The Effect of the Type of Respondent on Risk Estimates of Pesticide Exposure in a Non-Hodgkin's Lymphoma Case-Control Study

Geary W. Olsen, DVM, PhD
Kenneth M. Bodner, MSPH

ABSTRACT. There has been considerable controversy among epidemiologists whether proxy interviews provide agricultural pesticide use data comparable to that obtained from direct informants, who are assumed to be generally more knowledgeable about their use of pesticides. The purpose of this analysis was to determine whether odds ratios for pesticide use, in particular 2,4-dichlorophenoxyacetic acid (2,4-D), varied by the type of respondent in a resurvey of 310 subjects in the National Cancer Institute's Iowa/Minnesota Non-Hodgkin's lymphoma case-control study. Pesticides were grouped according to crop insecticides, animal insecticides, and herbicides (with and without 2,4-D), as well as 2,4-D itself. Using logistic regression, only animal insecticides had consistent risk estimates between the proxy and direct informants for the three categories of frequency of use employed in the analysis (1-4 days/yr., 5-9 days/yr. and 10+ days/yr.). Significant ($p < 0.05$) interaction terms (respondent type by each frequency of use category) were observed for crop insecticides at 1-4 days/yr., for herbicides including 2,4-D at 10+ days/yr. and for herbicides excluding 2,4-D at 10+ days/yr. use. For 2,4-D use, the proxy-derived odds ratio was 2.5 (95% CI 0.8-8.0) for the highest frequency of use (10+ days/yr.) compared to a direct informant-derived odds ratio of 0.7 (95% CI 0.3-1.9) (interaction term $p = 0.08$). The results from this analysis, as well as other published data, suggest that the type of respondent may act as an effect modifier in the association between cancer and pesticide exposure. We agree with others who have recommended that agricultural pesticide use risk estimates derived from case-control study data should be analyzed and presented separately by the type of respondent. (*Article copies available from The Haworth Document Delivery Service: 1-800-342-9678.*)

KEYWORDS. Cancer, epidemiological methods, herbicide, insecticide, non-Hodgkin's lymphoma, pesticide, 2,4-dichlorophenoxy-acetic acid, 2,4-D

Geary W. Olsen and Kenneth M. Bodner are affiliated with the Department of Epidemiology, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI.

Address correspondence to: Dr. Geary W. Olsen, 3M Occupational Medicine, 3M Center, Building 220-3W-05, P.O. Box 33220, St. Paul, MN 55133-3220.

Journal of Agromedicine, Vol. 3(1)1996

Chapter 1

Rationale for the Phenoxy Herbicide Benefits Assessment

ORVIN C. BURNSIDE[1]

Table of Contents

WHY THE ASSESSMENT WAS UNDERTAKEN

During the past several years a National Agricultural Pesticide Impact Assessment Program (NAPIAP) Task Force has conducted a biologic and economic assessment of benefits from the use of phenoxy herbicides in the United States. Phenoxy herbicides, such as 2,4-D, have provided economical, selective, postemergence control of broadleaf weeds in grass crops and noncropland for the past five decades (8, 12, 13). A major use of 2,4-D today is in combination with other herbicides because it economically enhances the weed control spectrum of many herbicide mixtures. The herbicide 2,4-D is registered for use on 65 crops in the United States and other phenoxy herbicides are registered on 25 crops, and these herbicides also have numerous noncropland uses. The chemical, 2,4-D, was first registered with the United States Patent Office for use as a herbicide in 1945 (12). Like other pesticides registered prior to November 1984, the phenoxy herbicides must be re-registered with the Environmental Protection Agency (EPA) by 1997 or all present registrations will be lost.

This benefits assessment of 2,4-D and other phenoxy herbicides encompasses all their cropland and noncropland uses in the United States, along with their biologic and economic benefits. This report will provide EPA personnel and others with benefits information about the phenoxy herbicides that will be useful in deciding whether this class of herbicides should be re-registered. Also, we are presenting a historical account of the development of phenoxy herbicides in the United States, and the impact that these herbicides have had on weed management technology and the development of the discipline of weed science.

[1] Prof., Dep. Agron. and Plant Genet., Univ. Minnesota, St. Paul, MN 55108.

ENVIRONMENTAL AND TOXICOLOGICAL INFORMATION

Environmental concerns and risk data development for 2,4-D re-registration was completed by the Industry Risk Assessment Task Force II in time to meet the December 1995 EPA deadline. Other Industry Task Forces have conducted risk research, as specified by EPA, for re-registration of other phenoxy herbicides used in the United States. A synopsis of environmental and toxicology information with the phenoxy herbicides is presented here, but in Chapter 3, epidemiological and toxicological information is presented in greater detail.

Based on dissipation studies, the acid, salt, and ester formulations of 2,4-D readily break down in the soil with an average half-life of 4 to 10 days (1). The herbicide 2,4-D can persist in detectable amounts in the environment for 1 to 4 weeks after application. Movement in soil is usually less than 6 inches downward from the site of application. In sandy soils with low organic matter content in California, 2,4-D has leached only 12 to 18 inches even after large amounts of water were applied (1). Thus, groundwater contamination with 2,4-D across the United States has remained very low (2), and new phenoxy herbicide management practices should further decrease the potential for groundwater contamination (4). Phenoxy herbicides decompose primarily by microbial activity, and degradation

increases with increased soil pH, moisture, organic matter, and temperature. Little genetically based weed resistance development has been observed despite the extensive use of the phenoxy herbicides during the past five decades (7).

Dissipation of 2,4-D is rapid in surface water and trace amounts are only occasionally detected (2), even though some ester and salt formulations of 2,4-D are registered and used to control aquatic weeds in lakes and ponds. The Health Advisory Level for 2,4-D that was established by EPA for potable water is 70 ppb (3). EPA has rated 2,4-D as "practically nontoxic" for both warm water and cold water fish, and monitoring studies indicate no evidence of bioaccumulation in fish or the environment (1). EPA has classified 2,4-D's bird-dietary-toxicity and honeybee-toxicity as "practically nontoxic" (1). Because there is little residue from phenoxy herbicides in the air, soil, or water and because residues in treated vegetation decline rapidly, exposure of wildlife to phenoxy herbicides has been low. The major effect phenoxy herbicides have on wildlife is habitat modification, because of reduction of broadleaf vegetation, but these effects may even be beneficial to wildlife (6).

The phenoxy herbicides are low in toxicity to humans and animals (1, 9). No scientifically documented human health risks, either acute or chronic, exist from the approved uses of the phenoxy herbicides. Acute toxicity to humans, based on oral, dermal, ocular, or inhalation administration, may vary with the 2,4-D formulation. However, phenoxy herbicides have been used widely by numerous individuals (9) from home owners to farmers and ranchers, and even with significant exposure, humans have shown essentially no acute toxicity (11).

In the late 1970's and the 1980's, questions were raised about the chronic effects to humans from repeated exposure to the phenoxy herbicides because of the occurrence of three rare forms of cancer (10). A comprehensive study of the scientific evidence relating to the safety of 2,4-D was published in 1992 (11). This report stated that: "The case-control epidemiological studies that have been the source of the cancer risk hypothesis are inconclusive. Problems in assessing exposure based on patients' memories make these studies difficult to interpret. Cohort studies of 2,4-D-exposed workers do not generally support the specific hypothesis that 2,4-D causes cancer. Taken together, the epidemiological studies provide, at best, only weak evidence of an association between 2,4-D and the risk of cancer." The report further states: "Historical exposures to 2,4-D by user groups, particularly farmers, forestry workers and commercial applicators, would be higher than those sustained under present rigorous standards for application which involve the use of protective clothing and other measures to reduce exposure. Proposed label changes indicate that in the future exposures will be even further reduced. Viewed in this context, the available data indicate that the potential public health impact of 2,4-D, including the risk of human cancer, was negligible in the past and would be expected to be even smaller in the present and future."

Another Advisory Panel convened by the EPA to evaluate toxicology studies on 2,4-D since the prior report in 1992 (5) stated: "The Committee concludes that the data are not sufficient to find that there is a cause and effect relationship between the exposure to 2,4-D and NHL (non-Hodgkins lymphoma)." Thus, the risk to humans from exposure to the phenoxy herbicides has been considered negligible. Therefore, a benefits assessment of the phenoxy herbicides in the United States, which is the objective of this report, would be very useful considering the limited evidence of any apparent adverse human health or environmental risks from the use of these herbicides.

HOW THE ASSESSMENT WAS CONDUCTED

An organizational meeting on November 4, 1992 was attended by Orvin Burnside, Ronald Davis,

Leonard Gianessi, Dennis Kopp, Craig Osteen, and Nancy Ragsdale. We decided to conduct a biologic and economical benefits assessment of phenoxy herbicide use in the United States with Burnside as the Task Force chair. A Task Force of eminent weed scientists and an economist would be selected by Burnside to prepare a benefits assessment of all uses of the phenoxy herbicides. The first meeting of the Task Force on January 13, 1993 was attended by Rodney Bovey, Orvin Burnside, Herman Delvo, Clyde Elmore, Leonard Gianessi, Larry Hammond, Ellery Knake, Dennis Kopp, Carole Lembi, John Nalewaja, Michael Newton, and James Parochetti. We then selected an established weed scientist from each state, who we contacted by telephone before sending them generic questionnaires (Appendix 1) for each registered phenoxy herbicide use in their state. Our subsequent meetings were generally held in conjunction with the Weed Science Society of America annual meetings to reduce travel expenses, because most Task Force members normally attended those meetings. At the February 9, 1993 meeting, Philip Szmedra became a part of the Task Force to replace Herman Delvo as our economist. Additional meetings were held February 7, 1994, May 3, 1995, and February 7, 1996 to update Task Force members, agree on solutions to concerns needing attention, and answer questions that did arise.

Questionnaires (Appendix 1) were sent out during early 1993 to our selected weed scientist in each state plus Puerto Rico, and they were requested to respond for each registered phenoxy herbicide use in each cropland or noncropland situation. If the weed scientist did not feel qualified to provide information about some phenoxy herbicide use in their state, they were instructed to contact an individual, or individuals, with the best knowledge of the needed inforand they were requested to respond for each registered phenoxy herbicide use in each cropland or noncropland situation. If the weed scientist did not feel qualified to provide information about some phenoxy herbicide use in their state, they were instructed to contact an individual, or individuals, with the best knowledge of the needed information. Separate questionnaires were furnished for each EPA registered use of

Questionnaires were returned to Herman Delvo and he worked with Leonard Gianessi in recording and summarizing the data which Philip Szmedra then analyzed. Missing data from states caused us to alert the appropriate expert on our Task Force who in turn contacted the state weed scientists in question. If no data were received on some specific use in any state, our Task Force expert then provided such data based on results from surrounding states or from personal knowledge of the situation. Thus, we either received data from all states or we extrapolated to 100% of a specific cropland or noncropland use in the United States.

We compared estimated use of phenoxy herbicides in the various cropland or noncropland areas with the actual sale of phenoxy herbicides for the 1992 use year. If there was a discrepancy between the two amounts, the Task Force member responsible for that cropland or noncropland use made appropriate contacts and revisions to make these two estimates agree within reasonable limits. Efforts were made throughout the data analysis process to avoid double counting phenoxy herbicides uses or to avoid omissions of use areas. Actual data analysis procedures are discussed in Chapter 4.

The return rate for these phenoxy herbicides questionnaires (over 90%) was among the highest received by NAPIAP assessments. We believe such high returns were due to picking reliable respondents, contacting them for their concurrence before the questionnaires were sent, and following up by letters and telephone calls. Also, respondents were interested in retaining the use of the phenoxy herbicides in the United States.

ADDITIONAL INFORMATION ABOUT THIS REPORT

Each chapter in this report is intended to "stand alone," having its own Abstract, Literature Cited, and so forth. The various chapters are written by a recognized expert in that area of phenoxy herbicide use, economics, epidemiology, or toxicology.

For phenoxy herbicides and other herbicides that are derived from an acid, use rates are conventionally expressed in terms of weight of acid equivalent (a.e.) per unit area. For all other herbicides, use rates are conventionally expressed in terms of weight of active ingredient (a.i.) per unit area. This is how herbicide use rates are expressed in this report, but to reduce repetition we have dropped the use of a.e and a.i., and we just express rate in pounds of herbicide broadcast over one acre, which we abbreviate lb/A. Other abbreviations or acronyms used in this report are given in Appendix 2.

We have used common names for plants and herbicides, to avoid cluttering the text with the Latin names of plants and the chemical names of herbicides included in this report. However, we have provided this information in Appendices 3, 4, and 5 for those who want more detailed information.

Crops mentioned in this report are listed by common name in Appendix 3. The term crops is used in a broad sense, and it includes, for example, such things as trees in forests and orchards, and grasses in turf and rangeland.

Weeds mentioned in this report are listed by common name in Appendix 4. The term weeds is also used in a broad sense, and it includes, for example, such things as woody species and plant species that would be considered crops, if they were growing where they were wanted.

Herbicides mentioned in this report are listed by common name in Appendix 5. There, in addition to the chemical names, we have included some proprietary names (i.e., registered trade names, registered trademark names, and brand names).

And finally, Appendix 6 is a glossary of terms used in weed science, most of which have been used in this report.

ACKNOWLEDGEMENTS

Dennis Kopp, administrative advisor to this Task Force, and Nancy Ragsdale, Director of the United States Department of Agriculture NAPIAP organization, were most helpful and encouraging during the entire assessment effort. They had the needed information, data, resources, advise, and counsel when and where it was needed. It was both an educational and enjoyable experience to work with such dedicated and capable individuals.

Task Force members provided yeoman efforts when it was needed to complete this assessment in a timely manner. We appreciated their capable efforts in this "labor-of-love" as they all donated their time and effort to assuring the continued use of these important phenoxy herbicides.

Our selected state contacts were most generous with their time and talents as they met our many deadlines for the data that they were most capable of providing. The assessment results are credible because we selected those most knowledgeable and actively engaged in the areas surveyed in each state plus Puerto Rico. We could not have done this assessment without them so they should accept our sincere thanks for a job well done. These individuals are listed in the front pages of this assessment under "Respondents to Our Questionnaires."

Herman Delvo sent out the questionnaires, Leonard Gianessi compiled the survey data, Philip

Szmedra analyzed the results, and team members added the expertise that explained and summarized the results received. Also, there were many reviews of the data and manuscripts as it went through the editorial process, which added to the creditability and value of this phenoxy herbicide assessment.

Finally, we want to thank our capable typist, Patricia Kessler, who typed the various chapters and made sure that we followed basic rules of composition and editorial format. Eric Ristau produced our computer generated pie chart figures. Providing uniformity among the various chapters and readability was an objective that we strived to achieve. Also, we want to thank the publishers at Richtman Printing Companies, 301 NP Avenue, Fargo, ND 58107 who took our material and produced the finished product.

We believe that we have produced an accurate and useful assessment of the biologic and economic benefits of the phenoxy herbicides in the Unherbicides in the United States during 1992. This publication is the collaborative effort of many individuals knowledgeable about the phenoxy herbicides, and it is dedicated to all individuals who have worked for the continued use of these efficacious and economical herbicides.

Table of Contents

LITERATURE CITED

1. Ahrens, W. H., ed. 1994. Herbicide Handbook. Weed Science Society of America, Champaign, IL. 352 p.

2. Anonymous. 1986. National Water Quality Inventory, 1986 Report to Congress. Office of Drinking Water, U.S. Environmental Protection Agency, Washington, D.C. EPA-44014-87-008. 185 p.

3. Anonymous. 1990. Drinking Water Regulations and Health Advisories. Office of Drinking Water, U.S. Environmental Protection Agency, Washington, D.C.

4. Anonymous. 1993. Agricultural Research to Protect Water Quality. Conf. Proc., Soil and Water Cons. Soc., Ankeny, IA. 755 p.

5. Anonymous. 1994. An SAB Report: Assessment of Potential 2,4-D Carcinogenicity, Review of the Epidemiological and Other Data on Potential Carcinogenicity of 2,4-D by the SAB/SAP Joint Committee. U.S. Environmental Protection Agency, Washington, D.C. Science Advisory Board, Report EPA-SAB-EHC-94-005.

6. Bramble, W. C. and W. R. Burns. 1974. A long-term ecological study of game food and cover on a sprayed utility right-of-way. Purdue University, Lafayette, IN. Bull. No. 918. 16 p.

7. Duke, S. O., ed. 1995. Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory, and Technical Aspects. Lewis Publishers, CRC Press, Boca Raton, FL. 416 p.

8. Gianessi, L. P. 1993. The Use of Phenoxy Herbicides in U.S. Agriculture. Resources of the Future, Washington, D.C. 32 p.

9. Harris, S. A., K. R. Solomon, and G. R. Stevenson. 1992. Exposure of home owners and bystanders to 2,4-dichlorophenoxyacetic (2,4-D). J. Environ. Sci. Health. B27(1):23-28.

10. Linet, M. S. 1986. Review: Agricultural Herbicide Use and Risk of Lymphoma and Soft Tissue

Sarcoma, and Other Epidemiologic Studies Examining the Association Between the Herbicide 2,4-D, Herbicides in General, Farming and Malignant Lymphoma. John Hopkins School of Public Health, Baltimore, MD.

11. Munro, I. C., G. L. Carlo, J. C. Orr, K. G. Sund, R. M. Wilson, E. Kennepohl, B. S. Lynch, M. Jabinske, and N. L. Lee. 1992. Comprehensive, integrated review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D. J. Amer. Coll. Toxicol. 11(5):559-664.

12. Page, D. L. 1995. The reregistration of 2,4-D, where in the world -- and the environment -- are we? p. 2-12. in Industry Task Force II on 2,4-D Research Data. DowElanco, Indianapolis, IN.

13. Pokorny, R. 1941. New compounds. J. Amer. Chem. Soc. 63:1768.

Chapter 2

The History of 2,4-D and Its Impact on Development of the Discipline of Weed Science in the United States

ORVIN C. BURNSIDE[1]

- Table of Contents

Abstract. Research in the early 1940's on 2,4-D in the United States and MCPA in England, their release after World War II, and their rapid acceptance by farmers put selective weed control in the "public spotlight" worldwide. Phenoxy herbicides have provided economical, selective, postemergence control of broadleaf weeds in grass crops and noncropland for the past five decades. Weed science technology has had a major impact during this herbicide era in increasing crop yields and reducing labor requirements for controlling weeds. Farmers rely heavily on 2,4-D and subsequently developed herbicides as a major component of their weed management program. Because the phenoxy herbicides were so effective, weed scientists initially concentrated their research and educational efforts on chemical control of weeds. This trend continues today and has impeded research on alternate weed management technology. Herbicide development will continue to be the focus of chemical company personnel because of the profit incentive. Consequently, publicly-funded weed scientists will need to emphasize preventive, cultural, mechanical, biological, and integrated control technologies so they can provide growers and land users with more balanced systems of weed management.

---

[1]Prof., Dep. Agron. and Plant Genet., Univ. Minnesota, St. Paul, MN 55108.

INTRODUCTION

Weeds are the number one pest problem in crop production because they reduce crop yields and increase production costs (3, 4, 9). Herbicides are the most widely used class of pesticides in the United States because they are effective and economical in controlling weeds, and because weeds are the most important pest in crop production. In addition, herbicides reduce manpower and horsepower requirements and erosion-producing tillage in crop production. No exact figure has been placed on the economical benefits provided to world agriculture by the introduction of the phenoxy herbicides, which started the herbicide revolution in developed countries, but the estimated value to humankind from reduced labor requirements alone is in the billions of dollars annually.

