



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 16 1988

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Third Peer Review of 2,4-Dichlorophenoxyacetic acid

From: Marcia van Gemert, Ph.D. *M. van Gemert 1/29/88*
Head, Section III
Toxicology Branch, HED (TS-769C)

To: Doug McKinney, Product Manager #69
Registration Division (TS-767C)

The Peer Review Committee met on January 13, 1988 to examine the issues raised by the Science Advisory Panel (SAP) with respect to the classification of the carcinogenicity of 2,4-dichlorophenoxy acetic acid.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Theodore M. Farber

Theodore M. Farber

William L. Burnam

William L. Burnam

Robert Beliles

Esther Rinde

Esther Rinde

John Quest

John A. Quest

Richard Levy

Richard Levy

Marion Copley

Marion Copley

Kerry Dearfield

Kerry Dearfield

Judith W. Hauswirth

Judith W. Hauswirth

Anne Barton

Anne Barton

2. Scientific Reviewers: (Noncommittee members responsible for presentation of data: signature indicates technical accuracy of Committee report)

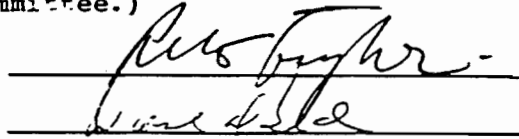
Marcia van Gemert

M. van Gemert

3. Peer Review Members in Absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Reto Engler

Diane Beal



B. Material Reviewed:

The SAP response memorandum of June 4, 1987, the submission from NTP's Jeffrey Collins to M. van Gemert received Oct. 6, 1987; the second Peer Review memo on 2,4-D dated Dec. 17, 1987; the newly published report of the subchronic rat study which appeared in Fundamentals and Applied Toxicology 9: 423-435, 1987. Acute Pharmacokinetic and Subchronic Toxicological studies of 2,4-Dichlorophenoxyacetic acid; S.J. Gorzinski, R.J. Kociba, R.A. Campbell, F.A. Smith, R.J. Nolan, D.L. Eisenbrandt.

C. Considerations:

The SAP did not agree with the Peer Review Committee's conclusion that the available oncogenicity data on 2,4-D should be classified as an interim category C oncogen. The SAP considered the increased incidence of astrocytomas in male rats exposed to 45 mg/kg to be equivocal evidence of oncogenicity. The Panel agreed with the Peer Review that further testing is required to resolve this issue, and recommended that a repeat rat bioassay be performed using two male rat control groups of 50 animals each and two male rat groups of the same size exposed to 45 mg/kg 2,4-D.

Issues:

1. Doses for Repeat Rat Bioassay Study

The 2,4-D Task Force has taken issue with EPA concerning an alleged approval of the doses for the mouse and rat bioassays on 2,4-D. Dr. H. Spencer was the reviewer for this chemical at the time (1980) the doses were selected for the bioassays, and he recalls a brief meeting with the Task Force where the 90-day rat data were discussed concerning rat kidney lesions. The selection of the doses for the bioassays was based on the kidney effects reported in the 90-day studies as relayed to the EPA by the Task Force. The severity and extent of these effects were not critically evaluated and thus the dose selection was the sole responsibility of the Task Force. Subsequently in 1985, NTP pathologists reviewed the slides from these 90-day rat studies as well as those slides from the interim sacrifices of both the rat and mouse chronic studies (as discussed in the second Peer Review memo on 2,4-D) and agreed unanimously that the kidney lesions were minimal in severity and clearly not life-threatening even at the highest dose of 150 mg/kg. Treated female rat kidneys did not differ appreciably from controls, and treated male rat kidneys only showed an increased incidence of epithelial regeneration at the higher doses. Treated male rat kidneys also displayed an altered tinctoral property of the tubular cell cytoplasm

at all doses which was not seen in controls. NTP speculated that this tinctorial alteration may reflect either decreased cytoplasmic a-2 globulin or an artifact of fixation or staining. After reviewing the 1-year, the interim sacrifice data from the rat and mouse chronic study, NTP stated that the "sacrifice data presented indicated minimal toxicity at the highest dose tested (45 mg/kg). Increased kidney weights were observed in high dose male rats and high dose female mice but even less kidney pathology was seen at 45 mg/kg after 52 weeks than had been evident at this dose in the previous 13-week subchronic studies." NTP stated "in summary, after analyzing the data presented by the 2,4-D Task Force which was utilized in selecting doses for the current 2-year chronic studies of this chemical, including direct examination of the relevant tissue sections from the subchronic studies, the consensus of the NTP participants is that the Task Force chronic studies are probably not being performed at an MTD."

The NTP's conclusions concerning the MTD issues and their evaluation of the subchronic and 1-year sacrifice slides were not available to the initial Peer Review Committee or the SAP at the time of their respective deliberations. Additionally, the 2,4-D Task Force has recently published the results and their interpretation of these results from the highest dose subchronic rat study. (Fundamental and Appl. Tox. 9: 423-435, 1987.) These conclusions differ dramatically from those arrived at by the NTP pathologists after examining the slides from this very study.

After evaluating both the results published in the 2,4-D Task Force paper and the NTP pathologists conclusions, the Peer Review Committee determined that the rat bioassay should be repeated using higher doses to achieve a level of exposure which allows an unequivocal assessment of 2,4-D's oncogenic potential.

2. Acceptability of the Mouse Oncogenicity Study:

At the initial Peer Review Committee meeting on 2,4-D the Committee had concluded that the high dose in the mouse bioassay was not sufficiently high to adequately test the oncogenic potential of this chemical. After examination of the NTP pathologists statement concerning the lack of effects seen at the high dose in the interim sacrifice, data which was not originally available to the first Peer Review Committee meeting and the SAP, the Committee concluded that an additional mouse bioassay should be performed using higher doses to achieve an MTD.

3. Categorization

The Peer Review Committee had originally classified 2,4-D as an interim category C oncogen. The basis for this decision was on increased incidence of astrocytomas seen in high dose male rats. The Committee had acknowledged in its first meeting that the data were weak, with a borderline p-value of 0.054. However, after re-examination of the data the Committee agreed with the SAP that the evidence for oncogenicity was not strong, and would categorize 2,4-D as a category D oncogen pending receipt of the repeat bioassays, and additional forthcoming epidemiological data.

To: M. V. Gemert

I do not wish to concur.
I believe that this complaint warrants
agency wide consideration

Robert Beble



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC 17 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Second Peer Review of 2,4-Dichlorophenoxy Acetic Acid
(2,4-D)

FROM: John A. Quest, Ph.D. *J.A. Quest 12/7/87*
Team Leader, Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

To: Doug McKinney, Product Manager #69
Registration Division (TS-767C)

The Peer Review Committee has evaluated the conclusions of the Science Advisory Panel (SAP) of June 4, 1987, and new information submitted by the National Toxicology Program (NTP) concerning the Maximum Tolerated Dose (MTD) for the chronic studies on 2,4-D. The Peer Review Committee concludes that the maximum tolerated dose had not been reached in either the mouse or rat chronic/oncogenicity studies on 2,4-D and recommends that both studies be repeated using a high dose of 90 mg/kg/day with an intermediate dose of 45 mg/kg/day, and further recommends that all tissues be evaluated histopathologically rather than just brain tissue as originally recommended.

1. Peer Review Committee (Signature indicates concurrence with the Peer Review unless otherwise stated.)

Anne Barton

Robert Beliles

Jerome Blondell

William Burnam

Reto Engler

Theodore M. Farber

Judith Hauswirth

Richard Hill

Richard Levy

John Quest

Esther Rinde

Anne Barton
Robert Beliles
Jerome Blondell
Wm. Burnam
Reto Engler
Theodore M. Farber
Judith Hauswirth
Richard Hill
Richard Levy
John A. Quest
Esther Rinde

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2. Scientific Reviewers: (Noncommittee members responsible for presentation of data: signature indicates technical accuracy of panel report.)

Marcia van Gemert (Section Head)

Marcia van Gemert

Material Reviewed:

Dr. Jeffrey Collins, chemical manager of 2,4-D for NTP submitted to EPA a package of information he presented to the NTP Toxicology Design Review Committee on October 10, 1985. This package (appended) provided a detailed description of the review by four NTP pathologists of the relevant tissue sections from the animals of the Industrial Task Force for 2,4-D Research Data subchronic studies, as well as the NTP's evaluation of the pharmacokinetic data utilized to set the chronic study doses.

NTP had concerns about the Task Force's dose selection rationale for their chronic studies of 2,4-D. NTP pathologists after reviewing kidney sections of male and female rats from the 0, 15, 60, 100 and 150 mg/kg dose groups of the subchronic study unanimously agreed that the kidney lesions were minimal in severity and clearly not life-threatening, even at the highest dose tested. Treated female rat kidneys did not differ appreciably from controls, and treated male rat kidneys only showed an increased incidence of epithelial regeneration at the higher doses. Treated male rat kidneys also displayed an altered tinctoral property of the tubular cell cytoplasm at all doses which was not seen in controls. NTP speculated that this tinctoral alteration may reflect either decreased cytoplasmic α_2 -globulin or an artifact of fixataion or staining. NTP pathologists did not consider this effect on the kidney to be compound-related.

NTP, after reviewing the pharmacokinetic data on 2,4-D concluded that the active transport system involved in elimination of 2,4-D is saturated at doses of 50 mg/kg. However, they stated that the glomerular filtration mechanism does not appear to be saturated at this dose and the saturation of the organic acid transport system doesn't appear to lead to any significant toxicity. In addition, the pharmacokinetic studies were by gavage, and are not directly comparable to the kinetics seen in oral feeding studies.

NTP also examined the 1-year interim sacrifice data for rats and mice at the high dose (45 mg/kg) in the chronic studies. They noted even less kidney pathology at 45 mg/kg at 52 weeks than was seen in the 13-week subchronic studies. At the final rat sacrifice a decrease in body weight in high dose females was claimed, but NTP scientists stated the data did not appear to support this conclusion.

The conclusion of the NTP scientists at the time of the appended documents writing and at present is that the Task Force chronic studies were probably not tested at the MTD.

The initial decision by the Peer Review Committee was that both the mouse and rat study be repeated using higher doses to achieve an MTD and only brains be examined. However, after reanalysis of the data the Peer Review Committee now recommends that both studies be repeated and all tissues be examined for histopathology. The SAP disagreed with the Peer Review Committee's classification of 2,4-D as an interim category C oncogen, giving it a category D classification. The Peer Review Committee still considers 2,4-D an interim category C oncogen, but will re-evaluate this position after the repeat oncogenicity studies in rat and mouse have been received by EPA.



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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October 6, 1987

National Institutes of Health
National Institute of
Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, N.C. 27709

Dr. Marcia van Gemert
U.S. EPA
Toxicology Branch (TS-769C)
Office of Pesticide Programs
401 M Street, SW
Washington, DC 20460

Dear Marcia:

Pursuant to our recent phone conversation, I have attached the relevant portions of two documents which clearly describe the National Toxicology Program's (NTP) previous evaluation of the data (particularly the histopathologic findings) generated by the subchronic studies of 2,4-Dichlorophenoxyacetic acid (2,4-D) carried out under the sponsorship of the Industry Task Force on 2,4-D Research Data (ITFRD), data which was used to select doses for the subsequent ITFRD-sponsored chronic studies of 2,4-D.

Attachment I is from the package of information which I presented to the NTP's Toxicology Design Review Committee on October 10, 1985, and provides a relatively detailed description of the review by four NTP pathologists of the relevant tissue sections from animals of the ITFRD's subchronic studies, as well as the NTP's evaluation of the pharmacokinetic data utilized to set the chronic study doses. Attachment II, which is derived from a package of materials submitted by me to the NTP on March 2, 1987 in support of my conversion from an Expert appointment to a permanent Civil Service position, further describes the history of NTP's consideration of the testing of 2,4-D, including a summary of our evaluation of the subchronic data used to select the ITFRD chronic study doses, as well as subsequent input from the EPA.

I believe the attached materials provide the information you requested. If I can be of any further assistance in this matter, please don't hesitate to contact me. I would appreciate your letting me know as soon as the EPA makes a final decision as to how they are to proceed with additional testing of 2,4-D.

Sincerely,

Jeffrey J. Collins, Ph.D.
Chemical Manager, 2,4-D

Attachments

cc: Dr. E. McConnell
Dr. J. Selkirk

Studies Sponsored by the Industry Task Force on 2,4-D Research Data:

The Industry Task Force on 2,4-D Research Data was established in 1980 in response to a Call for Data by the EPA. Over the past 5 years this Task Force has sponsored various acute, subchronic, chronic and special studies on 2,4-D in rats and mice. After reviewing summaries of the results provided by the Task Force, serious concerns arose with respect to the doses selected for the current chronic dosed feed studies of 2,4-D being carried out by the Task Force in F344 rats (0, 1, 5, 15, or 45 mg/kg) and B6C3F₁ mice (0, 1, 15, or 45 mg/kg). These doses were selected primarily on the basis of pharmacokinetic data and on the results of the Task Force's subchronic dosed feed studies, particularly the induction of kidney pathology in rats and mice at the higher doses tested (rats [two studies] - 0, 1, 5, 15, 45, 60, 100 or 150 mg/kg; mice [one study] - 0, 5, 15, 45 or 90 mg/kg). It should be noted that other than the kidney lesions and some alterations in certain organ weights, no signs of toxicity were apparent in rats or mice in the Task Force's subchronic studies.

In light of the concerns with the Task Force's dose selection rationale for their chronic studies of 2,4-D, a meeting was held at NIEHS on August 13, 1985 at which representatives of the Task Force presented data from their subchronic and pharmacokinetic studies of 2,4-D which were instrumental in selecting doses for the current chronic studies. In addition, some results from the 1-year interim sacrifices of rats and mice in the 2-year chronic study were presented. Lastly, the Task Force made available slides of the kidney lesions observed in their subchronic studies for examination by NTP pathologists.

This exchange of information appears to have validated the original concerns of the NTP with the doses selected for the Task Force's 2,4-D chronic studies. NTP pathologists reviewed kidney sections of male and female rats from the 0, 15, 60, 100 and 150 mg/kg dose groups of the subchronic study. They were unanimous in evaluating the kidney lesions as minimal in severity and clearly not life-threatening, even at the highest dose tested in the subchronic study. No appreciable differences were observed between the kidneys from control and treated female rats. An increased incidence of epithelial regeneration was present in males from the higher dose groups. In all treated male rats there was an altered tinctorial property of the cytoplasm of the tubular cells not present in controls, but this change was not useful for separating the different dose groups. The cause of this alteration was not determined, although it may reflect either decreased cytoplasmic α_2 -globulin in treated males or an artifact of fixation or staining. NTP pathologists did not consider this finding to be a chemical effect on the kidney.

The pharmacokinetic data presented did appear to support the conclusion that the active organic acid transport system involved in elimination of 2,4-D is saturated at doses ~50 mg/kg. However, the kidney (glomerular) filtration mechanism does not appear to be saturated at this dose and it was not clear that saturation of the organic acid transport system resulted in any significant toxicity. It should further be noted that the pharmacokinetic data presented was derived from gavage administration of a bolus of radioactively-labeled 2,4-D whereas the toxicologic testing of

2,4-D is being carried out by the dosed-feed route of administration. Thus, the doses being examined may not be directly comparable and given the intermittent feeding by rodents and the rapid clearance of 2,4-D via the urine it seems likely that the level of 2,4-D which the test animals are actually exposed to in the Task Force's 2-year chronic studies will be well below saturation of the active elimination system. While the Task Force stated at the meeting that results of studies with the related compound 2,4,5-T were also used in selecting doses for the 2,4-D chronic studies, this was not mentioned in the formal dosage selection rationales contained in the study protocols for the chronic studies provided previously by the Task Force.

The 1-year interim sacrifice data presented indicates minimal toxicity in rats and mice at the high dose tested (45 mg/kg). Increased kidney weights were observed in high dose male rats and high dose female mice but even less kidney pathology was seen at 45 mg/kg after 52 weeks than had been evident at this dose in the previous 13-week subchronic studies. Some data was presented on terminal sacrifice rats (mice are in the second year of the study) which indicated no effects on survival. While a decrease in final body weights in high dose female rats was claimed, the data did not appear to support this conclusion.

In summary, after analyzing the data presented by the 2,4-D Task Force which was utilized in selecting doses for the current 2-year chronic studies of this chemical, including direct examination of the relevant tissue sections from the subchronic studies, the consensus of the NTP participants is that the Task Force chronic studies are probably not being performed at the MTD. Considering that three dose levels are being tested in the mouse chronic studies and four were tested in the rat chronic studies, it is inexplicable why a broader range of doses was not examined, particularly in the latter case where significant toxicity at a higher dose would still have allowed a three dose study. In light of this conclusion, the course of action for NTP testing of 2,4-D described in the following section is recommended.

Rationale for Testing Recommendations:

Despite the considerable toxicity testing of 2,4-D which has previously been carried out (see Background), including that sponsored by the Industry Task Force on 2,4-D Research Data, it is clear that the possible carcinogenicity of this compound still remains undefined, in accord with IARC's earlier conclusion (1,47a). Thus, with the goal of providing adequate data for assessing the carcinogenicity of 2,4-D, it is recommended that the NTP perform prechronic testing of this compound. The purpose of the 14-day repeated administration and 13-week subchronic testing will be to ensure appropriate dose selection for possible 2-year chronic studies. However, it is recommended that no decision be made on whether the NTP will carry out 2-year chronic studies of 2,4-D until the final reports of the Task Force-sponsored chronic studies are available (estimated dates: F344 rats - 6/86; B6C3F₁ mice - 6/87). In light of the fact that the Task Force sponsored no 14-day repeated administration studies, but instead selected doses for the subchronic studies on the basis of a variety of acute toxicity studies, it is particularly important that

repeated administration studies be included in the NTP prechronic testing so as to facilitate accurate dose selection for the subsequent 13-week subchronic studies. It is quite possible that this approach will result in higher doses being selected for the subchronic studies than were used in the Task Force-sponsored 13-week studies. It is also proposed that neurobehavioral testing be included in the subchronic studies given the reported effects of 2,4-D on the central nervous system (24-26) and on neuromotor functions (14). It should be noted that the only Task Force-sponsored neurotoxicity studies of 2,4-D were carried out in F344 rats exposed for 3 weeks by the dermal route but that no dosed feed neurotoxicity studies have been performed.

Although human exposure to 2,4-D occurs by three primary routes, dermal, oral and inhalation (with an apparent order of importance of dermal > oral > inhalation [117]), it is proposed that testing be carried out by only one route since the available data demonstrates clearly that systemic exposure to 2,4-D can be achieved by all these routes of administration. Thus, the most convenient route providing systemic exposure has been selected and it is proposed to carry out the prechronic testing of 2,4-D by the dosed feed route of administration. Note that the Task Force-sponsored subchronic and chronic studies of 2,4-D have also used the dosed feed route of exposure.

5. 2,4-Dichlorophenoxyacetic acid (2,4-D)

Subsequent to the nomination of 2,4-D to the NTP by Dr. Rall, Director, NIEHS/NTP, I was asked by Dr. Kluwe to assume Chemical Manager responsibilities for this chemical in December, 1984. In the course of preparing a package on 2,4-D for presentation to the TQRC, I discovered the existence of an Industry Task Force on 2,4-D (denoted Industry Task Force on 2,4-D Research Data [ITFRD]), constituted in 1980 in response to a Call for Data by the EPA. I subsequently established an ongoing communication with a member of the ITFRD, Dr. David Eisenbrandt of the Dow Chemical Co. At my request, he provided me with certain materials which summarized the various acute, subchronic, chronic, and special studies of 2,4-D that the ITFRD had sponsored over the previous 5 years. Review of these materials raised concerns on my part as to the adequacy of the doses selected for the then current (mid-1985) ITFRD-sponsored chronic dosed-feed studies of 2,4-D being carried out in F344 rats and B6C3F₁ mice. Additional review by Dr. Montgomery, NTP, affirmed these concerns and concluded that to properly evaluate the dose setting criteria used for the chronic studies it would be necessary to examine directly the microslides of the kidney lesions from the 90-day studies which represented the major basis for the chronic doses selected.

Because of the above concerns, and after consultation with additional staff at NTP, including Drs. Bristol (then Acting Chief, CTEB) and Chhabra, I proposed to Dr. Eisenbrandt in July, 1985, that ITFRD representatives make a presentation to appropriate NTP staff in order to clarify the dose selection rationale used for their chronic studies of 2,4-D, including a presentation of data from their previous subchronic studies. In addition, data from the 1-year interim sacrifices of their current 2-year chronic studies in rats and mice could be presented. Lastly, I

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ITFRD-sponsored subchronic studies, which were instrumental in selecting doses for their chronic studies, be brought to NIEHS for evaluation by NTP pathologists.

After considerable negotiations, the ITFRD accepted my proposal and a meeting was held at the NIEHS on 8/13/85. Present were six members of the ITFRD (Drs. R. Fears, D. Eiserbrandt, R. Nolan, and R. Kociba of Dow Chemical Co., Dr. D. Serrone of Biotech, and Dr. R. Wilson of PBI/Gordon Corp.) and approximately 15 NTP staff members. The ITFRD members presented results from their subchronic and pharmacokinetic studies of 2,4-D (this was the first opportunity that I or other NTP staff had had to review actual data from the ITFRD studies), discussed the rationale for dose selection for their 2-year studies, and presented some interim results (1-year) from their ongoing 2-year studies of 2,4-D in rats and mice. In addition, that same day, but prior to the meeting, Drs. Boorman, Montgomery, Elwell and Uraih, NTP, examined slides of the kidney lesions observed in the ITFRD-sponsored subchronic studies, these lesions being among the major criteria used for dose selection for the ITFRD-sponsored chronic studies.

Based on the presentations and observations at the ITFRD-NTP meeting, as well as the histopathologic review conducted by NTP pathologists, my previous concerns as to whether the ITFRD-sponsored 2-year chronic studies of 2,4-D in rats and mice were being performed at appropriate dose levels (i.e., at doses approximating the MTD) were not only supported by other NTP staff, but, if anything, heightened. This was subsequently reflected in my recommendations to the TDRC on 10/10/85, which called for the NTP to proceed with prechronic testing of 2,4-D, including neurobehavioral studies, with no decision to be made on chronic testing until results of the ITFRD-sponsored 2-year studies were evaluated. While the TDRC approved the proposed study design, I was asked to obtain further input from the EPA before a final decision could be made as to whether or not the NTP should proceed with prechronic testing of 2,4-D.

I wrote to Dr. Henry Spencer of the EPA on 10/21/85 (with copies to Drs. T. Farber, J. Moore, and L. Rosenstein of the EPA) and requested that the EPA indicate whether they approved of the study design of the ITFRD-sponsored chronic studies of 2,4-D, particularly the doses being tested, and whether the results of these studies, whether positive or negative for carcinogenicity, would be accepted as valid by the EPA. In addition, if EPA agreed that further testing of 2,4-D was warranted, I requested input as to EPA's position as to the most appropriate means of performing such studies (e.g., the NTP, the ITFRD, other?). I also provided him with a copy of my 2,4-D package presented to the TDRC on 10/10/85.

~~Dr. Spencer of the EPA replied in a letter (dated 12/10/85) that while the EPA had no specific objections to the design of the ITFRD-sponsored chronic studies of 2,4-D, it was their general "policy that the responsibility of choosing dosages of test chemicals was solely that of the registrant."~~ Furthermore, the EPA proposed that no further testing of 2,4-D be conducted until the results of the ITFRD chronic studies could be reviewed and evaluated. If the study was deemed inadequate at that time, then the EPA would require the registrants to provide further testing. Based on this response from the EPA, Drs. Rall and McConnell decided on 12/17/85 that any plans for NTP studies of 2,4-D should be deferred until further notice, a decision which was reaffirmed to me by Dr. McConnell in September, 1986. It should be noted that preliminary evaluation (August, 1986) of the data from the ITFRD-sponsored 2-year studies of 2,4-D in F344 rats indicated a statistically significant increased incidence of brain tumors (astrocytomas) in high-dose (45 mg/kg) males. The EPA is currently conducting an independent histopathologic evaluation of this study.

In the meantime, I have submitted an expanded and modified version of my TDRC package on 2,4-D as a review article entitled, "The Toxicology of 2,4-Dichlorophenoxyacetic Acid (2,4-D)" (Appendix II-5) for publication in Reviews of Environmental Contamination and Toxicology. It is currently undergoing review by this journal. In addition, at the request of Dr. Canter, NTP, in October, 1986, I provided Dr. Fraumeni of the NCI with a package of material on 2,4-D, including my TDRC package, my letter to EPA of 10/21/85, EPA's response of 12/10/85, and the 8/86 summary of the ITFRD's rat chronic study results. Lastly, at Dr. McConnell's request, in January, 1987, I reviewed the WHO's Draft of Environmental Addendum for Environmental Health Criteria No. 29: 2,4-Dichlorophenoxyacetic Acid and provided comments to Dr. Mercier of the WHO.

7/3/87

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JUL 29 1987

MEMORANDUM

OFFICE OF
HEALTH, HAZARD AND TOXIC SUBSTANCES

SUBJECT: Peer Review of 2,4-Dichlorophenoxy Acetic Acid
(2,4-D)

FROM: John A. Quest, Ph.D. *JAK*
Team Leader, Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Doug McKinney, Product Manager #69
Registration Division (TS-767)

The Toxicology Branch Peer Review Committee met on April 23, 1987 to discuss and evaluate the weight-of-the-evidence on 2,4-D, with particular reference to its oncogenic potential.

A. Individuals in Attendance

1. Peer Review Committee: (Signature indicates concurrence with the peer review unless otherwise stated.)

Anne Barton

Anne Barton

Robert Beliles

Jerome Blondell

William Burnam

William J. Burnam

Reto Engler

Reto Engler

Theodore M. Farber

Theodore M. Farber

Judith Hauswirth

Judith W. Hauswirth

Richard Hill

Richard Hill

Louis Kasza

Louis Kasza

Richard Levy

Richard A. Levy

John Quest

John A. Quest

Esther Kinne

Esther Kinne

2. Scientific Reviewers: (Noncommittee members responsible for presentation of data; signature indicates technical accuracy of panel report.)

Marcia Van Gemert (Section Head) Marcia Van Gemert

3. Peer Review Members in Absentia: (Committee members who were not able to attend the discussion; signature indicates concurrence with the overall conclusions of the Committee.)

Diane Beal Diane Beal

4. Other Attendees: The following individuals from the Office of Pesticide Programs, Office of Research and Development, and Office of Program Planning and Evaluation attended the peer review meeting: David G. Anderson, David L. Bayliss, Henry M. Jacoby, Kathleen Knox, Doug McKinney, C. J. Nelson, and Robert P. Zendzian.

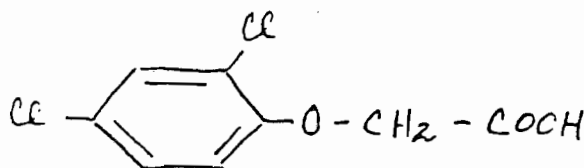
B. Material Reviewed

The material available for review consisted of a summary of toxicology data on 2,4-D (prepared by Dr. Van Gemert), DER's on rat and mouse oncogenicity studies, a 1984 WHO report on 2,4-D, consultant pathologist reports on 2,4-D, a memorandum dated 6/5/85 by Dr. Kasza on the oncogenicity of 2,4-D in Sprague-Dawley rats, information on, and reviews of the NCI epidemiology study of 2,4-D, and Toxicology Branch "one-liners" on 2,4-D.

C. Background Information

2,4-D is a growth regulator and herbicide that has been used for 40 years on broad leaf plants. Oncogenicity studies have been performed in CDF (F344/CRL-BR) rats and in B6C3F₁ Crl Br mice at Hazelton Laboratories. The primary focus of the Peer Review Committee was on the CDF rat study in which brain astrocytomas were observed and on the epidemiology studies. No significant increases in tumors were reported in the chronic study in mice.

Structure:



D. Evaluation of Oncogenicity Studies1. Rat Oncogenicity Study:

Reference: Combined Toxicity and Oncogenicity Study in Rats, 2,4-Dichlorophenoxyacetic Acid, Final Report. Hazleton Labs, 9200 Leesburg Turnpike, Vienna, Virginia, May 29, 1986

2,4-D (technical grade, 97.5% purity) was administered in the diet to groups of 60 CDF (F344/Crl-BR) rats of each sex at levels of 0, 1, 5, 15 and 45 mg/kg/day for 2 years. In each of the above experimental groups, 10 rats/sex were subjected to interim sacrifice at 53 weeks. Table 1 illustrates the increased incidence pattern of brain astrocytomas suggestive of a compound-related effect in male rats. No tumor response related to 2,4-D administration was observed in female rats.

Table 1. Incidence of Brain Astrocytomas in 2,4-D Treated Male CDF (F344/Crl-BR) Rats

Tumor Type	Dose (mg/kg/day)				
	0	1	5	15	45
Astrocytoma	1/60 ^a	0/60	0/60	2/58 ^b	6/60 ^{*,c}

* = Statistically significant ($p < 0.05$) positive dose-related trend.

a = Tumor was observed in control male that died on week 21.

b = Tumors were found in males killed in extremis on weeks 94 and 105.

c = Tumors were found in one male killed in extremis on week 93 and in five other males at final sacrifice at week 104.

2,4-D was associated with a statistically significant positive dose-related trend for astrocytomas in male rats. However, the increased incidence of tumors seen at the high dose level per se (i.e., 6/60) was not statistically significant when compared to the controls by the Fisher-Exact test. In addition to the pathology evaluation of brain tumors provided above by the test laboratory, two other independent pathologists also reviewed the brain slides of 2,4-D treated animals. Dr. Koestner (Michigan State University) differed from the original pathology diagnosis in that he believed that one of the astrocytomas seen in a high dose male rat actually had a mixed glial and mesenchymal cell population. Dr. Swenberg (CITC), however, confirmed the original pathology diagnosis as shown in Table 1.

One of the independent pathology consultants (Dr. Koestner) provided historical data on brain gliomas in Sprague-Dawley rats from studies on FD&C dyes. This information, which described a historical range of 0%-10% for gliomas, was not considered to be appropriate for comparison to the 2,4-D study where F344 rats were used. Instead, historical data from the NTP in male F344 rats was considered; the incidence of astrocytomas reported by the NTP for males was 9/2301, or 0.4 + 1.0% (no range data was available) (Toxicologic Pathology 12: 126-135, 1984). However, the NTP studies used considerably fewer slices/brain than the Hazelton study, making them inappropriate as historical controls.

Additional toxicological changes produced by 2,4-D in male rats included an increased incidence in brown pigment in renal tubular cells (seen at doses of 5, 15 and 45 mg/kg/day), an increased incidence of renal microcalculi (seen at doses of 15 and 45 mg/kg/day), and increases in liver weight, serum alanine aminotransferase levels, the albumin/globulin ratio and thyroid/parathyroid weights (seen at a dose of 45 mg/kg/day). Based on these changes, a MTD did not appear to be reached in male rats in the 2,4-D study. In the case of toxicological changes produced by 2,4-D in female rats, the findings were somewhat more marked and suggested that 45 mg/kg/day may have been closer to a MTD level. The changes seen in females at this dose included reduced body weight gain (approximately -5 to -10%), the above mentioned renal changes seen in male rats, and the more marked renal finding of an increase in the frequency and severity of fine vacuolation of the cytoplasm in the renal cortex (this latter change occurred only at the 53 week interim sacrifice period but not at the 104 week terminal sacrifice period in females, indicating that regression had occurred with this event).

The Committee recommended that a repeat, modified, oncogenicity study of 2,4-D be performed in F344 male and female rats using greater numbers of animals and higher doses of 2,4-D than previously employed. However, only the brain (numerous sections) should be examined for tumors. This recommendation was made because of concern that a MTD level may not have been attained in the previous study and, more importantly, because of the high population exposure and wide use of 2,4-D that is currently prevalent in the U.S.

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2. Mouse Oncogenicity Study:

Reference: Oncogenicity Study in Mice with 2,4-Dichlorophenoxyacetic Acid. Hazelton Labs, 9200 Leesburg Turnpike, Vienna, Virginia (undated report).

2,4-D (97.5% purity) was administered in the diet to groups of 60 B6C3F₁ CRL-BK mice of each sex at levels of 0, 1, 15 and 45 mg/kg/day for 24 months. In each of the above experimental groups, 10 mice/sex were subjected to interim sacrifice at 52 weeks. No oncogenic effects attributable to compound administration were noted in either male or female mice.

The Committee agreed that none of the doses tested in male or female mice reached an MTD level. The only toxicological changes seen with 2,4-D in this study were an increase in adrenal gland weights (mid and high dose males), an increase in kidney weights (mid dose females, and high dose males and females), and an increase in the cytoplasmic homogeneity of renal tubular epithelium which was due to a reduction in cytoplasmic vacuoles (mid and high dose males).

The Committee recommended that a repeat oncogenicity study of 2,4-D be performed in B6C3F₁ male and female mice using the standard protocol described in EPA's Subpart F Guidelines. The reasons for this recommendation were the same as those expressed above for requiring a repeat rat oncogenicity study.

E. Additional Toxicology Information:

1. Mutagenicity:

The 1984 WHO report on 2,4-D indicated that the studies available at present are not adequate for the evaluation of the mutagenic effects of 2,4-D and its derivatives in short term tests. Dr. Hill of the Peer Review Committee presented some NTP data on the mutagenicity testing of 2,4-D, wherein sister chromatid exchanges were increased in Chinese hamster ovary (CHO) cells, but no positive findings were observed for cytogenetics in CHO cells, for the Drosophila sex-linked recessive lethal test, or for the Ames test with and without metabolic activation.

2. Reproduction and Teratology:

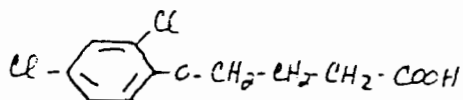
2,4-D was not teratogenic to the rat but did cause fetotoxicity (slight increase in delayed ossification) at an oral dose of 75 mg/kg, p.o. In a 2-generation reproduction study, 2,4-D administered to rats produced renal tubular degeneration in F₀ and F₁ generation males, and reduced pup weights in the F_{1b} generation. The lowest-effect-level for these changes was an oral dose of 20 mg/kg/day p.o.

3. Metabolism:

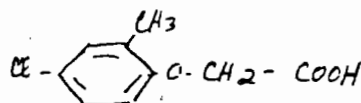
An extensive description of the pharmacokinetics of 2,4-D in animals and man following dermal and oral exposures (the two most important ones in terms of human toxicity), and also inhalation exposure, is provided in the 1984 WHO report. In brief, 2,4-D is not well absorbed through skin, but is fairly well absorbed orally and the volume of distribution is 20-50% of body mass as volume. 2,4-D does not appear to be significantly metabolized. However, the metabolite 2,4-dichlorophenol (2,4-DCP) can be found as a residue in ruminants, probably due to bacterial degradation of 2,4-D in the rumen. 2,4-D conjugates have also been found in urine of several species, including man. 2,4-D is mainly excreted in urine, and to a lesser extent in feces. Half-life in humans from a single exposure can be from 24 to 48 hours.

4. Structure Activity Relationships:

2,4-D is structurally related to the following six herbicides:

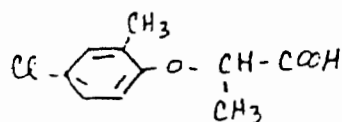


2,4-DE
(2,4-Dichlorophenoxy
acetic acid)

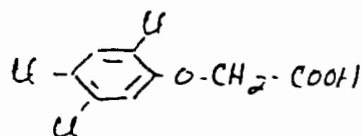


MCPA
(4-Chloro-2-methylphenoxy
acetic acid)

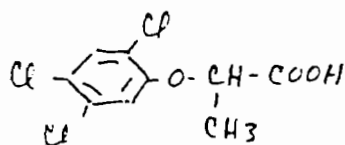
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Structure Activity Relationships: (Cont.)

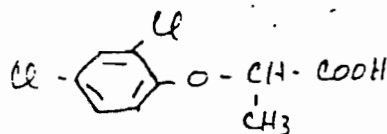
MCPP
(2,4-Dichloro-2-methylphenoxy
propionic acid)



2,4,5-T
(Trichlorophenoxyacetic
acid)



Fenoprop
(2,4,5-Trichlorophenoxy
propionic acid)



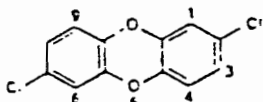
2,4-DP
(2,4-Dichlorophenoxy-2-
propionic acid)

The following information regarding oncogenicity testing of these chemicals was available to the Committee: (1) 2,4-DB, MCPA and MCPP have not been tested chronically in rodents; (2) 2,4,5-T was not found to be oncogenic in studies in rats and mice, but its contaminant TCDD did produce liver hyperplastic nodules and liver carcinomas in rodents; (3) Fenoprop was not oncogenic in rats; a mouse study on this compound was not available to the Committee; and (4) 2,4-DP was not oncogenic in mice or in one strain of rats (F344), but was reported to cause thyroid, pituitary and brain tumors in another strain of rats (Sprague Dawley). The latter study, however, contains inadequacies in pathology evaluations and data reporting which render it difficult to evaluate (see Dr. Kasza's memorandum of 6/5/85 on 2,4-DP).

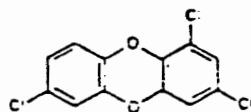
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5. Contaminants Found in 2,4-D:

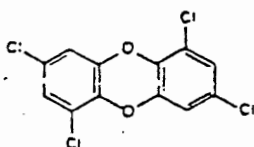
2,4-D formulation contain several potentially hazardous dioxin contaminants, including di-, tri-, and tetrachlorodibenzo-p-dioxins (structures shown below) and N-nitrosamines. Of the contaminant dioxins, only 2,7-dichlorodibenzo-p-dioxin has been tested for oncogenicity; this chemical was negative in the male and female rats, negative in female mice, and equivocal in male mice.



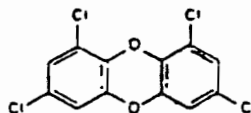
2,7-dichlorodibenzo-p-dioxin



1,3,7-trichlorodibenzo-p-dioxin



1,3,6,8-tetrachlorodibenzo-p-dioxin



1,3,7,9-tetrachlorodibenzo-p-dioxin

6. Subchronic and Chronic Toxicity Data:

A variety of short and long term toxicology studies have been performed in animals using 2,4-D. These studies have been evaluated in the 1984 WHO report on the chemical. Dr. van Gemert's summary document on 2,4-D has noted that the observed subchronic and chronic effects include vomiting, diarrhea, muscle weakness, muscle spasms (myotonia), reduced food and water consumption, weight loss, CNS depression, damage to myocardium, various hematological and blood chemistry changes, hepato- and nephrotoxicity and endocrine organ toxicity.

7. Epidemiology Data:

Information was available to the Committee regarding a population-based case control study conducted by the NCI in Kansas where a relationship was found between farm herbicide use (phenoxyacetic acids) and non Hodgkin's lymphoma (J.A.M.A. 256: 1141-1147, 1986), but not between herbicide use and soft tissue sarcoma or Hodgkin's disease. The information was presented by Mr. Blondell and discussed at length by individuals present at the meeting. The consensus reached was that the overall data was good with respect to phenoxyacetic acid herbicides, but insufficient in the specific case of 2,4-D. As a result of this, plus the fact that there were difficulties in interpreting the language used to classify epidemiology studies in the EPA guidelines, there was a difference of opinion as to whether the study provided "limited" or "inadequate"

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human evidence of carcinogenicity. The Committee was informed however that additional epidemiology data regarding the farm use of herbicides, including specific data on 2,4-D, would be available in the near future, and thus deferred a final epidemiological categorization of 2,4-D until the new data was available for review.

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on 2,4-D to be of importance in a weight of the evidence determination of oncogenic potential.

1. Administration of 2,4-D was associated with a statistically significant positive dose-related trend for brain astrocytomas in male CDF (F344/CRL-BR) rats. This original diagnosis was confirmed by two other consulting pathologists who reviewed the same data.

2. The increases in astrocytomas produced by the two highest dose levels of 2,4-D in treated male rats (i.e., 2/58 or 3.4% at 15 mg/kg/day; and 6/60 or 10% at 45 mg/kg/day) were not statistically significantly elevated per se when compared to control male rats (i.e., 1/60 or 1.6%) by the Fisher Exact test. However, the increased incidences observed in the treated animals (and also that observed in the controls) exceeded the historical control incidence of astrocytomas ($0.4 \pm 1.0\%$) in recent studies conducted by the NTP.

3. The highest dose level of 2,4-D tested in male rats (45 mg/kg/day) did not appear to approximate a MTD level. This dose produced minor renal changes (pigmented tubular cells and pelvic microcalculi) plus liver weight and enzyme increases. The same dose level in female rats (where no tumors occurred) appeared to be somewhat closer to a MTD level based on findings of body weight gain decrements (5% to 10%) and renal cortex cytoplasmic vacuolation at the middle but not at the end of the study. Because of the impression that higher doses of 2,4-D could have been tested in this study, and the fact that the chemical is widely used with high public exposure, the Committee recommended that a repeat study be performed in male and female rats using higher doses and more animals (but with examination of only the brain for the presence of tumors in the male rats).

4. 2,4-D was not oncogenic when administered in the diet to B6C3F₁ CRL-BR mice at dose levels ranging from 1 to 45 mg/kg/day. A MTD level was not reached in this test; because of this and the wide use and high exposure associated with 2,4-D, the Committee recommended that a repeat study be performed in male and female mice according to Subpart F Guidelines using higher dose levels of the compound.

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5. 2,4-D was negative in several short term assays for mutagenicity. The only positive mutagenicity results obtained involved an increase in sister chromatid exchanges in Chinese hamster ovary cells.

6. 2,4-D was not teratogenic in the rat, but did cause fetotoxicity manifested as a slight increase in delayed ossification. In a 2-generation reproduction study it caused renal tubular degeneration in P₀ and F₁ males and reduced weight in F₁₀ pups.

7. 2,4-D is structurally related to six other herbicides: 2,4-DB; MCPA; MCPP; 2,4,5-T; Fenoprop; and 2,4-DP. The first 3 chemicals have not been tested for oncogenicity in rodents. The fourth and fifth chemicals were tested in rodents and found to be negative; however, a dioxin contaminant (i.e., TCDD) of one of the chemicals (i.e., 2,4,5-T) did cause liver hyperplastic nodules and carcinomas in rodents but this contaminant is not found in 2,4-D. Finally, the sixth chemical was reported to cause thyroid and brain tumors in rats but numerous inadequacies in this study precluded its evaluation by the Toxicology Branch.

8. Epidemiology data from a population-based case control study in Kansas suggested that phenoxyacetic acid herbicide usage is associated with non-Hodgkins lymphoma in farmers. It was not possible to specifically identify 2,4-D as a causative agent in this study. The Committee deferred a carcinogenic weight of the evidence classification of the epidemiologic data using EPA Guidelines pending the receipt of further human data involving 2,4-D in the near future.

6. Classification of Oncogenic Potential:

The Committee concluded that the data available for 2,4-D provided only limited evidence of oncogenicity for the chemical in male rats. According to EPA Guidelines for Carcinogen Risk Assessment (Crk September 24, 1980), the Committee classified 2,4-D as a Category C oncogen (possible human carcinogen with limited evidence of carcinogenicity in animals). The Committee made this classification on an interim basis pending the receipt of additional data (see below). 2,4-D produced benign (although life-threatening) tumors incidences of ~~marginal-statistical-significance-in-one-sex-and-species~~ of animal in a single study that was inadequate in design, i.e., only a positive trend for brain astrocytomas in male CDF (F344; CRL-58) rats in a study where a MTD level did not appear to be reached. No compound-related tumors were observed in mice. In addition, mutagenicity data and structure-activity relationship information provided weak and

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relatively unconvincing support for the oncogenicity of 2,4-D. Epidemiology data on phenoxy acetic acid herbicides and non-Hodgkins lymphomas in farmers did not provide a definitive link between the use of 2,4-D per se and human oncogenesis. However, additional epidemiology data on 2,4-D were reported to be forthcoming. None of the criteria specified in the EPA Guidelines for classifying a chemical as a category B₂ carcinogen were met for 2,4-D based on the data available to the Peer Review Committee. The interim category C classification was assigned to 2,4-D pending the receipt of two additional oncogenicity studies in rodents (rats and mice) and additional epidemiology data in humans.

#19 5/19/87 sp
rew: 6/18/87

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001720

To: John H. Guest, Ph.D.

From: Robert F. Beliles RB 7/987

Subject: Peer Review of 2,4-Dichlorophenoxy Acetic Acid (2,4-D)

I do not feel that I can concur the weight-of-evidence finding on 2,4-D. I think that the brain tumors bear greater consideration. I submitted to your office a copy of the following publication:

GARMAN, R.H., SNELLINGS, W. M., and MARONFOT, R. R. (1985) Brain tumors in F344 Rats associated with chronic inhalation exposure to Ethylene Oxide. Neurotoxicol. 6:117-138.

Garman et al. (1985) have indicated that an incidence of granular cell tumors, glial cell tumors, and malignant reticulosis (tumors they reported) occurred at rates of 0.03, 0.78 and 0.05 percent among 5450 male F344 rats of untreated control groups in the National Toxicology Program/National Cancer Institute (NTP/NCI) carcinogenesis bioassays. In addition, Garman et al. (1985) have pointed out only two agents, propylene imine and propane sultone (both alkylating agents), have been associated with the development of brain tumors in rats among the compounds evaluated by the NTP/NCI bioassay program. In this bioassay series no chemical has produced brain tumors in mice. To this list may be added acrylonitrile, another alkylating agent that produced brain tumors in rats by both the oral and inhalation routes and ethylene oxide which produces brain tumors in rats, but not mice.

Brain tumors were reported in rats treated with 2,4-DP, a related compound.

RB

00720



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FILE COPY

APR 15 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review on 2,4-D

FROM: Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: David Bayless
Office of Health
and Environmental Assessment (RD-689)
and
Kathleen Knox
Office for Policy, Planning,
and Evaluation (PM-219)

Following up on our telephone conversation I am inviting you to participate as ad-hoc member of the peer-review group.

Attached is a package of the data evaluation on 2,4-D. The meeting is scheduled for Thursday, April 23, 1987, at 1:00 to 3:00 PM in Room 1119 of CM-2.

Attachment

cc: T. Farber
A. Barton
J. Blondell

007720

SAP Executive Summary

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7/26/86

007720

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

Consideration of Health Advisory on 2,4-D

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed review of the Office of Drinking Water's (ODW) Health Advisory for 2,4-D. The review was conducted in an open meeting held in Arlington, Virginia on June 26, 1986. All Panel members were present for the review, except Dr. Rosmarie von Rumker and Dr. Thomas W. Clarkson.

Public notice of the meeting was published in the Federal Register on Wednesday, June 4, 1986.

Oral statements were received from staff of the Environmental Protection Agency and from Mr. Chuck Pace, representing the National Audubon Society.

In consideration of all matters brought out during the meeting and careful review of all documents presented by the Agency, the Panel unanimously submits the following report.

REPORT OF SAP RECOMMENDATIONS

General Comments

The Panel agrees, in concept, with the EPA/ODW program of preparing health advisories on selected pesticides and other chemicals that have been or could be found in drinking water supplies. These health advisories could be useful to regional, state and local officials in assessing the severity of incidents involving drinking water contamination. However, the Panel also agrees with several ~~comments offered by the public that these documents, though prepared~~ by EPA only as "advisories," will be adopted as real or de facto laws and regulations by other agencies. Consequently, it is extremely important that these health advisories present the most scientifically defensible positions possible. Further, these documents should be reviewed and updated on a regular basis.

The Panel believes that health advisory documents should present multiple calculations of health advisories to show the extent of agreement between different studies and endpoints. These should include the use of human data, the most appropriate NOAEL, and the

most appropriate LOAEL for toxic effects in multiple species including man. The health advisories should then conclude which of the above is most appropriate and present the range upon which the conclusion is based. One approach that should be considered strongly is the presentation of the above data in a tabular form similar to the National Academy of Sciences drinking water document, 1977 Drinking Water and Health, Volume I, National Academy Press, Washington, D.C. Furthermore, specific criteria should be established for how and when each uncertainty factor, i.e., 5, 10, 100, 1000, or 10000, is to be used. Presently, the entire process appears to be proceeding in a rather whimsical manner. For instance in one example, even though several carcinogenicity studies on a chemical had been completed and deemed adequate, the lifetime health advisory was calculated using subchronic data and then divided by an additional factor of 10 for uncertainty. This resulted in a 100- to 200-fold lower health advisory than would have been calculated from the available chronic data. Since the health advisories are likely to become real or de facto regulations, much greater attention must be given to the scientific validity of these documents.

In some of the health advisories it was stated that "the chemical" may be classified as a Group B, C, etc., carcinogen. Will some of these compounds be classified by the Agency as of the effective dates of the health advisories? If so, this information should be included in the health advisories. In addition, when carcinogenic endpoints have been demonstrated for a chemical, upper and lower confidence limits and maximum likely estimates (MLE) should be included.

Although the Panel agrees with the potential utility of the 1-day health advisories, their reliability may be greatly affected by the types of data used to calculate them. Particularly, one must be certain that the endpoint, where an effect level is interpreted, is a toxic effect resulting from the chemical in question. It must be realized that the 1-day health advisories are subject to error if the effect endpoints reflect merely a physiological variation.

Lastly, the Panel recommends that the word "protective" be removed from the definition of the DWEL and substituted with a "no-effect-level."

2,4-D

The Agency requested the Panel to focus its attention upon a set of issues relating to the Health Advisory for 2,4-D and provide any comments on the scientific and technical merit of the document, focusing principally upon those sections of the document devoted to risk assessment, both qualitative and quantitative. There follows a listing of the specific questions the Agency asked the Panel and the Panel's response to each.

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1. Is it appropriate to derive allowable drinking water levels for lifetime exposure from an interim (first year's results) report of a 2-year (chronic) study?

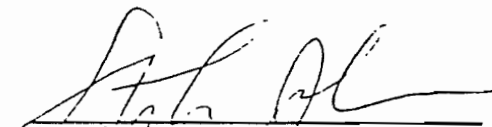
Panel Response:

It would be preferable to have data from lifetime studies (2-year studies in rats, for example), but the results from appropriate 1-year studies may be used in the absence of lifetime studies.

The Panel questions whether a thorough review of the toxicology data base on 2,4-D was accomplished in producing the health advisory.

FOR THE CHAIRMAN

Certified as an accurate report of Findings:


Stephen L. Johnson
Executive Secretary
FIFRA Scientific Advisory Panel

Date: 7/7/56

00,720

Reviewer's Peer Review Package for 1st Meeting

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

00720

APR 7 - 1987

FILE COPY

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review on 2,4-D

FROM: Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

Attached is a comprehensive package on 2,4-D prepared by Dr. M. Van Gemert for your review. In Appendix F you will also find an evaluation of epidemiological studies by Jerome Blondell.

A meeting to discuss and evaluate the weight-of-the-evidence on 2,4-D has been scheduled for Thursday, April 23, 1987, at 1:00 to 3:00 PM in Room 1119 of CM-2.

Attachment

ADDRESSEES:

T. Farber
W. Burnam
J. Quest
E. Rinde
R. Levy
L. Kasza
J. Hauswirth
J. Blondell
D. Anderson
H. Spencer
A. Barton
R. Beliles
M. Van Gemert
D. Beal
D. Barnes/R. Hill

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6/8/87

R Engler

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUN 8 - 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: 2,4-Dichlorophenoxyacetic Acid, Rat Study -
Quantitative Risk Assessment and Updated Qualitative
Risk Assessment of Combined Toxicity and
Oncogenicity Study in Rats. Caswell #315

FROM: C.J. Nelson, Statistician *C. Nelson*
Scientific Mission Support Staff 6-5-87
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Marcia van Gemert, Ph.D.
Chief, Section III
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Richard Levy, M.P.H., Leader-Biostatistics Team
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C) *Richard Levy*
6-8-87

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C) *Reto Engler*

Summary:

The Toxicology Branch Peer Review Committee met on April 23, 1987 and classified 2,4-dichlorophenoxyacetic acid (2,4-D) as an Interim Category C Oncogen. The potency estimate, Q_1^* , of 2,4-D is $1.9 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$ in human equivalents. This estimate was calculated using the Weibull 82 model and is based upon male rat astrocytomas.

Quantitative Risk Assessment:

Since there was significantly higher mortality in the control group than there was in the 1mg/kg and 5mg/kg dose groups, the potency estimate, Q_1^* , was obtained using the Weibull 82 model for extra risk. (Reference memo on Qualitative Risk Assessment of 2,4-Dichlorophenoxyacetic Acid - C. Nelson, 4/87). The resulting potency estimate in mg/kg/day was converted to human equivalents on the basis of an interspecies surface area adjustment as recommended by the EPA Cancer Guidelines.

The potency estimate based on astrocytomas in the male rat fed 2,4-D was $3.6 \times 10^{-3} \text{ (mg/kg/day)}^{-1}$, the human equivalent potency estimate was $1.9 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$. For comparison, the human equivalent potency estimate using the Crump multistage procedure was $1.7 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$.

Qualitative Risk Assessment Update:

The historical control data is not the most appropriate because the first set was from a different strain, Sprague-Dawley, and the NTP data was from a different lab. The NTP controls have fewer slices taken, and one would expect to find more tumors if more slices were taken. Since we considered the astrocytomas to be uncommon tumors, we used the Tarone adjustment to the control response. Previously we used the historical control data furnished by Marcia van Gemert. Using the astrocytoma rate from the NTP in male F344 rats of .4%, the probability⁺ of observing 6 or more such tumors out of 59 animals is less than 0.001.

+ Note: The probability was calculated using the binomial distribution with the background rate of 0.004 as the probability for an individual response.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

001720

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: The Weight of the Evidence Evaluation for the
Oncogenic Potential of 2,4-dichlorophenoxy acetic acid
(2,4-D)

From: Marcia van Gemert, Ph.D.
Head, Section III
Toxicology Branch, HED

M. van Gemert 3/31/87

Thru: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch, HED

Theodore M. Farber 3/31/87

Attached is a report prepared for the Peer Review Committee on
2,4-D. Data are provided so that a "Weight of the Evidence"
determination may be made regarding the oncogenic potential of
2,4-D.

Attachment

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- VIII. Summary of lifetime studies
 - 24-month chronic/oncogenicity rat study
 - 24-month oncogenicity mouse study
- IX. NCI epidemiology study

Appendices

- A. WHO report
- B. DERS for 2-generation reproduction study
- C. DER for teratology study
- D.
 - 1. Preliminary DER of combined toxicity and oncogenicity study in rats
 - 2. Pathology report from Dr. Koestner of Michigan State
 - 3. Review of revised pathology tables on Rat study

Appendices- cont.

D. 4. Dr. Swenberg's evaluation of brain slides

E. DER for mouse oncogenicity study

F. NCI epidemiology study reviews:

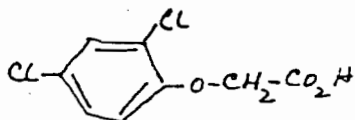
1. Summary of peer review comments
2. critical review of NCI study by Jerome Blondell, EPA
3. Review by Martha S. Linet, MD., MPH
4. Review by Leon F. Burmeister, Ph.D., U. of Iowa
5. Review by Donald P. Morgan, MD Ph.D., U. of Iowa
6. Review by Brian Macmahon, MD Ph.D.

G. Toxicology Branch "one-liners"

REPORT FOR THE PEER REVIEW COMMITTEE FOR ASSESSING
THE ONCOGENICITY OF 2,4-Dichlorophenoxy acetic acid

I. Summary:

The Industry Task Force for 2,4-D Research Data has submitted data in response to a data call in for 2,4-D. 2,4-D has been used extensively as a growth regulator and herbicide on broad-leaf plants for 40 years. A large WHO monograph was printed in 1984 summarizing all the available literature to that data and is appended for reference in Appendix A. The chemical structure is:



2,4-D contains some potentially hazardous dioxin contaminants including the di, tri and tetra chlorodibenzodioxins.

2,4-D is not well absorbed through skin, however, is fairly well absorbed orally and the volume of distribution is 20-50% of body mass as volume. 2,4-D does not appear to be significantly metabolized, however, the metabolite 2,4-dichlorophenol (2,4-DCP) can be found as a residue in ruminants, probably due to bacterial degradation of 2,4-D in the rumen. 2,4-D conjugates have been found in urine of several species, including man. 2,4-D is mainly excreted in urine, and to a lesser extent in feces. Half-life in humans from a single exposure can be from 24 to 48 hours.

2,4-D is structurally related to 2,4-DB, MCPA, Mecoprop, 2,4,5-T, fenoprop and 2,4,5-TP. 2,4,5-T and fenoprop both contain the carcinogenic dioxin TCDD. However, when both herbicides were tested in carcinogenesis bioassays with the TCDD contaminant present, the results were negative. 2,4,5-TP was found to be carcinogenic in rats but not mice.

~~Subchronic and chronic effects include vomiting, diarrhea, muscle weakness, muscle spasms (myotonia), reduced food and water consumption, weight loss, CNS depression, damage to myocardium, various hematological and blood chemistry changes, hepato- and nephrotoxicity and endocrine organ toxicity.~~

In the teratology study submitted by the Industry Task force there was a slight delayed ossification seen with no maternal effects seen. In a submitted 2-generation study in rats, nephrotoxicity was seen in F₁ and F₂ male parents with reduced pup weights seen in F₂ pups.

According to the recent (1984) WHO report the present available mutagenicity studies are inadequate to evaluate the genetic effects

effects of 2,4-D and its derivatives in short-term tests.

2,4-D was tested in the B₆C₃F₁ mouse for 24 months at doses of 0, 1, 15 and 45 mg/kg, and found not to increase the incidence of tumors. It was, however, nephrotoxic causing increased cytoplasmic homogeneity of the renal tubular epithelium due to a reduction of cytoplasmic vacuoles. Adrenal and kidney weights were effected by treatment.

A 2-year combined chronic/oncogenicity study in rats at doses of 0, 1, 5, 15, and 45 mg/kg/day revealed an increased incidence of a rare brain tumor, astrocytoma, in high dose males. The incidence in males was 1/60, 0/60, 0/60, 2/58, 6/60 for males and 0/60, 1/60, 1/60, 1/60, 1/60 for females in groups 1-5 respectively. Other effects seen included increased kidney weights, increased brown tubular pigment in kidneys from groups 3, 4, and 5, increased incidence of pelvic microcalculi in group 4 and 5 males and group 5 females. There was an increase in transitional epithelial hyperplasia of the kidney in group 5 females.

A population-based case control epidemiology study conducted by the NCI was done in Kansas where an association was found between herbicide use and non-hodgkins lymphoma. Other epidemiology studies have recently been completed and are in the process of being reviewed by Jerome Blondell and are considered negative by Mr. Blondell for an association between 2,4-D and non-hodgkins lymphoma or other tumors.

II. Contaminants:

Several potentially hazardous contaminants have been identified and recently more sensitive and specific methods have become available to quantitate them. These contaminants include the di-, tri-, and tetrachlorodibenzo-p-dioxin isomers (structures given below) and ^{Py 2A} N-nitrosamines. The most toxic CDD, namely 2,3,7,8-TCDD, is not normally found in 2,4-D products. However, there have been some cases where manufacturing equipment was used to produce both 2,4,5-T and 2,4-D resulting in cross contamination of 2,4-D with 2,4,5-T and 2,3,7,8-TCDD. Except for the NCI studies on 2,7-dichlorodibenzo p-dioxin (which was reported to be negative in rat and negative in female, equivocal in male mouse), the chronic toxicity of the CDDs found in 2,4-D has not been tested.

III. Metabolism:

This is a brief summary of the WHO text, pages 54-62 and the full WHO report is appended in appendix A for more detailed examination.

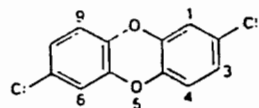
Absorption:

Inhalation: 2,4-D appears to be rapidly absorbed by inhalation, following first order kinetics with an absorption half time of 1.4 to 1.7 minutes in rats.

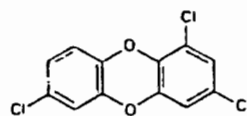
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pg 2A

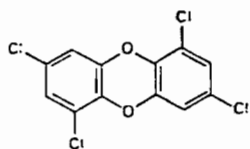
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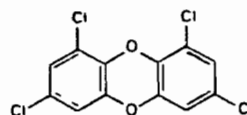
2,7-dichlorodibenzo-p-dioxin



1,3,7-trichlorodibenzo-p-dioxin



1,3,6,8-tetrachlorodibenzo-p-dioxin



1,3,7,9-tetrachlorodibenzo-p-dioxin

W-10-8 PMT

Fig. 2. Polychlorinated dibenzo-p-dioxin (CDD) byproducts.

CONTAMINANTS FOUND IN 2,4-D -
THESE CDDs HAVE NOT BEEN TESTED FOR CARCINOGENICITY

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Dermal: Some work has been done to indicate little absorption occurs through skin. One human volunteer study indicated that only 5.8% of the administered dermal dose of ^{14}C -labeled 2,4-D was excreted in urine. Occupational exposures have suggested a fairly efficient dermal exposure, however, surfactant properties of solvents have not been adequately evaluated concerning dermal penetration.

Oral: In human volunteers there was individual variation in rate and extent of absorption from the GI tract, however, absorption is fairly rapid and 2,4-D is completely absorbed from the human GI tract, and follows first order kinetics.

Distribution: There appears to be more than one physiological compartment for 2,4-D storage. The volumes of distribution appear to be 20-50% of the body mass in volume. 2,4-D is reversibly bound to plasma proteins, particularly albumins, and compete for binding sites with related compounds. Approximately 17% of an administered dose crosses the placenta in mammals.

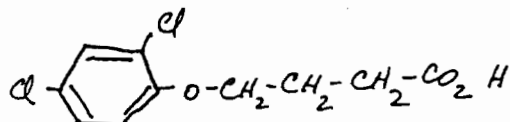
Metabolism: Rats and pigs appear to hydrolyse 2,4-D esters both in the gut and after absorption in the body. However, this finding is open to question. 2,4-D does not appear to be significantly metabolized in animals except for ruminants. The metabolite 2,4-dichlorophenol (2,4-DCP) has been found as a residue in ruminants and is probably there due to bacterial degradation of 2,4-D in the rumen. 2,4-D appears to form conjugates in the kidney tubules. Taurine and glycine conjugates have been found in urine of rats, pigs, chickens and the dogfish shark. In human urine conjugates have been found at up to 27% of the ingested dose.

Tissue residues: Highest tissue residue levels have been found in liver, kidney, lungs, spleen and heart in several mammals studied. However, there is no evidence for any significant bioaccumulation of 2,4-D or conjugates.

Elimination: 2,4-D is mainly excreted in urine and to a lesser extent in bile and feces. The half-life in human volunteers is about 24 hours, depending on circumstances, although estimates from single industrial exposures place the half-life between 35-48 hours.

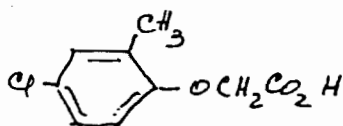
IV. Structure-activity relationships

~~There are six herbicides that are structurally related to 2,4-D.~~

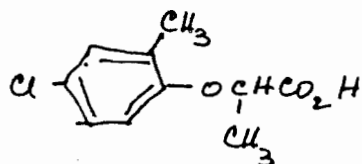


1. 2,4-DB - 2,4 dichlorophenoxy butyric acid: Has not been tested chronically.

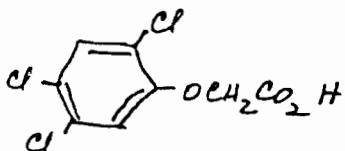
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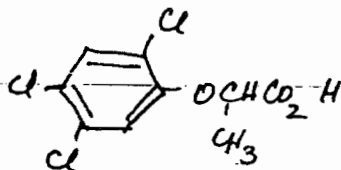
2. MCPA - (4-chloro-2-methylphenoxy acetic acid) has not been tested chronically



3. MCPP, Mecoprop, (2,4-dichloro-2-methylphenoxy propionic acid) has not been tested chronically

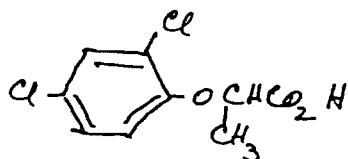


4. 2,4,5-T, (trichlorophenoxyacetic acid) was found not to be carcinogenic in rat and mouse. The contaminant TCDD was found by CAG to be a more potent carcinogen than aflatoxin, producing hepatocellular hyperplastic nodules and hepatocellular carcinomas. TCDD produced fetotoxicity and embryoletality and reproductive effects. 2,4,5-T mutagenicity was done by NIEHS according to Hank Spencer, and the results were negative. 2,4,5-T was not cancelled for all uses. There is still a registered use for rangeland, and vines in orange orchards.



5. Fenoprop, Sylvex (2,4,5-trichlorophenoxy propionic acid) is fetotoxic and teratogenic in rat but not carcinogenic in rat. Sylvex contains the same TCDD contaminant as 2,4,5-T. Not all uses were cancelled. It is still used for pear ripening.

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6. 2,4-DP, (2,4-dichlorophenoxy-2-propionic acid) was tested in
- A. Sprague Dawley rat and found:
 1. increased incidence and frequency in males by dosage of malignant tumor types compared to controls.
 2. increased incidence in males and frequency of three specific tumor types: pituitary, thyroid and brain carcinomas.
 3. A decrease in life span in male rats with pituitary and brain tumors.
 4. a shift with dose in the malignant tumor pattern in male controls to the malignant tumor pattern in the male treated groups. The treated groups had 85-86% of pituitary and thyroid malignant tumors whereas the controls had 37% of these tumor types.
 5. Increased tumor load with dose (number of tumors/rat) in male rats.
 - B. 18-month oncogenicity study in mice was negative for oncogenicity
 - C. Chronic/oncogenicity study in Fisher-F344 rats was negative for oncogenicity
 - D. Teratology study in rabbits- causes developmental toxicity
Teratology study in rats was negative.
 - E. Reproduction study in rats- causes increased mortality during lactation.
 - F. Mutagenicity: was found to be mutagenic in several tests when tested without S-9 activation.

V. Non-oncogenic effects:

Acute effects:

Skin and eye irritation: According to the WHO report 2,4-D does not appear to be an eye or skin irritant.

Skin sensitization: Adequate information on the skin sensitization potential of 2,4-D is not available.

LD50:

Species Sex LD50 (mg/kg B.W.)

mouse	m	375
mouse	m	368
rat	m	375
rat		666
guinea pig	m + f	469
guinea pig		1000
rabbit		800
dog		100
chicken	m + f	541

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Acute dermal: Several literature reports indicate that under extreme test conditions mice and rats may absorb lethal amounts of 2,4-D amine salts and esters through skin. However, no acute dermal LD₅₀ studies are reported on 2,4-D in the literature.

Acute inhalation: No reports are available

Subchronic and chronic effects: (summarized from the WHO report)

1. Observations of toxicity: These include diarrhea, vomiting dysphagia, decreased gut motility, irritation and necrotic changes. Characteristic signs of severe 2,4-D poisoning in mammals include muscular weakness, stiffness, stilted gait and muscle spasms (myotonia) especially in hind limbs of rodents. Muscular incoordination can progress to paralysis. At high doses 2,4-D may act as a central nervous system depressant, causing lethargy slowed respiration, stupor, coma and death.
2. Effects on food and water consumption: High concentrations of 2,4-D or derivatives may cause a reduction in food and water consumption and weight loss or reduced body weight gain in rats dogs, pigs, cattle, sheep, and rabbits.
3. Effects on CNS: 2,4-D is a central nervous system depressant at high concentrations and appears to be related to a partial breakdown of the blood-brain barrier, possibly as a result of damage to capillary vessels and a subsequent accumulation in the CNS.
4. Effects on the peripheral nervous system: More studies are needed on this subject. 2,4-D does not appear to cause peripheral neuropathy. The partial or complete paralysis seen in 2,4-D intoxication especially in hind limbs of rats may be a myotoxic rather than a neurotoxic mechanism.
5. Cardiovascular effects: 2,4-D at high doses may cause biochemical physiological and structural damage to the myocardium in vitro and in vivo, as part of its myotoxic action.
6. Hematologic effects: According to the WHO report, shifts have been reported in the number or types of erythrocytes, leukocytes bone marrow cells or changes in hemoglobin levels in a variety of laboratory and domestic animals from 2,4-D ingestion.
7. Blood chemistry: Some investigators have found 2,4-D administration changes various serum, plasma or erythrocyte enzyme activity levels, and electrolytes. Blood proteins and glucose as well as SGOT, SGPT and BUN changes may be secondary to myotoxic nephrotoxic or hepatotoxic effects seen with 2,4-D.
8. Nephrotoxicity: Many studies in the literature and in the submitted studies indicate that the kidney is a target organ for the structural, physiological and chemical effects of 2,4-D. Signs of toxicity at high doses include impaired kidney function, increased relative kidney weight and gross and histological

abnormalities (parenchymatous degeneration, hypertrophy and hyperplasia, cloudy swelling especially in the cells of the proximal convoluted tubules and glomerular lesions). Additional more detailed descriptions of 2,4-D induced nephrotoxicity are found in section IX in the 2 life-time rat and mouse studies submitted by the Industrial Task Force for 2,4-D Research Data.

9. Hepatotoxicity: After prolonged treatment with toxic doses of 2,4-D subacute toxic hepatitis is seen with congestion of hepatic blood vessels, and cloudy swelling, fatty infiltration, focal necrosis, degeneration or atrophy of hepatocytes, especially of the parenchyma in the centrilobular areas. High doses of 2,4-D have also been reported to induce peroxisome proliferation and increased levels of mixed function oxidases in liver cells of rats and hamsters.

10. Endocrine organ toxicity: Swelling and congestion of the thyroid were noted in cattle and sheep after fatal poisoning with various 2,4-D products. 2,4-D also appears to effect adrenal function.

VI. Reproductive and developmental studies:

The scientific literature provided studies of little value for regulatory purposes on this subject.

Submitted studies:

1. Rat teratology study dated 3/2/83 from Wil laboratories. Pregnant Fisher 344 rats were given 0, 8, 25, or 75 mg/kg of 2,4-D between days 6-15. No maternal effects were seen and at the high dose there was a slight increase in delayed ossification.

2. 2-generation reproduction study in rats dated 9/30/86 from Will research labs tested at doses of 0, 5, 20, or 80 mg/kg/day. Results indicated renal tubular degeneration of the males of the F₀ and F₁ generations. Cortical tubular degeneration (observed mostly in the proximal convoluted tubules) was confined to F₀ males. There was reduced pup weight in the F₁b pups.

VII. Mutagenicity:

According to the WHO report, the available studies are inadequate to evaluate the genetic effects of 2,4-D and its derivatives in short-term tests.

VII. Summary of lifetime studies submitted by the Industrial Task Force for 2,4-D Research Data.

1. 24-month rat combined chronic/oncogenicity study

Performing laboratory: Hazleton Laboratories, Vienna Virginia

Date: May 29, 1986.

2,4-D technical (97.5% purity) was administered to CDF (F 344/CRL-BR) rats at doses of 0, 1, 5, 15, and 45 mg/kg/day for two years. Each group contained 60/sex/dose with an interim sacrifice of 10/sex/dose at 53 weeks. Hematology, clinical chemistry and urinalysis were collected from 10 rats/sex/group at initiation and following weeks 26, 52 and 78. Clinical chemistry analyses were also performed on all animals surviving to termination of the study.

There was no treatment-related effect on survival. Male mortality at 24 months was 18, 7, 2, 8 and 12, and female mortality was 10, 13, 2, 8 and 12 for groups 1-5 respectively. Females of group 5 showed a statistically significant decrease in body weight gain for weeks 0-52 and 0-104 with an accompanying decrease in food consumption for weeks 0-52. There was a slight increase in albumin and a slight decrease in globulin at week 105 in group 5 males, increasing the AG ratio at weeks 79 and 105. There was an increase in serum alanine aminotransferase in males and females at week 105 in group 5. T₄ was slightly depressed at 105 weeks in group 5 females.

At 52 weeks, all kidney weight parameters measured for group 5 males were elevated along with absolute and kidney/body weight ratios for females of group 5. At terminal sacrifice group 5 females had increased kidney weight parameters and group 4 females also had increased kidney/body weight ratios. Male kidney weight ratios were elevated, but not significantly. There was a dose-related increase at 104 weeks in all male thyroid/parathyroid parameters with statistical significance in group 4 males and females and group 5 male thyroid/body weight ratios. Organ/body weight changes were seen in group 5 females for ovaries and brain stem and all female group 3 and 5 pituitary weight parameters were elevated.

At the 52 week sacrifice histopathologic examination revealed that there were general alterations in histopathological parameters in the kidneys of groups 3, 4 and 5 that appeared compound-related.

These consisted of:

1. an increased incidence in brown tubular cell pigment in the males of groups 3, 4, and 5 (9/10, 10/10, 10/10 respectively) and groups 3, 4, and 5 females (5/10, 6/10, and 7/10 respectively) when compared to control males (2/10) and control females (3/10).
2. An increased frequency and severity of fine vacuolization of cytoplasm in the renal cortex in group 5 females (8/10) when compared to control females (5/10) and an increase in severity in groups 3 and 4 females when compared with control females.

At the terminal sacrifice compound-related histopathologic alterations occurred in the kidneys of groups 3, 4, and 5 males and females. These included:

1. increased brown tubular cell pigment in the kidneys of groups 3, 4, and 5 males (8/47, 18/41**, and 18/36** respectively) and groups 3, 4, and 5 females (23/37*, 19/38**, 13/36 respectively) when compared to control males (2/32) and females (8/40).
2. Increased incidence of pelvic microcalculi in groups 4 and 5 males (8/41, 9/36 respectively) and group 5 females (28/36**) when compared to control males (2/32) and females (19/40).
3. A slight increase in frequency of transitional epithelial hyperplasia in group 5 females (6/36) when compared to controls (0/40).

Additional histopathology tables were requested and the submitted information concerning granulomatous prostatitis, kidney transitional cell hyperplasia and microcalculi are discussed in more detail on page 2 of appendix D.3.

The most significant finding in this study involved a rare, small brain tumor-astrocytoma (glioma) found in high dose males. In the original report the study text had stated that the tumors seen for the groups was:

Incidence of Astrocytomas as given in study text

mg/kg/day	group 1 2,4-D	2	3	4	5	1	2	3	4	5
	0	1	5	15	45	0	1	5	15	45
Unscheduled deaths	1/19	0/7	0/5	2/7	1/14	0/10	1/13	0/13	0/12	0/14
52-week sac.	0/10	0/10	0/5	0/10	0/10	0/10	0/10	0/10	0/10	0/10
104 wk sac.	0/32	0/43	0/43	0/41	5/36	0/40	0/37	2/37	1/38	1/36
All animals	1/60	0/60	0/60	2/60	5/60	0/60	1/60	2/60	1/60	1/60

Dr. Koestner of Michigan State re-reviewed the brain slides for the Industry Task Force (appendix D.2). He differed from the original pathology report on only one point. He felt that the high dose male animal # 23473, originally diagnosed as having an astrocytoma, actually had a mixed glial and mesenchymal cell population. He presented arguments concerning the biological criteria for evaluation of neurocarcinogens, summarized on page 2 of appendix D.2 and gave historical control incidences for glioma summarized on page three of this appendix. These historical controls are summarized below.

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VARIABILITY IN BRAIN GLIOMA INCIDENCE
IN CONTROL MALE SPRAGUE-DAWLEY RATS 1 YR. AND OLDER
(SELECTED FROM SWENBERG, J.A. 1986)

NUMBER	COLOR	LABORATORY	CONTROL 1(%)	CONTROL 2(%)	CONTROL 3(%)
1-5	Diff. Colors	IRDC	0/292 (0)	2/287 (0.7)*	2/137 (1.4)
6	Red No. 33	IRDC	3/57 (5.2)	0/59 (0)	2/58 (3.4)
7	Green No. 3	Biodynamics	0/52 (0)	5/55 (9)**	---
8	Blue No. 2	Biodynamics	0/59 (0)***	2/59 (3.4)	---
9-13	Diff. Colors	Biodynamics	2/290 (0.7)	2/289 (0.7)	4/231 (1.7)
14	Red No. 9	Litton	4/58 (6.9)****	6/60 (10)	2/57 (3.5)
15	Red No. 27	Litton	2/5 (3.7)	0/55 (0)	---
16	Red No. 36	Litton	2/57 (3.5)	1/59 (1.7)	0/53 (0)
17	Red No. 30	Hazleton	3/59 (5.1)	1/55 (1.3)	---

- * One of the rats died on day 350 with a glioma
 ** Additional sections resulted in 5/55 (10.9%)
 *** Additional sections resulted in 2/59 (3.4)%
 **** One glioma diagnosed at 12 mos. interim sacrifice

RANGE of historical
CONTROLS = 0-10%

*

Dr. James Swenberg of CIIT was asked by EPA to re-evaluate the brain slides originally diagnosed as having astrocytomas. For all but one animal, he agreed with the original study text diagnosis of astrocytomas. He disagreed with the astrocytoma diagnosis of female B23289 in the 5 mg/kg dose group. He diagnosed this lesion as a focal area of gliosis present near the center of the olfactory bulb with no neoplasm detected. The original study text had diagnosed this area as an astrocytoma. Dr. Swenberg also disagreed with Dr. Koestner's diagnosis of the high dose male B 23473. Dr. Koestner had diagnosed this male as having an area in the brain of mixed glial and mesenchymal cell population. Dr. Swenberg diagnosed this animal as having a small astrocytoma present in the ventral portion of one hemisphere of the forebrain. The final tabulation of Dr. Swenberg is below:

Astrocytoma Incidence as Diagnosed by Dr. Swenberg

Males					
Dose	0 mg/kg	1	5	15	45
animal:	B23025			B23376 B23377	B23473 B23476 B23479 B23492 B23500 B23505
Females					
		B23185	B23302	B23442	B23546

24-month mouse oncogenicity study:

Performing laboratory: Hazleton Laboratories, Vienna, Va.

Date: 1/15/87(?)

2,4-D (97.5% purity) was administered to 60 B₆C₃F₁ Crl Br mice/sex/group for 24 months with an interim sacrifice at 52 weeks of 10/sex/group. Doses administered were 0, 1, 15 and 45 mg/kg/day, administered in the diet. Body weight, food consumption and physical exam results were recorded weekly through week 14 and biweekly thereafter. Blood for hematology was collected from 10/sex/group after 52 weeks and at 104 weeks. Clinical chemistry, and urinalysis were not performed. Liver, heart, kidney, testes, ovaries, brain, adrenals and thyroids were weighed at the 52-week interim sacrifice and at the 104-week terminal sacrifice.

There was no treatment-related increase in mortality. The number of males that died on test or were sacrificed in extremis prior to terminal sacrifice were 10, 6, 10 and 11 for groups 1-4 respectively. Numbers of females that died on test were 12, 8, 17, and 15 for groups 1-4 respectively. There were no treatment-related effects on body weight or body weight gain, food consumption, hematology or gross pathology. At 52 weeks there was an increase in absolute combined kidney weights in group 4 females, and an increase in absolute and relative adrenal weights in males at all doses tested. At 104 weeks, combined female relative and absolute kidney weights were increased in group 4 and kidney/body weight ratios were also increased in group 3 females and group 4 males. Absolute and relative adrenal weights in males were elevated in group 3 and 4 males at 104 weeks. There were no associated histopathological changes in the adrenals at 52 and 104 weeks accompanying the weight increases. There was, however, an increase in cytoplasmic homogeneity of the renal tubular epithelium due to a reduction of cytoplasmic vacuoles in the mid and high dose kidneys in males at 52 and 104 weeks and in unscheduled deaths. Low dose males were also effected in the unscheduled death group. However, when these animals are combined with the scheduled deaths, no significance was noted. No treatment-related increased incidence in tumors was seen at 52 weeks, 104 weeks or in unscheduled deaths.

IX. Epidemiology study:

The study of interest is "Agricultural Herbicide use and risk of Lymphoma and soft tissue sarcoma. S. Hoar, A. Blair, F. Holms et al. J.A.M.A., 256, 1141-1147, 1986. This was the report of a population-based case-control study conducted by the National Cancer Institute in Kansas. The report stated that they found an association between farm herbicide use (phenoxyacetic acids) and non-hodgkins lymphoma (NHL) but did not find an association with soft tissue sarcoma (STS) or Hodgkin's disease(HD). Four reviewers were asked to evaluate this study and the results of their deliberations are in appendix F. Jerome Blondell, Health Statistician of EPA has summarized the key points from the Peer Review comments and these points are listed below and appended in appendix F.

1. The NCI study by Hoar et al. was well-designed, competently conducted and carefully analyzed.
2. Half of the respondents were next-of-kin who are unlikely to remember accurately which herbicides were used over 10 to 20 years ago.
3. Inconsistencies between subjects' reported use of herbicides and reported use in USDA and EPA surveys in the Kansas area suggest a very serious potential for inaccurate reporting of exposure.
4. Statistically significant findings, particularly those implicating 2,4-D, were based on a very small number of cases, usually 10 or less. Moderate amounts of exposure misclassification, described above, might easily make these findings nonsignificant.
5. The study found a significant association between non-Hodgkins lymphoma and fungicides even with or without exposure to herbicides. The study also found that five groups of herbicides exhibited higher odds ratios for NHL than did phenoxyacetic acids. These two findings cast serious doubt on the specificity of 2,4-D/NHL association.
6. Occupation was not controlled for in the analysis. Those who live on farms have lifestyles, diets, physical activity and exposure to viruses that greatly differ from the general population. These factors are known to affect many kinds of cancer and, as a result, are a potential source of confounding.
7. Reviewers had conflicting views on the strength of support from other studies. The Swedish studies by Hardell which seem to provide the strongest case for a chlorophenoxy herbicide/NHL association were characterized as having "important methodologic limitations" even by the most favorable reviewer.
8. One of the external reviewers felt that serious regulatory action to limit exposure to 2,4-D should be considered. Two other reviewers did not feel that the weight of evidence implicates 2,4-D as a cause of NHL.

9. The EPA Guidelines for Carcinogen Risk Assessment do not distinguish well enough between the categories "limited evidence of carcinogenicity" and "inadequate evidence" to say with certainty which category the NCI study belongs to.

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APPENDIX A

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Environmental Health Criteria 29

2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

Published under the joint sponsorship of
the United Nations Environment Programme,
the International Labour Organisation,
and the World Health Organization



World Health Organization
Geneva 1984

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The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organization, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by IPCS include the development of a knowledge base for cooperation with chemical accidents, coordination of safety testing, and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

ISBN 92 4 154089 3

World Health Organization 1984

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NOTE TO READERS OF THE CRITERIA DOCUMENTS

While every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication, mistakes might have occurred and are likely to occur in the future. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors found to the Manager of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda, which will appear in subsequent volumes.

In addition, experts in any particular field dealt with in the criteria documents are kindly requested to make available to the WHO Secretariat any important published information that may have inadvertently been omitted and which may change the evaluation of health risks from exposure to the environmental agent under examination, so that the information may be considered in the event of updating and re-evaluation of the conclusions contained in the criteria documents.

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FOR 2,4-DICHLOROPHOXYACETIC ACID

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ENVIRONMENTAL HEALTH CRITERIA FOR 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

Further to the recommendations of the Stockholm United Nations Conference on the Human Environment in 1972, and in response to a number of World Health Assembly resolutions (WHA23.60, WHA24.47, WHA25.58, WHA26.68) and the recommendation of the Governing Council of the United Nations Environment Programme, (UNEP/GC/10, July 3 1973), a programme on the integrated assessment of the health effects of environmental pollution was initiated in 1973. The programme, known as the WHO Environmental Health Criteria Programme, has been implemented with the support of the Environment Fund of the United Nations Environment Programme. In 1980, the Environmental Health Criteria Programme was incorporated into the International Programme on Chemical Safety (IPCS). The result of the Environmental Health Criteria Programme is a series of criteria documents.

The Environmental Health Directorate, Health Protection Branch, Department of National Health and Welfare, Canada (Director-General Dr E. Somers) was responsible, as a Lead Institution of the IPCS, for the preparation of the first and second drafts of the Environmental Health Criteria Document on 2,4-D. Dr D. Kiedel co-ordinated the work.

The Task Group for the Environmental Health Criteria for 2,4-D met in Ottawa from 4 to 11 July, 1983. The meeting was opened by Dr E. Somers. Dr A.B. Morrison, Assistant Deputy Minister, Department of National Health and Welfare, Canada welcomed the participants on behalf of the host government and Dr F. Valic, on behalf of the 3 co-sponsoring organizations of the IPCS (UNEP/WHO). The Task Group reviewed and revised the second draft criteria document and made an evaluation of the health risks of exposure to 2,4-D.

The efforts of all who helped in the preparation and the finalization of the document are gratefully acknowledged.

Partial financial support for the publication of this criteria document was kindly provided by the United States Department of Health and Human Services, through a contract from the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA - a WHO Collaborating Centre for Environmental Health Effects.

1. SUMMARY AND RECOMMENDATIONS FOR FURTHER STUDIES

1.1 Summary

1.1.1 Analytical methods

1.1.1.1 2,4-D, 2,4-D alkali metal salts or 2,4-D amine salts, and 2,4-D esters

The available analytical results concerning 2,4-dichlorophenoxyacetic acid (2,4-D) and its derivatives in herbicides and biological and environmental matrices were collected over a span of almost 40 years, by diverse and, until fairly recently, not sufficiently specific or sensitive methods. This makes comparison of most of the data reported in the literature difficult.

1.1.1.2 Contaminants in 2,4-D herbicides

Adequately specific and sensitive methods for the reliable identification of such potentially hazardous contaminants as the di-, tri-, and tetrachlorodibenzo-p-dioxin isomers and N-nitrosamines have only recently been developed. Available analytical data are limited to a few manufactured products.

1.1.2 Sources of environmental pollution

Most of the 2,4-D residues result from the production and use of 2,4-D herbicides. Other possible minor sources of 2,4-D include the use of 2,4-dichlorophenoxybutyric acid (2,4-DB).

Little information is available on the uses of 2,4-D products and the amounts used in various parts of the world.

The drifting of vapours of the more volatile short-chain 2,4-D esters may result in air pollution and crop damage, and these products are being replaced by less volatile long-chain esters or by amine salts.

The use of 2,4-D for aquatic weed control may lead to contamination of sources of irrigation and drinking-water. Environmental pollution also arises through inadequate disposal practice.

1.1.3 Environmental distribution and transformations

Various amounts of 2,4-D products applied to a target area may be distributed in the general environment, within a few hours or days, by the movements of air, water, or soil,

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particularly during periods of rain, high winds, or high temperature.

2,4-D and its derivatives are fairly rapidly broken down by hydrolysis, photolysis, and by biological action.

Persistence or accumulation of 2,4-D residues from normal use is occasionally possible, mainly under dry or cold conditions where there is little biological activity.

Nothing is known about the environmental fate of the impurities present in 2,4-D herbicides.

1.1.4 Environmental exposure levels

Available data indicate that residues of 2,4-D rarely exceed 1 mg/kg in soil, several µg/litre in water, several µg/m³ in air, and a few tens of µg/kg in food sources. Exceptions may occur in the vicinity of 2,4-D herbicide spills, in water treated with aquatic 2,4-D herbicides, in berries and mushrooms grown in treated right-of-way areas, or when the herbicide is used in quantities far in excess of the rates applied in normal agricultural or forestry practice. No information is available on the corresponding exposure levels for the contaminants present in 2,4-D herbicides.

Exposure to 2,4-D, in the work environment, of persons producing, handling, or using herbicides may result in absorption of detectable amounts of 2,4-D.

1.1.5 Uptake and fate of 2,4-D in the body

2,4-D and its derivatives can be absorbed via the oral, dermal, and inhalation routes. General population exposure is mainly by the oral route, but under occupational and bystander exposure conditions, the dermal route is by far the most important.

Distribution of 2,4-D occurs throughout the body, but there is no evidence that it is accumulated. Transformation in mammals appears to occur only to a slight extent and mainly involves the production of 2,4-D conjugates with sugars or amino acids. A single dose is excreted within a few days, mainly with the urine, and to a much lesser extent in the bile and faeces.

Little is known about the uptake and subsequent fate of the contaminants of 2,4-D other than 2,4-dichlorophenol.

1.1.6 Effects on animals

1.1.6.1 Acute toxic effects

Death may result in mammals and birds administered oral doses of 2,4-D exceeding approximately 100-300 mg/kg body weight.

The most characteristic signs of severe 2,4-D poisoning are those of myotonia, but various other physiological, haematological, biochemical, and histological changes have been described.

The no-observed-adverse-effect level for a single dose of 2,4-D in animals has not been clearly established for all species.

No adequately documented reports of acute accidental 2,4-D poisoning of mammals or birds have been found.

1.1.6.2 Chronic toxic effects

The no-observed-adverse-effect level for some of the chronic adverse effects of 2,4-D in mammals has not been established firmly.

1.1.6.3 Teratogenic and reproductive effects

The no-observed-adverse-effect level for the teratogenic, embryotoxic, or fetotoxic effects of 2,4-D in mammals and birds appears to be about 10 mg/kg body weight per day.

1.1.6.4 Mutagenic effects

Studies available at present are not adequate for the quantitative evaluation of the mutagenic effects of 2,4-D and its derivatives in short-term tests. However, the evidence does not suggest that 2,4-D derivatives are potent mutagens.

1.1.6.5 Carcinogenic effects

The carcinogenic potential of 2,4-D and its derivatives such as the amine salts and esters has not been adequately tested. The reports on animal bioassays carried out so far are either too brief for proper evaluation, or have become the subject of scientific controversy.

1.1.7 Effects on human beings

1.1.7.1 Acute toxic effects

2,4-D drug trials and studies on volunteers have shown that doses of between 5 and about 30 mg/kg body weight do not cause any acute toxic effects.

Accidental and intentional 2,4-D poisonings indicate that the toxic effects of 2,4-D are the same in human beings as in other mammals. The lethal single oral dose is uncertain.

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1.1.1.7.2 Chronic toxic effects

It is uncertain whether the chronic toxic effects of 2,4-D products reported in occupationally-exposed people are solely attributable to 2,4-D.

1.1.1.7.3 Teratogenic and reproductive effects

Scientifically valid studies have not shown any adverse reproductive effects in human beings accidentally or occupationally exposed to 2,4-D.

1.1.1.7.4 Mutagenic effects

The results of studies suggesting that occupational exposure to 2,4-D may result in chromosome abnormalities are equivocal.

1.1.1.7.5 Carcinogenic effects

The results of some epidemiological studies have suggested an association between exposure to phenoxy herbicides and increased incidences of malignant tumours and tumour mortality. It is not clear, at present, whether this represents a true association, and if so, whether it is specifically related to 2,4-D.

