UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review (4th) of 2,4-Dichlorophenoxyacetic acid (2,4-D)

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The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on July 17, 1996 to discuss and evaluate the weight-of-the-evidence on 2,4-D with particular reference to its carcinogenic potential. The CPRC concluded that 2,4-D should remain classified as a Group D - Not Classifiable as to Human Carcinogenicity. That is, the evidence is inadequate and cannot be interpreted as showing either the presence or absence of a carcinogenic effect.

SUMMARY

2,4-Dichlorophenoxyacetic acid (2,4-D) has been evaluated by the CPRC three times, and once each by the Science Advisory Panel (SAP) and by a Scientific Advisory Board (SAB)/SAP Joint Committee.

At its first meeting, the CPRC classified 2,4-D as an interim Group C - possible human carcinogen. This was based on an increase in brain astrocytomas in male F344 rats, which had a borderline significance at the highest dose (45 mg/kg/day) with a positive statistical significant trend. No apparent compound-related increases were reported for female rats or in either sex of B6C3F1 mice at doses up to 45 mg/kg/day. The highest dose in both the rat and mouse study were not considered adequate for assessing the carcinogenic potential of 2,4-D, and the Registrant was asked to repeat both studies at higher doses (subsequently, this became a requirement). Mutagenicity data were essentially negative and there was little support from SAR chemicals. The epidemiology evidence was not considered to provide a definitive link between the use of 2,4-D and human non-Hodgkin's lymphoma. The interim Group C was assigned pending receipt of repeats of the rodent studies and additional epidemiology data in humans.

The SAP then disagreed with the CPRC and concluded that 2,4-D should be classified as a Group D (Not Classifiable as to Human Carcinogenicity), based on what they considered was equivocal evidence in animals and humans.

The CPRC at its second meeting did not concur with the SAP and maintained the Group C classification; however, in its third meeting the CPRC agreed that the evidence was not strong, and classified 2,4-D as a Group D carcinogen pending receipt of the repeat studies in the rodents and additional epidemiology data. On April 2, 1993, the joint panel of SAB/SAP concluded that the evidence for 2,4-D in animals was equivocal, and that in humans, while there was some evidence that non-Hodgkin's lymphoma (NHL) may occur, the data were not sufficient to conclude a cause and effect relationship between exposure to 2,4-D and NHL.

The present meeting was convened to evaluate two new rodent studies which were conducted at higher doses, and additional epidemiology data.

2,4-D was administered in the diet to B6C3F1 mice; at doses up to 125 mg/kg/day in males and 300 mg/kg/day in females. There were no compound-related statistically significant increases in tumors reported for either sex. The CPRC agreed that dosing in this study was adequate to assess the carcinogenicity of 2,4-D in mice.

2,4-D was administered in the diet to Fischer 344 rats at doses up

to 150 mg/kg/day. There were no compound-related statistically significant increases in tumors reported for either sex. The CPRC agreed that dosing in this study was adequate to assess the carcinogenicity of 2,4-D in rats.

Further analysis of the epidemiology data and review of five additional studies of 2,4-D exposure and cancer were presented to the CPRC (details are given in section E.II). The CPRC concluded that these studies are not sufficient to change the conclusions drawn by the joint SAB/SAP.

The CPRC agreed that 2,4-D should remain classified as a Group D (Not Classifiable as to Human Carcinogenicity).

A. Individuals in Attendance at the meetings:

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

William Burnam

Marion Copley

Kerry Dearfield

Yiannakis Ioannou

Hugh Pettigrew

Esther Rinde

Richard Hill

Yin Tak Woo

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Jess Rowland[1]

Yiannakis Ioannou

Lori Brunsman

Jerome Blondell

Lucas Brennecke[2]
(PAI/ORNL)

3. Other Attendees: Albin Kocialski, Joycelyn Stewart, David Miller, Christina Scheltema, Bernice Fisher (HED) Jill Bloom (SRRD), Vincent Cogliano and Charalingayya Hiremath (ORD), Krishan Khanna (OW).

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

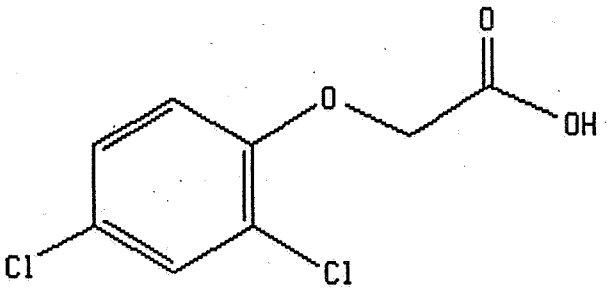
[2]Signature indicates concurrence with pathology report.

B. MATERIALS REVIEWED

The material available for review included: documents from the first, second, and third Carcinogenicity Peer Review Committee meetings; the Scientific Advisory Panel report; the report of the Joint Meeting of the SAB/SAP panel; Data Evaluation Records of the 1995 Carcinogenicity studies in mice and rats, Chronic toxicity study in dogs and Mutagenicity studies; and summaries of other data prepared by Jess Rowland and tables and statistical analysis by C.J. Nelson. The material reviewed is attached to the file copy of this report.