Sale of phenoxy herbicides in the United States was about $171 million in 1992, but the loss of these herbicides would increase alternative herbicide and non-chemical weed control costs by $947 million annually (10, 16). The phenoxy herbicides still represent over 10% of herbicide poundage used in the United States, and they are the most widely used family of herbicides worldwide. Our NAPIAP report will provide EPA personnel information about the phenoxy herbicides that will be useful in their deciding whether this class of herbicides should be re-registered for agricultural crops, forestry, pastureland, rangeland, roadsides, turfgrass, aquatics, and other noncropland uses in the United States (10).

The objectives of this chapter are: (a) to trace the discovery and development of 2,4-D, and (b) to discuss the impact of 2,4-D and subsequent herbicides on the development of the discipline of weed

science.

DISCOVERY AND DEVELOPMENT OF 2,4-D

The phenoxy herbicides, and 2,4-D in particular, ushered in the chemical weed control revolution in the mid 1940's. Even 51 years after its introduction, 2,4-D continues to be the most commonly and widely used herbicide worldwide. This discussion encompasses the numerous research and development pieces that completed the 2,4-D "puzzle" and its subsequent extensive utilization as a selective herbicide (Table 1). No one can give credit to all the personnel involved in the basic, applied, developmental, and marketing activities that brought 2,4-D use to fruition; however, that should not prevent an attempt at recognizing some major developmental and utilizational milestones. Table 1 is a chronological listing of some major advances in the history of 2,4-D development in the United States and worldwide, and when and where these developments occurred. Table 1.

Initial research on selective herbicides. The original research on selective herbicides was carried out by Bolley in the United States, Bonnett in France, and Schultz in Germany around 1900 (20). These researchers independently found that solutions of copper salts and other inorganic compounds would selectively control broadleaf weeds in cereals. Bolley, a plant pathologist, wrote in 1908 (6) that "When the farming public has accepted this method (selective weed control) of attacking weeds . . . the gain to the country at large will be much larger in monetary consideration than that which has been afforded by any other single piece of investigation applied to field work in agriculture." Bolley continued with, "If, therefore, this method of attacking weeds by means of chemical spray is one-quarter or one-half as successful in general operation as the writer is willing to vouch for, the money returns to the spring wheat growing states must far exceed the hopes of the most optimistic." This prophecy by Bolley regarding new methods of weed management actually came to fruition with the advent of the phenoxy herbicides in the mid-1940 era (32).

Basic research that led to 2,4-D. Botanists have long been intrigued with plant shoot and root growth and the mechanisms causing plants to respond to stimuli. Darwin from England in 1880 (33) reported that plants bend towards the light and that some influence from the tip of oat coleoptiles caused this bending or phototropic response. Boysen-Jensen from Denmark in 1911 (33) reported that when an oat coleoptile that was exposed to directional light was excised and reattached to unexposed oat tips, the bending still occurred. He concluded that the influence from coleoptile tips was thus chemical not physical. Went from the Netherlands in 1926 (33) collected the chemical from oat coleoptile tips and found it to be an active plant growth regulator. He developed a quantitative measurement of the regulator and thus stimulated considerable research in this area. Kögl and Haagen-Smit from the Netherlands in 1934 (33) reported the isolation of indoleacetic acid (IAA) from plants and human urine and identified it as the principal naturally occurring hormone (later called an auxin) in plants. When humans eat fresh vegetables they injest the hormone IAA (chemically very similar to the more stable 2,4-D) and excrete it in their urine.

Because IAA was unstable outside of plants, researchers began synthesizing and investigating the effect of IAA derivatives and homologs on plant growth activity. Zimmerman and Wilcoxson from the United States in 1935 (37) reported the discovery of phenylacetic acid and naphthylacetic acid, which affected plants by preventing premature fruit drop, inducing rooting, accelerating fruit ripening, and causing seedless tomatoes. Pokorny from the United States in 1940 (29) synthesized 2,4-D and 2,4,5-T while looking in vain for a fungicide. In June 1941, Pokorny described the chemical synthesis of 2,4-D (29). In 1942 Zimmerman and Hitchcock (36) reported that 2,4-D was 300 times more potent than indolebutyric acid, the major plant growth regulator at that time, for inducing seedless

tomatoes.

Monochloroacetic acid and 2,4-dichlorophenol were used to make 2,4-D (29). Subsequently, salts were made by adding the appropriate amine or inorganic hydroxide to the acid (1). Esters were synthesized by reacting 2,4-D with the appropriate alcohols. The discovery of 2,4-D could be considered more fortuitous than systematic, but one must recognize that discovery begets further discovery, and the process seems to gain momentum and often culminates in numerous and diverse scientific advances. Went stated that, "When I worked 25 years ago with the growth hormone (2,4-D), I had many wild ideas about what it might do once it was available in large quantities, but I never dreamed that it would lead to the development of weed killers. This is an excellent example, how fundamental research may lead to the solution of very practical problems." (2). Therefore, basic research on plant growth regulators during the 1930's and earlier (33) facilitated the development of selective phenoxy herbicides in the 1940's. Thus, the discipline of weed science evolved from the field of economic botany or more specifically the study of plant growth regulators.

Military interest in 2,4-D. Interest in 2,4-D within the scientific community seemed to lose momentum during World War II when both United States and England scientists initiated secret biological warfare research on plant growth regulators with the objective of destroying enemy crops. Kraus at the University of Chicago had observed since 1936 that certain growth regulators were phytotoxic (28). Kraus was aware of the inadequacy of existing herbicides, and in 1941 he was first to propose that growth regulators might work as herbicides, because they oftenwhen both United States and England scientists initiated secret biological warfare research on plant growth regulators with the objective of destroying enemy crops. Kraus at the University of Chicago had observed since 1936 that certain growth regulators were phytotoxic (28). Kraus was aware of the inadequacy of existing herbicides, and in 1941 he was first to propose that growth regulators might work as herbicides, because they often killed test plants. In late 1941, Kraus and other prominent scientists convinced Secretary of War, H. L. Stimson, of the potential dangers

In November 1942, the United States Army began developing Camp Detrick (later renamed Fort Detrick) in Frederick, Maryland, as the center for research and testing of chemicals for biological warfare with special emphasis on crop destroying chemicals. In March 1943, the United States Army paid the University of Chicago $3500 for herbicide research completed by Kraus (28). In January 1944, research at Camp Detrick was accelerated on crop destroying herbicides. W. B. Ennis, Jr. was one of a small group of military scientists assigned to Camp Detrick, and he provided the following information regarding research by the military with the phenoxy herbicides[2].

---

[2]Ennis, W. B., Jr. 1995. Personal communication from one of the military scientistsassigned to Camp Detrick, Maryland, to O. C. Burnside, Dep. Agron. and Plant Genet., Univ. Minnesota, St. Paul, MN.

While laboratory facilities were under construction at Camp Detrick, E. J. Kraus at the University of Chicago and J. W. Mitchell with the USDA at Beltsville, Maryland, were supported by contract to continue studies of the herbicidal effects of plant growth regulators. Somewhat later, a synthesis program was commenced under contract with A. S. Newman at Ohio State University.

In 1944, the biological warfare effort at Camp Detrick was stepped up by the Special Projects Division of the Chemical Warfare Service, U.S. Army under a heavy cloak of secrecy. Herbicide research with crop destruction as the objective was vigorously pursued under the direction of A.G. Norman by a team of about a dozen scientists drawn from other assignments in the services. Most were recent Ph.D. graduate students or graduates in agronomy or botany.

The research program was broadly conceived to throw light on the nature of plant responses, plant and herbicide specificities, environmental effects, effective rates of application, spray volumes and droplet size, co-agents and carriers, etc. Although attention was soon centered on halogen-substituted aryloxy acids, particularly the phenoxyacetics, many new compounds and derivatives were synthesized and screened for growth regulating properties. Sufficient amounts of the more active compounds, such as 2,4-D, 2,4,5-T and others, were procured for field trials, which included aerial application. Attention was also given to forest defoliants for the purpose of reducing cover of enemy defense positions in the expected assault on Japan.

All the research conducted at Camp Detrick was kept under military secrecy until the end of World War II. Then the scientists were free to publish results of their research. The entire June 1946 (Vol. 107) issue of the Botanical Gazette consisted of papers from Camp Detrick scientists. These papers reported well-designed experimental observations likely to be of general interest. Muchadditional information was obtained, but not all in the rigorous experimentaldetail justifying journal publication. Additional papers appeared later in the Agronomy Journal, American Journal of Botany, Science, Weeds, and other journals. The research at Camp Detrick formed a major part of the beginning of modern weed science and represented a significant part ofthe foundation knowledge on weed control with herbicides.

Among the accomplishments of the Camp Detrick scientists were the development of methods for evaluating over 1000 chemical compounds fortheir herbicidal properties, determining histological effects of 2,4-D and 2,4,5-T, cytological effects of propham, demonstration that weeds can becontrolled with ultra low-volume sprays, investigating theimportance of carriers for 2,4-D (solvents, surfactants, granules,etc.), defining the selective action of sprays on broadleaf plants,identifying the herbicidal effects of soil and water applications, anddetermining the dosages required.

The herbicide 2,4-D was patented by American Chemical Paint Co. with licensing provisions for production by Dow Chemical Co. and other companies. Commercialization followed in the mid to late 1940's.

After the war most of the scientists in the program returned to civilian life, some to universities to complete graduate programs and later to embark on careers that stemmed from their involvement in the Camp Detrick program. The Camp Detrick program, however, was not terminated. After a few months of reduced activity it was converted to a civilian staffed operation administered by the Civil Service. Security was reduced, yet certain aspects remained classified. A new staff was slowly assembled, including some of the wartime group. The program was less specifically targeted than previously and broadened to encompass various types of growth responses in plants. Synthesis and screeningwere continued and improved procedures for assessingherbicidal activity were devised. Camp Detrick research wassupplemented by contracts with universities, and research was initiated on abscission and the gibberellins. Leaf abscission and the testing of defoliants for woody species were pursued during 1961-72 under the direction of C. E. Minarik, which led to their military use that became so controversial in Vietnam operations.

All biocidal research activities were terminated in 1972, following adeclaration by President R. M. Nixon that the United States would nolonger conduct biological warfare research or develop biologicalweapons. The herbicide/defoliant research at Camp Detrick was then closed and its personnel dispersed. Some of the plant researchlaboratories and facilities were taken over by USDA-ARS scientists and personnel for research relevant to their ongoing programs."

The development of organic herbicides was greatly facilitated during World War II because of their military potential as biological warfare weapons (28). Throughout the war, United States Army research on the phenoxy herbicides was done in secrecy until January 3, 1946 when Secretary of War, R. P. Patterson, announced that more than 1000 different chemical agents had been tested on living plants. In May 1946, G. W. Merck (28), Director of the Civilian War Research Service, declared that, "...only the rapid ending of the war prevented field trials in an active theater that would, without injury to human or animal life, affect the growing crops and make them useless." Thus, herbicides were not used in chemical warfare until November 21, 1962 when the United States and South Vietnam military personnel sprayed 2,4,5-T plus 2,4-D (Agent Orange) in Vietnam to defoliate trees along military routes to reduce sniper activity (7, 11). Several Vietnam veterans in my subsequent weed science classes praised the use of Agent Orange because of the safety it provided from sniper fire for our military personnel. Antiwar protestors seized on the military's use of Agent Orange in their successful campaign to discredit this unpopular war. In the 1970's, following cessation of military hostilities, spokespersons for Vietnam veterans alleged that health problems and deaths of veterans were related to Agent Orange exposure in the military, and these allegations persisted. Thus, the public announcement on March 15, 1979 by EPA Administrator, W. D. Ruckelshaus, of an Emergency Suspension Order and Threat to Cancel further use of 2,4,5-T and silvex in the United States was strongly propelled by political pressure and not based upon scientifically developed risk assessment data. Unfortunately, on January 2, 1985 agriculturalists and foresters lost all registered uses of 2,4,5-T and silvex, effective and widely used woody plbiological warfare weapons (28). Throughout the war, United States Army research on the phenoxy herbicides was done in secrecy until January 3, 1946 when Secretary of War, R. P. Patterson, announced that more than 1000 different chemical agents had been tested on living plants. In May 1946, G. W. Merck (28), Director of

Commercial development of 2,4-D. Public plant scientists in the United States (22, 36) and England (5, 30) continued to do limited research with 2,4-D during World War II. Research in England stressed the development of MCPA, a herbicide similar to 2,4-D. MCPA was favored in England because of a plentiful supply of cresol extracted from coal and used to make MCPA versus a plentiful supply of phenol from oil refineries in the United States which was used to make 2,4-D.

In 1942, Zimmerman and Hitchcock (36) reported that the phenoxyacetic acids could induce seedless tomatoes and that they were potent synthetic plant hormones. Kraus and Mitchell tested 2,4-D at the University of Chicago and Beltsville, Maryland, but, because of the war, did not publish their results until 1944 (28). In June 1944, Mitchell and Hamner with the United States Department of Agriculture (USDA), Bureau of Plant Industry at Beltsville, Maryland, made the first public announcement of using 2,4-D as a herbicide that exhibited differential weed kill (22). Hamner then left the USDA and joined the Agricultural Experiment Station at Geneva, New York. In August 1944, Hamner and Tukey (17) caused considerable public interest when they reported that within 10 days after spraying field bindweed with 2,4-D, the weeds died. English researchers had worked with MCPA, 2,4-D, and other plant growth regulators during the early 1940's but delayed publishing their results until after World War II (5, 30).

Marth and Mitchell in 1944 (22) sprayed 2,4-D on a lawn infested with dandelions at Beltsville, Maryland, and achieved selective broadleaf weed control with no injury to the lawn grasses. Mitchell et al. (24) then conducted additional studies on a golf course in August and September 1944 and in October 1944 reported selective broadleaf weed control in turfgrass. Dayis in 1945 (12), with the United States Golf Association, was the first person to direct a developmental program that would

result in practical selective weed control with 2,4-D in turfgrass and lawns. Davis even sprayed 2,4-D on turfgrass of the National Capital Park Service including the White House lawn.

In 1945, workers at various state agricultural experiment stations began the first extensive field testing of 2,4-D (28). Also, the popular abbreviation, 2,4-D, first appeared in the literature in 1945. When the second annual North Central Weed Control Conference (NCWCC) workers met November 26 to 28, 1945 in St. Paul, Minnesota, Timmons indicated that data were reported from 30 cooperators with 140 experiments conducted in the United States and 36 experiments conducted in Canada (31). Kephart with the USDA chaired a panel discussion on new chemicals for weed control, and plant injury problems due to phenoxy herbicide drift were first reported (19).

Experiments in the late 1940's proved that Kraus had been right in 1941 when he first envisaged the use of plant growth regulators as herbicides (28). Military secrecy that covered the Camp Detrick research did not extend to all herbicide applications that scientists discovered. Thus, the original patent of 2,4-D and related compounds (U.S. Patent Number 2,322,761) was as plant growth regulators by John F. Lontz and assigned to E.I. du Pont de Nemours and Company dated June 29, 1943 (28). Franklin D. Jones with the American Chemical Paint Company (ACPC) filed on March 20, 1944 and received use patent 2,390,941 in December 1945 for 2,4-D as a herbicide[3]. In 1944, Mitchell paid $12.50 to ACPC and Davis paid $78.00 to Sherwin-Williams Company for a pound of 2,4-D, but the price dropped to less than $3.00 per pound in 1945 and $0.50 per pound in 1950 (12, 28). In June 1945, ACPC marketed 2,4-D under the brand name Weedone, which was the first selective, systemic herbicide produced and sold on a commercial scale. In 1945, Weedone sold so poorly that many dealers wanted to return their unsold stocks, but appropriate advertising in Better Homes (now Better Homes and Gardens) and the Reader's Digest stimulated enough sales for ACPC so that in 1946 150,000 acres were treated.[3] However, one must not overlook the tremendous impact of research by public weed scientists across the United States and Canada during 1945 and thereafter, which generated immediate farmer interest in selective weed control (12, 19, 31).

---

[3]Jones, F. D. 1964. Personal communication, from the individual who patented 2,4-D as a herbicide, to C. J. Willard, Dep. Agron., Ohio State Univ., Columbus, OH who was then editor of the journal Weeds.)

The Hamner and Tukey article about field bindweed control (17) precipitated such interest among regulatory personnel, farmers, and home owners in 2,4-D that manufacturers rushed to market the herbicide. Witman wrote [4] that the agricultural chemical industry in the UnitedStates had geared upchemical industry in the UnitedStates had geared up to merchandise 2,4-D when, " . . . the industry was thunderstruck on December 11, 1945 when U.S. Patent 2,390,941, on herbicides containing chlorophenoxyacetic acids, was issued to Franklin D. Jones and assigned to American Chemical Paint Company. However, cool-headed businessmen were able to negotiate licensing of the Jones Patent for a very small royalty fee for anyone who sought to make and sell chlorophenoxyacetic acid herbicides, and the 1946 season went forward to a spectacular success for

Sherwin-Williams Company lawyers filed a lawsuit against ACPC's patent of 2,4-D as aherbicide and the following judgement was rendered:[5] "Under this settlement, the Government and the Public are given a free license to make and use, but not to sell,compositions covered by the patents involved in the litigation. In addition, the same free license is given to theGovernment and the Public under the three other Jones' patents. Americansell,compositions covered by the patents involved in the litigation. In addition, the same free license is given to theGovernment and the Public under the three

other Jones' patents. American Chemical PaintCompany, Ambler, Pa., will, upon request grant licenses to any responsible party desiring tomanufacture and sell the patented compositions. The royalty to be charged has been fixed at amaximum of 2% of the net sale and price of patented compositions, with a minimum amountroyalty of $250 and a maximum total royalty for the lives of the patents of $10,000." Obviously,

---

[4] Witman, E. D. 1978. Personal communication from a product development specialistwith PPG Industries, Inc. to J. H. Dawson, Res. Center, Washington Agric. Stn., Prosser,WA who was then editor of the WSSA Newsletter.

[5] Press release. 1947. Announcement of the settlement on October 24, 1947 of Sherwin-Williams' litigation against the American Chemical Paint Company patent of 2,4-D as aherbicide.

USDA officials also recognized the importance of 2,4-D and ordered human toxicity studies in 1945 and all of these proved negative. Mitchell et al. (25) reported that treating pastures with twice the normal use rates of 2,4-D produced no toxic effects in sheep and cows grazing on them, and feeding a cow 5 1/2 grams of pure 2,4-D per day for 3 months produced no ill effects to the cow or her calf fed entirely on milk from that cow. Kraus even announced that he had personally eaten one-half gram of 2,4-D per day for 3 weeks with absolutely no ill effects (19).

Several 2,4-D esters with low volatility were patented (U.S. Patent Numbers 2,523,227 and 2,523,228) in 1950 by W.H. Mullison and assigned to Dow Chemical Company. The butoxyethyl ester of 2,4-D with low volatility was patented (U.S. Patent Number 2,543,397) in 1951 by W. W. Allen and assigned to Union Carbide.

The first year of widespread testing and sale of 2,4-D in the United States was 1945 and 917,000 pounds were produced (28). Production rose to 5,466,000 pounds in 1946 and 14, 36, and 54 million pounds in 1950, 1960, and 1964, respectively. At present, the annual production of 2,4-D for use in the United States is about 47 million pounds (10, 16), so 2,4-D is still one of the more widely used herbicides in the United States and also worldwide. This continued demand for 2,4-D shows that it has markedly advanced humankind's utopian dream of lifting the hoe from the farmer's hand and reducing much of the drudgery associated with crop production.