1.2 Recommendations for Further Studies

1.2.1 Analytical methods

Methods not requiring highly sophisticated and expensive equipment are available for the accurate, specific, and sensitive determination of 2,4-D residues in a wide variety of environmental and biological materials. However, it would be desirable to develop simpler but specific methods for the detection and quantification of dioxin contaminants.

1.2.2 Environmental exposure levels

Further studies should be undertaken to determine the total 2,4-D intake of various sub-populations in areas of 2,4-D use.

It would be desirable to monitor 2,4-D residues in aquatic organisms taken from lakes or rivers receiving discharges or treatment with 2,4-D.

Further work on the relationship between the factors influencing the dermal absorption of various 2,4-D formulated products in human beings and animals should be carried out.

1.2.3 Studies on animals

More animal studies are desirable to investigate the possible interactions between 2,4-D and other herbicides commonly used in conjunction with 2,4-D.

Further work is required to accurately define the no-observed-adverse-effect level for 2,4-D in long-term exposures.

Where unknown, the chronic toxicity of the alcohols and amines used in preparing 2,4-D derivatives, should be investigated.

More studies are needed to assess the mutagenic potential of 2,4-D derivatives.

1.2.4 Studies on human beings

In the case of occupationally-exposed workers further consideration should be given to the chemobiokinetics of 2,4-D under repeated exposure conditions.

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2. PROPERTIES AND ANALYTICAL METHODS

2.1 Physical and chemical properties of 2,4-D

2.1.1 Introduction

The structures of 2,4-dichlorophenoxyacetic acid (2,4-D) and of chemically-related phenoxy herbicides in common use are given in Fig. 1.

Some physical properties of 2,4-D and of the 2,4-D derivatives that are used in agriculture are summarized in Table 1.

2,4-D has growth-regulating and herbicidal properties in broad-leaved plants. Because of its solubility, 2,4-D is rarely used in the form of the acid; commercial 2,4-D herbicide formulations consist of the more soluble forms such as alkali salts, amine salts, or esters. These are combined with solvents, carriers, or surfactants and are marketed in the form of dusts, granules, emulsions, or oil and water solutions in a wide range of concentrations.

Table 1. Physical properties of 2,4-D

Molecular formula	Molecular weight
$\text{C}_8\text{H}_6\text{Cl}_2\text{O}_2$	221.0
Melting point	140-141°C
Solubility in water	slightly soluble
Solubility in organic solvents	soluble
Vapor pressure	52.3 Pa at 160°C
Boiling point	260-261°C

2.1.2 Synthesis of 2,4-D

2,4-D is commonly prepared by the condensation of 2,4-dichlorophenol with monochloroacetic acid in a strongly alkaline medium at moderate temperatures (Canada, NRC, 1978; Sittig 1980; Que Hee & Sutherland, 1981), or by the chlorination of phenoxyacetic acid, but this method leads to a product with a high content of 2,4-dichlorophenol and other impurities (Melnikov, 1971). Higher reaction temperatures and alkaline conditions during the manufacture of 2,4-D increase

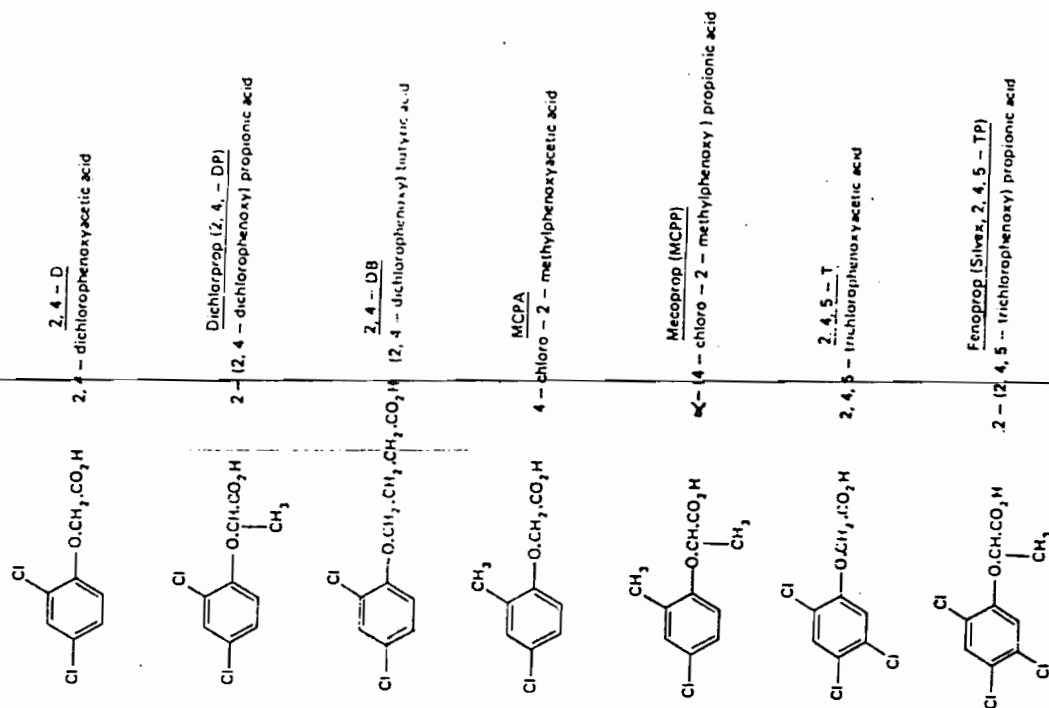


Fig. 1. Structures of 2,4-dichlorophenoxyacetic acid (2,4-D) and chemically-related herbicides.

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the formation of polychlorinated dibenzo-p-dioxin (CDD) byproducts (Fig. 2). The alkali metal salts of 2,4-D are produced by the reaction of 2,4-D with the appropriate metal base. Amine salts are obtained by reacting stoichiometric quantities of amine and 2,4-D in a compatible solvent (Que Hee & Sutherland, 1974, 1981). Esters are formed by acid-catalysed esterification with azeotropic distillation of water (Que Hee & Sutherland, 1981) or by a direct synthesis in which the appropriate ester of monochloroacetic acid is reacted with dichlorophenol to form the 2,4-D ester (Canada, NRC, 1978).

2.1.3 Important chemical reactions of 2,4-D

Pyrolysis converts various amine salts of 2,4-D to the corresponding amides (Que Hee & Sutherland, 1975). Pyrolysis of 2,4-D and its derivatives is likely to produce certain CDD isomers (Section 2.1.4). 2,4-D is readily photodegraded (Section 4.4.4).

2.1.4 Composition of technical 2,4-D materials

Technical 2,4-D may range in purity from less than 90% to 99%. Typical levels for impurities are listed in Table 2. Trace levels of CDDs have been found in amine and ester formulations (Table 3). It can be seen that the amine formulations tend to be less highly contaminated with di- and tetra-CDD than the ester products. The structures of these impurities are shown in Fig. 2.

The composition of technical 2,4-D depends on the manufacturing process and especially on the purity of 2,4-dichlorophenol when this is the starting material. During 2,4-D synthesis from monochloroacetic acid and 2,4-dichlorophenol, the latter compound as well as other ortho-chlorinated by-products can give rise to a wide variety of chlorinated by-products at a high temperature and high pH. Self condensation of 2,4-dichlorophenol may form 2,7-dichlorodibenzo-p-dioxin, while trichlorophenols may give rise to a mixture of 1,3,6,8- and 1,3,7,9-tetrachloro dibenzo-p-dioxins (but not 2,3,7,8-TCDD) by self-condensation, or to 1,3,7-trichlorodibenzo-p-dioxin by cross-condensation with 2,4-dichlorophenol.

A different type of toxic trace impurity, namely N-nitrosamines, can occur in amine formulations of 2,4-D, especially when nitrite is added as a corrosion inhibitor for containers. Dimethyl-N-nitrosamine has been found in some 2,4-D dimethylamine products at levels of up to 0.3 mg/litre (Ross et al., 1977; Cohen, et al., 1978).

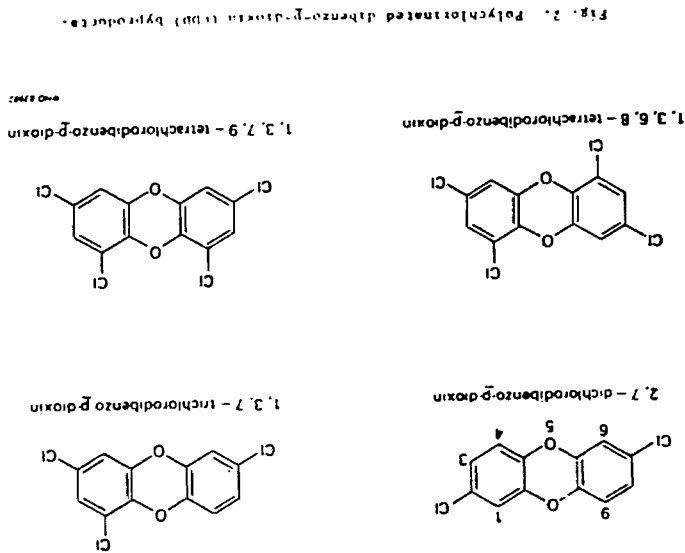


Fig. 2. Polychlorinated dibenzo-p-dioxin (CDD) byproducts.

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Table 2. Typical

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Figure 1 shows the results of a comparison of the two methods. The χ^2 values are given in the table. The χ^2 values are calculated for the two methods using the same data. The χ^2 values are calculated for the two methods using the same data. The χ^2 values are calculated for the two methods using the same data.

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Table 1	$2,2,2$ -trifluoroethane	$1,1,1,2$ -tetrafluoroethane	$1,1,1,2,2$ -pentafluoroethane
$2,2,2$ -trifluoroethane	$n_D^{20} = 1.269$ $n_D^{25} = 1.2615$	$n_D^{20} = 1.278$ $n_D^{25} = 1.270$	$n_D^{20} = 1.284$ $n_D^{25} = 1.276$

[illegible]

appears in form of 2, 4, 6, 8.

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2.1.5 Volatility of 2,4-D derivatives

2,4-D esters with short-chain alcohols are highly volatile (Table 1). This influences the effectiveness of their application to target crops, their effects on neighbouring crops, and the degree of contamination of the atmosphere. 2,4-D alkali salts or amine salts are much less volatile than

2.2 Determination of 2,4-D

2.2.2.1 General comments:

General comments on criteria for acceptable analytical methods and on other pertinent aspects of 2,4-D determination can be found in the publications of Gunther (1962), Currie ((1968), Kaiser (1973), Carl (1979), Katsman & Pijper (1981), Que Hee & Sutherland (1981) and Chau et al. (1982).

2.2.2.2 Analysis of technical and formulated 2,4-D products

In the past, the quality of 2,4-D products was assessed by an acid-base titration or by a total chlorine determination (Collaborative International Pesticides Analytical Council, 1970). These non-specific and thus inaccurate methods have been superseded by specific gas-liquid chromatography (GLC) or high pressure liquid chromatography (HPLC), making it possible to determine various by-products (Henshaw et al., 1975; Bontoyan, 1977; Skelly et al., 1977; Stevens et al., 1978; Cochrane et al., 1982). The isomer-specific HPLC method is now preferred by many 2,4-D producers and regulatory agencies. The chlorinated dibenzo-p-dioxins (CDDs) are usually produced only in trace amounts and are difficult to separate and identify; highly specialised equipment and skills are necessary (Crummett & Stehl, 1973; Huckins et al., 1978; Norström et al., 1979; Baker et al., 1981; Cochrane et al., 1981; Haas et al., 1981, and National Research Council of Canada Associate Committee on Scientific Criteria for Environmental Quality, 1981).

2.2.2.3 Determination of 2,4-D residues

All exposure determinations and risk assessments ultimately depend on accurate chemical analyses, and therefore, some critical aspects of analysis for 2,4-D residues have been included in the present document.

Before 2,4-D residues can be measured, they have to be quantitatively extracted and purified to remove substances that could interfere with the final residue determination. They must then be converted to a stable product (derivative) suitable for determination with a given type of detector.

When comparing analytical results, it should be kept in mind that the older methods of extraction and clean-up

contained considerable sources of errors, and that the early methods for measuring 2,4-D residues, such as colorimetry and spectrophotometry, were not as sensitive or specific as those developed in recent years.

2.2.3.1 Sampling, extraction, and clean-up

Methods for the sampling, extraction, and clean-up of 2,4-D residues in water, air, soil, and biological materials have recently been reviewed by National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality (1978) and by Que Hee & Sutherland (1981). Problems caused by the conjugate formation of 2,4-D with amino acids, proteins, sugars, or lipids, or the adsorption of 2,4-D onto container surfaces, including those of glass vessels, have been solved by Chow et al. (1971), Renberg (1974), Osadchuk et al. (1977), Løkke (1975), Jensen & Glas (1981), and Bristol et al. (1982). For sampling and extracting 2,4-D residues, the following references should also be consulted:

Air: Van Dyk & Visvesvariah (1975), Farwell et al. (1976a,b), Grover et al. (1976), Johnson et al. (1977), Gluck & Melcher (1980), and Grover & Kerr (1981); water: Suffet (1973a,b), Renberg (1974), Mierswa & Witek (1977), Chau & Thomson (1978); soil: Woodham et al. (1971); Smith (1972, 1976a), Foster & McKetcher (1973); food: Que Hee & Sutherland (1981), Bjerke et al. (1972), Jensen & Glas (1981), Løkke (1975); biological media: Smith (1976b), (blood, urine); Senczuk & Pogorzelska (1981).

2.2.4 Derivatization and quantification

At present, gas-liquid chromatography with electron-capture detection (GLC-EC) is the most commonly used and generally most sensitive method (picogram level) for measuring 2,4-D residues.

To improve the sensitivity of detection, the 2,4-D has to be transformed (derivatized), usually to a methyl ester by reacting with BF₃-methanol, diazomethane, or with concentrated sulfuric acid-methanol; the first method may give the best results (Munro, 1972; Horner et al., 1974; Olson et al., 1978).

For a recent review of derivatization methods and GLC columns for various substrates see Cochrane (1981).

Thin-layer chromatography (TLC) has been used for herbicide residue determination (Guardigli et al., 1971; Yip 1975). It has recently been recommended by Batora et al. (1981) as a simplified method for determining pesticide residues that requires a minimum of costly equipment. TLC is

suitable for food inspection and could be of use in the establishment of new residue laboratories in developing countries.

High-pressure liquid chromatography (HPLC) is less sensitive than GLC-EC i.e., nanogram (ng) versus picogram levels, but may be advantageous under some circumstances (Tuinstra et al., 1976; Arjmand et al., 1978; Connick & Simoneaux, 1982). Using mass fragmentography with deuterated internal standards it is possible to determine nanogram amounts of 2,4-D and related compounds in urine and plasma (De Beer et al., 1981); it is also suitable for chemobiokinetic studies on subtoxic doses of 2,4-D in blood.

2.2.5 Confirmation

The ultimate confirmatory technique is gas chromatography coupled with mass spectrometry and specific ion monitoring, with a sensitivity down to the femtogram level (Farwell et al., 1976a).

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4.4.3 Degradation in the aquatic ecosystem

A multitude of variables influence the partitioning and removal of phenoxy herbicides within an aquatic ecosystem. Detectable residues have been reported to persist for 4 weeks in some situations and up to 4 months in others (Frank & Cones, 1967; Wojcik et al., 1971; Schultz & Harman, 1974). Photolysis is an important means of degradation of 2,4-D in natural water and is more rapid than that of 2,4,5-T (Crosby & Wong, 1973). The partition of residues between water and sediment will have an effect on the rate of breakdown, as will temperature and intensity of light. Anaerobic conditions will favour microbial breakdown. The effects of some of these factors have been tabulated by the National Research Council of Canada Associate Committee on Scientific Criteria for Environmental Quality (1978).

4.4.4 Photochemical degradation

Photodecomposition of 2,4-D was studied in detail by Crosby & Tutasa (1966) and Roval & Smith (1973) and reviewed recently by Que Hee & Sutherland (1981). It leads to the formation of a variety of products but commonly involves reductive dechlorination of the acid, esters, and salts in aqueous or in organic solutions, with 2,4-dichlorophenol acting as a catalyst for the breakdown of 2,4-D, which may involve rupture of the aromatic ring. Que Hee & Sutherland (1981) studied the vapour and liquid phase photolysis of the n-butyl ester of 2,4-D and observed dechlorination at the second position with simultaneous reduction and re-arrangement to produce a variety of photoproducts. According to Roval & Smith (1973), carbon dioxide is the final oxidation product when aqueous solutions of 2,4-D undergo photodecomposition.

4.5 Bioconcentration

There is no evidence that bioconcentration of 2,4-D occurs through the food chain or in any compartment of the environment. This has been demonstrated by large-scale monitoring for 2,4-D residues in soils, foods, feedstuffs, wildlife, and human beings, and from examinations of the many routes of metabolism and degradation that exist in ecosystems (sections 5.1.3 and 5.1.4).

5. ENVIRONMENTAL LEVELS AND EXPOSURE

5.1 Levels of 2,4-D Residues in the Environment

Most of the available information on 2,4-D levels in the environment has been reviewed in detail (National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality, 1978; Ramel, 1978; Bovey & Young, 1980; Canada, Health & Welfare, 1980; Shearer & Halter, 1980; US EPA, 1980a). In comparing early and recent results, it should be kept in mind that the analytical procedures used before about 1965 were often unreliable and may have resulted in under- or overestimation of the actual levels of 2,4-D derivatives. No information is available on the levels of 2,4-D-related dioxin by-products in the environment.

5.1.1 In air

Some levels of 2,4-D in ambient air are shown in Table 4. These 2,4-D residues consist mainly of esters, particularly the highly volatile butyl esters (Bamezberger & Adams, 1966; Farwell et al., 1976b; Grover et al., 1976). Total 2,4-D residues in the air were found to decrease during periods of rain, suggesting a "washout effect" (Grover et al., 1976). In the majority of cases, the levels reported were those found shortly after spraying.

5.1.1.1 Field exposure

Concentrations of 2,4-D that occurred during and after herbicide use in the air of the work zone of people engaged in herbicide spray operations in various use situations, are given in Table 5. Workers involved in these operations were exposed to 2,4-D levels of up to 0.2 mg/m³ air during the period of actual application.

5.1.1.2 General environmental exposure

In large-scale studies in areas of intense 2,4-D use, about 40% of all air samples were found to contain between 0.01 and 0.1 µg 2,4-D/m³ (Grover et al., 1975). In a similar study undertaken by Que Hee et al. (1975), much higher levels were recorded in one urban location, reaching an average of 339 µg/m³ air during 3 days. However, Grover et al. (1976), in their subsequent work, showed that such concentrations could only be produced under artificial conditions that could not reflect environmental conditions. In a general programme of air monitoring undertaken in

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Table 3. Concentrations of 2,4-D in air and water

Herbicide product	Circumstances	Type of exposure monitoring	Time after spraying	Mean of 2,4-D concentration in air (µg/m ³)	Reference
2,4-dimethylamine salt (0.9% aqueous solution)	agricultural spray operations with tractor-drawn equipment	Analyses of air in tractor cabs	-	0.002	Thompson et al., 1971
2,4-D butoxyethanol ester, 2% emulsion in water	Exposure during forest spray operation with tractor driven equipment	Analyses of air in breathing zone of workers	-	0.002	Thompson et al., 1971
2,4-D isooctyl ester in diesel oil	3-day aerial spray operation with single engine aircraft	Analyses of air in breathing zone of pilot and ground crew	-	0.002-0.01	Thompson et al., 1971
2,4-D PGSE ester emulsion in water	Two 1-day aerial forest spray operation by helicopter	Analyses of air in breathing zone of ground crew	-	0.0000012	Thompson et al., 1971

4 Application using large spray propellers.
5 One flagman was recorded as being exposed to 0.001 µg/m³.

citrus-growing regions in the USA, only one out of 480 samples analysed was found to contain 2,4-D, at a level of 0.004 mg/m³. The sites were not chosen in relation to 2,4-D use (Stanley et al., 1971).

2.4-D in water

2,4-D, as well as chlorophenol residues resulting from the microbial transformation of 2,4-D, may occur in raw and finished supplies of drinking water (Frost & Alvi, 1967; EPA, 1976, 1980a; National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality, 1978; Reves & Young, 1980; Canada, Health & Welfare, 1980; Sheehan & Miller, 1980).

Information on 2,4-D-related dioxins in water are not available.

Drinking water in the USA is routinely analysed by EPA as part of the beverage-food group in their "market basket" analysis programme; 2,4-D has not been detected in these studies, where the limit of detection is 0.005 mg/litre for beverages (Table 4). This indicates that drinking water is not a significant source of human exposure outside directly sprayed areas.

The same conclusion can be drawn from the results of large-scale surveys of pesticide residues, including 2,4-D in surface waters in areas of 2,4-D use (Table 7).

Levels much higher than those found in these studies have been observed, but only in relation to local spills or direct contamination (Frank et al., 1970; Frank & Sironi, 1980). A very wide fluctuation has been found in water samples following treatment of bodies of water, shores, ditches, or stream banks with herbicides (Averitt, 1967; Frank & Sironi, 1980; Bartley & Hartup, 1970; Frank et al., 1970; Wojtalik et al., 1970; Frank, 1972; Whitney et al., 1973; Schultz & Harman, 1974; Schultz & Whitney, 1974; Padernova, 1975; Province of British Columbia, 1981). Occasional high contamination levels in samples of potable water have been reported following experimental treatments of reservoirs with 2,4-D (Wojtalik et al., 1973). However, the mean levels tended to remain below 2 µg/litre, even in samples of raw or processed water from 2,4-D-treated reservoirs (Smith & Brown, 1967; Wojtalik et al., 1971; Province of British Columbia, 1981). Generally, 2,4-D residues were < 0.1 µg/litre in two large-scale monitoring programmes of surface waters (Frank & Sironi, 1980; Gunner, 1980). This is not unexpected in view of the moderately rapid microbial degradation of 2,4-D in the environment (Robson, 1966; Averitt, 1967; Frank, 1972; Nesbitt & Watson, 1980a,b; Province of British Columbia, 1981).

Table 6. Dieldrin residues reported in market basket samples in the USA

Years	Types of samples analysed	Nature of samples containing residues	% of samples with residues	Residue levels (mg/kg)	References
1955-60	Total diets	sugars and adjuncts ^a	4.2	< 0.1	Duggan & Corneliussen (1972)
1956-60		leafy vegetables (1) low rats	1.1	< 0.1	
1961-67		leafy vegetables (2) oil fats (1)	1.7	< 0.1	
1965-68		dairy produce (1)	2.6	0.01-0.11	
1964-69		fruits (1), sugars (2)	0.1	< 0.2	
1970-70		leafy vegetables (1)	0.1	< 0.02	
		dairy produce (1)			
1970-71	Total diets	leafy vegetables (3)	-	0.01-0.02	Manske & Corneliussen (1972)
1971-72	Total diets	dairy products (1)	-	0.1	Manske & Johnson (1975)
1973-80	Total diets		0	< 0.01	Manske & Johnson (1979) Johnson et al. (1981a,b) Johnson et al. (1979)
1972-73	Portulaca from fields treated with herbicide	raw, bottled or tinned	-	< 0.01-0.12	Bristol et al. (1982)

^a % of positives not specified.

2,4-D and especially its transformation product, dichlorophenol, at levels exceeding 20 µg/litre will impart an objectionable odour and taste to contaminated water (Pal'mova & Galuzova, 1963; Faust & Suffet, 1966). This organoleptic effect may reduce the likelihood of highly contaminated water being ingested. It is noteworthy that public water supplies containing "traces" of 2,4-D, and wells contaminated with 2,4-D or other herbicides have been shut down because of objectionable odours or tastes (Turbanov, 1968; Krauer & Schmaland, 1974; Frank et al., 1979).

5.1.3 In soil

Most of the information available at present concerning 2,4-D and other chlorophenoxy herbicide residues in soils has been reviewed by the National Research Council of Canada Associate Committee on Scientific Criteria for Environmental Quality (1978), Bovey (1980a), and by Que Hee & Sutherland (1981). In highly acidic soils, or in soils in cold or arid regions, 2,4-D degradation is apparently slow (Lavy et al., 1973; Buslovich & Melchior, 1976; Ou et al., 1978; Motale & Van Bladel, 1980; Herbst & Watson, 1980b). However, even at about 20 - 200 times the normal agricultural application rates, little or no detectable 2,4-D was left in soils under temperate climatic conditions with prolonged winters, after intervals of 185-240 days (Young et al., 1974; Stewart & Gant, 1977; Bovey, 1980a). Furthermore, results of a laboratory study on 2,4-D degradation in the soil showed a half-life of 4 days (Altom & Stritzke, 1973). Several soil monitoring studies in North America, in areas with regular 2,4-D use, have shown residues in less than 10% of the samples, and at levels of less than 1 mg/kg (Stevens et al., 1979; Wiersma et al., 1972; Gown et al., 1976).

The available data are inadequate for establishing regional and seasonal profiles of 2,4-D soil residues and of direct population exposure, but it is likely that direct exposure would be minor, except during or soon after herbicide application. Indirect exposure through the transfer of 2,4-D residues from soil to air, or food sources is assessed separately.

5.1.4 In food sources

Although 2,4-D and its transformation products do not tend to accumulate in plants and plant products, detectable residues of 2,4-D on food plants may be consumed by human

beings or animals and may thus contribute to the overall exposure of the human population to this chemical.

The results of pertinent studies on 2,4-D residues on or in foods, and in food sources for human beings and animals, are summarized in Tables 8-11. Theoretically, some contribution to the reported 2,4-D residues may have been partly derived from other phenoxy herbicides, as 2,4-D undergoes beta-oxidation to 2,4-D in some plants and fish, and in cattle (Lisk et al., 1963; Guttenmann & Lisk, 1965; Sundström et al., 1979; Bovey, 1980a).

5.1.4.1 Residues in retail food supplies

The frequency of occurrence and the levels of 2,4-D residues in over 110 000 samples of a variety of different ready-to-eat foods, beverages, and infant and young children's diets, have been studied over the last 20 years in the USA (Lipscomb, 1958; Corneliusson, 1970, 1972; Duggan et al., 1971; Duggan & Corneliusson, 1972; Johnson et al., 1974, 1981, 82). The 2,4-D residues found in such samples are reported in Table 6. The theoretical daily intake resulting from these residues was variously estimated to be < 1-5 µg/person per day (Duggan & Corneliusson, 1972).

Studies undertaken since 1970 have failed to detect residues of 2,4-D in any of the US diet samples analysed, except for a single positive sample in the dairy product food group which was estimated at 0.01 mg/kg (Manske & Johnson, 1975).

5.1.4.2 Residues in fish and shellfish

Fish and shellfish may be exposed to 2,4-D as a consequence of aquatic herbicide use, or through the agricultural use of 2,4-D. The residues in the edible portions of such fish rarely exceed 1 mg/kg wet weight (Erne, 1974, 1975 and Table 8). Residues of 2,4-D have not been detected in retail samples of fish and shellfish analysed as part of the US "market basket" studies (section 5.1.4.1).

There is some evidence that the organoleptic properties of the 2,4-D residues may reduce the likelihood of the consumption of fish flesh contaminated with higher levels of 2,4-D (Gavrillova, 1965; Folmar, 1979).

5.1.4.3 Residues in wild fruits and mushrooms

Uncultivated fruits and mushrooms taken from areas where 2,4-D is used, or was likely to have been used, were examined

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Table 9. 2,4-D residues in wild berries and mushrooms collected in fields or forests following application of phenylalkanoic herbicides

Country	Year(s)	Sample	2,4-D application rate (kg a.i./ha)	Days after treatment	No. samples analysed	2,4-D residues (mg/kg)	References
Canada ^a	1979-dl	raspberries	1.1-3.9	2 14-35	124	2.6-31.0 0.1-3.3	Frank et al. (1982)
Finland ^a	1974-75	vaccinium berries jam mushrooms	2.5 not known	10-356 not known 14-100	44 1 28	2.2 < 0.05-1.1	Mukula et al. (1978)
Finland ^a	1975-76	vaccinium berries	0.25-2.25	365	not stated	< 0.05	Siltanen et al. (1981)
Sweden ^a	1979	raspberries vaccinium berries blueberries mushrooms	1.5-2.2 1.5-2.2 1.5-2.2 1.5-2.2	2-32 2-32 2-32 2-32	9 68 19 15	< 0.03-0.9 < 0.03-7.7 < 0.03-2.9 < 0.03	Erne & Von Haartman (1973) ^b
Sweden ^a	1971-79	vaccinium berries ^b raspberries blueberries cowberries mushrooms	0.25-2.25 not stated not stated not stated not stated	365 14 2 1-18 7	41 not stated not stated not stated 1	nd (< 0.05) nd-2.5 nd-10.0 nd-6.0 0.3	Erne (1980)
Sweden ^a		blueberries vaccinium berries raspberries	0.4-1.5 0.4-1.5 0.4-1.5	1-35 30-35 1-10	not stated not stated not stated	0.2-5.3 0.5-4.5 0.7-2.0	Ingelög et al. (1977)

^a Samples taken from areas treated with 2,4-D.
^b Samples entering factory for processing.
 Data from authors' Table 1.

Table 10. 2,4-D residues reported in samples of herbicide-treated forage or feed

Country	Year(s)	Type of sample	2,4-D application rate (kg a.i./ha)	Post-treatment interval (days)	No. samples examined & positive	2,4-D residues (mg/kg)	References
Canada	1971	corn silage	1.1-3.9	1-36	1	4.15-0.011	Cochrane & Russell (1975)
Finland	1963-69	grass, lucerne, clover and alfalfa	1.5-3.5	1-11 1-10	1 1	600-1.7 11-0.4	Finnish State Institute of Agriculture (1963-1969)
Finland	1974-76	grass leaves and straw alfalfa leaves and straw alfalfa leaves and straw alfalfa leaves and straw	2.5	60-90 60-90 60-90 60-90	12 16 16 16	30-0.1 31-0.1 31-0.1 31-0.1	Mukula et al. (1978)
Germany, Federal Republic of	1971	barley, oat, rye, oat straw, rye, oat straw	1.25-0.125	64-101	1	< 0.015-0.01 < 0.12-0.02	Mayer-Rode (1971)
Hungary	1971	silage corn	1.4-1.5	56-120	1	0.8-0.025	Bodai et al. (1974)
Sweden	1972-76	barley, oats, lucerne	2	1-10	3 2 2	6.7-0.1 0.1 1.4-0.7	Erne & Rucqvist (1979)
USA	1971	alfalfa silage	4.5-8	1	1	1.4-1.65	Erne & Farver (1980)
USA	1971	alfalfa silage	4.5-8	1	1	1.4-1.65	Erne et al. (1977)
USA	1971	alfalfa silage	4.5-8	1	1	1.4-1.65	Erne et al. (1977)
USA	1971	alfalfa silage	4.5-8	1	1	1.4-1.65	Erne et al. (1977)

Table 11. 2,4-D residues in game and domestic animals and animal products

Country	Year(s)	Species	2,4-D treatment rate (kg a.i./ha)	Post-treatment interval (days)	Type of samples examined	2,4-D residues (ppb/kg)	References
Sweden	1968-1972	moose (<i>Alces alces</i>) deer (<i>Capreolus capreolus</i>) hares (<i>Lepus lepus</i>) pheasants grouse	game animals found dead, or shot by hunters in herbicide-treated areas	0	liver and kidney from 200 animals found dead liver and kidney from 100 animals shot by hunters	< 0.05-0.6 (2,4-D and 2,4,5-T)	Ståhl (1973, 1974)
USA	1963	Jersey cow	50 ppm in dist. for 4 days	0	milk	< 0.1	Stevens et al. (1964)

Table 11 (contd.)

USA	1974(?)	adult beef cattle	2,4-D 2000 mg/kg feed for 28 days (0.1-1.0 mg/kg feed)	0	liver, kidney, muscle, fat	< 0.1-0.2 < 0.1-0.2 < 0.1-0.2 < 0.1-0.2	Stevens et al. (1974)
USA	1974(?)	adult sheep	2000 mg/kg feed for 28 days	0	muscle, fat, liver, kidney	< 0.1-0.2 < 0.1-0.2 < 0.1-0.2 < 0.1-0.2	Stevens et al. (1974)
USA	1965(?)	dairy cows	animals grazing on pasture sprayed with herbicide at 2.24 kg a.i./ha	2	milk	< 0.1-0.2	Stevens et al. (1965)
USA	1972	dairy cows	10 300-1000 mg/kg in feed for 2-3 weeks	0-10	milk	< 0.1-0.2 < 0.1-0.2	Stevens et al. (1972)
USSR	1973	"forest deer"			liver, kidney, muscle, fat	< 0.1-0.2 < 0.1-0.2 < 0.1-0.2 < 0.1-0.2	Stevens et al. (1973)

kidney of a few game animals shot by hunters, or found dead in or near areas sprayed with phenoxy herbicides (Table II, Erne, 1974, 1975). The residues in muscle tissue were not measured but would be lower than in the liver and kidney, as indicated by the data summarized in Table II.

On the whole, the available evidence indicates that 2,4-D is rarely detected in commercial foods and that residues in food taken from areas where 2,4-D has been sprayed will usually be < 1 mg/kg food. The liver and kidney from range animals are possible exceptions, but these contribute little to the total diet of the general population.

5.2 Occupational Exposure to 2,4-D During the Production, Handling, and Use of Chlorophenoxy Herbicides

During occupational exposure to 2,4-D, the chemical may be absorbed via the inhalation, oral, and dermal routes, but more than 90% of the total amount of 2,4-D or other chlorophenoxy compounds entering the body under these circumstances appears to be absorbed through the skin and excreted relatively quantitatively in the urine as the phenoxy acid and readily-hydrolyzed conjugates (Kolmodin-Gedman et al., 1979, 1980; Libich et al., 1981; Draper & Street, 1982; Franklin et al., 1982; Leng et al., 1982; Nash et al., 1982) (section 6).

Data from occupational exposure studies concerning the amounts of 2,4-D found on the clothing or on cloth patches worn by workers are not included in this review because the correlation between these amounts and amounts absorbed into the body and then excreted in urine is poor (Franklin et al., 1982; Lavy et al., 1982; Leng et al., 1982).

5.2.1 Industrial exposure

Several studies have been published on the levels of 2,4-D to which workers producing or packaging 2,4-D herbicides are exposed (Fetisov, 1966; Johnson, 1971; Juzwiak et al., 1973; Andreason et al., 1979). In every case the amount of 2,4-D absorbed by the workers was uncertain and, therefore, the data are inadequate for estimating industrial exposure to 2,4-D. Workers manufacturing 2,4-D were also exposed to other chemicals (Assouly, 1951; Bashirov & Ter-Bagdasarova, 1970).

5.2.2 Exposure related to herbicide use

The available studies on the occupational exposure to 2,4-D of workers during the use of 2,4-D herbicides are summarized in Table III. Studies on the exposure of back-pack

Table III. Occupational exposure to 2,4-D during the use of herbicides

Study	Exposure route	Exposure level (mg/kg body weight/day)	Reference
Franklin et al. (1982)	Inhalation, dermal	0.004 - 0.04	Franklin et al. (1982)
Kolmodin-Gedman et al. (1979, 1980)	Inhalation, dermal	0.004 - 0.04	Kolmodin-Gedman et al. (1979, 1980)
Libich et al. (1981)	Inhalation, dermal	0.004 - 0.04	Libich et al. (1981)
Draper & Street (1982)	Inhalation, dermal	0.004 - 0.04	Draper & Street (1982)
Nash et al. (1982)	Inhalation, dermal	0.004 - 0.04	Nash et al. (1982)
Leng et al. (1982)	Inhalation, dermal	0.004 - 0.04	Leng et al. (1982)
Lavy et al. (1982)	Inhalation, dermal	0.004 - 0.04	Lavy et al. (1982)

Table 12 (contd).

2,4-D PGBE ester	26	helicopter in forestry use	-	5	nd - 0.06 ^a nd - 0.02 ^b	Lavy et al. (1982)
2,4,5-T PGBE ester	7	Back-pack forestry use single exposures, one week apart	-	5	0.01 - 0.09 (2,4,5-T)	Leng et al. (1982)
2,4-D/2,4-DE and 2,4-D/picloram ^c	23	roadside and right-of-way ground equipment incl. mist blowers	< 0.01 - 8 (one usually high result of 31)	3	-	Libich et al. (1981)
2,4-DE	17	aircraft repeated exposure	-	7	0.006 - 0.02 ^d (mean values/day)	Nash et al. (1982)
2,4-D amine salt and ester	26	ground equipment (single exposure)	-	7	nd - 0.08	

^a No special precautions taken.

^b Protective clothing worn.

^c Preparation used not specified.

^d Mean values per day recorded for different individuals.

It is not possible to calculate the total 2,4-D excretion in urine from these data, because of individual variations in urine concentrations from day to day from sample to sample.

sprayers to 2,4-D have not been published. However, comparable exposure data are available for 2,4,5-T back-pack sprayers and they have been included in Table 12 for comparison. The levels of 2,4-D found in the air of the working zone in these and other studies have already been referred to in section 5.1.1.1 and Table 5.

In studies undertaken before 1980, only the amounts of 2,4-D in the air, on the clothing, or on the skin were determined, except for 2 urinary 2,4-D values reported by Shafik et al. (1971). Thus the amounts of 2,4-D actually absorbed cannot be reliably estimated from these early reports and are not included in Table 12.

After 1980, several detailed occupational exposure studies were carried out to determine the amounts of 2,4-D or other chlorophenoxy acids absorbed by various members of ground and aerial spray teams, using a variety of equipment for dispersing aqueous or oil solutions or emulsions (Kolmodin-Hedman et al., 1979; Kolmodin-Hedman & Erne, 1980; Libich et al., 1981; Draper & Street, 1982; Franklin et al., 1982; Lavy et al., 1982; Leng et al., 1982; Nash et al., 1982).

The total 2,4-D urinary excretion levels reported in Table 12 reflect a wide variety of uses and show that the excretion does not usually exceed 0.1 mg 2,4-D/kg body weight per day of exposure. However, so far, a comprehensive comparison of the relative exposures resulting from different methods of application and different 2,4-D derivatives (amine salts and esters) or formulations (aqueous, oil) cannot be carried out, because the available data are still incomplete. The amount of 2,4-D absorbed depends on the type of work performed, and on the degree of care taken to avoid direct dermal contact with the herbicide concentrate, spray solution, or spray. The most heavily-exposed workers tend to be the mixer-loaders, who handle the herbicide concentrate, and the spray personnel. However, if careful, they may be exposed to less 2,4-D than, for example, a pilot of a spray plane who is not careful (Franklin et al., 1982; Leng et al., 1982; Lavy et al., 1982; Nash et al., 1982). The reports by Libich et al. (1981) and Leng et al. (1982) on ground spray crews indicate that, even under unfavourable working conditions, the amount of 2,4-D absorbed may be greatly reduced simply by wearing clean gloves and overalls, and by making the workers more aware of the importance of safe work habits.

As the chemokinetic profiles of urinary 2,4-D output are reported in only a few of the studies, summarized in Table 12, it is not possible to estimate the total 2,4-D intake in all cases.

The results of the studies by Libich et al. (1981) and by Draper & Street (1982) suggest that using single-exposure studies to estimate the peak exposure levels received by workers exposed several days in succession may give an underestimation.

No information is available on the amounts of chlorinated dibenzodioxins, or other by-products or contaminants, absorbed as a consequence of occupational exposure to 2,4-D herbicides.

In one extensive occupational monitoring programme undertaken in 1979-82, about 3000 urine samples were analysed for herbicide residues (Simpson, 1982). The subjects included pesticide factory staff, pest control operators, farmers, park workers, and others potentially exposed to 2,4-D. During the first year of the study, no 2,4-D was detected (< 0.001 mg/litre) in 735 of 973 samples. Most of the other samples contained less than 0.1 mg/litre and only 27 contained more than 1 mg/litre. The highest value was 31 mg/litre. The study is continuing.

5.3 Exposure of Bystanders to 2,4-D

Aerial drift and other forms of pesticide transport, as well as the contamination of surfaces during or after herbicide production, distribution, or use, may bring 2,4-D into contact with bystanders, i.e., persons other than those who are occupationally exposed. Few studies of bystander exposure to 2,4-D or other chlorophenoxy herbicides have been published. Studies available for review included that of Lavy et al. (1982) concerning 9 supervisors and observers present at two helicopter forest spray operations using 2,4-D propylene glycol butylether (PGBE) ester, respectively, for unspecified durations. These people excreted a maximum of 1.3 µg 2,4-D/kg body weight. In a forest ground spray operation with tractor-drawn equipment, 2,4-D was not detected (< 0.05 mg/litre) in the urine of bystanders (Kolmodin-Hedman et al., 1980). Additional bystander exposure studies for various 2,4-D use patterns are desirable. However, the 2,4-D intake of bystanders is unlikely to exceed the 2,4-D intake during occupational exposure.

5.4 Estimated Exposure of the General Population in 2,4-D-Use Areas

Data useful for estimating the intake by the general population of 2,4-D residues in the environment including those in food sources have been generated. The present calculations of the intake of the general population in an area of 2,4-D use are based on these data and on a series of

stated assumptions aimed at obtaining a moderate overestimation rather than underestimation of the actual exposure.

5.4.1 Intake of 2,4-D residues from air

On the basis of available information, it can be assumed that the general population in areas of 2,4-D herbicide use would rarely be exposed to 2,4-D concentrations exceeding 0.1 µg/m³ air.

Assuming an air level of 0.1 µg 2,4-D/m³, a body weight of 60 kg, an air intake of 20 m³ per day, and a 100% retention of 2,4-D, it can be calculated that the respiratory intake would be 0.03 µg 2,4-D/kg body weight per day.

5.4.2 Intake of 2,4-D residues from potable water

The larger surveys of potable water (Table 7) show mean 2,4-D residues in surface water to be generally < 0.1 µg/litre, but for the present estimate, it is assumed that potable water from surface sources or from treatment plants, during a period of about 10 days after reservoir treatment, can contain an average 2,4-D residue level of 2 µg/litre (Wojtalik et al., 1971 and Table 7). Assuming a 2,4-D concentration in water of 2 µg/litre, a body weight of 60 kg, a water intake of 2 litres per day (Canada, Health & Welfare, 1980), and a 100% absorption of the ingested 2,4-D, it can be calculated that the 2,4-D intake of the general population in a 2,4-D use area resulting from water could approach 0.07 µg/kg body weight per day, which could occur for about 10 days.

Insufficient data are available to give a reliable estimate of 2,4-D intake from ground water sources, but it is likely to be lower than the above value.

5.4.3 Intake of 2,4-D residues from soil

2,4-D on soil particles ingested with food or water, or carried into the air and inhaled, is considered to be part of the exposure due to residues in air, water, or food and is therefore assumed to be completely covered in these exposure estimates.

5.4.4 Intake of 2,4-D residues from food

The data in Tables 8 - 11 indicate that there is unlikely to be any exposure of the general population to 2,4-D residues in retail food supplies. The possibility that individuals are exposed to contaminated local sources of food has been assessed in section 5.1.4. In the case of milk or muscle

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meat, it can be assumed that no individual will be exposed to levels in excess of 0.02 mg/kg of these foods, the limit of detection of the method of analysis used. Assuming a concentration of 0.02 mg 2,4-D/litre in milk, and a consumption of 1.5 litre per day, the maximum intake from this source would be 0.0003 mg/kg body weight per day for a 60 kg adult. Individuals who consume wild berries taken from 2,4-D-treated areas could be exposed through this food source. Assuming consumption of 100g of berries per serving and a maximum 2,4-D concentration of 1 mg/kg, the intake from this source would be 0.002 mg/kg body weight per serving.

5.4.5 Total exposure of the general population in a 2,4-D-use area

The above considerations suggest that the total daily 2,4-D intake of the population in use areas will not normally exceed about 2 mg/kg body weight per day during the application period (Table 13).

Table 13. Components of estimated exposure to 2,4-D

Exposure group	Estimated amount of intake (mg 2,4-D/kg bw/day)	Source of 2,4-D
<u>Occupational</u>		
i. Factory workers	Insufficient data	Mainly dermal contact
ii. Applicator crews	100 ^a	
iii. Bystanders	0	
<u>General population in areas with 2,4-D use</u>		
	0.03	air
	0.07	water
	0.5	milk
	ND	retail food
	2.0	wild berries, mushrooms etc.

^a Based on total urinary output after several days of exposure.
^b Unlikely to exceed occupational exposure.

5.4.6 Total exposure of persons occupationally exposed in agriculture

An accurate maximum occupational intake of 2,4-D cannot be determined on the basis of the limited studies undertaken. However, the available data suggest that work performed in the

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preparation of, and during, agricultural application of 2,4-D herbicide will probably result in an exposure of not more than about 0.1 mg 2,4-D/kg body weight per day, providing that minimum precautions are taken against excessive exposure.

5.4.7 Total exposure of the general population outside areas of 2,4-D use

Monitoring of air, water, and food outside areas of known 2,4-D use show that intake is below present detection limits.

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6. CHEMIOBIOKINETICS AND METABOLISM

With the exception of recent occupational exposure studies and studies on animals published in 1979 or later, the available information on the uptake, distribution, transformation, and excretion of 2,4-D by human beings and other mammals has already been reviewed by Long (1977), National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality (1978), Young et al. (1978), Bovey (1980a,b), Shearer (1980), and United States Veterans' Administration (1981).

6.1 Uptake via Different Routes of Exposure

6.1.1 Uptake by Inhalation

6.1.1.1 Animals

Burton et al. (1974) found that small amounts of 2,4-D instilled into the rat lung were rapidly absorbed, apparently by a nonmetabolizable process following first-order kinetics, with an absorption half time of 1.6 - 1.7 min. The kinetics of the absorption of 2,4-D vapours or aerosols in the respiratory tract of animals have not yet been studied.

6.1.1.2 Human beings

The uptake of 2,4-D and of 2,4-D derivatives via the human respiratory tract does not appear to have been studied under controlled conditions. However, the observations of Kolman-Hudman & Erne (1980), Libich et al. (1981), Franklin et al. (1982), and Lavy et al. (1982) on people occupationally exposed to 2,4-D indicated that only a small percentage of the total amount of 2,4-D absorbed via all routes of exposure was taken in through the respiratory tract.

6.1.2 Dermal uptake

6.1.2.1 Animals

Mice whose tails had been immersed in 2,4-D butyl or crotyl ester solutions, 4 h daily for 3-5 days, absorbed lethal amounts of the chemicals (Fetisov, 1966). However, the actual doses absorbed and other details were not given. In contrast, no major ill effects were reported in studies in which rabbits were treated percutaneously for 2 or 3 weeks with 130-180 mg/kg body weight/day of a 50% aqueous solution of 2,4-D octyl ester, or with unspecified amounts of solutions

of 2,4-D dimethylamine salt in water, or oil solutions of 2,4-D isooctyl or butyl ester (Vinokurova, 1960; Kay et al., 1965).

6.1.2.2 Human beings

Only 5.8% of a dilute solution of ¹⁴C-labelled 2,4-D in acetone applied at a dose of 4 µg a.i./cm² to the ventral forearm of adults was excreted in the urine compared with 100% of a small intravenous dose (Feldmann & Maibach, 1974) (Table 14). The 2,4-D excretion in urine is delayed and more prolonged after dermal application than after intravenous or oral administration (Feldmann & Maibach, 1975; Sauerhoff et al., 1977), and complete elimination may take about one week (Levy et al., 1982; Leng et al., 1982). Cases of acute occupational 2,4-D poisoning following combined dermal and inhalation exposures (Monarca & Divito, 1961; Tsapko, 1966; Paggiaro et al., 1974), as well as occupational exposure studies (Table 12), suggest a fairly efficient dermal absorption of 2,4-D. However, the importance of solvents, surfactants, and other ingredients of the herbicides in the uptake of 2,4-D via the dermal route still needs to be defined.

6.1.3 Oral uptake

6.1.3.1 Animals

The uptake of 2,4-D from the gut of rats, mice, guinea-pigs, cattle, pigs, and sheep appears to be similar in both rapidity and extent to that observed in human beings (Mitchell et al., 1946; Lisk et al., 1963; Bache et al., 1964a; Erne, 1968a,b; Milhaud et al., 1970; Shafik et al., 1971; Buslovich et al., 1973; Fedorova & Belova, 1976; Clark et al., 1975; Beniczuk & Pogorzelska, 1975, 1981; Van Peteghem & Heyndrickx, 1975). In some of the ungulates, 2,4-D acid and 2,4-D amine salts or esters are at least partially converted to 2,4-D in the rumen, before being absorbed (Gutenmann et al., 1963; Lisk et al., 1963). Some of the esters may be less well absorbed from the gut than the acid or its alkali or amine salts (Erne, 1966a; Buslovich et al., 1973), but the uptake mechanisms for 2,4-D and its salts or esters is not known, and thus deserves further study.

6.1.3.2 Human beings

Information on the uptake of 2,4-D by human beings via the oral route has been gathered in studies on two groups of 5-6 volunteers each, who ingested single doses of 5 mg 2,4-D/kg body weight (Table 14), and by chemiobiokinetic studies on

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Table 14. Chemobiokinetics of 2,4-D in human beings following administration under controlled conditions

Product	Dose and dosing schedule	Subjects	Observations	Toxic effects EL ₅₀ (mg/kg bw) single dose	References
14C-2,4-D (New England Co., and Amersham Searle Co.)	1) Intravenous injection: Dose (7 μ Ci) not stated as 2,4-D/weight unit 2) Dermal application: 1 x 4 μ Ci 2,4-D (in acetone)/cm ² of skin of forearm. Application site was not washed for 24 h	6 (sex & age not stated) 6 (sex & age not stated)	Scintillation counting 1) 100% of dose excreted in urine urine in 120 h. Mean $t_{0.5}$ = 13 h 2) 5.8% of applied dose excreted in 120 h.	?	Feldmann & Maibach (1974)
2,4-D, 99% pure (Dow Chemical Co.)	Oral administration: 1 x 2.3, or 5 mg/kg bw, in gelatin capsule, with water, following breakfast	6 (adult M)	gas chromatography of blood & urine samples; no ill effects; no changes in clinical parameters: blood pressure, pulse rate, Hb, WBC counts (total & differential); mean plasma clearance $t_{0.5}$ = 33 $\pm$ 3.1 h; peak plasma conc. at 7-24 h = 40 mg/litre; ~75% of dose excreted in urine in 96 h "no metabolic transformation at up to 5 mg/kg"	?	Khanna & Kohli (1977); Kohli et al. (1974)
2,4-D, analytical grade	Oral administration: 1 x 5 mg/kg bw as a slurry in milk, or in powder form, with some water, following breakfast	6 (adult M, 70-90 kg)	gas chromatography - mass sp. for metry of blood & urine samples: no ill effects; essentially all of the dose absorbed; peak plasma conc. = 10-30 mg/litre within 24 h; mean plasma clearance $t_{0.5}$ = 11.6 h; mean urinary excretion $t_{0.5}$ = 17.7 h; total total excreted amount ~82% of dose administered; 4.8-7.1% of excreted compound was conjugated	?	Sauerhoff et al. (1976, 1977)

2 NULL = No-observed-adverse-effect level.

individuals who, with suicidal intent, swallowed lethal or non-lethal amounts of various 2,4-D herbicides (Geldmacher-Von Mallinckrodt & Lautenbach, 1966; Rivers et al., 1970; Kohli et al., 1974; Sauerhoff et al., 1976, 1977; Khanna & Kohli, 1977; Young & Haley, 1977; Prescott et al., 1979) (Table 15). These results show that single doses of 2,4-D are fairly rapidly and completely absorbed from the human digestive tract, unless the dose is so large that toxic effects interfere with absorption. However, in the two studies on volunteers, considerable individual variation in the rate and extent of absorption from the digestive tract was observed. The absorption mechanism appears to involve first-order kinetics (Kohli et al., 1974; Khanna & Kohli, 1977) and may fit a single- or multi-compartment chemobiokinetic model, depending on individual characteristics (Sauerhoff et al., 1977).

6.2 Distribution and Transformation in the Body

6.2.1 Animals

The absorption and distribution kinetics and metabolism of pure 2,4-D and of a variety of pure or commercial 2,4-D or 2,4-DB amine salts and esters have been repeatedly studied both in vivo and in vitro in a wide variety of animals including rats, mice, rabbits, guinea-pigs, cattle, sheep, goats, pigs, chickens, fish, and spiny lobsters (Gutemann & Lick, 1965; Erne & Sperber, 1974; Guafino & Arnold, 1979; James, 1979; Koschier & Pritchard, 1979; Pritchard & James, 1979; Pritchard & Miller 1980). The considerable differences observed in the relative amounts of residues found in the cells and plasma of mouse, rat, and horse blood, after dosing animals or after in vitro addition of 2,4-D (Erne, 1966a; Jensen & Renberg, 1976), in different tissues of rats, mice, and sheep (Erne, 1966a,b; Lindquist & Uhlberg, 1971; Milhaud et al., 1970; Buslovich et al., 1973; Clark et al., 1973; Elo & Vitolalo, 1979), and in the soluble and particulate fractions of rat tissues (Khanna & Fang, 1974) support the idea that there is more than one physiological compartment for 2,4-D storage. The distribution volumes appear to be equivalent to the volume occupied by about 25-50% of the body mass (Erne, 1966a).

2,4-D is reversibly bound to blood plasma proteins, particularly albumins, possibly at sites for which it competes with related compounds. The same sites are apparently also binding sites for palmitic acid and thyroxine. The extent of 2,4-D binding depends, in part, on pH and 2,4-D concentration (Floresheim et al., 1963; Erne, 1966b; Kolberg et al., 1973; Haque et al., 1975; Mason, 1975; Uiberger, 1980a), and may

Products	Preparation	Yield	Characteristics	References
$\text{C}_{10}\text{H}_{16}\text{O}$	from $\text{C}_{10}\text{H}_{18}\text{O}$ by acid-catalyzed dehydration of menthyl ether	40% (100°C)	bp 100–101°C/10 mm Hg; n_D^{20} 1.4660; n_D^{25} 1.4630; d_4^{20} 0.8240; d_4^{25} 0.8200; d_4^{30} 0.8160; d_4^{35} 0.8120; d_4^{40} 0.8080; d_4^{45} 0.8040; d_4^{50} 0.8000; d_4^{55} 0.7960; d_4^{60} 0.7920; d_4^{65} 0.7880; d_4^{70} 0.7840; d_4^{75} 0.7800; d_4^{80} 0.7760; d_4^{85} 0.7720; d_4^{90} 0.7680; d_4^{95} 0.7640; d_4^{100} 0.7600; d_4^{105} 0.7560; d_4^{110} 0.7520; d_4^{115} 0.7480; d_4^{120} 0.7440; d_4^{125} 0.7400; d_4^{130} 0.7360; d_4^{135} 0.7320; d_4^{140} 0.7280; d_4^{145} 0.7240; d_4^{150} 0.7200; d_4^{155} 0.7160; d_4^{160} 0.7120; d_4^{165} 0.7080; d_4^{170} 0.7040; d_4^{175} 0.7000; d_4^{180} 0.6960; d_4^{185} 0.6920; d_4^{190} 0.6880; d_4^{195} 0.6840; d_4^{200} 0.6800; d_4^{205} 0.6760; d_4^{210} 0.6720; d_4^{215} 0.6680; d_4^{220} 0.6640; d_4^{225} 0.6600; d_4^{230} 0.6560; d_4^{235} 0.6520; d_4^{240} 0.6480; d_4^{245} 0.6440; d_4^{250} 0.6400; d_4^{255} 0.6360; d_4^{260} 0.6320; d_4^{265} 0.6280; d_4^{270} 0.6240; d_4^{275} 0.6200; d_4^{280} 0.6160; d_4^{285} 0.6120; d_4^{290} 0.6080; d_4^{295} 0.6040; d_4^{300} 0.6000; d_4^{305} 0.5960; d_4^{310} 0.5920; d_4^{315} 0.5880; d_4^{320} 0.5840; d_4^{325} 0.5800; d_4^{330} 0.5760; d_4^{335} 0.5720; d_4^{340} 0.5680; d_4^{345} 0.5640; d_4^{350} 0.5600; d_4^{355} 0.5560; d_4^{360} 0.5520; d_4^{365} 0.5480; d_4^{370} 0.5440; d_4^{375} 0.5400; d_4^{380} 0.5360; d_4^{385} 0.5320; d_4^{390} 0.5280; d_4^{395} 0.5240; d_4^{400} 0.5200; d_4^{405} 0.5160; d_4^{410} 0.5120; d_4^{415} 0.5080; d_4^{420} 0.5040; d_4^{425} 0.5000; d_4^{430} 0.4960; d_4^{435} 0.4920; d_4^{440} 0.4880; d_4^{445} 0.4840; d_4^{450} 0.4800; d_4^{455} 0.4760; d_4^{460} 0.4720; d_4^{465} 0.4680; d_4^{470} 0.4640; d_4^{475} 0.4600; d_4^{480} 0.4560; d_4^{485} 0.4520; d_4^{490} 0.4480; d_4^{495} 0.4440; d_4^{500} 0.4400; d_4^{505} 0.4360; d_4^{510} 0.4320; d_4^{515} 0.4280; d_4^{520} 0.4240; d_4^{525} 0.4200; d_4^{530} 0.4160; d_4^{535} 0.4120; d_4^{540} 0.4080; d_4^{545} 0.4040; d_4^{550} 0.4000; d_4^{555} 0.3960; d_4^{560} 0.3920; d_4^{565} 0.3880; d_4^{570} 0.3840; d_4^{575} 0.3800; d_4^{580} 0.3760; d_4^{585} 0.3720; d_4^{590} 0.3680; d_4^{595} 0.3640; d_4^{600} 0.3600; d_4^{605} 0.3560; d_4^{610} 0.3520; d_4^{615} 0.3480; d_4^{620} 0.3440; d_4^{625} 0.3400; d_4^{630} 0.3360; d_4^{635} 0.3320; d_4^{640} 0.3280; d_4^{645} 0.3240; d_4^{650} 0.3200; d_4^{655} 0.3160; d_4^{660} 0.3120; d_4^{665} 0.3080; d_4^{670} 0.3040; d_4^{675} 0.3000; d_4^{680} 0.2960; d_4^{685} 0.2920; d_4^{690} 0.2880; d_4^{695} 0.2840; d_4^{700} 0.2800; d_4^{705} 0.2760; d_4^{710} 0.2720; d_4^{715} 0.2680; d_4^{720} 0.2640; d_4^{725} 0.2600; d_4^{730} 0.2560; d_4^{735} 0.2520; d_4^{740} 0.2480; d_4^{745} 0.2440; d_4^{750} 0.2400; d_4^{755} 0.2360; d_4^{760} 0.2320; d_4^{765} 0.2280; d_4^{770} 0.2240; d_4^{775} 0.2200; d_4^{780} 0.2160; d_4^{785} 0.2120; d_4^{790} 0.2080; d_4^{795} 0.2040; d_4^{800} 0.2000; d_4^{805} 0.1960; d_4^{810} 0.1920; d_4^{815} 0.1880; d_4^{820} 0.1840; d_4^{825} 0.1800; d_4^{830} 0.1760; d_4^{835} 0.1720; d_4^{840} 0.1680; d_4^{845} 0.1640; d_4^{850} 0.1600; d_4^{855} 0.1560; d_4^{860} 0.1520; d_4^{865} 0.1480; d_4^{870} 0.1440; d_4^{875} 0.1400; d_4^{880} 0.1360; d_4^{885} 0.1320; d_4^{890} 0.1280; d_4^{895} 0.1240; d_4^{900} 0.1200; d_4^{905} 0.1160; d_4^{910} 0.1120; d_4^{915} 0.1080; d_4^{920} 0.1040; d_4^{925} 0.1000; d_4^{930} 0.0960; d_4^{935} 0.0920; d_4^{940} 0.0880; d_4^{945} 0.0840; d_4^{950} 0.0800; d_4^{955} 0.0760; d_4^{960} 0.0720; d_4^{965} 0.0680; d_4^{970} 0.0640; d_4^{975} 0.0600; d_4^{980} 0.0560; d_4^{985} 0.0520; d_4^{990} 0.0480; d_4^{995} 0.0440;	
Herberticide containing $\text{C}_{10}\text{H}_{16}\text{O}$ plus 20% 2,4-D and 2,4,5-T	synthesized by esterification of menthyl ether with herbicide	40% (100°C)	bp 100–101°C/10 mm Hg; n_D^{20} 1.4660; n_D^{25} 1.4630; d_4^{20} 0.8240; d_4^{25} 0.8200; d_4^{30} 0.8160; d_4^{35} 0.8120; d_4^{40} 0.8080; d_4^{45} 0.8040; d_4^{50} 0.8000; d_4^{55} 0.7960; d_4^{60} 0.7920; d_4^{65} 0.7880; d_4^{70} 0.7840; d_4^{75} 0.7800; d_4^{80} 0.7760; d_4^{85} 0.7720; d_4^{90} 0.7680; d_4^{95} 0.7640; d_4^{100} 0.7600; d_4^{105} 0.7560; d_4^{110} 0.7520; d_4^{115} 0.7480; d_4^{120} 0.7440; d_4^{125} 0.7400; d_4^{130} 0.7360; d_4^{135} 0.7320; d_4^{140} 0.7280; d_4^{145} 0.7240; d_4^{150} 0.7200; d_4^{155} 0.7160; d_4^{160} 0.7120; d_4^{165} 0.7080; d_4^{170} 0.7040; d_4^{175} 0.7000; d_4^{180} 0.6960; d_4^{185} 0.6920; d_4^{190} 0.6880; d_4^{195} 0.6840; d_4^{200} 0.6800; d_4^{205} 0.6760; d_4^{210} 0.6720; d_4^{215} 0.6680; d_4^{220} 0.6640; d_4^{225} 0.6600; d_4^{230} 0.6560; d_4^{235} 0.6520; d_4^{240} 0.6480; d_4^{245} 0.6440; d_4^{250} 0.6400; d_4^{255} 0.6360; d_4^{260} 0.6320; d_4^{265} 0.6280; d_4^{270} 0.6240; d_4^{275} 0.6200; d_4^{280} 0.6160; d_4^{285} 0.6120; d_4^{290} 0.6080; d_4^{295} 0.6040; d_4^{300} 0.6000; d_4^{305} 0.5960; d_4^{310} 0.5920; d_4^{315} 0.5880; d_4^{320} 0.5840; d_4^{325} 0.5800; d_4^{330} 0.5760; d_4^{335} 0.5720; d_4^{340} 0.5680; d_4^{345} 0.5640; d_4^{350} 0.5600; d_4^{355} 0.5560; d_4^{360} 0.5520; d_4^{365} 0.5480; d_4^{370} 0.5440; d_4^{375} 0.5400; d_4^{380} 0.5360; d_4^{385} 0.5320; d_4^{390} 0.5280; d_4^{395} 0.5240; d_4^{400} 0.5200; d_4^{405} 0.5160; d_4^{410} 0.5120; d_4^{415} 0.5080; d_4^{420} 0.5040; d_4^{425} 0.5000; d_4^{430} 0.4960; d_4^{435} 0.4920; d_4^{440} 0.4880; d_4^{445} 0.4840; d_4^{450} 0.4800; d_4^{455} 0.4760; d_4^{460} 0.4720; d_4^{465} 0.4680; d_4^{470} 0.4640; d_4^{475} 0.4600; d_4^{480} 0.4560; d_4^{485} 0.4520; d_4^{490} 0.4480; d_4^{495} 0.4440; d_4^{500} 0.4400; d_4^{505} 0.4360; d_4^{510} 0.4320; d_4^{515} 0.4280; d_4^{520} 0.4240; d_4^{525} 0.4200; d_4^{530} 0.4160; d_4^{535} 0.4120; d_4^{540} 0.4080; d_4^{545} 0.4040; d_4^{550} 0.4000; d_4^{555} 0.3960; d_4^{560} 0.3920; d_4^{565} 0.3880; d_4^{570} 0.3840; d_4^{575} 0.3800; d_4^{580} 0.3760; d_4^{585} 0.3720; d_4^{590} 0.3680; d_4^{595} 0.3640; d_4^{600} 0.3600; d_4^{605} 0.3560; d_4^{610} 0.3520; d_4^{615} 0.3480; d_4^{620} 0.3440; d_4^{625} 0.3400; d_4^{630} 0.3360; d_4^{635} 0.3320; d_4^{640} 0.3280; d_4^{645} 0.3240; d_4^{650} 0.3200; d_4^{655} 0.3160; d_4^{660} 0.3120; d_4^{665} 0.3080; d_4^{670} 0.3040; d_4^{675} 0.3000; d_4^{680} 0.2960; d_4^{685} 0.2920; d_4^{690} 0.2880; d_4^{695} 0.2840; d_4^{700} 0.2800; d_4^{705} 0.2760; d_4^{710} 0.2720; d_4^{715} 0.2680; d_4^{720} 0.2640; d_4^{725} 0.2600; d_4^{730} 0.2560; d_4^{735} 0.2520; d_4^{740} 0.2480; d_4^{745} 0.2440; d_4^{750} 0.2400; d_4^{755} 0.2360; d_4^{760} 0.2320; d_4^{765} 0.2280; d_4^{770} 0.2240; d_4^{775} 0.2200; d_4^{780} 0.2160; d_4^{785} 0.2120; d_4^{790} 0.2080; d_4^{795} 0.2040; d_4^{800} 0.2000; d_4^{805} 0.1960; d_4^{810} 0.1920; d_4^{815} 0.1880; d_4^{820} 0.1840; d_4^{825} 0.1800; d_4^{830} 0.1760; d_4^{835} 0.1720; d_4^{840} 0.1680; d_4^{845} 0.1640; d_4^{850} 0.1600; d_4^{855} 0.1560; d_4^{860} 0.1520; d_4^{865} 0.1480; d_4^{870} 0.1440; d_4^{875} 0.1400; d_4^{880} 0.1360; d_4^{885} 0.1320; d_4^{890} 0.1280; d_4^{895} 0.1240; d_4^{900} 0.1200; d_4^{905} 0.1160; d_4^{910} 0.1120; d_4^{915} 0.1080; d_4^{920} 0.1040; d_4^{925} 0.1000; d_4^{930} 0.0960; d_4^{935} 0.0920; d_4^{940} 0.0880; d_4^{945} 0.0840; d_4^{950} 0.0800; d_4^{955} 0.0760; d_4^{960} 0.0720; d_4^{965} 0.0680; d_4^{970} 0.0640; d_4^{975} 0.0600; d_4^{980} 0.0560; d_4^{985} 0.0520; d_4^{990} 0.0480; d_4^{995} 0.0440;	
Herbicide containing $\text{C}_{10}\text{H}_{16}\text{O}$ plus 20% 2,4-D and 2,4,5-T	synthesized by esterification of menthyl ether with herbicide	40% (100°C)	bp 100–101°C/10 mm Hg; n_D^{20} 1.4660; n_D^{25} 1.4630; d_4^{20} 0.8240; d_4^{25} 0.8200; d_4^{30} 0.8160; d_4^{35} 0.8120; d_4^{40} 0.8080; d_4^{45} 0.8040; d_4^{50} 0.8000; d_4^{55} 0.7960; d_4^{60} 0.7920; d_4^{65} 0.7880; d_4^{70} 0.7840; d_4^{75} 0.7800; d_4^{80} 0.7760; d_4^{85} 0.7720; d_4^{90} 0.7680; d_4^{95} 0.7640; d_4^{100} 0.7600; d_4^{105} 0.7560; d_4^{110} 0.7520; d_4^{115} 0.7480; d_4^{120} 0.7440; d_4^{125} 0.7400; d_4^{130} 0.7360; d_4^{135} 0.7320; d_4^{140} 0.7280; d_4^{145} 0.7240; d_4^{150} 0.7200; d_4^{155} 0.7160; d_4^{160} 0.7120; d_4^{165} 0.7080; d_4^{170} 0.7040; d_4^{175} 0.7000; d_4^{180} 0.6960; d_4^{185} 0.6920; d_4^{190} 0.6880; d_4^{195} 0.6840; d_4^{200} 0.6800; d_4^{205} 0.6760; d_4^{210} 0.6720; d_4^{215} 0.6680; d_4^{220} 0.6640; d_4^{225} 0.6600; d_4^{230} 0.6560; d_4^{235} 0.6520; d_4^{240} 0.6480; d_4^{245} 0.6440; d_4^{250} 0.6400; d_4^{255} 0.6360; d_4^{260} 0.6320; d_4^{265} 0.6280; d_4^{270} 0.6240; d_4^{275} 0.6200; d_4^{280} 0.6160; d_4^{285} 0.6120; d_4^{290} 0.6080; d_4^{295} 0.6040; d_4^{300} 0.6000; d_4^{305} 0.5960; d_4^{310} 0.5920; d_4^{315} 0.5880; d_4^{320} 0.5840; d_4^{325} 0.5800; d_4^{330} 0.5760; d_4^{335} 0.5720; d_4^{340} 0.5680; d_4^{345} 0.5640; d_4^{350} 0.5600; d_4^{355} 0.5560; d_4^{360} 0.5520; d_4^{365} 0.5480; d_4^{370} 0.5440; d_4^{375} 0.5400; d_4^{380} 0.5360; d_4^{385} 0.5320; d_4^{390} 0.5280; d_4^{395} 0.5240; d_4^{400} 0.5200; d_4^{405} 0.5160; d_4^{410} 0.5120; d_4^{415} 0.5080; d_4^{420} 0.5040; d_4^{425} 0.5000; d_4^{430} 0.4960; d_4^{435} 0.4920; d_4^{440} 0.4880; d_4^{445} 0.4840; d_4^{450} 0.4800; d_4^{455} 0.4760; d_4^{460} 0.4720; d_4^{465} 0.4680; d_4^{470} 0.4640; d_4^{475} 0.4600; d_4^{480} 0.4560; d_4^{485} 0.4520; d_4^{490} 0.4480; d_4^{495} 0.4440; d_4^{500} 0.4400; d_4^{505} 0.4360; d_4^{510} 0.4320; d_4^{515} 0.4280; d_4^{520} 0.4240; d_4^{525} 0.4200; d_4^{530} 0.4160; d_4^{535} 0.4120; d_4^{540} 0.4080; d_4^{545} 0.4040; d_4^{550} 0.4000; d_4^{555} 0.3960; d_4^{560} 0.3920; d_4^{565} 0.3880; d_4^{570} 0.3840; d_4^{575} 0.3800; d_4^{580} 0.3760; d_4^{585} 0.3720; d_4^{590} 0.3680; d_4^{595} 0.3640; d_4^{600} 0.3600; d_4^{605} 0.3560; d_4^{610} 0.3520; d_4^{615} 0.3480; d_4^{620} 0.3440; d_4^{625} 0.3400; d_4^{630} 0.3360; d_4^{635} 0.3320; d_4^{640} 0.3280; d_4^{645} 0.3240; d_4^{650} 0.3200; d_4^{655} 0.3160; d_4^{660} 0.3120; d_4^{665} 0.3080; d_4^{670} 0.3040; d_4^{675} 0.3000; d_4^{680} 0.2960; d_4^{685} 0.2920; d_4^{690} 0.2880; d_4^{695} 0.2840; d_4^{700} 0.2800; d_4^{705} 0.2760; d_4^{710} 0.2720; d_4^{715} 0.2680; d_4^{720} 0.2640; d_4^{725} 0.2600; d_4^{730} 0.2560; d_4^{735} 0.2520; d_4^{740} 0.2480; d_4^{745} 0.2440; d_4^{750} 0.2400; d_4^{755} 0.2360; d_4^{760} 0.2320; d_4^{765} 0.2280; d_4^{770} 0.2240; d_4^{775} 0.2200; d_4^{780} 0.2160; d_4^{785} 0.2120; d_4^{790} 0.2080; d_4^{795} 0.2040; d_4^{800} 0.2000; d_4^{805} 0.1960; d_4^{810} 0.1920; d_4^{815} 0.1880; d_4^{820} 0.1840; d_4^{825} 0.1800; d_4^{830} 0.1760; d_4^{835} 0.1720; d_4^{840} 0.1680; d_4^{845} 0.1640; d_4^{850} 0.1600; d_4^{855} 0.1560; d_4^{860} 0.1520; d_4^{865} 0.1480; d_4^{870} 0.1440; d_4^{875} 0.1400; d_4^{880} 0.1360; d_4^{885} 0.1320; d_4^{890} 0.1280; d_4^{895} 0.1240; d_4^{900} 0.1200; d_4^{905} 0.1160; d_4^{910} 0.1120; d_4^{915} 0.1080; d_4^{920} 0.1040; d_4^{925} 0.1000; d_4^{930} 0.0960; d_4^{935} 0.0920; d_4^{940} 0.0880; d_4^{945} 0.0840; d_4^{950} 0.0800; d_4^{955} 0.0760; d_4^{960} 0.0720; d_4^{965} 0.0680; d_4^{970} 0.0640; d_4^{975} 0.0600; d_4^{980} 0.0560; d_4^{985} 0.0520; d_4^{990} 0.0480; d_4^{995} 0.0440;	

affect the rate and extent of renal 2,4-D excretion (Pritchard & James, 1979; Pritchard & Miller 1980), and thus the toxicity of 2,4-D.

In pregnant mammals, up to about 17% of a single dose of 2,4-D may rapidly cross the placenta to reach the embryos or fetuses (Lindquist & Ullberg, 1971; Fedotova & Belova, 1974; Antonenko, 1977).

Pigs and rats hydrolyse 2,4-D esters both in the gut and after absorption in the body (Erne, 1966a,b). Observations from several studies indicate that 2,4-D is not significantly metabolized in animals, except in ruminants. No 14C-labelled 2,4-D was produced by rats given C¹⁴- or C¹⁴-labelled 14C-2,4-D (Khanna & Fang, 1966). No 2,4-dichlorophenol (2,4-DiP) was detected in the tissues of mice or rats dosed by various routes with 2,4-D (Zielinski & Fishbein, 1967; Shakil et al., 1971; Fedorova & Belova, 1974; Grunov & Bohme, 1975). However, residues of 2,4-DCP were detected in the milk of dairy cows fed 100 mg 2,4-D/kg diet for 3 weeks (Bjork et al., 1972; Leng, 1972), and in the livers and kidneys of cattle and sheep fed up to 2000 mg/kg diet for 4 weeks (Clark, 1975; Leng, 1972, 1977). These 2,4-DCP residues probably resulted from the bacterial degradation of 2,4-D in the rumen of the animals. Bacterial degradation may also account for the 2,4-DCP reported by Antonenko (1977) in pregnant or lactating rats and rat fetuses.

Other investigators did not detect 2,4-DGP in the tissue of mice or rats dosed with 2,4-D by various routes (Zilinski & Fishbein, 1967; Shafik et al., 1971; Fedorova & Belova, 1974; Grunov & Böhne, 1974).

Results of studies on experimental animals have suggested that 2,4-D conjugates are formed in the kidney tubules (Erne, 1966a,b; Erne & Speiser, 1974; Grunov & Böhme, 1976). Turine and chloride

and lysine conjugates, as well as various other unidentified conjugates of 2,4-D have been found in the urine of rats, pigs, chickens, and the dogfish shark (*Squalus acanthias*) (Erne, 1966b; Erne & Sperber, 1974; Grunov & Böhme, 1974; Koschier & Pritchard, 1979). However, in rats and pigs, only about 10-20%, and in the chicken, less than 5% of the total amount of 2,4-D appeared to be excreted in this form. In the dogfish shark, the taurine conjugate may be primarily formed in the tubular cells of the kidney (Koschier & Pritchard, 1979). The site and mechanism of 2,4-D conjugation seem to be unknown in the other species.

6.2.2 Human beings

Studies on human volunteers who ingested pure 2,4-D, and on cases of accidental or voluntary acute poisoning with various 2,4-D herbicides have shown that 2,4-D is very rapidly

absorbed from the gut and carried in the blood to cells and tissues throughout the body, but that is not extensively transformed (Tables 14, 15) (Curry, 1962; Herbach & Machata, 1963; Nielsen et al., 1965; Dudley & Thapar, 1972; Coutselinis et al., 1977). The kinetics following ingestion suggest a 1- or 2-compartment distribution, depending on individual characteristics (Sauerhoff et al., 1977; Young & Haley, 1977). Following absorption of purified 2,4-D, or of herbicides containing only 2,4-D, no transformation products, including 2,4-dichlorophenol, were found in blood or tissues. After ingestion of herbicides containing 2,4-D and other compounds, some 2,4-D metabolites or manufacturing by-products were detected in tissues, but were not identified (Geldmacher-Von Mallinckrodt & Lautenbach, 1966; Prescott et al., 1979). Unidentified 2,4-D conjugates were also found in urine following ingestion of pure 2,4-D. These conjugates represented up to 27% of the 2,4-D ingested (Sauerhoff et al., 1976, 1977). Of the 5 North American volunteers studied by these authors, only one did not produce a conjugate; in contrast, apparently none of the 6 Indian subjects studied by Kohli et al. (1974) and by Khanna & Kohli (1977) produced 2,4-D metabolites.

6.3 2,4-D Levels in Body Tissues and Fluids

6.3.1 Animals

2,4-D levels in the blood and organs of mammals have been determined, e.g., by Erne (1966a,b), Milhaud (1970), Ruslovich et al. (1973), Khanna & Fang (1974), Clark et al. (1975), Jensen & Renberg (1976), and Eto & Yitai (1979). The highest residue levels were usually found in liver, kidney, lungs, spleen, and heart. In a study by Fedorova & Belova (1974), 6-8% of the amount of 2,4-D administered was found in all of the tissues examined in rats dosed orally 26-35 days previously with this chemical. However, the 2,4-D residue levels quoted were close to the limit of detection for the analytical method used.

6.3.2 Human beings

In volunteers, each of whom ingested a single dose of 5 mg 2,4-D/kg body weight, the 2,4-D levels in blood plasma reached peaks of about 10-45 mg/litre within about 7-24 h, and then declined (Kohli et al., 1974; Khanna & Kohli, 1977; Sauerhoff et al., 1977). In one group of workers occupationally exposed to 2,4-D for one week while using ground equipment for spraying (Kolmodin-Hedman et al., 1979), plasma levels ranged from the detection limit (0.02 mg/l) to 0.2 mg/litre, while

urinary levels ranged from 1 to 14 mg/litre. Urinary 2,4-D levels reported in other occupational exposure studies are summarized in Table 12. However, it should be noted that analysis of single urine specimens is not adequate for estimating the dose absorbed by individuals, because excretion follows a diurnal pattern and continues for several days after dermal exposure (Leng et al., 1977; Sauerhoff et al., 1979; Lavy et al., 1982). Thus, levels found after several days of spraying will probably be higher than first day levels, as reported by Libich et al. (1981) and Draper & Street (1982). However, excretion of 2,4-D should be completed within one week following the last exposure (Feldmann & Maibach, 1974; Lavy et al., 1982; Leng et al., 1982).

The toxic and lethal levels of 2,4-D in human blood and tissues are still not well defined. A woman with reportedly 335 mg 2,4-D/litre plasma did not show any signs of poisoning; in general, the acute lethal levels of 2,4-D appear to lie between 447 and 826 mg/litre plasma (Herbach & Machata, 1963; Nielsen et al., 1965; Geldmacher-Von Mallinckrodt & Lautenbach, 1966; Coutselinis et al., 1977; Prescott et al., 1979). The lowest lethal 2,4-D levels in blood or tissues were recorded several days after the chemical was ingested, i.e., after most of the 2,4-D had probably been eliminated. Among the different organs examined post mortem in cases of fatal 2,4-D poisoning, liver and kidney tended to contain the highest concentrations of 2,4-D, while brain and other fatty organs, and muscle including the heart, usually had lower 2,4-D levels (Table 15) (Curry, 1962; Herbach & Machata, 1963; Nielsen et al., 1965; Geldmacher-Von Mallinckrodt & Lautenbach, 1966; Dudley & Thapar, 1972; Coutselinis et al., 1977). As in the case of blood, the values for the different tissues may vary according to the proportion of 2,4-D eliminated by the time death occurs.

6.4 Elimination and Biological Half-Life

The term "biological half-life" will be used to indicate the time required to eliminate one half of a single dose of 2,4-D, or to reduce 2,4-D residues in the body fluids or tissues to one-half of the peak concentration. Biological half-life, as defined here, is a useful concept with which it is possible to make rough comparisons of the elimination rate of 2,4-D with that of other toxic chemicals.

6.4.1 Animals

The half-life values recorded in mammals fall into the range observed in the rat (Erne, 1966a,b; Fedorova & Belova, 1974; Khanna & Fang, 1974), with the exception of the very low

2,4-D butyl ester in whole mouse carcasses was observed by Zielinski & Fishbein (1967). In the investigations conducted by these authors also it was found that prior dosing with 2,4-D ("priming") may increase the rate of elimination of 2,4-D butyl ester in mice, probably through stimulation of the renal excretory function.

An important correlation between diet and 2,4-D elimination rate was observed by Orberg (1980b) in the goat. A protein-poor diet reduced the 2,4-D plasma clearance rate by about 50%, possibly because of decreased renal size and renal blood flow.

The many species-related factors known to affect the rate of excretion make interspecies comparisons difficult, and often the published reports do not include such details as diet, age, sex, and body weight of the test animals, type and purity of the test compounds, and important environmental factors such as the ambient temperature, which are necessary for valid comparisons.

2.4.4 Human beings

The studies on volunteers and on cases of accidental or voluntary 2,4-D poisoning summarized in Tables 14 and 15 show that human beings excrete 2,4-D mainly in the urine, and that the blood plasma clearance times depend on the dose, individual characteristics, and the presence or absence of compounds that may competitively inhibit 2,4-D excretion. For example, oral doses of 2,4-D, the biological half-life in blood plasma is about one day, depending on the circumstances. However, forced alkaline diuresis may reduce this to as little as 3-7 h (Sauerhoff et al., 1977; Prescott et al., 1979).

In occupationally-exposed people, absorption of successive daily doses of 2,4-D makes its biological half-life difficult to determine, but for single occupational exposures it has been estimated to be 35-68 h (Nash et al., 1982).

2.4.5 Chlorinated dibenzo-p-dioxins (CDDs)

The metabolism in the rat of several dibenzo-p-dioxins, including the 2,7-CDD found in 2,4-D, was described by Tulp & Hutzinger (1978). The primary pathway of transformation appears to involve hydroxylation at the 2-, 3-, 7-, or 8-position in the molecule; some sulfur-containing conversion products have also been identified.

7. EFFECTS OF 2,4-D ON ANIMALS

7.1 General Introduction

Many studies of the toxicity of 2,4-D have been carried out before the possible toxicological importance of manufacturing by-products, such as 2,6-dichlorophenoxycetic acid, 2,4,6-trichlorophenoxycetic acid (2,6-D and 2,4,6-T), monochlorophenoxycetic acid, or N-nitroso compounds, was known or appreciated.

Furthermore, 2,4-D may be contaminated with several chlorinated dibenzo-p-dioxins (section 2.1.4). The toxicity of a number of these contaminants has not been examined in detail, but whether or not their presence would affect the toxicity of 2,4-D and its derivatives would depend on the amount of CDD present in the product, and on the inherent toxicity of the particular CDD isomers. The most toxic CDD isomer, namely 2,3,7,8-TCDD (Schwartz et al., 1973; McConnell et al., 1972; Leng, 1979; Kociba & Schwetz, 1982), is not normally found in 2,4-D products (see also section 2.1.4). However, there have been instances in which the same manufacturing equipment was used to produce both 2,4,5-T and 2,4-D, resulting in cross-contamination of 2,4-D with 2,4,5-T and 2,3,7,8-TCDD (US EPA, 1982).

Studies of structure-activity relationships using *in vitro* systems have shown that the CDDs that may be present in 2,4-D and its derivatives have a much lower biological activity than 2,3,7,8-TCDD (Poland & Glover, 1973; Poland & Kende, 1974; Poland et al., 1976; Bradlaw et al., 1980; Knutson & Poland, 1980). However, except for a study of the carcinogenic potential of 2,7-dichlorodibenzodioxin (2,7-DCDD) (US National Cancer Institute, 1979), the chronic toxicity of the CDD detected in 2,4-D and its derivatives has not been studied.

2,4-D has been in use as a herbicide for nearly 40 years, and during this time a great deal of literature on the toxicology of this chemical has accumulated. The extent to which its toxicity to various organisms has been tested, and the types of 2,4-D products used for such testing have varied over the years. Earlier 2,4-D products probably contained higher concentrations of trace contaminants than the 2,4-D in use today, and therefore it may have been found to be more toxic in earlier than in more recent studies. In addition, the generally accepted standards and protocols for pesticide toxicity tests have changed, making some of the older tests inadequate by present day standards. For these reasons, attention has been focused on the more recent studies. The older studies are, in many instances, only cited for completeness and should be used with caution, especially when

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Table 16. Absolute oral toxicity of 2,4,6-trichloro-3-nitrophenol, and data

[illegible]

3.2.3.1 Acute oral LD₅₀

7.2.3.1.1 Mammals

Published acute oral LD₅₀ values vary for different 2,4-D products and test species (Table 16). It appears that 2,4-D has a moderate acute toxicity for mammals (WHO, 1976).

7.2.3.1.2 Birds

Table 17 shows published oral LD₅₀ values for coccidiosis.

describing specific toxic effects to unspecified 2,4-D products and when establishing an effect level or no-observed-adverse-effect level for adverse effects of 2,4-D. The Task Group noted that a number of additional studies on 2,4-D are currently in progress. As the additional information becomes available, the present document will need to be updated.

7.2 Acute Effects

The reports of experimental studies to define the toxic and other effects of 2,4-D or its derivatives cover a wide range of organisms commonly referred to as "animals", including worms, molluscs, arthropods, lower vertebrates, birds, and mammals. Much of this information, especially on acute toxic effects, was recently tabulated by the National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality (1978), Schneider (1979), Bowey & Young (1980), Shearer & Halter (1980), and the Commission of the European Communities (CEC, 1981).

The Commission of the European Communities (CEC) has recommended that pesticides include assays for eye and skin irritation and for skin sensitization, and determinations of the acute oral, percutaneous, and parenteral lethal doses, or of the corresponding lethal concentrations in the air, diet, or water.

1.2.1 Skin and eye irritancy

2,4-D does not appear to be an eye or skin irritant (Schneider, 1979). Adequate tests of the potential irritative properties of 2,4-D derivatives have not been reported in the literature.

11.11.1971 16.11.26 11.11.25 7.7.71

No adequate published information is available on the formal acetalization potential of 2,4-D and its derivatives in mammals.

2.2.3 Lethal doses and concentrations (LD₅₀ and LC₅₀)

The lethal potential of a chemical is usually measured as the dose (mg/kg body weight), or as the concentration in the diet, or water (mg/kg, mg/l, mg/litre, respectively) that will kill 50% of the test animals in a specified time interval. These amounts are referred to as the LD50 or LD50₀₁. For 2,4-D and its derivatives, and for 2,4-D herbicidal formulations, these statistically estimated values vary depending on the test product, the test species, and the route and frequency of administration (Tables 16 and 17).

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Table 11. Acute toxicity for various 2,4-D derivatives in fish.

Derivative	Species	Concentration (mg/l)	Exposure Time (days)	Reference
2,4-D	Brook trout	100	14	Buslovich (1968)
2,4-D	Brook trout	100	14	Buslovich (1968)
2,4-D	Brook trout	100	14	Buslovich (1968)
2,4-D	Brook trout	100	14	Buslovich (1968)
2,4-D	Brook trout	100	14	Buslovich (1968)
2,4-D	Brook trout	100	14	Buslovich (1968)
2,4-D	Brook trout	100	14	Buslovich (1968)
2,4-D	Brook trout	100	14	Buslovich (1968)
2,4-D	Brook trout	100	14	Buslovich (1968)
2,4-D	Brook trout	100	14	Buslovich (1968)

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7.2.3.2 Acute dermal LD₅₀

7.2.3.2.1 Mammals

Reports by Buslovich (1968) and Feltus (1966) indicate that, under extreme test conditions, mice and rats may absorb lethal amounts of 2,4-D amine salts and esters through their skin, but no acute dermal LD₅₀ values were available for review.

7.2.3.3 Acute inhalation LD₅₀

No accurate measurements of acute inhalation LD₅₀ values for 2,4-D products were available.

7.2.3.4 Parenteral LD₅₀

The acute parenteral LD₅₀ values reported for various laboratory animals and 2,4-D products ranged from 20 to 100 mg a.i./kg body weight (National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality, 1978; Young et al., 1978; Schneider, 1973).

7.2.4 Acute toxicity in aquatic organisms

The available reports indicate great differences in acute LD₅₀ values obtained with different test species, and with different 2,4-D derivatives and test formulations. The species appear to be considerably more toxic than the water-soluble salts. For fish, the LD₅₀ for various 2,4-D isooctyl ester products may range from 5 to 68 mg/litre for bluegill sunfish (*Lepomis macrochirus*) and from 62 to 153 mg/litre for rainbow trout (*Salmo gairdneri*) (Schneider, 1979). This variability may, in part, be due to differences in test animal species and strain, in test conditions (temperature, pH, O₂ tension, mineral content of water), and, in part, to the effects of chemicals other than 2,4-D in the herbicide formulations, or of 2,4-dichlorophenol, which may occur in water as a decomposition product of 2,4-D (Holcombe et al., 1960). Discussions on this variability can be found in numerous reviews: Way (1969), Katz, et al. (1972), National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality (1978), and of Halter (1980), as well as in the reports of studies by Harrison & Rees (1966), King & Penfold (1966), Loper (1961), Hughes & Davis (1967), Butler (1965), Hiltbrun (1967), Mount & Stephan (1967), Alabaster (1969), Sanders (1970a), Andrushech (1972), Goble (1972), Shim & Self (1973), Fabacher & Chambers (1974), Meehan et al. (1974), Nishitani & Yoshida (1974), Reinhold et al.

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1972, Vaidia & Jeeve (1981), and particularly in the extensive and methodical investigation of Prasad (1973). Similar information on aquatic invertebrates is available in the review of Hutchinson & Kemp (1972) and in the review of Cooper (1978). Budak & Claff (1960), Brown et al. (1961), Wells (1962), Sanders (1970b), Klekowski & Zvirgava (1971), Wierzbicka (1975a,b), and Caldwell et al. (1972, 1973) and Palmer (1966) indicate that fish and aquatic invertebrates will be exposed to water containing toxic amounts of 2,4-D products.

2.3 Sub-chronic and Chronic Toxicity

2.3.1 Mammals

Most of the published long-term studies with mammals have already been reviewed by Bodyskin et al. (1969), Kay (1970), JAC (1977, 1982), National Research Council of Canada, A Scientific Committee on Scientific Criteria for Environmental Quality (1978), Fong et al. (1978), Bovey & Fong (1978), and Veterans' Administration (1981).