C. BACKGROUND INFORMATION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a chlorinated phenoxy herbicide widely used for broadleaf weed control in farming, forestry, power line maintenance, roadside brush control, aquatics, on home lawns, and for other end uses. The CAS Registry Number (CAS No.) is 94-75-7. The PC Code is 030001. The Tox. Chemical No. is 315. The chemical structure is:



The carcinogenic potential of 2,4-D has been evaluated three times by the Health Effects Division's Cancer Peer Review Committee (CPRC), once by the Science Advisory Panel (SAP), and once by a joint Committee of the Science Advisory Board (SAB) and the SAP. The CPRC, in their first meeting on April 23, 1987 classified 2,4-D as an *Interim Group C* Carcinogen; Possible Human Carcinogen. The SAP in their meeting of June 25, 1987 disagreed with the CPRC's classification and classified 2,4-D as a *Group D* Carcinogen; Not Classifiable as to Human Carcinogenicity. The CPRC, in their second meeting did not agree with the SAP's classification and reaffirmed their (CPRC's) original *Interim Group C* classification. The CPRC, in a third meeting on January 13, 1988, concurred with the SAP and classified 2,4-D as a *Group D* Carcinogen pending evaluation of the repeat bioassays in mice and rats as well as forthcoming epidemiological data. On April 2, 1993, a joint panel of SAB/SAP reviewed the existing data and concluded that there was

only equivocal evidence for carcinogenicity in animals and in humans while there was some evidence that non-Hodgkin's lymphoma (NHL) may occur in excess in populations which are likely to be exposed to 2,4-D, the data were not sufficient to conclude a cause and effect relationship between exposure to 2,4-D and NHL.

CHRONOLOGY OF 2,4-D CARCINOGENICITY PEER REVIEW

1. First Peer Review April 23, 1987

The CPRC, in this meeting, classified 2,4-D as an *Interim Group C* Carcinogen - Possible Human Carcinogen based on the following weight-of-the-evidence:

a. Evidence of Carcinogenicity in Rats

Reference: Serota, DG. *"Combined chronic toxicity and oncogenicity study in rats with 2,4-D acid"*. Hazleton Laboratories America, Inc. Report No. 2184-102. 5/29/86. ACCESSION. No. 263112-263114. HED Document No. 004498.

Groups of 60 male and 60 female Fisher 344 rats were fed diets containing 2,4-D (97.5%) at 0, 1, 5, 15, or 45 mg/kg/day for 104 weeks. There was a statistically significant ($p = 0.0026$) trend for brain astrocytomas only in male rats. A statistical significance was not observed in a two-tail Fisher Exact test ($p = 0.0702$) when the incidence at 45 mg/kg/day (6/60, 10%) was compared with that of the controls (1/50, 2%). The incidences in both the treated and control rats exceeded the historical control incidences for brain astrocytomas (0.4 ± 1.0%) in studies conducted by the National Toxicology Program. Tumor incidences are presented in Table 1.

Table 1. Incidence of Brain Astrocytomas in Rats Fed 2,4-D Acid.

	Males					Females				
(mg/kg/day)	0	1	5	15	45	0	1	5	15	45
Brain Astrocytomas	1[a]/60* 1.6%	0/60 0%	0/60 0%	2[b]/58 3.4%	6[c]/60 10%	0/60 0%	1/60 1.6%	2/60 3.3%	1/60 1.6%	1/60 1.6%

* = Statistically significant (p = 0.0026) positive dose-related trend.
a = Tumor seen in male that died on Week 21.
b = Tumors seen males sacrificed on Weeks 94 and 105
c = Tumors seen in 1 male sacrificed on Week 93 and in 5 at Week 104.

The CPRC concluded that the highest dose level tested (45 mg/kg/day) did not appear to attain an adequate level for carcinogenicity testing in males based on the renal lesions characterized as brown tubular cell pigment, pelvic microcalculi, and transitional epithelial cell hyperplasia, increases in kidney weights and ALT levels. The high dose was considered to be somewhat closer to an adequate level in females due to reduced body weight gain (-5 to -10%), renal lesions similar to those seen in males, and the more marked renal finding of an increase in the frequency and severity of fine vacuolation of the cytoplasm in the renal cortex. Consequently, the Committee recommended that a repeat, modified, carcinogenicity study be performed in Fischer 344 rats using higher doses of 2,4-D than previously used.

b. Evidence of Carcinogenicity in Mice

Reference: Serota, DG. *"Oncogenicity study in mice with 2,4-D acid"*. Hazleton Laboratories of America, Inc. Report No. 2184-101. 1/15/87. MRID No. 40061801. HED Document No. 005794.

No evidence of carcinogenicity was seen in male and female B6C3F1 mice (50/sex/dose) fed diets containing 2,4-D (97.5%) at 0, 1, 15 or 45 mg/kg/day for 104 weeks. Treatment-related changes were limited to the kidneys and included increases in absolute (males at 45 mg/kg/day) and relative kidney weights (females at 15 mg/kg/day and in both sexes at 45 mg/kg/day) and renal lesions. Kidney lesions characterized as cytoplasmic homogeneity of the renal tubule epithelium, were seen only in males at 15 and 45 mg/kg/day; the incidences were 11/60 (18%), 15/60 (25%), 48/60 (80% p <0.0001) and 58/59 (98%, p <0.0001) at 0, 1, 15 and 45 mg/kg/day, respectively.

This change was associated with the reduction of cytoplasmic vacuoles that are normally present in the renal tubular epithelium.

