appear during the period of the ACh-induced muscle fiber activity. This could also explain the prevention of necrosis by denervation due to immobilization of the denervated muscle.

It is generally accepted that two groups on the enzyme are essential for hydrolysis, the esteratic and anionic site. The anionic site interacts by ionic binding with the cationic head of the ACh molecule. There is good evidence that part of the chemical forces binding ACh to the enzyme are more or less similar to those between ACh and its receptor. It is therefore not surprising that drugs that inhibit ChE may, to some extent, also react with the ACh receptor. DFP, for instance, in higher concentrations than needed to inhibit enzyme activity has a curare-like action which may block neuromuscular transmission (128). In contrast to ChE inhibition, this latter effect is reversible on washing. Furthermore, it has not been shown as yet whether this higher concentration is due to direct action on the receptor or on the ionophore which controls the permeability once the receptor has been activated, an effect not unlike that of local anesthetic. The myopathic effects of the ChE inhibitors can be explained entirely on the basis of their inhibition of the enzyme and the increased action of ACh on pre- and postganglionic receptor sites. Other effects that have been described occur at a much higher concentration of the inhibitor.

Besides their inhibition of ChE, or interaction with the ACh receptor and or the ionic conductance modulator, organophosphates may have direct effects on the muscle. We have observed that paraoxon directly depolarizes muscle membranes (127), and subsarcolemmal abnormalities of muscle fine structure are occasionally seen not associated with an end-plate (95). This unspecific action as a cause of the myopathy could not explain the selectivity of the myopathy, since only 6–8% of the fibers are affected.

The importance of studying the myopathies produced by cholinesterase inhibitors derives not from the accurate reproduction of a specific human disease but rather from the insight they provide into the myopathic effects of altering the delicate

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nerve-muscle relationship. With this approach it is possible to follow a highly controllable disease process from its origin at the end-plate within 30 min after the drug, to its completion with necrosis and phagocytosis after 24 hr. The crucial questions may lie with the mechanisms which spare most fibers, rather than the disease process itself.

CONCLUSIONS

Within the last ten years there have been major developments in the field of experimental models of human muscle disease. Recently models of Duchenne's muscular dystrophy have been pursued most actively because of the particularly sinister nature of the disease. It is essential now to define carefully the mechanisms underlying the generation of these myopathies and to clarify major areas of controversy. This will require a multidisciplinary approach combining physiology, ultrastructure, and biochemistry, using the same drug under the same experimental conditions. Duchenne's dystrophy is a multisystem disease. The significance of pursuing the pharmacologic models rests with the development of drugs important to combating muscle degeneration.

The models for myotonia present a different problem. The pharmacology of these experimental myopathies has been examined in considerable detail. A review of the literature demonstrates that membrane physiology has been examined thoroughly in one model and biochemistry in another. Again, what is required is a multidisciplinary investigation of each model toward the end of defining whether a single common defective mechanism is responsible for the myotonic state.

Finally the myopathies induced by experimental alteration of nerve-muscle relationships have been developed to analyze the basic pathophysiology of muscle degeneration. The most actively studied of these have been the experimental myopathies induced by cholinesterase inhibitors. Muscles respond to insult with a limited arsenal of physiological and histological alterations. In-depth multidisciplinary analyses of the very earliest of these myopathic processes can provide insight into some of the basic mechanisms underlying muscle fiber degeneration.

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Milan, Jan 7, 1978

PRELIMINARY REPORT ON MORTALITY IN THE SEVESO AREA, 1975-77.*

by Berrino Franco

Introduction.

The Brianza di Seveso is an area of about 9,300 hectars, 20 Km North from Milan, subdivided in 11 small towns of 6 to 37,000 inhabitants, with a total population of 220,000 on Dec 31, 1976. The ICMESA plant is located in the territory of Meda, near the border of this town with Seveso (fig. 1).
The age and sex structures of the Oct 1971 (census year) populations of some towns of the area are shown in fig 2; these figures have never been properly updated; moreover an adequate description of the population distribution inside each town is still lacking, so that the best available denominators for the present study were the Dec 1975 populations of each town with the 1971 age structure.

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* This report is part of a feasibility study for the extension of the Cancer Registry of Lombardy to the Brianza di Seveso. At our knowledge so far no official study on general mortality has been conducted.

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According to the first epidemiological data on acute dermatitis in the emergency period and to the territorial distribution of chloracne cases in children, the towns of Seveso, Meda, Cesano Maderno and, to a lesser degree, Desio, seem to be the most heavily contaminated by TCDD; both acute skin lesions and chloracne,however,have been observed in all the towns of the zone. On the other hand, apart from these very rough crude incidence figures on dermatological diseases and a few data on obstetrical diseases , no meaningful population based morbidity rates have been produced.

Quality of information.

In most towns the available information on causes of death is of rather poor quality because the demographic offices do not regularly keep copy of the death certificates before sending them to the Central Institute of Statistics (ISTAT) in Rome, and the forms on which some information is recorded are not standardized and in some instances not regularly filled in. In some cases the quality of registration became somewhat better after Oct 1975, when new rules for burials were issued. So far no official recommandation has been given to improve and standardize the death registration. As the ISTAT statistics can be disaggregated only to the province level, it would be very important to get back from the Institute the original informations. As for the more interesting towns, fairly good information is available from Meda (after Jan 1976), where the three causes of death are routinely registered; only the "principal" cause is recorded in Seveso and only the terminal one in Cesano Maderno. Moreover, with few exceptions, the causes of the deaths occurred outside the town of residence are not registered and have to be searched for at the municipalities

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excellent death registration system exists in Seregno and a useful one in Desio and Saronno, where the main hospitals serving the zone are located and where most hospital deaths occur.

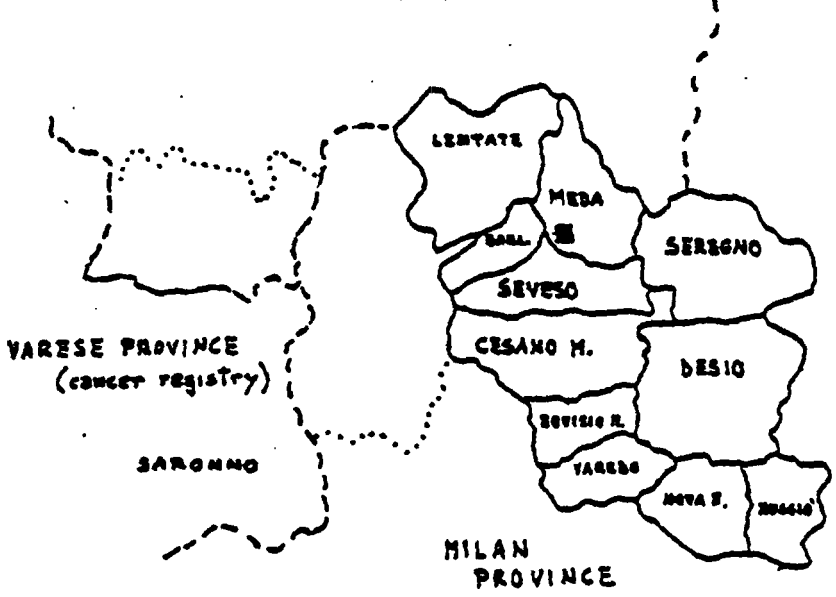
Results.