In the long-term as in the short-term studies on mammals, the test products were mainly administered orally. The composition of the test products was not adequately known by present standards, but many of the tests were carried out with more or less purified 2,4-D or its alkali salts. It is difficult to decide to what extent the available results reflect the toxicological properties of the present 2,4-D products, in which the maximum contents of CDB and other toxic by products are kept at very low levels.

A long-term feeding study in the rat (Hansen, 1971) was evaluated by FAO/WHO JMPR in 1971 (FAO/WHO, 1972). It was concluded that a no-observed-adverse-effect level in the rat was equivalent to 31 mg/kg body weight per day. However, new studies on mice and rats are in progress.

2.3.1.1 Clinical signs of poisoning

Signs of toxic effects on the digestive tract, such as diarrhoea, vomiting, dysphagia, decreased gut motility, irritation, or necrotic changes (in dogs including necrosis of oral tissues) are likely to appear in animals following the absorption of high doses of 2,4-D or its derivatives by the oral, dermal, or inhalation routes, and after parental injections (Bucher, 1946; Hill & Carlisle, 1947; Drill & Hirtzka, 1953; Rowe & Hymas, 1954; Thomassen, 1958; Björklund & Erne, 1966; Erne, 1966a; Palmer & Radeleff, 1969). Blood-tinged discharges from the nose, and in dogs also nasal and eye irritation and skin lesions may also occur (Bucher,

1946; Hill & Carlisle, 1947; Kosyan et al., 1974). Cattle may suffer from tympanitis, and may show signs of thirst (Björklund & Erne, 1966). However, some of the signs of 2,4-D poisoning reported in herbivores pastured on 2,4-D-treated vegetation may have been caused by the ingestion of inherently poisonous plants (Maclean & Davidson, 1970). Pigs may refuse to eat feed containing high amounts of 2,4-D (Strach & Bohosiewicz, 1964), but sheep have been reported to feed avidly on 2,4-D-treated vegetation (Sibakov et al., 1972). Characteristic signs of severe 2,4-D poisoning in mammals appear to be muscular weakness, stiffness, stilted gait, and possible spasms (myotonia), alleviated by exercise and exacerbated by rest. There may also be muscular incoordination progressing to paralysis especially in the hind limbs; in rodents, caudal rigidity has also been observed. These clinical signs are the result of the myotoxic action of 2,4-D discussed below, which, through its effects on the heart, may also lead to hypotension or hypertension, cardiac fibrillation and death. Prolonged inactivity may also occur and may be pulmonary congestion, emphysema, and pneumonia (Bucher, 1946; Hill & Carlisle, 1947; Drill & Hirtzka, 1953; Vinokurova, 1953; Björklund & Erne, 1966; Goshkov, 1972; Sibakov et al., 1972; Kosyan et al., 1974).

At high doses, 2,4-D and its derivatives may act as a central nervous system depressant and cause lethargy, slowed respiration, stupor, coma, and death (Bucher, 1946; Hill & Carlisle, 1947; Drill & Hirtzka, 1953; Vinokurova, 1960; Desi et al., 1962a,b; Desi et al., 1962a,b; Kosyan et al., 1974; Flo & Vitalo, 1977, 1979).

2.3.1.2 Effects on food and water consumption, and on body weight

Relatively high concentrations of 2,4-D or its derivatives in the diet or drinking water, or given by capsule, gavage, or parenterally, may cause a reduction in food and water consumption, and weight loss or reduced body weight gain in rats (Rowe & Hymas, 1954; Thomassen, 1958; Björklund & Erne, 1966; Chang et al., 1974; Chen et al., 1981; Gorshkov, 1972), as well as in dogs (Bucher, 1946; Drill & Hirtzka, 1953), in pigs (Strach & Bohosiewicz, 1964; Björklund & Erne, 1966), in rabbits (Loktionov et al., 1973), in cattle (Rowe & Hymas, 1954; Björklund & Erne, 1966; Palmer & Radeleff, 1969; McLennan, 1974), and sheep (Palmer & Radeleff, 1969). However, the same authors, as well as Guseva (1956) and Hansen et al. (1971) did not observe these effects at low doses or low dietary concentrations of 2,4-D. In fact, in the studies by Raul & Murnay (1948) and Strach & Bohosiewicz (1964) on rats and piglets fed low dietary concentrations of 2,4-D, an

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unimpaired appetite and an improved body weight gain was seen in some groups of animals. In these studies, the dosages ranged between 15 and 119 mg 2,4-D/kg body weight.

Observations of Thomson (1959), Strach & Babosiewicz (1960), Hattynov (1970), Gorskov (1972), Sadykov et al. (1972), and Erve (1974), on hares (*Lepus timidus*), European elk, pigs, and rats suggest that, given a choice, animals will refuse to eat food or to drink water containing more than a certain amount of 2,4-D and that this may, in part, be because of the strong characteristic odour and taste of this compound, or of oleaginous substances such as diesel fuel that are used in herbicide formulations or as diluents.

7.3.1.3 Effects on the central nervous system (CNS)

It has been suggested that the signs of central nervous system depression in animals severely poisoned with 2,4-D are related to a partial breakdown of the blood-brain barrier, possibly as a result of damage to capillary vessels, and a subsequent accumulation of 2,4-D in the CNS (Erve & Thomson, 1974; Hattynov, 1970, 1979).

If so, further studies are needed to clarify the mechanism by which 2,4-D acts on the central nervous system.

7.3.1.4 Effects on the peripheral nervous system

No peripheral neuropathy was attributed to 2,4-D in the available reports on short-term and long-term studies with 2,4-D in a variety of animals (Shillinger & Naumova, 1972; Stojanov, 1959). The partial or complete paralysis, especially of the hind legs, of 2,4-D poisoned animals reported already by Bucher (1946) and Hill & Carlisle (1947) may be a myotoxic rather than a neurotoxic effect of 2,4-D. Moreover, in severe 2,4-D poisoning, a general weakness may lead to inactivity that might be interpreted as paralysis.

No signs of neuropathy were reported by Kay et al. (1965) in rabbits given large percutaneous doses of 2,4-D (dimethylamine salt, 2,4-D butyl ester, or 2,4-D isooctyl ester). In similar studies in which rats or rabbits were exposed by the dermal route, Buslovich (1953) reported paralysis and death in rats given unspecified doses of 2,4-D amine salt or 2,4-D butyl ester, but no peripheral neuropathy, while Stojanov (1959) found 130-180 mg of a 50% aqueous emulsion of 2,4-D butyl ester/kg body weight to have "no systemic effect" on rabbits.

The neurotoxic effects in animals of 2,4-D and of other related compounds have not been adequately studied. Further research is required to elucidate the mechanism of the neurotoxic and myotoxic action of 2,4-D in animals.

7.3.1.5 Myotoxic effects

Most of the studies of the effects of 2,4-D on vertebrate muscles were carried out because the 2,4-D-induced abnormalities resemble a heritable muscle disorder in human beings, namely myotonia congenita (Rothmann et al., 1966; Laskowski & Bettbarn, 1977). As a rule, high doses of 2,4-D (1/4 to 1/2 LD₅₀) are required to obtain severe and prolonged myotonia. The effects of 2,4-D on muscle cells are complex, and include disturbances in: the activity of various enzymes (leading, for example, to increased lactate production); potassium levels; membrane resistance; and in chloride conductance. There are also shifts in calmodulin-binding sites, changes in muscle and nerve cell electrical potentials, and mitochondrial structural and ultrastructural degenerative changes in muscle (Vezaguirre et al., 1968; Funn & Stein, 1969, 1965, 1966; Herre 1966, 1975, 1977; Rothmann et al., 1966; Kenigsbort, 1968; Funn & Funn, 1968; Boden et al., 1971; Preiss & Rossner, 1971; Stojanov, 1971; Buslovich & Koldobskaya, 1972; Kridger et al., 1972; Senke & Kudei, 1972; Brady, 1973; Daman et al., 1973; Funn et al., 1973; Bretay & Caputo, 1978; Fox et al., 1978; Senke et al., 1979; Eisenstein & Gindoff, 1979; Mazarein et al., 1979a,b). Similar effects are produced by the 2,4-D analogue drug chlorbutate (atromid) (Priedes et al., 1973; Smals et al., 1977). None of the available studies on 2,4-D-induced myotonia were designed to establish non-observed-adverse-effect levels for the various myotoxic effects in intact animals, and therefore additional studies should be carried out for this purpose.

7.3.1.6 Cardiovascular effects

As part of its myotoxic action, 2,4-D and its derivatives may in high doses cause biochemical, physiological, and structural damage to the myocardium in vitro and in vivo (Budem et al., 1971; Preiss & Rossner, 1971; Kridger et al., 1972; Mazarein et al., 1979a,b).

7.3.1.7 Haematological effects

Shifts have been reported in the number or types of erythrocytes, leukocytes, or bone marrow cells, or changes in haemoglobin levels, in a variety of laboratory and domestic mammals given 2,4-D or 2,4-D derivatives (Bucher, 1946; Hill & Carlisle, 1947; Drill & Hratzka, 1953; Shillinger & Naumova, 1957; Shillinger, 1960; Björklund & Erve, 1966; Hansen et al., 1971; Sadykov et al., 1972; Loktionov et al., 1973; Konyan et al., 1974; and Halliup et al., 1980).

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7.3.1.8 Effects on blood chemistry

Rowe & Hymas (1954) were apparently the first investigators to monitor blood chemistry in 2,4-D-treated animals. They did not observe any adverse effects at 2,4-D dietary levels as high as 300 mg 2,4-D/kg in rats treated for 113 days.

Other investigators noted changes in various serum, plasma, or erythrocyte enzyme activity levels, and shifts in the levels of electrolytes, glucose, blood proteins, or other chemicals in response to treatment with 2,4-D or 2,4-D derivatives in rats, rabbits, cattle, pigs, or sheep. Of interest from such animals to which 2,4-D was added in vitro are the changes in transaminase (Sokol, Stoltz, and Stoltz, 1969), or glucose levels appeared to be secondary to a primary hepatotoxic, or hepatotoxic effects of high doses of 2,4-D.

Other biochemical effects observed in vivo or in vitro

sufficiently high doses of 2,4-D may induce changes in nutritional oxygen consumption and oxidative phosphorylation, in electrolyte, ascorbic acid, glycogen, lipid, and nucleic acid contents of organs, tissues, cells, or cell fractions from a variety of mammals (Sokol, 1969; Guseva, 1956; Baker et al., 1960; Kuhn & Smith, 1964; Beebe, 1966, 1967, 1968; Shevchenko, 1966; Butler & Pang, 1967; Kenigsberg, 1968; Dzhabarov & Tsilikov, 1969; Stanosz, 1969; Buslovich & Koldobskaya, 1972; Graft et al., 1972; Buslovich et al., 1973; Abu-gharwa & Hollingsworth, 1974; Chang et al., 1974; Nikandrov, 1974; Venkatar & Padulak, 1978).

7.3.1.9 Pulmonary effects

No pulmonary abnormalities were reported in studies with rats, rabbits, rats, or dogs conducted by Bucher (1966), Drill & Hirtzka (1963), Rowe & Hymas (1954), Schilling & Naumova (1969), Schilling (1960), or Bjorklund & Erne (1966). In contrast, Hill & Carlisle (1947) and Palmer (1971) noted congestion of pulmonary vessels, pulmonary petechial haemorrhages, and pulmonary edema or emphysema in mice, rats, pigs, cattle, or sheep that died of 2,4-D poisoning.

7.3.1.11 Hepatotoxicity

Rabbits, rats, mice, dogs, cattle, or sheep treated for a prolonged period with toxic doses of 2,4-D were found to develop a subacute toxic hepatitis with congestion of hepatic blood vessels, and cloudy swelling, fatty infiltration, local necrosis, degeneration, or atrophy of hepatocytes, especially of the parenchyma in the centrilobular areas (Bucher, 1947; Hill & Carlisle, 1947; Drill & Hirtzka, 1963; Rowe & Hymas, 1954; Bjorklund & Erne, 1966; Szekes et al., 1970; Palmer, 1972). High doses of 2,4-D may induce a deterioration of peroxisomes and increased levels of mixed function oxidases in liver cells of rats and hamsters (Buslovich et al., 1982; Vainio et al., 1982, 1983).

Changes in the levels of certain liver enzymes such as salivary or hepatic dehydrogenase, and in ascorbic acid or glycogen content of hepatocytes and production have also been reported (Schilling & Naumova, 1969; Baker et al., 1960; Dzhabarov & Tsilikov, 1969; Szekes et al., 1970; Buslovich et al., 1973; Chang et al., 1974; Nikandrov, 1974). Some of the results concerning liver glycogen levels in rats subjected to reverse trend at high doses, as Dzhabarov & Tsilikov (1969) found that a dose equivalent to 1/16 kg/kg per day lowered the liver glycogen content, while Chang et al. (1974) observed the opposite effect at doses of about 100 mg/kg per day.

7.3.1.12 Effects on the kidney

In early studies with high doses of 2,4-D, signs of impaired kidney function, increased relative kidney weight, and of gross and histological abnormalities (parenchymatous degeneration, hypertrophy, and hyperplasia, cloudy swelling especially in the cells of the proximal convoluted tubules, and glomerular lesions) were noted in mice, rats, and dogs (Bucher, 1946; Hill & Carlisle, 1947; Drill & Hirtzka, 1953; Rowe & Hymas, 1954). That the kidney is a target organ for the structural, physiological, and chemical effects of 2,4-D was confirmed repeatedly by later and more detailed studies on a wider range of test species including pigs, goats, and sheep (Schilling, 1960; Bjorklund & Erne, 1966; Erne, 1966a; Stanosz, 1969; Hunt et al., 1970; Milhaud et al., 1970; Goshkov, 1972; Palmer, 1972; Senczuk & Pogorzelska, 1975; Koschier et al., 1978; Öberg, 1980a). In a recent 13-week study on rats, the no-observed-adverse-effect level for histological changes induced with pure 2,4-D in mammalian kidney appeared to be 15 mg/kg body weight per day (Chen et al., 1981).

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7.3.1.13 Effects on endocrine organs.

Swelling and congestion of the thyroid were noted by Palmer (1972) in cattle and sheep fatally poisoned with various 2,4-D products. Effects of 2,4-D on iodide uptake by the thyroid gland were first described by Sos & Kertai (1958) and studied further by Florshaim & Velroff (1962), Florshaim et al. (1963), Tsilikov (1969), and Gorskov (1972). Their results indicate that doses of 5-250 mg 2,4-D sodium salt or equivalent/kg body weight per day have a stimulatory effect on thyroid function.

Effects of 2,4-D on adrenal function and its relationship to muscle carbohydrate metabolism and 2,4-D-induced myoglobinuria have been studied by Kenisberg (1968), and by Ruslovich & Koldobskaya (1972). 2,4-D-induced changes in adrenal or thyroid function may also be implicated in the abnormal temperature regulation in 2,4-D-treated rats described by Sudak et al. (1966). However, it is noteworthy that Ruslovich (1963) was unable to induce a change in the body temperature of rats by giving 15-20 oral doses of 2,4-D sodium salt, twice salt, or butyl ester at a level of 1/10 or 1/5 LD_{50/day}.

7.3.1.14 Effects on the digestive tract

Vomiting, diarrhea, hyperemia, bloody exudates in the gut, necrotic changes in the mucosa, and other non-acute toxic effects on the digestive tract have been reported after administration of high doses of 2,4-D by either the oral or parenteral route in mice, rats, and dogs (Butcher, 1970; Hill & Carlisle, 1947; Drill & Hirsatzka, 1953; Kossan et al., 1974). However, Hansen et al. (1971) did not observe any such effects in a 2-year feeding study at dietary levels of 2,4-D corresponding to about 37.5 and 67.5 mg 2,4-D/kg body weight per day, respectively, for rats and dogs. Their observations on dogs contrast with those of Drill & Hirsatzka (1953) who found repeated doses of 20 mg 2,4-D/kg body weight per day to be fatal in 3 out of 4 dogs studied by them.

7.3.2 Birds

The published reports on the toxic effects of 2,4-D and 2,4-D products in birds deal mainly with chickens (Gallus domesticus) and game birds. The available data on cumulative oral lethal doses for game birds have been summarized in Table 18; studies on the reproductive, embryotoxic, and teratogenic effects of 2,4-D are dealt with in section 7.3.6.

The clinical signs of 2,4-D poisoning in birds appear to be similar to those in mammals, and the kidney seems to be the

most sensitive organ. No adverse effects were reported in chickens fed dietary levels of 1000 mg/kg for 21 days (1967 mg/kg body weight) whereas kidney enlargement occurred in chickens fed 5000 mg/kg of diet for 21 days (Wittchen & Rottstein, 1972).

7.4.3 Cold-blooded animals

The literature on the chronic effects of 2,4-D and its derivatives on cold-blooded animals (poikilotherms) was reviewed in detail. The available reports indicate that aquatic vertebrates in general, 2,4-D ester are more toxic than 2,4-D amines, and that the overall non-observed-adverse effect level for toxic effects on fish for the esters is at or near 1 mg/litre (King & Pentimont, 1966; Zaitseva & Chasnovaya, 1973; Kabacher & Chambers, 1974; Schuchman et al., 1963; 2,4-dichlorophenol, which may occur in water as a by-product of transformation product of 2,4-D, is particularly toxic to fish (Schuchman et al., 1960).

7.4.4 Fetotoxicity, Teratogenicity, and Reproductive Effect

The scientific literature contains a fairly large number of reports on the fetotoxic, teratogenic, and reproductive effects of 2,4-D in livestock and in laboratory animals, however most of the observations on livestock are either too sketchy to be useful or neglect to take into account various confounding factors. Examples of this are studies by Björklund & Lane (1966) on a single pregnant pig, by Kadkov et al. (1972) on sheep grazing on pastures apparently containing a high percentage of poisonous plants, and the report by Boda et al. (1974), on reproductive disturbances in cattle feeding on either 2,4-D-treated vegetation possibly including poisonous plants, or on contaminated silage.

Some of the studies carried out with rodents under laboratory conditions also provide little useful information, either because it is difficult or impossible to determine the dose levels used in the studies (Weinmann, 1957; Schuchman, 1963, 1965, 1969; Schiller, 1964; Buslovich et al., 1976), or because the information provided is insufficient and the studies cannot be properly evaluated (Bucher, 1956; Hansen et al., 1971; King et al., 1971). The study by Weinmann (1957) can be considered invalid, as a high mortality occurred in both control and experimental animals, when they were exposed to cold stress because of construction work affecting the animal quarters during the winter months. Moreover, Weinmann (1957) and several other authors, including Schuchman (1963, 1965, 1969) Schiller (1964), Schilling (1960), and Schilling & Namova (1957) did not in fact examine the effects of 2,4-D, but rather the effects of foods or food

extracts prepared from crops that had been sprayed with 2,4-D, and, in some cases, also with other plant growth substances. As none of these authors appears to have carried out an analysis to demonstrate the presence of 2,4-D residues in the test material, it is questionable whether the test animals ingested any 2,4-D or 2,4-D residues, and these studies are therefore not reviewed in detail. Buslovich et al. (1976) tested only a single dose level (1/2 LD₅₀) and this reduces the usefulness of their study. Pertinent reports are discussed below.

7.4.1 Rats

7.4.1.1 Effects on adult rats

No deleterious effects on the health or fertility of rats receiving the maximum tolerated dose of 87.5 mg/kg body weight in terms of 2,4-D or its molar equivalent of the isooctyl ester or the propylene glycol butyl ether ester per day, on days 6 - 13 of pregnancy, were reported by Schwetz et al. (1971) and Unger et al. (1980). Even higher amounts of 2,4-D or its equivalent in 2,4-D derivatives were used by Björklund & Erne (1966), Hansen et al. (1971) and Khara & McKinley (1972) with similar results.

Reduced testis and prostate size, abnormal spermatogenesis (and also liver and kidney damage) were reported by Schilling (1960) in some of the male rats given 375 mg/kg body weight per day (about 1/4 LD₅₀) of a Soviet-made 2,4-D butyl ether formulation containing polyethylene glycol alkyl phenyl ether surfactant. These effects were not noted at 1/10 of this dose level, i.e., at 37.5 mg/kg body weight per day. Some of the toxic effects reported by Schilling (1960) may have been caused by the surfactant, but as a surfactant control group was not included in this study, this cannot be confirmed. Thus, the available studies suggest that the no-observed-adverse-effect level for reproducing adult rats lies between 37.5 and 87.5 mg 2,4-D/kg body weight per day.

7.4.1.2 Effects on offspring

Björklund & Erne (1966) gave pregnant rats a 2,4-D concentration in drinking-water of 1000 mg/litre during pregnancy, and for the following 10 months. No effects on reproduction were noted.

Hansen et al. (1971) fed male and female rats dietary levels of technical 2,4-D of 100, 500, and 1500 mg/kg (ppm), and the rats were bred through 3 successive generations. At dietary levels of 100 and 500 mg/kg, no effects were noted. However, at a dietary level of 1500 mg/kg, survival of pups to

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weaning was reduced. The number of pups surviving ranged from 79 - 972 in the control group and from 60 - 932 at the 100 and 500 mg/kg (ppm) dietary levels; survival at the highest dose ranged from 20 - 62.

Resorptions, reduced fetal weight and size, enlarged ventricles of the brain and haemoperitoneum were found by Buslovich et al. (1976) in the offspring of rats treated with two different 2,4-D derivatives at a dose of one-half LD₅₀ (value unspecified). Schwartz et al. (1971) dosed female rats from day 6 - 15 of pregnancy by gavage at dose levels of 12.5, 25, 50, and 87.5 mg/kg body weight per day with 2,4-D ester, 2,4-D butyl ester, and the isooctyl ester (10). At doses of 50 and 87.5 mg/kg, a decrease in fetal body weight was noted for all three compounds.

Subcutaneous edema, delayed ossification of sternbrae, sternbrae with split centres of ossification, wavy ribs, and lumbar ribs increased with increasing doses, for at least one of the agents studied. However, these anomalies were not significantly increased at the 12.5 or 25 mg/kg dose level for the 3 compounds. A small but significant increase in the incidence of subcutaneous edema was observed in fetuses from dams receiving 12.5 mg/kg molar equivalent of 10. The incidence of missing sternbrae was significantly increased at dose levels of 87.5 mg/kg for 2,4-D and 12.5 and 87.5 mg/kg for PCBE.

Unger et al. (1980), in a later study, using the same dosing regimens for 2,4-D 10 and 2,4-D PCBE did not observe the effects reported by Schwartz et al., except for a statistically significant increase in rib buds at the highest dose (87.5 mg/kg) tested for both compounds ($p < 0.05$).

Khura & McKinley (1972) dosed female rats from day 6 - 15 of pregnancy by gavage with 2,4-D, 2,4-D 10, 2,4-D butyl ester, 2,4-D butoxyethanol ester and 2,4-D dimethylamine salt. The butyl and lauroyl ester depressed fetal weight and decreased fetal viability at the highest dose of 150 mg/kg body weight. Wavy ribs, additional ribs, retarded ossification and splanchnic defects, fused ribs, small-sized distorted scapula and micromelia were observed as anomalies among the treated groups. A statistically significant increase in malformed fetuses was noted at 2,4-D levels of 25 mg/kg body weight ($p < 0.05$) and at levels of 150 mg/kg or more for the other compounds.

Konstantinova et al. (1975) reported hemorrhage into internal organs in the fetuses of rats treated with a 2,4-D level of 50 mg/kg body weight.

The results of all these studies suggest that dosage levels of less than 12.5 mg/kg body weight for the various 2,4-D derivatives do not cause fetotoxic or teratological

effects in rats, and the results of the more recent study by Unger et al. (1980) indicate that higher doses may be without deleterious effects on the fetuses.

Thus, at present, a daily dose level of 10 mg 2,4-D or 2,4-D acid equivalent/kg body weight can be considered to be without significant fetotoxic or teratogenic effects in rats.

7.4.2 Mice

The report by Courtney (1977) indicated that in CD-1 mice, doses of 1 mM/kg body weight of 2,4-D and its n-butyl and PCBE esters reduced fetal body weight, and increased fetal mortality. The compounds were also teratogenic, inducing cleft palates, at levels of 124 mg/kg body weight per day (2,4-D or acid equivalent) or more. However, the 2,4-D isopropyl and isooctyl esters appeared to be less teratogenic than 2,4-D, as they did not induce birth defects at a 2,4-D equivalent level of 124 mg/kg body weight per day. Furthermore, the 2 esters did not induce fetal death in CD-1 mice at doses of 2,4-D equivalent up to 221 mg/kg body weight per day.

7.4.3 Birds

In the 1950s and 1970s, a number of tests of the embryotoxic, teratogenic, and other reproductive effects of 2,4-D were carried out on birds' eggs and embryos. These results may not apply to mammals, because bird embryos develop in a closed environment different from that of mammalian embryos, and because birds differ anatomically from mammals.

Lutz-Ostertag & Lutz (1970, 1972) reported mortality and severe deformities in wild bird embryos exposed to 2,4-D amine salt, but they did not provide crucial experimental details, and other investigators (Dunachin & Fletcher, 1977, 1970; Kopischke, 1972; Grolleau et al., 1974; Gyrd-Hansen & Balgaard-Mikkelsen, 1974; Somers et al., 1974a,b,c; Hilbig et al., 1970a,b; Spittler, 1976) were unable to duplicate their results either with 2,4-D amine salts or esters. Some of the teratogenic effects attributed to 2,4-D by Lutz-Ostertag & Lutz (1970, 1974) resemble those induced by excessively high incubation temperatures (Nielsen, 1968), and may thus have been experimental artifacts.

In the whole, the available studies on bird embryos indicate that the no-observed-adverse-effect level for 2,4-D-induced embryotoxic and teratogenic effects lies near 0.5 mg active ingredient/egg (equivalent to about 10 mg/kg) and is thus similar to that in mammals.

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7.4.4 Cold-blooded animals

The available literature contained little information concerning the possible reproductive, embryotoxic, or teratogenic effects of 2,4-D or 2,6-D derivatives on cold-blooded animals.

7.4.4.1 Amphibians

Lopez (1991) noted that the motility of frog spermatozoa was not affected by low concentrations of 2,4-D or its sodium salt, and that the inhibition of movement, or the delay observed under some conditions were caused by changes in the pH of the test solution. Aqueous solutions of less than 0.002 mg/litre sodium salt did not induce any macroscopic abnormalities in developing frog eggs or embryos (Hosono & Koto, 1982). While Koto (1972) did not find either toxic effects of 2,4-D residues in frog tadpoles exposed for 1 or 2 days to up to 50 mg 2,4-D/litre. According to Sanders (1978a) a commercial 2,4-D dimethylamine salt had an LC₅₀ of 100 mg/litre in frog tadpoles.

7.4.4.2 Fish

Only three brief reports were available on the effects of 2,4-D on developing fish eggs and embryos. Andrusaitis (1972) found that 2,4-D reduced the oxygen consumption of 32-cell blastomeres, and increased the oxygen consumption in 64-128 cell blastomeres of *Misgurnus fossilis*. In a study by Mount & Stephan (1967), 2,4-D butoxyethanol ester (BEE) at a concentration of up to 0.31 mg/litre, or 2,4-D at 0.80 mg/litre did not reduce the reproduction rate in fathead minnows (*Pimephales promelas*), whereas 2,4-D BEE at 1.5 mg/litre killed minnow eggs in 48 h. Kehrwald et al. (1977) similarly found that 0.1 mg 2,4-D/litre did not have any adverse effect on reproduction in guppies.

These reports suggest that the no-observed-adverse-effect level of 2,4-D BEE for the reproductive or teratogenic effects of 2,4-D in fish may be about 1 mg a.i./litre water.

7.5 Mutagenicity and Related Effects

7.5.1 2,4-D and its derivatives

Studies on the mutagenicity of 2,4-D and its derivatives have been reviewed by Andersen et al. (1972), National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality (1978), Rameil (1978), Seiler (1978), Vachkov-Petrova (1978), Kas'yanenko & Kuroleva

(1979), Murthy (1979), Shearer (1980), US Veterans' Administration (1981), Waters et al. (1981), and Linnainmaa (1983).

A recent IARC Working Group (IARC, 1982) evaluated the activity of 2,4-D and derivatives in short-term tests. It was reported that 2,4-D induced unscheduled DNA synthesis in cultured human fibroblasts (Ahmed et al., 1977a), but not in rat hepatocytes (Probst et al., 1981). 2,4-D was not mutagenic in bacterial systems (Andersen et al., 1972; Sherasen et al., 1976; Zetterberg, 1977; Moriya et al., 1981). 2,4-D was mutagenic in yeast, when tested at low pH (Zetterberg et al., 1977), but was not active under other conditions (Zetterberg, 1977) or in a host-mediated assay (Zetterberg et al., 1977). Results of four studies on *Brasophila melanosoma* were reported to be positive (Rasmussen & Persson-Svahlén, 1978); results of three other studies were negative (Berin & Buslovich, 1971; Vogel & Chandler, 1972; Magnusson et al., 1977; Rasmussen & Svahlén, 1978).

2,4-D was reportedly mutagenic in cultured Chinese hamster ovary (CHO) cells (Ahmed et al., 1977b), but did not induce a statistically significant increase in sister chromatid exchanges (SCEs) in CHO cells in vitro (Linnainmaa, 1981). Chromosomal effects have been reported in plants (Khalatkar & Bhargava, 1982).

Chromosomal aberrations or SCEs were found in cultured human lymphocytes (Pillinskaya, 1974; Korte & Jatal, 1982), but chromosomal aberrations were not found in cultured embryonic bovine kidney cells (Bongao & Baarur, 1973).

In mice, single oral doses of 100 - 300 mg 2,4-D/kg body weight reportedly induced chromosomal aberrations (Pillinskaya, 1974), but micronuclei were not found in mice after ip injection of single doses of 100 mg 2,4-D/kg body weight (Jennawon & Renberg, 1976). 2,4-D was not active in a dominant lethal test in mice (Epstein et al., 1972), and did not induce SCEs in rats after oral administration of 2,4-D amine salt at daily doses of 100 mg/kg body weight for two weeks (Linnainmaa et al., 1983). Recent evidence (Buslovich et al., 1982; Vainio et al., 1982) suggests that 2,4-D may have an indirect effect on genetic material via the production of active oxygen radicals derived from peroxisome proliferation, which has been demonstrated in vivo and in vitro studies in liver cells of rats and hamsters (Reddy et al., 1980, 1982; Vainio et al., 1982; Gray et al., 1983).

At present, available studies are inadequate to evaluate the genetic effects of 2,4-D and its derivatives in short-term tests (IARC, 1982). No data on cell transformation are available.

7.6 Carcinogenic Effects on Experimental Animals

7.6.1 2,4-D and its derivatives

A number of studies have been carried out on mice and rats to assess the potential carcinogenic effects of 2,4-D and its derivatives. Two IARC Working Groups have reviewed these studies (IARC, 1977, 1982) and concluded that it was not possible to make an evaluation on the basis of the available data. 2,4-D and several of its esters were tested by oral administration and by a single sc injection in the necks of strains of mice (Jones et al., 1969); 2,4-D or its amine salts were also tested in rats by oral administration (Atkhypov & Kozlova, 1975; Hansen et al., 1971).

During 20 years, have passed since these studies were carried out and because of basic flaws in their design and execution, it is unlikely that further reviews of these studies will lead to generally accepted conclusions. The Task Force is aware that further long-term studies in mice and rats are in progress, and these should prove useful in the future evaluation of the potential carcinogenicity of 2,4-D.

7.6.2 Contaminants in 2,4-D

2,4-DDB was tested for carcinogenicity in mice and rats fed dietary levels of 5000 and 10 000 mg/kg for 90 weeks, followed by a short observation period of about 1 - 10 weeks, at which time all animals were killed. Sufficient numbers of animals survived to evaluate the development of late-appearing tumours. Although an increased incidence of hepatocellular adenomas and carcinomas was observed in treated male mice (20/50 in the low dose and 17/42 in the high dose compared with 8/49 in matched controls), it was noted that the incidence of liver tumours in historical controls ranged from 16 - 32%. No teratogenic effects were found in rats (National Cancer Institute, 1979).

8. EFFECTS ON MAN, CLINICAL AND EPIDEMIOLOGICAL STUDIES

The available clinical and epidemiological studies fall into 4 groups: (a) studies on patients treated with 2,4-D as an anticancer drug (Apfel, 1959a) or antibiotic (Seabury, 1963), (b) reports on acute 2,4-D poisoning due to voluntary or accidental ingestion of herbicides (Tables 19 and 20), (c) reports on workers (mainly men) overexposed to 2,4-D during the manufacture, processing, or use of 2,4-D herbicides, and (d) epidemiological studies on groups of people who were actually or potentially exposed as a result of herbicide spray programmes, or who lived in areas in which herbicides were used. With the exception of the case studies of Apfel (1959a) and Seabury (1963), almost all of the reports deal with mixed exposures to 2,4-D and other chemicals, and therefore it is often unclear to what extent 2,4-D, its alkali or amine salts, or its esters contributed to the effects reported by the authors of the studies.

Much of the literature on acute poisoning and on the health effects of occupational overexposure to 2,4-D or other chlorophenoxy compounds or their toxic by-products has been recently reviewed by Pocchiari et al. (1973), Slev & Young (1969), Huff et al. (1980), National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality (1981), CDC (1981), Kapper & Busch (1981), US Veterans' Administration (1981), Coggon & Acheson (1982), Hay (1982), IARC (1982,1983), and Jobrovoltski (unpublished data, 1983). However, most of these reviews concentrated on 2,4,5-T, Agent Orange, and other herbicides used in the Vietnam war, or on industrial accidents resulting in massive exposures to largely undefined mixtures of chlorophenols, chlorinated dibenzodioxins, and other reaction products. In contrast, the present review focuses mainly on 2,4-D herbicides.

In evaluating human exposure to mixtures of chemicals that include 2,4-D and various concentrations of contaminants of 2,4-D, it is in many instances difficult or impossible to determine whether any of the described effects can actually be attributed to the exposure to 2,4-D or its derivatives.

8.1 Acute Poisoning and Occupational Overexposure

Pertinent reports on acute poisoning with 2,4-D, or of the effects of occupational overexposure to 2,4-D herbicides are summarized or cited in Tables 19 and 20.

Signs and symptoms of acute over-exposure to 2,4-D or its derivatives occurred after ingestion or absorption of large amounts, or where poor occupational hygiene was practised leading to pronounced dermal absorption of the material. It

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Table 19. Acute toxicity of 2,4-D, fatal poisonings with herbicides containing 2,4-D

Product(s)	Circumstances	Sex of victim	Body weight or age	Dose ingested	2,4-D concentration in tissues (µg/kg)	Effects and outcomes	References
a) Fatal poisonings							
2,4-D diethyl ester (DIOGILX EXTRA)	not stated	F	59.8 kg	50-100 ml (1180-1770 mg DIOGILX, kg bw)	not stated	coma; death in about 2 days; degeneration of convoluted kidney tubules	Curry (1962)
"2,4-D" 400g a.i./litre	suicide	M	?	5-125 ml	15-100 µg/kg	loss of consciousness, coma, generalized muscular hypotonia, loss of all reflexes, hypotension, hyperglycaemia, proteinuria; death in 12 h	Dolan and Bartlett (1969)
"pure" 2,4-D acid in kerosene	suicide	M	55 kg	?	17,000-27,000	coma, convulsion, fever, pulmonary oedema and edema, liver necrosis, degeneration of kidney tubules; death in 5 days	Dudley and Thapar, (1972)
"2,4-D"	suicide	F	?	?	20-116	loss of consciousness, vomiting, uterine bleeding, tachycardia, and circulatory failure; death in about 10 h; edema and congestion of brain; fatty liver cell changes; fatty changes in kidney tubules; pulmonary hyperemia & edema with isolated haemorrhages	Geldewer-Van Mallickrodt & Lautensack, (1966)
"2,4-D"	suicide	M	?	"at least" 11.5 g		stiffness in legs, vomiting, loss of consciousness; death in 14 h; hyperemia and edema of brain; pulmonary edema	Hernández & Nájera (1963)

Table 19 (continued)

2,4-D diethyl ester (DIOGILX EXTRA)	suicide	M	59.8 kg	50-100 ml (1180-1770 mg DIOGILX, kg bw)	not stated	coma; death in about 2 days; degeneration of convoluted kidney tubules	Curry (1962)
"2,4-D" 400g a.i./litre	suicide	M	?	5-125 ml	15-100 µg/kg	loss of consciousness, coma, generalized muscular hypotonia, loss of all reflexes, hypotension, hyperglycaemia, proteinuria; death in 12 h	Dolan and Bartlett (1969)
"pure" 2,4-D acid in kerosene	suicide	M	55 kg	?	17,000-27,000	coma, convulsion, fever, pulmonary oedema and edema, liver necrosis, degeneration of kidney tubules; death in 5 days	Dudley and Thapar, (1972)
"2,4-D"	suicide	F	?	?	20-116	loss of consciousness, vomiting, uterine bleeding, tachycardia, and circulatory failure; death in about 10 h; edema and congestion of brain; fatty liver cell changes; fatty changes in kidney tubules; pulmonary hyperemia & edema with isolated haemorrhages	Geldewer-Van Mallickrodt & Lautensack, (1966)
"2,4-D"	suicide	M	?	"at least" 11.5 g		stiffness in legs, vomiting, loss of consciousness; death in 14 h; hyperemia and edema of brain; pulmonary edema	Hernández & Nájera (1963)

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TABLE 10 (Contd.)		
respiratory system	1) irritation, coughing	Assouly (1951); Belomestova & Karimova (1963); Belomestova (1967); Halli et al. (1970); Andreask et al. (1972)
	2) functional disorders	Belomestova & Karimova (1963); Kashirov (1969); Halli et al. (1970)
circulatory system	1) functional disorders - arterial hypotension - venous insufficiency	Belomestova & Karimova (1963); Belomestova (1967); Andreask (1972); Kashirov (1969); Kashirov et al. (1970); Andreask et al. (1972)
	2) circulatory disorders of central nervous system	Kashirov et al. (1969); Kashirov & Belomestova (1972); Kashirov et al. (1972); Lova et al. (1967); Pargiaro et al. (1974); Andreask et al. (1972)
	3) subjective symptoms	Kashirov et al. (1967); Kashirov (1969); Andreask et al. (1972)
digestive system	functional abnormalities	Belomestova & Karimova (1963); Belomestova (1967); Kashirov (1969); Andreask et al. (1972)
urinary system	functional abnormalities	Momchev & Ivanov (1961); Kojouhar-Gepova et al. (1962); Belomestova (1967); Kashirov (1969); Pargiaro et al. (1974); Andreask et al. (1972)
skin	1) irritation or allergic reactions	Belomestova (1967); Kashirov et al. (1967); Lomova (1970); Kozak (1970)
	2) desquamation	Kojouhar-Gepova et al. (1962)
	3) chloracne	Londono (1966)
reproductive system	functional abnormalities (uterine decreased volume) men- struence	Elina et al. (1975) Andreask et al. (1974) Kaper et al. (1970)

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is unlikely that, with good agricultural practice, good personal protection, and occupational hygiene, resulting in exposures to low concentrations of 2,4-D, any of the acute symptoms and signs reported below would be expected to occur.

The observations of Apfel (1959a) and Seabury (1963) on patients treated with 2,4-D suggest that the acute non-observed-adverse-effect level for biological effects in human beings may be as high as 36 mg of purified 2,4-D/kg body weight, or its equivalent in alkali or amine salts or esters. The lack of subjective or clinical effects in studies in which volunteers ingested low doses (5 mg/kg body weight) of purified 2,4-D also supports the idea that a dose of a few mg/kg body weight is unlikely to be toxic (Khanna & Kohli, 1977; Sauerhoff et al., 1977). However, it is less certain whether this is true also of the less pure commercial 2,4-D products. From the point of view of occupational and bystander safety, it is reassuring that no reports were found of fatal poisonings following dermal exposure or inhalation, though temporary unconsciousness and other severe acute effects have been attributed to massive combined dermal and inhalation exposures to 2,4-D herbicides.

Most poisonings with 2,4-D herbicides involve formulations containing more than one toxic ingredient, including solvents, surfactants, and other additives. The symptoms and clinical signs therefore tended to vary with different products.

M. I. I Neurotoxic effects of 2,4-D and related compounds

Some of the case reports cited below on poisoning with, and occupational overexposures to, herbicides indicate that 2,4-D and other chlorophenoxy compounds may affect both the central and peripheral nervous systems.

8.1.1.1 Effects on the central nervous system

In addition to subjective symptoms of the central nervous system, impaired coordination, impaired responses to environmental stimuli, unconsciousness, coma, and death have been observed in human beings mainly after absorption of lethal or near-lethal doses of 2,4-D. The acute effects on the central nervous system seem to resemble those produced by alcohol, sedative drugs, or aromatic chlorinated hydrocarbons rather than those produced by organophosphate or carbamate neurotoxic poisons (Geldmacher Von Mallinckrodt & Landelbach, 1966; Prescott et al., 1979). Acute cerebral demyelination occurred in a guinea pig injected a solution of 2,4-D in kerosene (Dudley & Thapar, 1972). Severe degeneration of brain ganglion cells was observed in another

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suicide who drank a herbicide product containing 2,4-D in the form of water-soluble dimethylamine salt (Nielsen et al., 1963). It is therefore possible that lethal doses of chlorophenoxy compounds may cause structural as well as functional damage to the brain.

Cranio-cerebral and peripheral functional nerve damage was noted by Bezuglyi et al. (1979) in a group of women occupationally overexposed to 2,4-D herbicides and other pesticides. Electroencephalographic (EEG) abnormalities were observed in tractor drivers spraying herbicides containing 2,4-D, MCPA, or mecoprop (Kontek et al., 1973), in individuals exposed to chlorophenoxy herbicides, including 2,4-D and MCPA (Bilicki & Madra, 1976) and in workers packaging 2,4-D sodium salt (Andreask et al., 1979). On the other hand, a case of neuropathy with EEG abnormalities and flaccid quadriplegia, originally attributed to occupational overexposure to MCPA, was diagnosed as being of viral origin (Hayes et al., 1978), and therefore some of the neurological damage attributed to 2,4-D may have been caused by viruses. In fact, some evidence indicates that 2,4-D herbicides may affect the sensory system, as Andreask et al. (1979) and Assouly (1979) reported intolerance to certain odours, hypersensitivity to noise, and other sensory abnormalities in workers producing or packaging 2,4-D herbicides, while Fetisov (1966) reported hypaesthesia and other sensory deficiencies in similarly employed workers.

8.1.1.2 Effects on the peripheral nervous system

"Peripheral neuropathy" and reduced peripheral nerve conduction velocities have been reported in workers producing 2,4-D and 2,4,5-F (Poland et al., 1971; Singer et al., 1962). More than one dozen other studies of persons overexposed to chlorophenoxy herbicides also indicated detrimental effects of 2,4-D products on the peripheral nervous system (Goldstein et al., 1977; Goldstein & Brown, 1960; Foissac-Gegoux et al., 1962; Todd, 1962; Berkley & Magee, 1963; Wallis et al., 1970; Bezuglyi et al., 1979). Long-lasting flaccid paraparesis or quadriplegia following skin contact with 2,4-D herbicides was reported by Goldstein et al. (1959) and Goldstein & Brown (1960). Abnormal tendon reflexes in these or similar cases were reported by the same authors and by Andreask et al. (1979), Berkley & Magee (1963), and Foissac-Gegoux et al. (1962). Cases of sensory neuropathy attributed to the ingestion of, or dermal exposure to, 2,4-D herbicides have also been reported by Monaco & Pivito (1961), Foissac-Gegoux et al. (1962), Todd (1962), Wallis et al. (1970), Sare (1972), and Bezuglyi et al. (1979). However, no signs of peripheral neuropathy were reported by Apffel (1959a), Seabury (1963),

Paggiaro et al. (1974), and Prescott et al. (1979), in similar cases of massive exposure to 2,4-D herbicide, and in patients given relatively large amounts of purified 2,4-D or 2,4,5-F salts or esters as drugs. One explanation for this may be great individual differences in susceptibility to poisoning with chlorophenoxy herbicides (Rosentherg, 1980), as attested by the case of a 58-year-old woman who "was fully conscious with no clinical evidence of toxicity" after ingesting enough herbicide to allegedly attain 2,4-D and mecoprop blood plasma concentrations of 335 and 400 mg/litre respectively (Prescott et al., 1979). By comparison, Herlich & Machata (1963) reported that a plasma concentration of 447 mg 2,4-D/litre caused death in a 46-year-old man.

Herbicide ingredients other than 2,4-D and related compounds might be at least partly responsible for the observed neurotoxic effects. In particular, organic solvents, emulsifiers, and ethylene glycol present in herbicide formulations have been mentioned in this connection (Goldstein & Brown, 1960; Goldwater, 1960). Solvents such as alcohols and trichloroethylene, used in the manufacture of the active herbicide ingredients, might account for some of the abnormalities observed in workers involved in the production of chlorophenoxy compounds (Assouly, 1951; Bashirov, 1969; Bashirov & Ier-Bagdasarova, 1970).

Some of the reported cases of central nervous system dysfunction or peripheral neuritis may have been merely coincidental to the herbicide exposure, as there are many known causes of neuropathy, such as nutritional and hereditary factors, infectious diseases, and many toxic chemicals, including alcohol (Freeman, 1975). Thus, alcoholism may have been a contributory factor in one case of "9,4-D polynuropathy" reported by Brandt (1971).

Further studies of the possible effects of 2,4-D and other chlorophenoxy compounds or their by-products on the human nervous system are desirable, including studies of behavioural effects measurable by recently-developed test batteries (Baker et al., 1983).

8.1.2 Myotoxic effects of 2,4-D

Muscle fibrillations, myotonia, myoglobinuria, muscular weakness and other indications of a myotoxic effect of 2,4-D have been reported in patients treated with large doses of purified 2,4-D products by Apffel (1959a) and Seabury (1963), as well as in cases of suicidal or accidental ingestion of 2,4-D herbicides or following occupational over-exposure (Herlich & Machata, 1963; Berwick, 1970; Dudley & Thapar, 1972; Prescott et al., 1979). In laboratory animals, myotonia and structural or biochemical muscle lesions can be reliably

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induced by doses in excess of about 100 mg 2,4-D/kg body weight. In human beings, the threshold dose for gross myotoxic effects certainly exceeds 5 mg/kg body weight per day, and may be above 30 mg/kg body weight per day (Seabury, 1963; Khanna & Kohli, 1977; Sanderhoff et al., 1977).

8.1.1 Cardiac, and cardiovascular effects

Myocardial dystrophy, myocarditis, cardiac arrhythmias or fibrillations, a slowed heart rate, or electrocardiographic (ECG) changes were observed in human beings who ingested herbicides containing 2,4-D (Dutic et al., 1979) or 2,4-D and mecoprop (Prescott et al., 1979), and also after occupational overexposure to chlorophenoxy herbicides (Belomyytaeva, 1975; Khibin et al., 1968; Zakharov et al., 1968; Paggiaro et al., 1974; Kaskovich & Sobolova, 1978; Andreask et al., 1979; Prescott et al., 1979). However, in other cases of acute poisoning with 2,4-D herbicides, the ECG was essentially normal (Berwick, 1970; Monarco & Divito, 1981).

Thus, further studies are desirable to determine the threshold 2,4-D doses at which electrophysiological cardiac abnormalities can be observed, and to determine whether they result from a direct effect on the nerve conducting system of the heart, or secondarily from a toxic action on the myocardium.

It has been suggested that allergic reactions and an increased sensitivity may be involved in 2,4-D-related cardiac arrhythmias (Winkelmann, 1960; Rea, 1978).

Both hypertension and hypotension have been reported following exposure to high doses of 2,4-D (Apfel, 1959a; Kaskovich & Sobolova, 1978; Bezugly et al., 1979), but no conclusions could be drawn from these studies.

8.1.4 Haematological effects

Monarca & Divito (1981), Todd (1962), Radionov et al. (1967), Bashirov (1969), Bashirov & Ter-Bagdasarova (1970), Brandt (1971), Andreask et al. (1979), and Bezugly et al. (1979) observed haematological changes such as mild anaemia, bone marrow depression, mono- or lymphocytosis or eosinophilia, changes in erythrocyte volume or size, or methaemoglobinemia following ingestion of, or overexposure to 2,4-D. However, these changes may have been due to other causes, as Apfel (1959a) did not observe either haematological changes or effects on haematopoiesis in patients given 0.1-0.3 g of purified 2,4-D per day as an anticancer drug.

8.1.5 Blood chemistry effects

Hyperglycaemia, hypercholesterolaemia, elevated levels of blood urea, transaminase (SGOT, SGPT), and creatine phosphokinase (CPK), or altered blood albumin, globulin, or phospholipid levels following acute poisoning with, or occupational overexposure to 2,4-D were reported by Bashirov (1969), Bashirov & Ter-Bagdasarova (1970), Berwick (1970), Lukashkina et al. (1970), Brandt (1971), Bezugly et al. (1979), Dutic et al. (1979) and Prescott et al. (1979). However, Bashirov (1969) reported hypoglycaemia (< 700 mg/litre) and abnormally slow return to renal values in glucose tolerance tests in about one-third of a group of workers producing 2,4-D. Increased activity of erythrocytic glycolytic enzymes was found in Polish workers packaging 2,4-D sodium salt (Andreask et al., 1979). In one case of intentional 2,4-D poisoning, there was perglycaemia (De Lattard & Barbaste, 1969), while in some cases of 2,4-D overexposure, blood glucose abnormalities were not observed (Goldstein et al., 1959). Apfel (1959a) never observed hyperglycaemia in his patients, on the contrary, daily doses of 1-1.25 g 2,4-D led to hypoglycaemia. Thus, under some circumstances, high doses of 2,4-D apparently can affect glucose metabolism, and produce hypo- or hyperglycaemia. Gamble (1975) proposed that 2,4-D might inhibit certain APT-dependent enzymes and thus affect lipid metabolism.

8.1.6 Pulmonary effects

Pulmonary emphysema, oedema, hyperaemia and haemorrhages were found in cases of fatal poisonings due to 2,4-D herbicide ingestion (Herbich & Machata, 1963; Geldmacher-Von Mallinckrodt & Lautenbach, 1966; Dudley & Thapar, 1972). It is not clear whether the acute pulmonary effects were caused by the 2,4-D preparations or by the solvents such as kerosene or fuel oil. However, it is unlikely that the pulmonary emphysema was caused by acute exposure to 2,4-D.

Dyspnoea or respiratory tract irritation were occasionally reported following occupational overexposure of 2,4-D production workers or herbicide sprayers (Asaouly, 1951; Belomyytaeva, 1964; Bashirov, 1969; Bezugly et al., 1979).

8.1.7 Hepatotoxic effects

Liver necrosis or fatty liver cell changes were observed in 2 fatal cases following 2,4-D herbicide ingestion (Geldmacher-Von Mallinckrodt & Lautenbach, 1966; Dudley & Thapar, 1972). In several non-fatal 2,4-D poisonings, no biochemical evidence of liver damage was noted, and neither

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Apfel (1959a) no. Seabury (1963) reported indications of liver damage in patients treated with up to 2.5 g/day of purified 2,4-D, salts, or esters. Hyperbilirubinaemia, elevated urobilinogen levels, or liver enlargement were reported in workers occupationally exposed both to 2,4-D herbicides and to other chemicals (Belomytseva, 1964, 1965; Bashirov, 1969; Bashirov & Ter-Bagdasarova, 1970; Kaskavich & Bobolova, 1978; Andreask et al., 1979).

8.1.8 Nephrotoxic effects:

Regeneration of, or fatty changes in kidney tubules, or proteinuria, increased blood urea levels, and other indications of a nephrotoxic effect were observed in cases of fatal or nearly fatal herbicide ingestion (Goldstein et al., 1969; Curry, 1962; Geldmacher-Von Mallinckrodt & Lautenbach, 1969; Brandt, 1971; Dudley & Thapar, 1972; Duric et al., 1979). Impaired renal function was reported in occupationally-exposed persons by Bashirov (1969), Bashirov & Ter-Bagdasarova (1970), Pazzolato et al. (1971), and by Andreask et al. (1979). On the other hand, neither Apfel (1959a) nor Seabury (1963) reported any evidence of kidney damage in their patients, some of whom received in excess of 2 g of pure 2,4-D per day.

8.1.9 Effects on the digestive tract

Vomiting, diarrhoea, nausea, and other indications of toxic effects on the digestive tract were observed by Apfel (1959a) in patients injected intramuscularly with large doses (up to 2.5 g) of purified 2,4-D products. The same effects have also been noted after ingestion of larger doses of 2,4-D herbicides or after combined inhalation and dermal overexposure (Goldstein et al., 1959; Monaco & Lavato, 1961; Nielsen et al., 1965; Tsapko, 1966; Radionov et al., 1966; Pazzolato et al., 1974; Dennis, 1976; Kuzyk, 1979; Prescott et al., 1979). However, no gastrointestinal symptoms were reported by volunteers who ingested a single dose of 5 mg pure 2,4-D/kg body weight (Khanna & Kohli, 1977; Sauerhoff et al., 1977). Thus, an intake of more than 300 mg 2,4-D per adult appears to be required to induce acute toxic effects on the gastrointestinal tract.

8.1.10 Effects on endocrine organs

Andreask et al. (1979) found an impaired iodine uptake by the thyroid, and decreased thyroxine, thyroxine clearance, and thyroxine iodine values in workers packaging 2,4-D sodium salt. Since these workers were exposed to a variety of chemicals, these results need confirmation.

8.1.11 Irritatory and allergenic effects

Chronic tonsillitis and paranasal sinusitis were reported in workers packaging 2,4-D sodium salt (Andreask et al., 1979).

Acute eye or skin irritation, as well as skin reactions of an allergic type, including anaphylactoid purpura (allergic angitis) and contact eczema have been reported in agricultural and forestry workers following occupational exposure to 2,4-D herbicides (Winkelmann, 1960; Radionov et al., 1967; Balu-Banga et al., 1973; Jung & Wolf, 1977; Kuzyk, 1979). Jung & Wolf (1977) found that exposure to the vapour of a 2,4-D/2,4,5-T formulation in diesel oil (SELEST 100) caused an acute allergic reaction in the skin of sensitized herbicide applicators, and that the allergic reactions were caused by the mixture of 2,4-D/2,4,5-T esters and not by the diesel oil.

8.2 Epidemiological Studies of the Chronic Effects of 2,4-D

Much concern has been raised about the phenoxo herbicides, including 2,4-D, especially in relation to birth defects and cancer in human beings.

Several episodes have also been reported in which defined populations were exposed to mixtures of 2,4-D and 2,4,5-T, in which the 2,4,5-T was contaminated with various amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) (Blasberg et al., 1964; Huff et al., 1980). It is now generally accepted that chloracne and porphyria cutanea tarda observed in these studies were caused by exposure to 2,3,7,8-TCDD and not by exposure to 2,4,5-T or 2,4-D (Kimbrough, 1980).

The following sections concentrate on epidemiological studies or other related studies on human beings in which actual or potential exposures to 2,4-D products alone or to mixtures of 2,4-D with other chlorophenoxy herbicides were demonstrated.

8.2.1 Reproductive, fetotoxic, and teratogenic effects

Although effects on reproduction have been demonstrated in animals with 2,4,5-T, 2,4-D and 2,3,7,8-TCDD, all of the attempts made to determine whether human beings suffer similar effects have been frustrated by the poor design of the studies, inadequate determination of exposure, or inadequate information about the background incidence of spontaneous abortions and other abnormal reproductive outcomes, by inadequate evaluation of confounding variables, by inadequate assessment of exposure, and by mixed exposures (Aldred et al., 1975; Lee, 1978; Field & Kerr, 1979; Brogan et al., 1980;

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Hanify, 1980; Carmelli et al., 1981). For these reasons they are not discussed in detail in this report.

Conclusive evidence of reproductive effects caused by 2,4-D in populations that might be exposed to chlorophenoxy herbicides is unlikely to be obtained from new epidemiological studies on indirectly-exposed populations living in, or adjacent to, areas in which phenoxy herbicides are used. Doses of 2,4-D absorbed by bystanders are far below those expected to be toxic, as shown by occupational exposure studies with 2,4-D and 2,4,5-T herbicides (section 5). Any effects induced by such small amounts would probably be obscured by more potent confounding factors (Janerich, 1973; Kärkinen-Jäskeläinen & Saxén, 1974; Saxén et al., 1974; Elwood & Rogers, 1975; Grantroth et al., 1977, 1978; James, 1977; Holmberg, 1979; Lappe, 1979; Schacter et al., 1979).

Additional studies on female workers occupationally exposed to significantly higher levels of 2,4-D than bystanders would be useful to clarify some of the uncertainties raised by past studies, if sufficiently large cohorts could be identified.

6.3 Studies on Mutagenic Effects in Workers Exposed to 2,4-D

Lymphocytes from ten workers exposed to 2,4-D esters during the manufacture of 2,4-D herbicides, or from 15 workers packaging 2,4-D sodium salt, did not show any chromosome abnormalities (Johnson, 1971; Andreasik et al., 1979). Chromosome or chromatid abnormalities in lymphocytes from some pesticide sprayers applying a variety of agricultural chemicals, including in some cases 2,4-D, were observed by Yoder et al. (1973) and Croasen et al. (1978). Högstedt et al. (1980) did not observe any significant increases in chromosome abnormalities in workers exposed to 2,4-D and other pesticides.

The induction of SCEs among workers occupationally exposed to the phenoxy herbicides 2,4-D and MCPA has been recently studied. The subjects used were only 2,4-D and MCPA or mixtures of the two for spraying, and the exposure levels were estimated by determining the urinary 2,4-D and MCPA excretion by the workers. No dose-related differences in the frequencies of SCEs could be found either in relation to the exposure level or to the length of the exposure (Linnainmaa, 1983b).

Although some studies suggest that occupational exposure to 2,4-D may result in chromosome abnormalities, the results are conflicting. Moreover, the possibility of mixed exposure and other confounding variables cannot be excluded in the studies with positive results.

8.4 Carcinogenic effects

8.4.1 Epidemiological studies

In two case-control studies of soft-tissue sarcoma (Hardell & Sundström, 1979; Eriksson et al., 1981) and one of lymphoma (Hardell et al., 1981), exposure to phenoxyacetic acids (mainly 2,4,5-T, 2,4-D, and MCPA) was associated with approximately 5-fold increases in the risk of soft-tissue sarcomas. Exposure to 2,4-D, either with or without MCPA exposure, also increased relative risks. In the study of malignant lymphomas, 7 cases and 1 control were apparently exposed to 2,4-D only (relative risk, 19.6; 95% confidence interval, 4.3-89.8).

In a different case-control study with a small number of cases and controls, no increased risk was observed (Smith et al., 1982).

A follow-up was also carried out on 3-8 railroad workers exposed for more than 45 days during the period 1957-72 to the herbicides 2,4-D, 2,4,5-T, atrazine, mecoprop, dichloropropionic acid, and amitrole (Axelson & Sundell, 1974). The authors found a significant increase in cancer mortality and morbidity among workers exposed to amitrole.

Axelsson et al. (1980) reported a further follow-up of these workers, up to October, 1978, accumulating 551 person-years. The herbicide exposure of the workers was analysed in terms of exposure to either amitrole, or phenoxy acids, or to a combination of the two. A 10-year lapse period from the first day of exposure was used as the induction latency. They found 15 cases of cancer versus 6.87 expected (relative risk, 2.2). In the cohort with combined exposure to amitrole and phenoxy acids, 6 cases were observed versus 1.78 expected (relative risk, 3.4); in the group exposed to amitrole alone, 3 tumours were observed versus 1.95 expected (relative risk, 1.5); and 6 cancers were observed versus 3.14 expected (relative risk, 1.9) in the phenoxy acid-exposed group. All cancers, as well as cancers of the stomach, occurred in statistically-significant excesses in the cohort as a whole. In the groups exposed to amitrole plus phenoxy acids, there was a significant excess of all cancers. In the group exposed only to phenoxy acids, stomach cancer occurred in significant excess (2 observed, 0.33 expected; relative risk, 6.1). No soft-tissue sarcomas were identified, but the statistical power of this study to detect an excess of a rare cancer was limited.

Högstedt & Westerlund (1980) conducted a retrospective mortality study on 142 forestry workers exposed to phenoxy pesticides and 244 unexposed forestry workers, comparing their mortality experience with national statistics. Work

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supervisors, who were more highly exposed to phenoxy herbicides than the others had a significantly elevated tumour mortality (5 observed, 1.4 expected). No particular tumour type predominated, and no soft-tissue sarcomas were observed, though the authors noted that the study had limited statistical power and was inconclusive, because of the relatively short follow-up period.

Riihimäki et al. (1982) reported on a prospective cohort study of 2,4-D and 2,4,5-T spray personnel which was in progress. Because of the small number of deaths and the brief follow-up period, no conclusions can so far be drawn from this study.

Follow-up studies on cohorts of pesticide sprayers, farmers, and agricultural workers occupationally exposed to a variety of chemicals, in some cases including phenoxy herbicides, have been reviewed by IARC (1983).

Follow-up studies on groups of industrial workers exposed to chlorophenols, 2,4,5-T, or other chlorophenoxy compounds, and to 2,3,7,8-TCDD or other dioxins, during the manufacture of chlorophenoxy herbicides, have recently been reviewed by IARC et al. (1980) and IARC (1983).

8.4.2 Evidence on the carcinogenicity of 2,4-D

The available studies suggest that an association exists between mixed exposure to phenoxy herbicides, chlorinated phenols, and chlorinated dibenzodioxins, and an increased incidence of soft tissue sarcomas and malignant lymphomas. It is not clear, at present, whether these findings represent true associations, and further studies are in progress (Huir & Wagner, 1981) to clarify this point. Since many of the tumour cases had been exposed to combinations of phenoxy herbicides and their contaminants as well as other chemicals, it is not known whether exposure to 2,4-D is specifically associated with the development of soft tissue sarcomas.

8.5 Treatment of Poisoning in Human Beings

Successfully treated cases of 2,4-D poisoning indicate that forced alkaline diuresis is helpful in reducing the level of 2,4-D in the blood and tissues (Young & Halsey, 1977; Prescott et al., 1979). Heart and kidney damage should be anticipated and counteracted in cases of severe poisoning (Duric et al., 1979).

In cases of acute poisoning due to 2,4-D, the nearest Poison Control Centre should be contacted for additional information on symptoms and recommended treatment.

9. EVALUATION OF HEALTH RISKS TO MAN FROM EXPOSURE TO 2,4-D

9.1 General Considerations

In areas of 2,4-D herbicide production, handling, or use, the highest exposure will be incurred by those who are directly involved in these processes, followed by bystanders indirectly exposed to 2,4-D vapour, dust, or droplets, or to contaminated vegetation, soil, or water. In these two groups, exposure will usually be via the skin. The general population in 2,4-D-use areas would be exposed to a lesser extent, mainly through food containing 2,4-D residues and to a lesser extent through 2,4-D residues in water. The contribution from air is negligible. As far as the general population is concerned, 2,4-D intake from any source, is negligible.

9.2 Estimated Intake of 2,4-D by the Population in a 2,4-D-use Area

The total contribution from air, food, and water is estimated to be 0.3 - 2 µg/kg body weight per day (table 13).

9.2.1 Intake by bystanders

Given the limited data available and the many uncertainties involved, an adequate estimate of 2,4-D intake by bystanders is not possible at this time, but it should generally be less than that for occupationally-exposed persons.

9.2.2 Occupational intake

Workers using 2,4-D may, on average, absorb about 0.1 mg 2,4-D/kg body weight per day. However, this level may be exceeded if good occupational hygiene is not practiced (section 5.2). Simple precautions against excessive exposure can reduce the amount of 2,4-D uptake.

9.3 Safety Factors

9.3.1 Definitions

For the present assessment, the safety factor is defined as the integer obtained by dividing the overall no-observed-adverse-effect level for a known adverse effect of 2,4-D (determined from all available information on human

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beings or animals) by the daily exposure value (absorbed dose of 2,4-D) for the various exposed groups.

9.3.2 Determination of safety factors

9.3.2.1 Acute poisoning

Based on clinical studies in which 2,4-D was injected into patients as a drug, the no-observed-adverse-effect level for signs and symptoms of acute 2,4-D poisoning in children and adults appears to be at or near 36 mg/kg body weight (section 6.1). Based on the available studies of the amounts of 2,4-D absorbed by occupationally-exposed person, bystanders, and populations in 2,4-D-use areas, the safety factors for acute 2,4-D poisoning are likely to be:

- (a) much greater than 1000 for the general population in 2,4-D-use areas;
- (b) at least 300 for occupationally-exposed spraying crews.

The margin of safety for persons with excessive occupational exposures would be smaller.

9.3.2.2 Chronic toxicity

Dose-effect relationships for the chronic toxic effects of 2,4-D or 2,4-D derivatives are available only from animal studies. The no-observed-adverse effect levels for certain chronic toxic effects of 2,4-D in animals have not been firmly established, and for this reason safety factors cannot be established (section 7.2.1) for all of the chronic effects of 2,4-D.

9.3.2.3 Embryotoxic, fetotoxic, and teratogenic effects

The no-observed-adverse-effect level for embryotoxic, fetotoxic, or teratogenic effects of 2,4-D in mammals appears to lie at 10 mg/kg body weight per day (section 7.3.1.2). Assuming that the same is true for human beings, then the corresponding safety factors for the various exposed groups are:

- (a) much greater than 1000 for the general population in 2,4-D-use areas;
- (b) 100 for occupationally-exposed spraying crews using precautions against excessive exposure.

9.3.2.4 Mutagenic effects

The available information was inadequate for an assessment of the mutagenic potential of 2,4-D in mammals.

9.3.2.5 Carcinogenic effects

Available animal bioassays and epidemiological studies are inadequate for an assessment of the carcinogenic potential of 2,4-D or of its derivatives.

5.4 Evaluation of Health Risks from 2,4-D Exposure

From the data available at present, the Task Group assumes that a possible health risk will exist, when the safety factor is less than 100.

9.5 Recommendations on Exposure

Results of recent exposure and occupational health studies suggest that excessive exposure to 2,4-D can be avoided by fairly simple measures of occupational hygiene, such as those recommended in two pertinent publications of the International Labour Office (ILO, 1977, 1979). Laundering procedures for 2,4-D-contaminated clothing have been published by Easley et al. (1983), and these should be considered.

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APPENDIX B

CASWELL FILE

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SEP - 8 1986

Subject: The Effects of 2,4-D in a Two-Generation Study
on Reproduction in Rats.

From: David G Anderson, PhD.
Toxicology Branch
Section VII
Hazard Evaluation Division (TS-769C)

David G. Anderson
9/5/86

To: Ms. Lois Rossi PM #61
Special Review Branch
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Thru: Albin B Kocialski, PhD.
Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ABK
9/8/86
11/11/86
9/8/86

The 2-generation rat reproduction feeding study on 2,4-D has been reviewed and classified as Core-minimum data. The calculated and nominal NOEL's and the LEL's with their respective effects are as follows.

F0 parental toxicity.

NOEL - 15(20) mg/kg/day.*

LEL - 58(80) mg/kg/day, reduced male body weight.

F1 parental toxicity.

NOEL - 4(5) mg/kg/day.

LEL - 14(20) mg/kg/day, reduced female body weight.

Developmental toxicity, dose level to dams.

NOEL - 7(5) mg/kg/day.

LEL - 26(20) mg/kg/day, reduced weight in Flb pups.

Nominal dose levels administered 0, 5, 20, or 80 mg/kg/day.

* Calculated lowest dose level within the range consumed by the animals at the nominal dose level administered (nominal dose level administered).

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Reviewed by: David G Anderson
Section VII, Tox. Branch (TS-769C)
Secondary reviewer: Albin B Kocialski
Section VII, Tox. Branch (TS-769C)

David G Anderson 9/5/86

DATA EVALUATION REPORT

STUDY TYPE: Effects of 2,4-D on Two-Generations of Reproduction
in Rats

TEST SUBSTANCE: 2,4-Dichlorophenoxyacetic Acid (2,4-D)

SYNONYMS: 2,4-D TOX. CHEM. NO. 315

ACCESSION NO.: 259442-6 (Study in 5 Volumes)

SPONSOR: Industry Task Force on 2,4-D Research Data (ITF)

TESTING FACILITY: Wil Research Laboratories, Inc. (WIL)
Ashland, OH 44805-9281

TITLE OF REPORT: A Dietary Two-Generation Reproduction Study
in Fischer 344 Rats with 2,4-Dichlorophenoxy-
acetic Acid.

AUTHORS: Stanley Kopp, Patricia L Leist, Michael D
Mercieca, Elaine J Tasker, Gabriela P Adam,
Mark D Nemec, Dean E Rodwell.

STUDY NO.: WIL-81137

TESTING PERIOD: November 16, 1982 to May 15, 1984

REPORT ISSUED: July 26, 1985

PURITY OF TEST SUBSTANCE: ITF analysis 97.5%
WIL analysis 95.8%

CORE GRADE: Minimum.

A. CONCLUSIONS ON THE EFFECT AND NO EFFECT LEVELS:

The effect levels and no effect levels are expressed as the lowest dose level consumed within a measured dose level range. The target or nominal dose levels administered, for reference purposes only, are enclosed in parentheses. Discussed more fully in the section on Study Design and Conduct. Dose levels are given in mg/kg/day.

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LEL and NOEL is expressed in mg/kg/day

F0 parental toxicity

LEL- 58(80), reduced body weight.

NOEL- 15(20)

F1 parental toxicity

LEL- 14(20), reduced body weight.

NOEL- 3.8(5)

Developmental toxicity

LEL- 26(20), Flb pup weight reduction.

NOEL- 7.2(5)

Target or nominal dose levels administered in the study are 0, 5, 20, or 80 mg/kg/day.

In designating the LEL and the NOEL, several considerations were applied. The lowest dose level in a range was used. Although it might be expected that the highest dose level within a range would initiate the toxicity, in a study on reproduction, where effects may be development stage or age specific as well as dose dependent, the highest dose level is not totally appropriate. In the study under consideration, the dose levels consumed varied widely during the study, and it was not always possible to determine adequately the dose level or the animal state at which the toxicity was initiated. Thus, it seems appropriate to select, for the LEL, the lowest dose level possibly resulting in the effect.

The NOEL is also designated as the lowest dose level in the range where no effects were observed. The upper dose level of the range was rejected because the animals did not continuously consume these levels. If they had, effects may have been demonstrated. Thus, for safety considerations, the lowest dose level within the range where no effects were observed is designated the NOEL.

The appropriate dose level range for the NOEL for the F1 female body weight reduction includes; a) the gestation and lactation for the Flb pups (the F1 females were selected from these pups), b) and the growth and development of the F1 females, c) and the gestation and lactation for the F2a and F2b litters, e) and for the 4 weeks of dosing after weaning the F2b litters. The lowest dose level consumed during these periods is considered to be the NOEL.

Similarly, the NOEL for the Flb pups is the lowest dose level consumed by F0 dams during the gestation and lactation for the Flb litters.

The effect and no effect levels from this study are also presented as the target dose levels and the range in the amount of test substance consumed. The target dose levels are the dose levels which were designed for the study and which the testing laboratory attempted to deliver to the animals. The amounts of test substance consumed are the actual dose-levels delivered to the animals, at least as best could be determined from the concentration of the test substance in the feed, food consumption, and the animal weight for the week concerned. Since the dose levels are calculated for 1 week before they are delivered, the actual delivered dose varied somewhat from these anticipated dose-levels during the study.

Effect and No Effect Levels, with ranges

F0 parental LEL and NOEL in mg/kg/day

- LEL- 80(58-94)(a)(b), F0 male body weight reduction.
- 80(71-86)(b), F0 female body weight reduction.
- 80(69-114)(c), F0 increase in length of gestation.
- NONE, F0 and F1 fertility.
- NOEL- 20(15-22)(b), No F0 male body weight reduction occurred.
- 20(18-21)(b), No F0 female body weight reduction,
- 20(18-35)(c) or no increased length of gestation occurred.

F1 parental LEL and NOEL in mg/kg/day

- LEL- 20(14-48)(d), F1 female body weight reduction.
- NOEL- 5(3.3-13.5)(d), No F1 female body weight reduction occurred.

Developmental toxicity in mg/kg/day to dams

- LEL- 80(112, gestation and lactation for the Fla litters)(e), Fla pup death.
- 80(103-133, gestation and lactation for the Flb litters)(e), Flb pup death.
- 80(69-112, gestation and lactation for the Fla litters)(e), Fla reduced pup weight.
- 20(26-48, gestation and lactation for the Flb litters)(e), Flb reduced pup weight.
- 80(103-114, gestation producing the Flb litters)(e), Flb skeletal anomalies, and reduced ossification, the only dose level studied.
- NOEL- 5(7.2-13.5, gestation and lactation for the Flb litters)(e), for all developmental effects.

Discounted effects and toxicity

- F0 male liver and liver/body weight ratio reduction at all dose levels.
- F0 female kidney and kidney/body weight ratio increase at all dose levels.

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The following dose levels were administered.

F0 males(f)	F0 females(f)
5(3.7-6.1)	5(4.2-8.6)
20(14.9-24.5)	20(17.8-29.5)
80(57.7-103.7)	80(70.7-124.5)
F1 males(f)	F1 females(f)
5(4.7-5.6)	5(4.5-6.0)
20(18-23)	20(19-24)
80(9)	

- (a) Target dose level(range in amount of test substance consumed, except during gestation and/or lactation, unless noted)
- (b) Includes test substance consumption prior to mating only.
- (c) Includes test substance consumption prior to mating, through gestation and lactation for the Fl_a litters and the gestation producing the Fl_b litters, the only gestation for which the effect was noted.
- (d) Includes test substance consumption throughout life time of the the Fl generation, which includes the Fl_b, F2_a, and F2_b litters.
- (e) Includes test substance consumption only during the period/s indicated.
- (f) Target dose levels administrated(range in test substance consumption for the test animals indicated, except for gestation and laccation) in mg/kg/day.
- (g) Due to excess toxicity the Fl_b litters, the highest dose level was not continued beyond weaning.

The dose levels were set at 50% of the pre-mating dose during the second week of lactation and 33% of the pre-mating dose during the third and fourth week of lactation. This somewhat arbitrary setting of dose-levels during midlactation and end lactation, has merit but needs evaluation for its impact on Agency assessment of reproductive effects. Also, the consequence of the reduced dosing to young animals when the study was initiated and just after weaning needs evaluation. Animals eat approximately twice as much food as they do as adults during the first 2-3 weeks post-weaning. Thus, they consumed less test substance in this study than would have if the concentration of the test substance in the feed had not been adjusted for body weight.

B. Conclusions

Toxicity was expressed in the Flb pups and in F1 females at dose levels lower than those administered to the F0 parents. Pup death occurred at birth and before lactation day 4 in Fla and Flb litters at the highest dose level which caused slight but statistically significant reduced weight gain in the F0 parents. Because of the toxicity to the pups at this target dose level of 80 mg/kg/day, this dose level was dropped from the study after weaning the remaining Flb pups. Reduced weight gain occurred in Flb pups during lactation at the middle dose level. At this same dose level, the Flb female pups, which became the F1 female generation, demonstrated a reduced body weight compared with controls during the last 4 weeks before sacrifice, but after weaning the F2b pups. No significant effects occurred in any pups or any animals at the lowest target dose level. No reduced food consumption occurred to explain any of these effects on weights.

At all dose levels, absolute and relative liver weights were statistically significantly less than controls in F0 males and absolute and relative kidney weights at all dose levels were statistically significantly greater than controls in F0 females. These statistically significant effects did not demonstrate "smooth" dose response curves, and the effects were not confirmed in the F1 generation or in the histological examination of these organs. The report did not consider them to be biologically significant.

The toxicological significance of these effects are discounted. The liver weight reduction was not seen in 90 day subchronic and chronic studies conducted in this species and strain of rats. The increased kidney weights are also discounted because the kidney weights of 5 female controls were lower than the kidney weights of the remaining control animals of the F0 generation by approximately 3 standard deviations. If these animals are excluded from the average, then the kidney weights of dosed animals are comparable to the kidney weights of the remaining animals in the control group.

The LEL for development is reduced pup weight compared to controls during gestation and lactation of F0 dams at a target dose level of 20 mg/kg/day or a dose level range of

26-48 mg/kg/day. At the highest dose level, pup viability was reduced in the Fl_a and Fl_b litters. The NOEL for the reduced pup weight in the Fl_b litters compared to controls is a dose range of 7.2-13.5 mg/kg/day during gestation and lactation of F₀ females.

Since the liver weight decrease in males, and the kidney weight increase in females is not considered biologically significant, the LEL in adults is in Fl females at the target dose level of 20 mg/kg/day, where statistically significant weight depression compared to controls occurred during the last 4 weeks before sacrifice, but after weaning the F_{2b} pups. The NOEL for Fl adults then would be the target dose level of 5 mg/kg/day, the same target dose level as the NOEL for the Fl_b pup weight reduction. However, the range of dose levels consumed differed (see LEL and NOEL above).

No effects were seen on fertility in the F₀ or the Fl males or females.

C. Study Design and Conduct

The study was conducted essentially according to the OPP guidelines proposed August 22, 1978, for a two-generation, two litters per generation study of reproduction. The quality assurance statement was signed the director of quality assurance, Ralph Anderson, on 7/26/85.

About 140 Fischer 344 rats per sex were obtained from Charles River Breeding Laboratories, Inc, Kinston, NY on November 3, 1982, and quarantined for 13 days. Assignment of 30 rats per group were based on random selection of rats in a block design for body weight stratification. Animals were housed individually under recommended conditions.

The F₀ generation was placed on diets designed to deliver dose levels of 0, 5, 20, or 80 mg/kg/day, respectively, to each group, each of 30 rats per sex, for 105 days prior to mating. Subsequently, the animals were dosed in an analogous manner during each mating, each gestation, and each lactation. The total dosing and continuous dosing period for F₀ animals was 40 weeks which included 2 weeks rest between the end of lactation for the Fl_a litters to the beginning of mating for the Fl_b litters and 30 days after weaning these latter litters.

The Fl generation, selected from the Fl_b pups, was exposed to the test substance in utero, and continuously via the

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milk or the feed for 125 days postnatally and prior to mating and through mating, gestation and lactation for the F2a litters. Dosing continued through a 2 week rest period and mating, gestation, lactation for the F2b litters and for at least 30 days after weaning the F2b litters.

The total period of continuous administration of the test substance, from initial dosing of the F0 generation to the end of the F1 generation, was 77 weeks. During this period, the test substance was administered to the F0 generation, Fla and Flb litters, the F1 generation (selected from Flb litters), including the F2a and F2b litters and for 30 days after weaning the F2b litters.

The test substance was administered in the feed at target dose levels of 5, 20, or 80 mg/kg/day. The concentration in the feed was adjusted weekly according to the food consumption during the previous week and the average body weight for that week. This regimen was followed in the F0 generation up to week 15 (105 days) or just prior to mating to produce the Fla litters. Except as indicated below, monthly adjustments were made after mating. During mating, males and females were exposed to the diet prepared for the females which was based on the concentration prepared for the week prior to mating (week 15 for the F0 matings). The same dietary concentration was used throughout mating, gestation, and the first week of lactation. During the second week of lactation, the dietary concentration was reduced by 50 percent and during the third and fourth weeks of lactation, the dietary concentration was reduced by 67 percent of diet concentrations used during the first week of gestation (a concentration based on week 15). A similar dosing regimen was followed in producing the Flb litters, except the dosing regimen was based on body weights for week 24 and food consumption for week 15. The actual dose level consumed during gestation and lactation are given in tables 1 and 2.

The report claimed that the food consumption for week 15 was actually for 6 days instead of 7, but that the average daily food consumption used for week 24 was incorrectly based on a 7 day week. Thus, the average daily food consumption for week 15 was calculated to be $86\% (6/7 = .86)$ of the actual daily average. This would result in the intended concentration of test substance in the feed during production of the Flb litters ~~to be 86% of the actual feed concentration used during this~~ period. The report did not make it clear whether or not this same error was made in the test substance concentration in the feed used during production of the Fla litters.

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Table 1.

Test substance consumed during gestation in F0 and F1 dams producing Fla, Flb, F2a, and F2b litters.

Target dose levels in mg/kg/day

0 5 20 80

Test substance consumed, in mg/kg/day, was calculated from the concentration, food consumption, and body weight.

F0 dams during the gestation					
producing the Fla	days 0-7	--	4.6	18.1	69.0
	days 7-13	--	5.0	20.5	79.6
	days 13-20	--	4.9	19.6	76.1
F0 dams during the gestation					
producing the Flb	days 0-7	--	7.2	26.4	103.4
	days 7-13	--	8.0	29.4	113.8
	days 13-20	--	7.5	28.4	106.9
F1 dams during the gestation					
producing the F2a	days 0-7	--	3.8	17.1	NC
	days 7-13	--	4.8	19.6	NC
	days 13-20	--	5.1	19.9	NC
F1 dams during the gestation					
producing the F2b	days 0-7	--	3.9	14.2	NC
	days 7-13	--	4.8	18.1	NC
	days 13-20	--	4.7	16.7	NC

NC-Testing of the F1 generation was not continued at this dose level after weaning.

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Table 2

Test substance consumed during lactation in F0 and F1 dams for Fla, Flb, F2a, and F2b litters.

		Target dose levels in mg/kg/day				
		<u>0</u>	<u>5</u>	<u>20</u>	<u>80</u>	
		Test substance consumption in mg/kg/day calculated from concentration, food consumption, and body weight. (a)				
F0 dams during lactation						
for Fla	days	1-7	--	8.9	34.7	112.3
	days	7-14	--	6.5	25.2	84.0
	days	13-20	--	4.9	19.6	76.1
	days	14-21	--	5.1	20.7	72.4
	days	21-28	--	6.9	27.6	82.9
F0 dams during lactation						
for Flb	days	1-7	--	13.5	47.8	132.7
	days	7-14	--	9.4	34.6	106.9
	days	14-21	--	7.3	26.4	90.9
	days	21-28	--	9.3	32.6	112.7
F1 dams during lactation						
for F2a	days	1-7	--	3.2	34.0	NC
	days	7-14	--	5.9	24.6	NC
	days	14-21	--	4.6	18.8	NC
	days	21-28	--	7.0	29.1	NC
F1 dams during lactation						
for F2b	days	1-7	--	7.6	28.5	NC
	days	7-14	--	5.6	20.6	NC
	days	14-21	--	4.3	17.5	NC
	days	21-28	--	6.6	25.2	NC

NC-Testing of the F1 generation was not continued at this dose level after weaning.

(a) Included in these values is the 50% reduction in the concentration of the test substance during the second week and the 67% reduction during the third and fourth week of lactation.

The Flb litters for the F1 generation remained on the reduced diet of lactation week 3 and 4 after weaning from the F0 dams. After selection for the F1 generation, pups were placed on test diets at target dose levels of 5 and 20 mg/kg/day, with weekly adjustments to week 53, the week prior to mating to produce the F2a litters. At this time, F1 males received the same diet as F1 females. Monthly adjustments were made to the diets after mating, except during the second week of lactation for the F2a and F2b litter when the concentration of the test substance was reduced by 50 percent and during the third and fourth weeks of lactation for the same litters when the concentrations of the test substance was reduced by 67 percent.

The study report did not specifically state how the doses were adjusted for the F1 generation but their dosing regimen can be calculated from the amounts of test substance consumed, and the body weights during the F1 generation for gestation and lactation for the F2a and F2b litters. The study report stated, "After selection, the F1 pups were placed on test diets at dose levels of 5 and 20 mg/kg/day." The study report also stated that adjustments were made to the diet based on food consumption and body weight.

These dosing regimens resulted in generally higher dose levels in the lactating F0 and F1 dams than for the premating target dose levels. The higher dose levels were greatest in F0 dams lactating for the Flb litters. Table 1 and 2 gives the amount of test substance consumed during gestation and lactation, respectively for Fla, Flb, F2a, and F2b litters. Consumption of test substance during gestation is included in table 1 because test substance consumption differed among these litters. The downward adjustment of the concentration in the feed during lactation did not occur during gestation. At other times the actual dose levels consumed were very close to the target dose levels.

The usual parameters were evaluated such as, fertility, duration of gestation, viability of pups at parturition and during lactation, the amount of food consumption, body weight, pup anomalies, and variations, in addition to histopathology on the testes, ovaries, kidneys, and livers. Organ weights were determined on the kidney, liver at necropsy and on testes after fixing in 10 percent formalin.

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The following organs and tissues were taken at sacrifice and preserved, but histopathology was conducted only as previously indicated.

1. Adipose tissue	19. Mammary Gland and Skin
2. Adrenals	20. Nasal turbinates
3. Aorta	21. Pancreas
4. Bladder	22. Parathyroids
5. Bone marrow	23. Pituitary
6. Brain	24. Prostate
7. Cecum, colon	25. Salivary Glands
8. Spinal cord	26. Sciatic Nerve
9. Epididymis	27. Seminal Vesicle
10. Esophagus	28. Skeletal Muscle
11. Eyes	29. Spinal Cord
12. Ovaries/Testes	30. Spleen
13. Heart	31. Sterum
14. Intestines	32. Stomach
15. Kidneys	33. Thymus
16. Liver	34. Thyroid
17. Lung/bronchi	35. Trachea
18. Lymph node-thoracic and mesenteric	36. Uterus/cervix
	37. Vagina

Statistical Methods

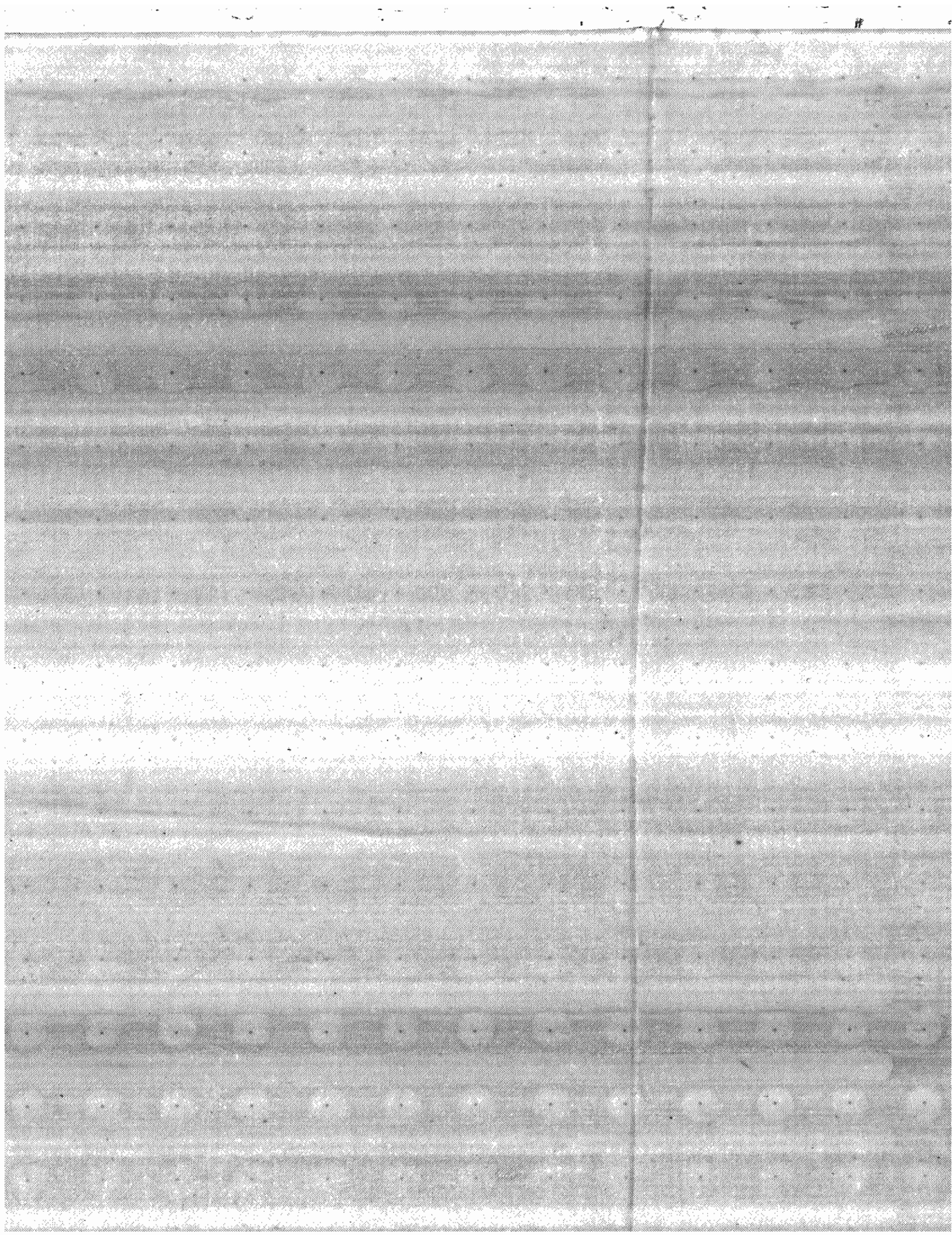
All analyses were conducted using two-tailed tests (unless otherwise specified).

1. Histopathological findings and incidence by sex were compared to control groups by Kalmogorov-Smirnov one-tailed test.
2. F0 and F1 male and female fertility indexes, Fla, Flb, F2a, and F2b pup sex ratios on lactation day 1, and Fla, Flb, F2a, and F2b pup survival indexes on lactation day 4, 7, 14, 21, and 28 for the control groups were compared to each treated group by the Chi-square test with Yates correction factor.
3. ~~Other effects in treated groups were compared to controls by analysis of variance followed by Dunnett's test.~~

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Summary of Study Conduct

1. Test substance administered continuously throughout all phases of the study.
2. F0 dosed continuously from approx. 5 to 6 weeks of age for 105 days prior to first mating (i.e., approx. 20 weeks of age).
3. F0 mated 1:1 for 10 days and if no evidence of sperm, second matings were allowed with a proven male for 5 days.
4. F0 continued for 3 weeks of gestation and 4 weeks to weaning of Fla litters. Pups reduced to 8 per dam on day 4 of lactation.
5. All Fla litters necropsied and discarded after weaning.
6. F0 rested 2 weeks between weaning Fla and mating for production of the Flb as in #3.
7. F0 continued for 3 weeks gestation and 4 weeks to weaning of Flb litters. Pups reduced to 8 per dam on day 4 of lactation.
8. Ten Flb pups per sex per dose level randomly selected for necropsy, after weaning.
9. One pup per sex per dam per dose level randomly selected from Flb litters for the F1 generation. Because of excess toxicity at the target dose level of 80 mg/kg, only controls, and the target dose level groups of 5 and 20 mg/kg were continued on study. All Flb pups at 50 mg/kg/day were sacrificed at the end of weaning.
10. All F0 animals were sacrificed on week 40 of the study.
11. Selected F1 pups were dosed via milk and in the feed for 125 days prior to mating to produce the F2a litters.
12. Dosing, mating, gestation, and weaning in the F1 generation producing the F2a and F2b litters followed procedures, including necropsy, similar to those followed for the F0 generation in producing the Fla and Flb litters.



