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JUL 29 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

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

FROM: John A. Quest, Ph.D. *JAS*
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

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 Rinne

Esther Rinne

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 Seal

Diane Seal

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.

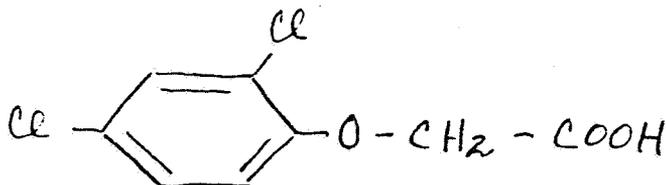
B. Material Reviewed

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/CRJ-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 Studies

1. Rat Oncogenicity Study:

Reference: Combined Toxicity and Oncogenicity Study in Rats, 2,4-Dichlorophenoxyacetic Acid, Final Report. Hazelton 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 (CIIT), 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.

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-BR 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:

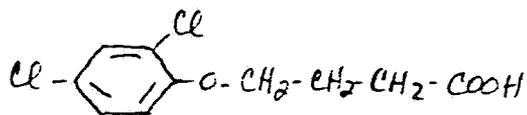
2,4-D was not teratogenic to the rat but did cause fetotoxicity (slight increase in delayed ossification) at an oral dose of 75 mg/kg, p.o. In a 2-generation reproduction study, 2,4-D administered to rats produced renal tubular degeneration in F₀ and F₁ generation males, and reduced pup weights in the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

3. Metabolism:

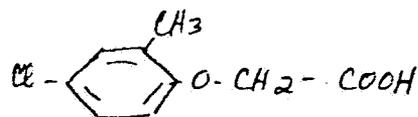
An extensive description of the pharmacokinetics of 2,4-D in animals and man following dermal and oral exposures (the two most important ones in terms of human toxicity), and also inhalation exposure, is provided in the 1984 WHO report. In brief, 2,4-D is not well absorbed through skin, but is fairly 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:

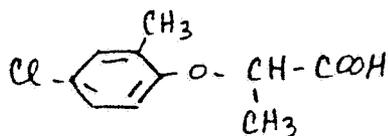
2,4-D is structurally related to the following six herbicides:



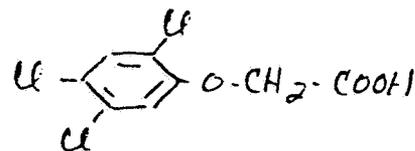
2,4-DB
(2,4-Dichlorophenoxy
butyric acid)



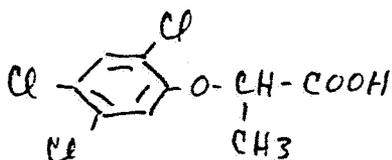
MCPA
(4-Chloro-2-methylphenoxy
acetic acid)

Structure Activity Relationships: (Cont.)

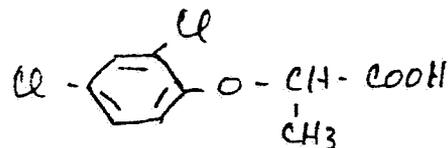
MCPP
(2,4-Dichloro-2-methylphenoxy
propionic acid)



2,4,5-T
(Trichlorophenoxyacetic
acid)



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

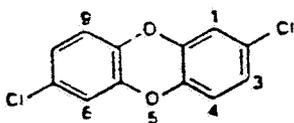


2,4-DP
(2,4-Dichlorophenoxy-2-
propionic acid)

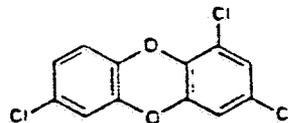
The following information regarding oncogenicity testing of these chemicals was available to the Committee: (1) 2,4-DB, MCPA and MCPP have not been tested chronically in rodents; (2) 2,4,5-T was not found to be oncogenic in studies in rats and mice, but its contaminant TCDD did produce liver hyperplastic 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).

5. Contaminants Found in 2,4-D:

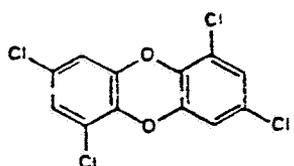
2,4-D formulation contain several potentially hazardous dioxin contaminants, including di-, tri, and tetrachlorodibenzo-p-dioxins (structures shown below) and N-nitrosamines. Of the contaminant dioxins, only 2,7-dichlorodibenzo-p-dioxin has been tested for oncogenicity; this chemical was negative in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



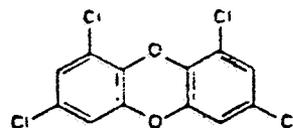
2,7-dichlorodibenzo-p-dioxin



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"

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 + 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.

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 F₀ and F₁ males and reduced weight in F_{1D} 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.

G. 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 (CFR September 24, 1986), 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/ CKL-BK) 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

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: 6/16/87

To: John A. Quest, 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 MARONPOT, 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.