Table of Contents

DEVELOPMENT OF THE WEED SCIENCE DISCIPLINE

Weed science is one of the younger academic disciplines in agriculture, first receiving significant recognition in the United States in the mid-1940's because of the introduction of the phenoxy herbicides (9, 20). Research in the early 1940's on 2,4-D in the United States and MCPA in England, their release after World War II, and their rapid acceptance stimulated selective weed control research and education worldwide. Extensive research on mechanical and cultural weed management before 1945 should have brought recognition to the discipline of weed science, but these efforts were generally identified as part of agronomic practices or crop production systems. Leighty (21), for example, wrote in the 1938 Yearbook of Agriculture that, "Weeds, aside from the noxious species, are not a serious problem on a well-organized diversified farm. Indeed, there is little excuse for such a farm to be weedy." And he goes on to say, "In some kinds of one-crop farming, weeds, while plentiful, do not prevent the production of good yields." Furthermore, even though the major benefit of tillage is weed control, many farmers tended to identify cultivation benefits as coming from tillage per se. Thus, many early agricultural scientists did not give adequate recognition to weeds and the

importance of their control until the advent of the phenoxy herbicides (9, 18).

Weed control results from 2,4-D and subsequent herbicides were so spectacular that weed workers concentrated their efforts on chemical control of weeds and this shift impeded research and education on alternative weed management technology (9, 34, 35). Also, this shift in weed research, development, education, and marketing efforts over the past five decades has impacted all areas of weed science.

The objectives of this section are: (a) to enumerate the economic importance of weeds and the extent of herbicide use, and (b) to discuss the impact of 2,4-D on the development of the herbicide industry, regulatory agencies, farmers, and publicly-funded weed scientists.

Monetary losses from weeds. In the early 1960's, USDA scientists estimated the relative proportion of total losses in productivity of United States agriculture from insect pests to be 10%, soil erosion 13%, livestock diseases 17%, plant pathogens 26%, and weeds 34% (3). In addition, farmer costs were about three times higher for controlling weeds than for controlling plant pathogens and insect pests combined. In 1971, after substantial use of pesticides for controlling pests (various biological organisms that reduce crop yields or quality) in agriculture, USDA scientists estimated annual United States losses to crop pests as follows: nematodes 3%, plant pathogens 27%, insect pests 28%, and weeds 42% of total pest losses in crop production (4). These relative loss comparisons probably have not changed greatly in the past two decades. Annual weed losses (mainly reduced crop yields and increased costs of control) in the United States were estimated to be over $20 billion in 1993[6]. Thus, weeds remain an important impediment to crop production because they are omnipresent, they reduce crop yields, and weed control often constitutes the major cost of producing a crop even though we have eliminated much of our reliance on manual weed control. It must be recognized, however, that crop losses from weeds, costs of control, and crop production costs in general would be much greater if farmers were forced to revert to crop management methods used prior to 1945.

Herbicide costs and usage. In 1991, pesticides were a $26.8 billion industry worldwide with sales totaling $6.4 billion in the United States or 24% of worldwide pesticide sales (23). Herbicides represented about 65% of total pesticides used in the United States in 1990 and 85% of pesticides used on cropland (13). Herbicide use on crops in the United States grew from 115 million pounds in 1966 to a peak of 500 million pounds in 1982 when over 90% of the acreage of major crops was treated with one or more herbicides (15). Since 1982, total pounds of herbicides used has decreased about 10% because of the introduction of compounds that are active at lower rates, reductions in use rates of older herbicides, and government sponsored acreage diversion programs (9). The phenoxy herbicides still represent about 10% of total herbicide usage in the United States.

---

[6] Bridges, D. C. 1993. Personal communication from the chairman of the Weed Science Society of America, Weed Loss Committee, to O. C. Burnside, Dep. Agron. and Plant Genet., Univ. Minnesota, St. Paul, MN.)

Impact of 2,4-D on the herbicide industry. The phenoxy herbicides stimulated chemical industry involvement in herbicide development because industry personnel recognized the weed control need in agriculture and noncropland and the profit potential of this technology. The spectacular results with 2,4-D stimulated an industry-wide search for additional herbicides, which ushered in the herbicide era that has lasted for five decades. Total numbers of hedevelopment because industry personnel recognized the weed control need in agriculture and noncropland and the profit potential of this technology. The spectacular results with 2,4-D stimulated an industry-wide search for additional

herbicides, which ushered in the herbicide era that has lasted for five decades. Total numbers of herbicides available in the United States as listed by Timmons (32) or in Weed Science Society of America journals (9) were 14 in 1940, 25 in 1950, 61 in 1960, 131 in 1970, 156 in 1980, and 145 in 1990. Thus, industry rapidly expanded personnel in herbicide development, manufacture, and sales in order to exploit the market potential of herbicides that increased rapidly from 1945 until about 1982 when herbicides use peaked in the United States (15). Since 1982 there has been increased competition among chemical companies because the herbicide market in the United States had matured and profits were decreasing as companies competed for the same market share rather than exploiting mainly untapped markets. T[7] projects that this consolidation may continue until only six full-service pesticide companies (synthesis of pesticides on through to sales) will remain worldwide.

---

[7]Carpenter, W. D. 1995. Personal communication from the retired Vice President andGeneral Manager, New Products Division, Monsanto Company, St. Louis, MO to O. C. Burnside, Dep. Agron. and Plant Genetics, Univ. Minnesota, St. Paul, MN.)

Impact of 2,4-D on the regulatory agencies. The phenoxy herbicides were vigorously promoted by personnel of various weed control regulatory agencies that were already in place when 2,4-D was first sold in 1945. For example, the Connecticut legislators enacted in 1726 a European barberry control law which was the initial United States program to aid in the control of black stem rust of wheat, but a more effective barberry control law was enacted in 1917 that encompassed 13 North Central and Western States (32). Federal and states' Ribes spp. Quarantine and Eradication Laws aimed at the control of white pine blister rust were initiated during 1912 to 1919 in 26 states. In 1914 Virginia passed a law requiring the eradication of red cedar as an aid in the control of apple rust, and similar legislation was also enacted in four other states. Numerous States and Canadian Provinces enacted Seed Laws beginning in 1821 in Connecticut and Noxious Weed Control Laws beginning in 1872 in Minnesota, and these regulatory personnel immediately recognized the importance of 2,4-D in carrying out their legislative mandates (32). However, 2,4-D did not turn out to be the panacea weed control herbicide that it was advertised to be, and thus no noxious weeds were eradicated (2).

Laws aimed at the prevention or spread of undesirable weeds such as Seed laws have been moderately effective in reducing the spread of weeds, Weed Control Laws more often than not have been ineffective, and Eradication Laws have been ineffective because eradication of a weedy species from a large area has proven to be an impossible objective (8). Thus, the impact of regulatory efforts in weed management and financial support of these regulatory programs has eroded over the past several decades. However, regulation of pesticides by the EPA and similar state agencies has increased over the past several decades.

Impact of 2,4-D on the farmer. The phenoxy herbicides raised weed management to a higher level, and producers accepted this new technology rapidly and with enthusiasm, because they were able to achieve better weed control with less labor and expense (20, 31, 32). Phenoxy herbicides provided effective, economical, and selective broadleaf weed control in small grains, corn, sorghum, pastureland, rangeland, and turfgrass.

I remember when farmers commonly lost crops to weeds such as quackgrass, Canada thistle, or wild mustard. Now such a farmer would be considered a poor steward of the land and would be the topic of conversation at the local coffee shop. I also remember having to get off the tractor and remove wild mustard plants, or they would clog my father's small grain binder. Then, 2,4-D was introduced, and we were all amazed at its ability to selectively control wild mustard in small grains at a very

economical cost (see picture on the cover page).

Farmers began expecting selective herbicides for numerous other cropping systems, and the agricultural chemical industry responded positively because of the profit potential. Herbicides such as 2,4-D and MCPA established the concept of postemergence, selective weed control; chloramben and atrazine established preemergence, selective weed control; and EPTC and trifluralin established preplant-soil-incorporated, selective weed control. Examples of changes in herbicide technology over the past five decades are inorganic to organic herbicides, nonselective to selective herbicides, postemergence to soil-applied (some incorporated) herbicides and now back again, mechanical to more chemical control of weeds, single herbicides to mixtures and even multiple applications, formulated herbicides used alone to utilizing various herbicide additives, and crops bred or transformed for tolerance to specific herbicides rather than herbicides being screened for crop tolerance (9).

The American farmer relies heavily on herbicides as the major method of weed control, and herbicides allow them to farm more and more acres (9, 14, 35). Herbicide use and farm equipment development has resulted in substantial decreases in number of farmers and farm laborers required for crop production in the United States (26). Adequate alternative methods of weed control are not currently available in the event of future restrictions on herbicide usage that may result because of public concern about herbicides in ground and surface water, toxicity to humans, chronic health effects, food safety, and wildlife mortality (9). Operators of large acreage farms will continue to demand more and better selective herbicides; whereas, an increasing group of organic and smaller acreage farmers are looking for alternative and more economical weed management methods (34, 35).

Impact of 2,4-D on publicly supported weed scientists. The phenoxy herbicides had such an impact on weed technology that most publicly-supported weed scientists became engrossed in chemical weed control research and education endeavors (9, 34). Thus, weed control methods have changed because weed scientists slighted or abandoned alternative weed management technology. We have witnessed five decades in which most of the weed science research in the United States has had some connection with herbicides. However, this herbicide era has markedly advanced our ability to manage weeds in cropland and noncropland, and greatly reduced labor requirements and crop losses in agricultural production.

University and USDA-Agricultural Research Service (ARS) administrators began hiring weed scientists after the advent of 2,4-D, but employment numbers never reflected the fact that weeds were a greater production hazard than either plant diseases or insect pests (2, 9, 27). Possibly administrators rationalized that the herbicide industry would support or supply many of the weed science research and educational needs. For example, federal funding for pest management research at universities in fiscal year 1991 was $61 million for Entomology, $50 million for Plant Pathology, $9 million for Nematology, and $6 million for Weed Science (27). In 1995, Ogg (27) contacted the business managers of the Entomological Society of America to learn that they had 8100 members and 6075 were publicly-funded, the American Phytopathological Society had 5000 members and 4250 were publicly-funded, and the Weed Science Society of America had 2200 members and 550 were publicly-funded. Such public-funding and hiring practices forced the few publicly-funded weed scientists to conduct research and education activities in the areas where they could make the most rapid progress and receive industry support, i.e., herbicide technology.

Is it any wonder that recent assessments of weed management technology (9, 14, 34, 35) have shown that farmers place an overwhelming emphasis on chemical weed control technology? If public weed

scientists do not conduct preventive, mechanical, cultural, biological, and integrated weed management research, it will not be done. We coverwhelming emphasis on chemical weed control technology? If public weed scientists do not conduct preventive, mechanical, cultural, biological, and integrated weed management research, it will not be done. We cannot expect to entice large numbers of chemical company weed scientists into researching alternative weed control technology. They must research, develop, and sell marketable products that return a profit or there will be no herbicide industry (9). Most alternative weed management methods do not have the profit potential to merit private-sect

FUTURE TRENDS IN WEED SCIENCE

One can predict that weed science will continue to show modest growth in public research, education, and herbicide regulation areas, as well as in the area of private consulting; however, the number of weed scientists within the chemical industry will decrease because of continuing consolidation of pesticide companies (9). Academic employment opportunities should increase for those trained in the areas of weed biology, ecology, and management. Weed control activities will move from the largely chemical approach to systems involving greater use of preventive, cultural, mechanical, biological, and integrated weed management activities. Herbicides will continue to be important in weed management, but they will be used more selectively and judiciously. The use of postemergence herbicides may increase as they will be used to "back up" alternative weed control methods so that farmers do not experience crop yield loss while initiating the use of new weed management technology. However, public weed scientists must redirect their activities after five decades of largely herbicide-focused research and undertake a major change in weed research objectives to develop basic information about weeds and problem solving research using integrated weed management systems (9, 34, 35).

If there is one thing that I have learned from 35 years of weed research, it is that there is no panacea weed control method. Thus, one must emphasize an integrated approach to weed management, as certain weed species have survived all individual weed control methods that humankind has so far devised. Greater use of on-farm research may aid in developing the needed weed management technology. Public weed scientists have exciting years ahead as they emphasize research and education directed toward weed biology and life histories, weed seedbanks, seed and bud dormancy, cover and smother crops, rotation of crops with different life cycles, weed-competitive crops, biological and ecological weed management, postemergence herbicide technology, precision herbicide applications that vary rates or herbicides based on soil type and climate and weed species, environmentally benign herbicides or mycoherbicides, spray equipment activated by the presence of weeds, interference modeling of crop-weed systems, reduced-tillage crop production systems, and the destruction or inactivation of weed propagules to develop integrated, sustainable, and environmentally safe weed management methods.

We should not abandon herbicides, but we can use them more judiciously (9). We need to conserve natural resources such as soil and fossil fuels while increasing the productivity, profitability, and competitiveness of agriculture in the United States. Also, a major challenge for us in the future is to optimize weed management systems to meet or exceed the water quality and environmental impact standards demanded by the public. Implementing alternative weed control methods, not more monitoring programs, is the way to solve problems of pesticide contamination of water or the environment. To accomplish these objectives, an increase in public funding for the development of weed science as a discipline must occur because development of alternative weed management methods will not attract substantial private funding.

Now weed scientists must settle down and attend to research and educational endeavors that have been put on the "back burner," while continuing to pursue the judicious use of herbicides. The future will be just as exciting as the past, as weeds do not give up their turf easily.

• Table of Contents

LITERATURE CITED

1. Ahrens, W. H., ed. 1994. Herbicide Handbook. Weed Sci. Soc. America, 1508 West Univ. Ave., Champaign, IL. 352 p.

2. Andersen, R. N. 1991. The North Central Weed Control Conference: Origin and Evolution. North Central Weed Sci. Soc., Champaign, IL. 206 p.

3. Anonymous. 1965. Losses in Agriculture. U.S. Dep. Agric., Washington, D.C. Handbook No. 291. 120 p.

4. Anonymous. 1971. The Economics of Agricultural Pest Control. USDA Pesticide Review, Econ. and Stat., Washington, D.C. Report No. 14. 60 p.

5. Blackman, G. E. 1945. A comparison of certain plant growth substances with other selective herbicides. Nature 155:500-501.

6. Bolley, H. L. 1908. Weed control by means of chemical sprays. North Dakota Agric. Exp. Stn. Bull., Fargo, ND. 80:541-574.

7. Bovey, R. W. and A. L. Young. 1980. Military uses of herbicides. pp. 371-403. in Bovey, R.W. and A.L. Young, eds. The Science of 2,4,5-T and Associated Phenoxy Herbicides. John Wiley and Sons, New York, NY.

8. Burnside, O. C. 1970. Progress and potential for nonherbicidal control of weeds through preventive weed control. pp. 464-483. in Holstun, J.T., ed. FAO International Conf. on Weed Control, Weed Sci. Soc. America, Champaign, IL.

9. Burnside, O. C. 1993. Weed science -- the step child. Weed Technol. 7:515-518.

10. Burnside, O. C., R. W. Bovey, C. L. Elmore, E. L. Knake, C. A. Lembi, J. D. Nalewaja, M. Newton, and P. Szmedra. 1996. Biologic and economic assessment of benefits from use of phenoxy herbicides in the United States. WSSA Abstr. 36:36.

11. Davis, D. E. 1979. Herbicides in peace and war. BioScience 29(2):84, 91-94.

12. Davis, F. F. 1945. What's been observed in the tests of 2,4-D on weeds. Golfdom 19:11- 17.

13. Delvo, H. W., compiler. 1990. Agricultural Resources - Inputs Situation and Outlook Report. Resour. Technol. Div., Econ. Res. Serv., U.S. Dep. Agric., Washington, D.C. AR-17. 59 p.

14. Duke, S. O. 1992. Weed science -- the need and the reality. Phytoparasitica 20:183-186.

15. Gianessi, L. P. 1992. U.S. Pesticide Use Trends: 1966-1989. Quality of the Environment Division, Resources for the Future, Washington, D.C. 55 p.

16. Hammond, L. E. 1995. New perspectives on an essential product: 2,4-D. Down to Earth. 50(2):1-5.

17. Hamner, C. L. and H. B. Tukey. 1944. The herbicidal action of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid on bindweed. Science 100:154-155.

18. Holm, L. 1975. The role of weeds in world food production. Proc. North Central Weed Control Conf. 32:18-24.

19. Kephart, L. W. 1945. Panel discussion on new chemicals for weed control. Proc. North Central Weed Control Conf. 2:68-75.

20. Klingman, G. C., F. M. Ashton, and L. J. Noordhoff. 1982. Weed Science: Principles and Practices. John Wiley & Sons, New York, NY. 449 p.

21. Leighty, C. E. 1938. Crop rotation. pp. 406-430. in Knight, H.G., ed. Soils and Men, 1938 Yearbook of Agric. U.S. Dep. Agric., Washington, D.C.

22. Marth, P. C. and J. W. Mitchell. 1944. 2,4-Dichlorophenoxyacetic acid as a differential herbicide. Bot. Gaz. 106:224-232.

23. McDougall, J. and M. Phillips. 1992. Agrochemical Overview 1991. County Natwest Woodmac, Kintore House, 74-77 Queen St., Edinburgh, Scotland. 5 p.

24. Mitchell, J. W., F. F. Davis, and P. G. Marth. 1944. Turf weed control with plant growth regulators. Golfdom 18:34-36.

25. Mitchell, J. W., R. E. Hodgson, and C. F. Gaetzens. 1946. Tolerance of farm animals to food containing 2,4-dichlorophenoxyacetic acid. J. An. Sci. 5(2):226-232.

26. Nalewaja, J. D. 1974. Energy requirements for various weed control practices. Proc. North Central Weed Control Conf. 29:19-23.

27. Ogg, A. G. 1995. Expanding the Weed Science Society of America beyond weed science. Weed Technol. 9:406-408.

28. Peterson, G. E. 1967. The discovery and development of 2,4-D. Agric. Hist. 41:243- 253.

29. Pokorny, R. 1941. Some chlorophenoxyacetic acids. J. Amer. Chem. Soc. 63:1768.

30. Slade, R. C., W. G. Templeman, and W. A. Sexton. 1945. Plant growth substances as selective weedkillers. Nature 155:497-498.

31. Timmons, F. L. 1945. Tuesday morning session opening remarks. Proc. North Central Weed Control Conf. 2:16-19.

32. Timmons, F. L. 1970. A history of weed control in the United States and Canada. Weed Sci. 18:294-307.

33. Went, F. W. and K. V. Thimann. 1937. Phytohormones. The Macmillan Co., New York, NY. 294 p.

34. Wyse, D. L. 1992. Future of weed science research. Weed Technol. 6:162-165.

35. Zimdahl, R. L. 1991. Weed Science -- A Plea for Thought. U.S. Dep. Agric., Coop. State Res. Serv., Washington, D.C. Symposium Preprint, 34 p.

36. Zimmerman, P. W. and A. E. Hitchcock. 1942. Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. Contr. Boyce Thompson Inst. 12:321-344.

37. Zimmerman, P. W. and F. Wilcoxson. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Contr. Boyce Thompson Inst. 7:209- 229.

Table 1. Chronology of selected developments in the history of phenoxy herbicides (generally 2,4-D) mainly in the United States but also worldwide.a

Year	Individuals or groups	Development
1880	Darwin	Studied tropisms in plants (33).
1908	Bolley, Bonnet, and Schultz	Selectively controlled broadleaf weeds in small grains with copper salts (20).
1911	Boysen-Jensen	Documented chemical control of tropisms in plants (33).
1926	Went	Measured quantitatively a natural plant growth regulator (33).
1934	Kögl and Haagen-Smit	Identified IAA as the natural plant growth regulator (33).
1935	Zimmerman and Wilcoxson	Discovered PAA and NAA to be plant growth regulators (36).
1940	Pokorny	Synthesized 2,4-D and 2,4,5-T (29).
1941	Kraus	Proposed that growth regulators might work as herbicides (28).
1942	Zimmerman and Hitchcock	Found 2,4-D to be the most active of the plant growth regulators (36).
1942	Stimson	Initiated biological warfare research in the U.S. with crop killing herbicides (28).
1942	U.S. Army	Established Camp Detrick for biological warfare research (28).
1943	Lontz, DuPont Co.	Patented 2,4-D as a plant growth regulator (28).
1944	Mitchell and Hamner	Made the first public announcement that 2,4-D was a selective herbicide (22, 28).
1944	Marth and Mitchell	Selectively controlled dandelions in turfgrass with 2,4-D (22).
1944	Hamner and Tukey	Selectively controlled field bindweed with 2,4-D (17).
1945	NCWCC scientists	Used the name "2,4-D" in the literature for the first time (28).
1945	ACP Co.	Were the first to market 2,4-D as a selective herbicide (28).
1945	Davis	Used 2,4-D first for commercial control of weeds in turfgrass (12).
1945	NCWCC scientists	Conducted extensive research with 2,4-D as a selective herbicide (19, 31).
1945	Mitchell, Hodgson, and Gaetzens	Reported that animal toxicity studies with 2,4-D were negative (25).