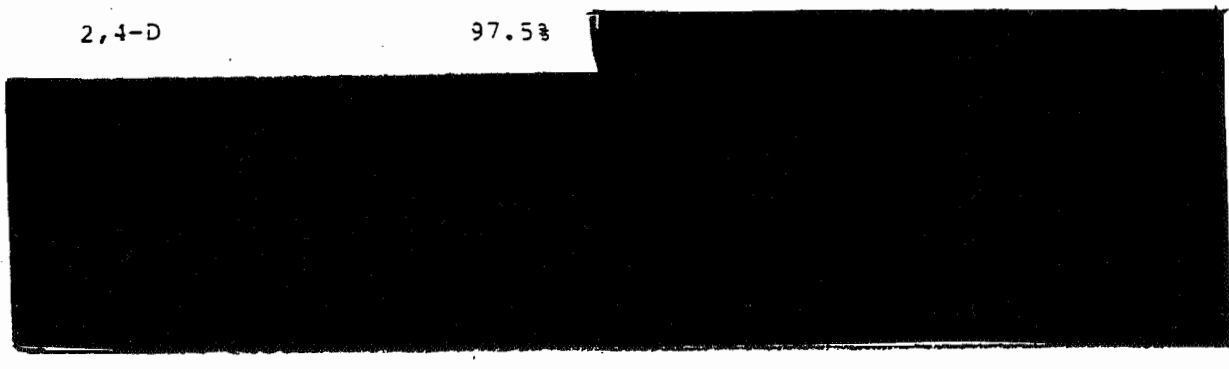
13. All F1 animals were sacrificed on week 77 of the study.
14. All F1a, F1b, F2a, and F2b dying prior to weaning were studied for malformations and variations.

D. Test Chemical Identity and Concentration in the Feed

The study report, presented an analysis conducted by Wil Research Laboratories, and the Industry Task Force analysis on 2,4-D. According to a Wil Research analysis, the test substance was 95.8 percent pure 2,4-D. The report presented the following analysis of the test substance by the task force, but no further analysis or explanation of the differences between the Task Force analysis of 97.5 percent, and the Wil Research analysis of 95.8 percent, was presented.

2,4-D

97.5%



ND = Not detected, (lowest level detectable).

Samples of the diets containing 2,4-D were collected for study weeks 0, 1, 2, 3, 4, 8, 13, 16, 22, 32, 45, 77. None of the sample diets were collected during weeks of gestation or lactation. The analyses after recovery of 2,4-D from the diets with the highest concentration were within 10 percent of the measured concentration. Analyses of 2,4-D in the diets at the middle dose level and the lowest dose level were always within 15 percent to 20 percent of the measured concentrations, except for three of the lowest dose levels which were 77 percent, 61 percent, and 55 percent of the measured dose levels. One was in a diet mixed on the 4th week of the study and two were for a diet mixed on the 13th week of the study. The 55 percent of the measured level was apparently a repeat analysis on a sample of the diet yielding the 61 percent of measured dose level.

E. Results

1. Fertility in F0 and F1 Males and Females.

No reduced fertility was expressed in males or females of the F0 generation in producing either the Fla or the Flb litters. However, a nonstatistically significant apparent reduction in male fertility occurred in producing the Flb litters (table 3). No reduced fertility was expressed in males or females of the F1 generation in producing the F2a and F2b litters. A second mating by a proven male was conducted when females demonstrated no evidence of sperm. The number of second matings producing the Fla/Flb pups were 0/6, 5/6, 1/2 and 0/2 for controls and the target dose levels of 5, 20, or 80 mg/kg/day, respectively. Second matings to produce F2a/F2b pups were 3/4, 2/1, and 4/4 for control and the target dose levels of 5, or 20 mg/kg/day.

The fertility index for production of the Fla and Flb litters is 70 to 79 percent in control F0 males and 70 to 79 percent in control F0 females (see table 3). The fertility index for males and females, respectively is the number of gravid females divided by the number males or females mated, respectively, adjusted to percent. These indexes ranged from 70 to 83 percent in treated males and 70 to 90 percent in treated females producing the Fla and Flb litters. Similarly, the fertility index for production of F2a and F2b litters is 60 to 70 percent in control F1 males and 64 to 72% in control F1 females (table 4.). These indexes range from 67 to 80 percent in treated F1 males and 64 to 80 percent in treated F1 females producing F2a and F2b litters. None were statistically significantly different from controls. The number of days required for mating ranged from 4.0 to 5.7 days of cohabitation to produce the Fla and Flb litters and 3.2 to 4.6 days of cohabitation to produce the F2a and F2b litters. These were no different from control values.

This failure to detect an effect on fertility is consistent with the lack of histopathological findings in the testes or epididymides of males and with the lack of histopathological findings in the ovaries or uteri of females from the F0 or F1 generation at terminal sacrifice. However, since the highest dose level was dropped from the study, fertility in the F1 generation was not evaluated at this dose level. Thus, the mid target dose level of 20 mg/kg/day should be considered the NOEL for fertility.

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Table 3

Fertility indexes for F0 male and females producing Fla and Flb litters.

Fertility Index (no. gravid/no. males or females mated) x 100

Target dose	<u>Producing Fla</u>				<u>Producing Flb</u>			
	No. of males	%	No. of females	%	No. of males	%	No. of females	%
0	21/30	70	21/30	70	23/29	79	23/29	79
5	25/30	83	26/30	87	25/30	83	27/30	90
20	24/30	80	24/30	80	23/30	77	23/30	77
80	21/30	70	21/30	70	21/30	70	21/30	70

Table 4

Fertility indexes for F1 male and females producing F2a and F2b litters.

Fertility Index (no. gravid/no. males or females mated) x 100

Target dose	<u>Producing F2a</u>				<u>Producing F2b</u>			
	No. of males	%	No. of females	%	No. of males	%	No. of females	%
0	21/30	70	21/30	72	18/30	60	18/28	64
5	24/30	80	24/30	80	20/30	67	20/30	67
20	22/30	73	23/30	77	20/30	67	20/30	67

2. Length of Gestation in F0 and F1 Females

The lengths of gestation was statistically significantly prolonged in F0 females producing the Flb pups only and only at the highest target dose level of 80 mg/kg/day. This increase in gestational lengths was due to a gestation length of 23 days in approximately one half of the dams from this group instead of the usual 22 days of gestation demonstrated by most F0 and F1 dams in all groups. The LEL is between 103 and 114 mg/kg/day and NOEL is between 18 and 35 mg/kg/day.

The effect could result from delayed implantation, hormonal imbalance, or parturition problems. The effect is considered biologically significant and undesirable.

3. Body Weights of the F0 and F1 Generations.

The mean body weights of F0 males and female rats were statistically significantly less than controls in the high dose group only. In F0 males, the reduced body weight (97 percent of controls) was consistent after the sixth week of test substance consumption and in F0 females the body weight was consistently reduced (96 percent of controls) by the twelfth week of test substance consumption. The failure to gain as much weight as controls could not be attributed to reduced food consumption. The food consumption, and the food consumption per gram body weight gain was slightly increased. Body weights of the F0 generation in the target dose groups of 5 or 20 mg/kg were similar to control weights throughout this study, but food consumption appeared to be slightly elevated (not statistically significant).

F0 dams producing Fla and Flb litters had statistically significantly lower body weights than control weights on day 20 of the gestation producing the Fla and Flb litters in the highest dose group (table 5). At this dose level, body weights of dams were reduced on day 7, 13, and 20 of the gestation producing Fla litters, but the body weights of dams producing Flb litters were statistically significantly reduced only on day 20. Thus, toxicity was expressed in F0 dams during gestation of the Fla and Flb litters.

On lactation day 7, F0 dams lactating for Fla litters, express significantly reduced body weights in the highest dose group (table 5). For these dams, the body weight per gram of food consumed was about one half the value when compared to other dose groups and controls (data not shown). Dams demonstrated toxicity during lactation for the Fla, and for the Flb litters. At the end of lactation for the Fla and Flb litters, the body weights were statistically significantly elevated.

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Table 5

FU Female Body Weight (g) during gestation and lactation for Fla and Flb litters.

		Target Dose Levels (mg/kg/day)			
		0	5	20	80
Body wt. of FU during gestation producing Fla					
Day	0	178	179	178	173
	7	190	191	191	181**
	13	208	208	206	196**
	20	246	252	249	232*
Body wt. of FU during lactation for Fla					
Day	0	189	191	191	184
	7	205	207	201	189**
	14	212	212	207	208
	21	216	213	219	212
	28	189	184	185	204**
Body wt. of FU during gestation producing Flb					
Day	0	200	205	202	197
	7	210	214	210	204
	13	226	232	230	218
	20	270	277	274	244**
Body wt. of FU during lactation for Flb					
Day	0	210	215	208	205
	7	226	233	225	211*
	14	228	237	233	224
	21	229	239	234	231
	28	203	197	193	226*

*p < 0.005, Dunnett's Test.

**p < 0.01, Dunnett's Test.

Table 6

F1 Female Body Weight (g) during gestation and lactation for F2a and F2b litters.

		Target Dose Levels (mg/kg/day)		
		0	5	20
F1 during the gestation producing F2a				
Day	0	201	198	198
	7	211	208	211
	13	234	227	228
	20	271	271	270
F1 during lactation for F2a				
Day	0	216	211	211
	7	228	221	222
	14	234	232	233
	21	232	233	236
	28	220	221	223
F1 during gestation producing F2b				
Day	0	222	221	214*
	7	234	229	224*
	13	250	248	241
	20	293	290	278
F1 during lactation for F2b				
Day	0	236	234	227*
	7	248	245	237
	14	260	245*	248
	21	255	252	250
	28	228	221	222

*p < 0.05, Dunnett's Test.

The body weights of the F1 generation, after selection, was comparable to control body weights, except in females at the target dose level of 20 mg/kg during weeks 74 to 77 where they were statistically significantly less than controls (97 percent of controls). The report stated that these body weight reductions in females were not biologically significant. No explanation was presented.

The body weights of F1 males during gestation and lactation demonstrated no consistently significant patterns during production or lactation for the F2a or F2b litters (table 6), however they were statistically significantly reduced on day 0 and 7 of the gestation producing the F2b litters, and on day 0 of lactation for the the F2b litters.

4. Pup Weights from Fla, Flb, F2a, and F2b Litters

Pup weights were significantly reduced over control weights in the Fla (table 7) and Flb (table 8) pups only. Both male and female Fla and Flb pup weights were less than control weights from birth to lactation day 28 in the 80 mg/kg target dose group. At the next lower dose level, both Fla and Flb male and female pup weights tended to be apparently lower than control weights toward the end of lactation. By day 20 of lactation, both male and female pups in the Flb litters only demonstrated a statistically significant decrease in body weight over control weights. The male pup weight in Flb litters in the lowest dose group which were statistically significantly reduced on lactation day 28 may not be biologically significant, since there were no apparent differences from control weights throughout the previous weeks of lactation.

None of the F2a or F2b pup weights were found to be different from control weights.

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Table 7

Summary of Fla litter weights (g)
males and females

			Lactation Days								
Group No.	Dose Level (mg/kg/day)	Males Mean S.D.	1	4	Before Selection	4	After Selection	7	14	21	28
1	0	Mean S.D.	5.5 0.83	7.7 1.50	7.7	8.0 1.06	11.9 1.15	22.5 1.72	32.6 2.81	51.8 6.33	
2	5	Mean S.D.	5.6 0.71	7.9 1.20	7.9	7.9 1.22	11.8 1.83	22.1 2.67	31.7 3.06	48.8 6.37	
3	20	Mean S.D.	5.6 0.61	7.9 0.71	7.9	7.9 0.71	11.8 0.80	21.3 2.26	30.9 2.59	48.0 5.62	
4	80	Mean S.D.	4.9* 0.46	6.4** 0.71	6.4** 7.22	8.5** 1.30	17.2** 2.10	26.7** 2.22	39.1** 5.24		
Females											
1	0	Mean S.D.	5.2 0.72	7.5 1.41	7.5	7.7 0.94	11.4 1.03	21.7 1.91	31.1 2.87	48.8 5.25	
2	5	Mean S.D.	5.4 0.73	7.7 1.24	7.7	7.7 1.25	11.5 1.76	21.5 2.55	30.5 2.74	46.0 5.47	
3	20	Mean S.D.	5.4 0.75	7.7 0.58	7.7	7.7 0.59	11.5 0.66	20.7 2.27	30.0 2.79	46.0 5.28	
4	80	Mean S.D.	4.7 0.39	6.3** 0.85	6.3** 0.85	6.3** 0.85	8.5** 1.46	17.0** 2.57	26.5** 3.10	39.3** 6.30	

* = Significantly different from control group at .05 level using Dunnett's test.

** = Significantly different from control group at .01 level using Dunnett's test.

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Table 8

Summary of F1b litter weights (g)
Males and females on
lactation days

Group No.	Dose Level (mg/kg/day)	Males									
		Mean S.D.	1	4	4	7	14	21	28		
1	0	Mean S.D.	5.8 0.43	8.5 0.77	8.5 0.77	12.4 1.17	23.9 2.31	34.6 3.58	56.0 8.87		
2	5	Mean S.D.	5.6 0.58	8.4 0.92	8.4 0.92	12.5 1.32	23.9 2.19	34.3 3.34	50.6* 5.17		
3	20	Mean S.D.	5.4 0.50	7.9* 0.63	7.9* 0.63	11.8 0.86	22.7 1.18	32.6 2.34	47.2** 7.26		
4	80	Mean S.D.	4.5** 0.44	5.2** 1.14	5.2** 1.16	7.2** 1.80	15.9** 3.57	26.3** 4.23	41.1** 6.58		
Females											
1	0	Mean S.D.	5.3 0.46	8.1 0.83	8.1 0.83	11.7 1.11	22.5 1.89	32.3 2.95	51.0 7.52		
2	5	Mean S.D.	5.3 0.59	8.0 0.83	8.0 0.84	11.9 1.19	22.9 1.79	32.6 2.59	47.4 4.56		
3	20	Mean S.D.	5.2 0.46	7.6 0.57	7.6 0.62	11.2 0.68	21.7 1.01	30.9 2.06	44.2** 6.77		
4	80	Mean S.D.	4.4** 0.54	5.6** 0.83	5.5** 0.80	7.2** 1.23	15.1** 1.00	25.0** 0.91	39.0** 1.47		

* = Significantly different from control group at .05 level using Dunnett's test.

** = Significantly different from control group at .01 level using Dunnett's test.

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The reduced Flb pup body weights in the mid dose level occurred from lactating dams demonstrating no statistically significant toxic signs at the time, although their body weights were apparently reduced from controls on lactation day 28. This may indicate that a change in the metabolism of 2,4-D occurred in F0 dams from production of the Fla to production of the Flb litters. Thus, dams exhibiting apparently no toxicity at the time, resulted in a reduction in pup weight over control weights.

5. Viability of Fla, Flb, F2a, and F2b Litters

The study demonstrated a statistically significantly reduced pup viability over controls only at the highest target dose level of 80 mg/kg (tables 9 and 10). The greatest reduction occurred in Flb pups at birth, with the mean litter size being about one half the control value due to deaths of portions and of entire litters. The mean litter size was reduced from five to three by day 14 of lactation, with no more deaths by lactation day 28 (table 10).

Some indication of reduced litter size was apparent in Fla litters of the target dose of 80 mg/kg, but the apparent decrease was not statistically significant (table 9). At birth however, there was a difference in the sex ratio of pups which was significant at the $p < 0.01$ level. From day 1 to day 28 of lactation, no further significant number of pup deaths occurred.

The study report stated that the decrease in female pups at births in Fla litters was not dose-related. I believe that it may be dose related, since at the highest test substance consumed by mothers producing Flb pups, where test substance consumption was higher than in dams producing the Fla litters, both male and female pup survival at birth were less in these Flb pups than the corresponding pup survival in the Fla pups. Thus, there appeared to be a dose response relationship.

Viability of the F2a and F2b pups was not affected.

6. Malformations and Variations

Flb pups which died before lactation day 28 were studied for malformation and variation. As can be seen from table 11, bent ribs, 14 the rudimentary ribs, malaligned sternbrae and unossified sternbrae were seen in the Flb pups. Since most of these pups died at birth or were dead by day 1 of lactation, the effects were seen primarily just after birth at the highest dose only and in the Flb pups only. This was the only group for which there were sufficient deaths, and animals could be necropsied. Only pups which died were available for necropsy except at weaning. These effects are

Table 9
Summary of Fla viability indexes

Group No.	No. Dead Pups	Sex Ratios Day 1 M:F	Live Litter		Gestation Survival Index	Day 4 Before Selection		Day 4 After Selection		
			No.	Size MEAN		No.	%	No.	%	
1	3	99:114	213/21	10.1	213/216	98.6	208/213	97.7	160/160	100.0
2	20	133:118	251/25	10.1	251/271**	92.6	247/251	98.4	191/191	100.0
3	3	121:116	237/24	9.9	237/240	98.8	237/237	100.0	183/183	100.0
4	9	109:71**	180/20	9.0	180/189	95.2	175/180	97.2	147/147	100.0
Group No.			Day 7		Day 14		Day 21		Day 28	
			No.	%	No.	%	No.	%	No.	%
1			156/160	97.5	156/160	97.5	156/160	97.5	156/160	97.5
2			190/191	99.5	190/191	99.5	190/191	99.5	190/191	99.5
3			183/183	100.0	183/183	100.0	183/183	100.0	183/183	100.0
4			146/147	99.3	143/147	97.3	143/147	97.3	143/147	97.3
1 - 0 mg/kg/day 2 - 5 mg/kg/day 3 - 20 mg/kg/day 4 - 80 mg/kg/day										

Survival ratios and sex ratios compared using chi-square test.
Mean number of viable pups compared using analysis of variance.

** = Significantly different from control at .01 level.

Live litter size = No. pup alive on day 1 of lactation/no. litters.

Gestation index = No. pups alive on day 1 of lactation/total no. pups born.

Viability indexes = No. pups alive on day 4 before selection/no. pups alive day 1.

= No. pups alive day n/no. pups alive day 4 after selection.

Table 10
Summary of F1b viability indexes

Group No.	No. Dead Pups	Sex Ratios Day 1 M:F	Live Litter Size		Gestation Survival Index		Day 4 Before Selection		Day 4 After Selection	
			No.	MEAN	No.	%	No.	%	No.	%
1	5	112:107	219/23	9.5	219/224	97.8	219/219	100.0	164/164	100.0
2	15	120:131	251/25	10.0	251/266	94.4	246/251	98.0	177/177	100.0
3	3	110:128	238/23	10.4	238/241	98.8	237/238	99.6	174/174	100.0
4	110**	23:28	180/20	5.1**	51/161**	31.7	44/51**	86.3	42/42	100.0
Group No.			Day 7		Day 14		Day 21		Day 28	
			No.	%	No.	%	No.	%	No.	%
	1		164/164	100.0	164/164	100.0	164/164	100.0	164/164	100.0
	2		177/177	100.0	177/177	100.0	176/177	99.4	176/177	99.4
3		174/174	100.0	174/174	100.0	174/174	100.0	174/174	100.0	
4			34/42**	81.0	30/42**	71.4	30/42**	71.4	30/42**	71.4
1 - 0 mg/kg/day			2 - 5 mg/kg/day		3 - 20 mg/kg/day		4 - 80 mg/kg/day			

Survival and sex ratios compared using chi-square test.

Mean number of viable pups compared using analysis of variance.

** = Significantly different from control at .01 level.

Live litter size, gestation index and viability indexes = see legend table 9.

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0.720

sometimes seen at dose levels causing maternal toxicity, but administration of many compounds do not cause these effects at maternally toxic dose levels.

The number of malformations and variations in these Flb pup dying prior to weaning were apparently not sufficient for statistical significance by the Fischer exact test. As can be seen from Table 11, 50 percent of the litters which died in the high dose group had, for example, malaligned sternbrae compared with 20 percent in controls. The adequacy of these statistical evaluations appear questionable and perhaps should be reevaluated by OPP. However, even if the number of anomalies and variations were significant in the high dose group, the failure to find significant numbers of these effects in five litters examined in each of the controls and the lowest dose group may indicate that these effects did not occur below the highest dose level.

If comparable examinations were conducted in all Fla pups, a dose relationship may have been apparent in the anomalies and variations. There is no indication that this was done. A detailed study on developmental effects on the Fla pups which died during lactation was conducted but these numbers were insufficient to establish a NOEL. If the Fla pups were preserved, it may have been useful to have examined them for a dose related response in developmental effects. However, by day 28 of lactation, all of the apparent effects analogous to those seen in the Flb pups shortly after birth may have disappeared.

Dose levels consumed by dams around the perinatal period were greater for the Flb litters than for the Fla litters. The week immediately before parturition, gestational day 13-20, the dams of the Fla pups consumed the test substance at a daily rate of 76.1 mg/kg, while the dams of the Flb pup, during the corresponding time period consumed 107 mg/kg. The daily consumption of test substance by dams during the first week of lactation for the Fla and Flb pups was 112 and 133 mg/kg, respectively, in the 80 mg/kg target dose level group.

Table 11.

Total Number of Pups and Litters with Developmental and Genetic Variations - Only Flb Pups Found Dead Lactation Days 0-28

Dose Group	Pups				Litters			
	1	2	3	4	1	2	3	4
Number Examined Externally Findings	5	15	3	103	5	5	3	18
	----None-----				----None-----			
Number Examined Viscerally Findings	5	15	3	103	5	5	3	18
	----None-----				----None-----			
Number Examined Skeletally	5	14	3	98	5	5	3	18
Sternebra #5 and/or #6 Unossified	0	0	0	7	0	0	0	5
Sternebrae #1, #2, #3 and/or #4 Unossified	0	0	0	1	0	0	0	1
Sternebrae Malaligned (slight or moderate)	1	0	0	23	1	0	0	9
14th Rudimentary Rib(s)	0	0	0	12	0	0	0	6
Bent Rib(s)	0	0	0	30	0	0	0	6
Reduced Ossification of the Vertebral Arches	0	0	0	2	0	0	0	2

None significantly different from control group using Fisher's Exact Test.

7. Organ Weights and Histological Studies

The absolute and relative liver weights were reduced at all dose levels in the F0 males (table 12). Only the liver/body weight ratios are presented. The absolute and relative kidney weights were increased at all dose levels in F0 females (table 12). The report did not consider the effects on organ weights to be dose-related in either sex. No explanation for this opinion or for these possible test substance related effects was presented. However, neither effect exhibited a smooth dose-related decrease or increase, respectively.

In the F1 generation relative kidney weight of the left but not the right kidney was significantly elevated in males at the 20 mg/kg target dose level only (table 13). The relative liver weights in males of this group were apparently elevated but not statistically. The relative liver weights were increased in F1 females of this dose group but the apparently slightly elevated kidney weights, probably, are not dose related (table 13). Thus, the possible organ weight effects in F1 generation failed to confirm the statistically significant organ weight effects seen in the F0 generation.

No organ weight effects or histopathology was seen in the testes from any dose level from any generation. No dose-related histological effects were seen in the ovary. Thyroids may have been saved but no histology was conducted on them. All the histological studies conducted failed to find any dose-related pathology in any of these organs in the F0 generation and the F1a, F1b, and F1 generation and F2a and F2b pups.

Two histological studies on the livers of the F0 animals were reported. One study was conducted by the testing facility (table 14), and the other was conducted by W. Ray Brown of Research Pathology Services, Inc., New Britain, P.A. (table 15).

When the livers from F0 males were examined histologically numbers of small foci of necrosis were found in all groups. This was initially diagnosed as Tyzzer's disease (table 14). This diagnosis was rejected because females were not affected, diarrhea was not detected, and survival was normal. Research Pathology Services found that small basophilic alterations in hepatocytes occurred at a slightly higher incidence in dosed animals (table 15). In females, these alterations occurred at a slightly higher incidence in controls. None of these histological findings were considered to be dose related by either pathologist.

Table 12.
F0 Terminal Body Weights and Relative Organ Weights

	Target Dose Levels mg/kg/day			
	<u>0</u>	<u>5</u>	<u>20</u>	<u>80</u>
F0 male bwt.	372.	373	368	354**
SD	15.4	17.8	18.3	19.5
F0 female bwt.	217	220	216	209**
SD	10.5	9.8	8.4	12.2
F0 male organ wt. per 100 g bwt.				
Lt Kidney	0.417	0.356**	0.421	0.435
SD	0.18	0.06	0.05	0.04
Rt Kidney	0.469	0.357**	0.420	0.429
SD	0.18	0.06	0.05	0.05
Liver	3.474	3.242**	3.337*	3.226**
SD	0.22	0.18	0.17	0.25
Testes	0.830	0.835	0.821	0.854
SD	0.04	0.04	0.05	0.08
F0 female organ wt. per 100 g bwt.				
Lt Kidney	0.351	0.471**	0.410*	0.425**
SD	0.14	0.12	0.05	0.04
Rt Kidney	0.361	0.476**	0.398	0.424
SD	0.15	0.11	0.06	0.04
Liver	3.477	3.663	3.608	3.627
SD	0.21	0.50	0.27	0.20

SD = Standard deviation; * = $p < 0.05$; ** = $p < 0.01$

Table 13.

F1 Terminal Body Weights and Organ Weight Ratios

	Target Dose Levels ^a mg/kg/day		
	0	5	20
F1 male bwt.	394.	388	386
SD	13.8	28.8	22.9
F1 female bwt.	238	231	231*
SD	9.8	9.0	11.1
F1 male organ wt. per 100 g bwt.			
Lt Kidney	0.394	0.381	0.411*
SD	0.025	0.03	0.02
Rt Kidney	0.390	0.378	0.402
SD	0.02	0.03	0.02
Liver	3.315	3.345	3.439
SD	0.25	0.25	0.17
Testes	0.865	0.857	0.861
SD	0.06	0.11	0.08
F1 female organ wt. per 100 g bwt.			
Lt Kidney	0.398	0.406	0.419
SD	0.03	0.04	0.03
Rt Kidney	0.402	0.400	0.415
SD	0.03	0.05	0.03
Liver	3.568	3.566	3.808**
SD	0.27	0.33	0.25

SD = Standard deviation; * = $p < 0.05$; ** = $p < 0.01$ ^aF1 at 30 mg/kg/day target dose level not dosed beyond weaning.

Table 14.

F0 histomorphological at terminal sacrifice.
 Summary incidence for the live.
 Testing laboratory summary.

Sex Dose group	Male				Female			
	1	2	3	4	1	2	3	4
Number of animals studied	30	30	30	30	29	29	30	29
Liver								
Total examined	30	30	30	30	29	29	30	29
Examined, unremarkable	6	3	9	13	20	22	20	17
Not examined	0	0	0	0	0	0	0	0
Cholangiofibrosis	21	20	19	14	5	3	5	5
Accessory lobe	1	1	0	3	2	1	0	3
Tyzzer's disease	4	18*	10	1	0	0	0	0
Nonspecific Kupffer cell granuloma	0	0	0	0	4	4	6	5
1= 0 mg/kg/day 2= 5 mg/kg/day 3= 20 mg/kg/day 4= 80 mg/kg/day								

* Significantly different from control at 0.05 level, using Kolmogorov-Smirnov, one-tailed test.

Table 15.

F0 histomorphological summary incidence
for liver, at terminal sacrifice.
Summary from Research Pathology Services

	Sex	Male				Female			
	Dose group	1	2	3	4	1	2	3	4
Number examined		30	30	30	30	29	29	30	29
Number normal		1	29	23	24	9	7	12	8
Multifocal bile duct proliferation		25	29	23	24	9	7	7	11
Focal necrosis		11	2	1	3	1	1	1	0
Multifocal necrosis		13	19	15	11	0	0	0	0
Focal cellular alteration									
Basophilic-cell focus/foci		0	4	3	6	8	9	3	1
Clear-cell focus/foci		1	0	0	2	0	0	0	0
Eosinophilic-cell focus/foci		0	0	0	0	0	0	1	0
Microgranuloma/s		2	5	4	2	6	3	7	9
Multifocal mononuclear cellular infiltration		6	2	4	3	6	10	6	8
Accessory lobe		1	1	0	3	2	1	0	3
Centrilobular hepatocellular vacuolation		0	0	0	1	0	0	0	0
Focal hepatocellular vacuolation		1	0	0	0	0	0	0	0
Congestion		0	0	0	1	0	0	0	0
Congenital anomaly		0	0	0	0	1	0	0	0

8. Summary and Discussion

- 1) The study reviewed is a 2-generation, 2 litter per generation study of the effects of 2,4-dichlorophenoxy-acetic acid (2,4-D) on reproduction in Fischer 344 rats.
- 2) The test substance, (97.5% 2,4-D by an I.T.F analysis; and 95.8% 2,4-D by a WIL analysis) was administered in the feed, ad libitum, to 30 rats per sex per group. The concentration of the test substance was adjusted in the feed weekly or monthly according to food consumption and body weight in an attempt to meet target dose levels of 0, 5, 20 or 80 mg/kg/day. During gestation and lactation the actual dose level administered was generally higher, see table 1 and 2, even with 50 percent reduction in concentration during week 2 and 67 percent reduction in concentration during week 3 and 4 of lactation.
- 3) No significant effects on fertility of males or females at any dose or in any generation was evident. This conclusion is supported by the failure to find dose related effects on the testes weight or on histological examination of the testes. No dose related histological effects were seen in ovaries. There was no dose related differences in the number of second matings or in the time required for cohabitation. The fertility of Fischer 344 rats is not high, 60-79 percent in controls, and the variability of the fertility probably would allow detection of only severe reductions in fertility.
- 4) The lengths of gestation was prolonged by 1 day in approximately one half the F0 dams producing F1b litters only in the highest dose group. This effect could result from delayed implantation, hormonal imbalances, or parturition problems.
- 5) The mean body weights of the F0 generation were statistically significantly reduced compared to controls prior to mating, at the highest dose level. ~~Since body weight gain per gram of food consumed was apparently nearly always less in the high dose group than in the other treatment groups or the controls, the body weight decrease cannot be explained by decreases in food consumption. At this dose level, food consumption was frequently statistically significantly~~

increased over control values. At the two lowest dose levels, food consumption was generally apparently increased, but it was seldom statistically significant. Thus, the weight reduction probably is real.

- 6) During lactation, the body weights of F0 dams in the high dose group were not consistently reduced and in the middle dose group in the F0 dams lactating for the Flb litters, there were no statistically significant reductions in body weight compared to controls. Note: It was in the mid dose group and during lactation for Flb litters, that the LEL for pup weight depression occurred.
- 7) The body weights of F1 females during gestation and lactation for F2a and F2b litters were infrequently significantly different from control weights (see table 6). After weaning of the F2b litters from week 44-77 were adult F1 female body weights significantly less than control weights for the target dose level of 20 mg/kg. The body weights of male F1 rats were not different from control weights at any time after weaning.
- 8) Pup body weights were significantly reduced over control weights in the Fla and Flb pups only. These reduced pup weights occurred at the highest dose throughout lactation and in the mid dose only toward the end of lactation, and only in the Flb pups. The NOEL was the lowest target dose level administered.
- 9) Pup viability was reduced at parturition and during the first day of lactation in Fla and Flb pups at the target dose level of 80 mg/kg (actual 76.1 to 133 mg/kg/day) only. A reduction in litter size probably also occurred in the highest dose group in the Fla litters. The apparent reduction probably was dominantly due to a decrease in number of female pups born, causing a significant difference in the sex ratio at birth.
- Pup viability was more severely and significantly reduced in the Flb litters than in Fla litters at birth and between birth and lactation day 1 in addition to the period between lactation day 1 and lactation day 4. The sex ratio in these Flb pups was normal, ~~probably because male, in addition to female pup viability, was less than in the Fla litters.~~

- 10) Anomalies and variations occurred in Flb litters of the high dose which died during lactation. This was the only group for which those effects could be determined because it was the only group apparently for which skeletal examinations were conducted. In addition, it was the only group in which a large number of nonscheduled pup deaths occurred.

These skeletal anomalies and reductions in ossification are generally consistent with similar effects produced by 2,4-D in the teratogenicity study in Fischer 344 rats. The NOEL for developmental effects in that study is 25 mg/kg/day.

- 11) The absolute and relative liver weights of F0 males were statistically significantly reduced at all dose levels at terminal sacrifice. The absolute and relative kidney weights of F0 females were statistically elevated over control weights at all dose levels. There was not a "clean" dose-response relationship and the report did not consider the effect on either sex to be biologically significant.

The liver weight reductions seen in the males may not be toxicologically or pharmacologically significant, and could be an artifact of the study.

- a) There was no "smooth" dose response relationship with the liver weight and the dose of the test chemical.
- b) F1 males and females demonstrated no liver weight reductions.
- c) No significant liver weight reductions occurred in a 90-day subchronic or a chronic study conducted at 1, 5, or 45 mg/kg/day in the Fischer 344 rat.
- d) The reductions probably are not due to the slight thyroid effects analogous to the thyroid effects seen in the subchronic and chronic studies, because only higher elevations of T4 than those seen cause glycogen depletion in the liver.

e) The reductions are not due to an interaction of 2,4-D with the liver histological findings seen. The liver weights in control animals with and without focal necrosis, multifocal necrosis, or basophilic alterations were each not different from each other. Similar comparisons failed to detect differences in the highest dose level group.

f) Food consumption apparently increased at all the higher dose levels, and in some cases the increase was statistically significant. Thus, the liver weight reduction is not due to a reduction in food consumption.

The statistically significant kidney weight increase in females of the F0 generation probably are not correlated with the kidney histopathology seen in the males and female of the subchronic and chronic studies. No kidney histopathology was seen in any animals in the reproduction study. In addition, the kidney weights of 5 females in control animals were an average of 0.18 g for the left or the right kidney, whereas the average kidney weights in the remaining control animals were 0.9 g for the left or the right kidney, approximately 5 standard deviations different. Thus, if these 5 animals are removed from controls, the kidney weights in dosed animals are comparable to controls.

It is concluded that the kidney weight increase is due to an anomaly in the kidney weights of 5 control females, and that it is not due to the test substance.

- 12) No significant dose-related histopathology occurred in any organ at any dose level in any generation.

A case-control study in Wisconsin suggested a higher frequency of non-Hodgkins lymphoma in farmers, particularly those producing small grains or wheat, and using insecticides on their crops (Int. J. Cancer 29:239, 1982). Specific associations with herbicide use were not investigated.

An analysis of death certificate information relative to agricultural practices and chemical usage in Iowa associated non-Hodgkins lymphoma with numbers of egg-laying chickens, milk and hog production, and herbicide use (unspecified) (Am. J. Epidem. 118:72, 1983). Multiple myeloma showed a stronger association with farming as an occupation than did non-Hodgkins' lymphoma.

Similarly, in New Zealand, excess mortality from non-Hodgkins lymphoma and multiple myeloma appeared to be associated with agriculture and forestry (Am. J. Epidem. 121:225, 1985). No effort was made to investigate chemical exposure. Statistical significance of the odds ratio evaluation in this study was certainly marginal. A follow-up of the study in 1986 (Br. J. Ind. Med. 43:75, 1986) indicated no excess risk in association with phenoxy herbicides. Suggestively elevated odds ratios were identified for employment in meat packing and fence construction.

A case-control study of leukemia in Iowa and Minnesota yielded slightly elevated odds ratios for past use of several specific insecticides, but apparently not herbicides (Am. J. Epidem. 122:535, 1985). A case-control study directed at non-Hodgkins lymphoma failed to associate farming with the disease, but did suggest an effect of high-volume pesticide use on frequency of small cell lymphocytic lymphoma. Several modern insecticides and herbicides seemed to be associated with prevalence of this disease, but 2,4-D was not identified among the presumed culprits (Am. J. Epidem. 122:535, 1985). A further case-control study of leukemia and non-Hodgkin's lymphomas in the same region yielded significant odds ratios for exposures to various industrial and household chemicals, including insecticides and herbicides (unspecified) (Am. J. Epidem. 124:535, 1986).

A case-control study of non-Hodgkin's lymphoma in Utah, using colon cancer controls indicated that farming, as an occupation, was a risk factor for non-Hodgkins lymphoma (J. Occ. Med. 27:580, 1976). Chemical exposure was not investigated.

A study of cancer among Danish manufacturers of plastic and other chemicals (mainly MCPA, some 2,4,5-T, but no 2,4-D) suggested an increased risk of soft tissue sarcoma, but not of malignant lymphoma in the group. The Danish investigators inquired carefully into the diagnoses and circumstances of specific cases, and concluded that the occupation-related excess mortality due to soft tissue sarcoma was apparent from superficial analysis, but was not verifiable. The skill with which the Danish investigators approached this matter should set an example for epidemiologists everywhere (Brit. J. Cancer 32:259, 1965).

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September 15, 1986

A case-control study in western Washington state (Am. J. Epidemiol. 124, 529, 1986) may have identified forest sprayers as being at risk for non-Hodgkin's lymphoma, but various other occupations involving phenoxy herbicide exposure did not involve excess cancer risk. Significantly elevated odds ratios were observed for past exposures to insecticides, solvents and metals.

This assortment of findings is what one might expect from epidemiologic inquiries that rely on memory of past chemical exposures, or worse, on best guesses by next of kin concerning work practices over past decades and the chemical exposures that might or might not have occurred. When odds ratio confidence intervals representing 95% assurance are reported as "significant", it is not surprising that a great jumble of inconsistent findings results.

Several features of the article by Hoar et al deserve comment.

One-half of the work practice and chemical exposure data on cases with a diagnosis of non-Hodgkins lymphoma and soft tissue sarcoma (plus controls) were obtained by telephone interview of next of kin. This kind of information cannot be accepted as reliable evidence of past chemical exposure.

For example, it is astonishing that of the 757 Kansas farmers studied relative to soft tissue sarcoma (cases plus controls), 75% are reported not to have used any herbicide at all. (For non-Hodgkins lymphoma, the figure is 74%). If, according to Hoar et al, "all but four subjects who lived or worked on farmland as adults grew at least one of the four specified crops" (wheat, corn, sorghum, or pasture), and if "these four crops constituted 94% of farm acreage and 87% of acres treated with herbicides", how does it happen that three-fourths of the Kansas farmers interviewed hadn't used any herbicide at all on their farms? I submit that the exposure information acquired by telephone interview was worthless.

The authors of the Hoar article claim that there is a graded response between the odds ratio for non-Hodgkin's lymphoma and indices of herbicide use (frequency and duration) (Table 1). But of the five odds ratios calculated for each index (frequency and duration) only two (at the highest frequency and duration) are significantly different from zero, based on the reported 95% confidence intervals of the ratios. Of the two "significant" ratios, that for usage frequency is 2.0 in an interval of 1.0 to 4.0. The odds ratio for duration of use is 6.0 in an interval of 1.9 to 19.5. This ratio is the only basis in the entire report tending to indict herbicides as a risk factor for non-Hodgkins lymphoma. This evidence rests on information obtained by telephone interviews from just seven cases, or their next of kin.

If odds ratios and their confidence intervals are to be believed, table 2 would appear to indict triazines, amides, trifluralin and "non-specified" as strongly as phenoxy herbicides as risk factors for non-Hodgkins lymphoma. Is it likely that so many diverse substances would all be risk factors for this disease?

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September 15, 1986

Table 3 analyzes odds ratios for development of non-Hodgkins lymphoma in relation to reported 2,4-D use. Of the 133 cases and 662 controls interviewed, only 87 Kansas farmers (11%) admitted to having used 2,4-D at least one day per year. The only "significant" odds ratios are for one category each of duration of use (16-25 years), frequency of use (>21 years), and first year of use (1956 - 1965). Evidence is based on not more than 9 interviews of cases and 23 interviews of controls in each category.

The meaning of the chi statistic for trend, used in all the tables, needs explanation. Although reported to be highly significant in many cases, trends are certainly not apparent from the data presented.

Table 4 analyzes odds ratios for non-Hodgkins lymphoma in terms of annual days of use of insecticides and herbicides, adjusting the ratios according to usage of the opposite class of chemicals. Odds ratios for insecticide use were not significant; the only significant odds ratio for herbicide use was for the "more than six days per year" category (OR = 2.3, interval 1.0-4.9). It was essentially unchanged when adjusted for insecticide use.

It is interesting that the data reported in the Hoar article fail to indict farming as a risk factor for soft tissue sarcoma, Hodgkins disease or non-Hodgkins lymphoma. (Table 1). For soft tissue sarcoma, the odds ratio is 1.0, for Hodgkins disease, it is 0.3 and for non-Hodgkins lymphoma, it is 1.4 (CI 0.9 to 2.1).

There is no substantive evidence from the Hoar et al article or from other reported epidemiologic or laboratory studies that 2,4-D or other herbicides causes soft tissue sarcoma, non-Hodgkins lymphoma or any other cancer.

Sincerely,

Donald P. Morgan
Donald P. Morgan, A.D., Ph.D.
Professor

DPM/mjm

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Review of Hoar et al¹ and related literature

This review is prepared in response to EPA Purchase Order 6W-3948-NASA dated September 10, 1986. According to Jerome Blondell's letter accompanying the purchase order: "The key question is: What does the 'weight of evidence' say about the risk of lymphoma for agricultural workers exposed to 2,4-D? Is 2,4-D a likely cause of lymphoma?" This question was prompted by the referenced publication of Hoar et al in the September 5, 1986 issue of JAMA.

Hoar et al

This is a population-based case-control study of all male cases of soft-tissue sarcoma (STS), Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL) identified in the State of Kansas over a 7-year period. 3 controls, matched for age and living-or-dead status, were selected - either by random digit telephone dialing (for living cases under 65 years of age), from Medicare files (for living cases 65 or older) or from Kansas state mortality files (for dead cases). Information on occupation and exposure to herbicides was obtained by telephone interview - with the case or control for half of the subjects with STS or NHL (and corresponding controls) and one-third of the HD cases and controls, and with the next of kin for the remaining, deceased subjects. This study shows every indication of having been carefully and competently carried out. I see no methodologic problems that are likely to have produced the reported positive association between use of herbicides (predominantly uracil and phenoxyacetic acids) and NHL. The strong and statistically

¹ Hoar SK, Blair A, Holmes FF et al. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA 1986; 256:1141-7.

significant increasing risk of NHL with increasing frequency of herbicide use (days per year) supports the idea that the association is real, but the weak, and barely significant association with years of use argues somewhat against it. There are some points of detail which should be noted, although none _____ jeopardize the principal findings, so far as I can judge:

- presumably to have series of the three tumors of approximately equal size (200, 173 and 200), the investigators selected a sample of 200 cases of NHL from the 297 available. The relevance of this sampling is that, if the investigators had had any inkling of what their results would be, they would probably not have discarded 93 cases of NHL, and it must be presumed that it was not an a priori hypothesis that an association would be found only for NHL.
- there is an unexplained, but statistically highly significant, difference between the three groups of cases in the proportion of identified cases which were interviewed. This is primarily due to the low proportion of NHL cases which were excluded, either because they were not confirmed histologically (i.e. were not eligible) or because, if eligible, they were not interviewed. The differential loss occurs at several levels. Thus, the percentages of SDS, HD and NHL cases not histologically confirmed were 19, 15 and 10 percent, respectively. Of the eligible cases, the percentages not interviewed were 4, 8 and 1, respectively. It is difficult to see any relevance of these differences ~~to the study conclusions, but it is curious that determination~~ of eligibility and success in interviewing were both more complete in the group of cases for which an association is found.
- for a high proportion of subjects (50% of cases of STS and NHL and

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References:

1. Subchronic toxicity study in Fischer 344 rats conducted by Hazleton Laboratories, Report No. 2184-102, dated September 12, 1983, for the Industry Task Force on 2,4-D Research No. 251474.
Feeding study conducted 90 days at dose levels of 0, 1, 5, 15, or 45 mg/kg/day.
2. Interim 52-week report on 2,4-D chronic feeding/ oncogenicity study in Fischer 344 rats. Conducted by Hazleton Laboratories submitted by the Industry Task Force on 2,4-D Research. Accession No. 256019.
Feeding study conducted at 0, 1, 5, 15, or 45 mg/kg/day.
3. Teratogenicity study of 2,4-D in Fischer 344 rats. Conducted at WIL Research Laboratories (WIL-81135) for the Industry Task Force on 2,4-D Research.
Study conducted at 0, 8, 25, or 75 mg/kg/day by gavage.
4. Reproduction study of 2,4-D in Fischer 344 rats. Conducted by WIL Research Laboratories (WIL-81137) for the Industry Task Force on 2,4-D Research. Accession No. 259442-6.

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APPENDIX C



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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AUG 7 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 3,C,2,5 data call-in on 2,4-D. Teratology of 2,4-D
and 2,4 Dichlorophenol with respect to PP# 3E2876
related action
CASWELL No. 315..

TO: Richard Mountford, PM #23
Herbicide Branch/RD (TS-767)

Lois Rossi, PM #61
SRB/RD (TS-767)

THRU: Robert B. Jaeger, Section Head *8/1/84*
Review Section #1
Toxicology Branch/HED (TS-769)

FROM: Henry W. Spencer, Ph.D. *not 6/29/84*
Review Section #1
Toxicology Branch/HED (TS-769) *16/10/83*
5/17/84

Conclusions and Recommendations:

1. The range-finding and teratology studies are sufficient to indicate that 2,4-D and 2,4-Dichlorophenol are not teratogenic at up to 75 mg/kg and 750 mg/kg, respectively.
2. Toxicology Branch considers the studies adequate to indicate a fetotoxic effect for 2,4-D at 75 mg/kg (LEL) and a NOEL of 25 mg/kg. Delayed ossification is the fetotoxic effect.
3. Toxicology Branch considers the 2,4-Dichlorophenol teratology studies adequate to indicate an LEL of 750 mg/kg and a NOEL of 350 mg/kg for delayed ossification. A teratogenic effect is not demonstrated in the study.

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4. Toxicology Branch recommends that these studies be added to the data base to support the registrations of 2,4-D.

5. It is noted that generally a maternally toxic dose should be attained in a teratology study. However, in these cases 2,4-D has been previously tested by a registrant, Dow Chemical Co. which demonstrated a slightly ~~or~~ maternal toxic effect at 87.5 mg/kg in rodents. These data were run in a manner to indicate a NOEL for only fetotoxicity.

As a result of previous studies in other species and strains of rodents, the Toxicology Branch continues to consider 2,4-D to be a teratogenic agent. These previous studies are in no way negated by the lack of a teratogenic finding in these current studies since they were run at much higher dosage levels.

Summary of Toxicity Studies Reviewed

I. 2,4-D acid

- a. Range finding study of 2,4-D for maternal toxicity:
(HDT) = 250 mg/kg
LEL for maternal toxicity = 150 mg/kg
NOEL for maternal toxicity = 100 mg/kg for reduction in feed consumption and body weight loss.
- b. Teratology study of 2,4-D
LEL for maternal toxicity NOT found
NOEL for maternal toxicity = 75 mg/kg = (HDT)
LEL for fetotoxicity (delayed ossif.) = 75 mg/kg
NOEL for fetotoxicity = 25 mg/kg
NOT teratogenic at up to 75 mg/kg (HDT)

II. 2,4-Dichlorophenol

- a. Range finding study with 2,4-Dichlorophenol:
Phase III LEL = 750 mg/kg (LDT)
Phase II LEL = 400 mg/kg (500 mg/kg = HDT)
NOEL = 300 mg/kg for mucous membrane effects (maternal)
Phase I LEL = 75 mg/kg (HDT = 150 mg/kg)
NOEL = 25 mg/kg for mucous membrane effects (maternal)
- b. Teratology Study with 2,4-Dichlorophenol Maternal
Toxicity LEL = 200 mg/kg (LDT) equivocal wt. gain effects.
Fetotoxicity LEL = 750 mg/kg
NOEL = 350 mg/kg as delayed ossification.
Not teratogenic at up to 750 mg/kg (HDT)

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Study:

Range-finding teratology study in Fischer 344 rats with 2,4-Dichlorophenoxy acetic acid by WIL Research Labs. Inc. dated May 17, 1983. WIL - 22002., Acc. No. 251032.

Material Tested:

2,4-Dichlorophenoxyacetic acid (Technical) from ITT Research Institute, Chicago, Ill. on March 15, 1982, 97.5% purity.

Animal Tested:

60 virgin, sexually mature female, Fischer 344 rats from Charles River Labs, Portage Michigan were used.

Methods:

After a 25 day quarantine, the females were bred if body wts. were greater than 170 g. Evidence of gestation day 0 was the finding of sperm from a vaginal smear. 10 rats were assigned to each dosage level. Dosages of 0, 75, 100, 150, 200 and 250 mg/kg were tested. The test material was mixed in corn oil and dosed at a volume of 4 ml/kg by gavage on days 6-15 of gestation.

Clinical observations were made daily on days 0-16 of gestation. Body wts. were also recorded on days 0-16 of gestation.

On day 16 of gestation the dams were sacrificed. The usual parameters were noted and included: number of corpora lutea formed, determination of viable and non viable fetuses and implantation status.

Statistical significance of changes was tested by using programs on a Digital Computer. Methods were obtained from: SMPD-79 Biomedical Computer Programs, Univ. of Calif. Press, Berkeley, Calif. 1979. PP. 612, 780, 781.

Results:

A statistically significant reduction in body wt. gain was noted for groups at (1) 150 mg/kg on days 15 and 16 of gestation and (2) 200 mg/kg and 250 mg/kg on days 10 through 16 of gestation. Mean body wt. changes were not statistically different from controls at 75 and 100 mg/kg throughout the study while significant changes were noted at 150 mg/kg and above.

Feed consumed on a mean group basis was found to be significantly reduced at 150 mg/kg and above.

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Dams treated in the study did not abort. However, 1/10 dam in the 200 mg/kg group and 3/10 dams in the 250 mg/kg group died during treatment. Total resorptions occurred in 2/8 pregnant females at 150 mg/kg and in 3/8 and 7/7 pregnant dams at 200 mg/kg and 250 mg/kg respectively.

A significant reduction in viable fetuses is seen at 150 mg/kg where 6.4 fetuses per litter were produced when compared to 9.1/litter in controls. No viable fetuses were found at 200 and 250 mg/kg. No effect on the number of viable fetuses at 100 mg/kg and below was seen. The LEL for resorptions, increased post implantation losses and implantation site effects is 150 mg/kg. A threshold NOEL is 100 mg/kg for fetotoxic and or lethal effects noted. It is based on the slight increase in litters displaying early resorptions even though no increase in numbers of litters effected was seen.

Liver-to-body wt. ratios and kidney wts./body wt. ratios in dams were increased above 150 mg/kg.

	Ratio	Right Ratio	Left Ratio
	Liver/bwt.	Kidney/bwt.	Kidney/bwt.
Control	.0436	347	359
75 mg/kg	.0412	361	370
100	.0414	372	367
150	.0440	374	369
200	.0473	384	386
250	.0524	394	399

Histopathological findings were reported as negative.

Conclusions:

This reviewer considers an LEL for maternal toxicity based on a reduction in feed consumption at 150 mg/kg. A NOEL is 100 mg/kg. The study is to determine a LEL for maternal toxicity but is of limited value because the evaluation only lasted for 16 days and not the 21 days as would be the case in the teratology study.

Core:

Supplementary data

Study:

A range finding Teratology Study/s in Fischer 344 rats with 2,4-Dichlorophenol. Phase I, II, III by WIL Research Labs. Inc. WIL-31133 dated March 31, 1983 for the Industry Task Force on 2,4-D Research Data. Acc. No. 251029.

Material Tested:

2,4-Dichlorophenol purity - approximately 99.2%. I.D. No.: AGR 132992.

Animal Tested:

Fischer 344 virgin female rats from Charles River Labs, Portage, Mich.

Methods:

After a quarantine period and an acceptable body wt. of 170 g or greater, animals were bred. Groups of ten animals were gavaged with 0, 750, or 1000 mg/kg in Phase III; 0, 200, 300, 400, or 500 mg/kg in Phase II; 0, 25, 75, or 50 mg/kg in Phase I. The test material was suspended in corn oil and dosed in a volume of 4 ml/kg.

Dosages were given on days 6 through 15 of gestation. Gestation day 0 was determined with the finding of sperm by a vaginal smear. Feed, Purina[®] rodent chow, and tap water were provided ad libitum. Temperature, humidity, and photoperiods were 72 ± 3° F, 40% and 12 hr. respectively throughout the study.

Clinical observations were made daily on days 0-16 of the study. Body wts. were recorded daily and calculated for 3 day intervals. Food and water intake values were recorded for the above intervals. Uterine and ovarian examination allowed for counting corpora lutea, implantations and locations, number of viable fetuses, and resorptions. Organ wts. of livers and kidneys were recorded.

Statistical evaluation was made using appropriate programs for a Digital Computer with significance for 2-tailed tests set at $p < .05$. The Mann-Whitney U-test was used to compare early and late resorptions, dead fetuses and post implantation losses. Other parameters were analyzed by a one way ANOVA and Dunnett's test.

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Results:

Phase III, (0, 750, 1000 mg/kg). Body wts. of treated dams were statistically reduced only on the last few days at 750 mg/kg and by day 9 at 1000 mg/kg. Food intake was significantly depressed at 750 mg/kg and markedly depressed at 1000 mg/kg after day 6 of study. No females aborted. However, 7/10 females and 1/10 at 1000 mg/kg and 750 mg/kg respectively died during the study. All dams dying were pregnant. One of 2 pregnant dams at 1000 mg/kg totally resorbed its embryos. Viable fetuses were reduced only at the highest dosage when compared to controls because one of two dams completely resorbed.

Implantation sites were not reduced in treated dams. However, early resorptions were increased at the HDT. No significant changes in liver and kidney were noted at 750 mg/kg when compared to controls. A slight increase in kidney wt./body wt. ratios was seen, ($p < .05$), but so few (3) dams were available for analysis, that the significance of this value is questionable. Red staining of the urogenital area was noted at 1000 mg/kg. Mucous membrane effects were also noted at 750 mg/kg.

Phase II: (0, 200, 300, 400, 500 mg/kg). All treated females exhibited a slight but non-significant reduction in weight gain values. Feed consumption was also only slightly reduced when compared to controls.

No females aborted in the study and none died prior to completion of the study. Total resorption of fetuses did not occur in treated dams. Only at 300 mg/kg was the number of viable fetuses reduced, but is considered to be only a spurious non-treatment effect since the effect was not seen at other dosage levels. Slight, nonsignificant increases in early resorptions and post implant losses in both groups treated with 400 and 500 mg/kg are noted. Only a relative organ wt. increase is noted in the liver of dams at the HDT (500 mg/kg).

Dried red discharges (LEL = 400 mg/kg, NOEL = 300 mg/kg) were increased over controls at 400 and 500 mg/kg. Some occurrences of these dried discharges appeared to be the result of cleaning other areas of the body. An LEL of 400 mg/kg is set for the dams using mucous membrane red discharges with a NOEL of 300 mg/kg.

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Phase I (0, 25, 75, 150 mg/kg)

Body wt. gains of treated dams were not different from controls during gestation. Values for feed intake per animal in each group were similar. No females died or aborted in the study. Group pregnancy rates were not significantly different within the study. Controls exhibited an unusually low number of implantation sites, and a subsequent lower number of viable fetuses than seen in the other treatment groups. Relative organ wts. were not different when comparing groups.

Clinical observations included urogenital staining in 3 and 4 30% and 50% of treated dams in groups respectively, exhibiting the effect. Red vaginal discharge was occasionally observed in groups at 25, 75, and 150 mg/kg with 20%, 30% and 10% females involved respectively.

There were no teratogenic or fetotoxic effects at doses up to and including 150 mg/kg (HDT). However, effects on the mucous membranes in the dams were evident at 75 mg/kg with no observable effects at 25 mg/kg.

The dried red discharge on the nares was noted in all groups with substantial increase in response seen at 75 and 150 mg/kg compared to controls.

These data (e.g. range-finding studies) are considered Core: Supplementary.

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Study:

Teratology study in Fischer 344 rats with 2,4-Dichlorophenoxy acetic acid by WIL Research Labs. Inc. dated March 3, 1983. Lab No. WIL - 81135, for the Industry Task Force on 2,4-D Research Data. Acc. No. 251031.

Material Tested:

2,4-D acid, Technical grade from IIT Research Institute, Chicago, Ill. 97.5% 2,4-D acid and related chlorophenoxy compounds,

Animal Tested:

Virgin, Fischer 344 rats from Charles River, Labs., Portage, Michigan.

Methods:

After acclimation and quarantine for a period of 1 week, test animals were individually caged, given Purina® rodent chow #5002 and municipal water ad libitum. Fresh air was at $72^{\circ} \pm 3^{\circ}$ F with 40% relative humidity and a 12 hr. light-darkness cycle. At breeding each female must have weighed greater than 170 g. Cohabitation with males was recorded and evidence of sperm by vaginal smear represented day 0 of gestation. Thirty five females were placed in each test group by randomized block determination.

The test material was suspended in corn oil and given in a dosage volume of 4 ml/kg by gavage so that groups were given either corn oil, 3, 25, or 75 mg/kg of the 2,4-D acid on days 6-15 of gestation. Clinical observations were recorded on days 0-20 of gestation. Observations were made 2X daily. Body wts. of dams were recorded on days 0, 6, 10, 12, 15 and 20 of gestation. At termination of the study, corpora lutea were enumerated, and fetuses were recorded as viable or nonviable. Resorptions were reported as early or late, and implantation sites were enumerated. Ammonium sulfide staining was performed on nongravid uteri.

Fetal Observations:

~~Individual body wts. and crown-to-rump lengths were determined for each fetus. External examination findings were recorded. Approximately one-half the number of fetuses born were fixed in Bouin's solution for Wilson sectioning. The other 50% of fetuses were fixed in 95% alcohol and prepared for skeletal examination by the Alizarin Red S dye method of Dawson.~~

Statistical Examination: Excerpted from study report.

Two-tailed tests were limited at the 5% p-value. "All statistical tests were performed by a Digital Computer with appropriate programming as referenced below.

1. The fetal sex ratios were compared by the Chi-square test⁷ with Yates correction factor.
2. The number of litters with malformations were compared by Fisher's Exact Test⁷.
3. The number of early and late resorptions, dead fetuses and post-implantation losses were compared by the Mann-Whitney U-test⁷.
4. Mean number of corpora lutea, total implantations, viable fetuses, mean fetal and maternal body weight at each interval and maternal body weight gain were analyzed by a one-way analysis of variance, and Dunnett's test.³

References for statistical tests above:

7. BMPD - 79 Biomedical Computer Programs, Univ. of California Press, Berkeley, California, 1979. pp. 612, 780-781.

3. S. S. Siegel, R.G.D. and Terrie, J.H., Principles and Procedures of Statistics, McGraw Hill, New York, New York, 1960 pp. 106, 107, 114."

Results:

Pregnancy status of rats treated with 2,4-D:

	<u>Number</u>	<u>Nonpregnant</u>	<u>Pregnant</u>	<u>% Pregnant</u>
Controls	34	5	30	85
8 mg/kg	34	5	30	85
25 mg/kg	35	7	28	80
75 mg/kg	35	8	27	77

One control and one group 1 (8 mg/kg) female delivered early. Treated animal pregnancy rates were not significantly different from controls.

Mean weight gains in the dams were slowed in the 6-10 day period and only slightly in the 10-12 day period in the group four (75 mg/kg) females. These changes are only equivocal.

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Data presented in the report indicate that no significant differences were apparent between treated and control groups when comparing the mean number of viable fetuses per litter for controls (9.0) vs. 9.3 in 75 mg/kg group litters (HDT). No increased numbers of born-dead fetuses are noted.

Fetal Parameters:

Fetal weights were not significantly different in the 75 mg/kg (HDT) group when compared to controls. In addition, crown-rump lengths were similar in all tested groups. Group 4 (75 mg/kg) early and later resorptions were not increased over control values.

External malformations occurred in a sporadic fashion. Cleft palate was seen in only 1 fetus of 1 litter at the HDT. One fetus in one litter of control exhibited exencephaly. One fetus in one litter at the HDT exhibited incomplete twinning. Microphthalmia and/or anophthalmia occurred in 1 fetus in each of two litters in control and 8 mg/kg groups, respectively; while 1 fetus in 1 litter at HDT dose was also found with ocular abnormality.

Visceral abnormalities in the 75 mg/kg group were limited to one fetus in one litter as kidney and/or ureter missing, one fetus with hydrocephaly, and one fetus with ovary and/or uterus absent. No significance to these incidences is noted.

Skeletal anomalies were limited to occurrences of severely malaligned sternbrae with one fetus in one litter at 25 mg/kg, one fetus in each of two litters at 75 mg/kg. In addition, 1 vertebral anomaly was noted in the control litters.

Developmental variations (feto toxicity) were seen to be slightly increased only at the HDT. These variations at the HDT included an increase in: 7th cervical rib to 4 occurrences in 3 litters, vs 0 in control; 14th rudimentary rib to 4 in 3 litters, vs 0 in controls; reduced ossification increased to 6 in 5 litters vs 2 in 1 control litter and; 15 fetuses in 10 litters with malaligned sternbrae vs 7 in 7 litters in controls. A slight increase in unossified sternbrae #5 and #6 was noted in 73 fetuses/22 litters (3.31 litter) at the HDT while 62 fetuses in 24 litters (2.58 aver/litter) exhibited the effects in controls.

Conclusion:

Toxicology Branch considers the study to exhibit slight fetotoxicity expressed as delayed ossification: LEL = 75 mg/kg
NOEL = 25 mg/kg

The study does not indicate a teratogenic effect at the 75 mg/kg (HDT). Core: Minimum.

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Study:

A teratology study in Fischer 344 Rats with 2,4-Dichlorophenol by WIL Research Labs Inc. Project No. WIL - 81134 dated March 31, 1983, for Industry Task Force on 2,4-D Research Data., Acc. No. 251030.

Material Tested:

AGR 182992, 99.2% of 2,4-Dichlorophenol.

Animal Tested:

Fischer 344 sexually mature, virgin, female rats from Charles River Labs, Portage Michigan.

Methods:

After a quarantine period of approximately 2 months 203 females were bred. Positive sperm findings on daily vaginal smears determined day 0 of gestation. Females with positive smears were randomly placed into 4 groups of 34 animals each. The groups were gavaged on days 6-15 of gestation with either 0, 200, 375 or 750 mg/kg of the test material suspended in corn oil at 4 ml/kg. Feed and water were supplied ad lib throughout the study. Photoperiods were 12 hrs., temperature was maintained at $72 \pm 3^{\circ} \text{F}$ with a relative humidity of 40%. Clinical observations of the individual dams were recorded daily through day 20 of gestation. Body wts. were recorded on days 0, 6, 10, 12, 15 and 20 of the study period. Uterine examination provided implantation sites, resorptions, post-implantation losses, and numbers of viable fetuses. Examination of the fetuses provided numbers of external malformations, anomalies and, crown-rump measurements. Visceral examination of approximately half the fetuses was carried out and the remaining fetuses were fixed in alcohol, stained and cleared for skeletal examination using Alizarin Red S.

Statistical evaluation of the parameters were carried out by the Laboratory as presented below: excerpted from study report.

"All statistical tests have been performed by a Digital Computer with appropriate programming as referenced below.

1. The fetal sex ratios were compared by the Chi-square test⁶ with Yates' correction factor.

2. The number of litters with malformations were compared by Fisher's Exact Test⁶.

3. The number of early and late resorptions, dead fetuses and post-implantation losses were compared by the Mann-Whitney U-test⁶.

4. Mean number of corpora lutea, total implantations, viable fetuses, mean fetal and maternal body weight at each interval and maternal body weight gain were analyzed by a one-way analysis of variance, and Dunnett's test⁷.

References to Statistical Tests in Submission:

6. BMPD - 79 Biomedical Computer Programs, University of California Press, Berkeley, California, 1979. pp. 612, 780, 781.

7. Steel, R. G. D. and Torrie, J. H., Principles and Procedures of Statistics, McGraw Hill, New York, New York, 1960. pp. 106, 107, 114."

Results:

Mean body wts. of only group 4 (750 mg/kg) were significantly reduced throughout the study following day 6 of gestation. However, mean body wt. changes within the 3 day-time intervals of 12-15 days were significantly reduced in all treated groups compared to controls ($p < .01$). Only 4/34 dams at the (HDT), 750 mg/kg, died during the study. These 4 dead females were also gravid. Pregnancy rates were similar in all groups. Total resorptions of litters were not increased compared to controls. Sex ratios of pups were similar in all groups. Mean numbers of viable pups per litter were reduced in all treated groups compared to controls but were not significantly reduced. Early resorptions were increased on a litter basis in group 4 to 1.2 ± 2.7 S.D. compared to 0.8 ± 0.9 S.D. in controls. Postimplantation losses were also slightly increased to 1.4 ± 0.9 S.D. and late resorptions were increased to 0.2 ± 0.4 S.D. compared to none in control. The values taken together suggest an embryotoxic effect at 750 mg/kg but were all nonsignificant. Developmental effects such as exencephaly, micro/or/anophthalmia or cleft palate, occurred in test groups at rates which were not dissimilar to those occurrences noted in controls. Skeletal effects such as malaligned sternebrae were not significantly different from controls. Development variations, with the exception of occurrences of unossified sternebrae #1 - #4, and reduced ossification of vertebral arches, were not significantly increased when compared to controls.

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Conclusion:

Fetotoxicity expressed as delayed or unossified bones exhibited an LEL of 750 mg/kg and a NOEL of 375 mg/kg. The test material was not teratogenic under the conditions of the study.

Maternal toxicity expressed as reduced body wt. gain was equivocal in all test groups except the HDT, which produced death in 4/34 as well as significant reductions in body wt. gains.

Core: Minimum.

TS-769L:SPENCER:sll:X73710:4/6/84: card

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APPENDIX D

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Preliminary Review of Combined Toxicity and
Oncogenicity Study in Rats on 2,4-Dichlorophenoxy-
acetic acid.

FROM: Marcia van Gemert, Ph.D. *M. Kaufman 5.30.86*
Head, Section III
Toxicology Branch, HED (TS-769C)

TO: Lois Rossi
Special Review Branch
Registration Division (TS-767C)

THRU: Theodore M. Farber, Ph.D. *Theodore M. Farber 6/30/86*
Chief
Toxicology Branch/HED (TS-769C)

Compound: 2,4-Dichlorophenoxyacetic acid

Tox. Chem. No.: 315

Registrant: Industry Task Force on 2,4-D Research Data

Accession No.: 030001

Action Requested:

Review the toxicology/carcinogenicity study submitted on
2,4-dichlorophenoxyacetic acid, possible 6(a)(2) action.

Conclusions:

The administration of 2,4-D appears to produce increased numbers of astrocytomas in brains of male rats at 45 mg/kg/day and is suggestive of a carcinogenic effect. The final determination of oncogenicity will come after a joint review with the Canadian Health Protection Branch, an evaluation of the brain and spinal cord slides by EPA officials, and presentation of the weight of evidence before the EPA Peer Review Committee.

The Task Force that submitted the study to EPA is presently re-evaluating the brain slides by an independent pathologist to confirm the diagnosis of astrocytoma, and will

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submit a report of this re-evaluation in July, 1986.

The task force should be requested to submit summary tables for the urinalysis data which were missing from the text of the study. They should also be asked to re-tabulate and submit clearer summary tables of the non-neoplastic and neoplastic lesions. Examples of summary incidence tables are appended to this memo for clarification. The Task Force should also be requested to submit all brain and spinal cord slides of control and experimental animals. Based on the non-neoplastic lesions seen in the kidney, (see DER)

the NOEL = 1 mg/kg/day and the LEL = 5 mg/kg/day.

Core Classification: Will be assigned pending receipt of the requested data.

6. 20

Reviewed by: Marcia Van Gemert, Ph.D. *M. Van Gemert 6.30.86*
Section 3, Tox. Branch (TS-769C) Section Head
Secondary reviewer: Theodore M. Farber, Ph.D. *Theodore M. Farber*
Chief, Tox. Branch (TS-769)

DATA EVALUATION REPORT

STUDY TYPE: Combined toxicity & oncogenicity TOX. CHEM. No.: 315

ACCESSION NUMBER: 263112-263114

MRID No.:

TEST MATERIAL: Dichlorophenoxyacetic acid

SYNONYMS: 2,4-D

STUDY NUMBER(S): 2184-103

SPONSOR: Industry Task Force on 2,4-D Research Data

TESTING FACILITY: Hazleton Labs, 9200 Leesburg Turnpike
Vienna, Virginia 22180

TITLE OF REPORT: Combined Toxicity and Oncogenicity Study in Rats
2,4-Dichlorophenoxyacetic acid, final report

AUTHOR(S): D.G. Serota, Ph.D. - Study Director

REPORT ISSUED: May 29, 1986

CONCLUSIONS: Increased astrocytomas in male rats at 45 mg/kg

NOEL = 1 mg/kg/day

LEL = 5 mg/kg/day based on kidney effects

Classification: Will be assigned pending receipt of the requested information.

A. MATERIALS:

1. Test compound: 2,4-D, Description of test material is on
appended pg.1 Purity 97.5%, contaminants: list in CBI appendix

2. Test animals: Species: rats, Strain: CDF(F344)/CRL-BR, .
Age: 7 wks.

Weight: 125.8-158.3, Source: Charles River Breeding Labs
94.4-118.5 Kingston, New York

B. STUDY DESIGN:

1. Animal assignment - 600 animals were assigned to the
following test groups:

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TABLE 1

Test Group	Dose in diet mg/kg/day	Main Study 104 wks.		Interim Sac. 53 weeks	
		male	female	male	female
1 Cont.	0	60	60	10	10
2 Low (LDT)	1	60	60	10	10
3 Mid-1 (MDT)	5	60	60	10	10
4 Mid-2	15	60	60	10	10
5 High	45	60	60	10	10

2. Diet preparation - Diet was premixed in 200 gms of basal diet and prepared weekly for 1st 14 weeks biweekly through week 18 then every 4th week thereafter and stored at room temperature. Samples of treated food were analyzed for stability and concentrations of 2,4-D in diet for weeks 1, 2, 3, 4, 17, 30, 43, 56, 69, 82, 95.

Results - Analysis of the diet indicated 2,4-D was stable in the diet for at least one month.

TABLE 2

Analysis of 2,4-D Concentrations

Groups	Percentage of Target Range		Mean & S.D.
	Low	High	
2	84.6	120.3	101.96 + 9.54
3	82.1	125.3	100.6 + 9.2
4	80.8	122.2	97.8 + 8.7
5	81.9	113.4	98.1 + 7.2

3. Animals received food (Diet + 2,4-D) and water ad libitum.
4. Statistics - The following procedures were utilized in analyzing the numerical data: (See appended pgs. 2&3).
5. Quality assurance was in compliance with EPA GLP regulations.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected twice/day for signs of toxicity and mortality.

Detailed physical exams for physical appearance, behavior tissue mass palpation and signs of abdominal distention were made weekly for 1st 14 weeks and biweekly thereafter.

Results - Toxicity - no treatment related effects on mortality (survival) were noted. (See appended pages 4 & 5).

TABLE 3

Mortality and (Percent Survival) at Month^a

	6	12	18	24
	<u>Males</u>			
Group 1	1 (98)	1 (98)	2 (95)	18 (64)
2	0 (100)	0 (100)	2 (95)	7 (85)
3	0 (100)	0 (100)	0 (100)	2 (96)
4	1 (98)	2 (97)	3 (94)	8 (84)
5	0 (100)	0 (100)	0 (100)	12 (76)
	<u>Females</u>			
1	1 (98)	2 (97)	4 (92)	10 (80)
2	0 (100)	0 (100)	1 (98)	13 (74)
3	0 (100)	0 (100)	0 (100)	2 (96)
4	1 (98)	2 (97)	3 (94)	8 (84)
5	0 (100)	0 (100)	0 (100)	12 (76)

- a. Percent survival based on 60, 60, 50 and 50 rats/sex/group at 6, 12, 18 and 24 months, respectively.

2. Body Weight - Animals were weighed at initiation of the experiment and weekly for 1-14 weeks then biweekly for remainder of experiment.

Results - Statistical analysis of absolute body weight at week 52, body weight changes at weeks 0-52 and 0-104 and growth rate data showed significantly decreased mean values for group 5 females. (see appended pages 6 & 7 for cumulative body weight gain.)

TABLE 4
MEAN CUMULATIVE BODY WEIGHT GAIN

0-52						0-104					
Weeks females			males			weeks males			females		
	N	Mean	S.D.	N	Mean	S.D.	N	Mean	N	Mean	S.D.
1	58	113.4	11.57	59	229.6	18.30	32	216.8	40	145.6	14.87
2	60	114.1	8.61	60	225.4	17.33	43	211.2	37	142.9	26.0
3	60	116.7	11.77	60	227.1	19.28	48	214.5	38	141.0	22.07
4	60	113.5	12.43	58	232.3	16.88	42	213.9	38	144.8	17.22
5	60	105.2*	11.31	59	227.9	14.05	37	206.5	36	132.8*	17.14

* Significantly different from control $p \leq 0.05$

3. Food consumption and compound intake - Consumption was determined and mean daily diet consumption was calculated. Food consumption was measured weekly for first 14 weeks and then biweekly for the remainder of the experiment.

Results - Food consumption - mean values for Group 5 females were significantly lower than control values at weeks 1 - 52. Also the mean value for Group 2 females was significantly higher than the mean value for Group 1 females at this time interval.

TABLE 5
MEAN TOTAL FOOD CONSUMPTION -Females

0-52 weeks				0-104 weeks			
	N	Mean	SD		N	Mean	SD
1	58	3114.9	169.52	40	5861.8	289.84	
2	57	3198.9*	171.65	34	5989.4	313.94	
3	56	3174.9	164.39	35	6022.7	319.78	
4	60	3115.7	166.14	38	5816.9	304.19	
5	60	3038.6*	140.29	35	5751.4	291.19	

*Significantly different from control $p \leq 0.05$

4. Ophthalmological examinations were performed at end of 52 weeks and at 104 weeks all animals.

Results - Ophthalmic exam revealed no ocular toxicity that could be associated with 2,4-D administration at any dose.

5. Blood was collected before treatment and at 26, 52 and 78 weeks for hematology and clinical analysis from 10 animals/sex/group. Clinical analysis was collected on all animals surviving to termination of study. The checked (X) parameters were examined.

a. Hematology -

X		X	
X	Hematocrit (HCT)*	X	Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Total Leukocyte count (WBC)*		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*		Mean corpuscular HGB conc. (MCHC)
X	Platelet count*		Mean corpuscular volume (MCV)
X	Reticulocyte count		

Results -

No treatment-related results on the hematological parameters measured were apparent.

b. Clinical Chemistry

X		X	
	Electrolytes:		Other
X	Calcium*	X	Albumin*
	Chloride*		Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
	Phosphorous*		Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
X	Alkaline phosphatase		Triglycerides
	Cholinesterase	X	Albumin/globulin ratio
	Creatinine phosphokinase*	X	Thyroxine
X	Lactic acid dehydrogenase	X	Total protein
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		

Results -

1. there was a slight ($p < .05$) increase in the albumin and a slight decrease ($p < 0.05$) in globulin at week 105 in males, increasing the A/G ratio at both 79 and 105 weeks ($p < 0.05$). (see appended pages 8 & 9)
2. There was slight ($p < 0.05$) increase in serum alanine aminotransferase in males and females at week 105 in Group 5. (see appended page 10)
3. T_4 was slightly depressed ($p < 0.05$) at 105 weeks in group 5 females. (see appended page 11)

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6. Urinalysis - Urine was collected from 10 rats/sex/group at initiation and following weeks 26, 52, and 78 weeks of treatment. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH		Blood*
	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

Results - Tables on mean values for urinalysis were missing from the text.

There appears to be a decrease in urinary protein at the highest dose level. Summary tables will have to be generated before this can be verified.

7. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

Digestive system	Cardiovasc./Hemat.	Neurologic
	Aorta*	XX Brain*
X Salivary glands*	XX Heart*	X Periph. nerve* (sciatic)
X Esophagus*	X Bone marrow*	X Spinal cord
X Stomach*	X Lymph nodes*	XX Pituitary*
X Duodenum*	X Spleen*	X Eyes (optic n.)*
X Jejunum*	X Thymus*	Glandular
X Ileum*	Urogenital	XX Adrenals*
X Cecum*	XX Kidneys*	Lacrimal gland
X Colon*	X Urinary bladder*	Mammary gland*
X Rectum*	XX Testes*	XX Parathyroids*
XX Liver*	XX Epididymides	XX Thyroids*
X Gall bladder*	X Prostate	Other
X Pancreas*	Seminal vesicle	X Bone* (sternum with marrow)
~~Respiratory~~	XX Ovaries	X Skeletal muscle*
X Trachea*	X Uterus*	X Skin
X Lung*		X All gross lesions and masses

Summaries of the pathology protocols for the 52-week sacrifice, unscheduled deaths, and the terminal sacrifices are appended on pages 12 and 13. The study states that "brain" sections (including at least one section of the forebrain, mid brain and hind brain) were examined microscopically by the study pathologist and then read blind by a second pathologist. Following these examinations remaining fixed brain tissue from each animal

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was processed and evaluated microscopically by the study pathologist. These observations were incorporated into the original findings to yield a composite incidence from both evaluations.

I called Dr. David Sorota of Hazelton Laboratories, the Study Director, and asked specifically how the brain was sectioned. He said originally only one section from fore, mid and hind brain was examined. But after finding some astrocytomas, they then sectioned all available brain tissue from each rat. We are in the process of formally writing to the Task Force for written confirmation of this statement.

Results -

a. Organ Weight

Interim sacrifice

Kidney weight parameters measured, eg. absolute organ weight, organ-to-body weight, and organ-to-brain weight were significantly elevated in the group 5 males. Females showed a slight increase in kidney weight parameters no other significant organ weight changes were noted. (see table 6 for details.)

TABLE 6

ORGAN WEIGHTS 52 WEEK SACRIFICE

Kidney		Absol. Wts		organ-to body wts.		organ-to- brain wts.	
		Mean	SD	Mean	SD	Mean	SD
Male	#						
1	10	2.44	.17	.693	.043	1.225	.061
2	10	2.43	.11	.684	.022	1.214	.041
3	10	2.46	.26	.698	.050	1.225	.107
4	10	2.61	.12	.738	.037	1.295	.053
5	10	2.66*	.15	.780*	.057	1.344*	.091
Kidney							
Female							
1	10	1.57	.10	.805	.090	0.876	.046
2	10	1.62	.13	.802	.067	0.901	.055
3	10	1.56	.10	.785	.052	0.873	.069
4	10	1.62	.05	.784	.036	0.892	.034
5	10	1.60*	.09	.829*	.037	0.884	.048

*Significantly different from controls $p \leq 0.05$

Terminal Sacrifice

At 105 weeks there was an increase in kidney weight parameters in groups 4 and 5 with statistical

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significance in the females ($p < 0.05$) in group 5 in all parameters. (Table 7) The increases in kidney weight values appear to be treatment-related. There appeared to be a dose-related increase at 104 weeks in all male thyroid/parathyroid parameters with statistical significance generally in groups 4 and 5. In female there appeared to be a trend of increased values in groups 3, 4, and 5 with group 4 having statistical significance. This appears to be a treatment-related effect. The other organ weights that were significantly different from control were noted in group 5. These organs include liver and thyroids/parathyroids in males, pituitary, brain with brain stem, and ovaries in females. Those changes in the pituitary, liver and ovaries appear treatment-related.

TABLE 7

ORGAN WEIGHTS 104 WEEK SACRIFICE

LIVER	N	Absolute Organ wt.		Organ-to-Body wt.		Organ-to-Brain wt.	
		Mean	SD	Mean	SD	Mean	SD
Male							
1	32	10.02	2.02	2.996	.664	4.846	.962
2	43	9.66	1.14	2.956	.390	4.702	.560
3	47	9.94	1.69	2.992	.538	4.827	.814
4	41	9.41	1.25	2.837	.337	4.609	.592
5	36	8.82	1.29	2.730	.543	4.277*	.658
LIVER Female							
1	40	7.14	1.35	3.072	.638	3.811	.658
2	37	7.16	0.95	3.102	.487	3.812	.483
3	37	7.07	1.20	3.099	.509	3.799	.626
4	38	7.04	1.15	3.061	.488	3.755	.578
5	36	6.73	1.23	3.066	.599	3.577	.620
KIDNEYS combined-Male							
1	32	2.78	0.35	.829	.111	1.345	.177
2	43	2.75	0.32	.840	.112	1.338	.169
3	47	2.74	0.31	.825	.090	1.333	.154
4	41	2.84	0.34	.860	.113	1.393	.162
5	36	2.85	0.26	.880	.111	1.383	.146
KIDNEYS combined-Female							
1	40	1.89	0.14	.813	.066	1.012	.081
2	37	1.95	0.13	.844	.095	1.037	.071
3	37	1.98	0.20	.871*	.108	1.064	.107
4	38	1.94	0.16	.843	.061	1.034	.088
5	36	2.07*	0.30*	.945*	.195	1.099*	.161
PITUITARY Male							
1	32	.022	.023	.0067	.0076	.0106	.0114
2	43	.016	.006	.0048	.0016	.0077	.0027
3	47	.024	.026	.0074	.0076	.0119*	.0124
4	41	.023	.066	.0092	.0252	.0139	.0351
5	36	.013	.015	.0055	.0045	.0086	.0075

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TABLE- 7 CONT.

PITUITARY Female

1	40	.016	.010	.0069	.0039	.0086	.0053
2	37	.023	.026	.0103	.0141	.0120	.0134
3	37	.040*	.071	.0180*	.0321	.0220*	.0386
4	38	.021	.033	.0087	.0121	.0112	.0177
5	36	.033*	.052	.0157*	.0278	.0176*	.0280

BRAIN w STEM Male

1	32	2.07	.06	.618	.041		
2	43	2.06	.06	.629	.033		
3	47	2.06	.07	.620	.042		
4	41	2.04	.08	.618	.052		
5	36	2.07	.11	.638	.062		

BRAIN w STEM Female

1	40	1.87	.06	.805	.063		
2	37	1.88	.06	.816	.088		
3	37	1.86	.08	.820	.082		
4	38	1.87	.06	.818	.068		
5	36	1.88	.06	.857*	.077*		

OVARIES

1	39	.108	.039	.0467	.0176	.0582	.0215
2	36	.105	.040	.0456	.0184	.0560	.0215
3	37	.125	.067	.0538	.0247	.0670	.0354
4	38	.115	.034	.0504	.0168	.0612	.0184
5	36	.131	.060	.0589*	.0260	.0693	.0320

THYROID/PARATHYROID Male

1	32	.027	.009	.0082	.0027	.0133	.0043
2	41	.031	.009	.0094	.0029	.0150	.0046
3	46	.032	.011	.0097	.0034	.0157	.0054
4	41	.033*	.007	.0100*	.0020	.0163*	.0036
5	36	.034	.014	.0106*	.0041	.0166	.0063

THYROID/PARATHYROID Female

1	40	.025	.006	.0106	.0028	.0131	.0033
2	37	.024	.007	.0105	.0037	.0128	.0038
3	37	.027	.005	.0117	.0023	.0143	.0027
4	38	.031*	.008	.0134*	.0035	.0164*	.0044
5	35	.027	.009	.0123	.0042	.0144	.0048

b. Gross Pathology

Inspection of detailed gross necropsy findings revealed that there were no differences in incidence of the findings between the control and treated animals with unscheduled deaths, at the 52 week sacrifice, or at the terminal sacrifice.

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c. Microscopic Pathology

1) Non-neoplastic

52-Week Sacrifice

There were general alterations in histopathological parameters in the kidneys of groups 3, 4, and 5 that appeared compound-related. These consisted of:

- 1) An increased incidence in brown tubular cell pigment in the males of groups 3, 4 and 5 (9/10, 10/10, 10/10 respectively) and groups 3, 4 and 5 females (5/10, 6/10 and 7/10 respectively) when compared to control males (2/10) and control females (3/10). (Note appended page 14 for details)
- 2) An increased frequency and severity of fine vacuolization of cytoplasm in the renal cortex in group 5 females (8/10) when compared to control females (5/10) and an increase in severity in groups 3 & 4 females when compared with control females. (see appended page 14 for details on increased severity.)

Unscheduled Deaths

No compound-related histopathologic alterations were found in the animals that died or were killed moribund prior to the terminal sacrifice.

Terminal Sacrifice

Compound-induced histomorphologic alterations occurred in the kidneys of groups 3, 4 and 5 males and females. (These are summarized on table 8.)

These were:

- 1) Increased brown tubular cell pigment in the kidneys of groups 3, 4 and 5 males (8/47, 18/41**, 18/36** respectively) and groups 3, 4 and 5 females (23/37*, 19/38**, 13/36 respectively) when compared to control males (2/32) and females (8/40). (Note appended page 15 for statistical analysis)
- ~~2) Increased incidence of pelvic microcalculi in groups 4 and 5 males (8/41, 9/36 respectively) and group 5 females (28/36**) when compared to control males (2/32) and female (19/40).~~
- 3) A slight increase in frequency of transitional epithelial hyperplasia in group 5 females (6/36) when compared to controls (0/40) however, the study pathologists considered this secondary to the increased frequency of microcalculi.

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TABLE 8

NON-NEOPLASTIC LESIONS IN RATS FED 2,4-D

Tubular Cell Pigment, increased kidney	Males					Females				
	1	2	3	4	5	1	2	3	4	5
UD*	0	1	1	0	1	0	1	0	1	2
IS**	2	2	9	10	10	3	3	5	6	7
TS***	2	0	8	18	18	8	9	23	19	13
Total	4	3	18	28	29	11	13	28	26	22
Transitional Epithelial Hyperplasia										
US	0	0	0	0	3	1	1	0	2	5
IS	0	0	0	0	0	2	1	1	3	3
TS	0	1	1	1	0	0	0	3	2	6
Total	0	1	1	1	3	3	2	4	7	14
Microcalculi Pelvis										
UD	0	0	1	0	2	0	2	1	2	7
IS	0	1	0	0	1	2	3	1	3	4
TS	2	2	3	8	9	19	9	14	21	28
Total	2	3	4	8	12	21	14	16	26	39
Fine cytoplasmic Vacuolization										
UD	0	0	0	0	0	0	0	0	0	0
IS	0	0	0	0	0	5	3	5	5	8
TS	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	5	3	5	5	8

*UD = unscheduled deaths

**IS = Interim sacrifice

***TS = Terminal sacrifice

2) Neoplastic

~~Astrocytomas were found in the brains of rats with~~
 unscheduled deaths and terminal sacrifice, including a
 group 1 male that died in week 21 and two group 4 males that
 were killed in extremis in week 94 and 105, and one group 5
 male that was killed in extremis in week 93. There were no
 reported astrocytomas found in the 52-week interim sacrifice
 but at the 104-week terminal sacrifice, 5 astrocytomas were
 found in group 5 males and none in the other four groups.
 The total astrocytomas found for male rats on test then
 totaled 1/60 for group 1 controls, with 0/60, 0/60, 2/58
 and 6/60 for groups 2,3,4 and 5 respectively. (See appended

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pages 17,18 and 19 for summary tables and individual animal data). According to the study text, "The incidence of astrocytomas in the brain of high-dose males is higher than that in control males, intercurrent mortality adjusted prevalence analysis indicates a positive trend at $p = 0.0026$ (one-tailed, uncorrected score test), and control versus high-dose group comparison is significant at $p = 0.0351$ (one-tail); but not at two-tail ($p = 0.0702$). (See appended page 20 for statistical analysis).

D. DISCUSSION

comments:

1. The administration of 2,4-D appears to produce astrocytomas in brains of male rats at 45 mg/kg/day dose level, and is suggestive of a carcinogenic effect. The task force that submitted the study for EPA review is presently re-reviewing the diagnoses of the brain slides and will submit another independent pathology report some time in July, 1986. This task force should be asked to submit summary tables for the urinalysis data and compile concise summary incidence tables for all the non-neoplastic and neoplastic histopathology data. They should also be requested to furnish EPA with all control and treated brain and spinal cord slides for our own independent analysis.

2. Based on the increase in frequency and/or severity of kidney lesions seen in groups 3, 4 and 5 male and female rats the NOEL for non-neoplastic lesions is 1 mg/kg/day, the LEL = 5 mg/kg/day.

TS-769:VAN GEMERT:6/25/86

cc. W. Burnam
T. Farber
A. Barton
J. Melone
J. Lamb
J. Moore

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2,4-D Peer Review

Page is not included in this copy.

Pages 168 through 189 are not included in this copy.

The material not included contains the following type of information:

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 - ☐ Identity of product impurities
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

CASWELL FILE

001720

OCT 20 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Review of Histopathology Tables submitted on 2,4-D

To: Ms Lin Vlier
Special Review
Registration Division TS-767C

From: Marcia van Gemert, Ph.D.
Head, Section III
Toxicology Branch, HED

M. van Gemert 10/16/86

Thru: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch, HED

10/20/86

Compound: 2,4-Dichlorophenoxyacetic acid

Tox Chem No.: 315

Registrant: Industry Task Force on 2,4-D Research Data

Accession No: 264933

Action Requested: Review submitted tables

The 2,4-D Task Force has submitted the revised histopathology tables according to our request as detailed in the Toxicology Branch memo of 6/20/86. Enclosed in this submission were:

1. The combined neoplastic and non-neoplastic tables which incorporated all the animals from the interim and terminal sacrifices, and the unscheduled deaths,
2. A set of tables which combined the histopathological findings by animal/organ system in order to assess if double counting was taking place,
3. A set of tables detailing the recent histopathology done to complete all the spinal cord sections,
4. The amended protocol concerning the original treatment of the spinal cord slides.

The submitted information and tables will be assessed in the order given above.

1. The combined neoplastic and non-neoplastic tables reveal

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as detailed in the June 30, 1986 memo, an increase in kidney tubule cell pigment, an increase in transitional epithelial hyperplasia, microcalculi and fine cytoplasmic vacuolization. Also noted in the new tables is an increase in testicular granulomatous prostatitis as noted below.

Testes	non-neoplastic lesions				
	groups				
	1	2	3	4	5
granulomatous prostatitis	1	1	1	1	4

However, this finding does not change the NOEL set in the 6/30/86 memo.

2. The combined animal/organ system histopathology tables may indicate a slight correlation between the increased frequency of transitional cell hyperplasia and the presence of microcalculi in groups 4 and 5 in females and group 5 in the males.

	males					females				
	1	2	3	4	5	1	2	3	4	5
transitional epithelial hyperplasia	0	1	1	1	3	3	2	4	7	14
Microcalculi pelvis	2	3	4	8	12	21	14	16	26	39
animals with combined hyperplasia and microcalculi	0	1	0	0	2	2	2	3	6	13

The matter of kidney hyperplasia and its possible cause by microcalculi will be a matter for discussion at the Peer Review Committee meeting where the histopathology data will be presented.