The CPRC concluded that a repeat carcinogenicity study in mice be performed since none of the doses tested in male or female mice reached an adequate level for fully assessing the carcinogenicity potential of 2,4-D.

c. Supporting Evidence: 2,4-D was negative in the Ames test with and without metabolic activation, for cytogenetics in Chinese hamster ovary (CHO) cells, and for the Drosophila sex-linked recessive lethal test. The only positive result was seen for sister chromatid exchange in CHO cells.

d. Epidemiological Evidence: A population-based case control study in Kansas (Hoar et al., 1986) suggested that phenoxyacetic acid herbicide usage was associated with NHL in farmers. It was not possible, however, 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 pending receipt of further human data involving 2,4-D in the near future.

e. Conclusion: Based on these observations the CPRC concluded that the available data on 2,4-D provided only limited evidence of carcinogenicity in male rats and classified 2,4-D as a *Group C* Carcinogen (Possible Human Carcinogen with limited evidence of carcinogenicity in animals). The CPRC made this classification on an interim basis pending the receipt of additional data. 2,4-D produced benign (although life-threatening) tumor 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 rats in a single study where an adequate level for fully assessing carcinogenicity potential did not appear to be reached).

2. Scientific Advisory Panel (SAP) Review

On June 25, 1987, the SAP reviewed the Agency's proposed classification of 2,4-D. The SAP disagreed with the *Interim Group C* Carcinogen classification and took the position that 2,4-D should be classified as a *Group D* Carcinogen for the following reasons:

- The SAP concluded that the mouse and rat studies were inadequate in design and conduct;
- The data were negative for carcinogenicity in female rats and both sexes of mice;

- The increased incidence of astrocytomas in male rats at 45 mg/kg/day was considered *equivocal* evidence of carcinogenicity, additional testing is required to resolve this issue, and this testing should specifically address the astrocytoma issue by repeating a carcinogenicity study;

- The Panel also concluded that the human epidemiology studies represent well-designed and conducted investigations that present *equivocal* data on 2,4-D carcinogenicity for humans. Additional studies are underway that should help clarify the issue;

- The SAP noted that *equivocal* evidence was different from *limited* evidence and that until additional data are developed, it was important to label 2,4-D as a carcinogen or a non carcinogen.

Consequently, the Panel concluded that the present data for animals and humans were inadequate for determining carcinogenicity and that 2,4-D should be classified as a *Group D* Carcinogen; Not Classifiable as to Human Carcinogenicity.

3. Second Peer Review of December 1987

In this meeting, the CPRC disagreed with the SAP's Group D classification, and reaffirmed the initial classification of *Interim Group C* Carcinogen, but decided to re-evaluate this position after the repeat carcinogenicity studies in rat and mouse have been evaluated by the Agency.

4. Third Peer Review of January 13, 1988

The CPRC after re-examination of the data and the issues raised by the SAP, concurred with the SAP that the evidence for carcinogenicity was not strong, and that would classify 2,4-D as *Group D* Carcinogen pending receipt of the repeat bioassays, and additional forthcoming epidemiological data.

5. The Meeting of the SAB/SAP Joint Committee

At the request of the Agency, a joint Committee of the SAB and SAP met on April 1-2, 1993 to review epidemiological studies and animal toxicology studies with regard to potential carcinogenicity of 2,4-D.

The Committee reported that while some case-control studies have shown a risk of NHL in association with farming, many of these studies did not control for exposure to other agents in addition to 2,4-D. Epidemiologic cohort studies have generally shown no increased risk of cancer albeit that all of the populations for which specific exposure to 2,4-D have been identified were small, and the follow-up period usually short. It was determined that current studies cannot distinguish whether observed risks reported are due to the use of 2,4-D. The Committee concluded that there is, at most, weak evidence for an association between exposure to 2,4-D and cancer in humans.

The Committee also reported that while astrocytomas of the brain were seen in one study in rats, other animal species have not shown this cancer, nor has it been reported in human studies. An on-going rat study at higher doses will clarify whether this finding is treatment-related or not. The chemical has not shown mutagenic changes under experimental situations. Although the Committee recognized that it is not necessary for a carcinogen to also be a mutagen, it would have strengthened the observations in humans if there were laboratory data, especially in toxicologic studies of animals, to support any observations in humans. The Committee felt that, at most, there was only equivocal evidence of carcinogenic activity in animals.

The Committee's overall conclusion was *"that while there is some evidence that NHL may occur in excess in populations which are likely to be exposed to 2,4-D, the data are not sufficient to conclude that there is a cause and effect relationship between the exposure to 2,4-D and NHL. The data, however, (are) sufficient to require continued examination of the issue through further studies."*

6. Data Call-In Notice

In 1988, the Agency required that rodent carcinogenicity testing with 2,4-D be repeated because a high enough dosing for assessing carcinogenicity had not been achieved in the

industry-sponsored studies. In the DCI of 1989, the Agency formally requested that the carcinogenicity testing in mice and rats be repeated at higher doses. The Industry Task Force II on 2,4-D Research Data conducted a carcinogenicity study in B6C3F1 mice and a chronic toxicity/carcinogenicity study in Fischer 344 rats. The new studies are discussed below in Section D.

D. EVALUATION OF CARCINOGENICITY EVIDENCE

I. EVIDENCE FROM ANIMAL STUDIES

1. Carcinogenicity Study in Mice

References: Male mice - Sott, WT, Johnson, KA, Gilbert, KS Ormand, JR, and Battjes, JE. *"2,4-Dichlorophenoxyacetic acid: Dietary Oncogenicity Study in Male B6C3F1 Mice - Two Year Final Report"* The Toxicology Research Laboratory, Dow Chemical Co. Study ID: K-002372-063M. 11/16/95. MRID No. 43879801.