1945	Kraus	Ate 1/2 gram of 2,4-D per day for 3 weeks with no ill effects (19).
1945	Kephart	Chaired a NCWCC symposium on 2,4-D in which drift damage was reported (19).
1945	Jones, ACP Co.	Patented 2,4-D as a selective herbicide (28).
1946	Patterson	Reported that the U.S. Army had screened over 1000 chemicals for their crop killing potential (28).
1946	U.S. Army scientists	Reported on their herbicide research at Camp Dietrick, filling the entire June issue of the Botanical Gazette (28).
1947	Sherwin-Williams Co.	Completed litigation against ACP's 2,4-D patent (28).
1950	Mullison, Dow Co.	Patented several low volatile 2,4-D esters (28).
1951	Allen, Union Carbide Co.	Patented butoxyethyl ester of 2,4-D (28).
1962	U.S. Army	Sprayed Agent Orange as a defoliant in Vietnam (7, 11).
1970	USDA-ERS	Estimated that restricting phenoxy herbicide use would cost U.S. farmers $290 million annually (4).
1979	Ruckelshaus, EPA	Suspended all uses of 2,4,5-T and silvex in the U.S. (7, 11).
1985	U.S. District Court, Washington, DC	Banned all use of 2,4,5-T and silvex in the U.S. (7, 11).
1988	Agriculture Canada	Reported that banning phenoxy herbicides would cost Canadian growers $420 to $488 million annually (16).
1990	EPA	Established the Health Advisory Level of 70 ppb of 2,4-D in potable water (16).
1992, 1994	EPA	Received reports from Advisory Committees of toxicologists indicating that 2,4-D was not a carcinogen (16).
1995	Industry Task Force II	Submitted the 2,4-D risk assessment re-registration data requested by EPA (16).
1996	NAPIAP	Presented a progress report of the phenoxy herbicide benefits assessment (10).

[a]Abbreviations used were 2,4-D [(2,4-dichlorophenoxy)acetic acid], IAA (indoleacetic acid), PAA (phenylacetic acid), NAA (naphthylacetic acid), 2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid], NCWCC (North Central Weed Control Conference), ACP (American Chemical Paint), USDA (United States Department of Agriculture), ERS (Economic Research Service), ppb (parts per billion), EPA (Environmental Protection Agency), and NAPIAP (National Agricultural Pesticide Impact Assessment Program).

Chapter 3

Risk Assessment of Phenoxy Herbicides:

An Overview of the Epidemiology and Toxicology Data

REBECCA A. JOHNSON and ELIZABETH V. WATTENBERG[1]

[1]Assist. Profs., Div. Environ. & Occup. Health, School Public Health, Univ. Minnesota, Minneapolis, MN 55455.

Table of Contents

Abstract. Despite the societal benefits of phenoxy herbicides, results from a series of epidemiologic studies have raised questions about possible human health risks associated with their use. However, the results from these studies have been inconsistent and may have been due to errors in the reporting of pesticide exposures. Recent methodologic research indicates that pesticide exposures may be reported inaccurately to such a degree that it may not be possible to interpret the results from epidemiologic studies where these data have been provided by study participants. Therefore, the evidence from such epidemiologic studies should not be given much weight when determining whether there is a causal association between cancer and phenoxy herbicides in general, or between cancer and 2,4-D specifically. Thus, the only conclusion that can be drawn from the previously conducted epidemiologic studies is that the data are insufficient to assess the carcinogenicity of phenoxy herbicides, or 2,4-D, in humans. Recent toxicologic research indicates that 2,4-D alone is not carcinogenic and suggests no known mechanism by which it could be carcinogenic. Rarely are chemicals carcinogenic in humans, but not in animals. Furthermore, a review of noncarcinogenic effects suggests that individuals who are exposed to 0.01 mg/kg/day of 2,4-D over their lifetimes would not experience an appreciable risk of toxic effects. Based upon data from exposure studies, the general public is exposed to levels of 2,4-D that are below this reference dose; therefore, the general public should not be at risk of adverse health effects due to 2,4-D exposure. Occupational exposures, however, may exceed the reference dose. To reduce risk, workers should use appropriate protective equipment.

INTRODUCTION

The societal benefits associated with use of phenoxy herbicides have been described in other chapters. Despite these benefits, possible risks also must be considered in the re-registration of phenoxy herbicides. In this context, risk is defined as the likelihood that some adverse effect to humans will occur (69). In general, pesticides may be thought to pose a certain amount of risk because: (a) most pesticides are synthetic chemicals, which are deliberately released into the environment; (b) pesticides are specifically designed to harm or kill living things, and they have the potential to be toxic to nontarget organisms, including humans; and (c) many humans may be exposed to pesticides, either occupationally, or from nonoccupational sources (47, 69). However, the degree of risk or lack thereof varies with the specific pesticide, as well as with the frequency, intensity, and duration of exposure (69).

Initially, much of the concern about pesticides was directed at adverse ecological effects. During the last few decades, however, the focus of concern has shifted from the environment to human health

(69). The purpose of this chapter is to review the epidemiology and toxicology data relating to phenoxy herbicides, or more specifically, 2,4-D.

EPIDEMIOLOGY

Epidemiology background. Epidemiology is the study of the occurrence of, and factors related to, states of health and disease in human populations. The potential for pesticide exposures to cause cancer has been the foremost human health effect of concern; therefore, this section of the review will focus on those epidemiologic studies that have evaluated phenoxy herbicides in relation to cancer. In the past decade, a number of reviews of the epidemiologic data relating to pesticides and cancer have been published (2, 4, 5, 7, 8, 10, 11, 12, 16, 20, 35, 38, 42, 48, 49, 55, 62). Thus, the summary of the epidemiologic literature presented here is, in part, drawn from these reviews. Furthermore, this review is not intended to be comprehensive, but instead, it includes select articles that reflect on the cancer risks associated with human exposure to 2,4-D or other phenoxy herbicides. In addition, the key methodologic issues surrounding these epidemiologic studies are discussed, as well as the results of epidemiologic studies designed to investigate these issues.

Study designs and risk estimates. In the late 1970's, the first analytic epidemiologic investigations of phenoxy herbicides and cancer were begun. Prior to that time, only descriptive studies had been done and these typically provide less useful information.

The analytic epidemiologic studies conducted have examined either specific types of cancer (i.e., case-control studies) or specific exposed populations (i.e., cohort studies). In these case-control studies, people who have a specific cancer (i.e., cases) and people who do not have the cancer (i.e., controls) are identified. Then, information about prior exposure to a suspected carcinogen is obtained for both the cases and controls. The odds of exposure among the cases is compared to the odds of exposure among the controls, and this risk estimate is called the odds ratio (OR). The way to interpret the value of the OR is as follows: (a) if the OR equals 1.0, the cases and controls have been exposed equally; (b) if the OR is less than 1.0, the controls have been exposed more than the cases; and (c) if the OR is greater than 1.0, the cases have been exposed more than the controls. If exposure is more common among the cases than the controls, the exposure may be associated with the occurrence of the cancer.

In a cohort study, groups of individuals with and without exposure are followed over time. The incidence of disease in the exposed group is compared to the incidence of disease in the unexposed group and this risk estimate is called the relative risk (RR). The way to interpret the value of the RR is as follows: (a) if the RR equals 1.0, the incidence of disease is equal among the exposed and unexposed; (b) if the RR is less than 1.0, the incidence of disease is higher for the unexposed than for the exposed; and (c) if the RR is greater than 1.0, the incidence of disease is higher for the exposed than for the unexposed. If the incidence of disease is more common among exposed persons than among those who are unexposed, the exposure may be associated with the occurrence of the disease. In addition, the OR and RR usually are provided with an interval that shows the variability in the risk estimate and specifies the probability that the interval includes the true risk. This interval is called the confidence interval (CI) and it is used to help determine whether the risk estimate is meaningfully different from 1.0. The risk estimate is said to be significantly different from 1.0 if the CI does not include 1.0.

Summaries of the case-control and cohort studies are presented separately below. The case-control studies are discussed first because findings from the early case-control studies prompted much of the epidemiologic research on phenoxy herbicides, including 2,4-D.

In the summaries and tables, reference is made to the following exposures: "pesticides," "herbicides," "phenoxy herbicides," and "2,4-D." These terms are presented as such to accurately reflect how the questions were asked in the epidemiologic studies. For example, in a particular study, participants may have been asked, "Did you ever use herbicides?" and "Did you ever use 2,4-D?"

Case-control studies. The majority of case-control studies have focused on the following types of cancer: (a) soft-tissue sarcomas (STS), (b) Hodgkin's disease (HD), and (c) non-Hodgkin's lymphoma (NHL). In Sweden, a clinical observation (28) of a number of patients with STS and prior exposure to phenoxy herbicides led to the first case-control study (30). In this case-control study in northern Sweden, a fivefold increase in risk for STS (OR = 5.3; 95% CI = 2.4 to 11.5) was observed for exposure to phenoxy herbicides. However, 2,4,5-T was frequently used by those who were exposed, and this phenoxy herbicide has been found to contain 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) as a contaminant. Another case-control study subsequently was undertaken in southern Sweden to try to confirm these findings (25). In southern Sweden, commonly used phenoxy herbicides included 2,4-D, MCPA, and others thought to be free of 2,3,7,8-TCDD. The risk for STS associated with exposure to any phenoxy herbicides found in this second Swedish study was nearly sevenfold (OR = 6.8; 95% CI = 2.6 to 17.3). A fourfold risk (OR = 4.2; CI not provided) was reported for exposure to phenoxy herbicides not contaminated by 2,3,7,8-TCDD.

Around the same time, a number of patients with malignant lymphoma and previous exposure to phenoxy herbicides was reported in Sweden (29). Once again, a case-control study was performed to see if there was a relation between this type of cancer and exposure to these herbicides (32). The results of this case-control study of malignant lymphoma showed almost a fivefold increase in risk (OR = 4.8; 95% CI = 2.9 to 8.1) with exposure to phenoxy herbicides. Separate results were not provided for HD and NHL, but were reported to be similar.

Due to the substantial risks observed in the early Swedish studies and the potential for widespread exposure to phenoxy herbicides, a number of case-control interview studies of STS, HD, and NHL followed in Sweden, New Zealand, Italy, United States, and Australia (21, 31, 33, 34, 53, 54, 56, 58, 59, 60, 61, 65, 70, 71, 74). The basic design features and results of these studies are summarized in Table 1 and are not discussed further.

Despite the strong associations observed by Swedish researchers, almost all of the other studies conducted elsewhere have failed to confirm these findings. In Kansas, Hoar et al. (34) found a significant association with NHL and use of phenoxy herbicides and 2,4-D. In addition, the risk of NHL significantly increased with the number of days of herbicide exposure per year. OR's of 1.4, 1.6, 2.6, and 6.0 were found for using herbicides for 1 to 5, 6 to 10, 11 to 20, and more than 20 days per year, respectively. Frequency of use data were not collected for specific pesticides such as 2,4-D. In Nebraska, this same researcher and colleagues (74) observed a nonsignificant elevation in risk for NHL attributable to 2,4-D. In addition, an association was suggested between increasing days of 2,4-D exposure per year and risk of NHL.

Cohort studies. Cohort studies that have examined cancer risk and phenoxy herbicides have included those persons potentially exposed during manufacturing or application. STS, HD, and NHL are relatively rare cancers and are believed to occur many years after initial exposure to a carcinogen; therefore, most of the cohort studies conducted to date have not had either a sufficiently large cohort or an adequate number of years of follow-up to be informative. The relevant cohort studies (14, 15, 24, 43, 44, 45, 52, 66, 67, 68) are summarized in Table 2 and are not described further. The findings from the cohort studies do not support those of the Swedish case-control studies.

Methodologic issues. Epidemiology is fraught with inconsistent studies; however, it is unusual for risk estimates to vary as greatly as those observed in the case-control studies of STS, HD, and NHL and phenoxy herbicides. Several papers (6, 7, 8, 9, 12, 13, 50) have been published about methodologic issues that may explain the inconsistent case-control study findings. Error in the measurement of exposure has been suggested as one of the possibleexplanations and continues to be the primary methodologic issue of concern (9).

Overview of exposure measurement concepts. Error in the measurement of exposure, also called exposure misclassification, is the difference between the measured exposure and the true exposure. Although exposure misclassification occurs to some extent in almost all epidemiologic studies, it is particularly problematic because it leads to bias in the risk estimate. This type of bias is called misclassification or information bias. Differential misclassification occurs when the exposure measurement error differs according to disease status; nondifferential misclassification is independent of disease status. Nondifferential misclassification usually results in an underestimate of the true risk. Differential misclassification can either underestimate or overestimate the risk and can make it impossible to draw any conclusions from the study results. Case-response or recall bias is a form of differential misclassification of exposure in which cases report exposures differently than controls. Compared to controls, cases may better recall or falsely report exposures in an attempt to identify what may have caused their disease (3).

Information about the validity and reliability of measured exposure data is needed to determine the extent and direction of exposure misclassification. Validity refers to the accuracy of a measure (i.e., whether it correctly measures the true exposure) and reliability refers to the reproducibility of a measure (i.e., whether it consistently provides the same results on two or more occasions). Long-term reliability refers to the reproducibility obtained when the measurements are made a long time apart and short-term reliability refers to the reproducibility obtained when the measurements are made a short time apart. Reliability is only important for what it reveals about validity. If an exposure measure is not reliable, it cannot be valid. A reliable exposure measure, however, does not imply a valid measure. Therefore, it is preferable to evaluate the validity of an exposure measure. In order to measure the validity of an exposure measure, however, the true exposure must be known. A measure of the true exposure usually is not available or is not practical to use; otherwise, this perfect measure would be used instead of the less accurate one. As a result, the "validity" of an exposure measure often refers to how the measure under study compares with a measure that is deemed to be the most accurate (i.e., a "gold standard") (3).

Percentages of agreement often are calculated to assess the reliability of an exposure measure. Sensitivity and specificity often are calculated to assess the validity of an exposure measure. Sensitivity is calculated as the percentage of truly exposed persons who report that they have been exposed. Specificity is calculated as the percentage of truly unexposed persons who report that they have not been exposed. Ideally, a valid exposure measure will have both high sensitivity and high specificity.

A number of questions have been raised regarding the exposure assessment procedures in epidemiologic studies of pesticides and cancer. STS, HD, NHL, and other cancers of interest occur infrequently and are characterized by long cancer induction periods following exposure. Consequently, studies of these cancers most often have used a case-control design to alleviate the need for large numbers of subjects and a long follow-up period. Therefore, information about past pesticide exposures usually has been obtained by interviewing subjects, or when necessary, proxy-respondents. The accuracy of these pesticide exposure measurements is dependent on the

memory or recall of the individuals being interviewed.

The ability to recall details related to pesticide use may decrease with time; thus, the accuracy of data regarding early uses of pesticides may be most suspect. Unfortunately, these data may be the most relevant with regard to initiation of cancer (6). Recall is hindered by the fact that subjects exposed to pesticides almost always have used a number of different pesticides during their lifetimes, and the available and recommended pesticides have changed over the years (8). Recall also is difficult because most subjects use pesticides relatively infrequently, often only seasonally (8, 12, 13).

Proxy-respondents (e.g., spouses, siblings, children, and parents) are interviewed on behalf of subjects when the subjects themselves are unable to be interviewed due to death, illness, dementia, or some other reason. In case-control studies of pesticides and cancers, the cancers being studied often have high death rates; thus, by necessity, proxy-respondents must be used. However, the proxy-respondents may never have known which pesticides the subjects used. It is commonly believed this lack of knowledge would cause proxy-respondents' recall of pesticide use to be less than study subjects' recall (8, 9), but it also is possible that proxy-respondents may be prone to case-response bias.

In the Nebraska study, the risk of NHL associated with 2,4-D was higher among subjects with proxy-respondents than among self-respondents (74). For proxy-respondents, OR's of 2.2, 2.2, and 2.4 were found for handling 2,4-D for 1 to 5, 6 to 20, and more than 20 days per year, respectively. For self-respondents, the corresponding OR's were 1.0, 1.6, and 1.4. Similarly, in a case-control study of NHL and leukemia conducted in Iowa and Minnesota (18, 21), the proxy-respondent OR's for use of 2,4-D for 1 to 4, 5 to 9, and more than 10 days per year were 0.8, 1.0, and 2.5, respectively (51). The self-respondent OR's for the same categories of use were 0.5, 0.2, and 0.7. Thus, in the case-control studies in Nebraska and in Iowa-Minnesota, there was evidence that case-response bias may have occurred.

Methodologic studies. The issues noted above illustrate the difficulties associated with retrospectively obtaining information about pesticide exposures and the need to assess the reliability and validity of reported use of pesticides. Given the complexity of pesticide exposures and the controversy surrounding the inconsistent findings from case-control studies of pesticides and cancer, it is remarkable how little methodologic research has been conducted in this area (17, 19, 30, 34, 39, 40, 70). Such research may not have been undertaken because it has been assumed that pesticide exposure misclassification always would reduce estimates of risk (i.e., that the misclassification is nondifferential) or because of the resources required. The few studies that have been done are summarized in Table 3 and described briefly below.

In the first case-control study of STS and pesticides conducted in Sweden, 50 employers were mailed a questionnaire to verify employment and use of phenoxy herbicides or chlorophenols reported by study subjects (30). Only a small proportion (40%) of the employers responded. Due to a lack of records regarding exposures to these chemicals, responses received mainly were based on employers' recall. The authors stated that the results obtained were uncertain and difficult to interpret. Despite these limitations, the authors concluded that the reported use of phenoxy herbicides probably was reliable because a high degree of agreement was observed for use of chlorophenols in sawmills or the pulp industry. The measure used to assess agreement and the actual agreement observed were not provided. In addition, possible differences in agreement for cases and controls were not evaluated.

A similar approach was used in the case-control study of STS and NHL in Washington state (70). In

this study, supervisors and coworkers were contacted by telephone to corroborate self-reported exposures to specific phenoxy herbicides, chlorophenols, or other chemicals for those jobs with potential exposures to these chemicals. The authors indicated that confirmation of subjects' responses was provided in essentially all instances in which the supervisors or coworkers were successfully reached. No response rate was given, but a greater number of supervisors and coworkers could be reached for recent occupations. In addition, the majority of verification contacts attempted (approximately 80%) were for recent occupations. The authors also stated that there were no significant differences in agreement for cases and controls. No agreement percentages were provided.

