12/17/87

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. JAQuest 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

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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 a2-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

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

October 6, 1987

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,

Jeff Collins

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

Attachments

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

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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 B6C3F$_1$ 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 phamacokinetic 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

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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; B6C3F$_1$ 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 TDRC, 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 adequancy 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 $B6C3F_1$ 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 ITRFD 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 suggested that representative slides of the kidney lesions observed in the

ITRFD-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 ITRFD accepted my proposal and a meeting was held at the NIEHS on 8/13/85. Present were six members of the ITRFD (Drs. R. Fears, D. Eisenbrandt, 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 ITRFD-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 preforming 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 (actrocytomas) 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.