Table 1a,b,c,d show the age distribution of deaths by six-months periods. The only significant increase in mortality after July 1976 occurred in Meda in the age group 55 tc '74. Such an increase is not apparent in the other towns. In Seveso and Cesano Maderno however a possible mouvement in mortality may have been masked by a marked excess of deaths occurred in the older age groups just before the ICMESA episode for unknown reasons, possibly following springtime complications of some winter epidemics (in Seveso these excess deaths have been coded mainly as ill defined cardiovascular diseases).

Table 2a and b show the causes of death in Meda and Seveso by groups of causes (intermediate A classification) and by six-months periods before and after the ICMESA episode. Five leukaemia deaths in 1977 in Meda are far in excess from expected (P=.02); 2 cases were specified as acute, 2 as chronic and 1 was not specified; the age at death was respectively 54, 72, 75, 81 and 35. Morbidity studies should be soon undertaken to find out the meaning of this observation. Both in Meda and in Seveso a significant increase in liver cirrhosis deaths has occurred, as if the intoxication had killed people with impaired liver function. This effect does not seem to be due to a changed attitude of phisicians towards death certification because liver cirrhosis diagnosis were

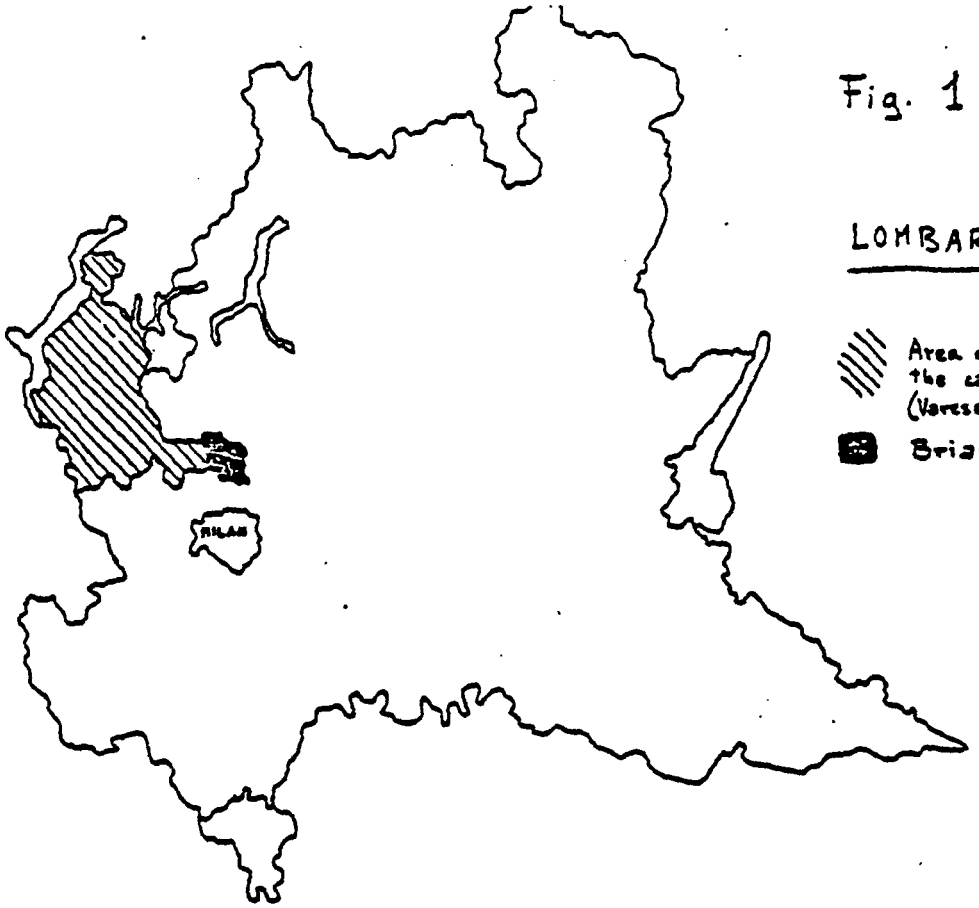
equally distributed among phisicians and no excess has been observed in Desio. Table 3 shows a comparison with the mortality for liver cirrhosis that would have been expected from the mortality statistics of the nearby Province of Varese, where the causes of deaths are routinely collected by the cancer registry.

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■ = ICMESA PLANT

Fig. 1



LOMBARDY

Area covered by the cancer registry (Varese Province)

Brianza di Seveso

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Fig. 2- 1971 age pyramids of 4 towns

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Table 1 a, b – Age specific mortality rates (/1000) by six-months periods,

a) MEDA, both sexes, 1976-77

Age	Dec 31, 75 Population	1976 Jan.-June n.	1976 Jan.-June rate	1976 July-Dec n.	1976 July-Dec rate	1977 Jan-June n.	1977 Jan-June rate	1977 July-Nov n.
0- 4	1688	2	1.2	7	4.1	4	2.4	1
5-14	3347	-	-	1	0.3	-	-	2
15-44	8804	6	0.7	9	1.0	3	0.3	5
45-54	1949	6	3.1	5	2.6	6	3.1	6
55-64	1779	8	4.5	15	8.4	8	4.5	9
65-74	1163	16	13.7*	36	30.8**	21	18.0	22
75+	466	28	60.1	30	64.4	24	51.5	36
Total	19199	66	3.4•	103	5.4••	66	3.4	81

Note: 95% confidence limits

* 7.8 - 22.1 ** 21.7 - 42.8

• 2.7 - 4.4 •• 4.4 - 6.5

b) SEREGNO

Age	Population	1976 Jan-June n.	1976 Jan-June rate	1976 July-Dec n.	1976 July-Dec rate	1977 Jan-June n.	1977 Jan-June rate	1977 July-Nov. n.
0-4	3213	4	1.2	4	1.2	2	0.6	3
5-	6055	2	0.3	-	-	2	0.3	-
15-	16581	5	0.3	6	0.4	19	1.1	7
45-	3913	10	2.6	15	3.8	13	3.3	12
55-	3558	27	7.6	29	8.2	22	6.2	21
65-	2437	50	20.5	54	22.2	40	16.4	50
5+	893	70	78.4	70	78.4	50	56.0	45
Total	36660	168	4.6	178	4.9	148	4.0	138