In the case-control study of malignant lymphoma and STS conducted in Kansas, pesticide suppliers were interviewed on behalf of 110 subjects with farming experience to see whether they could confirm the pesticide use reported by the farmers (34). For these interviews, the suppliers relied on their memories or written records, or both (6, 12). Overall agreement between the farmers' and suppliers' responses regarding use of pesticides was only about 50% (6). When only the previous 10 years were considered, agreement between suppliers and subjects was higher. Suppliers typically reported less pesticide use than the farmers (34). For all subjects, the percentage of agreement for herbicides was approximately 60%. Similar percentages were found for cases and controls (12, 13, 34). Agreement was considerably lower for specific years of exposure to herbicides (12, 13). For use of 2,4-D, agreement was 83% for NHL cases and 74% for controls (6). The number of cases and controls who reported using herbicides was multiplied by the percentage of verified herbicide use and the OR for NHL was recalculated. The recalculated OR was 1.8, which was higher than the OR of 1.6 calculated from only the interview responses (34). A recalculation of the OR was not performed for 2,4-D. The authors attributed the lack of agreement between the two sources to the large number of suppliers farmers could have used, the closing of suppliers, the lack of historical records about pesticide purchases at many suppliers, the long period of time during which farmers had purchased pesticides, and the number of pesticides purchased (6).

In Iowa, 95 Iowa male controls from the Iowa-Minnesota case-control study of NHL and leukemia (18, 21) were reinterviewed about selected agricultural pesticides that the farmer previously had reported using, and these responses were compared with responses provided by proxy-respondents (19). The percentages of agreement for 2,4-D use before and after 1960 were 97 and 95%, respectively. In each time period, proxy-respondents reported slightly more subjects as exposed to 2,4-D than the subjects reported themselves. Agreement for the usual number of days herbicides were used per year was 52%. Similarly, the agreement percentages relating to the average annual frequency of 2,4-D use before 1960 was 48%, while after 1960 it was 56%. Because the study included only controls, no assessment could be made of possible reporting differences for cases and controls. The authors concluded that agreement between the responses of subjects and proxies was excellent and that data from proxy-respondents will be sufficient for epidemiologic purposes, assuming that differential recall is minimal.

In another study, 270 male cancer cases were initially interviewed for a multicenter case-control study of STS, HD, NHL, and other cancers (17). For the cases who died during the 4-year study interval, proxy-respondents also were interviewed. Accuracy of data from proxy-respondents was assessed by calculating theother cancers (17). For the cases who died during the 4-year study interval, proxy-respondents also were interviewed. Accuracy of data from proxy-respondents was assessed by calculating the sensitivity and specificity (i.e., data from the self-respondents were assumed to be the "gold standard"). Agreement between self- and proxy-respondents for pesticide and herbicide exposure data was low (sensitivities ranged from 14 to 51% and specificities ranged from 95 to 100%). The sensitivity for herbicides used infarming was approximately 45%, and the specificity was

approximately 95%. For herbicideproducts containing 2,4-D, the sensitivity was 35% and the specificity was 100%. Becausethe study included only cases, no assessment could be made of possible reporting differen

As part of a large methodologic study done by one of us (39), proxy-respondents wereinterviewed for 328 Minnesota subjects from the Iowa-Minnesota case-control study of NHLand leukemia (18, 21) who died or became incompetent since the initial interview (40). Asquestions increased in detail, agreement percentages decreased. Agreement percentagesranged from 48 to 91% for specific pesticides, with the majority between 60 and 80%. Among the NHL cases, leukemia cases, and controls, the agreement percentages for allherbicides were 79, 70, and 71%, respectively. For these same groups of subjects, theagreement percentages for 2,4-D were 75, 61, and 64%, respectively. Exposuremisclassifications had varying effects on the OR's and 95% CI's. Generally, OR's calculatedfrom proxy respondent data were less than those from self-respondent data; however, severalexceptions occurred. For example, proxy-respondents had an OR for leukemia of 2.4 (95%CI = 0.9 to 6.0) for herbicides; whereas, the corresponding OR for self-respondents was 1.1(95% CI = 0.5 to 2.3). The leukemia OR for 2,4-D was 2.6 (95% CI = 1.1 to 6.3) forproxy-respondents and 1.0 (95% CI = 0.4 to 2.1) for self-respondents. However, for NHL,OR's for self- and proxy-respondents were more similar for ever using 2,4-D or anyherbicides. Due to the presence of nondifferential and differential misclassification, a numberof the self- and proxy-respondent OR's were markedly different from each other. Thus, thefindings from this component indicated that pesticide data provided by proxy-respondents willnot necessarily result in the same estimate of risk or lead to the same conclusion as dataprovided by self-respondents.

Another component of this large methodologic study (39) examined the long-termreliability of reported pesticide information by reinterviewing, 7 to 10 years later, 389 self-respondents and 369 proxy-respondents from Minnesota who participated in the earlier Iowa-Minnesota case-control study of NHL and leukemia (18, 21). Percentages of agreement for first and second interview responses were higher for self-respondents than for proxy-respondents. For 2,4-D, agreement percentages ranged from 78 to 81% for self-respondents and from 59 to 73% for proxy-respondents. OR's and 95% CI's, calculated using data from the first and second interviews, were variable for both types of respondents. The disparities in the percentages of subjects exposed as reported in the first and second interviews had varying effects on the OR's. For leukemia, the self-respondent OR's did not differ much by interview; whereas, for NHL, there were some differences in the self-respondent OR's by interview. There were a number of differences in the proxy-respondent OR's by interview for both leukemia and NHL. The findings from this component showed that self- and proxy-respondents may not provide reliable information about past pesticide exposures due to nondifferential and differential exposure misclassification.

The final components of the large methodologic study (39) were to evaluate the short-term reliability and validity of reported pesticide exposure data. Short-term reliability was assessed by interviewing a sample of 157 farmers twice, 3 to 10 months apart. For 42 of the farmers who participated in the initial interview, validity was assessed by comparing the interview responses to data about 2,039 pesticide purchases collected from 14 local suppliers. The percentage of agreement was used to compare first and second interview responses provided by the farmers. The agreement of the farmers' responses in the first interview with the suppliers' records was evaluated by calculating the percentage of agreement, sensitivity, and specificity. The information from the suppliers was assumed to be the "gold standard".

More general pesticide exposure data (i.e., ever use) were reported reliably; whereas, more definitive

pesticide exposure data (i.e., years of use, frequency of use, and duration of use) were reported less reliably. Agreement for ever use of any herbicides was 94%. For 2,4-D, the agreement percentage was 80%. Agreement percentages for 2,4-D for the average number of days used per year and the average number of hours used per day were 71 and 72%, respectively. As observed in this component of the study, the inability of respondents to reliably recall specific details of pesticide use is important because many of the positive associations between phenoxy herbicides and NHL reported to date have occurred among subgroups defined by frequency of exposure.

The validity of reported pesticide data was poor. The validity of pesticide use data is more critical than the reliability, because data may be repeatable, but inaccurate. For most pesticides, low values of sensitivity compared with those of specificity indicated that underreporting was a greater problem than overreporting; however, 2,4-D was a notable exception to this pattern. The overall agreement percentage for the farmers and suppliers was 81% for herbicides. In addition, the sensitivity was 89% and the specificity was 20%. For 2,4-D, the agreement percentage was 60%, the sensitivity was 63%, and the specificity was 53%. Findings from this component of the study demonstrated the potential for exposure misclassification and resulting errors in risk estimates for studies in which pesticide data are obtained by interview.

Epidemiology conclusion. In addition to the published reviews of the epidemiologic literature, a number of expert panels have weighed the evidence regarding the carcinogenicity of phenoxy herbicides, or 2,4-D alone. In 1987, a panel convened by the Canadian Centre for Toxicology (20) concluded that, "... there is limited evidence of carcinogenicity in man from exposure to phenoxy herbicides. In terms of exposure to 2,4-D specifically, the evidence must still be regarded as inadequate to classify it as a carcinogen." The International Agency for Research on Cancer Working Group reached the same conclusion for phenoxy herbicides (64). No distinction was made for 2,4-D. In 1989, a panel convened by the Harvard School of Public Health Center for Risk Analysis (35) concluded that, "Although a cause-effect relationship is far from being established, the epidemiological evidence for an association between use of 2,4-D and non-Hodgkin's lymphoma is suggestive and requires further investigation. There is little evidence of an association between use of 2,4-D and soft-tissue sarcoma or Hodgkin's disease, and no evidence of an association between 2,4-D use and any other form of cancer." Another panel sponsored by the Industry Task Force II on 2,4-D Research Data (49) concluded, "... the epidemiological studies provide, at best, only weak evidence of an association between 2,4-D and the risk of cancer." Finally, in 1993, a Special Joint Committee of the Science Advisory Board and the Scientific Advisory Panel of the EPA (64) judged that, "... at this time, the data are not sufficient to conclude that there is a cause and effect relationship between the exposure to 2,4-D and NHL."

In weighing the evidence, one must consider that the results of the epidemiologic studiesare only as good as the data on which they are based. In particular, OR's can be affected by differences in exposure misclassification for cases and controls. Thus, the reliability andvalidity of reported pesticide exposure data, and the comparability of such data obtained fromself- and proxy-respondents have been explored in methodologic studies.

Only one methodologic study, however, has demonstrated the effect of pesticide exposuremisclassification on the OR (39). Furthermore, this is the only study to systematicallyexamine the validity of pesticide exposure data, as well as to evaluate the short- and long-termreliability of such data. The combined results of this study demonstrated the potential for bothdifferential and nondifferential exposure misclassification and the potential for resulting errorsin risk estimates for studies in which pesticide data are obtained by interview. In particular,there was

substantial evidence regarding the poor quality of reporting exposures to 2,4-D.

As a result, findings from the previously conducted case-control studies should not begiven much weight when determining whether there is a causal association between phenoxyherbicides, or 2,4-D, and cancer. At this point in time, the only conclusion that can be drawnfrom the epidemiologic studies is that the data are insufficient to assess the carcinogenicity ofphenoxy herbicides, or 2,4-D, in humans.

Table of Contents

TOXICOLOGY

Toxicology background. The chemical structure of 2,4-D allows it to mimic the plant hormone, indoleacetic acid (49). Because of it's structural similarity to this plant hormone, absorption of 2,4-D through roots and leaves can result in altered plant metabolism, abnormal growth, and plant death.

The results from a series of epidemiologic studies, described in the preceding section, raised questions about the safety of 2,4-D for humans (49). A number of toxicologic studies have been conducted in response to these safety concerns, and to fulfill the EPA requirements for re-registration of 2,4-D (36, 49). In 1992, the manufacturers of 2,4-D commissioned an extensive review of these studies, which was published in the Journal of the American College of Toxicology (49). This report was reviewed by a panel of nonindustry experts in toxicology and epidemiology. In addition, the Science Advisory Board of the EPA has issued a report that reviews the epidemiologic and toxicologic data on the potential carcinogenicity of 2,4-D (64). Data related to the carcinogenicity of 2,4-D also was reviewed by an expert panel convened by the Harvard School of Public Health (35).

This section of our review presents a summary and update of toxicologic studies relating to the health risk assessment of 2,4-D, drawn mainly from the reviews already mentioned, and from summaries of the data collected for submission to the EPA for the reregistration of 2,4-D (36). The results from some of the key toxicologic studies are presented in Table 4.

Environmental fate. In 1989, the World Health Organization published a review and evaluation of the environmental effects of 2,4-D (73). The general conclusion from this review was that 2,4-D does not persist in the soil because it is rapidly broken down and detoxified by light and by microorganisms in the soil (73). Because most organisms rapidly excrete 2,4-D, this herbicide would not be expected to accumulate or remain stored in the tissues of animals (73).

Contaminants. The purity of technical grade 2,4-D can range from less than 90% to 99% (72). The forms of 2,4-D most commonly used in forestry and agriculture are the alkali salts, amine salts, or esters (73). Technical grade formulations of 2,4-D may contain solvents or "wetting agents" that help the herbicide stick to and cover the plants (73). The presence of other types of impurities depends on the purity of the starting material, the procedure used to manufacture the specific form of 2,4-D, and the storage conditions (72).

Reactions that take place at high pH and high temperatures tend to increase the formation of polychlorinated dibenzo-*p*-dioxins (PCDD's), some of which are toxic (72). A study done by researchers at Agriculture Canada in the early 1980's tested samples of 2,4-D for the presence of PCDD's (23). The levels of PCDD's differed depending on the form of 2,4-D. Out of 11 samples of 2,4-D acid analyzed, none had levels of PCDD's above the detection limit of 1 ppb. By contrast, eight out of 26 samples of 2,4-D amine salts had detectable PCDD levels, which ranged from 5 to 587 ppb.

The samples of 2,4-D esters had the most widespread PCDD contamination. PCDD's were detected in 20 out of 21 samples of 2,4-D esters, with levels ranging from 35 ppb to 23.8 ppm. Another study, however, detected 6.8 ppb 2,3,7,8-TCDD in a German formulation of 2,4-D (27). The Canadian Government limits PCDD contamination of 2,4-D formulations to 10 ppb (49). In the United States, the technical product of each manufacturer is analyzed for the presence of PCDD's, and the health risk posed by the contaminants is evaluated on a case-by-case basis.[2]

Nitrosamines, which can be carcinogenic, have been detected in amine formulations of 2,4-D (72). Nitrosamines may form when 2,4-D amine salts are stored along with nitrates inmetal containers. Nitrates have been added to metal containers to prevent corrosion. Thechange in packaging of 2,4-D from metal containers to plastic or epoxy-lined containers wouldbe expected to eliminate the formation of nitrosamines (49).

Few studies rigorously address the toxicology of complex mixtures. Therefore, the effectof solvents, additives, or impurities on the toxicology of 2,4-D remains largely unknown. Onestudy found, however, that when rats were fed 2,4-D for 13 weeks, technical grade 2,4-D acid(97.3% pure) was no more toxic than purified 2,4-D acid (26).

Estimated exposure levels. The highest levels of exposure to 2,4-D occur in occupationalsettings. The amount of 2,4-D absorbed from occupational exposures will vary depending onthe type of work and on the type of safety measures used. It has been estimated that, withoutprotective gear, commercial applicators may be exposed to 2 to 160 g of 2,4-D/kg/day and forestry workers may be exposed to 0.86 to 129 g of 2,4-D/kg/day (49). Workers whospray herbicides from a backpack may receive some of the highest doses of 2,4-D. It has beenestimated that workers using backpack sprayers and wearing protective clothing had internaldoses ranging from 30 to 244 g/kg/day (49). The doses received by workers involved inaerial spraying have been estimated to range from 0.53 to 8.54 g/kg/day (49). One studyestimated that farmers who do not wear protective gear received an average dose of 5.78 g/kg of 2,4-D during eachspray operation.

A variety of surveys indicate that commercial foods and public water supplies do notpresent a significant source of exposure of 2,4-D to the general public (72). Duringapplication periods, the general population in herbicide use areas usually is not exposed to doses of 2,4-D greater than 2 g/kg/day (72). Likewise, the dose of 2,4-D received by home gardeners has been estimated to be less than 2 g/kg/day (49).

---

[2] Personal communication. 1995. Stephen Funk, Chemistry Branch, EPA, Washington, D.C.

Absorption. Approximately 90% of exposure of agricultural workers to 2,4-D occurs through skin contact. The observation that 2,4-D can be detected in the urine 4 hours after dermal exposure suggests that 2,4-D is rapidly absorbed through the skin, and also rapidly excreted (49). Absorption of 2,4-D is slower through the skin than through ingestion. In addition, skin can serve as a reservoir for 2,4-D (49). The amount of 2,4-D absorbed through the skin depends on a number of factors, including the chemical form of 2,4-D, the condition of the skin, the site of dermal contact on the body, and the solvent in which 2,4-D is dissolved. For example, one study found that, when applied to the forehead of humans, approximately 58% of the applied dose of 2,4-D dimethylamine was absorbed in contrast to 6% of the applied dose of 2,4-D isooctyl ester (49). The interpretation of data from toxicity studies conducted in animals is difficult because the efficiency of absorption of 2,4-D varies across animal species. For example, whereas 2,4-D dimethylamine is absorbed relatively efficiently when applied to the foreheads of humans, only 20% of a dose of 2,4-D dimethylamine was

absorbed when it was applied to the shaved backs of rats over the course of 7 days (49).

Exposure to 2,4-D through inhalation may occur in some occupational settings. Inhalation has been estimated to account for approximately 2% of exposure for applicators who spray 2,4-D (35). Inhalation may be a significant route of exposure for workers involved in the manufacturing of 2,4-D. Unfortunately, there is little research data on the absorption of 2,4-D through inhalation (49).

Absorption of 2,4-D appears to be rapid and complete from the gastrointestinal tracts of humans and experimental animals (49, 72). Although ingestion is usually a relatively minor route of exposure, most toxicity testing of 2,4-D has been conducted by oral exposure. Because 2,4-D is absorbed more efficiently through the gastrointestinal tract than through the skin, 2,4-D should be more toxic when ingested than when applied to the skin. Therefore, using oral studies of 2,4-D to test toxicity may add some margin of safety when these data are used to predict risk from exposure to 2,4-D through skin contact.

Distribution through the body. Following absorption, 2,4-D is distributed throughout the body (49). Studies conducted in a variety of species show that, after oral dosing, 2,4-D is found in the liver, kidney, lung, and to a lesser extent in the brain (49). The distribution of 2,4-D through the human body appears to be similar to that in test species. When organs were examined following fatal poisonings, the highest levels of 2,4-D were found in kidney and liver, with lower levels found in brain, muscle, and heart (72). It also has been reported that up to approximately 17% of a dose of 2,4-D can cross the placenta in pregnant mammals (72). Transport of 2,4-D into tissues and across the blood-brain barrier probably occurs by a cellular transport system, such as the organic acid transporter (49). Because 2,4-D is water soluble it is excreted in the urine, and does not tend to be stored or to accumulate in tissues in contrast to fat-soluble compounds (49).

Binding of 2,4-D to plasma proteins can occur and may affect distribution (72). When 2,4-D is bound to the plasma proteins, it cannot reach tissues where it might cause damage. High doses of 2,4-D can saturate or use up all of the plasma protein binding sites, which could result in a dramatic rise in the concentration of "free" 2,4-D. Free 2,4-D may be excreted. At very high doses of 2,4-D, however, the rate of excretion may slow down, and therefore the concentration of 2,4-D that can reach tissues in the body may increase and therefore cause toxicity. Exposure to high doses of other compounds that compete with 2,4-D for binding to plasma proteins may also cause in a rise in the concentration of free 2,4-D.

Excretion. All species studied, including humans, excrete 2,4-D mainly in the urine (72). The rate of excretion depends on the dose of 2,4-D. Excretion of 2,4-D is through the organic acid transport system in the kidney (49). Low doses of 2,4-D are excreted more rapidly than high doses, which can saturate this active kidney transport system. This phenomenon was demonstrated in a pharmacokinetic study of 2,4-D in rats, designed to measure the rate at which 2,4-D is absorbed and excreted (26). In this study, rats were given single oral doses of 14C labeled 2,4-D at 10, 25, 50, 100, or 150 mg/kg. The concentration of labeled 2,4-D in the plasma and the urine was measured 6 hours later. The rats given 100 and 150 mg/kg 2,4-D exhibited a dramatic increase in the plasma concentration of labeled 2,4-D compared with rats given the lower doses. The rats in the two high-dose groups also exhibited a corresponding decrease in urinary excretion of labeled 2,4-D. Munro et al. (49) point out that the doses of 2,4-D that most humans are exposed to should be below doses that saturate active kidney transport. Therefore, they suggested that toxicologic results from animals treated with high doses of 2,4-D that saturate renal transport should be interpreted with caution because they may not be relevant to typical human exposures (49).

Metabolism. Many compounds are broken down or transformed by specific enzymes present in almost all tissues, but present in especially high concentrations in the liver. Although this type of metabolism can detoxify chemicals, sometimes these enzymes transform a compound into a chemical that is even more toxic than the original one. Studies indicate that 2,4-D is not extensively metabolized in humans or other mammals, and furthermore 2,4-D does not appear to be transformed into a more toxic compound by metabolic enzymes (49). In one study, five male humans were given an oral dose of 5 mg/kg 2,4-D acid (57). This study reported that 2,4-D was efficiently absorbed and then eliminated. Half of the initial dose of 2,4-D was eliminated by 11.6 hours. Approximately 95% of the administered dose of 2,4-D was found eventually in the urine. Depending on the individual, from 0 to 12% of the 2,4-D found in the urine was in the form of conjugates, compounds that have undergone a specific detoxification reaction. Conjugates containing 2,4-D also have been identified in the urine of rats and pigs following oral exposure to 2,4-D. More toxic or reactive forms of 2,4-D, as a result of metabolism, have not been identified (49).