Female Mice - Sott, WT, Johnson, KA, Gilbert, KS Ormand, JR, and Battjes, JE. *"2,4- Dichlorophenoxyacetic acid: Dietary Oncogenicity Study in B6C3F1 Mice - Two Year Final Report"* The Toxicology Research Laboratory, Dow Chemical Co. Study ID: K-002372-063F. 03/10/95. MRID No. 43597201. HED Document # 011934.

a. Experimental Design

In a carcinogenicity study with B6C3F1 mice, 2,4-D (96.4%) was administered in the diet at 0, 5, 62.5 or 125 mg/kg/day to males (50/dose) and at 0, 5, 150 or 300 mg/kg/day to females (50/dose) for 104 weeks. In addition, 10 mice/sex/dose were sacrificed at 12 months. Parameters evaluated were: survival, body weight, food consumption, clinical signs of toxicity, hematology parameters at 12, 18 and 24 months, and organ weights and histopathology at 12 and 24 months.

b. Discussion of Tumor Data

A variety of benign and malignant tumors commonly seen in this strain/age of mice were seen at different sites in both treated and control mice of both sexes, but none showed statistical significance in individual tumor types in any treated group.

c. Non-neoplastic Lesions

Treatment-related non-neoplastic lesions were observed at the interim and terminal sacrifices. Renal lesions were seen in both sexes at both intervals in the mid-and high-dose groups. In males at 62.5 and 125 mg/kg/day, renal lesions comprised a constellation of changes that involved five different diagnoses. The primary lesion, degeneration with regeneration of the descending limb of the proximal tubule, was seen at 12 and 24 months. Mineralization of the tubules and multiple cortical cysts were seen only after 24 months. Decreased vacuolation of the renal proximal tubules were seen after 12 and 24 months. In females, at 150 and 300 mg/kg/day, lesions of the kidneys comprised of hypercellularity of the descending portion of the proximal tubules and degeneration with regeneration of cortical tubules were seen at 12 and 24 months.

At 24 months, there was an increase in the aggregates of reticular endothelial (RE) cells frequently adjacent to degenerative or necrotic hepatocytes in male mice at all dose levels; the increase, however, reached statistical significance only at 125 mg/kg/day.

At 24 months, females at 300 mg/kg/day exhibited an increase in extramedullary hematopoiesis with a statistical significance only for the very slight grade but not for the slight or moderate grades. Since no anemia or other blood cell differences were seen at the 12, 18 or 24 months, the splenic hematopoiesis may be attributed to a secondary effect to treatment.

d. Adequacy of the Dose Levels Tested to Assess Carcinogenicity

In males, the high dose (125 mg/kg/day) did not cause any adverse effect on survival, body weight decrements, clinical signs, or alterations in hematology but did increase the absolute and relative kidney weights and induced histopathological lesions in the kidneys. In the original design of this study when male mice were fed higher doses (150 and 300 mg/kg/day), the study had to be "aborted" after 419 days due to significant decrements in body weight gain; 7 to 11% at 150 mg/kg/day and 20 to 27% at 300 mg/kg/day ($\leq$ 0.05). Thus, it is apparent that for males, while a dose of 300 mg/kg/day was definitely excessive, 150 mg/kg/day was also approaching, and possibly exceeding an adequate dose.

Therefore, based on the body weight data of the "aborted study" and the renal effects (dose-related increases in absolute/relative kidney weights and renal lesions) seen at 125 mg/kg/day in the present study, it is determined that the high-dose tested was adequate to assess the carcinogenicity of 2,4-D in male B6C3F1 mice.

In females, the high dose (300 mg/kg/day), did not alter survival, induce clinical signs or change hematology parameters, but decreased body weight gain by 14% at 3 months, 9% at 6 and 12 months and 5% at 20 months, increased the absolute and relative kidney weights, and induced renal lesions after 12 and 24 months of treatment. Renal effects were also seen at 150 mg/kg/day. Therefore, it is determined that the dose levels tested were adequate to assess the carcinogenicity of 2,4-D in female B6C3F1 mice.

2. Combined Chronic Toxicity and Carcinogenicity Study in Rats

Reference: Jeffries, TK, Yano, BL, Ormand, JR and Battjes, JE. "*2,4-Dichlorophenoxyacetic acid: Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats - Final*" The Toxicology Research Laboratory, Dow Chemical Co. Study ID: K-002372-064. 3/28/95. MRID No. 43612001. HED Document # 011934.

a. Experimental Design

In a combined chronic toxicity/carcinogenicity study, male and female Fischer 344 rats (50/sex/dose) were fed diets containing 2,4-D [96.4%] at 0, 5, 75 or 150 mg/kg/day for up to 24 months. In addition, 10/sex/dose were sacrificed at 12 months. Parameters evaluated were: survival, body weight, food consumption, clinical signs of toxicity, clinical pathology at 6, 12, 18 and 24 months, and organ weights and histopathology at 12 and 24 months.

b. Discussion of Tumor Data

A variety of benign and malignant tumors commonly seen at different sites in aging Fischer 344 rats were seen both in the treated and control animals, but none showed statistical

significance in individual tumor types in any treated group when compared to controls.

The present study was designed specifically to address the findings of astrocytomas of the brain observed both in the control and rats treated at much lower doses (15 or 45 mg/kg/day) of 2,4-D in a study conducted in 1986 as presented in Table 1. In order to ascertain this lesions, 8-9 sections of the brain were examined histologically in the present study (See DER for details/figure). The incidences of brain astrocytomas in the current study are presented in **Table 2.**

Table 2. Incidence of Brain Astrocytomas in the 1995 Study.