DOW 680101

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0003462

Table 1 c, d - Age specific mortality rates (/1000) by six-months periods,

a) SEVESO, both sexes, 1975-77

Age	Dec. 31, 1975 population	1975 Jan-June	1975 July-Dec	1976 Jan-June	1976 July-Dec	1977 Jan-June
0-4	1460	-	0.7	-	-	0.8
5-14	2863	-	0.3	0.3	-	-
15-44	7738	0.3	0.5	0.4	0.4	0.8
45-54	1728	1.7	3.5	4.1	1.2	2.9
55-64	1585	5.7	7.6	6.3	6.9	6.9
65-74	1149	22.6	16.5	30.5	19.1	15.7
75+	404	61.9*	47.0*	108.9**	74.3	59.4
Total	16927	3.8	3.7	5.3	4.0	3.8

Note: * weighted mean = 54.5, 95% confidence limits = 39.5 - 73.2
** 95% confidence limits = 79.1 - 146.4

b) CESANO MADERNO

Age	Population	1975 Jan-June	1975 July-Dec	1976 Jan-June	1976 July-Dec	1977 Jan-June
0-4	3325	0.6	1.2	0.6	0.6	0.6
5-	6374	0.2	0.5	0.2	0.2	1.2
15-	15830	0.6	0.8	0.3	0.6	0.6
45-	3218	2.5	4.7	5.3	3.4	4.7
55-	2836	9.2	8.1	4.2	8.5	9.5
65-	1497	21.4	20.0	24.7	28.1	20.0
75+	465	70.2	81.1	111.8	54.8	70.2
Total	33536	3.3	3.7	3.7	3.4	3.5

DOW 680102

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Table 2 a - Distribution of deaths by groups of causes and by six-months periods before and after the Icmesa episode (July 10, 1976), MEDA, both sexes.

		year	1976		1977		
	CAUSE	semester	1st	2nd	1st	2nd *	
21 A	1-44	Infectious diseases	-	1	1	2	x
A	45-60	Malignant tumours	(19)	(14)	(20)	(11)	
	45	lip, oral cavity & pharynx	-	-	1	-	
	46	esophagus	-	-	-	-	
	47	stomach	4	6	2	2	
	48	intestine except rectum	2	2	2	1	
	49	rectum	-	-	2	-	
	50	laryux	1	-	-	-	
	51	lung	3	1	3	2	
	52	bone	1	-	-	-	
	54	breast	1	2	2	-	
	56	uterus (ms)	1	-	-	-	
	57	prostate	1	-	1	1	
	58	other sites	5 (a)	3 (b)	2	4	
	59	leukemia	-	-	4	1	←
	60	lymphoma	-	-	1	-	
A	63-64	Endocrine diseases	1	1	4	1	
A	67	Anemia	-	1	-	-	
C A	67-79	Psychiatric & Nervous system d.	-	1	1	1	x
A	81	Reumatic heart d.	1	-	-	1	
A	82	Ipertension	5	-	1	1	
	83	Ischemic heart d.	9	12	9	6	
A	84	Other heart d.	11	5	11	9	
A	85	Cerebrovascular	12	10	13	11	
A	86	Other arterial d.	3	3	3	6	
A	87	Pulmonary embolism	-	-	-	-	
A	89,91,92	Respiratory infectious	1	1	-	-	
A	93	Chronic bronchitis, emphysema	2	1	3	1	
A	96	Other respiratory d.	1	-	-	1	
	98	Peptic ulcer	-	1	1	-	
A	101	Intestinal obstruction	-	-	-	1	
A	102	Liver cirrhosis	2	4	4	4	←
A	103	Cholecystitis, cholelithiasis	1	2	2	-	
A	104	Other liver diseases	-	1	1	2	
		Other Alimentary tract d.	1	-	2	1	
A	105-111	Genito urinary tract d.	2	3	2	1	x
A	127	Congenital heart d.	-	-	1	1	←
A	130	Other congenital malformations	-	1 (c)	-	-	x
A	134	New-born anoxia	1	-	1	-	
A	135	Other new-born d.	-	-	1	-	
A	136	Senility	2	-	1	2	
A	137	Ill defined diseases	1	-	-	1	
	N	Violent deaths	4	7	6	4	
Y	yet traced cause		5	5	-	3	

DOW 6801103

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* from July 10 to November 25 only

(a) 1 liver cancer n.o.s. and 1 cancer of the ampulla of Vater (b) 1 primary liver cancer

(c) birth date: AUG/16/1976

0003464

Table 2 b - Distribution of deaths by groups of causes and by six-months periods before and after the Icmesa episode (July 10, 1976), SEVESO, both sexes.

year	1975		1976		1977		
semester	1st	2nd	1st	2nd	1st	2nd*	
CAUSE							
A 21	-	-	-	-	-	1	
A 45-60	(8)	(11)	(17)	(11)	(12)	(9)	
45	1	-	-	-	1	-	
46	-	1	-	1	-	2	
47	1	3	3	2	1	1	
48	1	-	3	1	1	-	
49	-	-	1	-	-	3	
50	-	-	1	1	-	-	
51	2	-	3	1	5	-	
52	-	-	-	-	-	-	
54	1	1	1	2	-	1	
56	-	-	-	-	-	-	
57	-	-	-	-	1	-	
58	2	4	5 (a)	3	3 (b)	2 (c)	
59	-	1	-	-	-	-	
60	-	1	-	-	-	-	
A 63-64	3	2	1	1	1	1	
A 67	-	-	-	-	-	-	
A 70	-	-	-	-	1	-	
A 81	1	-	-	-	-	1	
A 82	2	3	-	-	1	2	
A 83	6	7	7	6	6	6	
A 84	5	3	13	2	2	2	⇦
A 85	8	13	14	12	8	8	
A 86	2	2	1	4	-	1	
A 87	-	-	-	1	-	-	
A 91-92	2	1	3	1	3	2	
A 93	3	1	1	1	3	-	
A 96	-	-	-	-	1	-	
A 98	-	1	-	-	-	1	
A 101	-	1	1	-	-	-	
A 102	5	2	2	4	2	6	←
A 103	-	-	-	-	-	-	
A 104 liver	-	-	-	-	-	1	
other	-	-	-	-	-	1	
A 105-111	-	-	2	2	-	1	
A 127							
A 130	-	-	-	-	-	-	
A 134							
A 135							
A 136	3	3	5	4	-	2	
A 137	5	1	12	5	10	9	⇦
N	-	2	5	2	1	2	
Not yet traced	*	18	12	12	9	6	
	65	71	96	68	60	62	

DOW 680104

7262

0003465

* from July, 10 to December 15 only

(a) 1 gallbladder cancer (b) 1 epatocellular carcinoma and 1 biliary ducts cancer

(c) 1 cholangiocellular carcinoma

Table 3 - Deaths for liver cirrhosis (ICD 571) in the Seveso Zone by 6 months periods before and after the ICMESA episode (July 10, 1976); males only. The expected numbers have been obtained from the age specific mortality rates observed in the nearby Province of Varese in the same period.