Acute toxicity. Acute toxicity usually refers to toxicity that occurs within a day or a few days after short-term, high dose exposure. Acute toxicity studies usually are intended to reflect a situation that would result in obvious poisoning. A common indicator of acute toxicity is the LD50, the dose that kills 50% of the test animals within a specified time interval. The lower the LD50, the more toxic the compound. The LD50 for 2,4-D varies depending on the form of the compound, the route of exposure (i.e., oral, skin, or inhalation), and the species to which it is administered. An early study determined an oral LD50 of 100 mg/kg in dogs, which appear to be the most sensitive species (72). A more recent study compared the oral LD50's of various forms of technical grade 2,4-D in rats (26). The test materials included: 2,4-D acid (95.0% pure), 2,4-D isoctyl ester (94.0% pure), 2,4-D dimethylamine salt (67.9% pure), 2,4-D isobutyl ester (96.1% pure), 2,4-D sodium salt (90.8% pure), 2,4-D butoxyethanol ester (97.1% pure), and 2,4-D butyl ester (98.6% pure). The LD50's ranged from 533 mg/kg for 2,4-D isobutyl ester in female rats (424 mg/kg 2,4-D acid equivalent) to 1090 mg/kg 2,4-D dimethylamine salt in male rats (619 mg/kg 2,4-D acid equivalent). Results from this study also indicate that acute exposure to 2,4-D through the skin is less toxic than acute oral exposure. The single dose dermal LD50's for all of the test materials was greater than 2000 mg/kg in rabbits. These results indicate that the acute dermal toxicity of 2,4-D is low.

The acutely toxic dose of 2,4-D in humans is difficult to determine. Death has resulted from ingestion of 80 mg/kg 2,4-D dimethylamine (72). An earlier report suggested that doses of 2,4-D as high as 36 mg/kg may not cause acute oral toxicity (72). In any case, workers should be able to avoid accidental exposure to acutely poisonous doses of 2,4-D through the use of proper occupational safety procedures.

Subchronic toxicity. Subchronic toxicity studies typically involve treating animals for approximately 3 months, and usually are intended to reflect a short-term exposure to a chemical. These studies usually report the highest experimental dose that does not cause toxicity, which is called the no-observed-adverse-effect-level (NOAEL). The NOAEL is an indication of a safe dose. These studies also usually report the lowest experimental dose that causes toxicity, which is called the lowest-observed-adverse-effect-level (LOAEL). Although most subchronic studies of 2,4-D have been oral studies, one subchronic study investigated the effects of dermal exposure to 2,4-D on both skin irritation and systemic (overall) toxicity (36). The results from this study support the conclusion from the acute study thintended to reflect a short-term exposure to a chemical. These studies usually report the highest experimental dose that does not cause toxicity, which is called the no-observed-adverse-effect-level (NOAEL). The NOAEL is an indication of a safe dose. These studies also usually report the lowest experimental dose that causes toxicity, which is called the

lowest-observed-adverse-effect-level (LOAEL). Although most subchronic studies of 2,4-D have been oral studies, one subchronic study investigated the effects of dermal exposure to 2,4-D on both skin irritation and systemic (overall) toxicity (36). The results from this study support the conclusion from the acute study that dermal exposure to 2,4-D has low systemic toxicity. This study also demonstrated that the doses that

The 2 ethylhexyl ester and the dimethylamine salt of 2,4-D were significantly more irritating to the skin than was 2,4-D acid. The highest dose of 2,4-D acid, 1000 mg/kg/day, did not cause dermal irritation. These results indicate a NOAEL for this effect of 1000 mg/kg/day, but a LOAEL could not be determined. By contrast the LOAEL for dermal irritation for 2,4-D ethylhexyl ester was 162.5 mg/kg/day with a NOAEL of 16.3 mg/kg/day. The results for 2,4-D dimethylamine salt were similar, yielding a LOAEL for dermal irritation of 180.1 mg/kg/day with a NOAEL of 18 mg/kg/day. These results indicate that 2,4-D ethylhexyl ester and 2,4-D dimethylamine induce local effects on the skin at much lower doses than those required to cause systemic toxicity.

A subchronic oral study (26) conducted in rats compared the toxicity of purified 2,4-D to technical grade 2,4-D (97.3% pure). The rats were fed either purified or technical grade 2,4-D acid for 13 weeks at doses of 0, 15, 60, 100, or 150 mg/kg/day. No overt signs of toxicity or behavioral changes were noted during daily observations. Microscopic examination of nervous system tissue revealed no treatment-related effects. Significant treatment-related effects of other tissues included altered levels of the thyroid hormone tetraiodothyronine (T4), increased liver weights, and kidney lesions.

Females were more sensitive than males when effects of 2,4-D acid on body weight, levels of serum T4, and the liver were examined. Both males and females appeared to be more sensitive to purified 2,4-D acid than to the technical grade. The LOAEL for decreased body weight gain in females fed purified 2,4-D was 60 mg/kg/day, with a corresponding NOAEL of 15 mg/kg/day. The LOAEL for males was 100 mg/kg/day, with a NOAEL of 60 mg/kg/day. The LOAEL for decreased body weight gain for animals fed technical grade 2,4-D was 150 mg/kg/day for both males and females, with a corresponding NOAEL of 100 mg/kg/day. Both purified and technical grade 2,4-D appeared to cause a decrease in serum T4 levels in females, but not in males. The LOAEL for purified 2,4-D for was 60 mg/kg/day, and the NOAEL 15 mg/kg/day. The LOAEL for technical grade 2,4-D was 100 mg/kg/day, with a NOAEL of 60 mg/kg/day. Microscopic examination of thyroid tissue revealed no treatment-related changes. Purified 2,4-D acid caused an increase in relative liver weights in both males and females. The LOAEL for females for 2,4-D was 60 mg/kg/day, with a NOAEL of 15 mg/kg/day. The LOAEL for males was 150 mg/kg/day, with a NOAEL of 100 mg/kg/day. Technical grade 2,4-D caused an increase in relative liver weights in females, but not males. The LOAEL for females fed technical grade material was 150 mg/kg/day, with a NOAEL of 100 mg/kg/day. Microscopic examination of liver tissue revealed minor, nonspecific effects primarily in the 100 and 150 mg/kg/day dose groups fed either purified or technical grade 2,4-D acid.

In this study, the kidney appeared to be the most sensitive target organ. In contrast to the other effects of 2,4-D acid, males were more sensitive to kidney effects than females. A significant increase in relative kidney weight was observed in all dose groups of males fed either purified 2,4-D or technical grade 2,4-D. The LOAEL for increased kidney weight was 15 mg/kg/day, but no NOAEL could be determined. The LOAEL for females fed purified 2,4-D was 60 mg/kg/day, with a NOAEL of 15 mg/kg/day. The LOAEL for technical grade 2,4-D in this study was 150 mg/kg/day, with a NOAEL of 100 mg/kg/day. Microscopic examination of kidney tissue also revealed treatment-related changes in males and females. Changes in kidney tissue were more extensive at 60 mg/kg/day and higher doses where the authors predicted that 2,4-D would saturate active kidney transport. This

study indicates a LOAEL of 15 mg/kg/day for oral subchronic exposure in rats based on effects on the kidney. A NOAEL could not be determined from this study.

More recent rodent studies indicate a NOAEL of 15 mg/kg/day for subchronic ingestion of 2,4-D.3 These studies, completed in 1991, examined the toxic effects of 1, 15, 100, or 300 mg/kg/day 2,4-D on rats or mice. The LOAEL for rats was 100 mg/kg/day based on histopathology, changes in body weight, and clinical pathology. The LOAEL in mice was 100 mg/kg/day based on decreased levels of glucose and T4, and increased kidney weights. The NOAEL for both rats and mice was 15 mg/kg/day.

Dogs appear to be more sensitive to the subchronic oral toxicity of 2,4-D toxicity than rodents (22). One dog study compared the toxicity of 2,4-D acid (96.7% pure), 2,4-D dimethylamine salt (66.7% pure), and 2,4-D 2-ethylhexyl ester (95.1% pure). Beagles were fed 2,4-D for 13 weeks at doses of 0, 1.0, 3.75, and 7.5 mg/kg/day. The 2,4-D acid also was given at an additional dose of 0.5 mg/kg/day for 13 weeks. No effects on hematology (blood), urinalysis, or ophthalmology were observed. All three forms of 2,4-D had significant effects on body weight gain, some parameters of clinical chemistry, and testes weight.

Although all three forms of 2,4-D caused a significant decrease in body weight gain, 2,4-D acid and 2,4-D 2-ethylhexyl ester appeared to be more potent than 2,4-D dimethylamine salt. The LOAEL for 2,4-D acid and 2,4-D 2-ethylhexyl ester was 3.75 mg/kg/day, and the NOAEL was 1 mg/kg/day. The LOAEL for 2,4-D dimethylamine salt was 7.5 mg/kg/day with a NOAEL of 3.75 mg/kg/day.

Dose-related effects were noted for 5 of the 17 clinical chemistry parameters examined. These included an increase in blood urea nitrogen (BUN), creatinine, and alanine aminotransferase; a decrease in alkaline phosphatase; and, in dogs treated with 2,4-D dimethylamine salt, an increase in aspartate aminotransferase. These results indicate a LOAEL of 1 mg/kg/day for 2,4-D dimethylamine salt and 2,4-D 2-ethylhexyl ester for changes in clinical chemistry. A NOAEL could not be determined. In the case of 2,4-D acid, the study established a LOAEL of 1 mg/kg/day for changes in alanine aminotransferase and creatinine, with a corresponding NOAEL of 0.5 mg/kg/day; and a LOAEL of 3.75 mg/kg/day for changes in BUN, with a NOAEL of 1 mg/kg/day. The authors state, however, that they did not consider these changes in clinical chemistry significant because they did not observe any treatment related microscopic changes in the kidney. In addition, progression in these effects was not observed in a chronic study of 2,4-D discussed below (22). Microscopic examination did reveal changes in the liver. The authors derived a LOAEL of 7.5 mg/kg/day and a NOAEL of 3.75 mg/kg/day for all three formulations based on liver effects.

Treatment with either of the three formulations resulted in a significant decrease in testes weight. The LOAEL for the effect of 2,4-D acid on testes weight was 3.75 mg/kg/day with a NOAEL of 1 mg/kg/day. The LOAEL for the 2,4-D amine and the 2,4-D ester was reported as 7.5 mg/kg/day with a NOAEL of 3.75 mg/kg/day. The authors did not observe significant treatment-related effects on the weights of other organs.

The authors concluded that these studies indicate a overall NOAEL of 1 mg/kg/day for subchronic ingestion or any of the three forms of 2,4-D. These data were reviewed by the EPA Office of Pesticide Programs, which concurred with the authors conclusion.[3] Collectively, these studies indicate a NOAEL of 1 mg/kg/day for subchronic ingestion based on dogs as the most sensitive species.

---

[3] Personal communication. 1995. Jess Rowland, Office Pesticide Programs, EPA, Washington, D.C.

Chronic toxicity. The results from oral chronic (i.e., long-term) studies conducted on rats and dogs are consistent with the results from the subchronic studies (22, 36). First, these studies indicate that dogs are more sensitive to chronic toxicity of 2,4-D than rodents. Second, the chronic dog study also supports a NOAEL of 1 mg/kg/day for 2,4-D (22).

A chronic ingestion toxicity study of 2,4-D acid in dogs was designed based on the results from the subchronic study discussed previously (22). The authors only studied 2,4-D acid, based on their conclusion that the subchronic toxicity of 2,4-D acid is similar to that of 2,4-D dimethylamine salt and 2,4-D 2-ethylhexyl ester. Beagles were fed diets containing 0, 1.0, 5.0, or 7.5 mg/kg/day 2,4-D acid (96,7% pure) for a year. Consistent with the findings from the subchronic study, the authors detected no significant alterations in hematology or urinalysis. In addition, microscopic examination revealed no treatment-related effects on bone marrow, lymph nodes, or spleen.

Treatment-related effects included decreased body weights, changes in clinical chemistry, and effects on the kidney and liver. Treatment with 2,4-D resulted in a decreased body weight gain for females, but not males. The LOAEL for females was 7.5 mg/kg/day, with a NOAEL of 5 mg/kg/day. The NOAEL for males was 7.5 mg/kg/day, but a LOAEL could not be determined. Changes in some of the clinical chemistry parameters were noted in both males and females. Dose-related changes in clinical chemistry included decreased glucose levels, and increases in BUN, creatinine, cholesterol, and alanine aminotransferase. The LOAEL for these effects was 7.5 mg/kg/day for both males and females, with a NOAEL of 5 mg/kg/day. Microscopic examination revealed effects on the liver and kidney in both males and females in the 5 and 7.5 mg/kg/day dose groups. These results indicate a LOAEL of 5 mg/kg/day and a NOAEL of 1 mg/kg/day based on the liver and kidney effects. These data were reviewed by the EPA Office of Pesticide Programs, which concurred with the NOAEL of 1 mg/kg/day.3

The results of chronic rodent studies are similar to those of the dog study. A 2-year chronic oral study conducted in rats, completed in 1984, supports the NOAEL derived from the chronic dog study.3 The LOAEL from this study was 5 mg/kg/day, with a NOAEL of 1 mg/kg/day based on kidney effects. More recent rodent studies indicate that dogs are more sensitive to 2,4-D than rats. A chronic study conducted on rats indicates a LOAEL of 75 mg/kg/day for 2,4-D acid based on decreases in body weights, with a NOAEL of 5 mg/kg/day (36). This study is discussed later in our "Carcinogenicity" section. A chronic oral mouse study, also discussed under "Carcinogenicity," indicates a LOAEL of 150 mg/kg/day based on renal effects and a NOAEL of 5 mg/kg/day (36).

Collectively, these studies suggest an overall NOAEL of 1 mg/kg/day for chronic exposure to 2,4-D, based on the observation that dogs are the most sensitive species and that liver and kidney are the target organs. The NOAEL from the dog study is used to calculate the reference dose (RfD) for 2,4-D currently used by the EPA Office of Pesticide Programs. The RfD is an estimate of the dose that humans, including sensitive subpopulations, could be exposed to daily, throughout their lifetime without appreciable risk of toxic effects. The calculation of the RfD is discussed in the "Toxicology conclusion" section at the end of this chapter.

Neurotoxicity. Some case reports have suggested an association between exposure to 2,4-D and the development of nervous system effects ranging from peripheral polyneuropathy and reduced nerve conduction velocity to depression, anxiety, and other symptoms of post-traumatic stress syndrome in Vietnam veterans (49). In test animals, doses of 2,4-D above 100 mg/kg can cause myotonia of the skeletal muscle (49), and oral doses above 150 mg/kg of 2,4-D can damage the blood-brain barrier (46). Toxicologic studies in rats and rabbits indicate, however, that neurotoxic effects of 2,4-D do not

occur below doses that saturate the kidney transport system. Therefore, neurotoxic effects would only be expected to occur at high doses of 2,4-D.

A short-term study conducted in rats investigated the neurotoxic effects of skin contact with 2,4-D (46). In this study, all four limbs of male rats were treated with a 12% or 24% solution of 2,4-D dimethylamine. The authors noted that when 2,4-D dimethylamine is used as an herbicidal spray it usually is diluted to final concentration of 0.5 to 2%. The rats were treated for 2 hours per day, 5 days a week. Treatment with 24% 2,4-D dimethylamine was stopped after 2 weeks because the animals developed severe dermatitis. Treatment with 12% 2,4-D dimethylamine was conducted for 3 weeks as planned, inasmuch as the animals exhibited only minimal skin change. The levels of 2,4-D measured in plasma were 323 g/ml after 2 weeks of treatment with a 24% solution, and 66.5 g/ml after 3 weeks of treatment with a 12% solution. The dramatic increase in plasma levels of 2,4-D at the higher dose was attributed to increased absorption through the damaged skin.

Dermal treatment with 2,4-D dimethylamine resulted in significantly decreased body weights and increased kidney weights, but no treatment-related effects on nervous system tissue (46). Decreased body weights were observed in animals treated with either 12% or 24% 2,4-D dimethylamine. Rats treated for 3 weeks with a 12% solution also exhibited an increase in kidney weights. Histology did not reveal any treatment-related effects on the kidney, suggesting that the increase in kidney weight may be an adaptive response to the active excretion of 2,4-D. One measure of neurotoxicity, grip strength, was increased after 3 weeks of treatment with a 12% solution. This result is consistent with other reports that indicate that 2,4-D can increase forelimb and hindlimb grip strength. The significance of the increased grip strength of the rats is not clear. Histology did not reveal any treatment-related lesions in central and peripheral nervous system tissues. This study indicates that short-term exposure to 2,4-D does not cause observable pathological effects on the nervous system.

To address the effects of prolonged exposure on the nervous system, the Industry Task Force II on 2,4-D Research Data conducted a 1-year oral neurotoxicity study on rats (36). Rats were fed diets providing 0, 5, 75, or 150 mg/kg/day of 2,4-D acid. The rats were evaluated by a functional observational battery (a series of observational tests to assess neurotoxicity), forelimb and hindlimb grip performance, landing foot splay, and an automated test of motor activity. Evaluation was conducted before exposure, and after 3, 6, 9, and 12 months of exposure. At the end of 12 months, central and peripheral nervous system tissues from the control and high dose group underwent microscopic examination.

A summary of the study results reported that the only significant neurological effects occurred in the high dose group. Retinal degeneration was noted in females treated with 150 mg/kg/day. Forelimb grip performance, normalized for body weight, was significantly increased in both males and females treated with 150 mg/kg/day. Systemic effects occurred at lower doses. Significantly decreased body weights were observed in the 75 and 150 mg/kg/day dose groups. The effect on body weight is consistent with the results of the rat subchronic study conducted by Gorzinski et al. (26). These results indicate a LOAEL of 150 mg/kg/day and a NOAEL of 75 mg/kg/day for neurotoxicity. This study also indicates a LOAEL of 75 mg/kg/day for a decrease in body weights, with a corresponding NOAEL of 5 mg/kg/day.

Reproductive and developmental toxicity. To examine the effects of 2,4-D on reproduction, the Industry Task Force II on 2,4-D Research Data conducted a two generation study in rats (36, 49). The rats were administered 0, 5, 20, or 80 mg/kg/day 2,4-D acid as a single dose from days 6 through 15 of gestation. The 80 mg/kg/day dose of 2,4-D caused excessive maternal toxicity, as indicated by

reduced maternal body weights and food consumption. The high dose group also exhibited decreased length of gestation, and reduced live-litter size. In addition to the maternal toxicity observed in the high dose group, decreased pup weight was observed in the 20 mg/kg/day group. The LOAEL for reproductive effects was determined to be 20 mg/kg/day based on the decreased pup weight. The NOAEL for reproductive effects was 5 mg/kg/day.

Studies conducted in rats and rabbits indicate that 2,4-D does not cause birth defects or effect development (36). In one study, female Fischer 344 rats were administered 2,4-D acid via stomach tube once a day from days 6 through 15 of gestation. The dose range in this study was 0, 8, 25, or 75 mg/kg/day. No teratogenic or embryotoxic effects were observed at these doses. There was a decrease in maternal body weight gain, however, in the 75 mg/kg/day dose group. This study indicated a LOAEL of 75 mg/kg/day for maternal toxicity based on effects on body weight gain, with a corresponding NOAEL of 25 mg/kg/day.