	Males				Females			
mg/kg/day	0	5	75	150	0	5	75	150
Brain Astrocytomas	0/50 0%	0/26 0%	0/18 0%	1[a]/50 2%	1[b]/50 2%	0/14 0%	0/11 0%	1[c]/50 2%

a = Tumor seen in a male sacrificed on Week 52
b = Tumor seen in a female found dead on Week 73
c = Tumor seen in a female sacrificed on Week 104

c. Non-neoplastic Lesions

Interim Sacrifice: Treatment-related non-neoplastic lesions were observed in the bone marrow, eyes, kidneys, liver, lungs, mesenteric tissue (adipose tissue), testes and thyroids in rats sacrificed at the 12-month interim sacrifice. Histopathology revealed: decreased hematopoiesis of the bone marrow in females at 150 mg/kg/day; bilateral retinal degeneration of the eyes, primarily in females at 150 mg/kg/day; degeneration of the descending portion of the proximal convoluted tubules of the kidneys in both sexes at 75 and 150 mg/kg/day; altered tinctorial properties in the liver of females at 75 mg/kg/day and both sexes at 150 mg/kg/day; multifocal alveolar histiocytosis in females at 75 mg/kg/day and in both sexes at 150 mg/kg/day; atrophy of the adipose tissue in females at 75 mg/kg/day and 150 mg/kg/day; atrophy of the testes in males at 150 mg/kg/day; and decreased secretory material in the thyroid follicles in females at 150 mg/kg/day. The other non-neoplastic lesions observed at 12 months were similar to those frequently seen in this strain/age of rats.

Terminal Sacrifice: Treatment-related non-neoplastic lesions were observed in the eyes, liver, lungs, and mesenteric fat (adipose tissue) at the 24-month terminal sacrifice. Eye lesions in both sexes at 150 mg/kg/day were slight to severe bilateral retinal degeneration and lenticular cataracts. Retinal degenerations were characterized by a decrease in the thickness of the retina due to the variable absence of the rod/cone and outer nuclear layer and occasionally the inner nuclear layer when involvement was severe. Liver lesions seen only at 150 mg/kg/day were characterized by an increase in the size of hepatocytes and was often accompanied by altered tinctorial properties that involved all hepatocytes within the hepatic lobule. Lung lesions in females at 75 mg/kg/day and in both sexes at 150 mg/kg/day were increases in subacute to chronic inflammation. Atrophy of the adipose tissue was increased in both sexes at 150 mg/kg/day. Other non-neoplastic lesions seen were similar to those occurring spontaneously in this strain/age of rats.

d. Adequacy of the Dose Levels Tested to Assess Chronic Toxicity/Carcinogenicity

The dose levels for this study were selected from a 90-day study which identified a LOEL of 100 mg/kg/day based on treatment-related effects on decreases in body weight gain and food consumption, alterations in clinical pathology parameters, changes in organ weights, and histopathological lesions in the eyes, liver, kidneys and thyroid.

In the present study, the highest dose tested (150 mg/kg/day) did not alter survival or cause any clinical signs, but manifested systemic toxicity as: decreases in body weight gains in both sexes (-17% in males and -48% in females) with a concomitant decrease in average food consumption (-4.7% in males and -11.6% in females); alterations in clinical chemistry parameters (increases in ALT, AST. AP and decreases in cholesterol); decreases in T_4 concentration; increases in absolute/relative weights of the thyroid glands; and histopathological lesions in the eyes, liver, lungs and adipose tissue. Treatment-related effects also seen only in the females at 75 mg/kg/day were: decreases in mean body weight (-14%), body weight gain (-24%), food consumption ((-4%) and T_4 concentration; increases in ALT, AST, and AP activities and absolute and relative thyroid weights; and lesions in the kidneys, liver, and lungs. Therefore, it is judged that the dose levels used in this study were adequate to assess the chronic toxicity and the carcinogenic potential of 2,4-D acid in rats.

E. ADDITIONAL TOXICOLOGY DATA ON 2,4-D

1. Metabolism

Reference: Timchalk, C., Dryzga, MD., and Brzak, KA. *2,4-Dichlorophenoxyacetic acid, Tissue Distribution and Metabolism of ^{14}C - Labeled 2,4-Dichlorophenoxyacetic acid in Fischer 344 rats.*" The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co. Michigan. December 5, 1990. Study No. K-2372-(47). MRID No. 41737302. HED Document No. 008561.

The metabolism of [phenyl-U-^{14}C]-2,4-D was studied in male and female Fisher 344 rats. The phenyl ring-labeled compound was administered as a single intravenous (i.v) dose of 0.95-0.97 mg/kg, as a single oral dose of 1.04-1.05 or 97.1-97.4 mg/kg, or as a single oral dose of 1.06 mg/kg following a 14-day pretreatment with unlabeled 2,4-D at approximately 1 mg/kg/day. Recoveries of radioactivity in urine, [85.5 - 93.7% of the dose] after oral dosing indicate extensive absorption of 2,4-D from the gastrointestinal tract. Total recovery of radioactivity 48 hours after treatment accounted for 98.0 - 99.5% of the dose in the orally dosed animals, and to 94.1 - 95.7% of the dose in the i.v treated rats. Among the orally dosed groups, approximately 85.5 - 93.7% of the dose was eliminated in urine and 3.6 - 10.5% of the dose was eliminated in feces.
At sacrifice, total radioactive residue in the carcass was less than 0.52 - 0.69% of the dose at the low oral dose and 1.17 - 2.57% at the high oral dose. No differences between the sexes were found as to the extent of absorption or excretion at any dose level. At the high dose level, however, it appears that a non-linear region (of decreased clearance) is being reached in the disposition of 2,4-D. Parent 2,4-D was the major metabolite found in urine, amounting to 72.9 - 90.5% of the dose among the orally dosed animals of the main experiment. Small amounts, of uncharacterized compounds A and B (0.6 - 1.3% and 0.0 - 0.7% of the dose respectively,) were found in the urine.