LIVER CIRRHOSIS DEATHS

Commune	Year	1975		1976		1977		Total after	p
	Semester	1st	2nd	1st	2nd	1st	2nd*	July 10, 76	value
Meda	obs.	n.k.	n.k.	2	4	3	3	10	
	exp.			1.81	3.61		1.20	4.82	<.05
Seveso	obs.	3	1	2	3	1	5	9	
	exp.		5.18		3.45		1.15	4.61	<.05
Seregno	obs.	n.k.	n.k.	2	3	7	2	12	
	exp.			3.61	7.23		2.41	9.64	NS
Desio	obs.	n.k.	n.k.	3	2	6	-	8	
	exp.			3.06	6.12		2.04	8.17	NS

* 4months only (July to October) n.k. = not known NS = not significant

DOW680105

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WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTÉ

CENTRE INTERNATIONAL DE RECHERCHE SUR LE CANCER
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

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JOINT NIEHS/IARC WORKING GROUP

on the

COORDINATION OF EPIDEMIOLOGICAL STUDIES

on the

LONG-TERM HAZARDS OF CHLORINATED DIBENZODIOXINS

AND CHLORINATED DIBENZOFURANS

Lyon, 10-11 January 1978

MN053305

AGENDA

1. Opening remarks - Dr J. Higginson
2. Appointment of Chairman and Vice-Chairman
3. Adoption of agenda and agreement to working procedures
4. Presentation and brief discussion of working papers
   (a) Chemistry and toxicology
   (b) Epidemiology
5. General discussion about epidemiology
6. Future research: recommendations and international coordination
7. General outline of meeting report

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ORGANISATION MONDIALE DE LA SANTÉ

CENTRE INTERNATIONAL DE RECHERCHE SUR LE CANCER
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

reviewed 4/15/78 M.L. Lang

RECEIVED
FEB 17 1978
REGISTRATION

M.L. from John D. papers attached

Joint NIEHS/IARC Working Group on the
Coordination of Epidemiological Studies
on the Long-Term Hazards of Chlorinated
Dibenzodioxins and Chlorinated Dibenzofurans

Lyon, 10-11 January 1978

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check availability of reference in number 11

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7266

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DOW372507

Jpn. J. Pharmacol. 28 1-13 1978
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IARC MEETING ON DIBENZODIOXINS AND DIBENZOFURANS

JANUARY 10-11, 1978

LYON, FRANCE

FROM: John H. Davidson 3/13/78

DOW 773180
MN053305

COMPARATIVE TOXICITY OF DIBENZODIOXINS AND DIBENZOFURANS

John A. Moore

TCDD

Chlorinated dibenzodioxin toxicity has been extensively characterized using the 2,3,7,8-isomer (TCDD) as the prototype. Toxicity in laboratory animals is characterized by a progressive wasting (mobilization of peripheral fat stores, increased triglycerides, fatty liver); and by hypoplasia of lymphoid tissues. Liver is also a target organ. The severity of effect varies between species with rats, mice, and rabbits markedly affected; hepatic effects in guinea pigs and Rhesus monkeys are minimal by comparison. Classic chloracne is not observed in laboratory species, but analagous integumentary effects are seen in monkeys, rabbits, and hairless mice. (Table 1)

TCDD is classified as a teratogen; however, the dominant prenatal effect is fetal toxicity or death. Existing reports on the mutagenicity of TCDD are not definitive. Recent studies have reported that TCDD is a carcinogen in rats. Additional lifetime studies in rats and mice and experiments in mice designed to determine cocarcinogenicity are nearing completion.

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Immune suppression has been demonstrated in rats, mice, and guinea pigs exposed to TCDD. The cell-mediated component is primarily affected, with humoral immunity also depressed at higher doses; low doses may be stimulatory. The developing fetus and pre-pubertal animal appear to be more susceptible. An increased susceptibility to bacterial (Salmonella) challenge is observed in mice that received 4 doses of TCDD that caused no gross or microscopic pathology.

DOW373181

TCDD is reported to have an extended half-life (24-31 days), liver and fat being the main body compartments. Significant species variability in the amount of TCDD stored in liver occurs with greatest liver storage found in rats and mice and lesser amounts in guinea pigs and Rhesus monkeys.

OTHER DIOXINS

The acute toxicity of other dibenzodioxin isomers has been systematically studied in guinea pigs and mice. The degree of toxicity is dependent on the location and number of halogens (Table 2). Extreme toxicity (LD 50/30 ± 1 mg/Kg) is found in tetra, penta, and hexachloro congeners that have halogens in the four lateral (2,3,7,8) positions.

The toxicopathologic pattern of disease caused by the chlorodibenzo-dioxins was found to be similar in a given species. Chronic toxicity (2 year) studies of the 1,2,3,6,7,8 and 1,2,3,7,8,9-hexachlorodioxin isomers in rats and mice are nearing completion.

7272

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The acute or chronic toxic dose for hepta or octachlorodibenzodioxin has not been established. Significant body burdens of these dioxins do occur as a consequence of prolonged exposure.

The interactive effects of subtoxic doses of various dibenzodioxin isomers have not been studied.

DOW 3731182

DIBENZOFURANS

The acute toxicity of 2,3,7,8-tetrachlorodibenzofuran has been evaluated in rats, mice, guinea pigs, and Rhesus monkeys. The LD 50/30 is similar to TCDD in guinea pigs and is about 40 fold higher (1 mg/Kg) in Rhesus monkeys. The toxic dose in rats and mice was not established using doses about 40 fold greater than for TCDD (Table 3).

Experiments in guinea pigs showed the acute LD 50/30 for 2,3,4,7,8-pentachloro and 2,3,7,8-tetrachlorodibenzofuran to be similar to the 2,3,7,8-tetrachlorodibenzodioxin and dibenzofuran.