Similar results were obtained from a study that investigated the potential developmental toxicity of 2,4-D triisopropanolamine, 2,4-D isopropylamine, and 2,4-D butoxyethyl ester (36). In this study, rabbits were administered 0, 10, 30, or 75 mg/kg/day acid equivalents of 2,4-D on days 7 through 19 of gestation. No treatment-related effects on embryo-fetotoxicity or teratogenicity were observed at any of the doses. Maternal toxicity was noted at 30 and 75 mg/kg/day for all three forms of 2,4-D. Maternal toxicity was indicated by a number of effects including decreased body weight gains, myotonia, and urine discoloration. This study indicates a NOAEL for maternal toxicity of 10 mg/kg/day, and a LOAEL of 30 mg/kg/day for all three forms of 2,4-D. These results indicate that rabbits are more sensitive to the general toxic effects of these forms of 2,4-D than to the general toxic effects of 2,4-D acid.

Carcinogenicity. The potential carcinogenicity of 2,4-D has been under review for over a decade. In August, 1980, the EPA requested that 2,4-D be tested for carcinogenicity in rats and mice (64). In 1987, the EPA classified 2,4-D under Group "D", "not classifiable as to human carcinogenicity," pending additional data (64). As previously discussed in the "Epidemiology conclusion" section, the Harvard School of Public Health convened a panel of 14 scientists (35) to, "...examine the weight of evidence on the potential carcinogenicity of 2,4-D." More recently, a public advisory group to the EPA, the Joint Committee of the Science Advisory Board and the Scientific Advisory Panel (SAB/SAP), met in 1993 to review the results from epidemiologic studies, carcinogen bioassays, and other relevant data concerning the potential carcinogenicity of 2,4-D (64). Both the SAB/SAP and the panel convened by the Harvard School of Public Health concluded that results from the rodent carcinogen bioassays available at the times of the reviews provided weak evidence that 2,4-D is carcinogenic (35, 64).

The SAB/SAP evaluated data from a variety of mutagenicity studies and concluded that 2,4-D does not appear to be mutagenic (64). The Ames test, mouse micronucleus assay, and unscheduled DNA synthesis assay were uniformly negative for various forms of 2,4-D. The SAB/SAP found that other types of mutagenesis assays that sometimes yielded positive results, such as tests of cytogenetics in human and animal lymphocytes, had significant experimental deficiencies. For example, 2,4-D was positive in some tests, but lacked a dose-response. Other reports of positive results did not specify the source and purity of the 2,4-D. These types of problems raised questions about the validity of the positive results. The SAB/SAP concluded that the available data suggests that 2,4-D is not genotoxic. The report from this committee also noted that although positive results would have strengthened the

case that 2,4-D is carcinogenic, negative results do not necessarily mean that 2,4-D is not carcinogenic.

The SAB/SAP and the panel convened by the Harvard School of Public Health evaluated data from the same two rodent carcinogenicity studies. A 2-year feeding study conducted in B6C3F1 mice was completed in 1987. The mice were fed 0, 1, 15, or 45 mg/kg/day 2,4-D (35, 49). The study reported no excess tumors in males or females in any of the dose groups (35). A second 2-year feeding study conducted in Fisher 344 rats was completed in 1986 (35, 49). The rats were fed the same doses of 2,4-D as the mice (35). The dose range was chosen on the basis of a 13-week subchronic study, which indicated that 60 mg/kg/day of 2,4-D produces kidney damage. The results from this study indicated no statistically significant increase in tumors in female rats. In male rats, however, there appeared to be a statistically significant increase in the occurrence of a type of brain tumor, astrocytomas, in the high dose group. One astrocytoma was identified among the 60 controls, none were observed in the 1 and 5 mg/kg/day dose groups, two were observed among the 58 rats in the 15 mg/kg/day group, and five were identified among the 60 rats fed 45 mg/kg/day.

The incidence of astrocytomas in the male rats was considered weak evidence of carcinogenicity by criteria used to evaluate potent neurocarcinogens such as methyl nitrosourea (35, 41, 49). For example, inbred rodents have a variable rate of spontaneous brain tumor incidence. Although there is not as much information on the F344 rats as other inbred rodents, it has been argued that the increase in astrocytoma incidence in the 2,4-D study is within the range of incidences of spontaneous brain tumor development. In addition, rats treated with 2,4-D did not appear to have signs of brain toxicity, preneoplastic lesions, or early neoplastic proliferations. On the other hand, the EPA Health Effects Division Carcinogenicity Peer Review Panel questioned whether this study used an adequate dose range. They specifically questioned whether the highest dose used in the study was a maximum tolerated dose. The maximum tolerated dose is the highest dose that will not decrease the lifetime of the animal due to causes other than carcinogenicity. This dose typically causes an approximately 10% decrease in body weight. The maximum tolerated dose is usually the highest dose used in carcinogen bioassays. To establish whether the incidence of astroycytomas was treatment-related, EPA personnel requested that additional rodent carcinogenicity studies be conducted at higher doses of 2,4-D (35, 64).

Two additional rodent carcinogenicity studies with 2,4-D were completed in March of 1995. One study was conducted in Fisher 344 rats. In this study, male and female rats were administered 0, 5, 75, or 150 mg/kg/day 2,4-D for either 12 months (1Fisher 344 rats. In this study, male and female rats were administered 0, 5, 75, or 150 mg/kg/day 2,4-D for either 12 months (15 animals/sex/dose) or 24 months (50 animals/sex/dose) (37). No treatment-related i[1] mice. The mice were fed 1, 5, 150, or 300 mg/kg/day for up to 24 months. The mid and high doses were excessively toxic to males. Therefore, the male mice were terminated at 1 year, and the study was continued with only the females. No treatment-related increase in tumors in female mice were noted up through 2 years. These recent rodent carcinogenicity studies support the suggestion that 2,4-D alone is not carcinogenic.

Toxicology conclusion.

Carcinogenicity. Recent rodent carcinogenicity assays indicate that treatment with 2,4-D alone does not cause an increase in tumor development. In addition to being negative in standard carcinogenicity bioassays, 2,4-D also was negative in a rat liver model for tumor promoters (1). These results indicate that 2,4-D is not carcinogenic alone, and furthermore is not synergistic with known mutagenic

carcinogens.

Noncarcinogenic effects. Studies of noncancer endpoints indicate that 2,4-D is not a developmental toxicant, and that 2,4-D does not cause neurotoxic effects below doses that saturate kidney transport. Dogs appear to be more sensitive to 2,4-D than rats with respect to general toxicity. This may be due to species differences in the capacity for excretion of 2,4-D.

The goal of most risk assessments for noncarcinogenic effects is the calculation of an RfD. An RfD is an estimate of the reference dose that humans, including sensitive subpopulations, could be exposed to daily, throughout their lifetime without appreciable risk of toxic effects. An RfD is calculated by dividing a NOAEL by an uncertainty factor. The NOAEL is usually obtained from the study with the most sensitive species and most sensitive target organ. The uncertainty factor is calculated by assigning numbers ranging from 1 to 10 to account for specific sources of uncertainty when using data from animal studies to estimate a safe dose for humans.

$$\text{RfD (mg/kg/day)} = \frac{\text{NOAEL (mg/kg/day)}}{\text{Uncertainty factor}}$$

Because dogs appear to be the most sensitive species to 2,4-D, the RfD will be calculated using the NOAEL from the chronic ingestion study conducted on dogs (22). The NOAEL from that study was 1 mg/kg/day based on the liver and kidney effects. Using conventional risk assessment methods, an RfD for 2,4-D would be derived by dividing the NOAEL of 1 mg/kg/day by an uncertainty factor of 100. The uncertainty factor of 100 is calculated by multiplying an uncertainty factor of 10 to account for interspecies extrapolation by another uncertainty factor of 10 to account for sensitive subpopulations. This would yield an oral RfD of 0.01 mg/kg/day. This RfD is consistent with the RfD for 2,4-D currently listed on the EPA's Integrated Risk Information System (63). This RfD should also be adequate for skin exposure, because absorption of 2,4-D via ingestion is very efficient and more rapid than the absorption of 2,4-D through the skin.

SUMMARY

Collectively, the epidemiologic and toxicologic data show that 2,4-D is not likely to be carcinogenic in humans unless it is acting through an unknown mechanism that is not evident in animals. Based upon the calculated RfD and data from exposure studies, the general public should not experience toxic effects from exposure to 2,4-D. Because workers involved in the manufacture or application of 2,4-D may be exposed to levels above the RfD, appropriate protective equipment always should be used.

- Table of Contents

LITERATURE CITED

1. Abdellatif, A. G., V. Préat, J. Vamecq, R. Nilsson, and M. Roberfroid. 1990. Peroxisome proliferation and modulation of rat liver carcinogenesis by2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, perfluorooctanoic acidand nafenopin. Carcinogenesis. 11:1899-1902.

2. American Medical Association Council on Scientific Affairs. 1988. Cancer risk of pesticides in agricultural workers. JAMA. 260:959-966.

3. Armstrong, B. K., E. White, and R. Saracci. 1992. Principles of ExposureMeasurement in Epidemiology. Monographs in Epidemiology and Statistics, Vol. 21. Oxford Univ. Press, New York, NY.

4. Axelson, O. 1987. Pesticides and cancer risks in agriculture. Med. Oncol.Tumor Pharmacother. 4:207-217.

5. Blair, A., H. Malker, K. P. Cantor, L. Burmeister, and K. Wiklund. 1985. Cancer among farmers: a review. Scand. J. Work Environ. Health. 11:397-407.

6. Blair, A., S.H. Zahm, K.P. Cantor, and P.A. Stewart. 1989. Estimating exposure to pesticides in epidemiological studies of cancer. p. 38-46. in Wang, R. G. M. C. A. Franklin, R. C. Honeycutt, and J. C. Reinert, eds. Biological Monitoring for Pesticide Exposure: Measurement, Estimation, and Risk Reduction. American Chem. Soc., Washington, DC.

7. Blair, A., O. Axelson, C. Franklin, O. E. Paynter, N. Pearce, J. Stevenson, E. Trosko,H. Vainio, J. Woods, and S. H. Zahm. 1990. Carcinogenic effect of pesticides. p.201-260. in Baker, S. R. and C. F. Wilkinson, eds. The Effects of Pesticides onHuman Health. Advances in Modern Environmental Toxicology, Vol.XVIII. Princeton Sci. Pub. Co., Inc., Princeton, NJ.

8. Blair, A. and S. H. Zahm. 1990. Herbicides and cancer: a review and discussion of methodologic issues. Recent Results Cancer Res. 120:132-145.

9. Blair, A. and S. H. Zahm. 1990. Methodologic issues in exposure assessment for case-control studies of cancer and herbicides. Am. J. Ind. Med. 18:285-293.

10. Blair, A. and S. H. Zahm. 1991. Cancer among farmers. Occup. Med.: State of theArt Rev. 6:335-354.

11. Blair, A., S. H. Zahm, N. E. Pearce, E. F. Heineman, and J. F. Fraumeni Jr. 1992. Clues to cancer etiology from studies of farmers. Scand. J. Work Environ.Health. 18:209-215.

12. Blair, A. and S. H. Zahm. 1992. Agricultural health: pesticides amp; cancer. Healthand Environ. Digest. 6:1-4.

13. Blair, A. And S. H. Zahm. 1993. Patterns of pesticide use among farmers:implications for epidemiologic research. Epidemiology. 4:55-62.

14. Bloemen, L. J., J. S. Mandel, G. G. Bond, A. F. Pollock, R. P. Vitek, and R. R.Cook. 1988. An update of mortality among chemical workers potentiallyexposed to the herbicide 2,4-dichlorophenoxyacetic acid and its derivatives. J.Occup. Med. 35:1208-1212.

15. Bond, G. G., N. N. Wetterstroem, G. J. Roush, E. A. McLaren, T. E. Lipps, and R.R. Cook. 1988. Cause specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-dichlorophenoxyacetic acid and related salts. Br. J.Ind. Med. 45:98-105.

16. Bond, G. G., K. M. Bodner, and R. R. Cook. 1989. Phenoxy herbicides and cancer: insufficient epidemiologic evidence for a causal relationship. Fundam. Appl. Toxicol. 12:172-188.

17. Boyle, C. A., E. A. Brann, and The Selected Cancers Cooperative Study Group. 1992. Proxy respondents and the validity of occupational and other exposuredata. Am. J. Epidemiol. 136:712-721.

18. Brown, L. M., A. Blair, R. Gibson, G. D. Everett, K. P. Cantor, L. M. Schuman, L.F. Burmeister, S. F. Van Lier, and F. Dick. 1990. Pesticide exposures and otheragricultural risk factors for leukemia among men in Iowa and Minnesota. CancerRes. 50:6585-6591.

19. Brown, L. M., M. Dosemeci, A. Blair, and L. Burmeister. 1991. Comparability ofdata obtained from farmers and surrogate respondents on use of agriculturalpesticides. Am. J. Epidemiol. 134:348-355.

20. Canadian Centre for Toxicology. 1987. Expert panel report on carcinogenicity of2,4-D. Canadian Centre for Toxicology, Guelph, Ontario, Canada.

21. Cantor, K. P., A. Blair, G. Everett, R. Gibson, L. F. Burmeister, L. M. Brown, L. Schuman, and F. R. Dick. 1992. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res. 52:2447-2455.

22. Charles, J. M., D. W. Dalgard, H. C. Cunny, R. D. Wilson, and J. S. Buss. 1996. Comparative subchronic and chronic dietary toxicity studies on 2,4-dichlorophenoxyacetic acid, amine and ester in the dog. Fundam. Appl. Toxicol. (In press).

23. Cochrane, W. P., J. Singh, W. Miles, and B. Wakeford. 1981. Determination of chlorinated dibenzo-*p*-dioxin contaminants in 2,4-D products by gaschromatography-mass spectrometric techniques. J. Chromatogr. 217:289-299.

24. Coggon, D., B. Pannett, P. D. Winter, E. D. Acheson, and J. Bonsall. 1986. Mortality of workers exposed to 2-methyl-4 chlorophenoxyacetic acid. Scand. J.Work Environ. Health. 12:448-454.

25. Eriksson, M., L. Hardell, N. O. Berg, T. Moller, and O. Axelson. 1981. Soft-tissue sarcomas and exposure to chemical substances: a case-referent study. Br. J. Ind. Med. 38:27-33.

26. Gorzinski, S. J., R. J. Kociba, R. A. Campbell, F. A. Smith, R. J. Nolan, and D. L. Eisenbrandt. 1987. Acute, pharmacokinetic, and subchronic toxicological studies of 2,4-dichlorophenoxyacetic acid. Fundam. Appl. Toxicol. 9:423-435.

27. Hagenmaier, H. 1986. Determination of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin in commercial chlorophenols and related products. Fresenius Z. Anal. Chem. 325:603-606.

28. Hardell, L. 1977. Soft-tissue sarcomas and exposure to phenoxy acids: a clinical observation. Lakartidningen. 74:2753-2754.

29. Hardell, L. 1979. Malignant lymphoma of histiocytic type and exposure tophenoxyacetic acids or chlorophenols (Letter). Lancet. 1:55-56.

30. Hardell, L. and A. Sandstrom. 1979. Case-control study: soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br. J. Cancer. 39:711-717.

31. Hardell, L. 1981. Relation of soft-tissue sarcoma, malignant lymphoma and coloncancer to phenoxy acids, chlorophenols and other agents. Scand. J. WorkEnviron. Health. 7:119-130.

32. Hardell, L., M. Eriksson, P. Lenner, and E. Lundgren. 1981. Malignant lymphomaand exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. Br. J. Cancer. 43:169-176.

33. Hardell, L. and M. Eriksson. 1988. The association between soft tissue sarcomas andexposure to phenoxyacetic acids: a new case-referent study. Cancer. 62:652-656.

34. Hoar, S. K., A. Blair, F. F. Holmes, C. D. Boysen, R. J. Robel, R. J. Hoover, and J.F. Fraumeni

Jr. 1986. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA. 256:1141-1147.

35. Ibrahim, M. A., G. G. Bond, T. A. Burke, P. Cole, F. N. Dost, P. E. Enterline, M. Gough, R. S. Greenberg, W. E. Halperin, E. McConnell, I. C. Munro, J. A. Swenberg,S. H. Zahm, and J. D. Graham. 1991. Weight of the evidence on the humancarcinogenicity of 2,4-D. Environ. Health Perspec. 96:213-222.

36. Industry Task Force II on 2,4-D Research Data. (No date). Unpublished results ofover 60 toxicology and 54 environmental toxicology studies with 2,4-D. Submitted to the U.S. Environmental Protection Agency to support re-registration of 2,4-D. Washington, D.C.

37. Jeffries, T. K., B. L. Yano, R. J. Ormand, and J. E. Battjes. 1995. 2,4-Dichlorophenoxyacetic acid: chronic toxicity/oncogenicity study in Fischer 344 rats -- final report. Unpublished report, DowElanco, Midland, MI.

38. Johnson, E. S. 1990. Association between soft tissue sarcomas, malignantlymphomas, and phenoxy herbicides/chlorophenols: evidence from occupationalcohort studies. Fundam. Appl. Toxicol. 14:219-234.

39. Johnson, R. A. 1993. The reliability and validity of pesticide use data obtained from interviews. Ph.D. Dissertation. Univ. Minnesota, Minneapolis, MN.

40. Johnson, R. A., J. S. Mandel, R. W. Gibson, J. H. Mandel, A. P. Bender, P. D. Gunderson, and C. M. Renier. 1993. Data on prior pesticide use collected from self-and proxy respondents. Epidemiology. 4:157-164.

41. Koestner, A. 1986. The brain-tumour issue in long-term toxicity studies in rats. Fd.Chem. Toxicol. 24:139-143.

42. Lilienfeld, D. E. and M. A. Gallo. 1989. 2,4-D, 2,4,5-T, and 2,3,7,8-TCDD: an overview. Epidemiol. Rev. 11:28-58.

43. Lynge, E. 1985. A follow-up study of cancer incidence among workers inmanufacture of phenoxy herbicides in Denmark. Br. J. Cancer. 52:259-270.

44. Lynge, E. 1987. Background and design of a Danish cohort study of workers inphenoxy herbicide manufacture. Am. J. Ind. Med. 11:427-437.

45. Lynge, E. 1993. Cancer in phenoxy herbicide manufacturing workers in Denmark, 1947-87 -- an update. Cancer Causes Control. 4:261-272.

46. Mattsson, J. L., K. A. Johnson, and R. R. Albee. 1986. Lack of neuropathologic consequences of repeated dermal exposure to 2,4-dichlorophenoxyacetic acid in rats. Fundam. Appl. Toxicol. 6:175-181.

47. Moses, M. 1992. Pesticides. p. 479-489. in Last, J. M., R. B. Wallace, and E. Barrett-Connor, eds. Maxcy-Rosenau-Last: Public Health and Preventive Medicine. 13th ed. Appleton & Lange, Norwalk, CT.

48. Moses, M. 1989. Pesticide-related health problems and farmworkers. AAOHN J. 37:115-130.

49. Munro, I. C., G. L. Carlo, J. C. Orr, K. G. Sund, R. M. Wilson, E. Kennepohl, B. S.Lynch, M. Jablinske, and N. L. Lee. 1992. A comprehensive, integrated review and evaluation of the scientific evidence relating to the study of the herbicide 2,4-D. J. Am.Coll. Toxicol. 11:559-664.

50. Olsen, G. W. 1992. Herbicides and cancer: a closer look at 2,4-D. Health and Environ. Digest. 6:4-6.

51. Olsen, G. W. and K. M. Bodner. 1996. The effect of the type of respondent on risk estimates of pesticide exposure in a non-Hodgkin's lymphoma case-control study. J. Agromed. 3:37:50.