3. The spinal cords were sectioned at the request of the Canadian government. These data are appended. The original protocol called for sectioning of only 10/sex/group. After examining the tables presented, there did not appear to be any increased incidence of astrocytomas associated with 2,4-D treatment.

4. The amended protocol, more clearly reflecting the original

histopathological treatment of the spinal cord slides is appended.

Conclusions

A. The submitted data have not resulted in a change in the NOEL which was stated in the 6/30/86 memo to be

NOEL = 1 mg/kg/day
LEL = 5 mg/kg/day

B. The new combined animal/organ system histopathology tables indicate only a slight correlation between the increased frequency of transitional cell hyperplasia in the kidney and the presence of microcalculi. This will be a matter for the Peer Review Committee to discuss.

C. After reviewing the histopathology tables on the new sections there did not appear to be an increase in incidence of astrocytomas in the treated animals.

2,4-D Peer Review

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Pages 193 through 198 are not included in this copy.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

CASWELL FILE

6.1720

JUL 22 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Pathology Report on 2,4-D from Dr. A. Koestner,
Michigan State University

To: Lois Rossi
Special Review Branch
Registration Division (TS 767C)

From: Marcia van Gemert, Ph.D.
Head, Section III
Toxicology Branch, HED

M. van Gemert 7.22.86

Thru: Theodore Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division

Theodore M. Farber 7/22/86

Compound: 2,4 Dichlorophenoxyacetic acid

Tox.Chem.No.: 315

Registrant: Industry Task Force on 2,4-D Research Data

Action Requested: Review Dr. Adalbert Koestner's pathology
report on 2,4-D.

Conclusions: Dr. Koestner has concluded that the incidence of astrocytomas in the brains of rats treated with 2,4-D is not a treatment-related phenomenon. His arguments for this conclusion are summarized below. These comments will be taken into consideration along with our own independent evaluation of the slides and peer review before reaching a final conclusion concerning the possible carcinogenicity of 2,4-D.

Discussion: ~~Dr. Koestner's conclusions concerning the tumor incidence from re-evaluating the tumor sections of the animals diagnosed as having astrocytomas are summarized in the table below:~~

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TABLE I
TUMOR INCIDENCE

	<u>Male</u>	<u>Female</u>
Controls	1/60	0/60
1 mg/kg	0/60	1/60
5 mg/kg	0/60	2/60
15 mg/kg	2/53	1/60
45 mg/kg	5/60	1/60

Dr. Koestner does not feel that the tumor seen in the high dose male (case # 23473) found after additional brain tissue was imbedded and sectioned, is actually neoplastic. His diagnosis is that it consists of a mixed glial and mesenchymal cell population, and he therefore did not include it in table I. In his experience, "most early astrocytomas are remarkably monomorphic and elicit no tissue reaction. In the animal # 23473, the tumor consists of pleomorphic perivascular and dispersed cell populations including granulocytes and lymphocytes in addition to glial tissue. Special stains in this case reveal reticulin and collagen formation which is a function of specific mesenchymal cells but not of astrocytoma cells." The tumors which were diagnosed as astrocytomas were all of glial origin, generally well differentiated with little tissue reaction by surrounding brain tissue. In some animals there were areas of necrosis with some subsequent repair responses evident. These tumors Koestner considered to be identical to glial tumors routinely found in aged rats.

BIOLOGICAL CRITERIA FOR EVALUATION OF NEUROCARCINOGENS

Depending on the experimental design, carcinogenic potential of the compound and availability of tissue samples at various stages during the experiment, some or all of the following criteria may apply to or are testable in any single case. Some according to Koestner, will always be present and he states will permit a distinction to be made between experimentally induced and naturally occurring brain tumors.

1. Increased incidence beyond expected control levels.
2. Shift of tumor appearance to a younger age (decreased survival time).
3. Demonstration of dose-effect relationship.
4. Higher tumor incidence after transplacental exposure.
5. Trend toward anaplasia.
6. Presence of preneoplastic lesions.
7. Multiplicity of tumors in individual animals.
8. Tumor occurrence in both sexes.
9. Tumor occurrence also in peripheral nervous system.
10. Tumor induction outside the nervous system.

QED

11. Genotoxicity, mutagenicity, chromosomal aberrations.

His arguments against any biological evidence for carcinogenesis are listed below in order.

1. Increased incidence beyond expected control levels:

Dr. Koestner presented a table of incidences of gliomas in control male Sprague-Dawley rats found by commercial laboratories with an incidence range of 0-10%.

TABLE II

VARIABILITY IN BRAIN GLIOMA INCIDENCE
IN CONTROL MALE SPRAGUE-DAWLEY RATS 1 YR. AND OLDER
(SELECTED FROM SWENBERG, J.A. 1986)

NUMBER	COLOR	LABORATORY	CONTROL 1(%)	CONTROL 2(%)	CONTROL 3(%)
1-5	Diff. Colors	IRDC	0/292 (0)	2/287 (0.7)*	2/137 (1.4)
6	Red No. 33	IRDC	3/57 (5.2)	0/59 (0)	2/58 (3.4)
7	Green No. 3	Biodynamics	0/52 (0)	5/55 (9)**	--
8	Blue No. 2	Biodynamics	0/59 (0)***	2/59 (3.4)	--
9-13	Diff. Colors	Biodynamics	2/290 (0.7)	2/239 (0.7)	4/231 (1.7)
14	Red No. 9	Licton	4/58 (6.9)****	6/60 (10)	2/57 (3.5)
15	Red No. 27	Licton	2/54 (3.7)	0/55 (0)	--
16	Red No. 34	Licton	2/57 (3.5)	1/59 (1.7)	0/53 (0)
17	Red No. 30	Hazleton	3/59 (5.1)	1/55 (1.3)	--

* One of the rats died on day 350 with a glioma

** Additional sections resulted in 5/55 (10.9%)

*** Additional sections resulted in 2/59 (3.4%)

**** One glioma diagnosed at 12 mos. interim sacrifice

In the F344 rat strain NCI carcinogenicity studies show an incidence range of gliomas in 4700 male and female rats of 0-3.3%. Dr. Koestner also quotes a reported incidence range of 2.8% in males and 1.6% in female F344 rats from a paper by Solerfeld, et al. Koestner emphasized that these incidences were reported for only 3 sections-per-brain-being-examined. Since these gliomas are "microtumors", the harder one looks for them, the more likely they are to be found. In the 2,4-0 study 7 blocks per brain were examined as compared to the standard 3. So these reported incidences may be under-estimating the actual historical control incidences.

2. Shift of tumor appearance to a younger age:

Dr. Koestner states that usually with space-occupying brain

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-4-

tumors in the restricting cranial cavity, there is a shortening of survival. However, this shortening of survival is not seen in 2,4-D exposed rats, but can be demonstrated in a number of neuro-oncogenic substances.

3. Demonstration of a dose-effect relationship:

Other neuro-oncogens such as ethyl nitrosourea and methyl methanesulfonate show a dose-response relationship. However, no such dose-effect relationship exists for 2,4-D. There is an unequal clustering of tumors in the high dose group only, according to Dr. Koestner.

4. High tumor incidence after transplacental exposure:

This criterion cannot be evaluated because it hasn't been tested.

5. Trend toward anaplasia:

The tumor spectrum, according to Dr. Koestner, in the 2,4-D study was comparable to that found in surveys of the spontaneous brain tumors in rats. He claims the tumors are primarily of mature and differentiated astrocytic cell populations. However, in other neuro-oncogens, such as MNU, 53% of the brain tumors produced were either unspecified gliomas (14%), anaplastic gliomas (14%) or gliosarcomas. In addition methyl methanesulfonate, a weak carcinogen, produced primarily malignancies. Five of the 7 tumor-bearing animals had malignant neurogenic neoplasms (most rats had several tumors).

6. Presence of preneoplastic lesions:

Neurocarcinogens such as ENU can elicit early preneoplastic glial proliferations with glial tumors appearing much later. Koestner believes that these early neoplastic glial proliferations are good indicators of chemical tumor induction as opposed to spontaneous neoplasms in older rats. None of these preneoplastic lesions were seen in the rats given 2,4-D.

7. Multiplicity of tumors in individual animals:

Koestner states that a multiplicity of neurogenic tumors is the rule rather than the exception for compounds such as ENU or MNU. However, no rat in the 2,4-D study had more than one neurogenic tumor.

8. Tumor occurrence in both sexes:

In the 2,4-D study Koestner claims that the incidence in male

-5-

rats is slightly higher than in female rats (2.6 to 1.6%), as is the case under natural conditions. Females in the 2,4-D study didn't show any increased tumor incidence.

9. Tumor occurrence in both the central and peripheral nervous system:

In the Nitrosourea studies, Koestner states, most of the longer living animals developed neurinomas. However, no tumors of the peripheral nervous system were seen in the 2,4-D study.

10. Tumor induction outside the nervous system:

Most systemic carcinogens produce extraneural tumors in addition to neurogenic tumors. These neoplasms include leukemias, lymphomas, carcinomas of various organs and sarcomas. However, no increased incidence of extraneural tumors was seen in the 2,4-D study.

11. Genotoxicity, mutagenicity, chromosomal aberrations:

2,4-D was negative when tested in the Ames test, the erythrocyte micronucleus test in mice, the dominant lethal in mice and in human lymphocytes, according to Koestner's assessment. 2,4-D only tested positive in Saccharomyces cerevisiae and gave mixed results in tests with fruit flies (Drosophila melanogaster). Koestner claims that overall, these results indicate little or no mutagenic potential for man.

Dr. Koestner claims that this clustering of gliomas in the high dose male group is most likely due to chance, and statistical analyses of these numbers are only meaningful when some or all of the above criteria are met. In this circumstance, the increased incidence of tumors in the high dose group is just another example of biological variation.

QNB

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DEC 15 1986

005634

MEMORANDUM

Subject: Dr. Swenberg's Evaluation of the Brain Slides for the
2,4-Dichlorophenoxyacetic acid rat study

To: Lynn Vlier
Special Review,
Registration Division TS-767C

From: Marcia van Gemert, Ph.D.
Head, Section III
Toxicology Branch, HED

Marcia van Gemert 12.11.86

Thru: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch, HED

Theodore M. Farber 12/15/86

Chemical: 2,4-Dichlorophenoxyacetic acid

Tox Chem #: 315

Project No: None

Dr. Swenberg met with Dr. Theodore Farber and myself on November 19, 1986 and conveyed both the box of brain slides and his histopathological diagnoses of these slides to us.

For all but one animal, he agreed with the original study text diagnoses of astrocytomas. He disagreed with the astrocytoma diagnosis of female B23289 in the 5 mg/kg group. He diagnosed this lesion as a focal area of gliosis present near the center of the olfactory bulb, no neoplasm was detected. The study text however had diagnosed this area as an astrocytoma. He also disagreed with Dr. Koestner's diagnosis of the male B2J473 of the 45 mg/kg group. Dr. Koestner had diagnosed the brain section in question of this animal as consisting of a mixed glial and mesenchymal cell population. Dr. Swenberg diagnosed this animal as having a small astrocytoma present in the ventral portion of one hemisphere of the forebrain.

Dr. Swenberg was asked if he felt this study provided sufficient evidence to classify 2,4-D an oncogen. He said the data were very equivocal for a number of reasons. Astrocytomas are not as uncommon as previously thought. These tumors are very small as a general rule, and more vigorous examination and sectioning tend to increase the numbers found. He had seen a similar circumstance when examining the slides from a rat study on Harvade. At that time he suggested that another study be performed to resolve the equivocal astrocytoma

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issue. This study should include a larger group size, two control and one high dose group, looking at brain sections only at termination of the experiment. He stated that this was his recommendation also for 2,4-D in order to attempt to resolve this astrocytoma issue.

Appended page 1 contains the original table of tumor incidences from the study text, and appended page 2 presents Dr. Swenberg's diagnoses in tabular form by animal number. Appended pages 3 and 4 present the study text's original diagnoses and appended pages 5 and 6 present Dr. Swenberg's diagnoses.

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2,4-D Peer Review

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Astrocytoma Incidence as Diagnosed by Dr. Swenberg

Males:

Dose:	0 mg/kg	1 mg/kg	5 mg/kg	15 mg/kg	45 mg/kg
Animal					
#	B23025		B23376	B23473	
			B23377	B23476	
				B23479	
				B23492	
				B23500	
				B23505	

Females:

Animal				
#	B23185	B23302	B23442	B23546

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NOV 18 1986

Summary of Peer Review Comments

Agricultural Herbicide Use and Risk of Lymphoma and Soft-Tissue Sarcoma. Shelia Hoar, Aaron Blair, Frederick Holmes, Cathy Boysen, Robert Robel, Robert Hoover, and Joseph Fraumeni. Journal of the American Medical Association (JAMA) 256: 1141-1147, 1986.

Summary prepared by Jerome Blondell, Health Statistician
Exposure Assessment Branch, Hazard Evaluation
Division (TS-769C)

I. Introduction

A population-based, case-control study conducted by the National Cancer Institute in Kansas, found an association between farm herbicide use and non-Hodgkin's lymphoma (NHL) but did not find an association with soft-tissue sarcoma (STS) or Hodgkin's disease (HD). Four reviewers were asked to evaluate this study, particularly the weight of evidence for an association between 2,4-D and NHL. In addition, the editorial in JAMA which reviewed the evidence from various studies was found to be useful and is included here. The reviewers are:

1. Brian MacMahon, M.D., Ph.D., Professor of Epidemiology and Head of the Department, Harvard University, School of Public Health.
2. Martha Linet, M.D., M.P.H., Assistant Professor of Epidemiology, Johns Hopkins University, School of Hygiene and Public Health.
3. Donald Morgan, M.D., Ph.D., Professor of Preventive Medicine and Environmental Health, University of Iowa, College of Medicine.
4. Leon Burmeister, Ph.D., Professor of Biostatistics, University of Iowa, College of Medicine.
5. Theodore Colton, Sc.D., Boston University School of Public Health (editorial in JAMA).

~~This summary of reviewer comments will focus on the possible association between 2,4-D and NHL. The overall quality of the study, problems with exposure assessments and analysis will be addressed. Support from other studies, especially the one by Hardell in Sweden will also be covered (Hardell L., et al. Malignant lymphoma and exposure to chemicals especially organic~~

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solvents, chlorophenols and phenoxy acids: a case-control study British Journal of Cancer 43:169, 1981). Finally, the main conclusions of each reviewer will be summarized.

II. Overall Quality of the Study

Reviewers disagree on the conclusions that could be drawn from the Hoar, et al. study but most agreed that it was of high quality and should serve as a basis for further research. MacMahon stated "This study shows every indication of having been carefully and competently carried out." Linet commented on "The overall excellent design and careful execution" and Burmeister noted that the study had "very high scientific validity." The editorial in JAMA praised the study for being "well designed and carefully executed." Hoar, et al. were commended for their population-based sample, use of histologically confirmed cases, high response rates, and the careful analysis of the results.

III. Exposure Assessment

Strong concerns were expressed by all the reviewers regarding at least one major aspect of the exposure assessment. Key areas of concern were the use of next-of-kin interviews, lack of specific data on 2,4-D exposure, and the apparent underreporting of herbicide use.

Half of the NHL cases and the matched controls had died before the study had started. Therefore, it was necessary to use next-of-kin telephone interviews to assess the use of herbicides. Linet, Morgan and MacMahon commented on the lack of reliability likely to occur with next-of-kin interviews. MacMahon noted "one must question surrogates' knowledge of what specific herbicides were used and on how many days of the year." Morgan felt that "This kind of information cannot be accepted as reliable evidence of past chemical exposure."

Recall bias may exist among both live subjects and next-of-kin that would tend to favor the memory of those herbicides which had been used most frequently, most recently and had the easiest names to remember (e.g. 2,4,-D). This is especially a problem when trying to remember the names of chemicals used 30 or 40 years ago. In addition, there may be recall bias between cases and controls such that cases will try harder to remember what pesticides they were exposed to. In order to assess recall bias a sample (14%) of the suppliers who sold the pesticides were contacted. The degree of confirmation by the suppliers is only vaguely described by Hoar, et al., "suppliers usually reported less pesticide use than subjects," and "there were no consistent differences between agreement rates for patients and controls." Additional data on suppliers' responses have been requested from NCI but have not yet been provided.

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2,4-D Peer Review

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Appendix

C-120

Histopathologic Examination of Brain Sections from Study 2184-103

<u>Animal Number</u>	<u>Number of Slides</u>	<u>Histopathologic Findings</u>
0.5mg/kg/day B23024 M	2	A small granular cell tumor is present in the cerebellar meninges that has invaded into the dorsal cerebellum.
0.5mg/kg/day B23025 M	2	A large cystic mixed glioma extends from the forebrain to the occipital lobe of one cerebral hemisphere.
1mg/kg/day B23185 F	3	A microscopic astrocytoma is present in the ventral medulla.
5mg/kg/day B23289 F	2	A focal area of gliosis is present near the center of the olfactory bulb. <u>No neoplasm was detected.</u>
5mg/kg/day B23302 F	3	The ventral forebrain contains an area of increased cellularity. This lesion is present in only one section but appears compatible with a microscopic astrocytoma.
15mg/kg/day B23376 M	5	There is an area of focal gliosis/early tumor present in the dorsal forebrain. In addition, the most anterior portion of the forebrain adjacent to the olfactory bulb has meningeal filtration of mononuclear cells having a similar astrocytic morphology. Diagnosis: Microscopic astrocytoma.
15mg/kg/day B23377 M	3	A large diffuse astrocytoma extends from the frontal region of the brain to the occipital lobe of the brain.
15mg/kg/day B23442 F	2	A small to moderate sized diffusely invading astrocytoma is present in one hemisphere of the forebrain.
15mg/kg/day B23473 M	3	A small astrocytoma is present in the ventral portion of one hemisphere of the forebrain.
4mg/kg/day B23476 M	2	The cerebellum contains an astrocytoma.
4mg/kg/day B23479 M	2	A small astrocytoma is present in the striatum of one hemisphere of the forebrain.

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Histopathologic Examination of Brain Sections from Study 2184-103 (cont'd)

<u>Animal Number</u>	<u>Number of Slides</u>	<u>Histopathologic Findings</u>
45mg/kg/day B22492 M	3	One hemisphere of the cerebellum contains a diffusely invading astrocytoma.
45mg/kg/day B23500 M	2	A diffusely invasive astrocytoma extends from the forebrain to the thalamus.
45mg/kg/day B23505 M	2	A small astrocytoma is present in the olfactory bulb of the brain.
45mg/kg/day B23546 F	2	The brain contains an astrocytoma extending from the thalamus to the midbrain with secondary hydrocephalus.

John A. Leachman, M.D., F.R.C.P.
11/19/86

007720

APPENDIX E

007720



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 27 1987

005794

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Review of the Mouse Oncogenicity Study on 2,4-Dichloro-
phenoxyacetic acid

From: Marcia van Gemert, Ph.D. *M. van Gemert 3/23/87*
Head, Section III
Toxicology Branch, HED (TS-769C)

To: Lin Vlier
Registration Division (TS-767C)

Thru: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch, HED

Theodore M. Farber 3/27/87

Compound: 2,4-Dichlorophenoxyacetic acid

Tox Chem No: 315

Registrant: Industry Task Force on 2,4-D Research Data

Accession No: 400618-01

Action Requested: The Toxicology Branch has been requested to review the recently submitted mouse oncogenicity study on 2,4-Dichlorophenoxyacetic acid (2,4-D). This review, along with the chronic/oncogenicity study in rats will be presented before the Peer Review Committee April 21, 1987. The conclusions reached from the review of this study are presented below.

Conclusions: 2,4-D at doses of 0, 1, 15 and 45 mg/kg were fed to B₆C₃F₁ mice for 104 weeks with a 52 week interim sacrifice. Effects were seen in absolute and relative kidney and adrenal weights at 15 and 45 mg/kg. Histopathology revealed an increase in the mid and high dose groups in cytoplasmic homogeneity of the renal tubular epithelium due to a reduction of cytoplasmic vacuoles.

NOEL = 1 mg/kg

LEL = 15 mg/kg based on treatment-related kidney and adrenal effects

Classification: Core minimum; batch number and dietary stability and homogeneity data were not provided.

Oncogenic NOEL = 45 mg/kg, (HDT)

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Reviewed by: Marcia van Gemert, Ph.D.
Head, Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C)

U. van Gemert 3/20/87

00720

Theodore M. Farber 3/27/87

005794

DATA EVALUATION REPORT

STUDY TYPE: Mouse Oncogenicity study

TOX. CHEM. NO.: 315

ACCESSION NUMBER: 400618-01

MRID NO.: 40061801

TEST MATERIAL: 2,4-dichlorophenoxyacetic acid

SYNONYMS: 2,4-D

STUDY NUMBER(S): 2184-101

SPONSOR: Industry Task Force for 2,4-D Research Data

TESTING FACILITY: Hazleton Laboratories America Inc.
9200 Leesburg Turnpike, Vienna Va. 22180

TITLE OF REPORT: Oncogenicity Study in Mice with 2,4-dichlorophenoxy-
acetic acid (2,4-D)

AUTHOR(S): D.G. Serota

REPORT ISSUED: Not dated, but compliance statement was signed and
dated by study director 1/15/87

CONCLUSIONS: 2,4-D at doses of 0, 1, 15, and 45 mg/kg were fed
to B₆C₃F₁ mice for 104 weeks with a 52 week interim sacrifice.
Effects were seen in absolute and relative kidney and adrenal weights
at 15 and 45 mg/kg. Histopathology revealed an increase in the
mid and high dose groups in cytoplasmic homogeneity of the renal
tubular epithelium due to a reduction of cytoplasmic vacuoles.

NOEL = 1 mg/kg

LEL = 15 mg/kg based on treatment-related kidney and adrenal effects.

Classification: core-Minimum, batch number and dietary stability
and homogeneity data were not provided.

Special Review Criteria (40 CFR 154.7)

005794

A. MATERIALS:

1. Test compound: 2,4-dichlorophenoxyacetic acid (2,4-D)
Description: beige powder
Batch # not given, Purity 97.5%, contaminants: listed in CBI appendix
2. Test animals: Species: mice, Strain: B₆ C₃F₁Cr1 Br
Age: 7 weeks at initiation of study
Weight: males: 16.4-24.2 gms, females: 14.8-20.9 gms.
Source: Charles River Breeding Laboratories Inc. Kingston, N.Y.

B. STUDY DESIGN:

1. Animal assignment

240 animals/sex were assigned randomly to the following test groups:

Test Group	Dose in diet mg/kg	Main Study 24 months		Interim Sac. 52 weeks	
		male	female	male	female
1 Cont.	0	50	50	10	10
2 Low (LDT)	1	50	50	10	10
3 Mid (MDT)	15	50	50	10	10
4 High(HDT)	45	50	50	10	10

2. Diet preparation

Diet was prepared weekly. The concentration of test material in the diet was adjusted weekly through the first 15 weeks and every 4th week thereafter. Reserve samples were taken from the initial batch received and from each mixed batch of test diet and sent to the sponsor. Additional reserve samples were taken from each mixed batch of diet and retained under refrigeration.

Results - ~~Stability~~ data were not provided. A statement in the study text was made that "the sponsor provided information that 2,4-D was stable in the diet for at least one month. Homogeneity of 2,4-D in the diet was established in previous studies conducted at Hazleton Biotechnologies Corp."

Routine analysis conducted during the test on 2,4-D mouse study gave the following results on table I.

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005794

TABLE I

group	sex	Percent of target range		percent of target mean + S.D.
		low	high	
2	male	73.99	136.1	96.48 + 14.824
2	female	77.92	121.2	97.57 + 10.677
3	male	85.17	111.5	97.56 + 6.490
3	female	80.91	110.0	95.43 + 8.234
4	male	80.24	103.4	95.61 + 4.966
4	female	78.79	101.9	94.26 + 5.341

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics - The procedures utilized in analyzing the numerical data are on appended pages 3-7.

5. Quality assurance statement was signed by Frederick G Snyder and dated 1/7/87.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. Detailed physical examinations for unusual appearance and behavior, and palpation of tissue masses and abdominal distension were performed weekly for weeks 1-14 and biweekly thereafter.

Mortality (survival) There were no statistically significant differences between treated and controls concerning mortality. Appended pages 8 and 9 graphically represent survival data. The numbers of animals dying on test are tabulated in table II.

TABLE II

Animals which died or were sacrificed in extremis prior to week 104

Group	males	females ^c
1	10	12
2	6	8 ^b
3	10	17
4	11 ^a	15

^a One found out of cage at week 30 and was removed from study.

^b One was found dead during week 105 prior to scheduled sacrifice

^c 3 animals (2 group 2, one group 3) were missexed and discovered at week 2 and removed from the study **218**

Toxicity: No treatment-related effects were seen.

2. Body weight

Animals were weighed at initiation of study and weekly for weeks 1-14, and biweekly thereafter.

Results: There were no treatment-related effects on body weight or body weight gains. Appended pages 10 and 11 graphically represent body weight data.

3. Food consumption and compound intake

Consumption was determined weekly for weeks 1-14 and biweekly thereafter. Data on food efficiency and compound intake were not given.

Results:

There did not appear to be any treatment-related changes associated with food consumption. There was a significant increase in food consumption in males at weeks 1-52 and 1-104 weeks in groups 2 and 4. Data are presented in Table IV below. Females in group 2 at 1-104 weeks showed an increase in food consumption. Females for weeks 1-52 showed a positive trend in food consumption.

TABLE IV

weeks	males				females			
	1	2	3	4	1	2	3	4
1-52	1361.8	1419.1*	1368.0	1418.9*	1485.0	1536.3	1532.2	1523.9
SD	91.97	87.51	84.19	104.6	91.79	119.66	109.61	109.74
N	56	59	54	50	53	47	50	56
1-104	2313.8	2439.0*	2349.6	2437.6*	2542.6	2668.2*	2568.3	2607.6
SD	137.41	139.7	134.81	170.54	142.91	146.68	178.84	178.09
N	31	35	35	32	30	29	27	33

* Significantly different from controls $p < 0.05$.

4. Ophthalmological examinations were not performed.

5. Blood was collected for hematology from the last surviving 10 mice/sex/group following 52 weeks of treatment and from the first 10/sex/group following 104 weeks of treatment. Clinical Chemistry analyses were not performed. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpuscular HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood Clotting Measurements	X	Cell morphology

* Required for subchronic and chronic studies

Results: No compound-related changes in hematological parameters were seen.

6. Urinalysis was not performed.

7. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X	Digestive system	X	Cardiovasc./Hemat.	X	Neurologic
X	Tongue		.Aorta*	XX	Brain*† at least 3 levels
X	Salivary glands*	XX	Heart*	X	Periph. nerve*#
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*#
X	Stomach*	X	Lymph nodes* ⁶	XX	Pituitary* ⁵
X	Duodenum*	X	Spleen*	X	Eyes (optic n.)* ⁷
X	Jejunum*	X	Thymus* (when present)		Glandular
X	Ileum*		Urogenital	XX	Adrenals* ⁵
X	Cecum*	XX	Kidneys*† ³		Lacrimal gland#
X	Colon*	X	Urinary bladder*	X	Mammary gland*#
X	Rectum*	XX	Testes*† ²		Parathyroids*††
XX	Liver*† ¹		Epididymides	XX	Thyroids*†† ⁴
	Gall bladder*#	X	Prostate		Other
X	Pancreas*		Seminal vesicle	X	Bone*# Sternum w marrow
	Respiratory	XX	Ovaries*† ⁵	X	Skeletal muscle*#
X	Trachea*	X	Uterus*	X	Skin*#
X	Lung*			X	All gross lesions
				X	3 coronal sections ⁸

Footnotes are on the next page

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- * Required for subchronic and chronic studies
- o Required for chronic inhalation
- # In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
- † Organ weights required in subchronic and chronic studies
- †† Organ weight required for non-rodent studies

- 1 weighed with gall bladder
- 2 weighed with epididymides
- 3 left and right (weighed separately, combined weight was calculated)
- 4 weighed with parathyroids post fixation, and sectioned together
- 5 weighed post fixation
- 6 all grossly enlarged or otherwise abnormal lymph nodes, or nodes draining known or suspected tumor sites.
- 7 with contiguous hardierian gland
- 8 coronal sections through head to include nasal cavity, paranasal sinuses, oral cavity, nasopharynx and middle ear

Gross examination included examination of :
external surface, all orifices, cranial cavity, carcass, nasal cavity and paranasal sinuses (not opened at necropsy but opened and examined post fixation, except for 10 animals/sex/group selected for histopathology exam of the spinal cord and coronal head sections), cervical tissues and organs, thoracic, abdominal and pelvic cavities and their viscera, cut surface of brain and external and cut surfaces of the spinal cord (examined post fixation during tissue trimming) according to the study text. All preserved tissues (except spinal cord, skeletal muscle and coronal sections of the head) from all mice were embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined microscopically. The spinal cord (cervical and thoracic) and coronal sections through the head were examined microscopically from the last 10 animals/sex/group surviving to study termination.

- a. Organ weight: Both kidneys and adrenals appeared to be affected by treatment.

Kidneys: absolute kidney weights, both left and right as well as combined, were significantly increased in group 4 females, and all revealed a significant positive trend. There were significantly increased kidney/brain weight ratios in group 4 females for left right and combined kidneys. At 104 weeks all absolute and relative kidney weight parameters were significantly increased weight in group 4 females with most significant positive trends seen, except kidney/brain weights for combined kidneys. Kidney/body weight ratios in group 3 females for left, right and combined kidneys were significantly elevated. Group 4 males also showed an increase in kidney/body weight ratios, possibly due to the drop in body weight seen at high dose. Data for the 52 week sacrifice are in table V, and data for the terminal sacrifice are in table VI.

Adrenals: Absolute and all relative male adrenal weights were decreased at all doses tested at week 54, with significant trends seen for all parameters. At week 104, all parameters in the mid and high dose groups were increased (rather than decreased at 54 weeks) with significant positive trends seen in all parameters for males. Data are given in table V and VI.

TABLE V

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ORGAN WEIGHTS 52 WEEK SACRIFICE

left kidney		Abs Wts		Organ-to body wts.		Organ-to Brain wts.	
#		Mean	SD	mean	S.D.	Mean	S.D.
male							
1	10	0.28	0.03	0.975	0.068	0.622	0.067
2	10	0.29	0.03	1.006	0.073	0.632	0.056
3	10	0.28	0.03	0.899	0.061	0.627	0.062
4	10	0.28	0.02	0.873* ^a	0.091	0.617	0.041
left kidney female							
1	10	0.20	0.02	0.763	0.065	0.436	0.033
2	10	0.20	0.02	0.742	0.092	0.420	0.036
3	10	0.20	0.01	0.749	0.076	0.435	0.029
4	10	0.22* ^a	0.02	0.825	0.116	0.471*	0.032
right kidney male							
1	10	0.29	0.02	1.014	0.048	0.647	0.064
2	10	0.31	0.02	1.074	0.093	0.673	0.039
3	10	0.30	0.03	0.952*	0.051	0.665	0.065
4	10	0.30	0.02	0.923 ^a	0.118	0.651	0.049
right kidney female							
1	10	0.21	0.02	0.794	0.063	0.455	0.045
2	10	0.22	0.02	0.801	0.079	0.454	0.031
3	10	0.22	0.01	0.802	0.083	0.466	0.027
4	10	0.24* ^a	0.02	0.893*	0.099	0.512* ^a	0.040
Combined kidneys males							
X		mean	S.D.	mean	S.D.	Mean	S.D.
1	10	0.58	0.05	1.991	0.111	1.270	0.125
2	10	0.60	0.05	2.085	0.169	1.307	0.084
3	10	0.58	0.05	1.845*	0.100	1.288	0.123
4	10	0.58	0.04	1.793	0.206	1.266	0.086
Combined kidneys females							
1	10	0.42	0.04	1.558	0.119	0.892	0.079
2	10	0.42	0.04	1.552	0.169	0.879	0.063
3	10	0.42	0.02	1.546	0.153	0.899	0.055
4	10	0.46* ^a	0.03	1.716	0.207	0.982*	0.061
Adrenal males							
1	10	0.007	0.002	0.0258	0.0068	0.0163	0.0039
2	10	0.005*	0.001	0.0189*	0.0047	0.0117*	0.0026
3	10	0.005*	0.001	0.0164*	0.0023	0.0114*	0.0016
4	10	0.006* ^a	0.001	0.0177* ^a	0.0047	0.0125* ^a	0.0030
Adrenal females							
1	10	0.008	0.001	0.0306	0.0041	0.0175	0.0023
2	10	0.008	0.001	0.0290	0.0036	0.0164	0.0009
3	10	0.008	0.001	0.0299	0.0060	0.0173	0.0027
4	10	0.008	0.003	0.0304	0.0137	0.0170	0.0065

* Significantly different from controls ($p \leq 0.05$)^a Significant trend ($p \leq 0.05$)

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TABLE VI

Organ Weights for the Terminal Sacrifice							
Left Kidney		Abs Wts		Organ-to Body Wts.		Organ-to Brain Wts	
males	X	Mean	S.D.	Mean	S.D.	Mean	S.D.
1	40	0.32	0.04	1.049	0.104	0.707	0.068
2	44	0.32	0.03	1.093	0.115	0.712	0.058
3	40	0.32	0.03	1.095	0.098	0.712	0.064
4	38	0.33	0.03	1.135*a	0.112	0.729	0.065
left kidney females							
1	38	0.22	0.02	0.819	0.090	0.469	0.044
2	38	0.23	0.02	0.857	0.091	0.486	0.051
3	31	0.23	0.02	0.880*	0.079	0.487	0.055
4	34	0.27*a	0.02	1.003*a	0.092	0.559*a	0.049
Right kidney males							
1	40	0.34	0.04	1.106	0.102	0.745	0.069
2	44	0.34	0.03	1.142	0.112	0.744	0.060
3	40	0.34	0.03	1.139	0.106	0.740	0.068
4	38	0.35 ^a	0.03	1.185*a	0.106	0.762 ^a	0.069
Right kidney females							
1	38	0.24	0.02	0.883	0.091	0.505	0.043
2	38	0.24	0.03	0.903	0.082	0.510	0.048
3	31	0.25	0.03	0.952*	0.122	0.527	0.079
4	33	0.28*	0.03	1.042*a	0.101	0.581*	0.048
Combined kidneys males							
1	40	0.67	0.07	2.153	0.180	1.450	0.122
2	44	0.66	0.06	2.236	0.216	1.457	0.109
3	40	0.66	0.05	2.234	0.194	1.453	0.123
4	38	0.68	0.06	2.322*a	0.208	1.492	0.129
Combined kidneys females							
1	38	0.46	0.04	1.701	0.178	0.973	0.084
2	38	0.47	0.04	1.762	0.167	0.998	0.094
3	31	0.48	0.05	1.832*	0.191	1.014	0.128
4	33	0.55*a	0.05	2.045*a	0.182	1.141*	0.092
Adrenals male							
1	40	0.005	0.002	0.0157	0.005	0.0107	0.0040
2	43	0.005	0.002	0.0182	0.0063	0.0118	0.0042
3	40	0.006*	0.002	0.0214*	0.0078	0.0138*	0.0047
4	37	0.006*a	0.002	0.0213*a	0.006	0.0135*a	0.0035
Adrenals female							
1	37	0.008	0.002	0.0283	0.0083	0.0164	0.0052
2	38	0.007	0.002	0.0280	0.006	0.0158	0.0037
3	31	0.007	0.002	0.0277	0.0075	0.0153	0.0043
4	35	0.008	0.002	0.0288	0.0073	0.0162	0.0044

* Significantly different from controls $p \leq 0.05$ ^a Significant trend $p \leq 0.05$

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b. Gross pathology: No treatment-related effects were noted in the unscheduled deaths, interim sacrifice and terminal sacrifice.

c. Microscopic pathology

1) Non-neoplastic:

Kidney: There was a compound related increase in histomorphologic alteration in the renal tubule epithelium of males. This was characterized as a cytoplasmic homogeneity, and was due to the reduction of cytoplasmic vacuoles that are normally present in the renal tubular epithelium. These effects are presented in Table VII. Other lesions seen were not treatment-related and were the result of normal spontaneous disease processes associated with the B₆ C₃F₁ mouse.

TABLE VII

Incidence of Cytoplasmic Homogeneity- Increased Tubular Epithelium

Group/sex	1m	2m	3m	4m	1f	2f	3f	4f
Unscheduled deaths								
N	10	6	10	11	0	0	0	0
Increased Tubular Epi.	3	5*	10***	10***	0	0	0	0
52-week sacrifice								
N	10	10	10	10	10	10	10	10
Increased Tubular Epi.	0	1	4*	10***	0	0	0	0
Terminal sacrifice								
N	40	44	40	38	33	38	31	35
Increased Tubular Epi.	8	9	34***	38***	0	0	0	0
Combined deaths								
N	60	60	60	59	60	58	59	60
Increased Tubular Epi.	11	15	48***	58***	0	0	0	0

* Significantly different from Controls $p < 0.05$

** Significantly different from Controls $p < 0.01$

*** Significantly different from Controls $p < 0.001$

Fishers Exact Test used by reviewer

Adrenal: No histopathological findings were noted in the adrenal at the interim sacrifice or terminal sacrifice, or in unscheduled deaths.

2) Neoplastic: No treatment-related increased incidence in tumors was seen at the 52 week sacrifice, in unscheduled deaths, at terminal sacrifice, or in combined scheduled and unscheduled deaths.

Discussion:

There were no significant treatment-related findings in toxicity, mortality, body weights, food consumption, hematology and gross pathology. Kidney and adrenal weights were effected by 2,4-D treatment. At 52 weeks there was an increase in high dose females in combined absolute kidney and kidney/brain weight ratios with a significant trend seen in absolute weights. All male adrenal weight parameters were elevated at all doses at 52 weeks with significant trends seen in all parameters. At 104 weeks, female and male kidney weights were elevated at the high dose with significant trends in female absolute kidney and organ/brain weight ratios and organ/body weight in males. Female organ/body weight ratios were elevated at the mid dose also. All adrenal organ weight parameters were elevated for males in both the mid and high dose groups at 104 weeks with significant trends seen in all parameters. Histopathology revealed a treatment-related histomorphologic alteration of the renal tubular epithelium of males seen mainly in the mid and high dose groups. The unscheduled deaths showed a marginal increase in tubular epithelium alteration at the low dose. No other treatment-related effects were evident and no increase in treatment-related tumors was seen. The adrenal weight data indicated significant increases in weight at all doses at the 52 week sacrifice. However, at terminal sacrifice the low dose animals appeared similar to controls. No histopathologic signs were evident either at 52 weeks or at terminal sacrifice, so the phenomenon may have been stress induced. Since the low dose showed no effects at terminal sacrifice, the 1 mg/kg was considered the NOEL.

NOEL = 1 mg/kg

LEL = 15 mg/kg based on treatment-related kidney and adrenal effects

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2,4-D Peer Review

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APPENDIX F

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APPENDIX F

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Key Points from the Summary of Peer Review Comments

NCI Kansas 2,4-D Study by Hoar et al.

Prepared by Jerome Blondell, Health Statistician

- ° The NCI study by Hoar et al. was well-designed, competently conducted and carefully analyzed.
- ° Half of the respondents were next-of-kin who are unlikely to remember accurately which herbicides were used over 10 to 20 years ago.
- ° Inconsistencies between subjects' reported use of herbicides and reported use in USDA and EPA surveys in the Kansas area suggests a very serious potential for inaccurate reporting of exposure.
- ° Statistically significant findings, particularly those implicating 2,4-D, were based on a very small number of cases, usually ten or less. Moderate amounts of exposure misclassification, described above, might easily make these findings nonsignificant.
- 2 ° The study found a significant associations between non-Hodgkins lymphoma (NHL) and fungicides. The study also found that five groups of herbicides exhibited higher odds ratios for NHL than did phenoxyacetic acids. These two findings cast serious doubt on the specificity of 2,4-D/NHL association.
- 3 ° Occupation was not controlled for in the analysis. Those who live on farms have lifestyles, diets, physical activity and exposure to viruses that greatly differ from the general population. These factors are known to affect many kinds of cancer and, as a result, are a potential source of confounding.
- 1 ° Reviewers had conflicting views on the strength of support from other studies. The Swedish studies by Hardell which seem to provide the strongest case for a chlorophenoxy herbicide/NHL association were characterized as having "important methodologic limitations" even by the most favorable reviewer.
- ° One of the external reviewers felt that serious regulatory action to limit exposure to 2,4-D should be considered. Two other reviewers did not feel that the weight of evidence implicated 2,4-D as a cause of NHL.
- ° The EPA Guidelines for Carcinogen Risk Assessment do not distinguish well enough between the categories "limited evidence of carcinogenicity" and "inadequate evidence" to say with certainty which category the NCI study belongs to.

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It is important to note that the frequency and duration of 2,4-D use was not specifically addressed by the Hoar, et al. study questionnaire. The questionnaire asks "About how many days per year were you usually exposed to herbicides . . .?" Table 3 in the Hoar, et al. article seems to suggest that the results were specific for 2,4-D use, although a careful reading of the text indicates that this table refers to total herbicide use and not just 2,4-D. NCI has submitted a request for a correction statement to JAMA regarding the title of Table 3 to show that while only users of 2,4-D are included, the numbers given for duration and frequency refer to all herbicide use.

Two reviewers commented on the apparent underreporting of herbicide use by both cases and controls. Dr. Morgan's criticism was the strongest. While quoting from the Hoar, et al. study, he stated: "If 'these four crops constituted 94% of Kansas farm acreage and 87% of acres treated with herbicides' [in 1978] how does it happen that three-fourths [71%] of Kansas farmers interviewed hadn't used any herbicides at all on their farms? I submit that the exposure information acquired by telephone interview was worthless." Dr. MacMahon raised a similar point in our phone conversation after he had already submitted his review.

To examine this possibility further, I have examined six USDA reports on pesticide usage (covering the years 1952, 1958, 1964, 1966, 1971, and 1976) and one EPA survey in 1974. In 1976, USDA reported the usage of herbicides in the Northern Plains area, consisting of North Dakota, South Dakota, Nebraska, and Kansas. The percent of planted acreage treated with herbicides was 41% for wheat, 66% for sorghum and 84% for corn. USDA reported that 39% of all Kansas corn acreage and 5% of the acreage in small grains was treated with herbicides in 1958. In Kansas the reported estimates of herbicide use in 1974 on the four crops studied was 6.6 million pounds for the triazines, 2.4 million pounds for phenoxyacetic acids and 16,000 pounds for the uracils. But the percentage of cases and controls who had ever lived or worked on a farm reporting use of these chemicals was in reverse order; just 1% for the triazines, 13% for phenoxyacetic acids and 17% for the uracils. It is possible that other family members or hired commercial applicators may have applied these herbicides over the 30-40 year period. But the inconsistencies between the subjects' reported use in the Hoar, et al. study and the reported use in USDA and EPA surveys suggests a very serious potential for inaccurate reporting.

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IV. Analysis

Although significant trends and associations were found between herbicide exposure (based on duration or frequency of use) and NHL, two of the reviewers noted that these trends and associations were based on very small numbers. Only 2 of the 9 odds ratios were significant for NHL and either duration or frequency of herbicide use among those who reported using 2,4-D. Regarding farmers who used herbicides, MacMahon commented that "in the most persuasive category (use of 21 or more days per year), where there are 7 cases, the expected number based on the controls would be about 2.3. It would take only 2 or 3 cases misclassified to this category (or controls misclassified out of it) to render the difference not statistically (or biologically) significant." The significant odds ratio of 7.6 for frequent use of herbicides among those who used 2,4-D was based on only 5 cases. With such small numbers, only moderate amounts of misclassification of exposure would be needed to produce this result. And, as described in the previous section, such misclassification might easily have occurred.

In addition to frequency and duration, the analyses for latency, protective clothing, and method of application supported an association between NHL and herbicides. Unfortunately, this association was not specific only to herbicides or only to phenoxyacetic acids. Fungicides were also implicated as a risk factor for NHL; even after adjustment for herbicide use. And phenoxyacetic acids were not the only herbicides implicated. MacMahon noted "Among 8 groups of herbicides . . . [the odds ratio] associated with phenoxyacetic acid is lower than that for any other group except the uracils."

The NCI study chose controls that were matched on age, sex, and vital status, but not occupation. The odds ratio for simply living or working on a farm was 1.4 (nonsignificant), and a number of other studies have found that farming is a risk factor for NHL. Farmers have lifestyles that differ considerably from the general population. Their diet, smoking and drinking habits, physical activity, and hygiene are all examples of factors that may confound results when comparisons are made with the general population. In particular, farmers may be more exposed to viruses (a possible cause of lymphoma) as a result of being outdoors and exposed to livestock. Controlling for farming as an occupation would have helped to alleviate this potential source of confounding.

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V. Evidence From Other Studies

Contradictory conclusions were reached by reviewers concerning support of the NCI findings by other studies, particularly the study by Hardell, et al. (see reference, page 1). Dr. Linet concluded "Although the Hardell, et al. study findings are greatly weakened by a number of substantial methodological problems, nevertheless the results support those of Hoar, et al." Dr. MacMahon reached an opposing conclusion: "I do not believe that the author's conclusion that the study confirms the reports from Sweden and several U.S. States that NHL is associated with farm pesticide use, especially phenoxy-acetic acids is justified." Dr. MacMahon explains part of his reasoning as follows:

"The important discrepancy is that the Swedish study found significant associations for all three tumors and the U.S. study only for one. Before concluding that the U.S. study is confirmatory of the Swedish one with respect to NHL, one must understand the reason for the discrepancy with respect to STS and HD. The reasons for these discrepancies--whether in the exposures studied, the method of study, or simply chance--are as cogent as is the agreement with respect to NHL. Until there is an adequate explanation for the discrepancies, one can have little confidence that the agreement represents reality." Dr. Morgan was also concerned about the reason for the discrepancy.

Dr. MacMahon goes on to conclude that "except when relative risks are high--and sometimes even then--no single study will establish an association between an exposure and an outcome. The acceptance of an association depends on a number of studies showing consistent results across populations and across different epidemiological methods. The study of Hoar, et al. is a strong study--strong enough on its own to establish a hypothesis of relationship of exposure to 2,4-D with some small proportion of cases of NHL."

Although Dr. Linet stated that the Hardell, et al. study was supportive, she felt that it was "plagued with a number of important methodologic limitations." Dr. Colton's editorial in JAMA supported this finding: "Selection bias, observation bias, and uncontrolled confounding loom as important points of vulnerability and, in fact, have emerged as the focus of sharp criticisms of the Hardell studies." Dr. Colton and Dr. Linet shared the concern raised by the Australia Royal Commission which reviewed Hardell's study and found that the intense media attention and Dr. Hardell's advocacy position in the media may have biased the results.

None of the other studies that were examined by the reviewers were felt to lend strong support to the findings of Hoar, et al.

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6 11 20

VI. Reviewers Conclusions

Listed below are the final conclusions of each reviewer relative to the 2,4-D/NHL association. Dr. Colton did not have a conclusion on this particular point.

Dr. MacMahon

"In my opinion the weight of evidence does not support the conclusion that there is an association between exposure to 2,4-D and NHL."

Dr. Linet

"In the opinion of this reviewer, the weight of the scientific evidence is beginning to lean towards possible causation between herbicide exposure, particularly 2,4-D, and development of non-Hodgkin's lymphoma in farmers . . . it would seem to be prudent to consider the possibility of temporarily substantially limiting or even banning the use of 2,4-D until ongoing studies have been completed."

Dr. Morgan

"I don't believe the "weight of evidence" indicates any excess risk of lymphoma among agricultural workers exposed to 2,4-D."

Dr. Burmeister

"It is my opinion that we can not say 2,4-D is a cause of lymphoma based on epidemiological studies. The "weight of evidence" should be limited to educating users about the cautions necessary to reduce to the likely risks of 2,4-D."

Reviewer's Conclusion

As both Drs. Linet and MacMahon point out, more than one study is needed to establish an association. These two reviewers disagreed on the level of support from other studies, particularly the Hardell study. Moreover serious questions have been raised about the accuracy of the exposure data, which when combined with the misclassification likely from next-of-kin interviews and the small numbers of 2,4-D users, could completely change the results. To be sure that herbicides and not some other lifestyle factor (e.g. viruses) were not responsible for the findings, the authors should have controlled for farming occupation in their analysis.

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In my opinion, the Hoar, et al. study does not provide sufficient evidence to support a credible causal interpretation concerning 2,4-D and NHL. Misclassification of exposure, chance and inadequate control of confounding factors might easily have created the apparent association. Other studies were not strong enough or consistent enough to support this finding of association. I cannot tell from the definitions given in the Guidelines for Carcinogen Risk Assessment whether this study should fall under category number two, "limited evidence of carcinogenicity," or category three, "inadequate evidence."



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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SEP - 5 1983

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Critical Review of NCI 2,4-D Study

FROM: Jerome Blondell
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Exposure Assessment Branch (TS-769C)

TO: Anne Barton, Deputy Director
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Attached is my review of the NCI 2,4-D study. I have also attached my earlier review of the protocol for this study which I sent to you on November 20, 1981, and my memo of concern relating to this study and others like it which I sent to John Melone June 2, 1983.

I have found that the present study does not provide sufficient evidence to conclude that 2,4-D is a likely cause of non-Hodgkin's lymphoma. However, the NCI study is, in many respects, a very strong study and I recommend that further studies of this kind be encouraged and supported. The evidence is currently inconclusive, but another well-done study might enable us to draw a much firmer conclusion.

Attachment

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Agricultural Pesticide Use and Risk of Lymphoma
and Soft-Tissue Sarcoma

A Critical Review

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Synopsis

A population based case-control study was performed in Kansas to determine if there was any relationship between agricultural pesticide use and soft-tissue sarcoma (STS), Hodgkin's disease (HD), or non-Hodgkin's lymphoma (NHL). Newly diagnosed cases of the three diseases occurring from 1976 to 1982 among white males, 21 years of age or older, were taken from a population-based registry covering the entire state of Kansas. A random sample of 200 of the 297 men diagnosed with NHL from 1979(?) through 1981 was drawn; while all of the 200 men with STS and 173 men with HD were taken. Pathology specimens were obtained for 87% of the cases and reviewed by a panel of three pathologists to confirm the diagnoses. Between 81 and 90 percent of the cases were confirmed for the three diseases. The total of histologically confirmed cases for each disease was: 139 cases of STS, 132 cases of HD, and 172 case of NHL. Controls were white men taken from the general population of Kansas and matched on age and vital status. Three controls were matched to each case. Some controls were used more than once when matched to more than one disease. Live controls were selected from Medicare files for those aged 65 years and older and by telephone using a random-digit dialing technique for those 21 to 64 years old. For deceased cases, controls were selected from Kansas State mortality files. Half of the STS and NHL cases and one-third of the HD cases had died before the study started. After this selection, process there were 443 cases and 1,005 controls.

Telephone interviews were administered to cases and controls and for deceased subjects, next-of-kin, from December, 1982 to January, 1984. Total interviews completed for each group were: 133 interviews for STS, 121 interviews for HD, 170 interviews for NHL and 948 interviews for controls. The overall response rate was 93 percent.

Ninety-five patients with STS (71%), 71 with HD (59%), and 133 with NHL (78%), reported living or working on a farm compared with 662 of the controls (70%). Odds ratios (ORs) and 95 percent confidence intervals were calculated for living or working on a farm for each disease as follows: STS-1.0 (95% CI, 0.7, 1.6), HD-0.3 (95% CI, 0.5, 1.2), and NHL-1.4 (95% CI, 0.9, 2.1).

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farm herbicide use on any of four specific crops (wheat, corn, sorghum, or pasture) was reported by 22 STS cases, 28 HD cases, 40 NHL cases and 192 controls. Calculations for odds ratios comparing cases to controls yield the following values: STS-0.9 (95% CI, 0.5, 1.6), HD-0.9 (95% CI, 0.5, 1.5), and NHL-1.6 (95% CI, 0.9, 2.6).

"There was a significant trend ($P=.02$) in risk of NHL with increasing years of herbicide use and with number of days of herbicide exposure per year ($P=.0004$) (Table 1). Persons exposed to herbicides more than 20 days per year [based on 7 cases] had an OR of 6.0 (95% CI, 1.9, 19.5)". NHL risk was found to increase with increasing time since first exposure. Higher risks of NHL were found for subjects who reported mixing or applying the herbicides themselves (OR, 1.9; 95% CI, 1.1, 3.3) and for those who did not use protective equipment or applied herbicides using backpack or hand sprayers.

Phenoxyacetic acid herbicide use was reported for 24 of the cases of NHL (out of 170 NHL cases). Three of these cases reported using 2,4,5-T. For those who reported using only 2,4-D (and not 2,4,5-T) the odds ratio for risk of NHL was 2.6 (95% CI, 1.4, 5.0). "There were significant, although inconsistent, increases in NHL risk in relation to the duration, frequency, and latency of 2,4-D use (Table 3)".

Risk of NHL was not associated with specific histologic types or the grade of the disease.

Insecticide use for more than six days per year was associated with a 2.3 fold increased risk in NHL (95% CI, 1.2, 4.5). But there was no NHL association with increasing years of insecticide use, mixing or applying insecticides, application method, or insecticide type. Thirty-two patients with NHL reported using fungicides which yielded a significant odds ratio of 2.1 (CI, 1.2, 3.7). Among the 32 patients the risk was elevated whether these farmers had used herbicides or not (although slightly more elevated with use of herbicides).

"Nonfarming pesticide use in home gardens and yards was not associated with NHL". Family history of cancer (especially lymphoma) was a significant risk factor for NHL. (OR, 2.3; 95% CI, 1.6, 3.2).

A sample of 130 subjects with farming experience had their pesticide suppliers contacted to help provide verification of insecticide and herbicide use. Suppliers generally reported less pesticide use than subjects. Level of agreement between suppliers and subjects is not reported. However, there were no consistent differences between cases and controls.

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Comment

Case-control studies of this kind are very difficult to conduct. This National Cancer Institute study has many good points to commend it. The cases were all drawn from a population based registry and therefore the results can be generalized to that population. All cancer cases were histologically confirmed by three independent experts. And a high response rate was obtained for the telephone interviews. Results were well analyzed, taking into account a variety of factors that might have confounded the analysis. The results were clearly presented.

The three major problems that any study of farmers, including this one, must confront are lack of appropriate controls, multiple exposures to chemicals, and poor data on actual exposure. This study chose controls that were matched on age, sex, and vital status, but not occupation. Farmers have lifestyles that differ considerably from the general population. Their diet, drinking and smoking habits, physical activity, hygiene, etc. all differ and may conspire to confound results when comparisons are made with controls from the general population. In this study, the odds ratio for simply living or working on a farm and NHL was 1.4 (non-significant) while it was 1.0 or less for the other two diseases. Therefore, lifestyle factors other than herbicide use may have confounded the results somewhat (though probably only slightly).

Multiple exposures to chemicals were observed among the farmers in this study and initial analyses did in fact find significant associations between NHL and triazines, amides, insecticides (used more than 6 days per year), and fungicides. However, further analysis, adjusting for herbicide or chlorophenoxy use, reduced the risk for these other chemicals. The other chemicals did not show the consistent pattern in terms of frequency of use, duration of use, years since first use, and application method. However, in many cases this may be due to the very small numbers involved. With only 14 cases exposed to triazines, 8 to amides, and 32 to fungicides, the statistical power to observe any association is going to be low. The authors should have pointed this out.

The authors claim that independent assessments of exposure (by self or next of kin and by pesticide supplier) yield similar estimates of risk. This claim is difficult to assess since the level of agreement between suppliers and cases is not reported. It should have been. There is little data supplied to show that

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reported use (particularly for the 24 users of 2,4-D) is a good surrogate for exposure. Memory by cases or next of kin will favor chemicals used most recently, most frequently and with the easiest names to remember. It is not reported how often suppliers consulted written records of sales or relied on memory to come up with their data.

Assessment of Causality

There are six or seven criteria commonly used when assessing epidemiologic data as evidence that a particular exposure causes a disease. These criteria were used, for example, by the Surgeon General when he determined that cigarette smoking causes lung cancer. My assessment for each of these criteria in regard to 2,4-D and NHL follows.

1. Dose-Response Relationship

For herbicide and 2,4-D use, the two surrogate measures of dose, frequency of reported use and duration of use, both demonstrated a statistically significant trend with NHL. These particular pieces of evidence get around the problem of lack of appropriate controls by comparing within the farming group. This is strong support for causality. One should note, however, that most of the individual categories for these two surrogate measures did not yield significant odds ratios.

2. Specificity of the Association

Given the multiple exposures to chemicals and the lack of statistical power in many instances, I disagree with NCI's statement that "The relationship between NHL and herbicide use was specific". There was also a relationship with fungicides and only two other cancer sites were studied. So I would characterize the association as only somewhat specific.

3. Consistency of the Association

Most of the analyses do support the association between herbicide use, 2,4-D use and NHL. NCI did a variety of appropriate analyses including duration, frequency, latency, protective clothing, and method of application, all of which supported the association with herbicide use. Duration, frequency and latency analyses performed for 2,4-D use also consistently found an association with NHL. As described above, I cannot agree that the independent assessments of pesticide exposure by self or next of kin and pesticide suppliers is evidence for consistency without seeing more data.

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4. Biological Plausibility

There is very little evidence for the biological plausibility that 2,4-D causes NHL. The only positive cancer study in rats was for a neurological cancer unlikely to be related to lymphoma. Also there are apparently no studies supporting an immunosuppressive effect for 2,4-D. The only argument, put forward by Hui, is that the older forms of 2,4-D were more likely to have been contaminated with impurities such as toxins which have been found to be both immunosuppressive and carcinogenic.

5. Strength of the Association

The odds ratio provides an estimate of the relative risk of getting a disease. Relative risk is the ratio of risk of disease among the exposed to the risk among the unexposed. In the current study the odds ratio for herbicide use in excess of 10 days per year was 6.0 (95% CI, 1.9, 17.5). The similar value for 2,4-D use (based on 6 cases), was 7.6 (95% CI, 1.6, 32.3). Note the relatively wide confidence intervals. In general, odds ratios of 6.0 to 7.6 could be characterized as estimating moderate or moderate-to-strong strength of association. For comparison cigarette smoking and lung cancer yield a ten-fold relative risk, definitely a strong association.

6. Temporal Sequence

This criteria simply addresses the timing of the disease in relation to the timing of the exposure. In the herbicide use and 2,4-D use had their strongest association with NHL for times well over 10 years before 1980. Given the lack of information on the latency period for NHL, this criteria is not evaluated as either supporting or denying causality.

7. Replication

Perhaps one of the most important pieces of evidence in epidemiology is replication of a study's results at different times and places with varying populations. I have not had the time to evaluate all the other studies that relate to the 2,4-D/lymphoma association, but will comment on a couple of studies I have in my possession.

Generally, a study of manufacturing workers is the most likely to reveal a relationship between pesticide exposure and cancer. Exposure levels are much higher and the frequency of exposure is

far in excess of what occurs on a farm. Recall that the high exposure group in the present study was only an excess of 20 days use per year. Also the problems of multiple exposures and finding appropriate controls is much reduced in studies of factory workers. Although you often have the problem that these workers are exposed to intermediate products and other related pesticides so that the specific exposure which caused the excess cancer cannot always be identified. The one sizable study published last year of Danish factory workers exposed to phenoxy herbicides including 2,4-D did not find a significant relationship between 2,4-D and NHL. (7 cases observed versus 5.4 expected).

One of the studies cited by NCI as demonstrating a link between agricultural workers who frequently use herbicides and NHL was published in 1966. However, a follow-up case-control study published this year interviewed 10 cases with NHL and did not find a significant association with phenoxy herbicide exposure.

To summarize, while there has been some replication of the findings of the NCI study, there are at least two studies which should have but did not replicate the finding of an association between 2,4-D and NHL.

Conclusion

From the standpoint of biological plausibility, and temporal sequence there is little evidence to support any true negative animal studies which would deny a causal relationship between 2,4-D and NHL although there is still some question about contaminants in 2,4-D. From the standpoint of replication, specificity, and strength of the association, the evidence is mixed. There is some evidence of a causal relationship but there is also some evidence that the relationship may be spurious. The strongest evidence for the causation were the consistent findings of the NCI analyses which implicated 2,4-D and the evidence of a dose-response relationship with the surrogate measures of dose: duration and frequency of use.

Therefore, I find that the evidence is insufficient at the present time to conclude that 2,4-D is a likely cause of NHL. Careful review of all related studies should be conducted by the Cancer Assessment Group and replication of the NCI study should be encouraged and supported.

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Review of "Agricultural Herbicide Use and Risk of Lymphoma and Soft
Tissue Sarcoma" (Hoar FK, Blair A, Holmes FF, et al) and Other
Epidemiologic Studies Examining the Association Between the
Herbicide 2,4-D, Herbicides in General, Farming
and Malignant Lymphoma

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October 17, 1986

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Review of the study entitled, "Agricultural Herbicide Use and Risk of Lymphoma and Soft Tissue Sarcoma"

This recently published case-control study, examining the relationship between agricultural herbicide use and non-Hodgkin's lymphoma, Hodgkin's disease, and soft tissue sarcoma, is an extremely important contribution to an expanding literature. The finding of an excess risk (OR=1.6) of non-Hodgkin's lymphoma associated with farm herbicide use, together with a very elevated odds ratio of 6.0 for substantial (greater than 20 days per year) herbicide exposure, and an even higher risk (OR=8.0) for frequent users who mixed or applied the herbicide themselves, a clear dose response relationship with both frequency of herbicide use, and to a smaller extent, duration of herbicide use, are of great concern. These findings, when taken in conjunction with earlier and somewhat more limited studies (due to use of study designs employing an ecologic approach or case-control designs in studies with a number of serious methodologic limitations) suggest to this reviewer that the weight of the scientific evidence is beginning to lean towards possible causation between herbicide exposure, particularly 2,4-D, and development of non-Hodgkin's lymphoma in farmers.

The findings are of special concern because of the overall excellent design and careful execution of the Hoar et al study. The strengths of the study are summarized below. First, the study was population based, and conducted in a state (Kansas) with documented high levels of use of herbicides, particularly 2, 4-D, as well as 2, 4, 5-T. In addition, the state of Kansas appears to have an excellent cancer registry with high levels of reporting. (Further documentation of this, with examples of other cancers, in addition to soft tissue sarcoma, would have been helpful.)

A second strength of the study is the use of histologically confirmed cases only, with all eligible cases having been reviewed by a panel of pathologists.

Third, careful attention was given to study design and statistical power considerations, with three controls, matched on age and vital status, selected for each case. In addition for deceased cases, controls were selected from mortality files in order to adjust for the use of next of kin responses and the attendant potential for recall bias.

Fourth, of special note were the very high response rates of both cases and, particularly, the random-digit-dial controls.

Fifth, the study was sharply focused with questions restricted to farming practices related to the four major crops which constituted the majority of farm acreage treated with herbicides.

Sixth, a very important component of the study was the effort given to exposure validation and the evaluation of potential observation bias through use of methods to corroborate self-reported pesticide exposures for a large sample of subjects with farming experience. The corroborative evidence consisted of validation of herbicide and insecticide purchases by subjects from suppliers.

Seventh, careful attention was given in the analysis to an examination of dose response relationships, although the precise rationale for defining exposure, with high exposure being greater than 20 days, and moderate exposure categories further subdivided into use of herbicides 1-5 days per years, 6-10 days per year, and 11-20 days per year, was not discussed. Additional consideration was given to total duration of herbicide use, the latency period from first use, the association with years of herbicide use, following adjustment for annual days of exposure, and the association of days of use.

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after adjustment for total years of exposure. Although the odds ratio for the association between non-Hodgkin's lymphoma and overall "ever-use" of farm herbicides was only 1.6, very high odds ratios were observed for individuals exposed more than 20 days per year (OR=6.0; 95% C.I.=1.9, 19.5); for frequent users who mixed or applied the herbicides themselves (OR=8.0; 95% C.I.=2.3, 27.9), and a clear dose-response relationship with odds ratios increasing with frequency of use. Those who applied herbicides infrequently had an estimated elevated risk or odds ratio of 1.3, which increased to a marked excess for those using herbicides more than 20 days per year having an odds ratio of 6.0. For duration of herbicide use, the odds ratio was 1.3 for 1-5 years of use, increasing to 2.0 for those using herbicides more than 16 years. In addition, farmers who used backpack or hand sprayers, with consequent higher levels of exposure, were also found to have higher risks (OR=2.3). It is of interest to note that the dose response trends were particularly marked for those who mixed or applied the herbicides themselves.

The number of cases in this study of non-Hodgkin's lymphoma was 170, compared to 1005 controls. Earlier studies of Hardell et al and Pearce et al, which are reviewed and compared with the Hoar et al study below, relied upon much smaller numbers of cases, 109 with non-Hodgkin's lymphoma in the Hardell et al study and 83 in the Pearce et al study.

~~Comparison of the Methodologic Features of the Hoar et al Study with those of the Hardell et al Study and the Pearce et al Study~~

~~All three studies were population-based although private hospital patients were excluded from the Pearce et al study. The impact of the exclusion of private hospital patients is not clear in that no data is provided about the~~

relative numbers or characteristics of cases reported to the Cancer Registry who were private hospital patients.

The Hoar et al study and the Hardell et al study both employed independent pathology review and confirmation of disease, while the Pearce et al study did not appear to make a special effort to do this.

The Hoar et al study is the largest of the three studies, as noted above, and thus, it had greater statistical power to detect differences.

In all three studies data was collected individually from cases and controls, or next of kin. For the Hoar et al study and Hardell et al studies there was reliance on next of kin reporting of exposure for cases and controls who were deceased. All data was collected by telephone interview in the Hoar et al study. For the Hardell et al study most of the data were collected through a mailed questionnaire, with a telephone follow-up interview of those subjects for whom the questionnaire information was either incomplete, contradictory or confusing. In the Pearce et al study, all interviews were conducted by telephone. All cases and controls interviewed in the Pearce et al investigation were alive. In the Hoar et al study, experienced and trained interviewers were used who employed a standardized predetermined questionnaire. For the Hardell study there was a single interviewer who was presumably unaware of the major hypothesis, but this is not completely clarified in the report. As noted previously, the majority of information for the Hardell et al study was obtained ~~from mailed questionnaires with the interviewer supplementing data collected from the mailed questionnaire for questions that were either incompletely answered or for which there were contradictory answers etc.~~ The interviewer in the Hardell et al investigation did not appear to use a standardized predetermined questionnaire, but apparently conducted a more

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probing type of interview to supplement answers given to the mailed questionnaire. In the Pearce et al study a single interviewer used a somewhat standardized questionnaire in which a stem question enquired about whether or not study subjects had worked in particular occupations in which there was potential for exposure to phenoxyherbicides or chlorophenols. If the response to the stem question was "yes", then a series of subsidiary questions was asked to clarify the work done and the actual potential for exposure. Thus, for the Pearce et al study, the interviews were not completely standardized, but were somewhat individualized, depending upon initial responses of subjects to stem questions.

For the Hoar et al study, interviewing occurred at a time when there was no particular local media attention to herbicide exposure and cancer risk among farmers, while with the Hardell et al study, interviewing occurred soon after extensive media attention in Sweden given to herbicide exposure, particularly 2,4-D, 2, 4, 5-T, Agent Orange and the carcinogenic potential of these chemicals. There appeared to be no particular media attention in New Zealand given to herbicide use and potential carcinogenicity at the time of the Pearce et al study.

The Hoar et al study was the only one of the three in which there was an effort to validate self reported (or next of kin reported) exposure through use of information obtained from a survey of suppliers. An important finding was that there appeared to be no differences found in the level of corroboration among controls and the three groups of cases, although, as the authors do point out, only a sample of subjects had validation of their exposure status. For both the Hardell et al study and Pearce et al study there was no attempt to validate self reported exposure.

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The Hoar et al study took into account potential confounding factors including exposure to other herbicides, insecticides, fungicides, and non-farming exposures which are known and suspected risk factors for each of the three malignancies.

The Hardell et al study did assess information about exposure to organic solvents (other than phenoxyacids and chlorophenols) including benzene, trichloroethylene, perchloroethylene, styrene, and other types of organic solvents but did not apparently obtain more detailed information about other known and suspected risk factors for non-Hodgkin's lymphoma.

The Pearce et al study obtained extensive information about employment in a variety of occupations and a variety of potential exposures in those occupations, but did not focus on other known and suspected risk factors for non-Hodgkin's lymphoma.

As noted above the Hoar et al study found an overall odds ratio for "ever-exposure" to herbicide among farmers of 1.3, whereas the Hardell et al study found an odds ratio for "ever-exposure" to phenoxyacids or chlorophenols of 6.0. For the Hardell et al study, an analysis restricted to those exposed to phenoxyacids, after exclusion of cases and controls exposed to chlorophenols demonstrated an odds ratio of 4.8; when exposure to chlorophenols was restricted to individuals, excluding cases and controls exposed to phenoxyacids, the odds ratio was found to be 4.3.

In the Hoar et al study there were consistent dose response relationships for both frequency of exposure and, to a lesser extent, for duration of exposure and with increasing time since first exposure. Farmers who began using herbicides before 1946 had odds ratios for "ever-exposure" of 3.3; for initial exposure occurring from 1946-55, odds ratios were 1.7; for those who

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initially started using herbicides between 1956-65, OR=1.7; for those who initially started using herbicides after 1965, OR=1.3.

No dose response relationships was found in the Hardell et al study. Dose response relationships were not examined by Pearce et al, since there was no statistically significant association found overall for "ever-exposure" to herbicides.

The Hoar et al study found that the association between exposure to herbicides and non-Hodgkin's lymphoma was specific to that cancer; these investigators found no statistically significant associations between exposure to herbicides and Hodgkin's disease or soft tissue sarcoma. In the Hardell et al studies there was an across-the-board elevated risk for all three cancers with exposure to phenoxyacids and chlorophenols. In addition, in the Hoar et al study there was a specificity of association between two herbicides, namely 2,4-D and triazene; no excess risk was found with use of uracil or other herbicides. Slightly elevated risks were found with use of insecticides, but no dose response relationship. Elevated risks as noted above were found for association with use of phenoxyacids, and chlorophenols, as well as a variety of other organic solvents in the Hardell et al studies.

In the Pearce et al study no significant association was found with exposure to phenoxyherbicides or chlorophenols although the odds ratio was elevated for performance of fencing work, employment in meat works and a statistically significant interaction was found for employment in both fencing and in meat works.

It is of interest to note in the Hoar et al study the substantially increased risk for farmers first using 2,4-D before 1946, with decreasing odds ratios for subsequent initial first years of use. The authors point out that

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this may indicate the presence of carcinogenic impurities in the early formulations of this substance with subsequent improvements in the manufacturing process, and thus decreasing risk of non-Hodgkin's lymphoma. On the other hand, this may represent a cohort effect with the true association due to other, unidentified exposures, since it is well known that non-Hodgkin's lymphoma continues to increase with age. Although adjustment was made for age in examination of this association, other cohort effects and/or other exposures experienced by farmers may be playing a role.

Among the weaknesses of the study by Hoar et al, it should be noted that half of the patients with non-Hodgkin's lymphoma and soft tissue sarcoma and one third of the patients with Hodgkin's disease had died before initiation of the study. Information about dead cases was supplied by next of kin, as was information about matched dead controls. The data for all analyses throughout the paper, however, is only provided for all subjects combined, both living and dead. It would be useful to see separate tabulations for the living cases and their matched controls, as compared with those of all cases combined and all controls combined, to see if the trends were similar for the living subjects and the combined case group of live and dead subjects. This would appear to be particularly important, since such a substantial proportion of cases had died; information about pesticide exposure may be somewhat less reliable for subjects who have died.

In addition, the information from the pesticides suppliers' survey is ~~presented in the aggregate for all 110 subjects.~~ It would be interesting to ~~know whether the corroboration of information about pesticide exposure between~~ live cases and the pesticide suppliers is similar to that for all cases combined; there was no mention about this in the manuscript.

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Similar criticisms can be applied to the study reported by Hardell et al in that information is not separately reported for living cases and matched controls separately from that of all cases. This criticism of course does not apply to the Pearce et al paper since only living cases and controls were included.

A second criticism of the Hoar et al study has to do with the sample size, although this is clearly the largest study examining non-Hodgkin's lymphoma and its association with herbicide exposures reported to date. These authors are well aware that non-Hodgkin's lymphoma consists of a heterogeneous group of diseases which may have different etiologies. Thus, ideally a much larger sample size would be a useful in order to determine whether the associations are substantially more marked with one subtype of lymphoma compared with others.

Previous literature suggests, in some reports, that the association of non-Hodgkin's lymphoma in farmers with herbicide use, found in earlier ecologic studies, was with histiocytic lymphoma, whereas other studies have indicated that some of the variants of lymphocytic lymphomas are, in fact, more highly associated.

The authors of the Hoar et al paper indicate that there were no differences in the odds ratios associated with herbicide use between different histologic types of non-Hodgkin's lymphoma. However, given the overall fairly small number (N=172) of non-Hodgkin's lymphoma cases, after categorization by cell type, the numbers in each of the cells would be fairly small. One method for increasing sample size would be to identify newly diagnosed cases going further back in time, but this would increase the proportion of dead cases, with the corresponding necessity of relying upon next of kin information. Another

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possibility would be to expand the geographic area included in the study to include perhaps other states where 2, 4-D use might be expected to be somewhat comparable to Kansas. However, it is recognized that, from the logistical point of view, those states may not have cancer registries that are as complete as that in Kansas, nor may it have been possible to develop cooperative arrangements to collaborate with investigators from those states. Thus, although larger sample sizes, especially when a number of histological types are involved, would be ideal, practically speaking, this may not have been possible.

The authors are well aware of the many other potential problems that can occur in epidemiologic studies that may affect the accuracy of the assessment of pesticide exposure. These are clearly detailed on page 1146 of the manuscript and include problems related to recall bias, the use of next of kin, vagueness of questions concerning exposure and the potential for additional pesticide exposures that the subject may not have recalled that may have been more residential, as opposed to occupational, as well as other potential confounding factors. However, as the authors note, and this reviewer agrees, the errors introduced by these factors are quite likely to be similar for cases and controls, particularly where there has not been extensive media attention drawn to the potential carcinogenicity of specific exposures being studied. In addition, as the authors note, the possible misclassification of exposure that might result from these circumstances might very well tend to dilute risk rather than result in spurious associations. The fact that no significant associations were found between herbicide use and soft tissue sarcoma or Hodgkin's disease suggest that, in fact, there is little likelihood of great inaccuracies in the assessment of exposure.

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Although the Hardell et al study does demonstrate a strong association, as noted above this study is plagued with a number of important methodologic limitations that limit the inferences that can be drawn. Among the most important limitations are the similar high odds ratios found with exposure to phenoxyherbicides and chlorophenols for three cancers in studies which were conducted during and shortly subsequent to a tremendous amount of media coverage. The data collection technique, namely mailed questionnaires, supplemented by non-standardized interviewer-obtained information, had shortcomings. It is not possible to determine from the Hardell et al reports to what extent there may have been variation in the effort used by the interviewer to obtain specific information between cases and controls. In addition, the definition of exposure used in the Hardell et al study was "exposure for at least one day to phenoxyacids and/or chlorophenols". In the Hoar et al study exposure was defined using a hierarchy of different durations, with heavy exposure being defined as exposure to herbicides of greater than 20 days per year. In the Hoar et al study, the frequency of use was also examined in the context of duration of use. Frequency was then examined separately after adjusting for duration of use, and duration was examined separately after adjusting for frequency. In the Hoar et al study, the dose response relationship continues to hold up no matter whether it is frequency of use that is the primary outcome being examined, or duration of use. In the Hardell et al study in contrast, the failure to examine dose response relationships and ~~the very limited definition of exposure that was used, relating to substances used by persons in a variety of occupations (that may have occurred more than 15-30 years ago)~~, suggests that both recall bias and interviewer bias may have played a major role in determining results. On the other hand, even given the

limitations in the Hardell et al study it is of interest that the Hardell et al study supports the findings of Hoar et al with respect to non-Hodgkin's lymphoma.

The greater potential for several types of bias just described and additional types of assessment bias in the Hardell study have been outlined in detail above and present much more methodologically important problems than appear to be present in the Hoar et al study.

The problems would probably be similar for the Pearce et al study as with the Hoar et al study, with the addition of the problem produced by the absence of the additional probing questions about exposure, through the restriction of questions to single stem questions if the subject initially replied in the negative. One also wonders if, given the possible tendency for subjects to have increasing awareness throughout the administration of the questionnaire, that if subjects answer stem questions negatively the overall length of the questionnaire and interview will tend to be shortened. If so, this may have unduly influenced the subjects to tend to falsely respond negatively to questions asked about exposure. The authors do not address the potential for this to occur in their discussion. If this were indeed a problem, it would tend to cause the findings of a study to be negative, even if a true positive association existed.

The absence of the association found by Pearce et al may be explained by ~~the fact that this study had the smallest number of cases of the three studies~~ and/or that different formulations of herbicides (compared to those studied by Hoar et al and Hardell et al) may have been involved, although this is somewhat unlikely. The use of the stem question approach with incomplete probing for

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specific details may also have played some role in the finding of no associations.

Additional Scientific Evidence Examining the Relationship Between Non-Hodgkin's Lymphoma and Herbicide Exposure

The rest of the literature which was supplied (studies by Cantor 1982; Burmeister et al, 1983; Schumacher 1985; Woods et al, 1986; Cantor et al, 1985) showing repeated associations between non-Hodgkin's lymphoma and employment in farming are inadequate to directly assess the evidence linking herbicide use and non-Hodgkin's lymphoma. It appears fairly definite that farmers are at increased risk of developing non-Hodgkin's lymphoma, but most of the studies included which demonstrate this relied on death certificates and/or did not obtain information about individual use of herbicides by cases and controls. For those studies which did attempt to examine overall herbicide use among farmers, a general ecological approach was used, relying upon analysis of non-Hodgkin's lymphoma among farmers in counties with high herbicide use versus low herbicide use. However, it is possible that the individual farmers who developed the non-Hodgkin's lymphoma in those studies, in fact, were not exposed to herbicides at all. Thus, while this evidence strongly suggests that farmers are at increased risk of developing non-Hodgkin's lymphoma, the reasons for this are not clarified, in my opinion, by any of these studies.

The follow-up studies of cancer incidence among workers employed in the manufacture of phenoxyherbicides (Lyng 1985) and among Swedish railroad workers (Axelson et al, 1980), are limited primarily, as noted by most of the authors by the small sizes of the cohorts and the relative rarity of the tumors being studied. None of the follow-up studies have shown an excess risk,

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although it is unlikely that any of these studies had adequate statistical power to detect such a risk even if it does exist. As Lynge points out, in order to test the hypothesis linking herbicide exposure and non-Hodgkin's lymphoma, it is necessary to have large enough cohorts with complete registration of all exposed persons. In addition to having a large enough cohort (in order to have adequate statistical power to detect differences in cancer outcomes between exposed and unexposed), it is necessary to also have very complete (and therefore probably very expensive) methods for tracing. In addition, it would be almost essential to have a very complete population-based cancer registry in order to link data about cancer outcomes with employment in specific occupations. As Lynge points out, even in Denmark, a country with excellent registration of cancer, and complete lists of workers in various occupations (supplemented by information about employed and retired workers from detailed pension records), it is very difficult to carry out an ideal study.

Summary and Evaluation of the Weight of the Evidence

In balance the Hoar et al study is methodologically strongest of the three case-control studies reviewed. Therefore the suggested association between use of herbicides, particularly 2,4-D and development of non-Hodgkin's lymphoma is of particular concern.

Although the Hardell et al study findings are greatly weakened by a number of substantial methodologic problems, nevertheless the results support those of Hoar et al. Pearce et al may have failed to detect a relationship due to small sample size and use of stem questions, which, if answered negatively, may have

resulted in less probing about pesticide exposures than occurred in the other two case-control studies.

Follow-up studies of cohorts occupationally exposed to herbicides (employed in herbicide manufacture and involved occupationally in spraying) are primarily limited, when rare neoplasm outcomes are being examined, because of the small population size and relatively small number of person-years of follow-up. Thus the negative findings must be interpreted with caution.

Clearly additional studies are badly needed to provide additional information about the relationship between herbicides (particularly 2,4-D) and non-Hodgkin's lymphoma. However, until additional studies underway and still to be executed are completed, the findings, particularly those of Hoar et al, and to a lesser extent those of Hardell et al, provide enough concern that risk assessments should be immediately undertaken to determine the potential number of cases of non-Hodgkin's lymphoma that would be expected to occur annually in the United States, if the estimated relative risks and attributable risks calculated by Hoar et al are correct.

In the opinion of this reviewer, the weight of the scientific evidence is beginning to lean towards possible causation between herbicide exposure, particularly 2,4-D, and development of non-Hodgkin's lymphoma in farmers.

This reviewer has not calculated the expected number of cases (using appropriate risk assessment techniques) or to weigh cost-benefit considerations. However, from the public health point of view, it would seem to be prudent to consider the possibility of temporarily substantially limiting or even banning the use of 2,4-D until ongoing studies have been completed.

The University of Iowa

Iowa City, Iowa 52242

College of Medicine
Department of Preventive Medicine
and Environmental Health



September 16, 1986

Jerome Blondell
Health Statistician
Exposure Assessment Branch
Hazard Evaluation Division (TS-769C)
United States Environmental
Protection Agency
Washington, DC 20460

Dear Dr. Blondell:

I have completed my review of the paper by Hoar, et al. entitled, "Agricultural herbicide use and risk of lymphoma and soft tissue sarcoma." The study appears to be of very high scientific validity and contributes important additional evidence to an already existing literature that indicates the carcinogenic risk of 2,4-D.

In spite of the scientific validity of this study, the fact that it is consistent with several previously published studies, and my own personal concern, it is my opinion that we can not say 2,4-D is a cause of lymphoma based on epidemiological studies. The "weight of evidence" should be limited to educating the users about the cautions necessary to reduce the likely risk of 2,4-D.

Thank you for the opportunity to review this paper. I hope I can be of similar assistance in the future.

If you have any questions concerning my reactions, please do not hesitate to call (319-353-6819).

Sincerely yours,

A handwritten signature in cursive script that reads "Leon F. Burmeister".

Leon F. Burmeister, Ph.D.
Professor of Biostatistics

LFB/ks

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September 15, 1986

Jerome Blondell
Health Statistician
Exposure Assessment Branch
Hazard Evaluation Division (TS-769 C)
U.S. Environmental Protection Agency
Washington, D.C. 20460

Re: Health Hazards of Herbicides

Dear Jerry:

This letter is in response to yours of August 27, 1986, requesting review and evaluation of epidemiologic literature bearing on the alleged long term health hazards of herbicides, particularly 2,4-D.

So that there will be no mistake about my final judgment, I will first answer your key questions: I don't believe the "weight of evidence" indicates any excess risk of lymphoma among agricultural workers exposed to 2,4-D. I don't believe 2,4-D is a likely cause of lymphoma, soft tissue sarcoma, or other cancer.

The first study to indict 2,4-D as a risk factor for cancer was published by Hardell in 1979. He thought he found a six-fold increase in risk of developing soft tissue sarcoma among workers exposed to chlorophenoxy and chlorophenolic compounds (Br. J. Cancer 39:711, 1979). Hardell wrote: "It is most unlikely that the results were influenced by uncontrolled confounding factors or other defects in the validity of the study." The most recently completed study by Hoar and six other authors (JAMA), did not confirm Hardell's original allegation. But possible reasons for this discrepancy receive scant attention from Hoar et al. Instead, they prefer to emphasize the degree of agreement between their work and Hardell's second allegation, namely, that exposure to chlorophenols, chlorophenoxy compounds, and solvents is a risk factor for malignant lymphoma (Hodgkins and non-Hodgkins types combined) (Br. J. Cancer 43:169, 1981). The Hoar article found no association with Hodgkins disease or soft tissue sarcoma, but claims to have found one with non-Hodgkins lymphoma.

Studies reported through the 1980's to date have yielded a melange of tenuous statistical associations. A follow-up study of Swedish railroad workers reported in 1980 tended to associate excess cancer deaths - particularly lung and stomach - with herbicide exposure, which included amitrol as well as phenoxy acids (Scand. J. Work Env. Hlth. 6:73, 1980).

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their controls), the exposure information was obtained from surrogates since the subjects themselves were dead. One would suspect that surrogate-supplied information on occupation would be reasonably accurate, but one must question surrogates' knowledge of what specific herbicides were used and on how many days of the year. Since any inaccuracy involved would presumably apply to both cases and controls this cannot be regarded as a possible explanation of the association noted. In fact, it would tend to reduce any true association that exists. One might even wonder, in fact, whether it could explain the lack of association found for STS and HD - tumors for which others have reported associations with phenoxyacetic acid exposures.

- although both years of herbicide use and days of use per year show statistically significant trends for NHL risk, it is useful to note the small numbers on which these trends rest. Only two individual categories show significant differences between NHL cases and controls - use for 16 years or more (based on 16 cases, RR 2.0 and of marginal statistical significance), and use for 21 or more days per year (based on 7 cases, RR 6.0 and more clearly significant). It is not stated to what extent these categories overlap - i.e. contain the same individuals - but it is noteworthy that, in the most persuasive category (use for 21 or more days per year), where there are 7 cases, the expected number based on the controls would be about 2.3. It would take only 2 or 3 cases misclassified to this category (or controls misclassified out of it) to render the difference not statistically (or biologically) significant.
- the authors' Table 1 shows that farmers for whom no use of herbicides were reported had RR higher than non-farmers (RR = 1.3) and while this

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is not formally significant (at p less than 0.05) it is approaching significance (the lower 95% confidence limit is 0.8) and is not much different from that for all farmers (1.4). The latter similarity results, of course, from the small size of the group of herbicide users that does show substantially increased risk.

- the paper's Table 2 shows risk ratios associated with ever-use of specific herbicides. Among 8 groups of herbicides (including a category "nonspecified"), the RR associated with phenoxyacetic acid is lower than that for any other group except the uracils. The RRs range from 1.3 to 12.5, that for the phenoxyacetic acids being 2.2. Besides the phenoxyacetic acids, RRs significantly above 1.0 are seen for triazines (2.5), amides (2.9), trifluralin (12.5) and non-specified" (5.8). The focus on the phenoxyacetic acids seems to stem from their frequency of use (second only to the uracils), rather than from the level of risk associated with their use.

In summary there are some questions and uncertainty in the data from this study - as there are in all epidemiologic studies - but, if there were no other evidence available, this study would stand as a good basis for the hypothesis that the risk of non-Hodgkin's lymphoma is increased by agricultural exposure to the phenoxyacetic acids - principally 2,4-D - and perhaps other herbicides. I would not accept this study as grounds for concluding that such associations do exist - only as a basis for hypotheses which must be tested in other data.

Other studies

Paradoxically, it is unfortunate that this study is not the only one to provide evidence on this topic. In fact this study was prompted by previous studies suggesting that STS, HD and NHL were all increased in persons exposed to phenoxyacetic acid and other herbicides. It is when one tries to fit the results of the Kansas study into the context of previous work that matters become difficult.

I do not believe that the authors' conclusion that "the study confirms the reports from Sweden and several US states that NHL is associated with farm pesticide use, especially phenoxyacetic acids" is justified. The Swedish studies of Hardell et al² showed elevated risks of 5- 6-fold for all three cancers investigated by Hoar et al. Exposure in the Swedish study was defined as "ever exposed" - principally on the basis of occupational history - and it is not possible to compare levels of exposure in the two studies to determine whether lower exposures could account for the lower RRs found in the US study (among all exposed). However, the important discrepancy is that the Swedish study found significant associations for all three tumors and the US study only for one. Before concluding that the US study is confirmatory of the Swedish one with respect to NHL one must understand the reason for the discrepancy with respect to STS and HD. The reasons for these discrepancies - whether in the exposures studied, the method of study, or simply chance - are as cogent as is the agreement with respect to NHL. Until there is an adequate explanation for the discrepancies one can have little confidence that the agreement represents reality.

² Hardell L, Eriksson M, Lenner P, et al. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. Br J Cancer 1981; 43:169-76.

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It is beyond the scope of this contract to review all the published relevant literature but perusal of the articles accompanying the review request does not lead to any clear impression of support for or evidence against the conclusion of Hoar et al. Pearce et al³ report a case-control study of 83 cases of NHL and conclude that there was no significant difference between cases and controls with regard to potential exposure to phenoxy herbicides. However, in this relatively small study, the results (RR 1.4, 90% CL 0.7-2.5) are not statistically incompatible with the RR (2.2) reported by Hoar et al for ever-use of phenoxyacetic acids.

Lynge⁴ reports a cohort study of persons exposed in the manufacture of various pesticides. The numbers are very small but are more suggestive of an association for STS (obs. 5, exp. 1.84) than for lymphoma (HN and NHL not distinguished) (obs. 7, exp. 5.37) among all employees, and there was no case of NHL among the 41 cancer deaths among persons employed in the manufacture and packing of phenoxy herbicides specifically. The total number of cancer deaths expected in this group was 41.46. Lung cancer showed a significant excess (obs. 12, exp. 6.11).

Other studies, because of small numbers, lack of specificity of exposure and/or other reasons, carry little evidential weight.

The key question

The key question in Mr. Blondell's letter quoted earlier is in fact two questions - what does the 'weight of evidence' say about the risk of lymphoma

³ Pearce NH, Smith AH, Howard JK, Sheppard, RA, Giles HJ, Teague CA. Non-Non-Hodgkin's lymphoma and exposure to phenoxyherbicides, chlorophenols, fencing work, and meat works employment: a case-control study. Br J Ind Med 1986; 43:75-83.

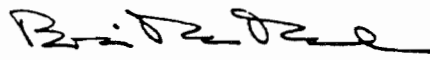
⁴ Lynge E. A follow-up study of cancer incidence among workers in manufacture of phenoxy herbicides in Denmark. Br J Cancer 1985; 52:259-70.

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for agricultural workers exposed to 2,4-D, and is 2,4-D a likely cause of lymphoma? The second question cannot be answered (except perhaps by animal experiment) until the first is answered, since without an association there is no causation.

In my opinion the weight of evidence does not support the conclusion that there is an association between exposure to 2,4-D and NHL. It is axiomatic that, except when relative risks are very high - and sometimes even then - no single study will establish an association between an exposure and an outcome. The acceptance of an association depends on a number of studies showing consistent results across populations and across different epidemiologic methods. The study of Hoar et al is a strong study - strong enough on its own to establish a hypothesis of relationship of exposure to 2,4-D with some small proportion of cases of NHL - a hypothesis that clearly deserves attempts at refutation or support in other populations. When one attempts to place the results of this study among the results of those published previously, the picture becomes very confusing - much more so than if Hoar et al had been the only study published. Taken as a whole, I believe that the weight of evidence indicates that an association between 2,4-D and NHL remains a hypothesis that is still to be tested. I am unwilling to speculate as to whether 2,4-D causes NHL (or some cases of NHL) until the evidence is clear that there is an association between them.