The pattern of toxicity of all the dibenzofuran isomers studied to date was found to be indistinguishable from that produced by the dibenzodioxins.

Chronic toxicity studies have not been conducted with the dibenzofurans.

TABLE 1
PATHOLOGY SUMMARY

	GUINEA PIG	MOUSE	MONKEY
THYMUS			
INVOLUTION	+ + +	+ + +	+ + +
SPLEEN			
REDUCTION (WHITE PULP)	+	+	+
BONE MARROW			
HYPOPLASIA	+ +	±	+
LIVER			
MEGALOCYTOSIS/DEGENERATION	-	+ + +	-
BILE DUCT			
HYPERPLASIA	±	±	+ + +
TESTICLE			
DEGENERATION	+ + +	+ +	ND (FEMALE)
RENAL PELVIS			
HYPERPLASIA	+ +	-	+
URINARY BLADDER			
HYPERPLASIA	+ +	-	-
ADRENAL			
CORTICAL ATROPHY (Z.G.)	+ +	-	-
HEMORRHAGE			
INTESTINAL	+	+	-
ADRENAL	+ +	-	-
ASCITES	-	+ +	+
CUTANEOUS LESIONS	-	-	+ + +

DOW373184

Table 2

Estimated Single Oral LD_{50-30} of CDDs *

	Guinea Pigs		Mice	
Chlorination	µg/kg	µ mole/kg	µg/kg	µ mole/kg
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[B]	0.178-0.255	1,250	3.19
1,2,3,7,8,9	60-100[B]	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] Spearman-Karber Method

[B] Estimated Range Due to Variability in Replicates.

*McConnell, E., Moore, J., Haseman, J., and Harris, M.: 1977

DOW373185

TABLE 3

GENERAL TOXIC EFFECTS OF HALOGENATED DIBENZOFURANS IN GUINEA PIGS[a] *

Chemical	Dose (ug/Kg)	Mortality	Mean Time to Death (days)	Comments
TCDF	0	0/9[b]	-	Normal growth
	1	0/6	-	Decreased body weight gain
	5	0/6	-	Body weight loss in 2; other decreased weight gain
	10	6/6	11.8 (9-16)[c]	Immediate & progressive weight loss
	15	6/6	14.5 (12-19)	Immediate & progressive weight loss
PCDF	0	0/9	-	Normal growth
	1	0/6	-	Decreased body weight gain
	3	0/6	-	Decreased body weight gain
	10	6/6	15.7 (13-19)	Immediate & progressive weight loss
	30	6/6	9.2 (8-11)	Immediate & progressive weight loss
TBDF	0	0/9	-	Normal growth
	.47	0/6	-	No effect
	1.58	0/6		No effect
	4.74	1/6	26	Decreased body weight gain; progressive weight loss in animal that died
	15.84	6/6	11.7 (10-13)	Immediate & progressive weight loss

[a]All animals received single oral dose in corn oil, controls received only corn oil.

[b]Numerator indicates number dead, denominator indicates number tested.

[c]Mean time to death; number in parenthesis is range.

DOW3743186

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DOW 731817

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IARC MEETING ON DIBENZODIOXINS AND DIBENZOFURANS

JANUARY 10-11, 1978

LYON, FRANCE

DOW 129547

MN 00161615

COMPARATIVE TOXICITY OF DIBENZODIOXINS AND DIBENZOFURANS

John A. Moore

TCDD

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TCDD is classified as a teratogen; however, the dominant prenatal effect is fetal toxicity or death. Existing reports on the mutagenicity of TCDD are not definitive. Recent studies have reported that TCDD is a carcinogen in rats. Additional lifetime studies in rats and mice and experiments in mice designed to determine cocarcinogenicity are nearing completion.

DOW 129548

Immune suppression has been demonstrated in rats, mice, and guinea pigs exposed to TCDD. The cell-mediated component is primarily affected, with humoral immunity also depressed at higher doses; low doses may be stimulatory. The developing fetus and pre-pubertal animal appear to be more susceptible. An increased susceptibility to bacterial (Salmonella) challenge is observed in mice that received 4 doses of TCDD that caused no gross or microscopic pathology.

TCDD is reported to have an extended half-life (24-31 days), liver and fat being the main body compartments. Significant species variability in the amount of TCDD stored in liver occurs with greatest liver storage found in rats and mice and lesser amounts in guinea pigs and Rhesus monkeys.

OTHER DIOXINS

The acute toxicity of other dibenzodioxin isomers has been systematically studied in guinea pigs and mice. The degree of toxicity is dependent on the location and number of halogens (Table 2). Extreme toxicity (LD 50/30 ± 1 mg/Kg) is found in tetra, penta, and hexachloro congeners that have halogens in the four lateral (2,3,7,8) positions.

The toxicopathologic pattern of disease caused by the chlorodibenzo-dioxins was found to be similar in a given species. Chronic toxicity (2 year) studies of the 1,2,3,6,7,8 and 1,2,3,7,8,9-hexachlorodioxin isomers in rats and mice are nearing completion.

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The acute or chronic toxic dose for hepta or octachlorodibenzodioxin has not been established. Significant body burdens of these dioxins do occur as a consequence of prolonged exposure.

The interactive effects of subtoxic doses of various dibenzodioxin isomers have not been studied.

DIBENZOFURANS

The acute toxicity of 2,3,7,8-tetrachlorodibenzofuran has been evaluated in rats, mice, guinea pigs, and Rhesus monkeys. The LD 50/30 is similar to TCDD in guinea pigs and is about 40 fold higher (1 mg/Kg) in Rhesus monkeys. The toxic dose in rats and mice was not established using doses about 40 fold greater than for TCDD (Table 3).

Experiments in guinea pigs showed the acute LD 50/30 for 2,3,4,7,8-pentachloro and 2,3,7,8-tetrachlorodibenzofuran to be similar to the 2,3,7,8-tetrachlorodibenzodioxin and dibenzofuran.

The pattern of toxicity of all the dibenzofuran isomers studied to date was found to be indistinguishable from that produced by the dibenzodioxins.

Chronic toxicity studies have not been conducted with the dibenzofurans.

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SUMMARY

1. The pattern of toxicity produced by chlorinated dibenzodioxins and dibenzofurans are indistinguishable.

   Other chemicals, notably certain halogenated napthalenes, biphenyls, and likely the azoxybenzenes, also cause a similar pattern of toxic response.

2. Toxicity is dependent on degree and location of halogens with halogenation at the 2,3,7,8 position required to cause marked toxicity. The interaction of various dibenzodioxins or dibenzofurans is unknown.