52. Ott, M. G., R. A. Olson, R. R. Cook, and G. G. Bond. 1987. Cohort mortality studyof chemical workers with potential exposure to the higher chlorinated dioxins. J. Occup.Med. 29:422-429.

53. Pearce, N. E., A. H. Smith, J. K. Howard, R. A. Sheppard, H. J. Giles, and C. A. Teague. 1986. Non-Hodgkin's lymphoma and exposure to phenoxyherbicides, chlorophenols, fencing work, and meat works employment: a case-control study. Br. J. Ind. Med. 43:75-83.

54. Pearce, N. E., R. A. Sheppard, A. H. Smith, and C. A. Teague. 1987. Non-Hodgkin's lymphoma and farming: an expanded case-control study. Br. J.Cancer. 39:155-161.

55. Pearce, N. and J. S. Reif. 1990. Epidemiologic studies of cancer in agriculturalworkers. Am. J. Ind. Med. 18:133-148.

56. Persson, B., A-M. Dahlander, M. Fredriksson, H. N. Brage, C-G. Ohlson, and O. Axelson. 1989. Malignant lymphomas and occupational exposures. Br. J. Ind. Med. 46:516-520.

57. Sauerhoff, M. W., W. H. Braun, G. E. Blau, and P. J. Gehring. 1977. The fate of 2,4-dichlorophenoxyacetic acid (2,4-D) following oral administration to man. Toxicology. 8:3-11.

58. Smith, A. H., D. O. Fisher, H. J. Giles, and N. Pearce. 1983. The New Zealand soft tissue sarcoma case-control study: interview findings concerning phenoxyacetic acid exposure. Chemosphere. 12:565-571.

59. Smith, A. H., N. E. Pearce, D. O. Fisher, H. J. Giles, C. A. Teague, and J. K.Howard. 1984. Soft tissue sarcoma and exposure to phenoxyherbicides andchlorophenols in New Zealand. J. Natl. Cancer Inst. 73:1111-1117.

60. Smith, A. H. and N. E. Pearce. 1986. Update on soft tissue sarcoma and phenoxyherbicides in New Zealand. Chemosphere. 15:1795-1798.

61. Smith, J. G. and A. J. Christophers. 1992. Phenoxyherbicides and chlorophenols: acase control study on soft tissue sarcoma and malignant lymphoma. Br. J. Cancer. 65:442-448.

62. Sterling, T. D. and Arundel A. V. 1986. Health effects of phenoxy herbicides: areview. Scand. J. Work Environ. Health. 12:161-173.

63. United States Environmental Protection Agency. 1986. Integrated Risk Information System file for 2,4-D dichlorophenoxyacetic acid (2,4-D). Prepared by theOffice of Health and Environmental Assessment, Environmental Criteria andAssessment Office, Cincinnati, OH for the Office of Drinking Water, USEPA, Washington, DC.

64. United States Environmental Protection Agency, Science Advisory Board. 1994. AnSAB report:

assessment of potential 2,4-D carcinogenicity, review of theepidemiological and other data on potential carcinogenicity of 2,4-D by the SAB/SAPJoint Committee. USEPA, Washington, DC.

65. Vineis, P., B. Terracini, G. Ciccone, A. Cignetti, E. Colombo, A. Donna, L. Maffi,R. Pisa, P. Ricci, E. Zanini, and P. Comba. 1986. Phenoxy herbicides and soft-tissue sarcomas in female rice weeders: a population-based case-referent study. Scand. J.Work Environ. Health. 13:9-17.

66. Wiklund, K., J. Dich, and L-E. Holm. 1987. Risk of malignant lymphoma inSwedish pesticide appliers. Br. J. Cancer. 56:505-508.

67. Wiklund, K., J. Dich, and L-E. Holm. 1988. Soft tissue sarcoma risk in Swedishlicensed pesticide applicators. J. Occup. Med. 30:801-804.

68. Wiklund, K., J. Dich, and L-E. Holm. 1989. Risk of soft tissue sarcoma, Hodgkin's disease, and non-Hodgkin's lymphoma among Swedish licensed pesticide applicators. Chemosphere. 18:395-400.

69. Wilkinson, C. F. and Working Group Chairpersons. 1990. Introduction andoverview. p. 5-33. in Baker, S. R. and C. F. Wilkinson, eds. The Effects of Pesticides onHuman Health. Advances in Modern Environmental Toxicology, Vol. XVIII. Princeton Sci. Pub. Co., Inc., Princeton, NJ.

70. Woods, J. S., L. Polissar, R. K. Severson, L. S. Heuser, and B. G. Kulander. 1987. Soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenoxyherbicide and chlorinated phenol exposure in Western Washington. J. Natl. Cancer Inst. 78:899-910.

71. Woods, J. S. 1989. Non-Hodgkin's lymphoma among phenoxy herbicide-exposed farm workers in western Washington State. Chemosphere. 18:401-406.

72. World Health Organization. 1984. 2,4-Dichlorophenoxyacetic acid (2,4-D). Environmental Health Criteria 29. Published under the joint sponsorship of theUnited Nations Environment Programme, the International Labour Organisation,and the World Health Organization, Geneva, Switzerland.

73. World Health Organization. 1989. 2,4-Dichlorophenoxyacetic acid (2,4-D) -- environmental aspects. Environmental Health Criteria 84. Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization, Geneva, Switzerland.

74. Zahm, S. H., D. D. Weisenburger, P. A. Babbitt, R. C. Saal, J. B. Vaught, K. P.Cantor, and A. Blair. 1990. A case-control study of non-Hodgkin's lymphomaand the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology. 1:349-356.

Table 1.

Summary of key case-control studies of phenoxy herbicides and soft-tissue sarcomas (STS), non-Hodgkin's lymphoma (NHL), and Hodgkin's disease (HD).

First author,reference, and year	Cases, number, and type	Controls, number, and type	Years encompassed for cases	Type of interview	Odds ratio (95% Confidence interval)	
					Phenoxy herbicides	2,4-D specifically
Hardell (30) 1979.	52 STS, hospital-based.	208, General population.	1970-1977	Mail with telephone supplement.	5.3(2.4 - 11.5)	---
Eriksson (25) 1981.	110 STS, population-based.	220, General population.	1974-1978	Mail with telephone supplement.	6.8 (2.6 - 17.3)	---
Smith (58, 59) 1984.	82 STS, population-based.	92, Other types of cancer.	1976-1980	Telephone.	1.3a (0.7 - 2.5)b	---
Hoar (34) 1986.	133 STS, population-based.	948, General population.	1976-1982	Telephone.	1.4c (---)d	1.3c (---)d
Smith (60) 1986.	51 STS, population-based	315, Other types of cancer.	1981-1982	Telephone.	0.7a (0.3 - 1.5)b	---
Vineis (65) 1986.	68 STS, population-based.	158, General population.	1981-1983	In-person and mail.	2.4e (0.6 - 10.3)b and 1.1f (0.2 - 5.1)b	--- 1.1f
Woods (70, 71) 1987.	128 STS, population-based.	694, General population.	1981-1984	In-person.	0.9g (0.4 - 1.9)	---
Hardell (33) 1988.	54 STS, population-based.	311, General population and 179, other types of cancer.	1978-1983	Mail with telephone supplement.	3.3h (1.4 - 8.1) and 2.2a (0.9 - 5.3)	---
Smith (61) 1992.	30 STS, hospital-based.	82, General population	1976-1987	In-person.	0.8h (0.2 - 3.7) and 2.5a	---

		and 82, other types of cancer.			(0.5 - 12.9)	
Hardell (32) 1981.	169 HD and NHL, hospital-based.	338, General population.	1974-1978	Mail with telephone supplement.	4.8 (2.9 - 8.1)	---
Hoar (34) 1986.	121 HD, population-based.	948, General population.	1976-1982	Telephone.	1.0c (---)d	1.0c (---)d
Hoar (34) 1986.	170 NHL, population-based.	948, General population.	1979-1981	Telephone.	2.2 (1.2 - 4.1)	2.3 (1.3 - 4.3)
Pearce (53) 1986.	83 NHL, population-based.	228, General population and 168, other types of cancer.	1977-1981	Telephone.	1.1h (0.6 - 2.2)b and 1.3a (0.7 - 2.2)b	---
Pearce (54) 1987.	183 NHL, population-based.	338, Other types of cancer.	1977-1981	Telephone.	1.0a (0.7 - 1.5)b	---
Persson (56) 1989.	54 HD, hospital-based.	275, General population.	1964-1986	Mail.	3.8 (0.7 - 21.0)b	---
Persson (56) 1989	106 NHL, hospital-based.	275, General population.	1964-1986	Mail.	4.9 (1.3 - 18.0)b	---
Woods (70, 71) 1989.	576 NHL, population-based.	694, General population.	1981-1984	In-person.	0.9 (0.5 - 1.5)	0.7 (0.4 - 1.3)
Zahm (74) 1990.	201 NHL, population-based.	725, General population.	1983-1986	Telephone.	Similar to risk estimate for 2,4-di	1.5 (0.9 - 2.5)
Cantor (21) 1992.	622 NHL, population-based.	1245, General population.	1980-1983	In-person.	1.2 (0.9 - 1.6)	1.2 (0.9 - 1.6)
Smith (61) 1992.	52 HD and NHL, hospital-based.	82, General population and 82, other	1976-1987	In-person.	0.8h (0.3 - 2.2) and 1.8a (0.5 - 6.0)	---

		types of cancer.				

[a] Odds ratio calculated using cancer controls.
[b] 90% confidence interval.
[c] Results not reported in original article. As cited by Blair et al. (7, 8).
[d] Confidence interval not provided.
[e] Odds ratio calculated using self-respondent subjects.
[f] Odds ratio calculated using next-of-kin for subjects.
[g] High exposure.
[h] Odds ratio calculated using population-based controls.
[i] Numeric results not reported in original article.

Top of Page
Next Table

Table 2. Summary of key cohort studies of phenoxy herbicides and soft-tissue sarcomas (STS), non-Hodgkin's lymphoma (NHL), and Hodgkin's disease (HD).

First author, reference, and year	Cohort population, number and type	Comparison population	Years of follow-up	Relative risk (95% Confidence interval)		
				STS	HD	NHL
Coggon(24) 1986	5,754 Phenoxy herbicide manufacturers and applicators.	England and Wales general population.	1947-1983	1.1 (0.0 - 5.9)	0.3 (0.0 - 1.6)	0.4 (0.0 - 1.3)
Ott (52) 1987	2,187 Phenoxy herbicide manufacturers.	U.S. general population.	1940-1982	2.5[a] (0.1 - 13.9)	0.9[a] (0.0 - 5.1)	1.9 (0.6 - 4.5)
Bond (15) 1988	878 2,4-D manufacturers.	U.S. general population and internal referents.	1945-1982	None observed.	2.7[b] (0.0 - 14.7)	3.9[b] (0.4 - 14.1)
Wiklund (66, 67, 68) 1989	20,245 Applicators.	Sweden general population.	1965-1984	0.9 (0.4 - 1.9)	1.5 (0.8 - 2.4)	1.1 (0.7 - 1.6)
Bloemen (14) 1993	878 2,4-D manufacturers.	U.S. General population and internal referents.	1945-1986	None observed.	Not reported.[c]	2.0[b] (0.2 - 7.1) and 3.0d (0.8 - 11.9)
Lynge (43, 44, 45) 1993	4,461 Phenoxy herbicide manufacturers, of which 2,119 were potentially exposed.	Denmark general population.	1947-1987	2.3[e] (0.6 - 5.8)	--	1.3[e] (0.4 - 3.3)

[a] Relative risk not reported in original article. As cited by Blair et al. (7, 8).

[b] Relative risk calculated using general population as comparison group.

[c] One case of HD had been observed in initial study (15), but was not reported separately in update.

[d] Relative risk calculated using internal referents as comparison group.

[e] Among cohort members with potential exposure to phenoxy herbicides.

Table of Contents
Previous Table
Next Table

Table 3.

Summary of completed studies on the reliability and validity of pesticide use data obtained from interviews.

First author, reference, and year	Study population	Comparison population	Study type	Methods used	Major findings	Notes
Hardell (30) 1979	STS[a] cases and controls.	Employers.	Validity.	Contacted employers of subjects who had worked in forestry, sawmill, or pulp industries to corroborate reported exposures to phenoxy herbicides and chlorophenols; information obtained via mailed questionnaires.	Agreement good for chlorophenols.	Methods not clearly defined; low employer response rate; reliance on memories of employers; employers may not be "gold standard"; no data provided fc overall level of agreement or specifically for cases and controls; reporting differences for cases and controls not assessed.
Hoar (6, 12, 13, 34) 1986	STS, HD[b], and NHL[c] cases and controls.	Suppliers.	Validity.	Contacted suppliers for 110 subjects who had farmed to corroborate reported exposures to herbicides and insecticides; information obtained via in-person interviews with suppliers and records	Overall agreement for use of pesticides was about 50%; agreement better for the last 10 years; suppliers usually reported less pesticide use than subjects; overall agreement for herbicides was about 60%, but was lower for years used; agreements for	Methods not clearly defined; unclear as to which pesticides were assessed fc agreement; reliance on memories of suppliers; suppliers may n be "gold standard"; recalculated OR not provided for 2,4-D; only limited data

				of suppliers.	herbicides was similar for cases and controls; recalculated OR[d] for NHL and herbicide use was higher; agree-ment for 2,4-D was 83% for NHL cases and 74% for controls.	provided in several publications.
Woods (70) 1987	STS and NHL cases and controls.	Supervisors and coworkers.	Validity.	Contacted supervisors and coworkers of subjects who had jobs with potential exposures to corroborate reported exposures to phenoxy herbicides, chlorophenols, and other chemicals; information obtained via telephone interviews.	Agreement obtained in essentially all instances in which supervisor or coworker was reached; agreement similar for cases and controls.	Methods not clearly defined; agreement assessed mostly for recent jobs; reliance on memories of supervisors and coworkers; supervisors and coworkers may not be "gold standard"; supervisor and coworker response rate nc provided; no dat provided for overall level of agreement or specifically for cases and controls.
Brown (19) 1991	Controls for NHL and leukemia cases.	Proxies.	Comparability of data from self- and proxy-respondents.	Interviewed 95 controls who had farmed and their proxies about controls' use of specific pesticides reported by the controls in earlier case-	Agreement about 95% for 2,4-D use; agreement better for more recent time periods; proxy-respondents reported more subjects as exposed than the subjects themselves;	Respondents asked only abou frequently used pesticides reported in earli study; could not assess reporting differences between cases and controls.

				control study.	agreement percentages for days per year of use for herbicides and 2,4-D specifically were about 50%.	
Boyle (17) 1992	STS, HD, NHL, liver, nasal, and nasopharyngeal cancer cases.	Proxies.	Comparability of data from self- and proxy-respondents.	Interviewed 270 proxies, for those cases who died during 4-year study period, about cases' use of pesticides, herbicides, and phenoxy herbicides containing 2,4-D.	For herbicides used in farming, sensitivity was about 45% and specificity was about 95%; for phenoxy herbicides containing 2,4-D, sensitivity was 35% and specificity was 100%.	Could not asses reporting differences between cases and controls.
Johnson (39, 40) 1993	NHL and leukemia cases and controls.	Proxies.	Comparability of data from self- and proxy-respondents.	Interviewed 328 proxies, for those cases and controls who died or became incompetent following interview in earlier case-control study, about use of pesticides, herbicides, and specific herbicides such as 2,4-D.	Agreement percentages for use of herbicides ranged from about 70 to 80%; for use of 2,4-D, agreement percentages ranged from about 60 to 75%; for both NHL and leukemia, OR's for herbicides and 2,4-D specifically were higher for proxy-respondents then self-respondents.	Proxy-responde interviewed several years aft self-respondents
Johnson (39) 1993	NHL and leukemia cases and controls.	Same.	Long-term reliability.	Reinterviewed 758 cases and controls from earlier case-control study about use of	Agreement percentages for 2,4-D use were about 80% for self-respondents and between 55 and 75% for	Reinterviews conducted up to decade after initial interviews

				pesticides, herbicides, and specific herbicides such as 2,4-D.	proxy-respondents.	
Johnson (39) 1993	Farmers.	Same.	Short-term reliability.	Interviewed 157 farmers on 2 occasions, less than 1 year apart, about use of pesticides, herbicides, and specific herbicides such as 2,4-D.	Agreement for use of herbicides was 94%; agreement for 2,4-D use was 80%; agreement for usual days per year of 2,4-D use was 71%.	Could not assess reporting differences between cases and controls.
Johnson (39) 1993	Farmers.	Suppliers.	Validity.	Abstracted data related to 2,039 pesticide purchases from 14 suppliers for 42 farmers to corroborate reported exposures to pesticides, herbicides, and specific herbicides such as 2,4-D.	Agreement, sensitivity, and specificity for herbicides were 81, 89, and 20%, respectively; for 2,4-D specifically, agreement was 60%, sensitivity was 63%, and specificity was 53%.	Suppliers may not be "gold standard"; could not assess reporting differences between cases and controls.

[a] Soft-tissue sarcoma.

[b] Hodgkin's disease.

[c] Non-Hodgkin's lymphoma.

[d] Odds ratio.

- Table of Contents
- Next Table
- Previous Table

Table 4.

Summary of NOAEL's[a] and LOAEL's[b] from key toxicity studies of 2,4-D.

Study type, species, and reference	Form of 2,4-D	NOAEL(mg/kg/day)	LOAEL(mg/kg/day)	Notes
21-day dermal, >toxicity, rabbit (36)	acid	1000	---	Systemic toxicity.
	1000	---	Dermal irritation.	
	2-ethylhexyl ester	1627.5	---	Systemic toxicity.
		16.3	162.8	Dermal irritation.
	dimethylamine	540.5	---	Systemic toxicity.
		18	180.1	Dermal irritation.
>				
13-week oral, toxicity, rat (26)	acid, purified	---	15	Kidney effects.
	acid, technical grade	---	15	Kidney effects.
Subchronic oral, toxicity, rat [c]	acid	15	100	Histopathology, body weight, and clinical pathology effects.
Subchronic oral, toxicity, mouse[c]	acid	15	100	Decreased glucose and T_4 levels and increased kidney weights.
13-week oral, toxicity, dog (22)	acid (96.7% pure)	1	3.75	Testes, body weight, clinical chemistry, and liver effects from all three forms.
	dimethylamine (66.7% pure)	1	3.75	
	2-ethylhexyl ester (95.1% pure)	1	3.75	
1-year oral, toxicity, dog (22)	acid	1	5	Liver and kidney effects.
2-year oral, toxicity, rat[c]	acid	1	5	Kidney effects.

2-year oral, toxicity and oncogenicity, rat (36)	acid	5	75	Decreased body weight, no indication of oncogenic effects.
2-year oral, oncogenicity, female mouse (36)	acid	5	150	Kidney effects, no treatment-related increases in the incidence of neoplasms.
1-year oral, neurotoxicity, rat (36)	acid	5	75	Decreased body weight.
		75	150	Retinal degeneration.
Two-generation oral, reproductive toxicity, rat (36, 49)	acid	5	20	Decreased pup weight.
Gavager[d], developmental toxicity, rat (36)	acid	25	75	Maternal toxicity. No teratogenic or embryotoxic effects observed.
Gavage, developmental toxicity, rabbit (36)	acid	30	90	Maternal toxicity for all four forms. No developmental effects observed for all four forms.
	triisopropanolamine	10	30	
	isopropylamine	10	30	
	butoxyethyl ester	10	30	

[a] No-observed-adverse-effect-level, which is the highest experimental dose that does not cause toxicity.

[b] Lowest-observed-adverse-effect-level, which is the lowest experimental dose that causes toxicity.

[c] Personal communication, Jess Rowland, Office of Pesticide Programs, EPA. 1995.

[d] Stomach tube.

- Table of Contents
- Previous Table