2. Mutagenicity

The mutagenic potential of 2,4-D has been extensively evaluated in a range of *in vivo* and *in vitro* assays that have included tests with human cells. The results of studies, those submitted to the Agency and open literature, are summarized in **Table 3.** Ames tests, with and without metabolic activation, were consistently negative. Negative results were

also seen in a mouse bone marrow micronucleus and UDS assays in rat hepatocytes. Conflicting results were obtained in *Drosophila*; positive effects were seen in larvae, while negative results were seen in adults after feeding or injection. Conflicting results were also seen in *in vitro* mammalian cell cytogenetics assays; 2,4-D was negative for structural chromosomal damage up to an insoluble level but positive in the presence of metabolic activation at high doses. The positive evidence, however, tends to be weak and generally not supported by the data from *in vivo* cytogenetic assays. 2,4-D also was nonactive in mammalian cell DNA repair assays. Overall, the pattern of responses observed both in *in vivo* and *in vitro* tests indicated that 2,4-D was not mutagenic (although some cytogenetic effects were seen).

Table 3. Summary of Mutagenicity Studies with 2,4-D Acid

Test system	Test object	Concentration	Results	Reference
Ames Test	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538	100 - 10000 μg per plate with S9; 66.7 - 6670 μg per plate without S9	Negative	Lawlor, 1990 MRID No. 41409801.
Ames Test	*Salmonella typhimurium* TA98, TA100, TA 1535, TA1537, TA1538	0 - 1000 g/plate	Negative	Rashid *et. al.*, 1984
Ames Test	*Salmonella typhimurium*	0 to 3333 μg/plate	Negative	Soler-Neidzieler, 1988
Ames Test	*Salmonella typhimurium* TA 97, TA 98, TA 100, TA 1535, TA 1537, TA 1538	0 - 1000 g/plate (Although, weakly positive in TA97a at 250 μg/plate with S9)	Negative	Kappas, 1988
Ames Test	*Salmonella typhimurium* TA 1538 and TA 1535	0 - 2000 μg/plate	Negative	Rashid and Mumma, 1986
Ames Test	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1538	up to 5000 μg /plate	Negative	Simmon *et al.*, 1977
E. Coli	K12, WP2	0 - 2000 g/plate	Negative	Rashid and Mumma, 1986
E. Coli	PQ 37	0 - 200 g/plate	Negative	Mersch-Sundermann *et al.*, 1989
SLRL mutation	*Drosophila melanogaster* larvae	0.1 - 10,000 ppm	Positive	Kale *et al.*, 1995
SLRL mutation	*Drosophila melanogaster* adults	1000 - 10,000 ppm (feeding) 10,000 ppm (injection)	Negative	Zimmering *et al.*, 1985
Hamster-HGPRT	V79 fibroblasts	10 - 100 μg/ml	Positive	Pavlica *et al.*, 1991

Test System	Test Object	Concentration	Results	Reference
Chromosomal Aberrations	CHO Cells	500 - 920 μg/ml	Negative without S9[a]	Galloway *et al.*, 1987
Chromosomal Aberrations	Embryonic bovine kidney and peripheral lymphocytes	1 - 1000 ppm	Negative	Bongso and Basrur, 1973
Chromosomal Aberrations	*In vitro* Human lymphocytes	0.125-0.35 mM 0.125-1.250 mM	Negative without S9 Positive without S9[b]	Mustonen *et al., 1986*
Chromosomal Aberrations	Human lymphocytes (occupational exposure)	(0.03 - 0.04 mg/m^3)[c]	Negative	Mustonen *et al., 1986*
Chromosomal Aberrations	*In vivo* Charles River Rat (bone marrow)	0 - 350 mg/kg i.p./4 or 24 hr; 3 replicates.	Equivocal[d]	Turkula and Jalal, 1987
Chromosomal Aberrations	*In vivo* Wistar Rat (bone marrow)	0, 17.5, 35 or 70 mg/kg/day/ i.p/2 times	Equivocal[e]	Adhikari and Grover, 1988
SCE	Rat lymphocyte	100 mg/kg	Negative	Mustonen *et al.*, 1989
SCE	CHO cells	50 - 299 μg/ml	Positive without S9	Galloway *et al.*, 1987
SCE	CHO cells	500 - 4200 μg/ml	Negative with S9	Galloway *et al.*, 1987
SCE	Human lymphocyte (occupational exposure)	Not reported	Negative	Linnainmaa, 1983
Micronucleus	ICR mouse (bone marrow)	40 - 400 μg/kg	Negative	Ivett, 1990; MRID No. 41409804, 41870101
Unscheduled DNA synthesis	Fischer 344 primary rat hepatocytes	0.969 - 2890 μg/ml	Negative	Cifone, 1990; MRID No. 41409807
DNA damage	Human Fibroblasts (PM2 DNA)	0 - 100 nmol/l	Negative	Clausen *et al.*, (1990)

a = Positive with S9 at 10.5 hrs after treatment, but not at 19 hrs.
b = Attributed to "unidentified clastogens"(i.e., contaminants) by investigators.
c = Present in urine at 0.09 -1.14 m/L (non smokers); 0.11-1.56 (smokers).
d = Positive $\leq$ 75 mg/kg but only in one replicate
e = Positive at top two doses but results were similar to DMSO solvent control

3. Structure-Activity Relationship

As shown in **Figure 1**, 2,4-D is structurally related to the following phenoxy herbicides: 2,4-Dichlorophenoxybutyric acid (**2,4-DB**); 2,4-Dichlorophenoxy-2-propionic acid (**2,4-DP; *Dichloprop***); 4-Chloro-2-methylphenoxyacetic acid (**MCPA;**); 2,4-Dichloro-2-methylphenoxypropionic acid (**MCPP; *Mecoprop***); 2,4,5-Trichlorophenoxyacetic acid (**2,4,5-T**); and 2-(2,4,5-Trichlorophenoxy) propionic acid (**2,4,5-TP; *Fenoprop***).