3. Toxicity caused by these chemicals is characterized by chronic wasting, fat mobilization, and altered lipid physiology; lymphoid hypoplasia and immune suppression; sebacious gland metaplasia and hyperkeratosis, and a hepatic toxicosis that is species dependent.

DOW 129551

TABLE 1
PATHOLOGY SUMMARY

	GUINEA PIG	MOUSE	MONKEY
THYMUS			
INVOLUTION	+ + +	+ + +	+ + +
SPLEEN			
REDUCTION (WHITE PULP)	+	+	+
BONE MARROW			
HYPOPLASIA	+ +	±	+
LIVER			
MEGALOCYTOSIS/DEGENERATION	-	+ + +	-
BILE DUCT			
HYPERPLASIA	±	±	+ + +
TESTICLE			
DEGENERATION	+ + +	+ +	ND (FEMALE)
RENAL PELVIS			
HYPERPLASIA	+ +	-	+
URINARY BLADDER			
HYPERPLASIA	+ +	-	-
ADRENAL			
CORTICAL ATROPHY (Z.G.)	+ +	-	-
HEMORRHAGE			
INTESTINAL	+	+	-
ADRENAL	+ +	-	-
ASCITES	-	+ +	+
CUTANEOUS LESIONS	-	-	+ + +

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Table 2

Estimated Single Oral LD_{50-30} of CDDs *

	Guinea Pigs		Mice	
Chlorination	µg/kg	µ mole/kg	µg/kg	µ mole/kg
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 [B]	0.178-0.255	1,250	3.19
1,2,3,7,8,9	60-100 [B]	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] Spearman-Karber Method

[B] Estimated Range Due to Variability in Replicates.

*McConnell, E., Moore, J., Haseman, J., and Harris, M.: 1977

TABLE 3

GENERAL TOXIC EFFECTS OF HALOGENATED DIBENZOFURANS IN GUINEA PIGS[a] *

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Chemical	Dose (µg/Kg)	Mortality	Mean Time to Death (days)	Comments
TCDF	0	0/9[b]	-	Normal growth
	1	0/6	-	Decreased body weight gain
	5	0/6	-	Body weight loss in 2; other decreased weight gain
	10	6/6	11.8 (9-16)[c]	Immediate & progressive weight loss
	15	6/6	14.5 (12-19)	Immediate & progressive weight loss
PCDF	0	0/9	-	Normal growth
	1	0/6	-	Decreased body weight gain
	3	0/6	-	Decreased body weight gain
	10	6/6	15.7 (13-19)	Immediate & progressive weight loss
	30	6/6	9.2 (8-11)	Immediate & progressive weight loss
TBDF	0	0/9	-	Normal growth
	.47	0/6	-	No effect
	1.58	0/6		No effect
	4.74	1/6	26	Decreased body weight gain; progressive weight loss in animal that died
	15.84	6/6	11.7 (10-13)	Immediate & progressive weight loss

i.p. 50 2378 dibenzofurans = skin on Rh monkey

[a]All animals received single oral dose in corn oil, controls received only corn oil.

[b]Numerator indicates number dead, denominator indicates number tested.

[c]Mean time to death; number in parenthesis is range.

*Moore, J., McConnell, E., Dalgard, D., and Harris, M.: 1977

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Strictly Confidential

Working paper for an IARC working group meeting on "Coordination of Epidemiological Studies on the Long-Term Hazards of Chlorinated Dibenzodioxins and Chlorinated Dibenzofurans".
Lyon, January 10-11, 1978

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MN0533095

Symptomatology, morbidity and mortality experience of chlorinated phenoxyacid herbicide (2,4-D; 2,4,5-T) sprayers in Finland. A clinical and epidemiological study.

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INTRODUCTION

In 1972 there were widespread rumours in Finland that chlorinated phenoxyacids used for fighting brush vegetation by roadside and railroad tracks, along powerlines and on forest plantation areas, had caused chronic ailments including cancer among the applicators. The Finnish National Board of Health established a working group to investigate the allegations and the Institute of Occupational Health was assigned to conduct a nationwide survey on the health effects of herbicides 2,4-D and 2,4,5-T.

Herbicides 2,4-D and 2,4,5-T have been extensively used in Finland since the latter part of 1950's. In 1971 consumption of both compounds was about 50 metric tons active ingredient but there has been a considerable decline in recent years. The regular preparation to fight brushwood contained 2,4-D and 2,4,5-T in a 2:1 ratio mostly as water soluble emulsified esters. Spraying took place mainly from a portable, manually operated or motor driven "knapsack" equipment, to a lesser extent from a moving vehicle. It can be postulated that exposure to the chemical must have frequently been quite large because the sprayers did not take special precautions to avoid inhaling aerosols nor to protect against skin contamination which often was heavy due to leakages in the equipment. Annually a population of

Approximately 500, almost exclusively males, was engaged in the brushwood spraying operations mentioned above. The work period lasted from a few weeks to two months per year.

There are few sources of information describing toxic manifestations in the context of occupational application of chlorinated phenoxyacids. Petisov (1966) relates that exposed personnel suffered from unusual fatigue, headache, dizziness, gastrointestinal disturbances and irritation on the skin and mucous membranes. In the end of 1950's as well as early 1960's a series of publications report on fairly acutely developed and sometimes incapacitating peripheral polyneuropathies from systemic and local cutaneous exposure to 2,4-D (Goldstein et al. 1959, Todd 1962, Poissac-Gegoun et al. 1962, Berkley and Magee 1963). There have been no further observations of the like since then which raises the possibility of the occurrence of a neurotoxic impurity. A large oral dose of chlorinated phenoxyacids administered to mammals causes gastrointestinal disturbances, apathy, incoordination of limbs, myotonia and ataxia (Drill and Hirzka 1953) and suicidal intake of 2,4-D has caused similar symptoms in man (Brandt 1971). In his case of a surviving patient toxic changes occurred in the liver and kidney function; when the patient recovered a reversible alteration in colour vision and impairment of memory were also observed as well as a persistent neuropathy in one limb.

A whole complex of pathological symptoms and signs including chloracne, liver disorders, neurological and behavioral changes, hypercholesterolemia and porphyria cutanea tarda are known to have occurred among 2,4,5-T and trichlorophenol manufacturing personnel (IARC 1977). These effects are now mainly attributed to a toxic byproduct and contaminant 2,3,7,8-tetrachlorodibenzodioxin (TCDD). In the manufacturing process of 2,4,5-T some TCDD will be invariably produced. In 1971 a maximum allowable

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concentration of TCDD in 2,4,5-T was set at 0.5 ppm in Finland. Prior to 1971 no monitoring of TCDD had been carried out in commercial 2,4,5-T containing herbicide preparations. To investigate TCDD contamination in retrospect in 1973, a search for old 2,4,5-T containing preparations was done and TCDD was analyzed. 8 commercial preparations were found, representing large lots and several manufacturing firms from the years 1957-71. TCDD content was found to be at most 0.47 mg/kg and generally much less[a] (Table 1). Analysis was done gas chromatographically according to Eldridge (1971) and although the sensitivity and specificity of the method do not meet modern requirements, the results are thought to deviate only to exaggerate true concentrations.