Brian MacMahon, M.D., Ph.D.

September 29, 1986

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Soft Tissue Sarcoma and Non-Hodgkins Lymphoma in Relation
to Phenoxy Herbicide and Chlorinated Phenol Exposure in
Western Washington

by James S. Woods, Ph.D. (Battelle) et al.

Reviewed by Jerome Blondell

Synopsis

All cases of Soft Tissue Sarcoma (STS) and Non-Hodgkins Lymphoma (NHL) diagnosed in males (aged 20-79) during the time period 1981 to 1984 were ascertained from a population based tumor registry in 13 counties in western Washington. Controls were selected from the same area and group-matched by vital status and 5 year age group. Live controls under age 65 were selected by using a random digit dialing method. Social security records were used to obtain live controls for age 65 and over and death certificates were used to select the deceased controls. Total subjects in the final analysis included 128 cases of STS, 576 cases of NHL and 694 controls. 29 percent of the cancer cases were deceased at the time the study was conducted. All subjects received an hour long in-person interview on their exposure to chemicals, family history of cancer, immune disease history and other potential risk factors. On average, cases were interviewed 1 year after first diagnosis. In cases where chemical exposure was reported, a supervisor or co-worker was contacted in an attempt to verify exposure status. A careful pathologic review was conducted of all STS cases to verify disease status, but no mention was made of a similar procedure for NHL cases.

The results indicated no significant risks for STS or NHL when compared with various levels of reported exposure to phenoxy herbicides or chlorophenols. However, elevated risks were observed for three selected occupations. Farmers exhibited a significant odds ratio of 1.33 for NHL (95% CI 1.03-1.7). Forestry sprayers exhibited a significant odds ratio of 4.80 for NHL (95% CI 1.2-19.4). And lumber graders exhibited a significant odds ratio of 2.66 for STS (95% CI 1.1-6.4). Further analysis of the farmers exposed to either 2,4-D, 2,4,5-T or phenoxy herbicides did not reveal any elevated risks. For example, for farmers exposed to 2,4-D, the odds ratio was 0.68 (95% CI 0.3-1.5). On the other hand, forestry workers who reported using phenoxy herbicides were still at significantly increased risk, though based on only seven cases. For the entire study population reporting use of 2,4-D the odds ratio was a nonsignificant 0.73 (95% CI 0.4-1.3). Verification of exposure to chemicals was generally quite good by the co-workers or supervisors of the study subjects. Analysis for duration of exposure did not reveal increased risks for NHL or STS among

those that worked with phenoxy herbicides for 20 years or more. Analysis for latency period effects did find a significant risk for NHL of 1.71 (95% CI 1.04-2.8) among those with cumulative exposures to phenoxy herbicides of more than 15 years during the period preceding 15 years before diagnosis (thus assuming a 15 year latency period).

Comment

Nearly all aspects of this study indicate great care was taken to avoid biasing the results. The data appear to be carefully and thoroughly analyzed. While there was a significant excess risk for farmers, there was not a significant or even an elevated risk for farmers who used 2,4-D. The authors of the study concluded that their study did not support the finding of substantial risk found in Swedish studies. They argue that the Swedish workers probably experienced higher exposures to phenoxys, especially to the contaminants chlorinated dibenzodioxins and furans. Alternatively, the Swedish workers may have exposure to an unknown factor other than chemical exposure which interacts with phenoxy herbicides to produce NHL or STS.

Only two of the many analyses performed in this study suggest that phenoxy herbicides are a risk factor for NHL. Among forestry workers who used phenoxy herbicides there were 7 cases and 0 controls which yields an infinite risk estimate. And among all workers who used phenoxy herbicides for 15 or more years (and taking into account a 15 year latency period) there was a marginally significant odds ratio of 1.71 (95% CI 1.04-2.8). However, none of the analyses that were specific for 2,4-D exhibited significant excess risk of NHL. In conclusion, I do not find that the present study supports an association between either NHL or STS and exposure to 2,4-D. If exposure to 2,4-D is causing any NHL or STS, it is apparent from this study that only certain subsets of workers with high exposures (and allowing for a long latency period) are affected.

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Mortality Analysis of Ontario Hydro Forestry Tradesman
Cohort, 1950-1982
By L.M. Green

Reviewed by Jerome Blondell

Synopsis

A total of 1,222 persons who worked for the forestry industry for 6 months or more between 1950 and 1982 were followed to determine if they had any excess risk of death when compared to the general male population of Ontario. Application of the herbicides 2,4-D and 2,4,5-T has occurred since 1950, mainly to control vegetation around rights-of-way for power transmission lines. Age-sex-cause specific Standardized Mortality Ratios were calculated for the 100 workers in the cohort who have died so far. There were no significant findings of excess death due to a particular disease. Twenty-four deaths were due to cancer but none of these were either soft-tissue sarcoma or non-Hodgkin's lymphoma.

Comment

This study involved considerable effort and was well conducted. Carefully laid out procedures were followed to ascertain all members of the exposed cohort and to determine their vital status and cause of death. Given the very small numbers the statistical power to detect significant excess cancer deaths is weak. The study properly acknowledges that the power was insufficient to detect a significant increase in STS and just adequate to detect a sevenfold increase in risk of NHL. I support the authors recommendation that this study be extended for at least another ten years.

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Herbicide Exposure and Cancer

The case-control study in Kansas reported by Hoar et al¹ in this issue has importance beyond its population of agriculture workers to the highly controversial and adversarial issue of health effects associated with herbicide exposure in Vietnam. The well-designed and carefully executed Kansas study adds substantially to the cumulating body of evidence concerning the following question: Does human exposure to phenoxyacetic acid herbicides increase the risk of soft-tissue sarcomas (STS), Hodgkin's disease (HD), and non-Hodgkin's lymphoma (NHL)? The findings among Kansas farmers are in accord with "no" answers to the first two malignancies and a "yes" answer to the third.

See also p 1141.

Most relevant to the Kansas study are the series of case-control investigations undertaken by Hardell and colleagues²⁻⁴ in rural Sweden in the mid- and late 1970s. Hardell's studies found highly significant relative risks, of magnitude fivefold and sixfold, for exposure to phenoxyacid herbicides in association with STS, HD, and NHL. Among the published epidemiologic studies, many regard the Hardell studies as the strongest in study design and certainly as providing the strongest available evidence of an association of herbicide exposure with the occurrence of cancer. However, selection bias, observation bias, and uncontrolled confounding loom as important points of vulnerability and, in fact, have emerged as the focus of sharp criticisms of the Hardell studies. In addition to critiques in the scientific literature, Hardell's studies have garnered considerable airing in the harsh give-and-take of the courtroom. Most recently, Hardell provided testimony in Australia to the Royal Commission on the Use and Effects of Chemical Agents on Australian Personnel in Vietnam. The commission's Final Report⁵ states:

This absence of replication, [I discuss this below] the absence of specific outcome (ie, 12 types of soft tissue sarcoma, non-Hodgkin's malignant lymphoma and Hodgkin's Disease), admitted information bias, the presence of significant confounding factors, the unreliability of the exposure data and the other factors detailed

above all indicate that the statistical associations asserted by Dr. Hardell are suspect. The Commission cannot, on the balance of probability, accept them as supporting an inference of causal connection between soft tissue sarcoma, malignant lymphoma and exposure to phenoxy herbicides.

With regard to STS, one other case-control study has appeared, namely, that of Smith et al⁶ in New Zealand. Smith's findings conflict with those of Hardell and yield a negative result with an estimated relative risk of 1.3. Smith's study was designed to have adequate statistical power to detect a relative risk of roughly 3, if such a risk truly existed.

What now does the study by Hoar et al add to the evidence? Perhaps most noteworthy are the negative findings for STS and HD. As a crude tally, this makes a score of two negative (Smith and Hoar) vs one positive (Hardell) study for STS and a tie of one each negative (Hoar) and positive (Hardell) study for HD. With regard to NHL, the score stands at two positive studies (Hoar and Hardell).

More important and relevant, however, are how the Hoar and Hardell studies compare methodologically. Does the Hoar study better withstand the critical onslaught that has besieged Hardell's studies? Of course, the Hoar study has undergone only the intensity of JAMA's peer review process and has not (at least to my knowledge) been subject to the rigors and scrutiny of courtroom debate. Let me attempt to probe some methodological contrasts of the two studies.

The Hoar study, like Hardell's, has the strength of case ascertainment with a population-based tumor registry along with independent pathological review and confirmation of disease. The Hoar study is considerably larger than Hardell's, with nearly twice the number of cases of malignant lymphoma and three times the number of controls. Thus, Hoar's study has considerably greater statistical precision and statistical power to detect differences. Both studies employed population-based controls, using living controls for living cases and deceased controls for deceased cases. As such, both studies had to rely in part on next of kin's reporting of exposure status.

In contrast to what has surfaced regarding the procedure

for conducting the interviews in the Hardell studies, the Hoar study involved experienced and trained interviewers who followed a detailed, predetermined interview protocol (Dr Hoar, oral communication, April 1986). The interviewers knew that the study concerned chemical exposure, but were unaware that the study's major hypothesis dealt with exposure to phenoxyacetic acid herbicides. Although the Hoar study intended blind interviews in regard to the subject's status as a case or control, information provided during the conversation often indicated clearly to the interviewer the subject's disease classification. Another advantage of the Hoar study is that the interviewing, conducted mainly in 1983, occurred at a time when there was no particular local media attention to herbicide exposure and cancer risk. Although there was considerable national concern at that time with Agent Orange exposure in Vietnam, the media in Kansas in 1983 did not shine the public spotlight on farmers' herbicide exposure and their risks of these three malignancies. This is very much in contrast to the timing of the Hardell studies during a period of intense media attention in Sweden and Dr Hardell's prominent role in the media with his taking of an advocacy position. Clearly, the interview methodology and the climate of the Hoar study make information bias a much more unlikely explanation for their findings compared with the Hardell studies.

Another strength of the Hoar study is the validation of a sample of self-reported exposure by a survey of suppliers. No differences were found in the corroboration among controls and each of the STS, HD, and NHL cases. Of course, not all subjects had such validation of their exposure status. But, what was done suggests the unlikely event of large differences between cases and controls in their accuracy of reporting exposure.

The Hoar study considered and reasonably ruled out confounding effects of exposure to other herbicides, insecticides, fungicides, nonfarming exposures, and both the known and alleged risk factors for each of these three malignancies. Another strength of the Hoar study is the consistent dose-response relationship for NHL according to the nature, intensity, and duration of exposure. The specificity of the findings of an association with one cancer and no association with the other two is another relative strength of the Hoar study compared with the Hardell studies, in which the results were an across-the-boards elevated relative risk for each of the three cancers.

Limitations of the Hoar study include those inherent with case-control studies that rely on subjects' and next of kin's recall of exposure status. Faulty recall leads to misclassification. If this occurred equally among cases and controls, the study would underestimate and thus possibly miss a true increase in risk. If it occurred differentially, this could lead to bias. As indicated above, however, bias due to differential recall of cases and controls seems unlikely in the Hoar study.

Another limitation characteristic of observational epidemiologic studies is the possibility of uncontrolled confounding, particularly with diseases whose epidemiology is unclear. This, of course, remains a possibility and to some extent constitutes the frontiers of our knowledge of the etiology of these three malignancies.

Also, one cannot rule out entirely the possibility of chance as an explanation for the findings.

All in all, however, the Hoar study does indeed provide a much firmer and tighter body of scientific evidence than do the Hardell studies.

What supporting or contradictory evidence exists from other epidemiologic studies? None of the several industrial cohort mortality studies of dioxin exposure has resulted in a noted perturbation in the frequency of deaths from these cancers. The one published cohort study of Agent Orange exposure in Vietnam, namely, the US Air Force Ranch Hand Study,⁷ has to date not detected any increase in incidence or mortality from these cancers. But, the industrial studies generally entail extremely small sample sizes that almost totally lack adequate statistical power to detect meaningful increases in risk. Even the Ranch Hand Study with its defined cohort of roughly 1200 exposed individuals lacks statistical power to detect an increased risk of these malignancies, whose incidence rates in the general population are low. Thus, the negative findings of the published cohort studies, though at first blush are corroborative of the negative case-control studies, provide little substantiation because of their inherent small sample size and limited statistical power.

Several proportional mortality studies have appeared in "bonus" states (ie, those states that offered a bonus in terms of a financial incentive to men who enlisted for Vietnam service). Most notable is the Massachusetts study⁸ that found nine deaths from STS among Vietnam veterans compared with only one expected. Interestingly, the Massachusetts study lacked sufficient numbers to investigate proportional mortality for NHL (Richard Clapp, MPH, oral communication, March 1986). In contrast to case-control studies, proportional mortality studies have even greater vulnerability to forces of selection, observation, and confounding biases. Furthermore, these state studies have three additional serious limitations: (1) the quality and accuracy of the death certificate diagnosis of these malignancies, (2) the effects of migration resulting from the sizable numbers of veterans who, subsequent to their military service, emigrated from the state and thus do not appear on the state's death tapes, and (3) the fact that none of these studies determined decedents' herbicide exposure but dealt only with their service in Vietnam. Thus, whatever the findings of the proportional mortality investigations, they add little if any substantive analytic evidence to the fundamental question of cancer risk as a result of herbicide exposure.

Is more pertinent evidence on these cancer risks in the offing? Yes! To my knowledge two additional case-control studies of these cancers are under way, each involving a considerably larger number of cases than any of the studies presently at hand, including the study by Hoar. Both studies in progress involve herbicide exposure in Vietnam as assessed by the Environmental Support Group of the Armed Forces with the use of available military records on troop locations in conjunction with records of herbicide sprayings. The first of these studies, tentatively scheduled for completion in the summer of 1986, is a joint effort of the Veterans Administration and the Armed Forces Institute of Pathology (Han Kang, PhD, oral communication, March 1986). It involves STS only and entails nearly 300 confirmed cases ascertained through the Armed Forces Institute of Pathology and approximately 600 controls. The study was designed to have good statistical power to detect a twofold increase in

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APPENDIX G

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Study/Lab/Study #/Date	Material	Accession No.	Results: LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	TOX Category	CORE Grade/Doc. No.
Teratology - rat	Acid		NOEL = 50 mg/kg LEL = 150 mg/kg		
Teratology - rat; Wil Research Labs; WIL-22002; 5/17/83	Tech 97.5%	251032	RANGE FINDING Maternal LEL = 150 mg/kg (reduction in food consumption and body wt loss) Maternal NOEL = 100 mg/kg Levels tested by gavage in Fischer 344 strain - 0, 75, 100, 150, 200 and 250 mg/kg in corn oil during 6 15 days of gestation	N/A	Supplementary 003887
Teratology - rat; Wil Research Labs; WIL-81135; 3/2/83	97.5% 2,4-D technical in corn oil	251031	Teratogenic NOEL > 75 mg/kg (HDT) Maternal NOEL > 75 mg/kg (HDT) Fetotoxic NOEL = 25 mg/kg Fetotoxic LEL = 75 mg/kg (for delayed ossif.) Levels tested by gavage in Fischer 344 strain - 0, 8, 25 and 75 mg/kg in corn oil on days 6 - 15 of gestation; A/D ratio => 75/25 => 3	N/A	Minimum 003887
Teratology - mice	Acid		NOEL = 147 mg/kg (single dose tested)		
Teratology - hamster	Acid		Terata NOEL = 40 mg/kg Terata LEL = 60 mg/kg		
Reproduction - rat	Acid		Equivocally positive for reduced viability to weanlings at 100 ppm (5 mg/kg)		
90 - Day feeding - rat; Hazleton Labs. Inc.; #2184-102; 9/12/83	97.5% a.i. 2,4-D	251474	NOEL < 1 mg/kg (histopathological changes in renal cortical tubules and increased thyroid wt) Levels tested in Fischer 344 strain 0, 1, 5, 15 and 45 mg/kg	N/A	Supplementary 003888

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Study/Lab/Study #/Date	Material	No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	Category	Doc. No.
90 Day feeding - mice; Hazleton Labs. Inc; #2184-100; 9/12/83	97.5% a.i. 2,4-D	251473	NOEL < 5 mg/kg (LDT) (histopatho- logical changes in renal tubules in both males and females) Levels tested in B ₆ , C ₃ , F strain - C, 5, 15, 45 and 90 mg/kg	N/A	Supplementary 003888
2-Year feeding - dog	Acid		NOEL = 500 ppm (12.5 mg/kg/day)		
2 Year feeding/oncogenic - rat	Acid 98.5% a.i. 2,4-D	251474	Systemic NOEL = 1250 (62.5 mg/kg/day)		
Acute oral LD ₅₀ - rat; Dow Company; 10/03/74	Dowco 290 2,4-D	243804	LD ₅₀ = 3730 mg/kg (male) LD ₅₀ = 2830 mg/kg (female)	III	Guideline 000458
Acute dermal LD ₅₀ - rabbit; Dow Company; 10/3/74	Dowco 290 2,4-D	243804	LD ₅₀ > 3980 mg/kg (male and female; single dose tested; slight to moderate ery- thema)	III	Minimum 000458
Acute inhalation LC ₅₀ - rat; Dow Company; 10/03/74	Dowco 290 2,4-D	243804	Actual concentration must be submitted. Nominal concentration was 5.03 mg/L		Supple- mentary 000458
Primary eye irritation - rabbit; Dow Company; 10/03/74	Dowco 290 2,4-D	243804	Corneal opacity, iris irritation and conjunctive irritation at day 1 and persisted through day 7.	I	Minimum 000458
Primary dermal irrita- tion - rabbit; Dow Co.; 10/03/74	Dowco 290 2,4-D	243804	Slight erythema and edema.	III	Minimum 000458
Acute oral LD ₅₀ - rat; Kalttech; #757246; 1/17/80	2,4-D MCPP	243956	LD ₅₀ > 5 g/kg	IV	Guideline 000447
Acute dermal LD ₅₀ - rabbit; Kalttech; #757246; 1/17/80	2,4-D MCPP	243956	LD ₅₀ > 5 g/kg	III	Guideline 000447

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Study/Lab/Study #/Date	Material	No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	Category	Doc. No.
Primary dermal irritation - rabbit; Raltech; #757246; 1/17/80	2,4-D MCP	243956	PIS = 0.8/8.0 Slight erythema and edema at abraded site.	IV	Guideline 000447
Acute oral LD ₅₀ - rat; Raltech; #759035; 2/15/80	2,4-D MCPA Dicamba	243672	LD ₅₀ > 5 g/kg	IV	Guideline 000452
Acute dermal LD ₅₀ - rabbit; Raltech; #759035; 2/15/80	2,4-D MCPA Dicamba	243672	LD ₅₀ > 2 g/kg Slight erythema, edema and desquamation.	III	Guideline 000452
Primary eye irritation - rabbit; Raltech; #759035; 2/15/80	2,4-D MCPA Dicamba	243672	Corneal opacity observed at 24 hrs; all irritation clear by day 7.	II	Guideline 000452
Primary dermal irritation - rabbit; Raltech; #759035; 2/15/80	2,4-D MCPA Dicamba	243672	PIS = 1.0/8.0	IV	Guideline 000452
Acute oral LD ₅₀ - rat; Int. Res. Dev. Corp.; 8/22/78	2,4-D + Banvel 4S		LD ₅₀ = 1847 mg/kg	III	Guideline 000045
Acute dermal LD ₅₀ - rabbit; Int. Res. Dev. Corp.; 8/22/78	2,4-D + Banvel 4S		LD ₅₀ = 11,892 mg/kg	III	Guideline 000045
Acute inhalation LC ₅₀ - rat; Int. Res. Dev. Corp.; 6/27/78	2,4-D + Banvel 4S		LC ₅₀ > 21.1 mg/L (4 hours)	IV	Guideline 000045
Primary eye irritation - rabbit; Int. Res. Dev. Corp.; 8/22/78	2,4-D + Banvel 4S		Corrosive	I	Guideline 000045
Primary dermal irritation - rabbit; Int. Res. Dev. Corp.; 8/22/78	2,4-D + Banvel 4S		PIS = 1.5 (Draize)	III	Guideline 000045

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Study/Lab/Study #/Date	Material	Accession No.	Results: LD ₅₀ , IC ₅₀ , PIS, NOEL, IEL	TOX Category	CORE Grade/Doc. No.
Primary eye irritation - rabbit; Kalsech Scientific Service Inc.; #721399; 4/25/79	2,4-D Dicamba 2.95% 0.48%	Unaccessioned	Minimal corneal opacity cleared at 72 hrs. and all other irritation cleared by day 4.	III	Guideline 000037
Acute oral LD ₅₀ - rat; Kalsech; #759034; 11/30/79	2,4-D Dicamba MCP 1.15% 1.00% 1.06%	242865 243675	10 < LD ₅₀ < 20 gm/2,4-D Slight erythema and edema PIS = 0.1/8.0	IV	Guideline 0000456
Acute oral LD ₅₀ - rat	Acid		LD ₅₀ = 375 mg/kg	II	
Primary eye irritation - rabbit; Kalsech Scientific Service Inc.; #721399; 4/25/79	2,4-D Dicamba 2.95% 0.48%	Unaccessioned	Minimal corneal opacity cleared at 72 hrs. and all other irritation cleared by day 4.	III	Guidelines 000037
Acute oral LD ₅₀ - rat	2,4-D 1.15% 2-(2-Methyl-4-chlorophenoxy acid 1.15%	242865	10 < LD ₅₀ < 20 gm/kg		Supplementary
Acute oral LD ₅₀ - rat	2,4-D 1.15% 2-(2-Methyl-4-chlorophenoxy acid 1.15%	242865	LD ₅₀ > 5 g/kg	IV	Minimum
Acute dermal LD ₅₀ - rabbit	2,4-D 1.15% 2-(2-Methyl-4-chlorophenoxy acid 1.15%	242865	LD ₅₀ > 21 g/kg	IV	Minimum
Primary dermal irritation - rabbit	2,4-D 1.15% 2-(2-Methyl-4-chlorophenoxy acid 1.15%	242865	PIS = 0	IV	Minimum

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD50, LC50, PIS, NOEL, LEL	TVX Category	CORE Grade/Doc. No.
Primary eye irritation - rabbit	2,4-D 1.15% 2-(2-Methyl-4-chlorophenoxy) acid 1.15%	242365	No corneal opacity or any irritation.		Supplementary
Primary eye irritation - rabbit; Kalthed Scientific Service Inc.; #772285; 3/19/80	2,4-D 1.15% MCPA acid 0.55%	Unaccessioned	Corneal opacity and iris irritation at day 7.	II	Minimum 000035
Acute oral LD50 - rat;	2,4-D ... 0.6%	243006	LD50 > 20 g/kg (M)		
Acute dermal LD50 - rabbit; Stillmeadow; #1335-79; 10/23/79	2,4-D 1.455% MCPA 0.612% Dicamba 0.129%	243058	LD50 > 2005 mg/kg (single dose tested)	III	Guideline 000836
Primary eye irritation - rabbit; Stillmeadow; #1336-79; 10/15/79	2,4-D 1.455% MCPA 0.612% Dicamba 0.129%	243058	Corneal opacity at day 21 (unwashed eyes). All washed eyes were cleared by 72 hours.	II	Guideline 000836
Primary eye irritation - rabbit; Stillmeadow; #1376-79; 10/17/79	100 mg of untreated vermiculite	243058	No corneal opacity; all irritation cleared by 72 hours.	IV	Guideline 000836
Primary dermal irritation - rabbit; Stillmeadow; #1337-79; 10/10/79	100 mg of untreated vermiculite	243058	PIS = 1.36/8.0	IV	Guideline 000836
Primary eye irritation - rabbit; Kalthed; #772286; 3/19/80	Not identified	243007			Invalid 000842
Acute oral LD50 - rat; WARE; #6021914; 2/12/76	Substance unidentified P-7645	243006			Invalid 000846

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results:		TOX Category	OHR Grade/ Doc. No.
			LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL			
Acute dermal LD ₅₀ - rabbit; WARF; #6021914; 2/12/76	Substance unidentified F-7645	243006				Invalid 000846
Primary eye irritation - rabbit; WARF; #6021914 2/12/76	Substance unidentified F-7645	243006				Invalid 000846
Primary eye irritation - rabbit; Raltech; #772287; 3/21/80	Substance unidentified F-7645	243006				Invalid 000846
Primary dermal irrita- tion - rabbit; WARF; #6021914	Substance unidentified F-7645	243006				Invalid 000846
Primary eye irritation - rabbit; Raltech; #772284; 3/19/80	Substance unidentified	243008				Invalid 000845
Primary eye irritation - rabbit; Midwest Res. Inst.; #4823-B(2); 1/2/80	"EH595"	244753	Corneal opacity in 5/6 animals with clearing by day 14 (unwashed eyes). Redness, chemosis and discharge.	II		Minimum 001174
Primary eye irritation - rabbit; Midwest Res. Inst.; #4823-B(2); 1/2/80	"EH601"	244753	Corneal opacity through day 21 (unwashed eyes). Corneal opacity with clearing by day 14 (washed eyes).	I		Minimum 001174
Primary eye irritation - rabbit; Midwest Res. Inst.; #4823-B(1); 11/12/79	881 iso-octyl ester 881 dimethyl- amine 2,4-D dimethyl- amine 2,4-D iso-octyl ester	244753	Corneal opacity through day 21.	I		Guideline 001174

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Study/Lab/Study #/Date	Material	Accession No.	Results: L ₅₀ , LC ₅₀ , PIS, NOEL, IEL	TOX Category	CORE Grade/Doc. No.
Primary eye irritation - rabbit; Midwest Res. Inst.; #4823-B(1); 11/12/79	881 iso-octyl ester 881 dimethylamine 2,4-D dimethylamine 2,4-D iso-octyl ester	244753	Corneal opacity through day 21.	I	Guideline 001174
Acute oral LD ₅₀ - rat; Environmental Health Tox. Labs of Std. Oil of California; SOCAL #1779; 5/1/81	MCPP, DMA salt 34.0% 2,4-D, DMA salt 31.8% (239-EUIL)	245070	LD ₅₀ = 1.7 g/kg (male) LD ₅₀ = 0.89 g/kg (female)	III	Minimum 001248
Acute dermal LD ₅₀ - rabbit; SOCAL #1778; 4/15/81	MCPP, DMA salt 34.0% 2,4-D, DMA salt 31.8% (239-EUIL)	245070	LD ₅₀ > 5.0 g/kg (male) (single dose used) LD ₅₀ = 3.1 g/kg (female) (95% conf. = 1.1 - 8.5 g/kg)	III	Minimum 001248
Primary eye irritation - rabbit; SOCAL #1777; 4/20/81	MCPP, DMA salt 34.0% 2,4-D, DMA salt 31.8% (239-EUIL)	245070	Corneal opacity through day 21 (unwashed eyes and washed eyes)	I	Guideline 001248
Primary eye irritation - rabbit; Stillmeadow; #1528-80; 2/20/80	2,4-D ... 23.1% MCPP ... 24.7% Dicamba .. 6.8% (Gordon's Trimec) 450 herbicide	245537	Corneal opacity, iris irritation, conjunctival redness, chemosis persisted through day 21 (unwashed eyes). Corneal opacity persisted through day 21 (washed eyes).	I	Guideline 001214
Primary eye irritation - rabbit; Stillmeadow; #1376-79; 10/17/79	Untreated vermiculite	245537	No corneal opacity - iris irritation, redness.	III	Guideline 001214

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	TOX Category	CORE Grade/Doc. No.
Primary eye irritation - rabbit; Stillmeadow; #137679; 10/17/79	Untreated vermiculite	243058	Redness and chemosis. No corneal opacity.	IV	Guideline 001214
Primary eye irritation - rabbit; Stillmeadow; #1336-79; 10/15/79	DMB #2	243058	Iris irritation, redness and chemosis (unwashed eyes) at 24 hrs. Mild corneal opacity developed 7 days post-treatment.	III	Guideline 001214
Primary eye irritation - rabbit; MRI #4823-B; 11/12/79	EH-596 2,4-D 2.6% MCPP 4.6% Dicamba . 0.5%		Corneal opacity with irritation and conjunctival erythema with clearing by day 7.	II	Minimum 001214
Primary eye irritation - rabbit; MRI #4823-B(1); 11/12/79	EH-594 2,4-D 5.3% MCPP 9.35% Dicamba . 1.17%		Corneal opacity exhibited through day 21. Erythema, edema and discharge observed through day 21.	I	Guideline 001214
Primary eye irritation - rabbit; Hilltop Lab.; 7/1/79	2,4-D ... 23.1% MCPP 24.7% Dicamba . 6.18%	240893	Corneal opacity through day 21.	I	Guideline 001214
Primary eye irritation - rabbit; MRI #4823-B; 11/12/79	EH-595 2,4-D 3.9% MCPP 6.7% Dicamba . 0.75%		Iris irritation and conjunctival erythema. No corneal opacity	III	Minimum 001214
Primary eye irritation - rabbit; Stillmeadow; #1336-79; 10/15/79	EH-576		Corneal opacity with corneal stippling, iris irritation, redness.	I	Minimum 001214
Primary eye irritation - rabbit; MRI #4823-B; 1/2/80	EH-601	244753	Corneal opacity, iris irritation, conjunctival redness, chemosis and discharge (unwashed eyes). Corneal opacity with clearing by day 14 (washed eyes).	I	Minimum 001214

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Study/Lab/Study #/Date	Material	Accession No.	Results: LD50, LC50, PIS, NOEL, LEL	TOX Category	CURE Grade/ Doc. No.
Primary eye irritation - rabbit; MRI #4823-B; 11/12/79	2,4-D ... 23.9% MCP ... 12.3% Dicamba ... 5.7%	244753	Corneal opacity examined through day 21. Iris irritation and conjunctival redness.	I	Guideline 001214
Primary eye irritation - rabbit; Stillmeadow; #1344-29; 10/17/79	2,4-D ... 23.5% MCP ... 15.69% Banvel ... 3.9%	244753	Corneal opacity through day 21 con- junctival redness, chemosis and discharge, corneal swelling and chemosis around eye.	I	Guideline 001214
Primary eye irritation - rabbit; Stillmeadow; #1340-79	2,4-D ... 20.7% MCP ... 20.7% Dicamba ... 3.5%	241332	Corneal opacity observed through day 21. Corneal swelling and stip- pling.	I	Minimum 001214
Primary eye irritation - rabbit; MRI #4823-B(3); 7/31/80	EH-552 -2,4-D MCP & Dicamba	245537	Corneal opacity with clearing by day 13. Redness, discharge and chemosis (unwashed eyes).	III	Minimum 001214
Primary eye irritation - rabbit; MRI #4823-B(3); 7/31/80	2,4-D ... 6.3% MCP ... 3.0% Dicamba . 0.69%	245537	Corneal opacity, redness, chemosis with clearing by day 7.	III	Guideline 001214
Primary eye irritation - rabbit; Stillmeadow; #2121-81; 4/16/81	EH-680, BK-800 2,4-DP ... 21.5% 2,4-D ... 21.5% Dicamba . 5.38%	245537	Corneal opacity observed in 1/6 animals 7 days after treatment and persisted for 21 days (unwashed eyes). Chemosis, discharge and redness (washed eyes).	II	Guideline 001214
Primary eye irritation - rabbit; Stillmeadow; #2126-81; 4/16/81	EH-681 2,4-DP ... 11.3% 2,4-D ... 11.3% Dicamba . 11.3%	245537	Corneal opacity observed in both unwashed and washed eyes by day 21.	I	Guideline 001214
Primary eye irritation - rabbit; Stillmeadow; #2131-81; 4/20/81	EH-683 2,4-D ... 33.2% Dicamba . 4.9%	245537	Corneal opacity observed in both unwashed and washed eyes by day 21.	I	Guideline 001214

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Study/Lab/Study #/Date	Material	Accession No.	Results: LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	TOX Category	CORE Grade/Doc. No.
Primary eye irritation - rabbit; MRI #48230-B(3); 8/27/80	EH-552 2,4-D ... 7.05% MCP ... 3.7% Dicamba ... 0.47%	245537	Corneal opacity persisted through day 7 (unwashed eyes). Corneal opacity with clearing by day 7 (washed eyes).	II	Minimum 001214
Primary eye irritation - rabbit; MRI #4823-B(1); 10/15/79	EH-590 2,4-D ... 5.78% MCP ... 5.79%	245537	Corneal opacity persisted through day 7.	II	Minimum 001214
Primary eye irritation - rabbit; MRI #4823-B(1); 10/15/79	EH-591 2,4-D ... 9.59% MCP ... 9.60%	245537	Corneal opacity, iris irritation, chemosis persisted through day 7 (unwashed eyes).	I	Minimum 001214
Primary eye irritation - rabbit; MRI #4823-B(1); 10/15/79	EH-597 2,4-D ... 5.15% MCP ... 5.1% Dicamba ... 1.27%	245537	Corneal opacity, iris irritation, redness persisted through day 7.	I	Minimum 001214
Primary eye irritation - rabbit; MRI #4823-B(1); 10/15/79	EH-598 2,4-D ... 5.6% MCP ... 5.6% Dicamba ... 1.3%	245537	Corneal opacity, iris irritation, redness, chemosis persisted through day 7.	I	Minimum
Acute oral LD ₅₀ - rat; Raltech Scientific Ser.; RT#900075; 10/29/81	2,4-D ... 14.25%	247246 247247		IV	Supplementary 002823
Acute dermal LD ₅₀ - rabbit; Applied Biological Sciences Lab.; #18836; 3/3/80	2,4-D ... 14.25%	247246 247247	LD ₅₀ > 2 g/kg	III	Guideline 002823
Primary dermal irritation - rabbit; Raltech Scientific Ser.; RT#900075; 10/29/81	2,4-D ... 14.25%	247246 247247	At 24 hrs. 4/6 had slight to moderate erythema and edema. At 72 hrs. 6/6 had slight to well defined erythema and 5/6 slight to well defined edema. PIS = 2.9	III	Guideline 002823

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Study/Lab/Study #/Date	Material	Accession No.	LD ₅₀ , LC ₅₀ , PTs, NOEL, LEL	Results:	TOX Category	CORE Grade/ Doc. No.
Acute dermal LD ₅₀ - rabbit; Raltech Scientific Ser.; RT#862787; 10/2/81	2,4-D .. 0.680% MCPP ... 0.680% Dicamba 0.027%	246283	LD ₅₀ > 2 g/kg		III	Guideline 003137
Primary eye irritation - rabbit; Raltech Scientific Ser.; RT#862787; 10/2/81	2,4-D .. 0.680% MCPP ... 0.680% Dicamba 0.027%	246283	6/9 corneal opacity (2/9 = 2.5, 4/9 = 5.0) and iris irritation present, but had cleared by day 4 conjunctive irritation present and persisted through day 14.		II	Guideline 003137
Primary dermal irritation - rabbit; Raltech Scientific Ser.; RT#862787; 10/2/81	2,4-D .. 0.680% MCPP ... 0.680% Dicamba 0.027%	246283	Slight to well defined erythema and slight edema at 24 hours post-treatment, slight erythema and edema at 72 hours.		IV	Guideline 003137
Acute oral LD ₅₀ - rat; Raltech Scientific Ser.; RT#862788; 10/2/81	2,4-D .. 1.370% MCPP ... 1.370% Dicamba 0.055%	246283	LD ₅₀ > 5 g/kg		IV	Guideline 003137
Acute dermal LD ₅₀ - rabbit; Raltech Scientific Ser.; RT#862788; 10/2/81	2,4-D .. 1.370% MCPP ... 1.370% Dicamba 0.055%	246283	LD ₅₀ > 2 g/kg		IV	Guideline 003137
Primary eye irritation - rabbit; Raltech Scientific Ser.; RT#862788; 10/2/81	2,4-D .. 1.370% MCPP ... 1.370% Dicamba 0.370%	246283	9/9 corneal opacity (1/9 1.25, 1/9 2.5, 4/9 = 5, 2.9 = 7.5, 1/9 = 12.8)		II	Guideline 003137
Primary dermal irritation - rabbit; Raltech Scientific Ser.; RT#862788; 10/2/81	2,4-D .. 1.370% MCPP ... 1.370% Dicamba 0.370%	246283	Slight to well defined erythema and slight edema at 24 hours post-treatment. Slight erythema and edema at 72 hours.		IV	Guideline 003137

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Study/Lab/Study #/Date	Material	Accession No.	Results:	TOX Category	CORE Grade/ Doc. No.
Primary eye irritation - rabbit; Raltech Scientific Ser.: #759034; 12/18/79	2,4-D ... 1.60% MCP ... 1.06%	243675	Corneal opacity in 6/6 unwashed eyes & 3/3 washed eyes. Iris & conjunctive irrit. present. Corneal opacity present through day 21 in 1/6 unwashed & through 48 hrs 3/3 washed.	I	Guideline 000456 002027
Primary eye irritation - rabbit; Cannon Labs.; 5/28/80	2,4-D ... 1.60% MCP ... 1.06%	243675	Corneal opacity in 6/6 unwashed eyes and 2/3 washed eyes. (1/6 = 5, 1/6 = 10, 1/5 = 15, 1/6 = 20) (1/3 = 5, 1/3 = 15). Iris and conjunctive irritation. Corneal opacity persisted through day 19 in unwashed group and through 72 hours in washed group.	I	Guideline 000456 002027
Primary eye irritation - rabbit; WIL Res. Labs.; #80122; 5/28/80	2,4-D ... 1.60% MCP ... 1.06%	243675	Corneal opacity in 4/6 unwashed eyes. No corneal opacity in washed group. Conjunctival irritation present. Corneal opacity clear by day 10 in all animals except 1.	II	Guideline 000456 002027
Primary eye irritation - rabbit; Raltech; #757246; 1/17/80	2,4-D ... 0.68% MCP ... 0.68%	243956	Corneal opacity, corneal epithelial peeling, iris irritation, chemosis, purulent discharge at 24 hours. Corneal opacity in 1/6 at day 21.	I	Guideline 000447 002028
Primary eye irritation - rabbit; Cannon Labs.; #UF-7287; 5/30/80	2,4-D ... 0.68% MCP ... 0.68%	243956	At 24 hours, 7/9 had corneal opacity (1/9 = 5, 3/9 = 10, 1/9 = 20, 1/9 = 30, 1/9 = 40) Conjunctive irritation; pannus also observed at day 21, 3/9 had corneal opacity (2/9 = 5, 1/9 = 10) with pannus in 1/9. Washed eyes: PIS = 0 by day 4.	I	Guideline 000447 002028

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Study/Lab/Study #/Date	Material	Accession No.	Results: LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/Doc. No.
Primary eye irritation - rabbit; WIL; #80123; 7/9/80	2,4-D ... 0.68% MCPP 0.68%	243956	At 24 hours, 5/9 had corneal opacity (4/9 = 5, 1/9 = 10) conjunctive irritation. All irritation clear by day 10.	II	Guideline 000447 002028
Acute oral LD50 - rat; Hazleton Raltech; RT#917207; 3/4/82	2,4-D ... 0.58% MCPP 0.58%	247086	LD50 > 5 g/kg	IV	Guideline 002819
Acute dermal LD50 - rabbit; Hazleton Raltech; RT#917207; 3/4/82	2,4-D ... 0.58% MCPP 0.58%	247086	LD50 > 2 g/kg only dose tested	III	Guideline 002819
Primary eye irritation - rabbit; Hazleton Raltech; RT#917207; 3/4/82	2,4-D ... 0.58% MCPP 0.58%	247086	4/9 animals had corneal opacity (1/9 = 5, 1/9 = 7.5, 1/9 = 10, 1/9 = 20 at 24 hours, 6/9 had iris irritation (6/9 = 5), 9/9 reiness (2/9 = 1, 1/9 = 1.5, 1/9 = 2, 4/9 = 2.5, 1/9 = 3); 7/9 chemosis (1/9 = 1, 5/9 = 2, 1/9 = 2.5); 5/9 discharge (1/9 = 1, 3/9 = 2, 1/9 = 3). All irritation had cleared by day 14.	III	Guideline 002819
Primary dermal irritation - rabbit; Hazleton Raltech; RT#917207; 1/11/82	2,4-D ... 0.58% MCPP 0.58%	247086	Slight erythema and edema. Primary Irritation Score = 1.0	IV	Guideline 002819
Acute oral LD50 - rat; Hazleton Raltech; RT#917208; 2/20/82	2,4-D ... 0.99% MCPP 0.99%	247086	LD50 > 5 g/kg only dose tested	IV	Guideline 002819
Acute dermal LD50 - rabbit; Hazleton Raltech; RT#917208; 2/20/82	2,4-D ... 0.99% MCPP 0.99%	247086	LD50 > 2 g/kg only dose tested	III	Guideline 002819

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Study/Lab/Study #/Date	Material	Accession No.	Results: LD50, LC50, PIS, NOEL, IEL	Tox Category	CURE Grade/Doc. No.
Primary eye irritation - rabbit; Raltech Scientific Ser.; #70904c; 1/15/79	2,4-D MCP	247086	At 24 hrs., 7/9 had corneal opacity (6/9 = 5, 1/9 = 10); 6/9 iris irritation (6/9 = 5); 9/9 redness (5/9 = 1, 4/9 = 2), chemosis (6/9 = 1, 3/9 = 2), and discharge (5/9 = 1, 4/9 = 2). All corneal opacity and other irritation had cleared by day 7.		Supplementary 002819
Primary eye irritation - rabbit; WARF Institute; #7082554	2,4-D MCP	247086	No irritation in washed or unwashed group.	IV	Guideline 002819
Primary dermal irritation - rabbit; WARF Institute; #7082554	2,4-D MCP	247086	No erythema or edema at 24 or 72 hours post-treatment. PIS = 0.0 .5 gm applied for 24 hrs.	IV	Minimum 002819
Acute oral LD50 - rat; Stillmeadow; #2088-81; 4/3/81	2,4-D . MCP . . Dicamba	245103	LD50 > 5050 mg/kg (ODT)	IV	Guideline 002106
Acute dermal LD50 - rabbit; Stillmeadow; #2089-81; 4/10/81	2,4-D . MCP . . Dicamba	245104	LD50 > 2100 mg/kg (ODT)	III	Guideline 002106
Primary eye irritation - rabbit; Stillmeadow; #2090-81; 3/20/81	2,4-D . MCP . . Dicamba	245105	No corneal opacity. Other irritation cleared by day 7.	III	Guideline 002106
Primary dermal irritation - rabbit; Stillmeadow; #2091-81; 3/27/81	2,4-D . MCP . . Dicamba	246106	PIS = 1.25 Slight to well defined erythema and slight edema at 24 hours. Slight erythema and edema at 72 hours.	IV	Guideline 002106
Primary eye irritation - rabbit; Raltech; # 757246 1/17/80	F9387	243956	Corneal opacity in unwashed eyes at 24 hours; 3/6=5, 1/6=10; 2/6=12.5; all animals with corneal epithelial peeling	I	000474

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Study/Lab/study #/Date	Material	No.	LD50, LC50, PIS, NOEL, LEL	Category	Unit of study/ Doc. No.
Acute inhalation LC50 - rat; Toxicogenics; #420-0664; 7/28/81	Atrazine 17.39% Atrazine relat- ed compounds 0.91% Metolachlor 31.80%	246035	LC50 > 2.34 mg/L	III	Guideline 002087
Primary eye irritation - rabbit; Bioresarch Lab; #1682-B; 7/20/81 7/20/81	Atrazine 17.39% Atrazine relat- ed compounds 0.91% Metolachlor 31.80%	246035	At 24 hours, 4/6 corneal opacity 3/3 corneal opacity (1/6 = 5) (1/3 = 10, 1/3 = 20, 3/6 = 40) Conjunctive irritation present - all irritation had cleared by day 14 except for 1/6 animals had redness (1/6 = 1)	II	Guideline 002087
Primary dermal irrit. - rabbit; Bioresarch Lab; #1682-A; 6/29/81	Atrazine 17.39% Atrazine relat- ed compounds 0.91% Metolachlor 31.80%	246035	PIS = 0.21	IV	Guideline 002087
Dermal sensitization - guinea pigs; Bioresarch Lab; #1682-E; 8/31/81	Atrazine 17.39% Atrazine relat- ed compounds 0.91% Metolachlor 31.80%	246035	Non sensitizing		Guideline 002087

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STUDY/IND/STUDY #/DATE	PRELIMINARY	NO.	IND, NOEL, LEL, LEL	Category	Doc. No.
Dissemination chemical - metabolite or impurity or contaminant or salt or photodegradant or etc.					
2-Year feeding/oncogenic - rat; Hazleton Laboratories; #2184-103; June 11, 1984	97.5% pure 0.9% 2,4-6	254872 256619 254708 263112 263113 263114	Caswell #314C 2,4-dichlorophenol NOEL = 1 mg/kg/day. LEL = 5 mg/kg/day renal effects (increased tubular brown pig- ment. Increased vacuolization of cytoplasm of renal cortex. Levels tested in CDR(F344)/CRL-BK strain- 0, 1, 5, 15, and 45 mg/kg Oncogenic NOEL = equivocal		Supplementary 004498 WITHHOLD 005234 006534
		264983	Amendment I Data indicated only a slight corre- lation between the increased fre- quency of transitional cell hyper- plasia in the kidney and the presence of microcalculi. There did not appear to be an increase in in- cidence of astrocytomas in the treated animals.		005555

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Study/Lab/Study #/Date	Material	Accession No.	LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/Doc. No.
2-Gen. Reproduction - rat; Wil Russ. Labs. Inc; # Wil- 81137; Sept. 30, 1986	2,4-D 97.5% a.i.	259442 259443 259444 259445 259446 265489	AMENDMENT Dose levels expressed in mg/kg/day to adults. FO parent toxicity; LEL - 19.9(20) mg/kg/day, (degeneration of male kidney tubules) NOEL - 5(5) mg/kg/day F1 parent toxicity LEL - 14(20) mg/kg/day, (degeneration of male kidney tubules, and female body wt. gain reduction). NOEL - 3.8(5) mg/kg/day Developmental LEL - 26(20), mg/kg/day (F1b pup wt. gain reduction). Developmental NOEL - 7.2(5) mg/kg/day Target or nominal dose levels administered in the study were: 0.5, 20, or 80 mg/kg/day. LEL and NOEL expressed in mg/kg/day (nominal dose level, mg/kg/day).	N/A	Minimum 005446 005684 005754
Dermal sensitization - guinea pig; Hazleton Labs., America; #852190; 5/9/84	2,4-Dichlorophenoxyacetic acid 1.22% 2-(2-methyl-4-chlorophenoxy) propionic acid 1.22% Dicamba .08%	253321	Non-sensitizing		Guideline 005480

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

R. Engler
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APR 7 - 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: 2,4-Dichlorophenoxyacetic Acid, Rat Study -
Qualitative Risk Assessment of Combined Toxicity and
Oncogenicity Study in Rats. Caswell #315

FROM: C.J. Nelson, Statistician *C.J. Nelson*
Scientific Mission Support Staff *4/7/87*
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Marcia van Gemert, Ph.D.
Chief, Section III
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Richard Levy, M.P.H., Leader-Biostatistics Team
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C) *Richard Levy*
4/8/87

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C) *Reto Engler*

Summary:

Significant survival differences were found between the controls and the dose groups in the two-year chronic oral study of male rats fed 2,4-dichlorophenoxyacetic acid. Using the Peto Prevalence method, adjusted time-to-death-with-tumor analyses were performed and statistically significant dose related trends were found for the astrocytoma tumors. However the pairwise comparison between the control and high dose group astrocytoma prevalence was not quite significant.

There were no significant survival or tumor differences or trends in the female rats.

Background:

This study was conducted at Hazelton Labs on male and female CDF (F344)/CRL-Br rats. 2,4-Dichlorophenoxyacetic acid (2,4,-D) was administered to both sexes of rats at 1 mg/kg, 5 mg/kg, 15 mg/kg, and 45 mg/kg in the diet. There was also a concurrent control group. All groups had 60 animals. There was an interim sacrifice at 53 weeks of 10 animals per group.

Mortality Analysis:

Tarone's (1975) extensions of Cox's test for life table data (1972) indicated no significant trend for male rats with increasing dose ($P=.31$) but there was a significant departure from trend ($P=.001$). This was due to the higher mortality in the controls, Table 1-A. Cox's test (1972) on pairwise comparisons indicated significantly higher mortality in the controls than in the 1 mg/kg group ($P=.02$) and the 5 mg/kg group ($P=.0004$). The 15 mg/kg group had nearly significantly lower mortality than the controls ($P=.09$).

The female rats had no significant mortality trend with dose ($P=.52$) and no departure from trend ($P=.94$) using Cox's test for life table data Table 1-B. There were no significant differences between any pairwise comparisons of groups.

Table 1. 2,4-Dichlorophenoxyacetic acid - Rat Study, Mortality Rates⁺ and Cox or Generalized K/W Test Results

A. Males

Dose (mg/kg)	WEEKS			TOTAL
	0-52	53 ^a -91	92-105	
0	1/60	6/49	11/43	18/50 (36)
1	0/60	4/50	3/46	7/50 (14) *
5	0/60	1/50	2/49	3/50 (6) **
15	2/60	3/48	4/45	9/50 (18)
45	1/60	3/49	10/46	14/50 (28)

B. Females

Dose mg/kg	WEEKS			TOTAL
	0-52	53 ^a -91	92-105	
0	2/60	3/48	5/45	10/50 (20)
1	0/60	4/50	9/46	13/50 (26)
5	0/60	6/50	7/44	13/50 (26)
15	0/60	5/50	7/45	12/50 (24)
45	0/60	7/50	7/43	14/50 (28)

+ Number of Animals Died/Number of Live Animals

() Percent

a Ten rats sacrificed at week 53 are not in this table.
Final sacrifice was at 105 and 106 weeks.

Note - The above survival tables are broken into aggregate time intervals for display purposes only.

Significance of Trend Analysis denoted at control.

Significance of pairwise comparison with control denoted at dose level.

* p < .05

** p < .01

Tumor Analysis:

Since there were no survival disparities in the females, an unadjusted tumor analysis was done using the Cochran-Armitage Tests for Trends (1971) and the Fisher's exact test for pairwise comparisons. There were no pairwise differences in astrocytoma tumor rates between control and dosed groups, and no dose related trends. No further analyses were done on females.

For the males, Fisher's exact test showed a nearly significant ($p=.054$) increase in astrocytomas in the 45mg/kg dose group over the controls. The Cochran-Armitage trend test was highly significant ($p<.00005$). Since there was significantly higher mortality in the controls than there was in the 1mg/kg and 5mg/kg dose groups two different analyses were done. First, the Peto Prevalence test was run which assumes the astrocytomas are not fatal in context and the test was highly significant for a dose related trend. Second, the methods of Cox and Gehan-Breslow (1977), which assume that the tumors are fatal in context, revealed that there was a significant trend ($p<.0001$) by Cox's test for life table data. None of the pair wise comparisons were significantly different.

A Tarone (1982) adjustment to the control response, using historical control data furnished by Marcia van Gemert, was applied to the Cochran-Armitage test. This agreed with the previous Cochran-Armitage test that there was a highly significant trend ($p<.00005$). Using the preceeding adjusted control response as an estimate of background tumor rate, the probability⁺ of observing 6 or more such tumors out of 59 animals is .002.

The question was also asked, "What if the response in the controls was 2 astrocytomas out of 60 instead of 1?" The Fisher's Exact test showed no significant difference between the controls and 45mg/kg group ($p=.131$) but there was still a highly significant trend ($p < .00005$), based on the Cochran-Armitage trend test.

In summary, there is an increasing dose related trend for astrocytomas in male rats fed 2,4,-D. The trend remains whether the tumors are considered in a fatal or non-fatal context, whether the controls are adjusted for historical values, or whether one more tumor is added to the concurrent control.

⁺ Note: The probability was calculated using the binomial distribution with the background rate of 0.0208 as the probability for an individual response.

Table 2. 2,4-Dichlorophenoxyacetic acid - Rat Study, Astrocytoma Rates⁺ and Peto's Prevalence Test Results

A. Males				
Dose mg/kg	WEEKS			TOTAL
	21 ^a -91	92-105	Sacrifice	
0	1/7	0/11	0/32	1/50 (2)**
1	0/4	0/3	0/43	0/50 (0)
5	0/1	0/2	0/47	0/50 (0)
15	0/4	2/4	0/41	2/49 (4)
45	0/3	1/10	5/36	6/49 (13)

a. First tumor occurred at 21 weeks in the controls.

B. Females				
Dose mg/kg	WEEKS			TOTAL
	0-88	89 ^b -105	Sacrifice	
0	0/4	0/6	0/40	0/50 (0)
1	0/3	1/10	0/37	1/50 (2)
5	0/5	0/8	1/37	1/50 (2)
15	0/4	0/8	1/38	1/50 (2)
45	0/6	0/8	1/36	1/50 (2)

+ Number of Rats with Tumor/Number of Rats Examined.

() Percent

b. First tumor occurred at 89 weeks in 1mg/kg group.

Ten rats were sacrificed at 53 weeks, none had tumors.

Note - ~~Significance of Trend Analysis denoted at control.~~
Significance of pairwise comparison with control denoted
at dose level.

* p < .05

** p < .01

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Miscellaneous

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Federal Register / Vol. 53, No. 56 / Wednesday, March 23, 1988 / Notices

ENVIRONMENTAL PROTECTION AGENCY

[OPP-30000/57; FRL-3388-1]

2,4-D, 2,4-DB, and 2,4-DP Proposed Decision Not To Initiate a Special Review**AGENCY:** Environmental Protection Agency (EPA).**ACTION:** Notice; Proposed Decision Not To Initiate a Special Review.

SUMMARY: This document announces EPA's proposed decision not to initiate a Special Review of 2,4-D, 2,4-DB, and 2,4-DP based on carcinogenicity. The Agency's decision is based on a consensus of opinion from EPA scientists, national experts on epidemiology, and the FIFRA Scientific Advisory Panel, that existing epidemiologic data are inadequate to assess the carcinogenic potential of 2,4-D. In addition, the Agency has concluded that existing laboratory data provide insufficient evidence of carcinogenicity. Therefore, EPA has determined that a Special Review is not appropriate at this time.

DATE: Comments on this Notice must be received by May 23, 1988.

ADDRESS: Submit three sets of written comments, bearing the document control number [OPP-30000/57] by mail to:

Information Services Branch, Program Management and Support Division (TS-767C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St. SW, Washington, DC 20460.

In person, bring comments to:

Rm. 236, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA.

Information submitted in any comment concerning this Notice may be claimed confidential by marking any part or all of that information as "Confidential Business Information" (CBI). Information so marked will not be disclosed except in accordance with procedures set forth in 40 CFR Part 2. A copy of the comment that does not contain CBI must be submitted for inclusion in the public docket. Information not marked confidential may be disclosed publicly by EPA without prior notice to the submitter. The 2,4-D public docket, which contains all non-CBI written comments and the corresponding index will be available for public inspection in Rm. 236 at the Virginia address given above from 8 a.m. to 4 p.m., Monday through Friday, excluding legal holidays.

FOR FURTHER INFORMATION CONTACT:
By mail:

W. Michael McDavit, Special Review Branch, Registration Division (TS-767C), Office of Pesticide Programs, Environmental Protection Agency, 401 M. St. SW, Washington, DC 20460. Office location and telephone number: Rm. 1006, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. (703-557-1787).

SUPPLEMENTARY INFORMATION: This Notice announces EPA's proposed decision not to initiate a Special Review of 2,4-dichlorophenoxyacetic (2,4-D), 2-(2,4-dichlorophenoxy) butyric acid (2,4-DB), and 2-(2,4-dichlorophenoxy) propionic acid (2,4-DP), and sets forth the rationale for that proposed decision. In summary, EPA has re-evaluated the concerns raised in the September 22, 1986, and December 3, 1986, preliminary notifications to registrants and applicants in light of other relevant information that, in part, has become available since issuance of the preliminary notifications. Based on this review, EPA has determined that a Special Review of 2,4-D, 2,4-DB, and 2,4-DP is not warranted at this time.

I. Introduction**A. Regulatory Background**

The common name for the herbicide 2,4-dichlorophenoxyacetic acid is 2,4-D. The herbicides 2,4-DB or 2-(2,4-dichlorophenoxy) butyric acid and 2,4-DP or 2-(2,4-dichlorophenoxy) propionic acid are structural analogs of 2,4-D. Including the various derivatives of these three chemicals (esters and salts), over 1500 registered pesticide products contain 2,4-D, 2,4-DB, or 2,4-DP as active ingredients.

The active ingredient 2,4-D, first registered in 1948, is a popular, systemic herbicide widely used for controlling broadleaf weeds on a large number of food and non-food crops.

It is also used as a growth regulator on citrus. The majority of 2,4-D is used to control weeds in wheat, field corn, grain sorghum, sugar cane, rice, barley, and range and pastureland. In addition, 2,4-D is used for aquatic weed and forest management, as well as weed control around the home.

The herbicide 2,4-DB is a selective, systemic herbicide used for postemergence weed control. The majority of 2,4-DB is used to control broadleaf weeds in soybeans, alfalfa, and peanuts.

The herbicide 2,4-DP is a selective, systemic herbicide used to control broadleaf weeds, annual grasses, and woody plants. The majority of 2,4-DP is used to control pest plants in turf (ornamental, golf course and lawn areas), non-bearing citrus fruit, rights-of-

way (utility, railroads, highways, etc.), and forestry.

On August 28, 1980, after reviewing all available health effects information on 2,4-D and consulting with the Scientific Advisory Panel (SAP), the Agency issued a Data Call-In notice (DCI) pursuant to section 3(c)(2)(B) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) to the registrants of 2,4-D. This notice required registrants to submit studies on the following areas: acute toxicity, oncogenicity in the rat and mouse, reproductive effects, teratogenicity (birth defects), neurotoxicity, and metabolism. Since that time, all of these required data have been received and reviewed by the Agency.

The Agency has also recently reviewed a number of epidemiology studies relevant to these pesticides, including a new study conducted by the National Cancer Institute and University of Kansas that found an association between farm herbicide use and non-Hodgkin's lymphoma. Published in the *Journal of the American Medical Association* on September 5, 1986, the authors of this study concluded that the use of phenoxy herbicides, including 2,4-D, was linked to an increased cancer risk among farmers handling such herbicides.

Based on this epidemiological evidence, on September 22, 1986, the Agency issued a preliminary notification of Special Review to the registrants of 2,4-D pursuant to 40 CFR 154.21. On December 3, 1986, the Agency issued a similar preliminary notification of Special Review to the registrants of 2,4-DB and 2,4-DP because the Agency believed that these compounds were toxicologically similar to 2,4-D and should be reviewed at the same time.

B. Legal Background

A pesticide product may be sold or distributed in the United States only if it is registered or exempt from registration under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) as amended (7 U.S.C. 136 *et seq.*). Before a product can be registered it must be shown that it can be used without causing "unreasonable adverse effects on the environment" [FIFRA section 3(c)(5)]. The term "unreasonable adverse effects on the environment" is defined in FIFRA section 2(bb) as "any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide." The burden of proving that a pesticide meets this standard for registration is, at all times, on the

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proponent of initial or continued registration. If at any time the Agency determines that a pesticide no longer meets this standard, the Administrator may cancel this registration under section 6 of FIFRA.

The Special Review process provides a mechanism to permit public participation in EPA's deliberations prior to issuance of any Notice of Final Determination describing the regulatory action which the Administrator has selected. The Special Review process, which was previously called the Rebuttable Presumption Against Registration (RPAR) process, is described in 40 CFR 154, published in the Federal Register of November 25, 1985 (50 FR 49015).

Prior to formal initiation of a Special Review, a preliminary notification is sent to registrants and applicants for registration pursuant to 40 CFR 154.21 announcing that the Agency is considering commencing a Special Review. In this case, that notification was issued on September 22, 1986 for 2,4-D, and on December 3, 1986 for 2,4-DB and 2,4-DP. Registrants and applicants for registration were given 30 days to comment on the Agency's proposal to commence a Special Review. Most registrants responded to the notifications by concurring on one particular comment provided in response to the September 22, 1986, 2,4-D notification on behalf of the Industry Task Force on 2,4-D Research Data. A few unique comments were also received in response to the December 3, 1986 notification. These comments will be briefly addressed in Unit IV of this Notice.

If the Agency determines, after issuance of a notification pursuant to 40 CFR 154.21, that it will not conduct a Special Review, it is required under 40 CFR 154.23 to issue a proposed decision to be published in the Federal Register. This Notice is being issued under 40 CFR 154.23. That regulation requires that a period of not less than 30 days be provided for public comment on the Proposed Decision Not To Initiate a Special Review. Subsequent to receipt and evaluation of comments on the Proposed Decision Not To Initiate a Special Review, the Administrator is required by 40 CFR 154.25 to publish in the Federal Register his final decision regarding whether or not a Special Review will be conducted.

II. Risk Concerns Underlying 40 CFR 154.21 Notification

A. Epidemiologic Evidence

The preliminary notifications under 40 CFR 154.21 were issued as a result of

Agency concerns raised by the findings of a new epidemiological study of Kansas farmers. The researchers had found an association between farm herbicide use and non-Hodgkin's lymphomas in farmers based on a population-based case control study (Vol. 25, No. 9, Journal of the American Medical Association, pp. 1141-1147) conducted by the National Cancer Institute and the University of Kansas.

This study was performed to determine if there was any relationship between agricultural herbicide use and soft-tissue sarcoma, Hodgkin's disease, or non-Hodgkin's lymphoma. Newly diagnosed cases of the three diseases were taken from a population-based registry covering the State of Kansas and compared with control groups from the general population of Kansas using Medicare and mortality files. Telephone interviews were then conducted with living individuals (or next-of-kin for deceased individuals) belonging to case and control groups. Numerous questions were asked with respect to farming practices and the use of pesticides. An attempt was made to corroborate information from telephone interviews with records or knowledge of pesticide use by local suppliers of pesticides.

In summary, the study found that Kansas farmers who used certain types of herbicides had an excess risk for developing non-Hodgkin's lymphoma. No association was found between farm herbicide use and soft-tissue sarcoma and Hodgkin's disease. Farmers exposed to the herbicides for more than 20 days each year had six times the risk of developing non-Hodgkin's lymphomas when compared to controls. Among these frequent users, those who mixed or applied the herbicides themselves had eight times the risk. These excess risks were reportedly associated with the use of phenoxyacetic herbicides, including 2,4-D.

EPA scientists and four epidemiology experts, requested by EPA to review the new evidence, generally agreed that the NCI/Kansas study was well conducted and that the study served as a good basis for a hypothesis of a non-Hodgkin's lymphoma and phenoxy herbicide association. Notwithstanding the lack of specificity for 2,4-D, the study was regarded by the reviewers as the most relevant epidemiological study of its kind that pertains to 2,4-D as a pesticide and provides a sound foundation for further inquiries.

A number of critical problem areas, common to many epidemiology studies, have been noted by reviewers. Some of the key areas of concern are the lack of appropriate controls, exposure to multiple chemicals, and insufficient

information on actual exposure to 2,4-D and other pesticides.

In order to evaluate the occurrence of non-Hodgkin's lymphoma cases among farmers with exposure to 2,4-D, appropriate study controls should be used. Farmers have different lifestyles (e.g., diet, exposure to animal viruses) than the general population. Differences in habit and lifestyle may confound results when comparisons are made with controls from the general population. In this case, the study did not choose controls based on occupation and, therefore, it is quite possible that lifestyle factors other than herbicide use may have confounded the results.

Farmers are frequently exposed to other chemicals or potentially tumorigenic agents which could account for some or all of the non-Hodgkin's lymphoma cases. Researchers did report some positive associations with the use of other types of pesticides, such as fungicides and insecticides. In addition, before the phenoxy herbicide, 2,4,5-T, was suspended by the Agency in 1979 based on part on the risk posed by the presence of the carcinogenic impurity, 2,3,7,8-tetrachlorodibenzo-p-dioxin, farmers in Kansas used pesticide products containing 2,4,5-T. Fertilizers, fuel, and other environmental toxicants, as well as biological agents (e.g., viruses) may also present some risk of non-Hodgkin's lymphoma for farmers. Unless exposure variables associated with farming are better controlled, it is difficult to reach conclusions on any contribution of 2,4-D or other specific phenoxy herbicides to the onset of non-Hodgkin's lymphoma in farmers.

The information obtained on 2,4-D use and exposure is incomplete. Reported use, particularly when that use occurred many years previously, is not necessarily a good surrogate measure of exposure. This type of information is useful, but substantially less reliable than some quantitative measure of exposure. Information obtained from next-of-kin should be used with caution. Living farmers and next-of-kin frequently have incomplete recall with respect to specific pesticide names or work practices. Although researchers tried to verify the veracity of the information gathered from telephone interviews by contacting a sample of pesticide suppliers, only about half of the contacted suppliers were able to confirm respondents' answers concerning use of 2,4-D (personal communication, Blondell 1987).

Some reviewers noted an apparent underreporting of all herbicide use. U.S. Department of Agriculture records indicate that significantly more

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pesticides were used in Kansas than was suggested by the results of the telephone survey. This discrepancy alone introduces substantial uncertainty in the pesticide use information obtained from and relied on in this study.

Taken together, these problem areas or uncertainties make it impossible to pinpoint 2,4-D alone as the causative agent in these particular non-Hodgkin's lymphoma cases. As previously mentioned, uncertainties of these kinds are typically present to some degree in all epidemiology studies. Nonetheless, findings of epidemiology studies are frequently insightful and, on occasion, such insight is sufficient for policy-making and regulation. In this case, the extent and degree of these weaknesses limit the usefulness of the study for regulatory purposes.

A number of other epidemiological studies pertaining to 2,4-D were also evaluated by the Agency. Some of the existing epidemiologic studies on 2,4-D and related compounds indicate an association with cancer in humans and others do not. Those studies finding a relationship with cancer in humans were determined to be inadequate for establishing a specific association between cancer risk and 2,4-D use. As mentioned above, the NCI/Kansas study, while relevant to farmers handling phenoxy herbicides, was also determined to be inadequate for establishing a specific association between 2,4-D and non-Hodgkin's lymphoma.

In addition, a recently published epidemiologic study designed to address the same issue at the NCI/Kansas study, that is, the relationship between occupational exposure to phenoxy herbicides and cancer in humans, did not confirm the NCI/Kansas study's conclusions with respect to non-Hodgkin's lymphoma. Woods, *et al.* (Soft Tissue Sarcoma and Non-Hodgkin's Lymphoma in Relation to Phenoxyherbicide and Chlorinated Phenol Exposure in Western Washington, *Journal of the National Cancer Institute* 1987; 78: 605-610) studied male farmers handling a variety of phenoxy herbicides and chlorinated phenols, including 2,4-D and found "small but significantly increased risks of developing [non-Hodgkin's lymphoma] in association with some occupational activities where phenoxyherbicides have been used in combination with other types of chemicals, particularly for prolonged periods." However, the investigators did not find a positive association between increased cancer risks and exposure to 2,4-D.

B. Laboratory Evidence

In response to the Data Call-In notice issued in 1980, the Industry Task Force on 2,4-D Research Data sponsored, among other things, oncogenicity studies in the rat and mouse. The rat study found equivocal evidence of oncogenicity and the mouse study found no treatment-related oncogenic responses.

In the rat, 2,4-D (97.5 percent purity) was administered in the diet to male and female rats at levels of 0, 1, 5, 15, and 45 mg/kg/day for 24 months. At an interim sacrifice of 53 weeks, an apparent treatment-related increased incidence of brain tumors (astrocytomas) was observed in male animals. No tumor response related to 2,4-D administration was observed in female rats.

The results of the final rat study were subjected to two statistical evaluations. Using the Fisher-Exact test, the increased incidence of tumors seen in male animals at the high dose level was not statistically significant when compared to control male animals. Using the Cochran-Armitage trend test, 2,4-D administration was found to be associated with a marginally statistically significant positive dose-related trend for astrocytomas in male rats. Thus, neither evaluation found strong statistical evidence of oncogenicity in the rat.

In the mouse, 2,4-D (97.5 percent purity) was administered in the diet to male and female animals at levels of 0, 1, 15, and 45 mg/kg/day for 24 months. No oncogenic effects attributable to 2,4-D administration were found in either male or female mice.

Although there were no oncogenic effects observed in either sex of the mouse and only marginally statistical oncogenic effects observed in the male rat, the Agency still does not believe there are adequate laboratory animal data to unequivocally assess the carcinogenic potential of 2,4-D. This is predicated on the Agency's conclusion that a Maximum Tolerated Dose (MTD) was apparently not achieved in either test animal. (A MTD, usually the highest dose tested in an oncogenicity study, is a level slightly below the level which resulted in significant life-threatening toxicity in a subchronic study. The level should not be selected too far below a life threatening level because the highest dose tested in an oncogenicity study should elicit significant toxicity without substantially altering the normal lifespan of the test species from effects other than tumor formation.)

Based on the Agency's most current review of the chronic studies and on the

results of subchronic studies with 2,4-D, the highest dose tested (45 mg/kg) in both the rat and mouse oncogenicity studies did not achieve a MTD (45 mg/kg is estimated to be only one-third to one-half of the MTD).

In 1985, scientists from NIH's National Toxicology Program (NTP) questioned the dose levels selected for the oncogenicity studies based on their evaluation of tissue slides taken from the subchronic studies and an interim sacrifice of test animals in both two year oncogenicity studies. The NTP pathologists concluded unanimously that the various kidney lesions observed in the subchronic studies, which were used to estimate the MTD and to support the dose levels used in the oncogenicity studies, were minimal in severity and clearly not life-threatening even at the highest dose of 150 mg/kg. They also concluded that the interim sacrifice data from the oncogenicity studies showed only minimal toxicity at the highest dose tested (45 mg/kg). Their overall conclusion was that the oncogenicity studies on 2,4-D were probably not being conducted at a MTD.

Having now evaluated the two year oncogenicity studies and having considered the scientific opinion of the NTP scientists, the Agency has decided to require, under authority of FIFRA section 3(c)(2)(B), additional oncogenicity testing in the rat and mouse to ensure that a MTD is achieved.

Additional toxicological information, including more detailed reviews of the rat and mouse studies, is available in the 2,4-D public docket.

In April 1987 the Agency concluded that the rat evidence provided limited evidence of oncogenicity in animals. Furthermore, the Agency concluded that although the NCI/Kansas study was well conducted, it provided "inadequate" evidence of cancer in humans attributable specifically to 2,4-D. Given these two conclusions, the Agency tentatively classified 2,4-D as Interim Category C (possible human carcinogen), based on the Agency's "Guidelines for Carcinogen Risk Assessment", and subsequently presented its conclusions and all available information regarding 2,4-D's potential to cause cancer to the FIFRA Scientific Advisory Panel for consideration in June 1987.

III. Scientific Advisory Panel Review

On June 25, 1987, the FIFRA Scientific Advisory Panel (SAP) met to review the data base supporting EPA's preliminary decision to classify 2,4-D as an Interim Class C carcinogen. The Panel was asked, "Does the Panel agree with the

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[Agency's Internal] Peer Review Committee's conclusion concerning the Interim Category C classification of the available 2,4-D oncogenicity data?"

On behalf of the Industry Task Force on 2,4-D Research Data, a number of expert witnesses provided written and oral comments to the Panel in an attempt to rebut the validity of the epidemiological and laboratory evidence. Comments concerning the epidemiological evidence were similar to those discussed in Units II.A. and IV of this notice. Regarding the laboratory evidence, one witness commented that the tumors noted in the rat study were not treatment-related because they failed to display certain commonly associated characteristics of this tumor type. The commentator also argued that the tumors in the high dose group were spontaneous in origin based on the presence of a brain tumor in one control animal.

In conclusion, the Panel issued the following written response on July 8, 1987:

The SAP does not agree with the [Agency's Internal] Peer Review Committee's conclusion that the available 2,4-D oncogenicity data should be classified as an Interim Category C (Possible Human Carcinogen). The Panel believes that the rat and mouse oncogenicity studies are adequate in design and conduct. The data are negative for oncogenicity in female rats and both sexes of mice. The increased incidence of astrocytomas in male rats exposed to 45 mg/kg 2,4-D was considered equivocal evidence of oncogenicity. The Panel believes that additional testing is required to resolve this issue. This testing should specifically address the astrocytoma issue by repeating an oncogenicity study. The study design should include two male rat control groups of 50 each and two male rat groups of the same size exposed to 45 mg/kg 2,4-D.

The Panel also believes that the human epidemiology studies represent well-designed and conducted investigations that present equivocal data on 2,4-D's oncogenicity for humans. Additional studies are underway that should help clarify the issue.

The Panel notes that equivocal evidence is different from limited evidence and that until additional data are developed it is improper to label 2,4-D as a carcinogen or a noncarcinogen. The Panel therefore concludes that the present data for animals and humans are inadequate for determining oncogenicity and that 2,4-D should be classified in Group D (Not Classifiable as to Human Carcinogenicity).

Dated: July 8, 1987

Stephen L. Johnson,

Executive Secretary, FIFRA Scientific Advisory Panel.

The Agency's Peer Review Committee has deliberated on the scientific issues involving 2,4-D since the SAP meeting. The Agency now concurs with SAP's conclusions regarding the classification

of 2,4-D with respect to carcinogenicity. The human and animal evidence of carcinogenicity is insufficient and, therefore, 2,4-D should be considered unclassifiable with respect to carcinogenicity (Category D). The Agency agrees with the SAP that the absence of strong statistical evidence in the rat does not support a finding of "limited" evidence of carcinogenicity. The Agency also agrees with SAP that additional testing is necessary in the rat in order to assess accurately 2,4-D's oncogenic potential in that species. This need is further supported by the concern that a MTD was not reached in the rat and mouse studies. For that reason, the Agency will also require additional testing in the mouse.

IV. Comments Received on the Preliminary Notifications

Comments were received in response to the preliminary notifications on 2,4-D, 2,4-DB, and 2,4-DP from most registrants. The majority of these commentators concurred on a detailed comment received from the Industry Task Force on 2,4-D Research Data. In addition, several parties prepared comments on behalf of certain registrants regarding the benefits of 2,4-D as a growth regulator on citrus crops.

Although the comment received from the Task Force focused primarily on the NCI/Kansas epidemiology study, it also provided comments on existing animal data and other human epidemiology studies. The Task Force's basic position was that, "the Kansas study does not demonstrate an association between 2,4-D and non-Hodgkin's Lymphoma." Criticisms given by the Industry Task Force were for the most part similar to those raised by the Agency and independent reviewers. These included, but were not limited to, limitations in information collection regarding exposure to 2,4-D, limitations on the general accuracy of next-of-kin information, and the fact that the authors did not adequately examine confounding factors or discuss other possible causative agents (such as viruses).

Upon closer examination, the Agency and most reviewers agree that the NCI/Kansas study provides the basis for additional research, but that on its own, it does not provide a conclusive argument for associating farmer exposure to 2,4-D and non-Hodgkin's lymphoma. Some additional research is now underway at NCI. This research will hopefully help resolve this issue and determine whether such an association exists for 2,4-D.

Most commentators on the 2,4-DB and 2,4-DP preliminary notifications

generally argued that these compounds should not be included in a review of 2,4-D. They pointed out basic differences in metabolism and the ostensible absence of 2,4-DB and 2,4-DP use in the Kansas study area.

The Agency generally agrees that 2,4-DB and 2,4-DP are sufficiently dissimilar toxicologically from 2,4-D to allow for a chemical-by-chemical type of evaluation. Therefore, the Agency will review these compounds individually and evaluate 2,4-D, 2,4-DB, and 2,4-DP in the reregistration process as separate compounds. Separate guidance documents for the reregistration of these pesticides are scheduled to be issued in 1988. However, within these pending evaluations, the Agency will continue to group the esters and salts of each active ingredient with the parent chemical.

The decision to issue separate guidance documents does not preclude the Agency from conducting a joint review of these compounds if at a later time data suggest metabolic or toxicologic similarities which would warrant such a simultaneous review.

V. Agency's Decision Regarding Special Review

Subsequent to the issuance of the preliminary notifications pursuant to 40 CFR 154.21, the findings of a NCI/Kansas epidemiologic study reporting an association between exposure to 2,4-D and human cancer was reviewed, at the Agency's request, by four National experts on epidemiology. These experts concluded independently that the study did not implicate 2,4-D alone as the causative factor for the Non-Hodgkin's lymphoma observed in this study, but rather indicated an association with phenoxy herbicide use in general. In addition, the FIFRA Scientific Advisory Panel reviewed this study, as well as the entire oncogenicity data base and concluded that 2,4-D should not be classified as a carcinogen or noncarcinogen at this time. Instead, the Panel recommended that 2,4-D be classified in Category D. Not Classifiable as to Human Carcinogenicity.

The Agency agrees with the external reviewers and SAP that the epidemiologic evidence as provided in the NCI/Kansas study does not raise as great a concern regarding 2,4-D as originally thought. The available human evidence, now considered inadequate by EPA on the basis of confounding factors and bias, does not establish a credible, causal relationship between 2,4-D and non-Hodgkin's lymphoma among Kansas farmers. Based on this conclusion, the Agency has determined

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that it will not conduct a Special Review of 2,4-D, or its structural analogs, 2,4-DB and 2,4-DP at this time.

The Agency now agrees with SAP that 2,4-D should be classified in Category D with respect to carcinogenicity (Not Classifiable as to carcinogenicity) based on the inadequate evidence of cancer in humans and laboratory animals.

Since the Agency is still interested in the results of further epidemiologic and laboratory studies on 2,4-D, the Agency may initiate a Special Review at a later time depending on the findings of such studies. In particular, NCI is currently evaluating human cancer cases and pesticide use in several other states in the U.S., which may have bearing on the continued registration of 2,4-D. In addition, the Agency will require additional testing in the rat and mouse. The Agency will also issue individual reregistration guidance documents on

2,4-D, 2,4-DB, and 2,4-DP in 1988, which will among other things involve intensive scrutiny of the entire available data bases and data deficiencies of these pesticides.

VI. Public Comment Opportunity and Public Docket

The Agency is providing a 60-day period to comment on this Notice. Comments must be submitted by May 23, 1988. All comments and information should be submitted in triplicate to the address given in this Notice under **ADDRESS**. The comments and information should bear the identifying notation OPP-30000/57. After receipt and evaluation of comments on this Notice, the Agency will publish a final decision in the **Federal Register** regarding whether or not a Special Review will be conducted.

The Agency has established a public docket (OPP-30000/57) for this proposal not to initiate a Special Review of 2,4-D, 2,4-DB, and 2,4-DP. This public docket will include this Notice; any other Notice; pertinent to the Agency's decision regarding the Special Review of 2,4-D, 2,4-DB, and 2,4-DP; non-CBI documents and copies of written comments or other materials submitted to the Agency in response to the pre-Special Review registrant notifications and this Notice regarding Special Review of 2,4-D, 2,4-DB, and 2,4-DP; and a current index of materials in the public docket.

Dated: March 14, 1988.

John A. Moore,

Assistant Administrator, Office of Pesticides and Toxic Substances.

[FR Doc. 88-8293 Filed 3-22-88; 8:45 am]

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January 18, 1988

Reto Engler, Ph.D.
Hazard Evaluation Division
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Dear Dr. Engler:

Enclosed is a copy of Barbara Tilley's epidemiology review as assigned under Task 3-03, Special Project--2,4,-D.

Dr. Tilley has expressed interest in publishing this report (see letter attached). Please advise us regarding requirements for publishing, or call Dr. Tilley direct at (313) 876-7563.

Sincerely,



Sharon M. Ambrose
Deputy Program Manager

cc: Richard Levy
3-03 Task file

Task no 3-03

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Henry Ford Hospital

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January 13, 1988

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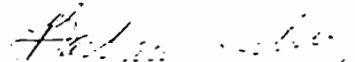
Dear Dr. Weir:

I have enclosed the report assessing the papers on studies of herbicide exposures and cancer. The report was prepared by Christine Cole Johnson, Ph.D, Senior Epidemiologist, with the assistance of Marcia Feingold, Ph.D, Senior Research Biostatistician and myself.

Thank you for the opportunity of preparing this evaluation. We apologize for our delay in getting this to you. The extra time allowed us to conduct a more detailed review than we otherwise might have accomplished. We hope we can be of similar assistance in the future.

We would like to submit this report in a manuscript format for publication. Is that possible? Please get back with us if there are any special requirements or problems. If you have any questions concerning the report, please do not hesitate to call either Dr. Johnson or myself at (313) 876-7563.

Sincerely,



Barbara Tilley, Ph.D
Division Head
Division of Biostatistics
& Research Epidemiology

BT/gcp
Enclosure

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Phenoxy Acid Herbicides, Chlorophenols and Cancer:
an Assessment of the Epidemiologic Studies of
Soft Tissue Sarcoma and the Lymphomas

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January 13, 1988

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INTRODUCTION

The purpose of this report is to assess collectively the results of the epidemiologic studies which have appeared in the literature concerning the associations between phenoxy acid pesticides and chlorophenols and the occurrence of soft tissue sarcoma, lymphoma, and multiple myeloma. Representative risk estimates from individual studies are compared, and where possible, risks associated with differential levels of exposure to these substances are evaluated.

In 1979, case-series and case-control studies from Sweden indicted these chemicals as human carcinogens. The reports from Sweden were followed by a series of epidemiologic studies of populations from many different areas in the world. Because of the widespread use of these substances among agricultural and lumbering occupations and in military endeavors, particularly in Vietnam, this research has been conducted in a relatively politicized atmosphere. While a detriment in the data collection phase of studies, the advantage in this situation for the purpose of this report is that negative and inconclusive as well as positive results have been published. Often when comparing published studies, the selection bias toward the publication of only manuscripts with positive results can make such an effort meaningless. Because of the attention focused on these chemicals and the fact that so many negative or inconclusive studies have been published, publication bias may be less of a problem here.

All observational studies on human beings - in other words,

all traditional epidemiologic studies - have inherent difficulties. Such studies rely on data gathered for other purposes or based on interviews from subjects and in almost all instances people have not been randomly assigned to be exposed or not exposed to a variable under question. Nevertheless, such research is the only available method to directly look at human exposures; comparable studies done in animal populations are not always generalizable to the human organism. Moreover, despite these difficulties many well-accepted relationships between risk factors and disease have been unquestionably demonstrated in epidemiologic studies. This stated, the remainder of this discussion will consider the following studies within the standards of epidemiology rather than debate the value of epidemiologic research.

This report will present a summary of the studies and aggregate analyses followed by a discussion and conclusion. Most of the published papers dealing specifically with these issues since 1970 have been obtained and reviewed. The analyses presented in this paper include only cohort and case-control studies. Case series reports and proportional mortality studies were not included in the tabular presentations. A summary table of studies relating exposures to all cancers is included as Table 1, but with this exception the cancers of interest are considered separately.

SUMMARY ASSESSMENT OF THE STUDIES

1. Cohort Studies

Cohort studies on the association between the phenoxy acid herbicides (PAH), chlorophenols (CP) and human cancer have come from two different sources; 1) industrial exposures involved in the manufacture of such products and 2) exposures involving the application of these substances. The studies from industry have included the follow-up of several cohorts who routinely came into contact with these substances as a part of their work as well as numerous groups of workers who experienced high intensity exposures of short duration due to accidents. Several cohort studies of other occupational groups exposed due to the application of the products have also been reported. In all these studies, exposure was relatively well documented through personnel records; however the number of exposed subjects is small in each case and statistically conclusive results cannot be determined on an individual study basis.

Table 1 presents a summary of the cohort studies of exposures associated with PAH and CP and overall cancer mortality or incidence. Reviewed studies that were more general with regard to exposure (e.g., all pesticides) or with only a short follow-up period were not included. Table 2 presents a similar summary specific to soft tissue sarcomas, lymphomas and multiple myeloma. These three cancers were selected because they were identified in the positive studies and have been the focus of other studies of agricultural exposure.

When presented in a study, relative risk estimates and confidence intervals were abstracted and recalculated for error checks, or, if not presented, calculated if the necessary data were available. Most studies reported data or results with a latency period, usually of 5 years, considered in the analysis, and these were the estimates selected for Tables 1 and 2.

Focusing on soft tissue sarcomas (STS) on Table 2, some risk estimates were elevated, but none were statistically significant. The same held for the various categories of lymphomas. Lymphomas are infrequently occurring cancers, with an estimated age-adjusted incidence rate in U.S. white males of 14.3 per 100,000 (14). Soft-tissue sarcomas are very uncommon, with a corresponding rate of 2.4 per 100,000 (14). In order to increase the sample size, it would be desirable to somehow combine the studies. The studies varied in exposure criteria, although all persons were considered exposed in some degree to phenoxy acid herbicides or chlorophenols. Some studies had disease incidence endpoints; others measured mortality. All the studies were limited to white males with the exception that 3.2% of the Michigan study subjects were American Indian, black and Hispanic males (5). No age-specific person-years of follow-up are available from the reports, as would be expected. However few of the studies present even the total number of person-years of follow-up. Many of the reviewed publications were different reports on overlapping groups of subjects, not always immediately obvious, particularly in the chemical industry studies. Close

attention and careful reading of the chemical industry studies, related articles and letters were required to discern the actual counts of STS cases in these cohorts (15-19).

Table 3 presents a combined analysis that involves the calculation of a crude PMR across all studies for deaths from the specific cancers of interest. The subject groups selected for this analysis are mutually exclusive. As this analysis measures mortality, studies with only incidence data were excluded. The percentage of all cancer deaths in the combined cohorts due to the specific cancer categories was compared to the same percentage calculated for white males aged ≥ 20 years as derived from the 1973-1981 SEER registries combined (14). Younger-aged males were excluded from the comparison population as all of the studies were of employed and retired males.

Two columns are presented in Table 3 for the soft-tissue sarcomas. The pathology material from four cases occurring in three chemical worker cohorts (5, 7, 9) along with three other cases were reviewed by the Armed Forces Institute of Pathology and another independent pathologist (19). Two of the first four cases were found to be incorrectly classified as STS. These cancers are difficult to classify, attested to by the fact that even the two expert reviewers only agreed 71% of the time as to histological subtype and in only two of seven cases did these reviewers and the original pathologist agree. In the case-control studies dealing with this issue that reported results for pathological reviews, a substantial number of subjects originally

classified with STS were reclassified to non-STs diagnoses, e.g. 6.3% (29), 9.3% (31), 19% (38) and 23.2% (33). Thus, any expected numbers for STS generated from the general population cancer registries can be assumed to also include a large number of misclassified cases. It is methodologically incorrect, therefore, to compare rates in study cohorts with pathology material undergoing expert review to general population rates. Calculations are presented here for both the number of cases before review, and the number of cases after pathologic review. The unreviewed PMR is most appropriate since general population rates are being used to compute expected values.

2. Case-Control Studies

The first epidemiologic study reporting an association between a cancer and phenoxy acids/chlorophenols was a case-control study. There is generally more possibility for systematic biases to affect a case-control study compared to a cohort study. However, when rare diseases such as STS and ML are investigated, the case-control approach is the only possible method to employ to obtain timely results (within a 5 year study) for a reasonable cost. Not unexpectedly the majority of the reports of the cancer-herbicide hypothesis have been case-control studies.

Table 4 summarizes the results of 17 epidemiologic studies that are relevant to the issue at hand. Odds ratios and confidence limits were recalculated (with some exceptions as noted) so that all the confidence intervals are computed using

the normal approximation to the logarithm of the odds ratio as suggested by Woolf (52). Unknowns were excluded from analysis. The studies vary with respect to selection of living or deceased cases, the source of cases, and the selection of controls. The classification of a case as exposed varies both by the source of data (ie. personal interview or from available documents) and the criteria for exposure (i.e. job titles versus specific contact with a substance). Studies in which pathological material was not reviewed probably resulted in the misclassification of some percentage of the cases with respect to disease, particularly for the soft-tissue sarcomas. It is also likely that there was a good deal of misclassification among exposure categories-particularly where death certificate occupations were used as surrogate measures of exposure. However, if both types of misclassification occur uneffectively among the cases versus the controls and the exposed versus the nonexposed, respectively, odds ratios estimates are biased toward one.

Most of the studies in Table 4 have moderately increased risk estimates, although a few studies reported high and statistically significant estimates in the 4.0 - 5.0 range with relatively narrow confidence limits. Table 5A presents studies where the appropriate data could be abstracted for the specific cancers in order to attempt the calculation of a summary odds ratio. Studies where the only disease data was a death certificate or where raw data were not available were excluded (20,27,30,21,28,37). The data for one 2x2 contingency table per

study were included. For each study where different exposure categories were analyzed, the data representing combined exposures to the putative substances were selected. If data using both occupational titles and specific chemical exposure were available, the specific chemical exposure was used. If only occupational titles were used in a study but the data were stratified by exposure potential (eg. residence in a high phenoxy acid herbicide use county) the criteria for the highest potential exposure were selected for the summary analysis.

In some instances, data were matched in the reported analyses. Sometimes the authors noted that unmatching the data did not affect the results and presented unmatched analyses; for others this procedure could be accomplished from data included in the manuscripts. Unmatching the data never resulted in a substantial change in the results. Where applicable, the matching was dissolved for the studies included in Table 5A.

Table 5B presents the summary statistics by disease category. All three diseases yielded moderately elevated statistically significant risk estimates; however the Breslow-Day test, which tests for the homogeneity of the individual study odds ratios, revealed that the individual estimates for soft tissue sarcomas and lymphomas were disparate. The Swedish studies that initially reported these associations had the highest estimates and have also received the most criticism (16,39-42). These studies were removed from the summary analysis with results as depicted on the bottom half of Table 5B. No

association was evident for soft tissue sarcomas. A statistically significant, slightly elevated indication of risk was evident for the lymphoma category. (In both categories, the Breslow-Day test did not reject the hypothesis that the strata-specific odds ratios were equal.)

3. Dose-Response Assessment

Table 6 presents risk estimates excerpted from earlier tables or the original reports where results were derived for presumably low or general exposure categories as well as high exposure groups within a population. A higher exposure for this table was defined by intensity as indicated by methods of exposure (spraying or mixing the material, or exposure during an accident), clinical evidence of heavy exposure (a history of chloracne), a longer duration of exposure, or other measures constructed by the authors.

For soft tissue sarcomas, three of the seven presented study locations in Table 6 (4,7,9,33) display an increased risk with increased exposure levels. In addition, the category in the Washington State study (37) implying definite and intense exposure, (as indicated by a history of chloracne), had a relatively higher estimate of 3.3. In the Michigan cohort (5), the one case that occurred was in the high exposure category. The exposure categories were relatively less reliable in the remainder of the studies (10, 31). In sum, a dose-response trend for soft tissue sarcomas cannot be ruled out.