2,4-DB: No evidence of carcinogenicity was seen following dietary administration of 2,4-DB (97.7%) to groups of male and female CD-1 mice (50/sex/dose) at 0, 25, 250, or 750 ppm for 78 weeks or to groups of male and female CD rats (50/sex/dose) at 0, 60, 600, or 1800 ppm for 24 months (MRID No(s). 40257501 & 41936201).

2,4-DP: No evidence of carcinogenicity was seen following dietary administration of 2,4-DP (95%) to groups of male and female CD-1 mice (50/sex/dose) at 0, 25, 100, or 300 ppm for 18 months or to groups of male and female Fischer 344 rats (50/sex/dose) at 0, 100, 300, 1000 or 3000 ppm for 24 months (Accession No(s). 242035 & 255729).

MCPA: No evidence of carcinogenicity was seen following dietary administration of MCPA (94.6%) to groups of male and female B6C3F1 mice (50/sex/dose) at 0, 20, 100, or 500 ppm for 24 months or to groups of male and female Wistar rats (50/sex/dose) at 0, 20, 80, or 320 ppm for 24 months (MRID No(s). 40792301 & 40634101)

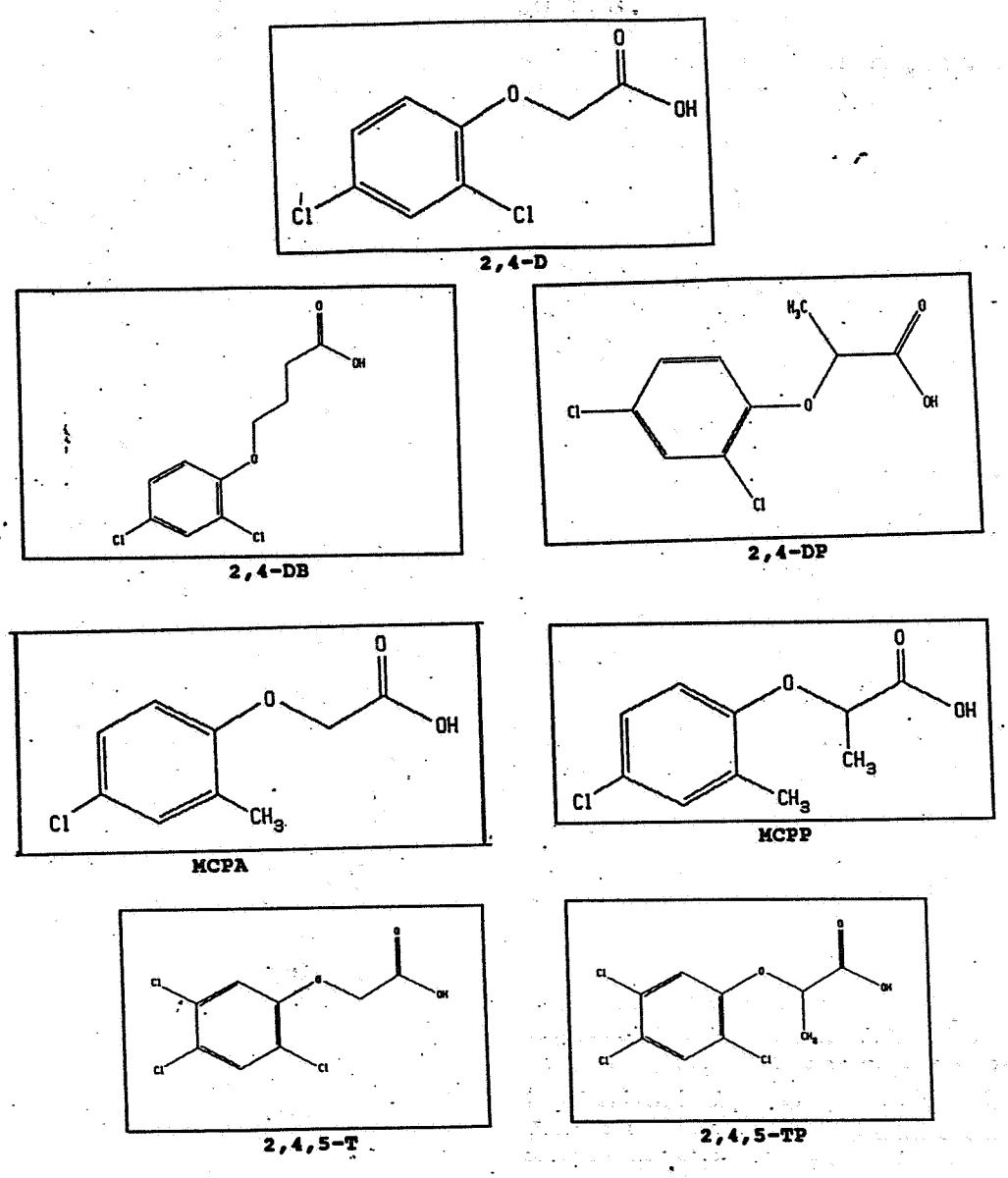
MCPP: No studies are available to assess the carcinogenic potential of MCPP.