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SYMPTOMATOLOGY EXPERIENCED BY 2,4-D AND 2,4,5-T APPLICATORS

In a nationwide hunt for reports on adverse health effects from brushwood killing herbicide exposure the public was informed to send their data to the Institute of Occupational Health. A further inquiry was then performed by the investigators with a through questionnaire. The questionnaire was also widely distributed by several employers to their personnel engaged in herbicide application. The investigators received nearly 200 reports of adverse effects. The leading symptom was headache which was reported as having occurred during the spraying operations by every second respondent; in half of them headache had persisted for longer periods of time post-exposure. Approximately one in ten respondents mentioned transient symptoms like dizziness, fatigue, abdominal complaints and irritation on mucous membranes and the skin. Skin

[a]Courtesy of P. Paasivuo, M.Sc., The State Institute of Agricultural Chemistry

affection was also of fairly common occurrence (7 per cent of respondents) and under closer scrutiny refered to obvious toxic reactions. There were no reports of chloracne but a few notified cases of persistent papular skin disorder are presently being reinvestigated. There were no personal histories reminiscent of the symptomatic peripheral neuropathies described by Goldstein and coworkers (1959) and others.

A survey of subjective symptoms associated with brushwood spraying produced a fairly uniform symptom complex. There were no clear indicators of a TCDD effect which could be anticipated to manifest itself as chloracne. There may be several plausible explanations to the common occurrence of headache chemical and non-chemical, and the factors may interact: physical strain, heat load, compression in the shoulder-neck area by straps, exhaust gases and unpleasant odour to mention the most obvious non-herbicidal reasons. Yet, the investigators were left with an impression that in many, if not in most cases the most suspect agent was the herbicidal preparation itself, generally emulsified esters of 2,4-D and 2,4,5-T. Clinical investigations were directed primarily to the nervous system, accordingly.

CLINICAL INVESTIGATIONS

Two groups of herbicide applicators were clinically examined. One, encompassing 29 males and one female, constituted the most heavily exposed group in a certain railroad maintenance district and the persons were selected on the basis of the duration of exposure ("local group"). The other comprised 18 males of 50 years of age or younger who were selected from the nearly 200 who reported subjective symptoms from exposure ("symptom group"). "Symptom group" persons were individually chosen according to symptomatology

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paying special attention to 1. severe acute cutaneous and neurologic symptoms and 2. unexplained, persistent neurologic and behavioral symptoms. Due to the urgency of the examinations and voluntary stoppage of brushwood spraying in 1972, nearly a year and in many cases a much longer period of time had elapsed since the last exposure.

Clinical investigations included a routine physical examination supplement with a neurological examination by a specialist neurologist. EEG was recorded and electroneuromyography (ENMG) was performed (Seppäläinen 1975). Clinical chemistry included E.S.R., Hgb, Hcr, WBC with a differential count, GOT, GPT, alkaline phosphatase, serum creatinine, urinary albumin and glucose, 2 hour sugar loading-test, serum PBI and T_3 binding capacity, serum cholesterol. Spirometry was performed and ECG (12 leads) recorded. Thorax and cervical spine were X-rayed. Several examinations in the battery were adopted to enhance differential diagnostics. As expected, some persons were found clinically ill (elevated blood pressure, CHD, cardiac insufficiency, emphysema) but these illnesses are hardly attributable to toxic agents. There were no notable skin disorders and the signs of pathology in the function of the liver, kidneys, blood forming organs and endocrines (sugar metabolism and thyroid function) were singular and mild.

A special emphasis was placed on a thorough neurological examination, EEG and ENMG. There were several observations of subclinical pathology in both groups which did not, however, constitute any uniform clinical picture nor conform to established clinical observations of a marked neurotoxic effect. EEG and ENMG findings were compared with those representing an industrial population occupationally nonexposed to neurotoxic compounds (Seppäläinen and Tolonen 1974). In the "local group" ENMG abnormalities occurred with the same frequency as with

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the controls, in the "symptom group" slightly more frequently. EEG abnormalities, which for the most part were classified as "light", occurred in both groups approximately two times more frequently than among the controls. The difference was not statistically significant ($0.05<p<0.10$; binomial distribution). It is obvious that there cannot be any conceivable control population for a population deliberately chosen for symptoms. Therefore, the findings of the "symptom group" must be evaluated individually in the light of possible underlying factors and between the individuals of the group. EEG abnormality found in the "local group" bears more weight and was thought to warrant a medical follow-up which is being performed through inquiries to the persons and local health authorities. Not a single person in either group was found to exhibit unequivocal signs of neurotoxicity. Since the examinations took place long after the last exposure to herbicides, only late and persistent sequelae can be thought of becoming detectable. Medical histories did not suggest, however, that clinically manifest neurological disability would have actually occurred in the early post-exposure period, either.

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MORTALITY STUDIES

Coinciding with our studies an epidemiological investigation in Sweden on the mortality of the Swedish State Railways herbicide applicators was being carried out. This personnel was exposed to mixtures of herbicides, for the most part combinations of chlorinated phenoxyacids with atrazine and aminotriazole with diuron or monuron. The results of this investigation pointed to an increased risk of respiratory cancer among the aminotriazole exposed workers while those exposed to phenoxyacids and combinations did not exhibit increased cancer mortality (Axelson and Sundell 1974). A recent re-analysis of data has indicated

however, that there may be an increased risk of cancer also from the exposure to phenoxyacids, and a follow-up study has been undertaken (Axelson and Sundell 1977). It was regarded necessary also in Finland to include an epidemiologic study into the investigation on the health effects of the herbicides.