Six of the seven study locations dealing with lymphomas

indicate a dose-response pattern. In the Michigan and W. Virginia cohorts, as well as the Wisconsin, Iowa and Kansas case-control results, higher exposures were associated with increased risk. In the Washington case-control study, a slight trend is seen for PAH but not CP exposure, but again, the group with definite exposure demonstrated by a history of chloracne had the highest risk estimate. The one study presented for multiple myeloma indicated a positive association between exposure and risk.

A second approach was used to assess risk associated with varying levels of exposure. Additional data were provided to us on herbicide use in the U.S. for one year, and information was obtained on state farm acreage from census publications (53). These data have been combined in Figure 1 with the results of those studies that were done in the U.S. that were associated with agricultural exposures. Risk estimates are plotted against estimated exposure in each state. Exposure is defined as the total pounds A.I. of 2,4-D use in each state in 1974 divided by the state's 1974 farm acreage. Only estimates pertaining to non-Hodgkin's lymphoma (NHL) were plotted as there were only two appropriate studies for STS (see Appendix-Table 1 for data.) No dose-response relationship for NHL is evident.

This approach suffers in that it is a comparison based on ecologic characteristics. While true associations are sometimes reflected in such studies, spurious relationships may occur or associations may be masked when using grouped data in which

exposures are not linked to individuals. An additional limitation in this instance is that the studies cover various exposure periods of differing lengths. The herbicide use in 1974 may not be representative of use throughout the time that PAH and CP have been available.

A third approach was utilized under the assumption that manufacturing processes and environments have improved over time and have been accompanied by a decrease in contaminants and lower exposure levels for workers. Figures 2a-b display risk estimates against the midyear year of eligibility of the cases, specifically, the midyear of the time period when a case could be ascertained either through a diagnosis or death due to the cancer under study. These graphs were constructed to see if changes in manufacturing processes (and thus decreases in contaminants) would affect the outcomes. Figure 2a includes results for soft tissue sarcomas; 2b relates to lymphomas. Case-control and cohort studies, including both manufacturing and application exposures, are included. Since survival for the tumors under study is relatively poor, the graph does not distinguish between cases identified at death versus diagnosis.

The highest estimates were found in earlier midyears for both Figures 2a and 2b - before 1980 for STS and before 1965 for ML. However neither graph shows a definite trend of increased risk estimates inversely associated with case ascertainment midyear. One difficulty with this approach is that the case ascertainment period for many of the studies covered wide ranges

of years.

DISCUSSION

In the interpretation of a collection of studies reporting on similar disease-exposure relationships, epidemiologists consider a series of six concepts that were first presented in published form in the Surgeon General's first report on smoking (54). These concepts include; 1) the biological plausibility of an association; 2) the specificity of associations; 3) the temporal sequence of exposure and disease; 4) the strength of associations; 5) the consistency of associations; and 6) dose-response trends. This report has emphasized the last three considerations while taking the second and third into account. In this discussion, all but the first concept -- the biological plausibility -- will be addressed. As stated earlier, the biological plausibility of dioxin contaminants behaving as tumorigens has been demonstrated in several animal studies identifying this substance as a general chemical carcinogen (43-46). Recent reviews conclude, however, that the evidence for carcinogenicity of the phenoxy herbicides irrespective of their dioxin contaminant is inadequate (47-49).

The specificity of an association is increased as the exposure and/or disease become more narrowly defined. Considering the associations only for separate disease categories as was done here permits an assessment of specificity as related to disease. Both the exposure and disease categories in previous reports have been less specific than has been the case in many

other exposure-cancer associations. The exposures have included a number of different herbicides (eg. 2,4-D, 2,4,5-T, amitrol, MCPA, MCPP) and these exposures have been related to several types of cancer (eg. soft tissue sarcoma, non-Hodgkin's and Hodgkin's lymphomas, multiple myeloma as well as nasopharyngeal neoplasms). This lack of specificity was one of the main criticisms of the first Swedish studies. One author points out, however, that soft tissue sarcomas and lymphomas originate from tissues that are embryonically related (42). Moreover, dioxins have been shown to affect the immune system, which could hypothetically lead to increased susceptibility to more than one cancer (46). These analyses did not focus as closely on the specificity of exposure - particularly with respect to separating the effects of the individual phenoxy herbicides. Such analyses could be pursued; however the data would become sparse if both disease and exposure categories are specified precisely.

Particularly in case-control studies the temporal sequence of exposure to disease requires close attention. It is not always simple to assure that a suspect exposure precedes disease. In a number of the studies in this series, it was evident that exposure periods overlapped with the time periods where cases could occur. Most of the studies attempted to address this problem by presenting analyses that imposed a latency period--disease had to occur after a designated time span after first exposure occurred. When available, the data that incorporated a latency period were selected for the analyses in this report.

Many studies used 5 years or 10 years, however, which may be much too short a time period. The generally accepted average latency period for cancer is 20 years. The length of time between first exposure and the diagnosis of cancer in the two soft tissue sarcoma cases verified by pathological review in the occupational cohorts were 32 and 30 years (16,7). It is impossible to recalculate estimates using different latency periods without the raw data.

The strength of an association is measured by the magnitude of risk estimates. A few studies (4,22,26,25) from Sweden and Finland reported consistently high estimates in the 3.0 - 6.0 range. Most of the positive studies detected relatively moderate increases in risk falling between 1.2 and 3.0. The summary estimates were also only moderately elevated. In many cases, however, the risk estimates were statistically significant. Thus a weak association, in particular for lymphomas, may be indicated.

With this level of risk these exposures would likely account for few of the total of these cancers in a population. The attributable risk is a measure which estimates the maximum proportion of a disease that can be attributed to a putative characteristic assuming that other etiologic factors are distributed equally. Considering the summary odds ratio of 1.2 for the lymphomas as shown on Table 5B, if the prevalence of exposure in the population is 10%, these exposures account for 2.0% of all the lymphomas that have occurred in that population.

If the exposure prevalence to PAH and CP is 20%, the attributable risk percent is 3.8. These relatively low attributable risks are consistent with estimated population risks for exposure to 2-4-D that were based on theoretical projections from animal carcinogenic studies (49). Unfortunately, no estimate is readily available regarding the actual proportion of the population who are exposed to these substances (46).

This report's primary focus is the assessment of the consistency across studies. Flaws can be found in any individual epidemiologic study, but when similar results are found across studies using varying methodologies in different populations, the case for an association is strengthened. Such an evaluation is difficult to conduct analytically, particularly when published reports are the only available data. Termed "meta-analyses", Tables 3, 5a and 5b present such analyses.

Even combined (Table 3), the cohort studies give equivocal results. The proportion of deaths due to the cancers under study is higher than expected in all cases. The confidence intervals, however, include 1.0. Furthermore, the confidence intervals do not cover a wide range of values which would be indicative of inconclusive results. In addition, it is generally accepted that PMR's are difficult to interpret.

One other case of STS (a reviewed and confirmed malignant fibrous histiocytoma) occurred among one of the chemical worker cohorts (5) but was not included in the reported study or on Table 3. This subject died in 1983 and the last day of follow-up

selected for this SMR study (published in 1987) was 12/31/82. This case was, however, diagnosed and known to the authors earlier than March 1981 (16). The duration between onset of exposure and death was 32 years. Including this case among those in the STS "unreviewed" yields 4 observed versus 0.85 expected for a PMR of 4.71 (95% confidence limits of 1.28 and 12.05). The bias introduced with respect to the crude PMR by adding this death is that other cancer deaths occurring in 1983 are not included in the total, which, if large in number, would tend to increase the expected number of STS deaths and decrease the PMR. In this cohort, 81 cancer deaths occurred between 1940 and 1982. If all occurred in the last ten years, the average would be 8 per year which would change the total number of cancer deaths to 177. The expected number of deaths would rise from 0.85 to 0.88, resulting in a negligible change in the PMR and confidence limits (4.55, 1.24-11.64). If 48 additional cancer deaths (for a total of 217) occurred in 1983 this estimate would remain statistically significant.

The inclusion of the fourth soft tissue sarcoma case resulted in a statistically significant PMR. With this crude analysis, however, it is difficult to reach any firm conclusions.

The case-control study summary results are more rewarding. After excluding the studies that were inappropriate in some manner, the cases and controls were combined in an analysis that was stratified by study. When the initial studies were excluded -- much like initial cases or clusters that stimulate a

hypothesis are not counted in an individual study -- the summary odds ratio for soft tissue sarcoma was 1.1 with a tight confidence interval. The slightly elevated risk for the lymphomas was sustained.

Figures 3a and 3b show odds ratios from the case-control studies (both significant and not) plotted against the number of cases in each study for STS and ML, respectively. These plots were done to see if the odds ratios tend to cluster around a central point, with larger samples more closely clustered around a single true population value (if such exists). Figure 3a, for STS, is not very informative. Most of the studies included less than 300 cases; of the two estimates based on the one larger study, one estimate could be considered as strong negative evidence ($OR = 1.1$, 95% CI of 0.8-1.5). The other estimate indicated a slightly higher risk ($OR = 1.7$, $p < .05$, 95% CI of 1.0-2.9). The next largest sample sizes ranged from between 250-300 cases; both of these odds ratios were less than 1.0. The results of the other studies, all with a case number less than 150, had widely disparate results.

Figure 3b for estimates related to the lymphomas is more what one would expect if a moderate association is present. As the number of cases increases, the estimates become less disparate. The values tend to cluster about a point between risks of 1.0 and 1.5, again indicating an association that is positive but of very moderate magnitude.

The results presented on Table 6 are suggestive of a dose-

response effect, particularly for the lymphomas, although some reports imply the same for soft tissue sarcomas. It is of particular interest that moderately high risks were found in the Western Washington State study to be associated with a history of chloracne (3.3 for STS and 2.1 for lymphomas). Chloracne indicates definite exposure to TCDD, the dioxin contaminant of 2,4,5-trichlorophenol and 2,4,5-trichlorophenoxy acetic acid. Risk was highly elevated for two other groups of people with diagnosed chloracne. the workers exposed via accidents in Michigan (12) and W. Virginia (7). In the W. Virginia accident, 1 case of STS and 1 lymphoma were diagnosed in a group of 121 workers. (see Table 1). In the Michigan accident group, 3 subjects died of cancer versus 1.6 expected in a cohort of 61 employees followed from the date of the accident, 1964, through 1978. One subject died of glioma, another of adenocarcinoma with an unknown primary site, and the third died of a fibrosarcoma, which was later reclassified as a renal clear-cell carcinoma (19). However another subject in this accident group died of a histologically confirmed soft tissue sarcoma in 1983, as was noted earlier in this discussion (5).

CONCLUSION

The two most recent studies in Kansas and Washington, (Hoar, et al. and Woods, et al., respectively), appear to be well-conducted studies that were designed to avoid the problems encountered in earlier studies. The Kansas study found associations only for lymphomas, specifically the non-Hodgkin's

lymphomas. The authors also reported strong indications that higher doses and longer exposure periods were directly associated with risk of NHL. The overall results of the Woods' study were negative. However, several subgroups that were evaluated because of findings in previous studies had elevated and significant risks for NHL; including farmers (see Table 4) as well as forestry herbicide applicators (OR=4.8) and men with a longer period of exposure (15 years) to phenoxy herbicides at least 15 years prior to diagnosis (OR=1.7) (37).

The hematopoietic cancers have been associated with farming as an occupation in many other studies (50-51). Most of the persons exposed in the majority of the case-control studies were exposed through farming-related work. These studies together point to a moderate association between lymphomas and farming. A common occupational exposure for farmers in the past decades has been PAH and CP. It should be realized, however, that another type of farming exposure positively correlated with herbicide exposure could be a true etiologic factor, with herbicide exposure an indirect association.

As more studies are conducted and reported, the specificity of exposure as well as disease could be enhanced. In many of the previous studies it is difficult to separate 2,4-D from 2,4,5-T exposure (49). Most of the studies involve presumed exposure to 2,4,5-T and its associated contaminant dioxin either alone or in combination with other substances (Tables 2 and 4).

In summary, carcinogenicity in humans from exposure to the

phenoxy herbicides and chlorophenols cannot be ruled out by the studies reported to date. The summary analyses presented here, and the evidence of previous studies of agricultural occupations, give greater credence to the possibility of a relationship between these exposures and the lymphomas.

TABLE 1: SUMMARY OF COHORT STUDY RESULTS FOR TOTAL CANCER MORTALITY AND INCIDENCE**

Location(Ref)	Exposure	Sample Size	Type of Cancer	Risk Estimate*	95% CI	Comments
Sweden (1) Axelson and Sundell 1974	phenoxy acids amitrol	207 ⁺ 152 ⁺	all all non-resp	1.8 3.7* 3.1	0.6-4.1 1.5-7.6 1.0-7.2	railroad workers exposed > 45 days from 1957-1972; followed 1957-1972; 5 yr latency incorporated.
Sweden (2) Axelson et al 1980	phenoxy acids & amitrol	348	all non-stomach/ lung	3.4* 3.2	1.2-7.3 0.9-8.2	same as (1) followed 1957-1978; 10 yr latency incorporated; no lymphomas but 1 STS in group exposed to amitrol; combined exposure has greater effect than individual exposure.
	phenoxy acids	207	all non-stomach/ lung	1.9 1.8	0.7-4.2 0.5-4.7	
	amitrol	152	all non-stomach/ lung	1.5 1.5	0.3-4.5 0.2-5.3	
Finland (3) Riihimaki et al 1982	phenoxy acids	1926	all non-lung	0.8 0.6	0.5-1.3 0.3-1.2	herbicide applicators exposed ≥ 14 days from 1955-71, followed 1972- 1980; 10 yr. latency incorporated.
Denmark (4) Lyngbe 1985	phenoxy acids & cresol	3390 ⁺	all	1.0	0.8-1.2	2 herbicide manufactur- ing plants; all employees from 1947- 1982; followed from 1947-1982; no latency incorporated.

(con't)

Location(Ref)	Exposure	Sample Size	Type of Cancer	Risk Estimate*	95% CI	Comments
Michigan (5) Ott et al 1987	2,4,5-T (with dioxin)	2187	all	1.0	0.8-1.3	chemical workers employed 1940-1980; followed from 1940-1982; no latency incorporated; includes 61 subjects involved in a 1964 chloracne accident.
Sweden (6) Hogstedt & Westerlund 1980	phenoxy acids	161 16	all (highly exposed)	0.7 2.9	0.1-2.1 0.3-10.3	forest workers employed > 5 days from 1954-1967; followed from 1954-1978; 10 yr latency incorporated.
W. Virginia (7) Zack & Suskind 1980	2,4,5-T	121	all (with dioxin)	0.7*	0.5-1.0	chemical workers accidentally exposed and diagnosed with chloracne in 1949; followed from 1949-1978; followed from 1955-77; no latency incorporated.
Germany (8) Theiss et al 1982	2,4,5-T (with dioxin)	74	all	1.7	0.7-3.5	chemical workers accidentally exposed in 1953, followed to 1979; no latency incorporated; no STS or ML.
W. Virginia (9) Zack & Gaffey 1983	2,4,5-T (with dioxin)	884	all	1.1	0.8-1.6	plant chemical workers with potential exposure; > 1 yr. employment from 1955-77; followed from 1955-77; no latency incorporated.

* p < 0.05

+ incidence rather than mortality study

** expected numbers based on respective national population rates in all studies; CI = confidence interval, STS = soft tissue sarcoma, ML = malignant lymphoma.

TABLE 2. SUMMARY OF COHORT STUDIES AND STANDARDIZED MORTALITY RATIOS FOR SOFT TISSUE SARCOMAS, LYMPHOMAS, AND MULTIPLE MYELOMA⁺⁺

Location(Ref)	Exposure	Sample Size	O/E	SMR	95% CI	Comments
SOFT TISSUE SARCOMAS						
Finland (3) Riihimäki et al 1982	phenoxy acids	1926	0/.1	-	-	See Table 1; incon- clusive, if one case found, SMR=10, (0.3-55.7).
Sweden (10) Wiklund & Holm 1986	phenoxy acids	354620 ⁺	nc	0.9	0.8-1.0	Relative risk in Swedish agricultural and forestry workers in 1960 compared to other Swedish employed men; followed 1961- 79; high exposure category included those in silviculture.
		7215 ⁺⁺	nc	0.8	0.3-1.9	
Denmark (4) Lyngø 1985	2,4-D phenoxy acid & cresol	3390 ⁺ 940 ⁺⁺	4/1.09 1/0.16	3.7 6.3	1.0-9.4 0.2-34.8	Same as Table 1 except 10 yr latency incorporated; of 4 STS, there are 2 leiomyosarcomas, one dermatofibro- sarcoma of the back and one sarcoma of the retroperitoneum. See Table 1.
Michigan (5) Ott et al 1987	2,4,5-T (with dioxin)	2187	1/0.4	2.5	0.1-13.9	
Sweden (6) Hostedt & Westerlund 1980	phenoxy acids	161 ⁺	0/.1	-	-	See Table 1; same comments as for first study on this Table. (con't)

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Location(Ref)	Exposure	Sample Size	O/E	SMR	95% CI	Comments
W. Virginia (7) Zack & Suskind 1980	2,4,5-T (with dioxin)	121	1/.05***	22.2	0.6-123.8	See Table 1; number expected for STS was grouped with "other cancer sites".
W. Virginia (9) Zack & Gaffey 1983	2,4,5-T (with dioxin)	884	1/.18***	5.7	0.1-31.8	See Table 1; number expected for STS was grouped with "other cancer sites"

(con't)

<u>Location (Ref)</u>	<u>Exposure</u>	<u>Sample size</u>	<u>Disease</u>	<u>O/E</u>	<u>SMR</u>	<u>95% CI</u>	<u>Comments</u>
LYMPHOMAS + MULTIPLE MYELOMA							
Finland (3) Riihimäki et al 1982	phenoxy acids	1926	NHL MM	0/.8 1/0.2	- 5.0	- 0.1-27.9	See Table 1; inconclusive - if one case had occurred the SMR would be 1.25 (0.3, 7.0)
Denmark (4) Lyngø 1985	2,4-D phenoxy acid & cresol	3390 ⁺	ML	4/3.04	1.3	0.4-3.4	Same as Table 1 except 10 yr
		940 ⁺⁺	ML	0/0.46	-	-	latency incorpo- rated; inconclusive- if one case had occurred in the in the high exposure subset the SIR would have been 2.2 (0.06, 12.1).
Michigan (5) Ott et al 1987	2,4,5-T (with dioxin)	2187	NHL HD MM	5/2.6 1/1.1 2/1.0	1.9 0.9 2.0	0.6-4.5 0.02-5.1 0.2-7.2	See Table 1.

(con't)

<u>Location (Ref)</u>	<u>Exposure</u>	<u>Sample Size</u>	<u>Disease</u>	<u>O/E</u>	<u>SMR</u>	<u>95% CI</u>	<u>Comments</u>
Sweden (6) Hostedt & Westerlund 1980	phenoxy acids	161 ⁺	ML	0/0.30***	-	-	See Table 1; number expected was not given.
W. Virginia (7) Zack & Suskind 1980	2,4,5-T (with dioxin)	121	ML	1/.33***	3.0	0.1-16.7	See Table 1; number expected for ML was grouped with all hematopoietic cancers.
W. Virginia (9) Zack & Gaffey 1983	2,4,5-T (with dioxin)	884	ML	1/1.30***	0.8	0.02-4.3	See Table 1; number expected for HD was grouped with all hematopoietic cancers.

+ incidence rather than mortality study
* higher exposed subgroup

++ SMR = standardized mortality ratio, SIR = standardized incidence ratio, CI = confidence interval, O/E = observed/expected, STS = soft tissue sarcoma, ML = malignant lymphoma; HD = Hodgkin's disease, NHL = non-Hodgkin's lymphoma; IS = non-Hodgkin's lymphoma or lymphosarcoma subtype; RCS = non-Hodgkin's lymphoma of reticulum cell sarcoma subtype; MM = multiple myeloma; nc = not calculated as necessary data not presented; "-" indicates that zero (0) cases were observed so an SMR was not calculated.

*** Crude risk estimates calculated (since expected numbers not available in the papers) based on the information that 0.5% of the total cancer cases ascertained in the study could be expected to have STS and 3.7% to have ML, as calculated from SEER registry data (14).

Table 3: Crude proportional mortality ratio (PMR) based on 7 combined cohorts of white males exposed to phenoxy acids or chlorophenols compared to all white male deaths, 10 SEER registries combined, 1973-1977.**

Ref*	# in cohort	# deaths	# Cancer Deaths	Causes of Death					
				# STS	# STS Reviewed	Total # Lymphomas	# NHL	# HD	# MM
2	207	20	6	0	0	1	0	1	0
3	1926	144	26	0	0	0	0	0	1
5	2187	370	81	1	0	6	5	1	2
6	161	29	5	0	0	0	0	0	0
7	121	32	9	1	1++	1	0	1	0
8	74	21	7	0	0	0	0	0	0
9	884	163	35	1	0	1	0	1	0
TOTAL	OBSERVED		169	3	1	9	5	4	3
	EXPECTED+++			0.85	0.85	6.25	4.90	1.35	2.37
	PMR			3.53	1.18	1.44	1.02	2.96	1.27
Lower 95% CI				0.73	0.03	0.66	0.33	0.81	0.26
Upper 95% CI				10.31	6.55	2.74	2.38	7.59	3.70

+++ Expected calculated using percentage of all cancer deaths, NCI's SEER program, 1973-1977, white males ≥ 20 yrs, which is; 0.5% for STS, 3.7% for malignant lymphomas combined, 2.9% for NHL, 0.8% for HD, and 1.4% for MM (14).

* Reference 1 was superseded by an extended follow-up report in reference 2. Reference 5 superseded three earlier reports (11-13). References 4 and 10 reported only cancer incidence with no data on mortality.

++ The specific diagnosis was malignant fibrous histiocytoma, confirmed by expert pathology review. The duration between first exposure and death was 30 years.

** STS = soft tissue sarcoma, NHL = non-Hodgkin's lymphoma, HD = Hodgkin's disease, MM = multiple myeloma, CI = confidence interval.

TABLE 4 SUMMARY OF CASE CONTROL STUDIES RELATING CANCER TO HERBICIDE AND CHLOROPHENOL EXPOSURE⁺⁺

Location(Ref)	Exposure	Disease	No. of Cases	No. of Controls	Risk Estimate	95% CI	Comments ⁺
Washington and Oregon (20) Milham 1971	farming occupations on death certificate	HD	506	506	1.1	0.7-1.7	Decedent cases and controls dying between 1960-1967, extensive matching.
		NHL	584	584	1.2	0.8-2.0	
		MM	522	522	1.7*	1.1-2.5	
Israel (21) Abramson et al, 1978	history of working with wood or trees	HD	397	397	1.1	nc***	Incident cases from 1960-72, population-based matched controls, extensive matching.
		HD-mixed cellularity	119	119	5.2*	nc	
Sweden (22) Hardell & Sandstrom 1979	chlorophenols & phenoxy acids	STS	52	206	5.7*	2.7-11.8	hospital cases admitted from 1970-77, general population controls, extensive matching.
	phenoxy acids	STS	46	201	5.3*	2.4-11.5	Positive exposure if > 1 day and 5 years preceding diagnosis.
	chlorophenols	STS	40	193	6.6*	2.1-20.9	(con't)

<u>Location(Ref)</u>	<u>Exposure</u>	<u>Disease</u>	<u>No. of Cases</u>	<u>No. of Controls</u>	<u>Risk Estimate</u>	<u>95% CI</u>	<u>Comments</u>
Sweden (26) Eriksson et al, 1981	chlorophenols	STS	110	219	4.7*	2.3-9.5	All incident cases in 5 counties from 1974-78; positive exposure if > 1 day at least 5 years preceding diagnosis.
	phenoxy acids	STS	92	207	17.0*	2.1-140.0	
	chlorophenols	STS	96	214	3.3*	1.3-8.6	
Sweden (25) Hardell et al, 1981	phenoxy acids & chlorophenols	HD+NHL	169	335	5.3*	3.3-8.7	Hospital cases admitted from 1974-78, general population controls. Positive exposure if > 1 and at least 5 yrs preceding diagnosis.
	phenoxy acids	HD+NHL	149	327	4.8*	2.9-8.1	
	chlorophenols	HD+NHL	144	319	4.3*	2.6-7.1	

(con't)

<u>Location(Ref)</u>	<u>Exposure</u>	<u>Disease</u>	<u>No. of Cases</u>	<u>No. of Controls</u>	<u>Risk Estimate</u>	<u>95% CI</u>	<u>Comments</u>
Wisconsin (27) Cantor, 1982	farming occu- pations on death certificates	NHL	774	1651	1.2	(1.0-1.5)	Decedent cases from 1968-76, other deaths frequency matched for controls (smoking related diseases excluded). Farm laborers considered not exposed.
	farmers dying < 65 years	NHL	343	746	1.7*	(1.1-2.5)	
	young farmers in counties with high herbicide use	NHL	nc	746	2.1	(1.0-4.3)	
Iowa (28) Burmeister et al, 1983	farming occu- pations on death certifi- cate	MM	550	1100	1.5*	nc	Decedent cases from 1964-78; other deaths for controls matched on age, yr of death and county of residence. Farm laborers considered exposed.
	farmers < 65 at death	NHL	447	894	0.7	nc	
	farmers > 65 at death	NHL	654	1308	1.8*	nc	
		MM	400	800	1.5*	nc	
	farmers born after 1900 from high herbicide use areas	MM	nc	nc	2.4*	nc	

(con't)

<u>Location(Ref)</u>	<u>Exposure</u>	<u>Disease</u>	<u>No. of Cases</u> nc	<u>No. of Controls</u> nc	<u>Risk Estimate</u> 3.4*	<u>95% CI</u> nc	<u>Comments</u>
New Zealand (29) Smith et al, 1984	farmers born before 1890 from high herbicide use areas phenoxy acids	NHL STS	82	92	1.2	0.6-2.6	Incident cases from 1967-1980, registry cancer controls matched on yr of diagnosis and age. Unmatched in analysis. Positive exposure if > 1 day and 5 yrs preceding diagnosis.
	chlorophenols	STS	82	92	1.5	0.5-4.7	
Wisconsin (30) Cantor + Blair,	farming occupations on death certificates	MM	411	926	1.4	1.0-1.8	Same as above study by Cantor (27). No associations with residence in high herbicide use areas.
	farmers < 65 at death	MM	136	277	1.1	0.6-1.9	
	farmers ≥ 65	MM	175	649	1.5	1.1-2.0	
New York State (31) Greenwald et al 1984	Vietnam Service	STS	281	281	0.5	0.2-1.2	Incident cases from 1962-80, 18-29 yrs old; controls from motor vehicle license listing and mortality files, partial path review.
	Agent Orange	STS	252	252	0.7	0.3-1.8	

(con't)

<u>Location(Ref)</u>	<u>Exposure</u>	<u>Disease</u>	<u>No. of Cases</u>	<u>No. of Controls</u>	<u>Risk Estimate</u>	<u>95% CI</u>	<u>Comments</u>
England (32) Balarajan + Acheson, 1984	agricultural and forestry occupations	STS	1961	1961	1.1	0.8-1.5	Incident cases > 15 yrs old from 1968-76. Cancer controls. Occupation at diagnosis from registry. Matching factors not stated but matched analysis.
	farming ^a	STS	1961	1961	1.7*	1.0-2.9	
Italy (33) Vineis et al, 1986	agricultural occupations in high herbicide use area: males & females	STS	68	158	1.8	1.0-3.2	Incident cases ≥ 20 yrs old from 1981-83. Controls selected from voting list and mortality files.
	surviving women with high herbicide exposure	STS	20	56	2.7	0.6-11.1	
New Zealand (34) Pearce et al, 1985	agricultural occupations	ML + MM HD < 65 MM < 65 LS + RCS < 65 Other NHL <65	734 107 82 136 88	2936 1652 1652 1652 1652	1.3* 1.1 2.2* 1.1 1.8	1.0-1.6 0.6-1.0 1.3-3.8 0.7-1.9 1.3-3.0	Incident cases from 1977-81, ≥ 20 yrs; registry cancer controls; exposure measure was occupation on cancer registry.

(con't)

<u>Location(Ref)</u>	<u>Exposure</u>	<u>Disease</u>	<u>No. of Cases</u>	<u>No. of Controls</u>	<u>Risk Estimate</u>	<u>95% CI</u>	<u>Comments</u>
Utah (35) Schumacher, 1985	farming occupation on death certificate	NHL NHL dx before 1978	228 186	261 203	1.5 2.1	0.8-2.8 1.0-4.4	Deaths from cancer registry 1967-82, 35-74 yrs; controls were registry colon cancer deaths.
New Zealand(36) Pearce, et al, 1986	phenoxy herbicides + chlorophenols phenoxy herbicides chlorophenols	NHL (exc LS+RCS) NHL(exc LS+RCS) NHL (exc LS+RCS)	82 83 83	396 228 228	1.2 1.2 1.0	0.7-2.0 0.6-2.2 0.4-2.2	Subset of prev- study (34); in- cluded inter- views; excluded deceased subjects & those too ill for interview; these results for population and cancer controls for combined ex- posures, pop- ulation controls otherwise.
Washington (37) Woods et al, 1987	phenoxy herbicides 2,4-D 2,4,5-T occupations with exposure to phenoxy herbicides	NHL NHL NHL NHL STS	576 576 576 576 128	694 694 694 694 694	0.9 0.7 1.0 1.1 0.8	0.5-1.5 0.4-1.3 0.5-2.0 0.8-1.4 0.5-1.2	Incident registry cases 1981-84, 20-79 yrs; pop- ulation control group matched on age and vital status; interview data.

Location(Ref)	Exposure	Disease	No. of Cases	No. of Controls	Risk Estimate	(con't)		Comments
						95% CI		
Kansas (38) Hoar et al, 1986	occupations with exposure to chlorophenol	NHL	576	694	1.0	0.8-1.2		
	farmers	STS	128	694	1.0	0.7-1.5		
		NHL	576	694	1.3*	1.0-1.7		
		STS	128	694	1.3	0.8-1.9		
	Hx Chloracne	NHL	576	694	2.1	0.6-7.0		
		STS	128	694	3.3	0.8-14.0		
	farmers	STS	133	948	1.1	0.7-1.6		Incident cases from 1976-82, ≥ 21 yrs. old. Controls from general popula- tion; random digit dialing, Medicare lists and mortality files; telephone interviews, path review.
		NHL	170	948	1.6*	1.1-2.3		
		HD	121	948	0.6*	0.4-0.9		
	herbicide use (largely 2,4-D)	STS	133	948	0.8	0.5-1.3		
		NHL	170	948	1.2	0.8-1.8		
		HD	121	948	1.2	0.8-1.9		
	herbicide use > 20 days/yr	NHL	138	795	3.5*	1.3-9.0		
	sprayed/mixed herbicides [‡]	NHL	170	948	1.9*	1.1, 3.3		
	sprayed/mixed [‡] herbicides	NHL	170	948	8.0*	2.3-27.9		
	> 20 days/yr.							

*** nc = not calculated as necessary data not presented.

* P < .05

** Borderline significance

+ unless otherwise noted, studies are limited to white males

++ STS= soft tissue sarcoma, ML= malignant lymphoma; HD= Hodgkin's disease, NHL= non-Hodgkin's lymphoma; LS= non-Hodgkin's lymphoma of lymphosarcoma subtype; RCS= non-Hodgkin's lymphoma of reticulum cell sarcoma subtype; MM= multiple myeloma; CI = confidence interval.

[‡] & could not recalculate odds ratios and confidence limits, reported as stated in manuscript.

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TABLE 5A. DATA USED FOR SUMMARY ODDS RATIO ANALYSES*

Exposure (Ref.)	No. Cases	No. Controls	No. Cases Exposed	No. Controls Exposed	No. Cases Non-exposed	No. Controls Non-exposed	OR & 95% CI**
SOFT TISSUE SARCOMAS							
phenoxo acids + chlorophenols (22)	52	206	19	19	33	187	5.7 (2.7-11.8)
phenoxo acids + chlorophenols (26)	110	219	25	13	85	206	4.7 (2.3-9.5)
phenoxo acids (29)	82	92	18	17	64	75	1.2 (0.6-2.6)
phenoxo acids (Agent Orange) (31)	252	252	7	10	245	242	0.7 (0.3-1.8)
phenoxo acids; largely 2,4-D (38)	133	948	22	192	111	756	0.8 (0.5-1.3)
agri- cultural & forestry occupations (32)+	1961	1961	93	82	1868	1879	1.1 (0.8-1.5)
							(con't)

Exposure ____(Ref)____	No. Cases	No. Controls	No. Cases Exposed	No. Controls Exposed	No. Cases Non-exposed	No. Controls Non-exposed	OR & 95% CI
agri- cultural occupations in high phenoxy herbicide use area (33)	68	158	29	47	39	111	1.8 (1.0-3.2)
TOTAL			213	380	2445	3456	
LYMPHOMA ⁺⁺							
HD farming (20) [†]	506	506	43	40	463	466	1.1 (0.7-1.7)
NHL farming (20) [†]	584	584	40	33	544	551	1.2 (0.8-2.0)
ML farming (25)	169	335	61	32	108	303	5.3 (3.3-8.7)
ML+MM agri- cultural occupation (34)	734	2936	118	390	616	2546	1.3 (1.0-1.6)
NHL farming (35)	228	261	24	19	204	242	1.5 (0.8-2.8)
NHL phenoxy (except acids+ LS+RSC) chlorophenols (36)	83	396	26	109	57	287	1.2 (0.7-2.0)

(con't)

Exposure (Ref)	No. Cases	No. Controls	No. Cases Exposed	No. Controls Exposed	No. Cases Non-exposed	No. Controls Non-exposed	OR & 95% CI
NHL phenoxy acids 170 (mostly 2,4-D) (38)		948	40	192	130	756	1.2 (0.8-1.8)
HD phenoxy acids 121 (mostly 2,4-D) (38)		948	28	192	93	756	1.2 (0.8-1.9)
TOTAL		380	1007	2215	5907		
MULTIPLE MYELOMA							
MM farming (20) ⁺ 522		522	60	38	462	484	1.7 (1.1-2.5)
MM+ML agricultural 734 occupations (34)		2936	118	390	616	2546	1.3 (1.0-1.6)
TOTAL			178	428	1078	3030	

* studies could only be included if numbers of cases and controls by exposure status could be determined from report.

** individual confidence intervals are calculated using the normal approximation to the logarithm of the odds ratio as suggested by Woolf(52); OR = odds ratio, CI = confidence interval.

+ HD = Hodgkin's disease, NHL = non-Hodgkin's lymphoma, ML = malignant lymphoma, MM = multiple myeloma, LS = non-Hodgkin's lymphoma, lymphosarcoma subtype, RSC = non-Hodgkin's lymphoma, reticulum cell sarcoma subtype.

TABLE 5B. SUMMARY STATISTICS FOR CASE-CONTROL STUDIES*

Disease Category	χ^2_{MH}	Adjusted Odds Ratio	95% Confidence Limits	Breslow-Day Test
Soft tissue sarcoma	na	na**	na	p < .001
Lymphomas	na	na	na	p < .001
Multiple Myeloma	8.1 (p<.004)	1.3	1.1 - 1.6	p < .26

After Exclusion of Initial Swedish Studies (22,25,26)

Soft tissue sarcoma	0.7 (p<.42)	1.1	0.9 - 1.4	p < 0.25
Lymphomas	7.5 (p<.006)	1.2	1.1 - 1.4	p < 0.99

* For the summary statistics, the Mantel-Haenszel χ^2 is presented; the confidence limits are calculated using Woolf's method.
 **na = not applicable as the calculations are not appropriate due to Breslow-Day test results indicating that the odds ratios vary across strata and should not be combined.

Table 6. Summary of Study Results by Degree of Exposure**

<u>Location (Ref.)</u>	<u>Method</u>	<u>Degree of exposure</u>	<u>Risk Estimate</u>	<u>95% Confidence Interval</u>
SOFT TISSUE SARCOMAS				
Sweden (10)	cohort	low	0.9	0.8-1.0
		high	0.8	0.3-1.9
Lyngne (4)	cohort	general	3.7	1.0-9.4
		high	6.3	0.2-34.8
Michigan (5)	cohort	low	-	-
		moderate	-	-
		high	(1 obs/ 0 exp)	-
West Virginia (7,9) [†]	cohort	general	5.7	0.1-31.8
		high (accident)	22.2 ⁺⁺	0.6-123.8
New York State (31)	case-control	general	0.5	0.2-1.2
		high	0.7	0.3-1.8
Italy (33)	case-control	general	1.8	1.0-3.2
		high	2.7	0.6-11.1
Washington (37)	case-control	none (PAH)	1.0	-
		low (PAH)	0.6	0.3-1.1
		moderate (PAH)	1.0	0.6-1.7
		high (PAH)	0.9	0.4-1.9
		none (CP)	1.0	-
		low (CP)	0.9	0.5-1.6
		moderate (CP)	0.9	0.6-1.5
		high (CP)	0.9	0.5-1.8
		high (chloracne)	3.3	0.8-14.0
				(con't)

<u>Location (Ref)</u>	<u>Method</u>	<u>Degree of exposure</u>	<u>Risk Estimate</u>	<u>95% Confidence Interval</u>
LYMPHOMAS				
Lyngø (4)	cohort	general high	1.3 -	0.4-3.4 -
Michigan (5)	cohort	low moderate high	0.8 1.7 -	0.02-4.3 0.2-6.0 -
W. Virginia (7,9) ⁺	cohort	general high (accident)	0.8 3.0	0.02-4.3 0.1-16.7
Wisconsin (27)	case-control	general high	1.2 2.1	1.0-1.5 1.0-4.3
Iowa (28)	case-control	general high	1.3* 3.4*	nc nc
Washington (37)	case-control	none low (PAH) moderate (PAH) high (PAH)	1.0 0.9 1.0 1.2	- 0.6-1.3 0.7-1.3 0.8-1.9
		none low (CP) moderate (CP) high (CP)	1.0 1.0 0.9 0.9	- 0.7-1.3 0.7-1.2 0.9-1.4
		high (chloracne)	2.1	0.6-7.0
Kansas (38)	case-control	moderate high	1.9* 8.0*	1.1-3.3 2.3-27.9

(con't)

<u>Location (Ref)</u>	<u>Method</u>	<u>Degree of exposure</u>	<u>Risk Estimate</u>	<u>95% Confidence Interval</u>
MULTIPLE MYELOMA				
Iowa (28)	case-control	general	1.5*	nc
		high	2.4*	nc

* p < .05

** nc = not calculated as necessary data not presented; "--" indicates that zero (0) cases were observed, so an SMR was not calculated.

+ crude risk estimates calculated, since expected numbers not available in the papers, using the SEER registry data (14) showing that 0.5% of the total cancer cases ascertained in the study could be expected to be soft tissue sarcomas and 3.7% to be malignant lymphomas.

++ in this paper the soft tissue sarcoma case was misclassified in the "skin cancer" category.

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Appendix-Table 1 - Data for Figure 1
(Risk Factor = Farming occupation)

Ref	State	acres	agric. pop.	Dose x 10 ³	Risk	CI
Soft Tissue Sarcoma						
37	Washington	16.7	1,562,738	94	1.3	0.8-1.9
38	Kansas	47.9	2,386,575	50	1.1	0.7-1.6
Non Hodgkin's Lymphoma						
20	Wash/Oreg	34.9	2,712,738	78.0	1.2	0.8-2.0
27	Wisconsin	17.6	374,200	21.0	1.2	1.0-1.5
28	Iowa	33.0	903,198	27.0	1.3*	nc
35	Utah	10.6	10,200	1.0	1.5	0.8-2.8
37	Washington	16.7	1,562,738	94.0	1.3*	1.0-1.7
38	Kansas	47.9	2,386,575	50.0	1.6*	1.1-2.3

37A

Appendix Table 2 - Data for Figures 2a and 2b⁺

<u>Exposure⁺⁺</u>	<u>Eligibility yrs (ref)</u>	<u>Disease</u>	<u>Risk</u>	<u>CI</u>
2	1972-80 (3)	STS	-	-
2	1961-79 (10)	NHL	-	-
1	1947-1982 (4)	STS	0.9	0.8-1.0
1	1940-1982 (5)	ML	3.7	1.0-9.4
2	1954-1978 (6)	STS	1.3	0.4-3.4
1	1949-1978 (7)**	NHL	2.5	0.1-13.9
2	1955-1977 (9)**	STS	1.9	0.6-4.5
1	1960-67 (20)	ML	-	-
2	1960-72 (21)	STS	22.2	0.6-123.8
2	1974-78 (26)	ML	3.0	0.1-16.7
2	1974-78 (25)	STS	5.7	0.1-31.8
2	1968-76 (27)	ML	0.8	0.02-4.3
2	1964-78 (28)	NHL	1.2	0.8-2.0
2	1967-80 (29)	HD	1.1	0.7-1.7
2	1962-80 (31)	HD	1.1	nc
2		STS	5.7	2.7-11.8
2		STS	4.7*	2.3-9.5
2		STS	5.3*	3.3-8.7
2		NHL	1.2	1.0-1.5
2		NHL	1.3*	nc
2		STS	1.2	0.6-2.6
2		STS	0.5	0.2-1.2

(con't)

Appendix Table 2 - Data for Figures 2a and 2b⁺ (cont.)

Manufacturing (1) vs. Applications Exposure	Eligibility (ref)	Disease	Risk	CI
2	1968-76 (32)	STS	1.1	0.8-1.5
2	1981-83 (33)	STS	1.8	1.0-3.2
2	1977-81 (34)	ML + MM	1.3*	1.0-1.6
2	1967-82 (35)	NHL	1.5	0.8-2.8
2	1981-84 (37)	NHL	1.1	0.8-1.4
2	1976-82 (38)	STS	0.8	0.5-1.2
		STS	1.1	0.7-1.6
		NHL	1.6*	1.1-2.3
		HD	0.6*	0.4-0.9

* p < .05

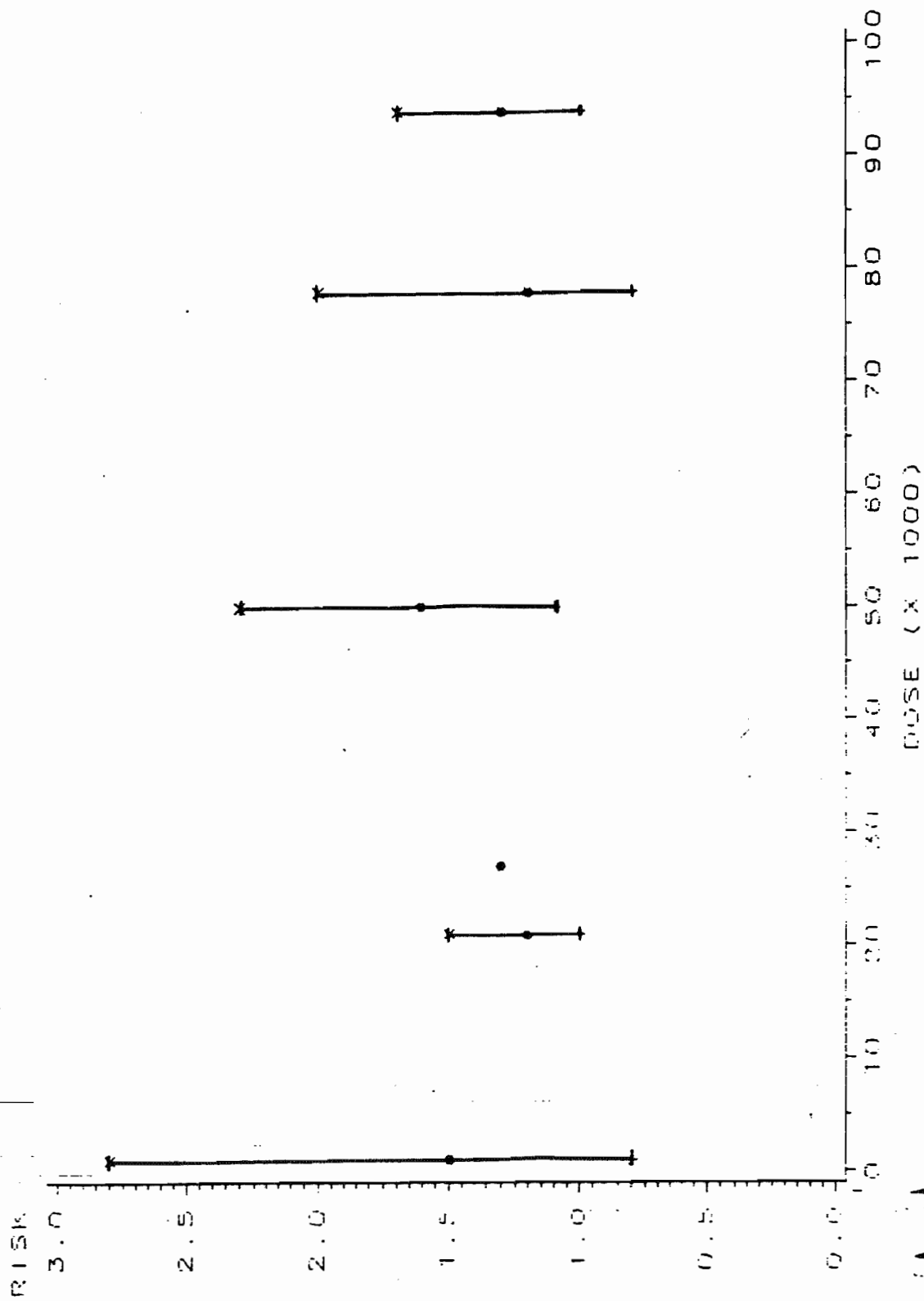
** Crude estimates calculated based on the estimate that 0.5% of the total cases could be expected to have STS and 2.9% could be expected to have NHL, as calculated from SEER registry (14).

+ See abbreviations as in Table 2

++ 1=manufacturing exposure; 2=applications exposure

37B

FIGURE 1: RISK ESTIMATES BY STATE HERBICIDE LEVELS, NHL



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FIGURE 2A: RISK ESTIMATES BY MIDYEARS, STS

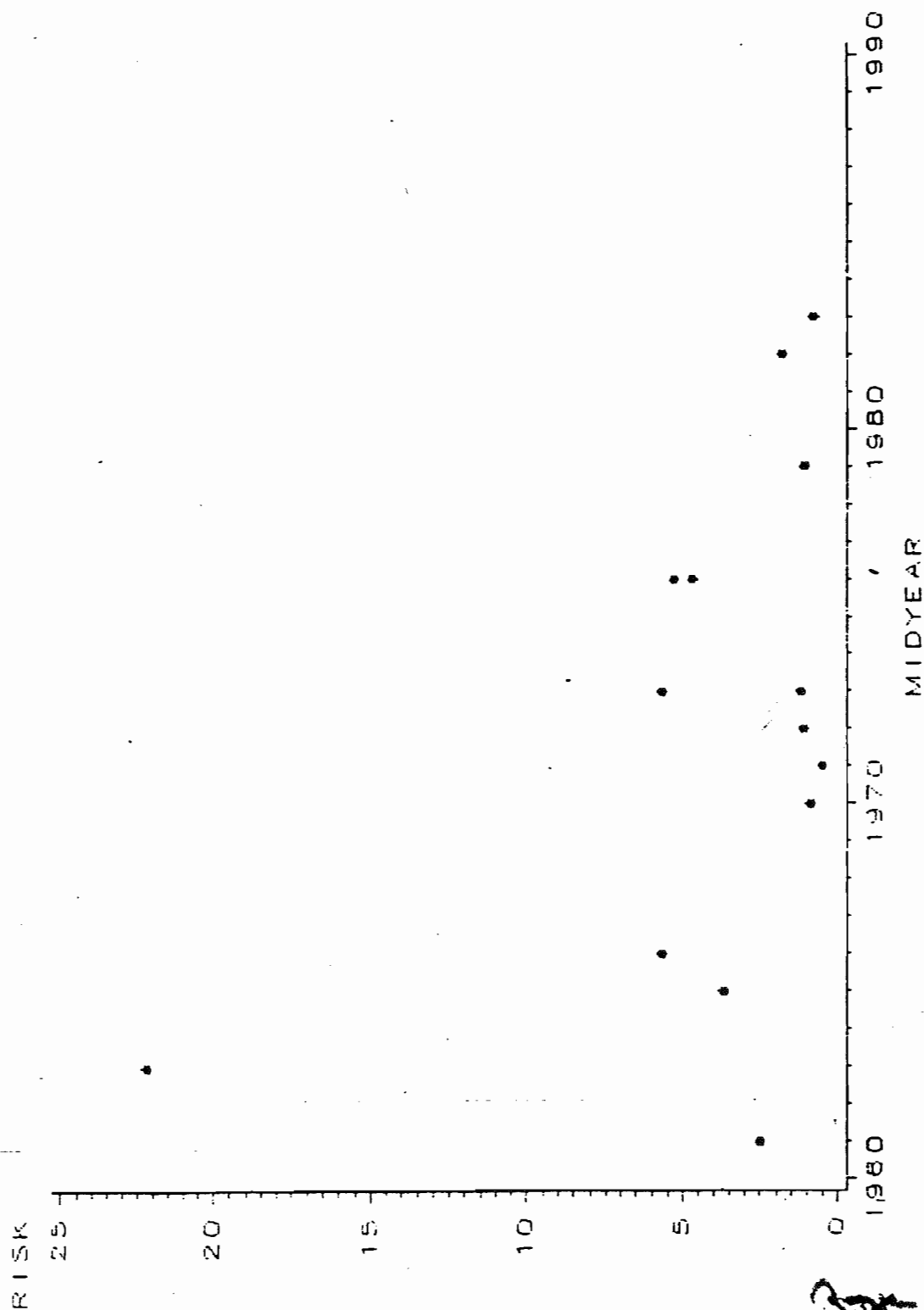


FIGURE 2B: RISK ESTIMATES BY MIDYEARS, LYMPHOMAS

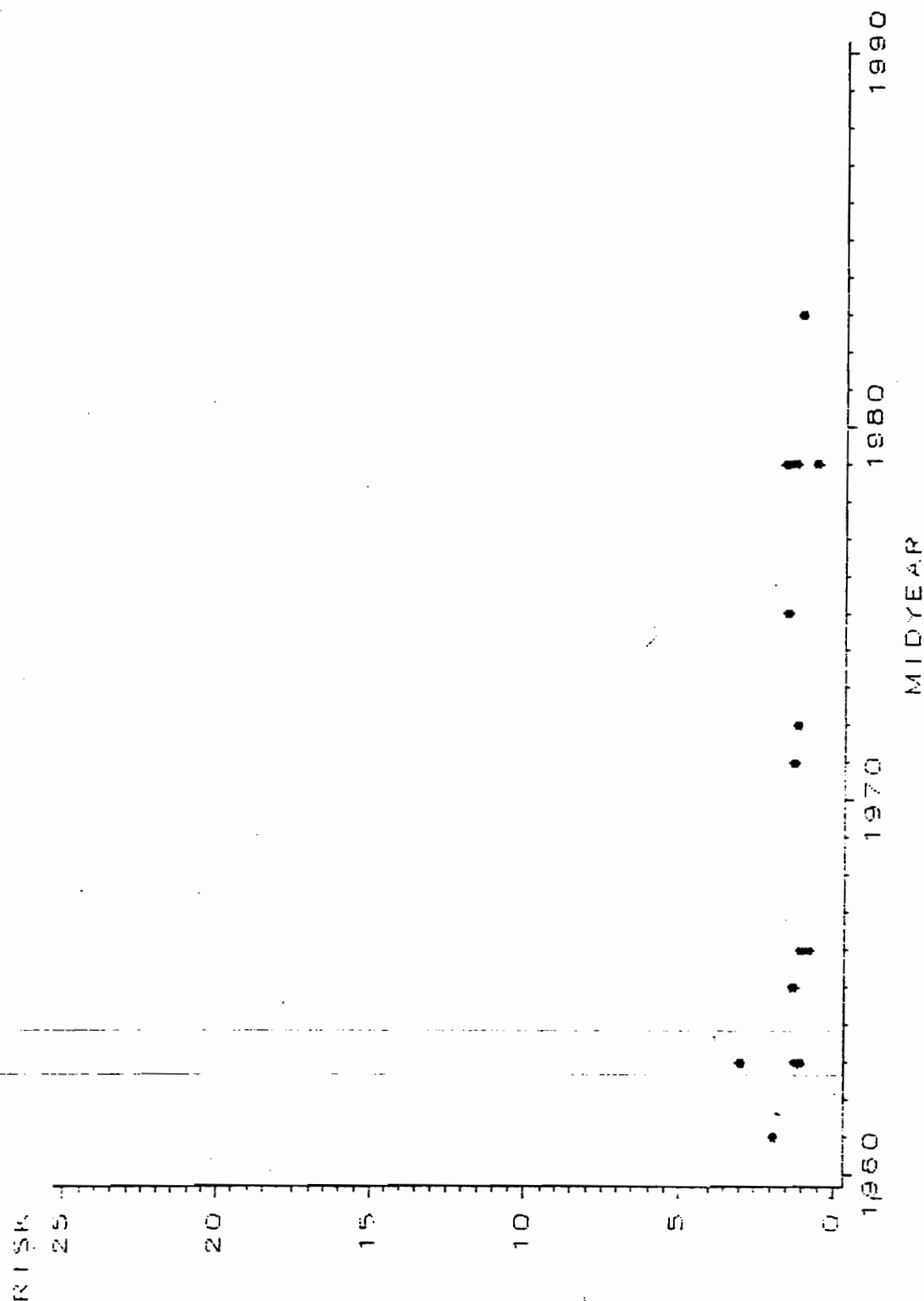
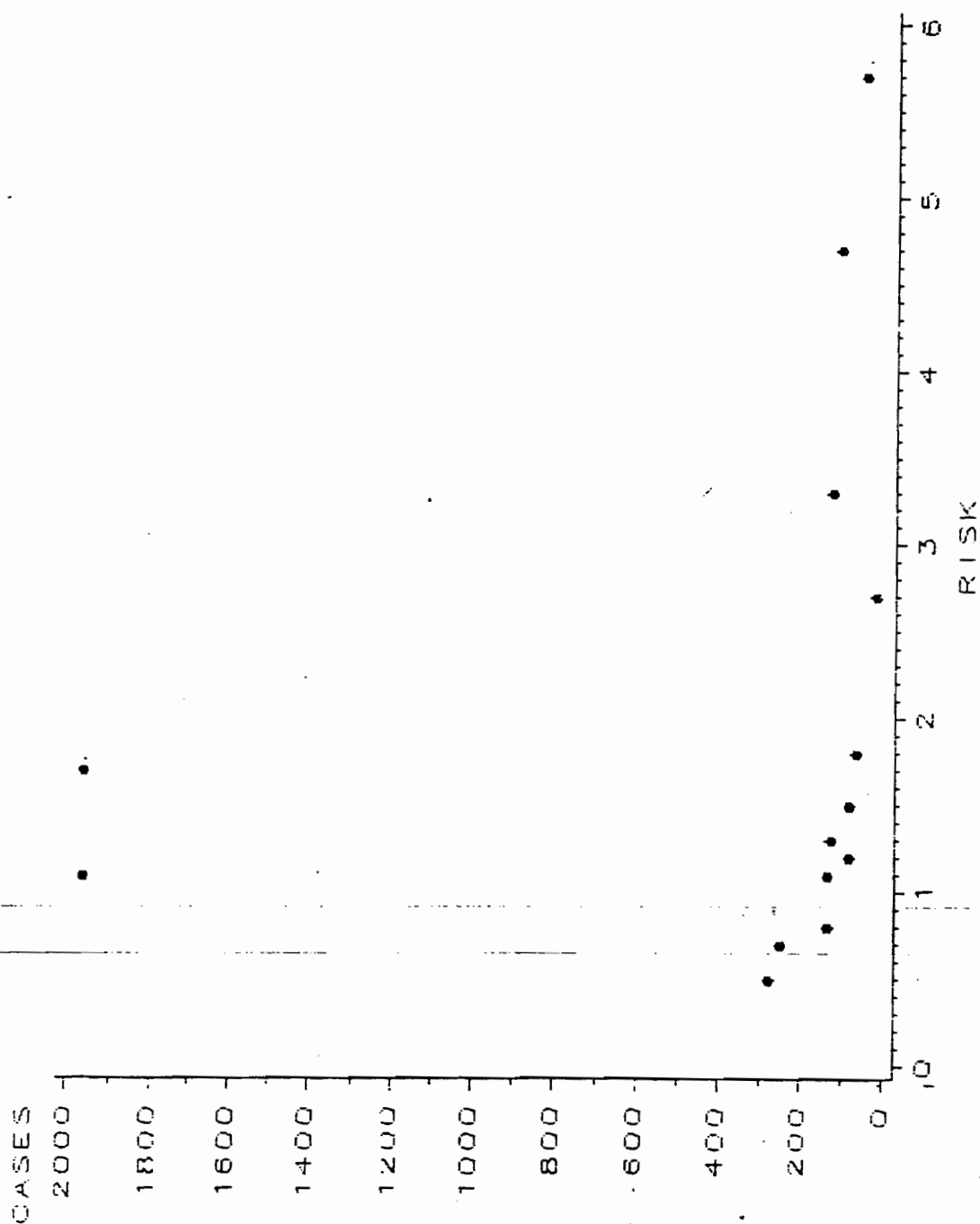
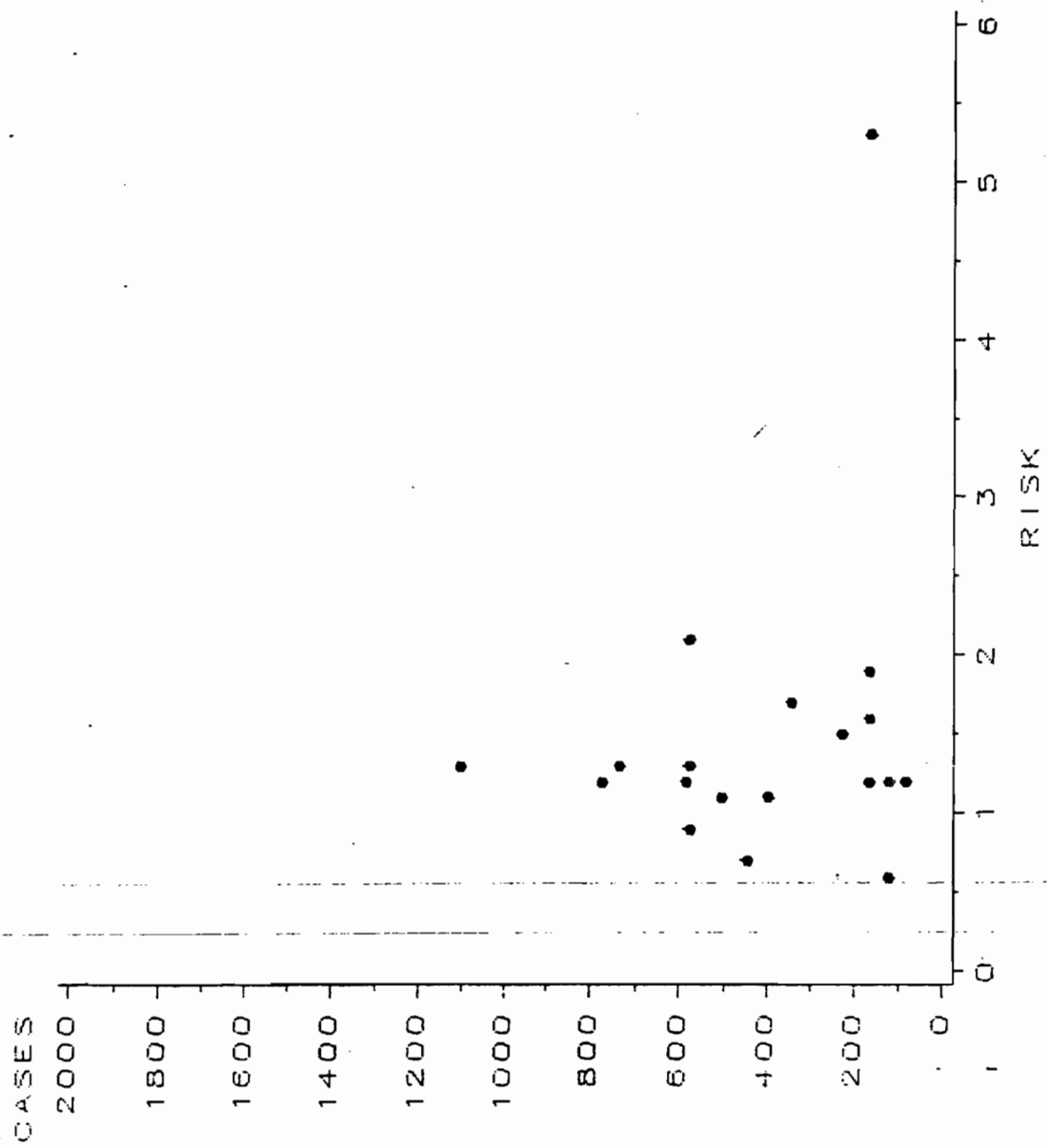


FIGURE 3A: NUMBER OF CASES BY RISK, STS



007720

FIGURE 3B: NUMBER OF CASES BY RISK, LYMPHOMAS



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12/15/87

RISK ESTIMATES FOR CARCINOGENS

007720

DEC 1

Chemical: 2,4-Dichlorophenoxy acetic acid (2,4-D)

Cas No.: 94-75-7

Preparation Date: 12-15-87

A. U.S. EPA Classification and Basis

Classification: "B1" probably carcinogenic to humans. This classification is based on epidemiologic evidence only. Animal cancer bioassays are inadequate.

1. HUMAN DATA

The epidemiologic evidence for the carcinogenicity of 2,4-D in humans should be regarded as "limited" and classified as B1 according to the EPA guidelines for risk assessment. The evidence consists of the Swedish studies of Hardell et al (1981), Hardell and Sandstrom (1979) and Eriksson et al (1979, 1981), the Danish study by Lynge (1985) and more recently a U.S. study by Hoar et al (1986). Exposures occurred to 2,4-D in all of these studies as well as to other phenoxy herbicides. In the Hardell and Eriksson studies, significantly elevated risks of soft tissue sarcoma (STS) and/or lymphomas were noted. An elevated risk of STS occurred to male employees of a phenoxy herbicide manufacturing plant in the Lynge study but not non-Hodgkin's lymphomas. In the Hoar et al (1986) study, a significantly elevated risk of non-Hodgkin's lymphomas was seen that appeared to be dose-related. An elevated risk of STS was not observed in this study. However, all of these studies are not without limitations that preclude a determination of a causal relationship. Chief among these is lack of good evidence of exposure. Furthermore, several other epidemiologic studies have not demonstrated an association between exposure to 2,4-D and a risk of cancer, specifically STS and non-Hodgkin's lymphomas. These studies have severe limitations as well i.e. no direct evidence of exposure to 2,4-D, lack of consideration of latent effects and/or little power to detect a site-specific carcinogenic risk.

2. ANIMAL DATA

Several studies have been conducted to investigate the carcinogenic potential of 2,4-D in rats and mice. The evidence is inadequate. Bionetics Research Laboratories (1969) conducted a bioassay of 2,4-D by oral or single subcutaneous injection in C57Bl/6 mice. In the oral study the compound was suspended in 0.5% gelatin and

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administered orally to two groups of 18 male and 18 female mice from 7-28 days of age, followed by dietary administration for 10-24 months. Groups of 36 male and 36 female received 0.540 gelatin or no treatment at all. No statistically significant increase in tumor incidence over controls was found. This study is considered inadequate because of small the number of animals, limited nature of histopathologic examination and no evidence of MTD dosing.

BRL (1969) Subcutaneous Injection Study: 18 male and 18 female mice received a single S.C. injection of 215 or 464 mg 2,4-D/kg bw dissolved in DMSO rat age 28 days and observed for 78 weeks. No statistically significant increase in tumor incidence over controls was found when compared with controls. This study is also considered inadequate because limited number of animals were used and inappropriate route of administration.

Hansen et al (1971) conducted a 2-year feeding study in Osborne-Mendel rats. Groups of 25 male and 25 female 3-week old rats were fed technical grade 2,4-D at levels of 0, 5, 25, 125, 625 or 1250 ppm. Authors observed a statistically significant (trend) in the dose response. Tumors observed, however, were not associated with any specific organ and were of the types usually observed in aging Osborne-Mendel rats.

Reuber (1979) reexamined original histopathology sections from Hansen et al (1971) study and reported a greater number of lymphosarcomas in treated rats of both sexes and found a significant ($P < 0.05$) increase in the incidence of this tumor among female rats at all five dose levels. The difference in tumor incidence reported by Hansen et al (1971) and Reuber (1979) might be resolved if an independent reexamination of the tissue sections performed. Until then the study could be considered a limited value to assess the carcinogenic potential for 2,4-D.

Archipove and Kozlova (1974) conducted a feeding study to investigate the carcinogenic potential of 2,4-D ammonium salt in rat and mice. No tumorigenic evidence was observed.

3. SUPPORTING DATA

2,4-D has been tested for mutagenicity in a variety of assays e.g. plant, bacteria, yeast, fruit flies in vitro and in vivo mammalian systems. The results were negative. Toxicokinetic studies have shown that most of the 2,4-D administered orally to animals is excreted in the urine unchanged within 24-48 hrs.

B. ORAL QUANTITATIVE ESTIMATE

Not available.

C. INHALATION QUANTITATIVE ESTIMATE

Not available.

D. DOCUMENTATION AND REVIEW

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U.S. EPA Drinking Water Criteria Document for 2,4-D, Feb., 1987.

2. REVIEW

° The carcinogen Assessment Group reviewed 2,4-D: _____

° Agency Work Group Review: _____

° Verification Date: _____

3/23/87

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**EXPERT PANEL REPORT ON
CARCINOGENICITY
OF 2,4-D**

March 23, 1987

**Canadian Centre for Toxicology,
Guelph, Ontario, Canada**

1. SUMMARY AND EVALUATION

The herbicide, 2,4-Dichlorophenoxyacetic acid (2,4-D) was introduced commercially in Canada during the 1940's. It is widely used as a herbicide in forestry, agriculture, turf maintenance and for weed control in parks and rights-of-way. It has extensive applications in the home and garden market as well. Several formulations of 2,4-D are registered for use in Canada, normally as the amine salt or as esters of the acid. The total volume of 2,4-D sold in Ontario during 1986 was approximately 500,000 kg active ingredient.

Concern over the safety of 2,4-D first arose in the 1960's when it was recognized that certain 2,4-D formulations were contaminated with dioxins. Although chlorinated dioxins have been identified as contaminants in 2,4-D products and formulations, current Canadian regulations allow only low concentrations (<10 ug/L) to be present. Moreover, the highly toxic 2,3,7,8-tetrachlorodioxin has not been identified, nor would it be expected in 2,4-D products and formulations. The dioxins that have been identified in 2,4-D products and formulations (2,7- or 2,8-dichlorodioxin, 1,3,7- or 1,3,8-trichlorodioxin, and 1,3,6,8- or 1,3,6,9-tetrachlorodioxin) are not considered highly toxic (Ontario Ministry of the Environment, 1985).

N-nitrosodimethylamine, a carcinogenic nitrosamine, also has been detected at low levels in certain samples of commercial 2,4-D particularly when nitrite was added as a rust inhibitor. The levels detected have ranged from 0.3-5 mg/L in the formulated product. A risk assessment on nitrosamines in 2,4-D conducted by the U.S. National Academy of Sciences indicated that the amounts found in 2,4-D formulations pose, at most, a negligible risk to human health.

Pharmacokinetic studies conducted on 2,4-D in experimental animals and humans indicate that 2,4-D is absorbed via all routes of exposure. It appears that 2,4-D is distributed widely among the tissue of the body; the highest concentrations are found in parenchymal and excretory organs. There is no evidence that 2,4-D is metabolized to reactive intermediates which might bind to tissue macromolecules such as DNA. In all mammals examined, including humans, 2,4-D is rapidly excreted in the urine, largely unchanged in chemical form, though a small but variable amount may be conjugated in the kidney prior to excretion. These conjugated forms, which are highly polar and rapidly excreted, would be expected to be even less toxic than 2,4-D.

The available pharmacokinetic studies indicate that there is a proportional and constant relationship between exposure, uptake and urinary elimination of

2,4-D in workers exposed over several days and who have achieved steady state pharmacokinetics. These studies further indicate that, in workers who use 2,4-D regularly, the amount excreted in the urine over a 24-hour period is a reliable measure of the absorbed systemic dose. This finding facilitates the measurement of dose received by workers employed in occupations involving 2,4-D exposure.

Exposure studies have been conducted on 2,4-D in Ontario or in areas with similar climate and use pattern. These indicate that hydro-line workers may be exposed to the 2,4-D in amounts from 0.005 - 5 mg/person/spraying day. Exposure in this setting is highly variable and depends upon the nature of the work performed (mixer-loader, sprayer, flagger, etc.) and the extent to which precautionary procedures are followed and protective gear is worn. Commercial lawn applicators were found to receive a daily dose of approximately 0.3 mg/person/spraying day while farmers were estimated to receive about 0.5 mg/person/spraying day. Variation between these groups is due to differences in spray equipment, terrain and to the degree to which the operators come into contact with concentrated formulations. The latter is the most probable reason for the low levels of exposure in commercial lawn applicators who were reported to take appropriate precautions when mixing the formulation.

Genotoxicity studies on 2,4-D have included in vitro studies in bacteria, yeasts and cultured mammalian cells. In vivo genotoxicity studies have been conducted in rats, mice, hamsters and Drosophila. In addition, some limited studies have been carried out in humans exposed occupationally to 2,4-D.

The results of tests conducted in in vitro systems indicate that 2,4-D is not mutagenic in Salmonella or E. coli; however, some conflicting data have been reported in B. subtilis. This test correlates poorly, if at all, with carcinogenicity. There is an isolated report that 2,4-D "fluid" induced unscheduled DNA synthesis in human fibroblasts; however, no such effects were noted in more definitive studies in human embryonic lung cells and cultured rat hepatocytes. No information was given on the composition of the 2,4-D "fluid" and thus the significance of this positive report cannot be evaluated.

It has been reported that 2,4-D induced mutations in yeast but positive effects were noted only below pH 4.5, leading the authors of these studies to conclude that effects were dependent entirely on pH of the culture media.

There are studies indicating that 2,4-D produces sister chromatid exchanges (SCE's) in cultured human lymphocytes but not in hamster embryo cells.

The significance of these findings is questionable in light of the fact that several in vivo studies involving rats, mice, hamsters and humans have not shown any effects on SCE's in lymphocytes or bone marrow cells when 2,4-D was administered by appropriate routes at up to toxic doses. In addition, SCE's in vitro cannot be viewed as reliable predictors of carcinogenicity. There is one report that 2,4-D induced chromosomal aberrations in mouse bone marrow cells; however, the significance of this finding is questionable because the animals were given a dose corresponding to the LD₅₀.

Conflicting data exist on the mutagenic activity of 2,4-D in Drosophila. Unstable strains appear to show weakly positive effects while more stable strains appear to be resistant even at very high dietary concentrations (e.g. 1,000 ppm). A micronucleus test and a dominant lethal assay conducted in mice at doses of 100-125 mg/kg produced negative results.

In summary, in vitro studies on the genotoxicity of 2,4-D, in some cases, produced conflicting results, however, there is no convincing evidence that 2,4-D produces mutagenic effects when it is tested in in vivo systems. Overall, the pattern of responses observed in both in vitro and in vivo tests indicates that 2,4-D is not genotoxic.

The carcinogenicity of 2,4-D has been studied in two recently completed, long-term cancer bioassays, conducted in the United States under the auspices of the Industry Task Force on 2,4-D Research Data. In one study, groups of male and female Fischer 344 rats were given 0, 1, 5, 15, or 45 mg 2,4-D(acid, 97.5% pure)/kg body weight/day for 2 years. The results of this study indicated an increased incidence of brain tumours (astrocytomas) in male rats of the high-dose group. No treatment-related increase in brain tumours was noted in female rats or at any other site in either males or females.

The incidence of brain tumours in male rats treated at the high dose was significantly increased compared to concurrent untreated controls. The incidence in the high dose group also exceeded that of tumors observed historically in untreated male rats of the same strain.

~~While it is not possible to discount this evidence for carcinogenesis, the characteristics generally attributed to a brain carcinogen were not present in this experiment.~~ There was no evidence of decreased tumor latency, the increase was limited to high-dose males, no preneoplastic lesions such as gliosis were present in treated animals, all tumors were solitary, and the tumors in treated animals were not more advanced (anaplastic) than generally seen in control animals.

Considering this, the Panel concludes that there is insufficient evidence to be certain that the brain tumors were related to 2,4-D exposure. This conclusion is supported by the large body of negative genotoxicity data on 2,4-D. In addition, there is no evidence to indicate that 2,4-D forms reactive intermediates in the liver or other tissues or forms adducts with DNA.

In the industry-sponsored 2,4-D mouse study, groups of male and female B6C3F1 mice were treated with the compound at dose levels of 0, 1, 15 or 45 mg 2,4-D(acid, 95% pure)/kg body weight/day in the diet for 106 weeks. The Panel considered that, in this study, a higher maximum dose could have been used; however, the highest dose used exceeded the highest estimated average occupational exposure by a factor of about 600. The results of this study did not indicate any relationship between 2,4-D exposure and tumour incidence in either male or female mice.

Epidemiological studies conducted on phenoxy herbicide-exposed workers have involved both case-control and cohort type studies. In one series of case control studies conducted in Sweden, an increased relative risk of soft tissue sarcoma and malignant lymphoma from exposure to phenoxy herbicides was noted; however, this was not found in similar studies conducted in New Zealand. In a cohort study in Denmark, however, an excess of soft tissue sarcomas in phenoxy-exposed workers was found. Several other cohort studies have been negative. An excess of Non-Hodgkins Lymphoma, but not soft-tissue sarcoma was observed in a case-control study conducted on herbicide-exposed farmers in Kansas.

Based on the available epidemiological studies 2,4-D cannot be exonerated as a reason for the excess cancer risk seen in studies involving the phenoxy herbicides conducted in the U.S., Denmark and Sweden, but neither can these studies identify 2,4-D as being the causative agent. Overall, the epidemiological evidence indicates that a relationship between an increased risk of soft-tissue sarcoma and non-Hodgkins lymphoma with phenoxy herbicide exposure is tenable; however, in regard specifically to 2,4-D, the evidence for human carcinogenicity must be considered as inadequate.

The evaluation of the validity and health significance of existing data pertaining to the carcinogenicity of 2,4-D is difficult. Because the epidemiological studies were conducted on persons exposed to several herbicides, it is not possible to identify the role, if any, of 2,4-D on the putative relationship between phenoxy herbicide exposure and increased risk of soft-tissue sarcoma and non-Hodgkins lymphoma. The epidemiological studies, by themselves,

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cannot be used to assess the possible carcinogenicity of 2,4-D. Other studies of 2,4-D carcinogenicity have demonstrated an increased incidence of brain tumors in male rats given 2,4-D at 45 mg/kg body weight/day. However, as discussed above, there is insufficient evidence to conclude that the tumors were related to 2,4-D exposure. Overall, the Panel concludes that the existing animal and human data are insufficient to support the finding that 2,4-D is a carcinogen and, consequently, finds insufficient evidence to conclude that existing uses of 2,4-D in Ontario pose a significant human health risk.

2. INTRODUCTION

The chlorophenoxy herbicides were developed during the early 1940s and the compounds 2,4-dichlorophenoxyacetic acid (2,4-D,) 2,4,5- trichlorophenoxyacetic acid (2,4,5-T) and (2-chloro-2-methylphenoxy)acetic acid (MCPA) were commercially introduced in the mid 1940s. By the mid 1960s, the chlorophenoxy herbicides were the most widely used class of herbicides. They are generally formulated either as the amine salts or as esters, the latter commonly of isooctyl and butoxyethanol alcohols. As a herbicide, 2,4-D is used for the selective control of a large number of broad-leaf weeds in cereals, grains and turf crops as well as the control of shrubs, broad-leaf weeds and trees in rangeland, forests and rights-of-way. Rates of application may vary from 0.25 kg/ha in grain crops to 16 kg/ha in spot treatment of trees and bushes in rights-of-way. Average use of 2,4-D in the USA for the years 1978-1983 was 10 million kg (range 14.3-6.2 million kg) while use in Canada was reported to be 3.5 million kg for the years 1974-1976 (IARC, 1987). Total sales of 2,4-D during 1986 in Ontario were reported as 0.5 million kg (DHS, 1987).

Concern over the human health implications of exposure to the phenoxy herbicides focussed initially on 2,4,5-T in the late 1960s with the discovery that some formulations containing this chemical were contaminated with dioxins and, in particular, 2,3,7,8-tetrachloro-p-dibenzodioxin (2,3,7,8-TCDD). Restrictions were placed on the use of 2,4,5-T in a number of jurisdictions (including Ontario) and it is now no longer manufactured in North America. This concern also resulted in what was probably the most thorough and extensive review of the scientific literature on the subject (Veterans Administration, 1981a and b; 1983; Royal Commission, 1985).

The recent discovery of dioxins in certain formulations of 2,4-D (Cochrane et al., 1981) raised concern for this product as well and prompted the establishment of regulations limiting dioxin levels in 2,4-D products in Canada. Recent epidemiological evidence, especially the so-called "Kansas study" (Hoar et al., 1986a and b) and the report that chronic feeding with 2,4-D increased the incidence of astrocytomas in male rats (Hazleton, 1986) have further raised the level of concern for the human health implications of this compound. These were the primary reasons for the commissioning of this report.

The terms of reference of the Expert Panel were as follows:

1. To assess the validity and health significance of existing experimental and epidemiological data on the carcinogenicity of 2,4-D.
2. To determine, on the basis of the existing data on carcinogenicity, whether any of the existing uses of 2,4-D in Ontario pose a significant health risk.

In light of these terms of reference, the panel has concentrated its efforts on the question of carcinogenicity and possible related effects. Only brief reference is made to other possible adverse effects.

3. ACUTE AND SUB-CHRONIC TOXICITY

The acute and sub-chronic toxicity of 2,4-D is well documented and was recently reviewed by the World Health Organization (WHO, 1984). A summary of the acute and sub-chronic toxicity data on 2,4-D is presented in Table 1.

The available evidence indicates that 2,4-D is of moderate acute toxicity to mammals and birds. Clinical signs of acute exposure to high doses of 2,4-D include effects on the gastrointestinal tract, muscular weakness, muscle spasms and depression of the central nervous system (CNS). CNS depression is thought to be due to alterations in the blood-brain barrier at very high exposure levels (Elo and Ylitalo, 1977 and 1979). The myotoxic effects of acute exposure to 2,4-D are reported to include changes in a number of physiological and biochemical processes in muscle (WHO, 1984). Direct myotoxic effects of 2,4-D may have been mistakenly interpreted as symptoms of peripheral neuropathy in some animals and WHO (1984) stated that the existing data are inadequate to assess the possible role of 2,4-D in the development of peripheral neurotoxicity. More recent studies have not reported any evidence of peripheral neurotoxicity. Mattsson *et al.* (1986a) did not find peripheral neuropathy in male and female Fischer 344 rats treated dermally with 12% 2,4-D amine for two hours daily, five days a week for three weeks (equivalent to ca. 110 mg 2,4-D acid/animal/day). A similar lack of effect was also noted in male Fischer 344 rats treated with 24% 2,4-D amine for two weeks in the same manner (Mattsson *et al.*, 1986b).

There have been some reports of peripheral neuropathy attributed to 2,4-D exposure in humans. Symptoms associated with exposure to phenoxy herbicides were said to include: Reduced peripheral nerve conduction velocity, long-lasting flacid paraparesis (incomplete paralysis) or quadriparesis, abnormal

tendon reflexes, sensory neuropathy. The relationship between 2,4-D exposure and peripheral neuropathy in humans has been questioned. No indications of similar effects were reported in persons exposed to "massive" exposure to 2,4-D or in patients given pure 2,4-D or 2,4,5-T (and their esters) as drugs (WHO, 1984). It is possible that the effects reported were associated with exposure to other agents such as solvents, nutritional or hereditary conditions, infections and alcoholism and it has been suggested that further studies should be carried out using more modern methods (WHO, 1984).

Table 1. Acute and chronic toxicity of 2,4-D in animals.

Compound/ formulation	Species	Sex	LD ₅₀ or criterion
<u>ORAL TOXICITY</u>			
2,4-D acid	Mouse	M	375-368 mg/kg body weight
	Rat	M	375-666
	Guinea-pig	MF	469-1000
	Rabbit	NA	800
	Dog	NA	100
	Chicken	NA	541
Various ester formulations	Mouse	MF	380-570
	Rat	MF	620-1500
	Guinea-pig	MF	550-848
	Cat	NA	820
	Rabbit	MF	1420
	Chicken	NA	900-2960
Sodium salt	Mouse	NA	375
	Rat	F	805-2000
	Guinea-pig	M	551
	Rabbit	NA	800
	Chicken	F	655
<u>SUB-CHRONIC STUDIES</u>			
Acid or sodium salt	Rat	NA	31 mg/kg body weight/day NOEL.
<u>REPRODUCTIVE EFFECTS</u>			
Acid or sodium salt	Mammals and birds	NA	10 mg/kg body weight/day NOEL for teratogenic, fetotoxic or embryotoxic effects.

Source: WHO, 1984

3.1. Contaminants in 2,4-D

3.1.1. Polychlorinated dibenzo-p-dioxins

Although Norström *et al.* (1979) reported that no polychlorinated dibenzo-p-dioxins were detected (detection limits were 0.01 to 0.05 mg/L for di- to hexachloro isomers) in samples of 2,4-D and 2,4-D ester produced in 1965 or

earlier, one sample contained 0.06 mg/L of pentachlorodibenzofuran. He also reported that no di-, tri-, penta-, or hexachlorodibenzofurans were detected.

The presence of dioxins in 2,4-D was first reported by Cochrane *et al* (1981). Samples of technical and formulated products containing 2,4-D esters and amines were analyzed by gas chromatography/mass spectrometry. Isooctyl formulations of 2,4-D contained 2,7- or 2,8-dichlorodioxin in concentrations ranging from 104 to 4,200 ug/L. Some isooctyl ester samples also contained 1,3,7- or 1,3,8-trichlorodioxin (346 to 2,079 ug/L) and 1,3,6,8-tetrachlorodioxin (226 to 1,752 ug/L). Two of three samples of 2,4-D mixed butyl esters and four of seven samples of 2,4-D dimethylamine salts showed the presence of dioxins.

A later study of dioxin contaminants, conducted after regulations of dioxin contamination had been promulgated (limiting the levels of dioxins to less than 10 ug/kg), revealed low dioxin concentrations (Cochrane *et al*, 1982). Of one hundred and ninety-nine samples that were analyzed, only 0/72, 7/78, and 2/49 samples of 2,4-D acid, amines, and esters, respectively exceeded the regulatory limit of 10 ug/kg.

The highly toxic 2,3,7,8-tetrachlorodioxin has not been identified and nor would it be expected in 2,4-D products and formulations. The toxicity of the dioxins identified in 2,4-D products and formulations (2,7- or 2,8-dichlorodioxin, 1,3,7- or 1,3,8-trichlorodioxin, and 1,3,6,8- or 1,3,6,9-tetrachlorodioxin) has been recently reviewed and is considerably less than 2,3,7,8-TCDD (Ontario Ministry of the Environment, 1985).

3.1.2. Nitrosamines

Concentrations of *N*-nitrosodimethylamine (NDMA) in pesticide products have been determined (Cohen *et al.*, 1978). Samples of dimethylamine, which may be used in amine salt formulation, showed NDMA concentrations of 27.5 to 53 mg/L. The NDMA content of formulations of 2,4-D dimethylamine salts ranged from not detectable to 6 mg/L. Studies have revealed *N*-nitrosamine contamination (up to 0.3 mg/L) in amine formulations of 2,4-D, particularly when nitrite was added as a corrosion inhibitor (WHO, 1984). *N*-nitrosodimethylamine has also been detected in dimethylamine salts of 2,4-D in Canada (Reid, 1984). Of one hundred and twelve 2,4-D samples analyzed, ninety-two, sixteen, and four samples contained ≤ 1 mg/L, 1 to 5 mg/L, and ≥ 5 mg/L *N*-nitrosodimethylamine, respectively.

Based on a risk assessment conducted by the National Academy of Sciences (NAS, 1981), and the exposures estimated in this document, the amounts of N-nitrosodimethylamine in 2,4-D pose, at most, a negligible risk.

4. PHARMACOKINETICS AND METABOLISM OF 2,4-D IN HUMANS AND EXPERIMENTAL ANIMALS

The absorption, distribution kinetics and metabolism of 2,4-D (in a variety of formulations) has been extensively reviewed (WHO, 1984) with the general conclusion that 2,4-D is not significantly metabolized in animals although a certain proportion of the absorbed dose may be conjugated prior to excretion. These studies are pivotal to the assessment of human exposure and are reviewed in more detail below.

4.1. Animal studies

The distribution and elimination of orally administered 2,4-D amine (commercial formulation, triethanolamine salt), 2,4-D K-Na salt ("pure acid"), and 2,4-D ester (commercial formulation, butyl ester) were studied in rats, pigs, calves, chicks, and chickens (Erne, 1966a and b). After single oral doses of 2,4-D amine, amounting to 50, 100, or 200 mg 2,4-D/kg, peak plasma concentration was reached within 2 h after dosing in chickens and 4 to 7 h after dosing in the mammalian species studied. Plasma half-life of 2,4-D after a single oral dose of 100 mg/kg was determined in several species:

<u>Compound</u>	<u>Species</u>	<u>Half-life, h</u>
2,4-D amine	Rat, male	2.9 ± 0.4
"	Rat, female	3.3 ± 0.5
"	Pig	12 ± 2
"	Calf	7.5 ± 0.8
"	Chicken	7.7 ± 0.7
2,4-D K-Na	Rat, male	3.5 ± 0.5
"	Calf	8.0 ± 0.6
2,4-D ester	Rat, male	6 ± 1
"	Pig	10 ± 0.8
"	Calf	10 ± 1

Repeated oral doses of 50 mg/kg/day 2,4-D amine and ester were given to pigs. In most animals, plasma 2,4-D concentrations declined steadily, and no evidence of accumulation was seen; however, in one pig given 2,4-D amine and in another pig given 2,4-D ester, plasma 2,4-D concentrations rose, and both animals showed signs of intoxication. Tissue 2,4-D concentrations were also measured after giving a single oral dose of 2,4-D. In general, tissue 2,4-D concentrations were highest in parenchymal organs (liver, kidney, lung). In

male rats given 2,4-D amine (100 mg 2,4-D/kg), plasma concentrations were 150 and 2 ug/g at 6 and 24 h after dosing; liver concentrations 90 and 5 ug/g at 6 and 24 h; kidney concentrations 250 and 27 ug/g at 6 and 24 h. In pigs given 2,4-D amine (100 mg 2,4-D/kg) orally, plasma 2,4-D concentrations at 6, 24, 48 and 72 h after dosing were 210, 55, 10 and 3 ug/g; brain concentrations at 6, 24, and 48 h after dosing were 12, 3, and 1.5 ug/g. In chickens given 2,4-D amine (200 mg 2,4-D/kg) the plasma and brain concentrations 6 h after dosing were 100 and 1.5 ug/g, respectively. In pigs fed 500 ppm 2,4-D amine in their feed for 2 months, plasma, liver, kidney and brain 2,4-D concentrations were 22, 6, 12 and 2 ug/g respectively. It was found that 0 to 18% of the 2,4-D present in urine after giving single or repeated oral doses of 2,4-D amine was present as an acid-hydrolyzable conjugate the structure of which was not identified. The ester of 2,4-D was found to undergo very rapid hydrolysis to 2,4-D (Erne, 1966a and b).

Metabolism of orally administered [^{14}C]2,4-D has been studied in rats (Khanna and Fang, 1966). Radiolabelled carbon dioxide was not detected as a metabolite of 2,4-D. In rats given 1 to 10 mg 2,4-D per rat (males: 350 to 400 g; females: 225 to 275g body weight), 93 to 99% of the administered radioactivity was excreted in the urine in 48 h. When 20 to 100 mg 2,4-D/rat was given, a smaller fraction of the dose was recovered in the urine over a 12 day period; i.e.:

Dose/rat (mg 2,4-D)	20	40	60	80	100
Recovery in urine (%)	91.3	87.1	87.4	77.9	75.5

In rats given 1 mg 2,4-D/rat, the highest brain concentration of 0.7 ug/g dry tissue was found 6 h after dosing. In rats given 80 mg 2,4-D/rat, the following concentrations were found:

Hours after treatment	4	8	24	41
Blood concentration (ug/g)	414	805	268	7
Brain concentration (ug/g)	20	66	16	1

The presence of a urinary metabolite of 2,4-D, which accounted for 0.25% of the administered radioactivity, was detected, but not identified. No evidence for the incorporation of 2,4-D or metabolites into tissue constituents was found.

In male rats dosed orally with 2,4-D (200 mg/kg; 97.9% pure dissolved in peanut oil), a total of 1.4% of the dose was eliminated as a glycine conjugate, and 1.4% as a taurine conjugate (Grunow and Böhme, 1974).

In studies designed to evaluate the effects of 2,4-D intoxication on the distribution of 2,4-D, adult male Sprague-Dawley rats (200 to 290 g) were given 0 or 250 mg 2,4-D/kg (sodium salt, 97% pure) by subcutaneous injection (Elo and Ylitalo, 1977; 1979). Three and a half to 4.5 h after treatment, the rats were given 8.8 mg of [^{14}C]2,4-D (98% pure) intravenously. Starting 30 min. later, the cerebrospinal fluid was collected for 1 h. Plasma and tissue samples were collected immediately after the termination of the cerebrospinal fluid collection period. Although absolute plasma or tissue 2,4-D concentrations were not reported, in saline-treated controls the brain and cerebrospinal fluid values amounted to 2.3 and 0.4% respectively, of the plasma values; after intoxication with 250 mg 2,4-D/kg, the brain and cerebrospinal fluid values rose to 15 and 9.4% of the plasma values. The administration of 250 mg 2,4-D/kg increased brain and cerebrospinal fluid tissue/plasma ratios of ^{14}C 6.5- and 23.5-fold, respectively; smaller increases, which amounted to 1.6 to 3.3-fold, were seen in liver, testis, lung, heart, and muscle tissue. The authors concluded that 2,4-D intoxication either increases the influx of 2,4-D into the brain or decreases the efflux of 2,4-D out of the brain.