MATERIAL AND METHODS

The cohort study carried out by our institute consists of two parts, a retrospective follow-up study which covers the years 1955-1971, and a prospective follow-up study covering the years 1972-1976. The data were collected in 1972 from the personnel records of four employers which are the main brushwood killing herbicide users in the country, the Finnish State Railways, the State Highway Authority, the State Forestry Authority, and a state owned electric power company. The material includes workers who have sprayed brush vegetation for at least two weeks' time during the years 1955-1971, irrespective of their employment status at the time of the investigation. Included in the study are also those who have retired or changed work later. The data were collected by the employers. They were obtained from old payrolls which unfortunately were not always complete enough, for instance, they did not always reveal the precise length of time in exposure. Therefore, in some cases we had to rely on the memory of the foremen and the clerks who keep the records. However, after checking the data it was reasonably certain that no systematic bias would affect the results. The material consists of 1960 men. The list of names and dates of birth was collated with the population data register in the National Pensions Fund where every living Finnish citizen is registered. The death certificates of persons found to be dead were obtained from the files of the Central Statistical Office of Finland. Nine persons could not be traced. The mortality of the herbicide

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sprayers was compared with the mortality of the total Finnish male population in the corresponding time periods. The material was divided into two cohorts according to the years when the exposure had occurred for the first time. The categories used were years 1955-1965 and 1966-1971. We have no information of their exposure to herbicides after 1971. Separate analyses were made for both cohorts and the whole material. Statistical calculations are based on person-years.

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RESULTS

The number of deaths during the retrospective follow-up of 1955-1971 was 45, and during the prospective follow-up of 1972-1976 it was 49. The deaths and the corresponding person-years are presented in detail in tables 4 and 5. The observed numbers of deaths from cancer in both cohorts follow quite closely the expected mortality during the retrospective study period, in the prospective study period they remain significantly lower. The mortality from all natural causes is in both cohorts and in both study periods only about a half of that of the male population in Finland. Deaths from unnatural causes are at the same level as those of the general population. The mortality of the cohorts do not differ remarkably from each other.

In the prospective follow-up the mortality differs between the workers of different employers (Table 6). Among the employees of the State Railways the frequency of deaths from cancer was about the same as the expected number, whereas there occurred no deaths from cancer among the personnel of the Forestry Authority although six had been expected. This group had also a much lower mortality from all natural causes than the other populations, only one fourth of the expected number. The number of violent deaths was about the same as expected among the employees of all four employers. In the retrospective follow-up the

differences between employers were less pronounced although a similar tendency could be observed. The workers of the State Railways were on the average over 10 years older than those of the Forestry Authority (Table 2).

DISCUSSION

This mortality study did not reveal any increased mortality from cancer or from natural causes on the whole. In fact, the considerable undermortality in comparison with the general male population seems somewhat puzzling. The spraying of brushwood by foot carrying a heavy gear as most of the subjects had done, particularly the employees of the Forestry Authority, must be considered physically heavy work. It is not uncommon that working populations, particularly when they are highly selected by work requirements, present during the occupationally active age considerably lower mortality than the general population (the so-called "healthy worker-effect" (McMichael 1976)). In an other investigation by the institute on the mortality of clerks and engine drivers at the State Railways, these groups presented figures closer to the death rates of herbicide sprayers than those of the general population (Järvinen 1976). Of all natural causes of death, cancer mortality could be expected to be little affected by the healthy worker-effect. This appears to be consistent with the findings from the observation period 1955-1971. The lack of cancer deaths among the Forestry Authority spraying personnel during 1972-1976 is unexplained. This group represents the youngest and largest population (Table 3) in the study.

Recently it was reported as a clinical observation in Sweden that in a material of 87 patients with mesenchymal tumours treated by a regional clinic for malignant diseases over the years 1970-76, 7 cases were thought to be exposed

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to chlorinated phenoxyacids some time previously (Hardell 1977). The localisation of malignant neoplasms observed in our study do not reveal a single mesenchymal tumour and present a distribution rather typical of cancers in these age groups in Finland.

The findings of the epidemiological mortality study must be critically examined in the light of known factors in chemical occupational carcinogenesis. Although the exposure to chlorinated phenoxyacids during spraying has probably been frequently heavy, total length of exposure has in most cases been a few weeks only. Furthermore, the follow-up period since the last exposure is at most 20 years and in most cases much shorter. In the conditions concerned, only strong carcinogenic effects of exposing compounds could be anticipated of becoming manifest. Weaker effects could present themselves after a much longer period of latency. We cannot, therefore, exclude the possibility of anything less than strong carcinogenic effects and a further follow-up of the population is warranted.

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Table 1. TCDD contamination in some 2,4,5-T containing herbicide preparations applied in Finland in the years 1957-1971.

Preparation	Year of application	Active ingredients	TCDD contamination mg/kg (calculated for 2,4,5-T)
Vesikontuho 500	1967	2,4-D + 2,4,5-T	0.31
Tributon DT	1971	- " -	0.20
Brushpon	1968	- " -	0.10
Spray 201 E/3	1960	- " -	0.31
Vesakonhävite F 500	1966	- " -	0.14
Brushpon	1957	- " -	0.23
Hormoslyr	1965	2,4,5-T	0.21
Regulan	1962	- " -	0.47

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Table 2. Age distribution of sprayers by employer in the beginning of 1972.

Employer	Median, years	Interquartile range, years
State Railways	47.8	38.0 - 56.6
State Highway Authority	38.0	29.0 - 47.0
State Forestry Authority	33.5	24.1 - 44.8
Power company	38.3	34.6 - 49.2

Table 3. Person-years by employer during the follow-up period of 1972-1976.

Employer	Person-years
State Railways	2050
State Highway Authority	2410
State Forestry Authority	4561
Power company	433

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Table 4. Mortality of 2,4-D and 2,4,5-T exposed workers during the time period 1955-1971. Expected numbers of deaths are based on the death rates of the total Finnish male population.

Cause of death (age, years)	Exposure started in the years						Total		
	1955-1965			1966-1971					
	Observed number of deaths (O)	Expected number of deaths (E)	Person-years	(O)	(E)	Person-years	(O)	(E)	Person-years
Malignant neoplasms									
15-24	-	0.1	1030	-	0.1	1110	-	0.2	2140
25-34	1	0.3	1780	-	0.1	750	1	0.4	2530
35-44	3	1.1	2048	1	0.4	868	4	1.5	2916
45-54	1	3.2	1560	-	1.2	653	1	4.4	2213
55-64	4	6.7	1060	2	1.8	292	6	8.5	1352
65-74	1	1.5	112	-	0.0	1	1	1.5	113
75 and over	-	-	-	-	-	-	-	-	-
Total	10	12.9	7598	3	3.6	3674	13	16.4	11272
All natural causes of death									
15-24	-	0.4		-	0.3		-	0.8	
25-34	1	1.7		-	0.5		1	2.1	
35-44	7	6.2		3	2.5		10	8.6	
45-54	5	14.4		4	6.1		9	20.5	
55-64	7	25.8		3	7.2		10	33.0	
65-74	6	6.3		-	0.0		6	6.4	
75 and over	-	-		-	-		-	-	
Total	26	54.8		10	16.6		36	71.4	

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