Male Fischer 344 rats were given single oral doses of 10, 50 or 150 mg or intravenous doses of 5 or 90 mg ring labelled [^{14}C]2,4-D (>99% radiochemical purity) and plasma and urine content of 2,4-D measured for 72 h (Smith *et al.*, 1980). The rate constant for absorption of orally administered 2,4-D was 1.4 per hour and it was rapidly cleared from the blood in a biphasic manner: The $t_{1/2}$ (α) for the plasma clearance of intravenously and orally administered 2,4-D was 0.92 and 1.01 h respectively; the $t_{1/2}$ (β) for the plasma clearance of intravenously and orally administered 2,4-D was 14.4 and 18.0 h, respectively. At doses >50 mg 2,4-D/kg there was a disproportionate increase in plasma ^{14}C concentrations and a decrease in the ^{14}C content of urine. The K_m for the saturable clearance of 2,4-D from the plasma was about 79 $\mu\text{g/ml}$: at concentrations below the K_m , plasma clearance of 2,4-D follows first-order kinetics. The authors conclude that the saturable clearance of 2,4-D is attributable to the saturable urinary excretion of 2,4-D.

The pharmacokinetics of dermally administered ring labelled [^{14}C]2,4-D propylene glycol butyl ether ester (2,4-D PCBE ester; 97.6% chemically pure; 99.4% radiochemically pure) was studied in rats (Smith *et al.*, 1981). The ester

(5 mg/kg) was applied in a single dermal application of an acetone solution to male Fischer rats and elimination of ^{14}C studied for 120 h after treatment. Absorption of 2,4-D PGBE ester through the skin followed first-order kinetics ($t_{1/2} = 19.7$ h) and an average of 85% of the applied radioactivity was recovered in the urine after 120 h. The authors concluded that the pharmacokinetics of 2,4-D PGBE ester are similar to that of 2,4-D acid.

Frantz and Kropscott (1984) studied the pharmacokinetics of the 2-ethylhexyl ester in rats. Male and female Fischer 344 rats were given a single oral dose of 130 mg 2,4-D (2-ethylhexyl ester) in corn oil, and blood and urine sampled for 72 h. The ethylhexyl ester could not be detected in blood or urine for 72 h after treatment, although 2,4-D was present in both blood and urine. A total of $4.8 \pm 9.2\%$ and $84.3 \pm 4.5\%$ of the dose was recovered in the urine of male and female rats, respectively, in 72 h. The authors concluded that 2,4-D 2-ethylhexyl ester is very rapidly hydrolyzed to 2,4-D, which is excreted in the urine.

Similar pharmacokinetic studies of 2,4-D have also been conducted in the mouse (Hazleton Laboratories, 1984). Male B6C3F1 mice were given 5, 45 or 90 mg orally as an aqueous solution or 90 mg ring labelled [^{14}C]2,4-D (98% pure) intravenously. Urinary and fecal elimination of ^{14}C was measured for 168 h after dosing. Urinary excretion of 2,4-D amounted to 53 to 71% of the dose; fecal excretion of 2,4-D to 5 - 15% of the dose and a greater fraction of the ^{14}C was found in the feces of mice in the higher dose groups. The $t_{1/2}$ for absorption of 2,4-D from the gut was approximately 0.013 h. K_m and V_{max} for the elimination of 2,4-D were dose dependent, i.e.:

Dose (mg/kg)	5	45	90
K_m	4.5	15.4	58.0
V_{max}	7.3	20.7	28.2

The authors concluded that the elimination of 2,4-D in the mouse was dose dependent and deviated from classical Michaelis-Menten kinetics.

In summary, results of animal studies on 2,4-D absorption, distribution, excretion and pharmacokinetics indicate that 2,4-D, its salts and esters are well absorbed from the gastrointestinal tract and peak plasma concentrations are reached soon after dosing. In addition, 2,4-D is rapidly cleared from the plasma and is excreted largely unchanged in the urine, although small amounts of the glycine and taurine conjugates have been detected. Pharmacokinetic

studies have revealed that the plasma clearance of 2,4-D is saturable. The chemical is distributed widely among the tissues of the body; the highest concentrations being found in parenchymal and excretory organs. Brain 2,4-D concentrations are generally low but may increase if 2,4-D is administered at intoxicating doses. These conclusions agree with those of others (Gehring and Betso, 1978; Leng, 1977; Mullison, 1986; Veterans Administration, 1981a and b; World Health Organization, 1984).

4.2. Human studies

The percutaneous absorption of [^{14}C]2,4-D (purity not stated) and the urinary excretion of [^{14}C]2,4-D after dermal or intravenous administration was studied in male subjects (age and weight not stated) (Feldman and Maibach, 1974). After intravenous administration of a tracer (1 uCi) dose of [^{14}C]2,4-D, $100 \pm 2.5\%$ of the dose was excreted in the urine in 5 days; the half-life for excretion was 13 h. After dermal administration of [^{14}C]2,4-D (1 uCi; 4 ug/sq. cm.), $5.8 \pm 2.4\%$ of the dose was excreted in the urine.

The pharmacokinetics of 2,4-D was studied in five male subjects aged 29 to 40 years and weighing 70 to 90 kg (Sauerhoff *et al.*, 1977). Each subject ingested 5 mg/kg of analytical grade 2,4-D either as a slurry in milk or as the powder followed by water. Blood samples were collected at 1, 4, 8, 12, 24, 36, 48, 72, 96, 120, and 144 h and urine samples at 12-h intervals after dosing. Average $t_{1/2}$ for absorption of 2,4-D was 3.8 h (range 1.67 to 4.20 h), $t_{1/2}$ for clearance from plasma averaged 11.6 h and $t_{1/2}$ for the urinary elimination averaged 17.1 h (range 10.2 to 28.4 h). Most (82%) of the 2,4-D was excreted unchanged, but 13% was excreted as conjugates. The clearance of 2,4-D from the plasma and the urinary elimination of 2,4-D followed first-order kinetics, which could be described by a one-compartment model although one subject showed biphasic clearance of 2,4-D. The authors concluded that 2,4-D was rapidly absorbed from the intestinal tract and rapidly excreted in the urine and would not be expected to accumulate in the body after repeated oral dosing at usual levels of exposure.

Plasma and urine 2,4-D concentrations have been measured in occupationally exposed workers (Kolmodin-Hedman and Erne, 1980). Four male subjects (mean age of 39 years) involved in spraying an emulsion containing 2% 2,4-D (neither purity nor amine or ester content was stated) and kerosene were studied. Blood samples were collected before the start of spraying, immediately after spraying, and at the end of the exposure week; urine samples were

collected at the start and at the end of 10 days of exposure or, in another study, at 12-h intervals during the exposure period. Concentrations of 2,4-D in air ranged from 0.1 to 0.2 mg/m³. Plasma 2,4-D concentrations ranged from 0.02 to 0.2 ug/ml and varied considerably because of intermittent exposure; after exposure, plasma 2,4-D concentrations dropped overnight to near the detection limit (0.02 ug/ml). Urine 2,4-D concentrations ranged from 3 to 14 ug/ml and the mean peak was 8 ug/ml. Mean 24-h excretion of 2,4-D amounted to 9 mg. The authors concluded that 2,4-D may enter the body by inhalation or dermal absorption and that urinary elimination of 2,4-D is rapid.

Taskar *et al.* (1982a and b) measured serum and urine 2,4-D concentrations in 11 male subjects aged 19 to 31 years (weights not stated), who applied 2,4-D (DMA-4, Dow Chemical Co.). Body surface exposure was estimated by measuring the 2,4-D content of gauze pads (620 cm²: 0.67 ft²) attached to the chest and back of each subject and of a paper cap (406 cm²: 0.44 ft²) worn by the subjects. Blood samples were collected before and after exposure. Urine was collected before and after exposure, during the day of exposure (3 h after spraying) and twice a day for four to seven days after exposure. At the end of the exposure period, 2,4-D residues of 564, 532, and 319 ug/m² were found on the head, chest, and back, respectively, of the subjects. Serum 2,4-D concentrations (expressed as "amount of 2,4-D measured as phenolics in serum") at the end of exposure averaged 167 ng/ml (range 15.6 to 484.2 ng/ml). The serum 2,4-D concentrations measured 1, 2, and 3 days after exposure varied considerably. In some subjects a progressive decline in serum 2,4-D concentrations was noted, but in other subjects the 2,4-D content of samples taken on the second day after exposure was greater than that in samples taken on the first or third day after exposure. In some subjects, serum 2,4-D concentration was highest on the third day after exposure. Urine 2,4-D levels were apparently measured for 84 h after exposure, but no units of concentration were reported in the paper; hence the urine data cannot be evaluated. The authors asserted that serum residues correlated positively with the 2,4-D concentration in the air and with the amount of 2,4-D deposited on the head and back of the subjects. However, no air 2,4-D concentrations were reported in the paper and the results of statistical analyses were not presented.

Absorption and urinary excretion of 2,4-D was studied in 36 applicators (sex, age, and weight not stated) occupationally exposed to a 2,4-D/picloram (4-amino-3,5,6-trichloropicolinic acid) mixture (Dow Chemical Tordon 101) or a 2,4-D/dichloroprop (2-(2,4-dichlorophenoxy)propanoic acid) mixture (Pfizer

Chemical) (Libich *et al.*, 1984). In one study conducted in 1979, urine 2,4-D concentrations averaged 6.17 ± 7.69 (range 0.27 to 32.74) mg/kg (L) in one group of workers and 3.16 ± 2.85 (range 0.63 to 12.35) mg/kg (L) of urine in another group of workers. In a second study conducted in 1980, improved working procedures were introduced and workers were instructed on the procedures that were required; moreover, air 2,4-D concentrations were also measured. In three groups of workers, urine 2,4-D concentrations averaged 1.42 ± 1.76 (range = 0.04 to 8.15), 1.72 ± 1.50 (range = 0.15 to 5.45), and 2.55 ± 1.72 (range = 0.44 to 5.07) mg/kg (L); respective air 2,4-D concentrations were 7.1 ± 4.9 (1.0 to 19.5), 13.5 ± 7.6 (0.4 to 35.3), and 55.2 ± 30.7 (16.2 to 91.3) $\mu\text{g}/\text{m}^3$. Finally, a model was devised that related urine 2,4-D concentrations to daily exposure levels of airborne 2,4-D and allowed the use of urine 2,4-D concentrations as exposure guides.

The urinary elimination of 2,4-D after direct and indirect exposure of forestry workers to 2,4-D has been studied (Frank *et al.*, 1985). Seven subjects (5 males and 2 females) weighing 51 to 84 kg and aged 20 to 38 years who applied formulations containing 2,4-D isooctyl esters (Estron LV-600 and Pfizer 2,4-D Ester LV600) were used. In an experiment conducted in 1981, three workers were directly exposed to 2,4-D for 65 h during an 11 day period; for the three workers, the highest daily excretion of 2,4-D in the urine was 0.30, 0.94, and 9.59 $\mu\text{g}/\text{kg}$ body weight. In an experiment conducted in 1982, three workers were exposed to 2,4-D for 50, 51, and 39 h during an 18 day period; the highest daily excretion of 2,4-D in the urine for each of the workers was 7.73, 8.37 and 22.2 $\mu\text{g}/\text{kg}$ body weight/day. For one subject who was sprayed directly, it was estimated that 0.44% of the applied dose was absorbed, and the highest daily excretion of 2,4-D in the urine was 4.75 $\mu\text{g}/\text{kg}$ body weight/day. Urinary excretion of 2,4-D was followed for 8 days in this subject; a half-residue decline of 16 h determined. Further studies showed that 2,4-D persisted in urine samples collected during the post-spray period. Analysis of surface swabs of vehicles, helicopters, and living quarters revealed contamination of all internal surfaces with 2,4-D which were probably the source of the 2,4-D detected in urine samples collected in the post-spray period. The authors calculated that, assuming a half-life of 18 h for 2,4-D, in the worker excreting the most 2,4-D (22.2 $\mu\text{g}/\text{kg}$ body weight/day), the maximum dose of 2,4-D absorbed amounted to 60 $\mu\text{g}/\text{kg}$ body weight/day.

Dermal absorption and urinary excretion of 2,4-D were studied in four subjects (ages 24 to 57 yr, sex and weight not stated) exposed under field

conditions to 2,4-D amine salts (the commercial formulations used were not described) (Grover *et al.*, 1986). Urine samples were collected before, during and for 4 to 7 days after the spray operations; dermal exposure was estimated by measuring the 2,4-D content of patches fastened both outside and underneath the clothing and by measuring the amount of 2,4-D removed by washing the hands with a sodium bicarbonate solution after the spray exposure. In all subjects, the urinary excretion of 2,4-D increased after each spraying operation. The amount of 2,4-D excreted was a function of the number of consecutive exposures, the number of days between exposures, and the time elapsed since the last exposure. The cumulative total amounts of 2,4-D excreted were a function of the number of consecutive exposures, the number of days between exposures and the time elapsed since the last exposure. The cumulative total amounts of 2,4-D excreted ranged from 215 to 6,258 ug. Statistical analysis revealed a positive correlation between the amount of 2,4-D applied and the cumulative amount of 2,4-D excreted. In addition, there was a positive and significant correlation between the amount of 2,4-D deposited on the hands and the amount of 2,4-D excreted in the urine.

4.3. Evaluation of pharmacokinetics and metabolism

Studies on the physiological disposition of 2,4-D in human subjects show that 2,4-D is absorbed after oral or dermal administration and is rapidly and almost completely eliminated from the body by urinary excretion. The half-life for clearance of 2,4-D from the body is <24 h. After occupational exposure to 2,4-D, dermal absorption appears to be the major route of entry into the body. These conclusions agree with those of others (IARC, 1987; Veterans administration, 1981a and b; World Health Organization, 1984).

The relevant pharmacokinetic studies provide evidence of a reliable and constant relationship between exposure, uptake and urinary elimination of 2,4-D in workers exposed over several days and who have achieved steady state pharmacokinetics. These studies also indicate that, in workers who use 2,4-D regularly, the amount excreted in the urine over a 24-hour period is an reliable measure of the absorbed systemic dose.

5. EXPOSURE ASSESSMENT

5.1. Occupational Exposure to 2,4-D.

Frank *et al.* (1985) reported a study on exposure to 2,4-D, by measurement of urinary excretion for 2 sets of workers. Crew 1, which

included 2 mixer-loaders and a supervisor, wore full protective gear (including respirator) and worked over an 11-day period. Approximately 2,200 ha were sprayed at an application of 2 kg/ha. Crew 2, included 1 mixer-loader, 1 mixer-balloon (flagger) man, and 1 balloon man, also wore protective gear but did not consistently wear respirators. The spray period was 18 days. Approximately 4,000 ha were sprayed with 1.3 kg/ha. Highest daily excretion for crew 1 ranged from 0.30 to 9.59 ug/kg body weight while that for crew 2 was considerably higher, ranging from 7.73 to 22.2 ug/kg body weight. Total excreted doses ranged from 136 ug (supervisor) to 1,600 ug (mixer-loader).

In a study by Lavy *et al.* (1982), urinary excretion data were utilized to estimate total absorbed dose of 2,4-D for 3 crews involved in aerial application of the herbicide to 40 ha tracts of forest in Washington State. The herbicide was applied at the rate of 2.1 kg/ha. Crews were involved in two applications each: one in which conventional clothing was worn (no protective gear), the other in which protective gear was worn (without respirator). Monitoring was performed in such a way as to allow estimates of exposure resulting from dermal and inhalation routes, results of which indicated that exposure was primarily due to dermal contact. This and a later study (Lavy *et al.*, 1987) were not considered further because conditions were not typical of Ontario.

Libich (1981) and Libich *et al.* (1984) reported studies to assess exposure to those who spray roadsides, power lines and rights-of-way for Ontario Hydro. Spraying was done by backpack (handheld gun or mist blower) or from vehicle (truck or all-terrain vehicle, using handheld gun), and mixtures of either 2,4-D and 2,4-dichloropropanoic acid (2,4-DP) (1:1) or 2,4-D and picloram (4:1) were used. Little information was given concerning the amounts of herbicide used, but a recent survey (DHS, 1987) indicates that Ontario Hydro applies 2,4-D/2,4-DP mixture at rates up to 5.5 kg 2,4-D/ha and 2,4-D/picloram mixture at a rates up to 22.7 kg 2,4-D/ha. Information on use of protective clothing was also limited to statements that there was general use of gloves, that clean coveralls were issued daily and that wash-up facilities were provided. Average daily intake, approximated by average excretion on Thursday of each spray week, was 3.53 mg, 3.45 mg, and 4.86 mg for rights-of-way sprayers using handheld gun, roadside sprayers using handheld gun, and rights-of-way sprayers using mist blowers, respectively.

Occupational exposure to 2,4-D was assessed for commercial lawn care

specialists who apply 2,4-D in combination with other herbicides including MCPP [2-(2-methyl-4-chlorophenoxy)propanoic acid] and dicamba (Yeary, 1986). The diluted mixture contained 2,4-D, MCPP and dicamba in a ratio of 12:6:1. Personnel had been involved in application of herbicides for 3 weeks prior to this analysis and, therefore, were assumed to exhibit steady-state body burdens of 2,4-D. Exposure was estimated by analysis of urinary excretion with the assumption that, at steady-state, the 24-hour urinary excretion of 2,4-D was a reasonable estimate of daily absorbed dose. While mixing herbicide, employees wore protective clothing including gloves, apron or coveralls, face protection, and rubber boots. However, during actual spraying, wearing of gloves and eye protection was optional while rubber boots and clean uniforms (long pants, short-sleeve shirt) were considered standard apparel. Solutions were applied at a rate of 16 L per 93 m² (1.3 kg 2,4-D/ha), and 3,000 to 4,500 L were applied daily (adequate treatment for 2 to 3 ha). Personnel from five locations were monitored; however, in one of these locations spray activities were interrupted by rain. For the other four spray areas, mean urinary excretion values were reported to be 1.4, 3.2, 6.3, and 2.5 ug/kg body weight/day. Average exposure for the four groups combined was 3.3 ug/kg/day.

Grover *et al.* (1986) assessed exposure in farmers, exposed multiple times in one season to 2,4-D under field conditions in Saskatchewan. Farmers used their own tractors and ground rigs and applied 2,4-D at rates applicable to crop and control needs. Dermal exposure was estimated by use of patches and handwashes and total exposure by urinary excretion values. Subjects wore garments consisting of two layers of cotton but did not use respirators. Number of exposures per subject ranged from 1 to 7, with those having more exposures excreting greater amounts of 2,4-D in terms of kilogram of herbicide applied. Application rates ranged from 0.35 kg/ha to 0.63 kg/ha, averaging 0.43 kg/ha. Cumulative urinary excretion ranged from 0.2 mg to 6.3 mg 2,4-D with an average value of 1.6 mg excreted. One subject who was monitored for only one exposure excreted a total of 0.3 mg as a result of spraying 71 ha at a rate of 0.35 kg/ha.

5.2. Estimation of Exposure

Data on urinary excretion of 2,4-D from each of the above reports were used to estimate absorbed dose under various work conditions most typical of the Ontario use pattern of 2,4-D. These estimates are contained in Table 2.

For each of the above studies, an average daily dose was estimated. Information on days per year exposed and number of years exposed (Table 2) were derived from data in a recent survey (DHS, 1987). If such data were not available, values for these parameters were assumed using best professional judgement.

Table 2. Occupational exposure of persons involved in the application of 2,4-D by various methods

Method of Application	Occupation	Average daily intake (mg/person)	Days/year Exposed	Years Exposed	Total Intake (mg)
Frank <i>et al.</i> , 1985					
Helicopter	mixer-loader	1.04	12	20	250
	mixer-flagger	0.46	12	20	111
	flagger	0.34	12	20	80
Airplane	mixer-loader	0.15	12	20	36
	supervisor	0.005	12	20	1
Libich, 1981					
Packsprayers handheld gun	rights-of-way sprayer	3.5	60	10	2107
	roadside sprayer	3.4	60	10	2070
Mist blower	rights-of-way sprayer	4.9	60	10	2895
Yeary, 1986					
Hand and tank	commercial sprayer	0.29	66	13	245
Grover <i>et al.</i> , 1986					
Tractor	farmer	0.48	14	25	166

In the study by Frank *et al.* (1985), total urinary excretion data were reported (including post-spray excretion) and average daily dose was approximated by dividing the total urinary excretion by the number of reported spray days. In contrast, the study by Libich (1981) reported urinary excretion on (often non-consecutive) spray days, but not on post-spray days. Libich (1981) indicated that, for successive daily exposure, urinary excretion after the fourth day should approximate the daily exposure. Also, the reported measurements were made generally during weeks in which spraying activities were conducted Monday through Friday (personal communication from Libich). Therefore, average daily urinary excretion reported on Thursdays and Fridays was assumed to approximate average daily intake. Information on exposure to farmers spraying 2,4-D (Grover *et al.*, 1986) was expressed as total urinary

excretion of 2,4-D for the total number of spray operations (all occurring within a short period of time). Average daily intake was approximated by dividing total urinary excretion per farmer by the number of spray operations performed by that farmer. The study of Yeary (1986) reported 24-hour urinary excretion of 2,4-D for five groups of commercial herbicide sprayers, in units of mg 2,4-D/kg body weight. The sprayers had been working almost daily for three weeks, so that this 24-hour sample was used as an approximation of average daily intake. Each study reported the average percent of 2,4-D recovered by the chromatographic method utilized, and reported amounts of 2,4-D excreted were corrected for less than complete recovery. It was assumed that the studies utilized reported relatively normal spray practices, i.e. that parameters such as rate of 2,4-D applied and use of protective clothing fell within normal limits. If body weight and total urinary output were not supplied, values of 70 kg and 1.4 L/day were utilized where needed.

5.3. Evaluation of Exposure

It is clear that exposure to 2,4-D is dependent on the rate of application of the herbicide. Rights-of-way sprayers use 2,4-D at the highest rate of application and their daily exposure was higher than other groups. Hydro-line workers may be exposed to the 2,4-D in amounts from 0.005 - 5 mg/person/spraying day. Exposure in this setting is highly variable and depends upon the nature of the work performed (mixer-loader, sprayer, flagger, etc.) and the extent to which precautionary procedures are followed and protective gear is worn. Commercial lawn applicators were found to receive a daily dose of approximately 0.3 mg/person/spraying day while farmers were estimated to receive about 0.5 mg/person/spraying day. Variation between these groups is due to differences in spray equipment, terrain and to the degree to which the operators come into contact with concentrated formulations. The latter is the most probable reason for the low levels of exposure in commercial lawn applicators who were reported to take appropriate precautions when mixing the formulation. Lifetime exposure to 2,4-D is related to the number of days of spraying and the number of years in the occupation.

6. GENOTOXICITY

It is widely accepted that tests of genetic activity, conducted using appropriate in vitro and in vivo systems, may indicate possible carcinogenic activity. Positive results in genetic toxicity tests; however, cannot be said to unequivocally predict carcinogenicity since these tests only measure a limited number of events putatively associated with the carcinogenic process. Similarly, negative results in tests of genotoxicity cannot be considered to provide conclusive evidence against carcinogenicity since agents may act to produce cancer through processes not detected by the currently available short-term tests.

Positive results for genotoxic activity obtained in in vitro systems such as prokaryotes, fungi and cultured mammalian cells may suggest that similar effects could occur in animals in vivo. It is therefore important to assess the genetic effects of chemicals in vivo to observe if similar effects are observed in animals exposed under appropriate doses and routes of administration. Positive results in in vivo test systems provide important additional evidence that the chemical is genotoxic and are regarded as being of greater relevance than effects in lower order organisms (IARC, 1987).

Attempts to relate genotoxic effects to carcinogenicity must take into consideration possible mechanisms of action and evidence which describes the relationship between the test system and carcinogenicity in whole animals or humans. In this regard it is important to note that correlations between genotoxicity and carcinogenicity are far from perfect and, indeed, are considered by some investigators (Mendelsohn, 1985) to be of limited value for regulatory purposes. It is therefore essential that the interpretation and regulatory use of the results of short-term genotoxicity tests encompass a critical scientific evaluation of the data, keeping in mind its possible relevance to human exposure conditions.

The genotoxicity of 2,4-D has been studied extensively in a wide variety of in vitro and in vivo test systems. As 2,4-D is a highly active and toxic herbicide, it is inappropriate to include those genotoxicity studies conducted on plants and plant tissues. The pivotal studies concerning the potential genotoxicity of 2,4-D are discussed below.

6.1. In Vitro Studies

The salient in vitro genotoxicity studies on 2,4-D are summarized in Table 3. Mutagenicity of 2,4-D has been tested repeatedly in several strains of

Salmonella typhimurium (Klopman *et al.*, 1985; Zetterburg *et al.*, 1977; Anderson *et al.*, 1972; Shiraus *et al.*, 1978; Mortelmans *et al.*, 1984; Rashid and Mumma, 1983). Concentrations of up to 10 mg/plate, a level which produced toxicity in several strains of Salmonella, failed to induce an increase in mutation frequency. In an assay measuring differential toxicity of 2,4-D to Bacillus subtilis, Waters *et al.* (1982) noted positive results, suggesting that 2,4-D may inhibit DNA repair in this test system. However, no effect on DNA repair, as measured by differential toxicity, was noted in Escherichia coli polA⁻ strain (Waters *et al.*, 1982) and Shirasu *et al.* (1976) failed to obtain positive results in the B. subtilis assay. Ahmed *et al.* (1977a) reported that a formulation of "2,4-D-Fluid" (purity and composition not given) induced unscheduled DNA synthesis (UDS) in cultured human fibroblasts; however, Probst *et al.* (1981) found no increase in UDS in primary rat hepatocyte cultures and Simmon (1979) found no effect of 2,4-D on UDS in human embryonic lung cells at concentrations up to 100 mg/L.

Table 3. In vitro studies on 2,4-D.

<u>Test Organism</u>	<u>Result</u>	<u>Reference and comments</u>
<u>Salmonella typhimurium</u> histidine reversion assay	-	Klopman <i>et al.</i> (1985). Neither the purity nor the concentration of the 2,4-D was given in the paper.
<u>Salmonella typhimurium</u> histidine reversion assay	-	Zetterburg <i>et al.</i> (1977). No effects at pH values of 4.3 or 6.8.
<u>Salmonella typhimurium</u> histidine reversion assay	-	Anderson <i>et al.</i> (1972). pH not stated but is probably that of the medium. Spot test system with concentration of 50 ug/plate.
<u>Salmonella typhimurium</u> reversion assay	-	Shirasu <i>et al.</i> (1976).
<u>Salmonella typhimurium</u> reversion assay in TA98, TA100, TA1535 and TA1567 strains.	-	Mortelmans <i>et al.</i> (1984). Used five concentrations over 2 orders of magnitude of 2,4-D acid, n-Butyl, isooctyl esters and amine with and without activation by rat S9 microsomes
<u>Salmonella typhimurium</u> reversion assay in G46, TA98, TA1000, TA1535, TA1537, TA1538, D3052 and C3076 strains.	-	Probst <i>et al.</i> (1981). Used 10000-fold concentration gradient replicated 4 times of 2,4-D acid with and without activation by Arochlor 1254 induced rat S9 microsomes
<u>Salmonella typhimurium</u> reversion assay in TA97, TA98, TA100, TA1535 and TA1538 strains.	-	Rashid and Mumma (1983). No effects with the alanine, aspartic acid, leucine, methionine and tryptophan conjugates of 2,4-D at concentrations of 10, 100 and 1000 ug/plate
<u>Salmonella typhimurium</u> reversion assay in TA97, TA98, TA100, TA1535, TA1537 and TA 1538 strains.	-	Moriya <i>et al.</i> (1983). Dose response was determined up to a concentration of 5000 ug/plate.

<u>Escherichia coli</u> DNA repair assay.	-	Waters <u>et al.</u> (1982).
<u>Escherichia coli</u> WP 2 tryp. reverse mutation assay.	-	Nagy <u>et al.</u> (1975). Spot test with undefined concentrations of 2,4-D.
<u>Escherichia coli</u> reversion assay	-	Shirasu <u>et al.</u> (1976).
<u>Escherichia coli</u> WP 2 tryp. reverse mutation assay.	-	Moriya <u>et al.</u> (1983). Dose response was determined up to a concentration of 5000 ug/plate.
<u>Bacillus subtilis</u> DNA repair assay.	+	Waters <u>et al.</u> (1982).
<u>Bacillus subtilis</u> recombination assay.	-	Shirasu <u>et al.</u> (1976).
<u>Saccharomyces cerevisiae</u> mitotic recombination assay.	+-	Zetterburg <u>et al.</u> (1977). Effects only observed at pH < 4.5, possibly because of lack of uptake of dissociated form of molecule.
<u>Saccharomyces cerevisiae</u> mitotic recombination assay.	-	Klopman <u>et al.</u> (1985). Comments as for above.
<u>Saccharomyces cerevisiae</u> host mediated mitotic recombination assay.	-	Zetterburg <u>et al.</u> (1977).
<u>Saccharomyces cerevisiae</u> RAD 18 strain reverse mutation assay.	+-	Zetterburg (1978). Again, effects only observed at pH < 4.5, possibly because of lack of uptake of dissociated form of molecule.
Mitotic gene conversion assay in <u>Saccharomyces cerevisiae</u> D4 strain.	+	Seibert and Lemperle (1974). 2,4-D increased convertants 5 to 6-fold at pH 4.5. (See comments on Zetterburg <u>et al.</u> , 1977 above)
Unscheduled DNA synthesis in human fibroblast cells.	+	Ahmed <u>et al.</u> (1977a). Assay with and without rat S9 microsomal activation was positive at all concentrations tested. The 2,4-D formulation used was not clearly identified except as 2,4-D fluid. The structure was shown to be that of the acid but it was stated that the material was water soluble, suggesting that the amine or the salt may have been used. The possible involvement of mutagenic contaminants cannot be ruled out.
Unscheduled DNA synthesis in human embryonic lung cells.	-	Simmon (1979). Cells of the WI-38 strain exposed to concentrations ranging from 0.1 to 100 mg/L in presence and absence of microsomal activation
Unscheduled DNA synthesis in rat hepatocyte cells.	-	Probst <u>et al.</u> (1981). Hepatocytes from Fischer 344 rats did not show increased UDS at concentrations up to 1,000 mg/L.
Forward mutation in Chinese hamster V79 cells.	+	Ahmed <u>et al.</u> (1977b). A 12-fold increase in forward mutation at a concentration of 4 mg/L at which 60% of cells were killed. The 2,4-D formulation used was not clearly identified except as 2,4-D fluid. The structure was shown to be that of the acid but it was stated that the material was water soluble, suggesting that the amine or the salt may have been used. The possible involvement of mutagenic contaminants cannot be ruled out.

Thymidine kinase gene mutation assay in mouse lymphoma cells (LS178Y).	-	Klopman <i>et al.</i> (1985). Neither the purity nor the concentration of the 2,4-D was given in the paper.
SCE in human lymphocytes.	+	Korte and Jalal (1982). Increases in SCEs at concentrations of 2,4-D $\geq$ 10 mg/L in <i>in vitro</i> system.
SCE in human lymphocytes.	+-	Turkula and Jalal. (1985). Statistically significant increase only at 50 mg/L, none at 100 and 250 mg/L. No dose response was observed.
Mitotic index in fetal bovine kidney cells.	+-	Bongso and Basrur (1973). Increase in mitotic index up to 10 mg/L with a decrease at concentrations up to 1000 mg/L. Some indication of interference with spindle formation. Formulation of 2,4-D not specified.
Mitotic index in bovine peripheral blood cells.	+-	Bongso and Basrur (1973). Increase in mitotic index up to 10 mg/L with a decrease at concentrations up to 1000 mg/L. No other indications of interference cell division. Formulation of 2,4-D not specified.
Chinese hamster ovary cells, SCE.	-	Linnainmaa (1984). Both pure (Acid) and commercial formulations of 2,4-D and MCPA (amine of 2,4-D and isooctyl ester of MCPA) at non-toxic concentrations of 10^{-3} , 10^{-4} and 10^{-5} M caused some increases in the presence or absence of activation by rat liver S9 fraction. Although these increases were, in some cases, statistically significant, they did not show a dose response and, in most cases, resulted from somewhat lower rates in the control groups. The author concluded that neither of these compounds appeared to act directly on DNA.

In tests employing yeasts, Zetterburg *et al.* (1977) noted a dose-dependent increase in the frequency of mitotic gene conversion and mitotic recombination in *Saccharomyces cerevisiae*; however, this effect was found only at pH 4.5 and 4.3 and not at higher pH values. At lower pHs survival was markedly affected. Zetterburg (1978) concluded that the pH dependency on mutation frequency was due to the fact that, at lower pHs, 2,4-D is in an molecular form which is readily taken up cells in culture. This conclusion is supported by Zetterburg's observation that 2,4-D did not produce mutagenic affects in *S. cerevisiae* when tested in a host-mediated assay using mice.

Studies by Korte and Jalal (1982) and Turkula and Jalal (1985) were said by these authors to demonstrate that 2,4-D produced sister chromatid exchanges (SCEs) in cultured human lymphocytes. Effects noted in these studies were, in most cases, marginal, occurred at near-toxic doses and failed to meet accepted criteria for positive results (Waters *et al.*, 1982). In addition, Waters *et al.* (1982) in a definitive series of tests, did not observe any effect of 2,4-D on SCE frequency in CHO cells while Linnainmaa (1984) observed only a

marginal effect of 2,4-D on SCE frequency in CHO cells which failed to meet accepted criteria for positive results.

In conclusion, it may be stated that there is no firm evidence that 2,4-D induces SCE's in cultured mammalian cells. This view is substantiated by the results of in vivo tests in animals and man.

6.2. In Vivo Studies

6.2.1. Animal Studies

Studies on the genotoxicity of 2,4-D conducted in animals are summarized in Table 4. As pointed out above the marginally positive results for SCE reported in in vitro systems have not been noted in whole animal studies. Linnainmaa (1984) did not find any increase in SCEs in circulating lymphocytes of rats treated for one week with 2,4-D at a daily dose (close to toxic) of 100 or 200 mg/kg. Similarly, Linnainmaa (1984) failed to demonstrate an increased frequency of SCEs in the bone marrow of hamsters treated with 100 mg/kg/day of 2,4-D for 7 days. Lamb et al. (1981) likewise noted no increase in the frequency of SCEs in the bone marrow of mice given 2,4-D. Pilinskaya (1974); however, reported a slight effect of 2,4-D on chromosomal aberrations in the bone marrow of mice treated at intoxicating doses of 100 or 300 mg/kg but no effects were noted at 10 or 50 mg/kg. A mouse micronucleus test conducted at an i.p. dose of 100 mg/kg produced negative results (Jenssen and Renberg, 1976). A dominant lethal assay in ICR Ha Swiss mice given 125 mg/kg 2,4-D was likewise negative (Epstein et al., 1972).

Table 4. Mutagenicity of 2,4-D in animal systems.

<u>Test Organism</u>	<u>Result</u>	<u>Reference and comments</u>
Male Wistar rats, peripheral lymphocyte SCE.	-	Linnainmaa (1984) Pure (acid) 2,4-D and MCPA did not cause increases in peripheral lymphocyte SCE when fed for one week at doses of 100 and 200 mg/kg body weight/day by gavage.
Chinese hamster bone marrow cell SCE.	-	Linnainmaa (1984) No elevations were observed in the case of 2,4-D when both were dosed by gavage at 100 mg/kg body weight/day for 7 days.
Mouse testicular DNA synthesis.	→	Seiler (1978). A 29% reduction in thymidine uptake after oral dosing of mice with 200 mg/kg. The dose is near to the toxic and results may be general toxic response. No dose-response measured.
C57BL/6N mouse bone marrow SCE.	-	Lamb <u>et al.</u> (1981) failed to demonstrate significant elevations in mice fed diets resulting in daily doses of 40 and 20 mg each of 2,4-D, 2,4,5-T (containing TCDD) /kg body weight/day for 8 weeks.

Mouse bone marrow cells, chromosomal aberrations.	+	Pilinskaya (1974). Only observed at doses of 100 and 300 mg/kg body weight in combination with intoxication. Not observed at 10 and 50 mg/kg body weight.
Mouse micronucleus test.	-	Jenssen and Renberg (1976). No effects observed at an IP dose of 100 mg/kg body weight.
Nondisjunction assay in <u>Drosophila melanogaster</u>	-	Magnusson et al. (1977). No effect at 100 ppm 2,4-D in diet.
Chromosome loss assay in <u>Drosophila melanogaster</u>	-	Magnusson et al. (1977). No effect at 100 ppm 2,4-D in diet.
Recessive lethal assay with Karsnas and Muller 5 strains of <u>Drosophila melanogaster</u>	+	Magnusson et al. (1977). Effect with 2,4-D statistically significant at 1000 ppm in diet.
Recessive lethal assay with Berlin K strain of <u>Drosophila melanogaster</u>	-	Vogel and Chandler (1974). No effects at dietary concentrations of 500 and 1000 ppm.
Genetic mutations in stable and unstable strain of <u>Drosophila melanogaster</u>	+-	Rasmuson and Svahlin (1978). Positive results in the unstable strain, negative in the stable strain.
Dominant lethal assay in ICR Ha Swiss mice.	-	Epstein et al. (1972). Single injection (IP) of 2,4-D at 125 mg/kg body weight.

There is some evidence from studies in Drosophila melanogaster that 2,4-D may produce mutations; however, the evidence in this regard is conflicting. Magnusson et al. (1977) noted negative results in a nondisjunction assay and a chromosome loss assay in Drosophila given a diet containing 100 ppm 2,4-D, but noted a statistical increase in a recessive lethal assay in Drosophila given a diet containing 1000 ppm 2,4-D. Magnusson et al. (1977) concluded that the effect was weak, amounting to only 2-3 times the control levels. Rasmuson and Svahlin (1978) reported an enhanced somatic mutation frequency in unstable but not in stable strains of Drosophila; however, the response reported was weak. Compared to the classical mutagen ethyl methane sulphonate, which induced a 3.28% increase in mutation frequency, 2,4-D induced only a 0.69% increase. The control mutation frequency was 0.075%. Other studies in Drosophila (Vogel and Chandler, 1974) have failed to demonstrate any significant effect of 2,4-D on the frequency of recessive lethal mutations.

The available animal studies do not provide convincing evidence that 2,4-D is genotoxic. Marginally positive results, when reported, have occurred at near-toxic concentrations. The relevance of these positive findings to risk assessment is questionable, particularly in view of the fact that most studies have produced negative results.

6.2.2. Human Studies

There is no evidence that, under conditions of manufacture or use, 2,4-D produces genotoxic effects in humans. In a review on 2,4-D, WHO (1984) reported five studies on workers exposed to 2,4-D under manufacturing or use situations. In no case were any chromosomal abnormalities noted.

Linnainmaa (1983a and b and 1982) compared the incidence of lymphocyte SCEs in herbicide workers with that in controls and found no exposure related effects. Comparison of 2,4-D levels in the urine of exposed workers and frequency of SCEs failed to show a dose response. In both the control and exposed group, SCEs were significantly elevated in those workers who smoked although no differences between control and exposed were observed in the degree of elevation. Elevation of SCEs in smokers would be expected and, as such, is a positive control for the study. The results failed to demonstrate synergism between 2,4-D exposure and smoking.

Mustonen et al. (1986) reported no difference between frequency of chromosomal aberrations in lymphocytes from workers exposed to 2,4-D and control workers. Exposure was confirmed by urinary analysis. As reported above, the frequency of aberrations was higher in smoking than non-smoking workers in both exposed and control groups.

Several older studies of chromosome aberrations and SCEs in humans exposed to 2,4-D were reviewed recently by IARC (1987). The results of these studies have been uniformly negative.

6.3. Evaluation of Genotoxicity

There is no evidence that 2,4-D is mutagenic in the Ames Salmonella test or in Escherichia coli; however, some conflicting data have been reported in other bacterial systems. Both positive and negative results have been noted in differential toxicity studies in B. subtilis. The significance of these positive results is questionable in view of the fact that this test correlates poorly, if at all, with carcinogenicity. A formulation of 2,4-D "fluid" of unspecified composition has been reported to induce unscheduled DNA synthesis in human fibroblasts; however, other studies with pure formulations of 2,4-D have been unable to confirm these findings in tests involving human embryonic lung cells and cultured rat hepatocytes. It is possible that the positive results reported in human fibroblasts with 2,4-D "fluid" may represent a true finding; however, the possibility that the effects noted were due to impurities or other anomalies in the test cannot be discounted. They also reported that 2,4-D induced

mutations in yeast but positive effects were noted only at a pH below 4.5, leading the authors of these studies to conclude that effects were dependent entirely on pH of the culture media.

There are studies indicating that 2,4-D produces SCEs in cultured human lymphocytes but not in hamster embryo cells. The significance of these findings is questionable in light of the fact that several in vivo studies involving rats, mice, hamsters and humans have not shown any effects on SCEs in lymphocytes or bone marrow cells when 2,4-D was administered by appropriate routes at up to toxic doses. In addition, SCEs in vitro cannot be viewed as reliable predictors of carcinogenicity (Brusick et al., 1983). There is one report that 2,4-D induced chromosomal aberrations in mouse bone marrow cells (Pilinskaya, 1974); however, the significance of this finding is questionable because the animals were given a dose corresponding to the LD₅₀.

Conflicting data exist on the mutagenic activity of 2,4-D in Drosophila. Unstable strains appear to show weakly positive effects while more stable strains appear to be resistant even at very high dietary concentrations (e.g. 1,000 ppm). A mouse micronucleus test and a dominant lethal assay in mice conducted at doses of 100-125 mg/kg produced negative results.

In vitro studies on the genotoxicity of 2,4-D, in some cases, produced conflicting results; however, there is no convincing evidence that 2,4-D produces mutagenic effects when it is tested in in vivo systems. Overall, the pattern of responses observed in both in vitro and in vivo tests indicates that 2,4-D is not genotoxic.

7. PATHOLOGY AND CARCINOGENICITY

The potential carcinogenicity of chemicals is determined primarily by epidemiological studies in humans or by long-term animal experiments. This section deals with studies in experimental animals chronically exposed to 2,4-D. Several animal experiments have been conducted using mice and rats, but most of these were completed over a decade ago and do not meet current standards for determining carcinogenicity (Innes et al., 1969; Hansen et al., 1971; Arkipov & Koxlova, 1974). Rueber (1983) also published an interpretation of the Innes et al. and Hansen et al. studies (1983). Two working groups of the International Agency for Research on Cancer have reviewed all of this data and considered them inadequate for an assessment of carcinogenicity (IARC, 1977, 1982). These studies have not been reviewed in detail for this report

Animal experiments in rats and mice have recently been conducted for the Industry Task Force on 2,4-D Research Data at Hazleton Laboratories America, Inc. in Vienna, Virginia. These studies were completed in 1986 and were conducted according to current good laboratory practices (GLP) requirements. The final study reports and selected rat brain sections, including all brain gliomas, were examined and form the primary basis for the assessment in this section.

7.1. Industry Task Force 2,4-D Rat Study

Groups of 60 male and 60 female weanling Fischer 344 rats were treated with 2,4-D (Acid, 97.5% pure) in the diet at levels of 0, 1, 5, 15 or 45 mg/kg body weight/day for up to 104 weeks (Hazleton, 1986). At 52 weeks, 10 animals in each group were sacrificed and subjected to pathological examination. The doses were selected based upon results of 13 week subchronic experiments in rats and mice which showed that doses of 60 mg/kg/day or higher produced damage to renal tubular epithelium in rats. Also, at 50 mg/kg/day and higher, there was a break in linear pharmacokinetics whereby excretion did not remain proportional to intake, suggesting a saturation of renal excretory capacity.

No treatment-related effects on animal survival, clinical observations or gross pathological findings occurred at either the 52 or 104 week sacrifices. However, in high-dose females, there were significant decreases in bodyweight gains at both intervals.

There were compound-related increases in histopathological lesions in 2 tissues. At all dose levels except 1 mg/kg/day, there was an increase in brown tubular cell pigment in the kidneys of both males and females. At levels of 15 mg/kg/day and 45 mg/kg/day in males and 45 mg/kg/day in females there was also an increase in renal pelvic microcalculi and there was a slight increase in renal pelvic transitional cell hyperplasia in the 45 mg/kg/day females. The hyperplasia was considered secondary to the microcalculi.

The nature of the brown pigment was not determined. However, all of these kidney changes occur spontaneously in aging control animals, and it is questionable whether the increases observed represent potential safety concerns at the levels of human exposure.

A summary of all tumor incidences is presented in Appendix 1. The only neoplastic finding of concern was an increase in astrocytomas in the brains of high-dose male rats. There was no increase in treated females. Based upon

the standard 3 coronal brain sections examined. (forebrain, midbrain, and hindbrain) the incidence of astrocytomas in male groups was as follows:

Table 5. Initial astrocytoma incidence in male rats.

Control	1 mg/kg	5 mg/kg	15 mg/kg	45 mg/kg
1/60	0/60	0/60	2/58	4/60

The tumor in a control male had the earliest onset at 21 weeks and apparently was responsible for death of the animal. No gliomas were found in the spinal cords of male rats.

Because of the greater incidence of tumors observed in high-dose animals, the remaining preserved brain tissues from all animals were sectioned, giving a final total of 6-8 brain sections per animal. Two more astrocytomas were found in high-dose male rats in the additional brain tissue. No further tumors were found in other male groups. One additional astrocytoma was found in a 5 mg/kg group female. The final over-all incidence of astrocytomas in males was, as follows:

Table 6. Final astrocytoma incidence in male rats.

Control	1 mg/kg	5 mg/kg	15 mg/kg	45 mg/kg
1/60	0/60	0/60	2/58	6/60

Statistical analysis of adjusted data using a 1-tailed Fisher-Irwin Exact test indicated the incidence of astrocytomas in male rats was increased in the high-dose group ($p=0.05$). Also, a Cochran-Armitage Test indicated a statistically significant positive trend ($p<0.01$; Hazleton, 1986). Based upon these statistical analyses, the contracting laboratory report states "While the characteristics of these tumors did not conform to published characteristics of chemically induced brain tumors, this finding is, nevertheless, suggestive of a possible carcinogenic effect at a dose of 45 mg/kg/day" (Hazleton, 1986).

It is generally accepted that statistical analysis alone should not be the basis for interpreting a biological experiment (Interdisciplinary Panel on Carcinogenicity, 1984; OSTP, 1985). There are many non-quantitative, biological factors to be considered when assessing the evidence for a carcinogenic effect in animals. These include:

1. High variability of spontaneous incidence in control animals of the same strain in concurrent and earlier studies (historical controls).
2. Decreased time-to-tumor development (latency) in treated vs. control animals.
3. A dose-related increase in incidence in more than 1 experimental group or in both sexes.
4. A greater degree of tumor growth (neoplastic progression) in treated vs. control animals.
5. The presence of preneoplastic or toxic lesions in the putative target tissue.
6. The presence of multiple tumors in the putative target tissue.
7. Positive genotoxicity of the test substance.

These factors apply to evaluation of suspected carcinogens at any site, including the central nervous system, as discussed by Koestner (1986) and by Ward and Rice (1982).

The characteristics generally attributed to a brain carcinogen were not present in this experiment. There was no evidence of decreased tumor latency, the increase was limited to high-dose males, no preneoplastic lesions such as gliosis were present in treated animals, all tumors were solitary, and the tumors in treated animals were not larger or more anaplastic than generally seen in control animals. In fact, the largest and most lethal tumor was the one in a control male. There is a final consideration, i.e. most, if not all known brain carcinogens show clear genotoxicity in mutational assays (Federal Register, 1983 and Kleihues *et al.*, 1982), whereas 2,4-D is negative in most such assays.

The absence of preneoplastic and toxic lesions in the brains of the high-dose male rats warrants particular attention. Generally, when a group of inbred test animals is exposed to a carcinogenic level of a chemical throughout the course of an experiment, the different stages of carcinogenicity are repre-

sented in many of the animals. It would be unusual for 6 animals to exhibit fully developed tumors while the remaining 54 fail to develop even the earliest stages of neoplasia or other signs of toxicity in the putative target cells.

All of these considerations do not, however, totally preclude the possibility that 2,4-D may be a weak neurocarcinogen. Although known animal neurocarcinogens are either relatively potent, genotoxic chemicals or oncogenic viruses, the central nervous system could be a target tissue for relatively weak promotional or indirect carcinogenic effects as reported in other tissues. Pathological evidence of neurotoxicity has not been identified; however, clinical signs of neurotoxicity have been reported in animals exposed to high levels of 2,4-D, indicating that the chemical may functionally affect the central nervous system (See section 3). Since little is known of neurocarcinogenic mechanisms, further consideration of this area would be speculative.

The over-all incidence of brain tumors of similar type (gliomas) reported in 2,320 historical control male Fischer 344 rats up to 116 weeks of age in the U.S. National Toxicology Program (NTP) was 0.8% (Solleveld et al., 1984). Among 529 animals allowed to live out their life-span (median age 28 months) the incidence was 2.9%, indicating an increase in incidence with advancing age. If one assumed there was no compound-related effect in the 2,4-D study, the over-all glioma incidence in male rats, combining all groups, was 3.6%. This is considerably higher than the NTP figure for 2 year studies, and slightly higher than the incidence for control rats with a median age of 28 months. It must be pointed out; however, that historical control data is generally based upon 3 brain sections per animal rather than the 6-8 examined in the 2,4-D study. Since most gliomas in rat brains are found only upon microscopic examination, the number of tumors reported is probably a function of the amount of brain tissue examined histologically. Thus, the 4 tumors initially found in 2,4-D treated high-dose male rats may be more appropriate for comparison to historical control rates. This would give an over-all glioma incidence in male rats of 2.8%.

The interpretation of animal tumor data where there is high variability in tumor incidences among different control groups must take into consideration the possibility of statistical false positive findings. Solleveld et al. (1984) report that astrocytomas in Fischer rats are one of the tumor types in the NTP testing program that exhibit statistically significant intergroup variability.

7.2. Industry Task Force 2,4-D Mouse Study

Groups of 60 male and 60 female weanling B6C3F1 mice were treated with 2,4-D (Acid, 97.5% pure) in the diet at levels of 0, 1, 15 or 45 mg/kg body weight/day for 106 weeks (Hazleton, 1987).

No treatment-related effects on animal survival, clinical observations, bodyweights or gross pathological findings occurred. The only histological alteration found to be treatment-related was increased cytoplasmic homogeneity of renal tubular epithelium in male mice receiving 15 mg/kg body weight/day and 45 mg/kg body weight/day. Untreated and low-dose animals had cytoplasmic vacuoles in the epithelium. The significance of this finding is uncertain.

A summary of all tumor incidences in the mouse study is presented in Appendix 2. There were no treatment-related increases at any site.

In summary, there was no clear evidence of toxicity as the result of exposure to 2,4-D under the conditions of the experiment, and no carcinogenicity was evident. One may question whether a maximum tolerated dose was employed in the mouse study. However, the doses administered were 600-fold higher than the maximum reported human exposure (Libich *et al.*, 1981).

7.3. Evaluation of Pathology and Carcinogenicity

Two recent animal experiments with rats and mice conducted for the Industry Task Force on 2,4-D Research Data were adequate to detect potential chronic toxicity or carcinogenicity. The only finding of concern was an increase in the incidence of astrocytomas in the brains of male rats administered 45 mg/kg/day of 2,4-D for 104 weeks. There is insufficient evidence to conclude that these tumors were related to 2,4-D exposure. Although there was a statistically significant increase in treated animals, an assessment of biological factors suggests the tumors were spontaneous rather than compound-related.

8. EPIDEMIOLOGY

The available epidemiological studies of persons potentially exposed to 2,4-D include cohort and case control studies. In cohort studies, the experience of exposed individuals is followed in comparison with an unexposed group often drawn from the general population. If the exposure is well characterized the incidence of a number of different diseases in relation to exposure can be assessed. Often mortality may be used as a substitute for incidence. In case control studies, individuals with specific diseases are iden-

tified and comparable controls and their past exposures ascertained, usually by means of interviews. As these two different classes of studies have different methodologic aspects and potential concern for biases, they will be considered separately. It should also be pointed out that the classification of soft-tissue sarcomas is controversial. As it was not possible to review the data upon which tumors in the epidemiological studies were classified, the reports of the authors have been taken as published. The findings of these studies are summarized in Table 7 and their salient points discussed in the following section.

Table 7. Summary of epidemiology studies.

<u>GROUP</u>	<u>FINDINGS</u>	<u>REFERENCE</u>
<u>COHORT STUDIES</u>		
Employees manufacturing 2,4-D and 2,4,5-T in Denmark. Two groups sized 3,844 and 615 exposed from 1947/1951 to 1982	Positive: Four cases of soft tissue sarcoma (STS) with only 1.09 expected. Negative for malignant lymphomas.	Lynge, 1985.
A group of 348 Swedish railway workers who used several herbicides for at least 40 days/year.	No indication of STS or non-Hodgkins lymphoma (NHL). Lacked power to indicate or exonerate 2,4-D.	Axelsson and Sundell, 1974 and Axelsson et al., 1980.
A group of 1,971 male herbicide applicators from Finland exposed to herbicides for >2 weeks/year from 1955 to 1971.	No excess of cancer, no cases of NHL or STS.	Riihimäki et al., 1982.
A group of 1,222 Ontario Hydro sprayers in which exposures to phenoxy herbicides were high, had worked in the forestry trade for >6 months between the years 1950-1982.	No cases of NHL or STS have yet been identified but latent period short and group size was small.	Green, 1986.
A group of 354,620 Swedish agricultural or male forestry workers born between 1891 and 1940 compared to a reference cohort of ca. 2,000,000 men with other occupations.	In men working in land and/or animal husbandry, 253 cases of STS were observed with a relative risk of 0.9. In timber cutting, 49 cases observed with a relative risk of 1.0. A large study with sufficient power but with poor identification of exposure.	Wiklund and Holm, 1986.
<u>CASE CONTROL STUDIES</u>		
Group exposed to phenoxy herbicides in agriculture and forestry from 1950 to the mid 1970s in Sweden	Relative risk for STS of 5.3.	Hardell, 1981; Hardell and Sandstrom, 1979.
South Swedish group of workers exposed to phenoxy herbicides.	Relative risk for exposure to all phenoxy herbicides of 6.8 for STS. Relative risk for 2,4-D was 4.2.	Eriksson et al., 1981.

Swedish workers exposed to herbicides and chlorophenols.	Relative risk of malignant lymphoma with exposure to phenoxy herbicides or chlorophenols was 6 and for exposure to phenoxy herbicides alone, 4.8. No dose response relationship for phenoxy herbicide exposure.	Hardell <u>et al.</u> , 1981.
New Zealand study on workers exposed phenoxy herbicides > 1 d/year.	Non significant risk for STS of 1.3 and 1.5 for NHL but information on exposure may not be accurate.	Smith <u>et al.</u> , 1984.
A group of Vietnam veterans who were possibly exposed to various phenoxy herbicides in between 1962 and 1971.	Odds ratio (OR) of 0.53 for Vietnam service, OR of 0.7 for exposure to Agent Orange. Short observation period may limit power.	Greenwald <u>et al.</u> , 1984.
STS in men and women in an area in Northern Italy.	Exposure included 2,4-D, MCPA and 2,4,5-T. No excess risk of cancer associated with phenoxy herbicide exposure in men. Among living women relative risk was 2.7 (90% CI 0.59-12.37). In women alive at time of interview, <75 years old, exposed in 1950-55 period, age adjusted odds ratio was 15.5 (90% CI 1.3-180.3). The study was of low power.	Vineis, <u>et al.</u> , 1987
60 cases of primary mesothelial ovarian tumors and 127 living controls with non-ovarian malignancies.	Cases and controls from same hospital based cancer file. Herbicide exposure determined as definite if the subject or next of kin described personal use of herbicides, probable exposure if a farmer resided in areas with known herbicide usage. Eight cases gave a definite history, no controls, combining definite and probable, relative risk for herbicide exposure was 4.38 (1.90-16.07). Series relatively small and recall bias cannot be excluded. Study not informative in terms of 2,4-D exposure.	Donna <u>et al.</u> , 1984

Conducted on male cases with STS, Hodgkins Disease (HD) and NHL from Kansas (139 STS, 132 HD, 172 NHL).

No significant association between farming and/or herbicide use and STS or HD. For NHL and farming the OR was 1.4. Farm herbicide use on any of wheat, corn, sorghum, or pasture gave an OR of 1.6 for NHL. Increasing risk of NHL with increasing duration of herbicide use and/or increasing time since first exposure. Higher risk also noted with greater herbicide use without protective equipment. Use of 2,4-D was not determined directly and exposure to other chemicals may confound analysis.

Hoar et al.,
1986a and
1986b

Case control study in Western Washington State including 128 cases of STS, 575 of NHL and 694 population controls.

All data obtained by personal interview. No excess risk for past occupational exposure to phenoxy herbicides for either STS or NHL. Elevated risk of NHL among men who had been farmers (RR of 1.33; 95% CI 1.03-1.7), forestry herbicide applicators, 4.8 (95% CI: 1.2-19.4) and those potentially exposed to phenoxy herbicides for 15 years or more during the period prior to 15 years before cancer diagnosis 1.71 (95% CI: 1.04-2.8). Also increased risk of both STS and NHL in individuals reporting prior occurrence of chloracne.

Woods, et
al., 1987

8.1. Cohort Studies

In spite of the apparent superiority of the cohort design in many aspects, cohort studies have many deficiencies which makes them less informative than case control studies. One of the important reasons for this is that cancer such as STS and NHL are relatively rare compared to other cancers and also probably occur only after a long latent interval. A cohort study therefore has to be very large and the observation period very prolonged for a negative finding, a feature of the majority of them, to be of much assurance. In addition, there are potential difficulties with regard to exposure, though these are shared with case control studies.

One class of cohort study which has been used in the interim evaluation of the potential for phenoxy herbicides to increase cancer risk is identification of individuals in occupations with potential exposure to these substances such as agricultural and forestry workers. Because exposure is not individually identified in such studies, substantial obscuring (dilution) of potential effects

may occur. The value of these studies is very small and, with one exception, studies using occupation to indicate exposure have been ignored. However, many of the other cohort studies suffered from a similar deficiency, in that, even though based on specific groups of workers, details of individual exposure were unknown. An example of this is the recent Ontario Hydro Study (Green, 1986).

The other major difficulty is that nearly all cohort studies, at least in part, depended on review of historically available records with follow-up of identified individuals to the present day. Such historical cohort studies present major difficulties in the identification of individual exposure, particularly in terms of intensity. Thus it is very difficult to evaluate a dose response relationship, a critical consideration in human carcinogenesis. Although some of the cohort studies have incorporated a protocol for follow-up, in nearly every instance this has been of short duration and has not helped to achieve much resolution of the exposure issue.

In common with the case control studies, there are few groups who are known to have been exclusively exposed to 2,4-D. In the majority of instances, exposure has been to 2,4,5-T as well, often in excess of 2,4-D and, even in a positive study, it may not be clear which agent was potentially responsible.

Potentially the most informative of cohort studies are those on workers involved in the manufacture of specific chemicals in specific plants. The available studies of this type in the United States have only reported on workers involved in the manufacture of 2,4,5-T and are therefore not considered further. There has, however, been one study of this type from Denmark (Lynge, 1985) in which 3,844 workers in one plant and 615 in another, employed from 1947 and 1951 respectively were followed to December 31, 1982, by linkage with the Central Population Register for Death and the Danish Cancer Register. Exposure appeared to be largely to 2,4-D or other chemicals of a non-phenoxy type, although a small amount of 2,4,5-T was made between 1951 and 1959 in the larger plant. In this study an excess of soft tissue sarcoma was found: — Restricting attention to those where the latent period exceeded 10 years, there were 4 observed cases with 1.09 expected (95% confidence intervals [CI] of 1.00-9.39). A corresponding analysis for malignant lymphomas indicated 4 observed, compared to 3.04 expected, a non-significant difference. This is the only cohort study which has shown an excess of soft tissue sarcomas, but it is also one of the largest which had relevant exposures.

Part of the difficulty in interpretation is the exposure to 2,4,5-T and, for this reason, it cannot be concluded that the excess was due to 2,4-D.

All the other cohort studies so far reported were negative. One of the earliest was the study of Axelson (Axelson and Sundell, 1974; Axelson, *et al.*, 1980). This was a small cohort study of Swedish railroad workers, who were selected for having been exposed on at least forty days (per year) to herbicides. Although an initial apparent excess of tumors in those exposed to another unrelated herbicide (amitrole) was reported, this finding was reversed in the second report. However, a non-specific excess of tumors in those exposed to phenoxy herbicides or phenoxy herbicides and amitrole was noted (two stomach cancers, vs. 0.33 expected in the group with a latency of 10 or more years). There were no soft tissue sarcomas (STS) or non-Hodgkins lymphoma (NHL) in this group. This study only involved 348 persons, and lacked the power to clearly indicate the role of herbicides in the causation of stomach cancer as these could have arisen by chance.

Riihimäki *et al.*, 1982, reported on 1,971 males who were exposed to 2,4-D and 2,4,5-T as herbicide applicators in Finland for at least two weeks per year from 1955 to 1971. They were only followed for nine years through to 1980 and no excess of cancer with no cases of STS or NHL were noted.

In the Ontario Hydro Study (Green, 1986), the numbers of exposed workers was also small. However, exposures to phenoxy herbicides were known to have been high. This study is potentially of importance, not so much in the fact that it has been negative, (no cases of NHL or STS have yet been identified) but because it has the potential for further prolonged follow-up. However, in common with other cohort studies, exposures were to other herbicides in addition to 2,4-D and it would be unable to specifically identify a risk related to 2,4-D.

The work of Wiklund and Holm (1986) is of note because it relates to a very large cohort of 354,620 men born between 1891 and 1940 and described as agricultural or forestry workers in the Swedish population and housing census of 1960. These men, together with a reference cohort of nearly two million men with other occupations, have been followed by linking to the Swedish Cancer Environment Registry for 1961 through 1979. The cohort was sub-categorized according to presumed exposure to phenoxy herbicides by the named occupations. Large numbers of STSs occurred but, in the final analysis, there was no significant excess in any of the sub-groups. For example, in those working in land and/or animal husbandry, 253 cases were observed with

a risk of 0.9 relative to the control population (95% CI: 0.8-1.1). Similarly, for timber cutting, 49 cases were observed with a relative risk of 1.0 (95% CI: 0.7-1.3). Both these groups had presumed exposure to 2,4,5-T and 2,4-D. Although this was a large study with sufficient power to identify the increased incidence of STS (excesses) noted in the case control studies conducted in the same country, there was undoubtedly some dilution effect because of the characterization of workers by job titles. There was also a potential shortage of person years of risk for periods where an excess might be expected to occur. Nevertheless, the authors have calculated that, even with dilution, their studies should have been capable of detecting a relative risk of 1.5 which they believe would have been compatible with the relative risks in excess of five noted in the Swedish case control studies (considered below). In many respects this report can be regarded as a reasonably powerful negative study. The study has the potential to further resolve the issue, particularly if further details of exposure are obtained (possibly through a case control approach which is reported to be in the planning stage).

In summary therefore, the majority of cohort studies are of limited usefulness in terms of their size and therefore relative lack of power as well as their difficulties in characterizing exposure. The one positive cohort study, that of Lynge (1985) has to be given substantial weight, even though the only excess noted was of soft tissue sarcoma. However, it cannot be used to indicate that the excess was due to 2,4-D alone.

8.2. Case Control Studies

The case control studies have been largely responsible for identifying potential risks of cancer following phenoxy herbicide exposure in man and have so far been reported from Sweden, New Zealand, Italy and the United States. Swedish studies pointed to potentially important relative risks in (>5) for STS and NHL. The studies in New Zealand have been negative or shown small and nonsignificant excess of these two tumors. Of the studies conducted and so far reported in the United States, one was negative but two were positive in relation to NHL.

Before proceeding to a detailed review of each study, a comment on issues of methodology is in order. Substantial criticism has been levelled against many of the case control studies, particularly those from Sweden, because of the possibility for bias in the recall of information. It is accepted that recall bias is possible in any case control study (a bias in which cases are

more likely to recall relevant exposures than controls because they have the disease and the incentive to try and find a reason for developing it). Because of the potential for recall bias in any case control study, considerable attention is paid to specific points of detail in order to ensure that it is minimized. Although preferable, this is not always achievable. Interviewers who collect the information should be kept unaware (blinded) as to case and control status. questions should be asked in a pre-assigned format and those relating to specific exposure be only part of a series of questions relating to exposure to a number of different substances. The reports of the Swedish case control studies suggest that sufficient care may not have been taken on these points. Hardell (1981) addressed this issue specifically and, in addition, discussed a suggested bias apparently first proposed by Dr. Philip Cole in testimony in 1980 to the Environmental Protection Agency of the United States. His suggestion was that cases might not always directly record employment in agriculture or forestry, but first remember exposure to phenoxy herbicides and then, conditionally on that recall, also remember earlier jobs. Such a bias might invalidate some of the approaches taken by Hardell and his colleagues to obtain detailed occupational exposures. Hardell (1981) claimed that this putative bias was largely discounted by an investigation of colon cancer conducted using the identical approach as in his other studies. In this study a risk related exposure to phenoxy herbicides was not identified; however, this may not discount the possibility of a bias as discussed further below.

In any observational epidemiologic inquiry a systematic bias may occur. There may be a systematic bias in the Swedish case control studies which persists throughout all the studies as they were essentially performed using the same technique. However, there may also be a systematic bias in the New Zealand case control studies which has led to those studies tending to be negative. In both sets of studies it was impossible to be certain of the nature of the exposure the cases and controls encountered. However, in view of the high relative risks observed in the Swedish studies, they have to be considered seriously in the present context.

Hardell and Sandstrom (1979) reported a case control study of STS which arose from the observation by Hardell in a series of cases under his care that exposure to phenoxy herbicides appeared to be reported more frequently than expected. The case control study largely confirmed this observation with a relative risk of 5.3 in those exposed to phenoxy herbicides in agriculture and

forestry from 1950 to the mid 1970s. A further analysis conducted by Hardell (1981) confirmed this finding.

In a second study in South Sweden using a completely different case series, Eriksson *et al* (1981) found a relative risk of STS for exposure to all phenoxy herbicides of 6.8 (95% CI: 2.6-17.3). It was possible to characterize exposure to non-2,4,5-T phenoxy herbicides including 2,4-D where the relative risk was 4.2. In these studies, as in the other Swedish case control studies, cases were identified from the Tumour Registry and were pathologically confirmed. For live cases, live controls were identified from the Population Registry and for dead cases, dead controls from the Death Registry. Each case was matched with 2 controls. Exposure data were obtained from a mailed questionnaire with questions relating to phenoxy herbicides as part of a series of questions relating to exposure to specific chemical substances. Where there was doubt over the answers received the answers were supplemented by telephone interviews conducted blind according to case or control status. With this design, the extent to which recall bias will occur, is dependent on the extent to which telephone interviews were indeed used when answers were uninterpretable. If there was a tendency to use more telephone interviews for cases than controls and this occurred in all studies then a systematic bias could in fact have occurred, leading to elevated relative risks. The negative finding in the case control study of colon cancer (Hardell, 1981) is to a certain extent reassuring, providing the use of telephone confirmation was not less for the colon cancer cases than for the STS or NHL cases. Also reassuring is the fact that when analyses were restricted to the data obtained from the mailed questionnaire, similar relative risks were obtained. Hardell *et al*. (1981) used the same method to evaluate malignant lymphoma. Once again, the study arose from the observation of a case series where exposure to phenoxy herbicides appeared to be greater than expected. The cases in this study possibly included the case series which prompted the hypothesis and included 60 cases of Hodgkins Disease and 105 of NHL, together with four unclassifiable lymphomas for a total of 169 cases with two controls, each derived in the same method as for the earlier studies. Relative risk for exposure to phenoxy herbicides or chlorophenols was 6 (95% CI: 3.7-9.7); for exposure to phenoxy herbicides alone, 4.8 (95% CI: 2.9-8.1). There was no dose response relationship for phenoxy herbicide exposure.

The studies in New Zealand were conducted because of the Swedish observations and because phenoxy herbicides are extensively used in that

country. In each instance, the cases and controls were drawn from the National Cancer Registry. Both cases and controls were restricted to those from public hospitals as there appeared to be some difficulty in contacting and interviewing the relatively small numbers of cases in private hospitals. Data was derived from telephone interviews of cases and controls. The interview schedule, though not given in any of the papers, appeared to be less extensive and potentially less specific in terms of phenoxy herbicide exposure than the schedule used in Sweden. This might contribute to a negative finding though the authors claim that their use of cancer controls tends to overcome the potential problem of recall bias in Swedish studies.

The first study related to STS (Smith *et al.*, 1984) in which there were 82 cases and 92 controls. The relative risk for those potentially exposed to phenoxy herbicides of one day (per year, but not in the five years before cancer registration) was 1.3 (90% CI: 0.6-2.5). In the second study (Pearce *et al.*, 1986), NHL was evaluated by a similar design. There were 86 cases and 228 population controls. A relative risk of contracting NHL of 1.5 (95% CI: 0.8-2.7) was observed for those using any agricultural spray. The relative risk was 1.3 (95% CI: 0.8-2.1) for cancer controls and 1.6 (95% CI: 0.3-3.1) for population controls for those who ever sprayed an agricultural chemical involving exposure to phenoxy herbicides, including both 2,4,5-T and 2,4-D.

These studies clearly do not demonstrate the absence of risk. There is a small, though not significant, elevation of risk for both diseases and the confidence intervals would not exclude a relative risk of two or more, depending on the case series. If an effect were really present, it is likely that the risk may have been underestimated because of the difficulty of obtaining exposure information.

In the United States, Greenland *et al.* (1984) attempted to evaluate whether Vietnam service had increased the risk of STS by identifying cases from the New York Tumour Registry. Population controls for living cases were obtained from driver licence files and controls for diseased cases from the Death Certificate Register for men with potential Vietnam service i.e. those aged eighteen to twenty-nine, between 1962 and 1971. The cases had to have been diagnosed between 1962 and 1980. Data were obtained from personal interviews of cases or next of kin. The odds ratio for Vietnam service was only 0.53 and, for reported exposure to Agent Orange, 0.70 (95% CI: 0.17-2.92). Agent Orange is a 1:1 mixture of 2,4,5-T and 2,4-D. This study therefore showed no association though the width of the confidence intervals indicates

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that it was not powerful enough to exclude an elevation of risk approximating to three. Further, the analysis appears to have ignored the issue of latency and may not be an appropriate test of the hypothesis.

Reports of two case control studies have appeared from Italy. Both relate to potential exposure to phenoxy herbicides in women, in the one case to STS and, in the other, to ovarian tumors.

Vineis, *et al.* (1987) studied soft tissue sarcomas diagnosed in men and women in an area in Northern Italy where rice is grown. Women are traditionally employed as rice weeders and during a period commencing in 1950 they were exposed to increasing amounts of phenoxy herbicides that were being used initially to experimentally control weeds. This exposure included 2,4-D, MCPA and 2,4,5-T; however, use of the latter was banned in 1970.

The case series was identified in 1981-1983 and a random sample of controls was drawn from the population. In addition, diseased subjects were chosen as controls for the 37 of the 135 cases who had died at the time of interview. No excess risk of cancer associated with phenoxy herbicide exposure was found for men. Among living women the relative risk was 2.7 (90% CI 0.59-12.37) but when attention was restricted to women alive at the time of interview, less than 75 years of age, exposed in 1950-55 period an age adjusted odds ratio of 15.5 (based on only 15 cases with a 90% CI of 1.3-180.3) was determined. Similarly when exposure to phenoxy herbicides among living females who had regular jobs in agriculture was considered the age adjusted odds ratio for women known to be exposed was 3.

Although this study is of low power it suggests an excess risk of STS in women known to have been occupationally exposed to phenoxy herbicides.

Donna, *et al.* (1984) studied a series of 60 cases of primary mesothelial ovarian tumors and 127 living controls with non-ovarian malignancies, drawn from the same hospital based cancer file. Ten of the cases of ovarian tumors were dead and next of kin were interviewed. These cases were studied because of preliminary data suggesting that some of the herbicides widely used in the rural areas from which the cases were drawn were carcinogenic in animals. Herbicide exposure was determined as definite if the subject or next of kin described personal use of herbicides and was familiar with the various commercial brand names, probable exposure if a farmer resided in areas where there is known herbicide usage. Eight cases and no controls gave a definite history of exposure combining definite and probable, the relative risk for herbicide exposure was 4.38 (1.90-16.07).

This was not a population based study. The case series was relatively small, controls were drawn from a cancer file, little detail is given. It is possible that there was an undue emphasis on herbicide exposure so recall bias cannot be excluded, even though the authors claim that it is unlikely with a cancer control series.

However, there was no attempt to characterize herbicide exposure according to nature of agent and thus this study is relatively noninformative in terms of 2,4-D exposure.

A recent case control study (Hoar et al., 1986a and b) conducted by a group from the environmental epidemiology branch from the U.S. National Cancer Institute, was designed to avoid many of the potential methodologic problems of other case control studies. The study was conducted in Kansas, known to be a wheat producing area and where herbicides were used more frequently than insecticides. Of the herbicides, 2,4-D was more commonly used; 2,4,5-T was also used "along with myriad other chemicals".

It was decided to study male cases with STS, Hodgkins Disease (HD) and (NHL). Cases were identified through the University of Kansas Cancer Data Service which is a population based registry covering the state of Kansas for the years 1976 to 1982. There were 200 males with STS; 173 with HD; 297 with NHL of which 200 were selected at random for study. A pathology review was conducted with confirmation of the diagnosis in 81%, 85% and 90% of the cases respectively (139 STS, 132 HD, 172 NHL). These appear to have been regarded as the eligible cases for the study. Three controls per case were selected by random digit dialing for those less than 65 years of age and from medicare files for those more than 64 if the corresponding case was alive. If the case was dead, controls were selected from Kansas State mortality files. Controls were matched to the case by age (plus or minus two years) and vital status and, for the deceased controls, by year of death or the case. A deceased individual was not eligible to be a control if they died of a STS, HD, NHL or a malignancy of an ill-defined site. Deaths resulting from homicide and suicide were also excluded.

Half of the cases of STS and NHL and one third of the cases with HD had died before the study commenced. Their next of kin were interviewed, as were the corresponding deceased controls. All interviews were conducted by telephone and were completed in 96% of the eligible STS cases, 92% of the eligible HD cases, 99% of the eligible NHL cases and 94% of the controls.

In the analysis, individuals were considered as "farmers", if they had reported as "having worked or lived on farm-land". An unmatched analysis was used as a "matched analysis yielded results similar to those provided by the unmatched approach". The same set of controls were used for analysis of all sites although, as the major findings were restricted to Non-Hodgkins Lymphoma, the detailed analyses were conducted by comparing that set of cases with all controls. No significant association was found between farming and/or herbicide use and STS or HD. Significant associations were found with NHL and further discussion will be restricted to these.

For NHL there was an association of borderline significance for farming (odds ratio (OR) of 1.4, 95% CI: 0.9-2.1). Farm herbicide use on any of the four specific crops (wheat, corn, sorghum, or pasture) was reported by 40 cases with NHL compared to 192 controls, for an OR of 1.6 (95% CI: 0.9-2.6). There was a significant trend in risk of NHL with increasing duration of herbicide use to an OR of 2.0 for sixteen or more years use, for frequency of herbicide use (with an OR of 6.0 for twenty-one or more days use per year). Risk of NHL also increased with time since first exposure, the greatest risk being found for farmers who started using herbicides before 1946 (OR of 3.3). This trend was diminished by controlling for frequency of herbicide use but, after this control, farmers who began use before 1946 still had an excess risk (OR of 2.2). This latter trend is reported only in the text, numbers are not provided and it is not indicated whether the elevated risk for use before 1946 is significantly different from the risks for use between 1946 and 1965 which was 1.7.

Subjects who usually mixed or applied herbicides themselves had increase risk of NHL with an OR of 1.9 (95% CI: 1.1-3.3). Among those who did not mix herbicides, the OR was 1.1 i.e. not elevated. Higher risk was also noted with greater herbicide use without protective equipment.

This appeared to be a well conducted study although, as for any study, some questions can be raised. Phenoxy herbicide use was said to be synonymous with 2,4-D use. However, the questionnaire did not address dates and frequency of use of each specific herbicide though, given the nature of the data collection including information obtained from proxies of both cases and controls (by design), it seems unlikely that detail of this type could have been anticipated. There has to be concern with the possibility of recall bias, associated with knowledge of the disease in cases. Indeed it is possible that, at the time the study was conducted there already was some information available

to farmers and next of kin of the possible association of herbicide use with cancer from the reports in Sweden. With the use of proxy cases and controls, the opportunity for misclassification of exposure was substantial although, in general, this would normally be expected to reduce risks towards zero and not produce spurious increases in risk.

Although the authors clearly indicate that the study is supportive of the possibility that 2,4-D was the responsible agent for the NHL excess, as pointed out by MacMahon (1986, in a review conducted for the U.S. EPA) previous studies had suggested that all three tumors were likely to show increased risk and this study only shows increased risk for one of them. However it is difficult to perceive of recall bias preferentially applying only to NHL. Also, the authors did not analyze data on individuals exposed only to 2,4-D and not other chemicals.

Nevertheless, it is questionable as to whether 2,4-D could be regarded as the responsible agent. Apart from the fact that the estimates of phenoxy herbicide exposure may not reflect use of 2,4-D alone, it is possible that exposure in the early part of the historical period covered by this study was to substances such as 2,4,5-T, likely to be contaminated with more toxic dioxins. That contaminated herbicides may be responsible is suggested by the highest risk for those exposed prior to 1946. However, it is not clear yet whether this risk is in fact significantly different from the risk in the succeeding twenty year period. The risk from recent exposure would be expected to be lower than for early exposure, possibly because of greater care in use as well as lack of expiration of the relevant latent period.

Woods, *et al.* (1987) conducted a population based case control study in Western Washington State that included 128 cases of STS, 575 of NHL and 694 population controls. All data were obtained by personal interview. There was no excess risk for past occupational exposure to phenoxy herbicides for either STS or NHL. However, there was an elevated risk of NHL among the following groups: Men who had been farmers with a relative risk of 1.33 (95% CI 1.03-1.7), forestry herbicide applicators, 4.8 (95% CI; 1.2-19.4) and those potentially exposed to phenoxy herbicides in any occupation for 15 years or more during the period prior to 15 years before cancer diagnosis 1.71 (95% CI; 1.04-2.8). Although these risks came from sub-group analyses, the sub-groups were evaluated because of positive findings in other studies and because of knowledge on latent period effects in relation to carcinogenicity. The significant excesses are compatible with the expectations from other studies are

therefore important. An additional confirmation was that increased risk of both STS and NHL was observed among those individuals reporting prior occurrence of chloracne. This presumably indicates either those who had severe exposure or might have been unduly susceptible to the toxic effects of phenoxy herbicides.

The authors also discussed why lower risks might be found in studies in the United States compared to Sweden. They point out that exposures in Sweden tends to be concentrated over a much shorter time period than for herbicide exposure in the United States. They have calculated a mean maximum daily dose of 45 ug/kg for American workers exposed to 2,4,5-T compared to mean of 90 ug/kg for Swedish workers. They also considered the possibility that Scandinavians might have undue susceptibility to the effect of phenoxy herbicides. They found that when their analysis was restricted to persons from Scandinavia only, the risk estimates for STS in relation to past occupational chemical exposures were substantially greater than those observed among the study population as a whole. This was true both for high level phenoxy herbicide exposure (relative risk of 2.8, CI; 0.5-15.6) and for high level chlorophenol exposure. This analysis was; however, based only on 15 STS cases.

This seems to have been a carefully conducted study and, although overall no increased risk was found for phenoxy herbicide exposure and STS or NHL, the sub-groups where increased risk was demonstrated are compatible with a true biological effect. It is important to recognize that when case control studies are performed in a general population group, it is likely that excesses will only be found among sub-groups who have had the opportunity for relevant exposures at an appropriate time period. This applies even if the area for study has been selected as one where it is known that herbicide exposure occurs.

Another case control study is being conducted by the Environmental Epidemiology Branch of the National Cancer Institute in Iowa and Minnesota involving 594 Leukemias, 690 cases of NHL and 1,245 controls (Cantor and Blair, 1986). Preliminary results indicate no overall increased risk for NHL associated with living or working on farming. However, persons reporting the use of 2,4,5-T had a two-fold risk of NHL (OR = 2; 95% CI; 0.7, 5.2) while those using 2,4-D had only a slightly elevated risk (OR = 1.2; CI; 0.9, 1.8). Unfortunately, information was not gathered on the number of days per year of pesticide use. Since this was the variable that showed the strongest as-

sociation risk of NHL in the study of Hoar, *et al.* (1986), the investigators have decided to recontact subjects to try and obtain this information.

S.3. Evaluation of Epidemiology Studies

An important feature of studies of potential carcinogenicity is consistency. The difference between the results in New Zealand and Sweden indicates lack of consistency; however consistency has been observed in the finding of an excess of STS in the studies in Sweden and Italy using a case control approach and in Denmark in a cohort approach. Further consistency has been observed in finding an excess of NHL in Sweden and in the United States in case control approaches. Although it is clear that 2,4-D cannot be exonerated as a reason for the excess, especially from the results of the Danish cohort study and the U.S. case control study, neither can these single studies, of themselves, be used to classify the risk as being confirmed.

Using IARC terminology, it may be 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.

9. RISK ASSESSMENT OF 2,4-D

The panel was of the view that it would be useful to give some indication of the theoretical risk to humans from exposure to 2,4-D if it is assumed that 2,4-D is carcinogenic. The human data are not adequate to support such a calculation because, amongst other limitations, the lack of information of specific human exposures to 2,4-D in epidemiological studies. Accordingly, cancer risk was assessed under the theoretical assumption that the increased incidence of astrocytomas observed in male rats in the Industry Task Force 2,4-D study was caused by exposure to 2,4-D. These estimates are not intended necessarily to be accurate estimates of risk nor should the fact that these calculations were made be interpreted as implying that the panel believes that 2,4-D is a carcinogen.

~~The theoretical risk of cancer at doses experienced in humans was were obtained by fitting the multistage mathematical dose response model (Crump, 1984) to the data on astrocytomas in male rats. The multistage dose-response model (Crump *et al.*, 1977; Crump, 1984) has been widely used by the U.S. Environmental Protection Agency (EPA, 1983) and the U.S. Occupational Safety and Health Administration. (OSHA, 1983a). The multistage model is used to~~

calculate statistical 95% upper confidence limits based upon an assumed linear relation between 2,4-D and cancer risk (i.e. that additional cancer risk is proportional to dose). Risk estimates to humans were made assuming that a given dose rate expressed in mg/kg/day gives the same risk in animals and humans. This assumption is supported by a recently completed study that compares animals and human data for 23 carcinogens (Allen *et al.*, 1987). Table 8 contains the resulting estimates of risk based upon the estimated human exposures from Table 2.

To help place the theoretical risks estimated for 2,4-D into perspective, cancer risks estimated for exposures to known carcinogens are also tabulated in Table 8. Risks from these carcinogens were computed using methodologies similar to that used for 2,4-D (e.g., a linear relations between exposure and risk was assumed in all cases and risk estimated from animal data were made to apply to humans).

Risks from occupational exposure to inorganic arsenic, ethylene oxide and benzene assume exposures at recently promulgated or proposed OSHA standards (OSHA, 1983b). Risk estimates are provided for an exposure duration commensurate with that estimated for occupational exposure to 2,4-D. The effect of intermittent exposure was accounted for in the same way in estimates for both 2,4-D and non-2,4-D exposures. Risk estimates are also presented for conditions which may not generally be regarded as "risky" by the general public: eating peanut products, having a chest X-ray, spending a week in the Rocky Mountains and smoking a single cigarette.

The methods used to estimate the theoretical risks contained in Table 8 involve many uncertainties and should be viewed only as crude indicators of risk based upon the stated assumptions. Nevertheless, theoretical risks for 2,4-D and from other sources were obtained using similar assumptions and the panel believes that these estimates may provide useful comparisons. These comparisons suggest that, even if 2,4-D were a carcinogen, the risk to persons exposed occupationally would be:

- 1) Considerably less than those for workers exposed to carcinogens at levels recently set by OSHA.
- 2) Less than those from some activities that the general public may regard as safe.

Table 8. Estimated risks per million persons under various exposure conditions.

PART I. Hypothetical risk from exposure to 2,4-D based on the theoretical assumption of rat brain carcinogenicity.

Application	Occupation	Days/year Exposed	Years Exposed	Risks per million
Helicopter	mixer-loader	12	20	0.6
	mixer-flagger	12	20	0.3
	flagger	12	20	0.2
Airplane	mixer-loader	12	20	0.09
	supervisor	12	20	0.003
Packsprayers	handheld right-of-way	60	10	5
	gus sprayer			
	roadside	60	10	5
	sprayer			
mist blower	right-of-way	60	10	8
	sprayer			
Hand and tank	commercial sprayer	66	13	0.6
Tractor	farmer	14	25	0.4

PART II. Occupational cancer risks at recently established or proposed OSHA levels.

Inorganic arsenic ^a	240	45	8000
(16 ug/m ³)	60	10	400
Ethylene oxide ^b	240	45	3000
(1 ppm proposed)	60	10	180
Benzene ^c	240	40	3000
(1 ppm proposed)	60	10	190

PART III. Other risks

Eating peanut products ^d (aflatoxin, U.S. average)	11
Having a chest X-ray ^e (Lung cancer)	1.5
Spending a week at 3050 m in the Rocky Mountains ^e (Cancer from terrestrial and cosmic radiation)	0.9
Smoking one cigarette (Lung cancer only) ^f	0.6

^a Risks reported in OSHA (1983b)

^b Risks reported in OSHA (1983a)

^c Risks reported in Crump and Allen (1984)

^d Average exposure to aflatoxin in peanut products obtained from FDA (1979). Risk estimates based on animal data in Wogan (1973); Wogan et al. (1974); Nixon et al. (1974) and Alfin-Slater et al. (1969).

^e Based on linear-quadratic model and exposure estimates contained in the National Academy of Sciences BEIR report (NAS, 1980).

^f Estimated from Doll and Peto (1978).

10. GLOSSARY OF TERMS

2,4-D	2,4-dichlorophenoxyacetic acid.
2,4-DP	2,4-dichlorophenoxypropanoic acid.
2,4,5-T	2,4,5-trichlorophenoxyacetic acid.
Adducts	Chemical compounds, usually between a small highly reactive molecule and a large molecule such as a protein or nucleic acid.
Ames	Usually referring to a test for genotoxicity.
Anaplastic	A process involving a loss of differentiation of cells usually seen in certain types of tumor.
Arochlor ^R	A commercial brand of polychlorinated bi-phenyl.
Astrocyte	A type of cell found in the central nervous system.
Astrocytoma	A tumor composed of astrocytes, usually in the brain.
BEIR	Biological Effects of Ionizing Radiation.
Biphasic	In two parts or two mechanisms.
Chlorophenol	A phenol in which one or more of the hydrogen atoms are replaced by chlorine.
CHO	Chinese Hamster Ovary, usually referring to cells.
Chromatid	A portion of the chromosome consisting of a strand of DNA attached to the centromere.
CI	Confidence Interval
Ci	Curie, a unit of radioactivity.
CNS	Central Nervous System.
Dermal	Referring to the skin such as in skin toxicity.
Embryotoxic	Toxic to the embryo.
EPA	Environmental Protection Agency of the U.S.
F	Female.
Fetotoxic	Toxic to the fetus.
Fibroblast	A connective tissue cell.
Genotoxic	Expression of toxicity which results in changes in the genetic material.
Glioma	A tumor of the glial cells of the nervous system, as well as any tumor of the brain or spinal cord.
Gliosis	An excess of astrocytes in a damaged part of the central nervous system.
GLP	Good Laboratory Practice, usually referring to a set of guidelines which must be followed in conducting experiments.
Hepatocyte	Liver cell.

Histopathological	Pathology at a microscopic or cellular level.
HD	Hodgkins Disease.
Hodgkins Disease	A malignant condition of the lymph nodes, spleen and lymphatic system.
Hyperplasia	An abnormal increase in the number of normal cells in a tissue.
IARC	International Agency for Research on Cancer.
ICR	Referring to a specific strain of laboratory rats or mice.
IP	Intraperitoneal, into the body cavity.
LD ₅₀	The dose, per animal required to kill 50% of the animals so treated.
LV	Low Volume, usually referring to pesticide sprays.
M	Male.
Macromolecules	Very large molecules, usually of biological origin such as proteins or nucleic acids.
MCPA	2-chloro-2-methylphenoxyacetic acid.
MCPP	2-(2-methyl-4-chlorophenoxy)propanoic acid.
Mesothelial	Pertaining to cells of mesodermal origins.
MF	Male and Female.
Microcalculi	Small abnormal concretions, usually of mineral origins.
Micronucleus	A subcellular organelle in the nucleus which is the site of synthesis of ribosomal RNA.
Microsome	A subcellular organelle usually associated with metabolism.
Myotoxic	Toxic to muscles.
NDMA	N-nitrosodimethylamine.
Neoplasm	A new growth of tissue in which the multiplication of cells is uncontrolled.
Neurocarcinogen	A substance which causes cancer in the tissues of the nervous system.
ng	nanogram, 10 ⁻⁹ of a gram.
NHL	Non-Hodgkins Lymphoma.
NOEL	No Observable Effect Level: The highest level, usually in food, at which symptoms of toxicity are not observed.
Non-Hodgkins Lymphoma	A malignant condition of the lymphatic system which does not possess the characteristics of Hodgkins Disease.
Nondisjunction	The failure of bivalent chromosomes to move apart during the process of cell division.
NTP	National Toxicology Program.
OR	Odds Ratio.

OSHA	Occupational Safety and Health Administration, of the U.S.
OSTP	Office of Science and Technology Policy, of the U.S.
Parenchymal	Referring to organs in the body cavity.
Paresis	Muscular weakness.
Percutaneous	Through the skin.
PCBE	Propylene Glycol Butyl Ether ester of 2,4-D.
pH	Acidity of a solution.
Pharmacokinetic	Referring to the distribution and movement of a chemical in an organism.
ppm	Parts per million.
Preneoplastic	Referring to events occurring before development of a tumor.
Prokaryotes	Organisms without a distinct nucleus.
Quadriparesis	Muscular weakness in all four limbs.
Radiolabelled	Containing a radioactive isotope.
Sister Chromatid	A chromatid from a homologous pair.
Sister Chromatid Exchange	Exchange of genetic material of sister chromatids as a result of genetic damage and as revealed by changes in banding patterns.
St	Sister Chromatid Exchange.
STS	Soft Tissue Sarcoma, a tumor of the connective tissues.
Subchronic	Toxicity studies where exposure is less than 0.25 of the lifespan.
TCDD	tetrachloro-p-dibenzodioxin.
Teratogenic	Causing developmental defects in the embryo or fetus.
Tryp.	tryptophan.
Tumor	A new growth of tissue in which the multiplication of cells is uncontrolled.
uCi	Unit of radioactivity.
UDS	Unscheduled DNA synthesis.
ug	microgram.

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