2,4,5-T: The carcinogenic potential of 2,4,5-T containing less than 0.05 ppm of dioxin was tested in two strains of mice, namely, C3Hf and XVII/G following administration in the water at 1000 mg/L for 2 months beginning at 6 weeks of age, and thereafter in the diet at 80 ppm (12 mg/kg/day) until death or when the mice were sacrificed *in extremis*. In treated C3Hf mice there was a significant ($p < 0.03$) increase in the incidence of total tumors found in female mice and a significant ($p < 0.001$) increase in total nonincidental tumors in each sex, which the authors interpreted as life-threatening. No significant difference was seen in the XVII/G strain between treated and control mice (Muranyi-Kovacs *et al.*, 1976).

No evidence of carcinogenicity was seen in groups of male and female Sprague-Dawley rats (50/sex/dose) fed diets containing 2,4,5-T (99%, no dioxins) at 0, 3, 10, or 30 mg/kg/day for 2 years (Kociba *et al.*, 1979).

2,4,5-TP: No studies are available to assess the carcinogenic potential of 2,4,5-TP.

Figure 1. Structurally Related Phenoxy Herbicides



4. Subchronic and Chronic Toxicity

a. Subchronic Toxicity studies

(i) Mice

Reference: Serota, DC. *"Subchronic toxicity study in mice with 2,4-D acid"*. Hazleton Laboratories America, Inc. Report No. 2184-100. 10/12/83. Accession No. 131303. HED Document NO. 003888.

Groups of male and female B6C3F1 mice (20/sex/dose) were fed diets containing technical 2,4-D (96.1%) at 0, 5, 15, 45, or 90 mg/kg/day for 13 weeks. No treatment-related effects were seen on survival, clinical signs, body weight, ophthalmology, hematology or gross pathology. Increases in the organ weights of pituitary, adrenals in both sexes, and kidneys in females only were observed. The effect on the kidneys correlated with histopathological lesions in that organ. Treatment-related histopathologic alterations in the kidneys of both sexes were observed; renal lesions were seen in 3, 9, 18 and 20 males and in 1, 4, 6, 12, and 14 females at 0, 5, 15, 45 and 90 mg/kg/day dose groups, respectively. These effects were characterized by increased homogeneity and altered tinctorial properties of the cytoplasm and decreased intracellular/ intraluminal vacuolization in the renal cortex of the males and increased homogeneity and altered tinctorial properties of the cytoplasm with or without cytoplasmic swelling in the renal cortex of the females. Since renal lesions occurred in a dose-related manner, including the lowest dose tested, **a NOEL was not established and the LOEL was 5 mg/kg/day.** Based on the results of this study, the dose levels selected for the 2-year carcinogenicity study in mice were 0, 1, 15, and 45 mg/kg/day.

Reference: Schultze, GE. *"Subchronic toxicity study in mice with 2,4-Dichlorophenoxyacetic acid"*. Hazleton Laboratories America, Inc. Report No. 2184-117. 8/16/91. MRID No. 41991502. HED Document No. 008754.

In a subchronic toxicity study conducted to select the dose levels to be used for the repeat carcinogenicity study, groups of 10 male and 10 female B6C3F1 mice were fed diets containing technical 2,4-D (96.1%) at 0, 1, 15, 100, and 300 mg/kg/day for 13 weeks. Treatment had no adverse effect on survival, body weight, body weight gain, food consumption, ophthalmology, hematology, clinical chemistry, and gross

pathology at 1, 15, and 100 mg/kg/day dose levels. Treatment-related changes at 100 mg/kg/day included significant ($p \leq 0.05$) decreases in glucose and thyroxine (T_4) levels in females and males, respectively, and increases in mean absolute and relative kidney weights in females and a liver lesion in one female. Treatment-related changes at 300 mg/kg/day included: transient decreases in food consumption (only up to Week 7); decreases in glucose and T_4 levels in females and males, respectively; a significant decrease in kidney-to-brain weight ratios in males; histopathological lesions in the kidneys of males characterized as karyomegaly, loss of brush border, and decreased size of tubular lining cells; and histopathological lesions in the liver of both sexes of mice characterized as nuclear hyperchromatism, and decreased glycogen in periportal hepatocytes. **The NOEL was 15 mg/kg/day and based on renal toxicity, the LOEL was 100 mg/kg/day.** Based on these findings the dose levels selected for the repeat carcinogenicity study were 0, 1, 5, 150 and 300 mg/kg/day. The dose levels for male mice, however, were changed to 0, 5, 62.5 and 125 mg/kg/day and a new study was conducted due to the severe toxicity observed in this sex.

(ii) Rats

Reference: Serota, DG. *"Subchronic toxicity study in rats with 2,4-D acid"*. Hazleton Laboratories America, Inc. Report No. 2184-102. 10/12/83. Accession No. 131304. HED Document No. 00388.

Groups of male and female Fischer 344 rats (20/sex/dose) were fed diets containing technical 2,4-D (97.5%) at 0, 1, 5, 15, and 45 mg/kg/day for 13 weeks. No treatment-related effects were seen on survival, clinical signs, body weights, food consumption, ophthalmoscopic findings, or hematology parameters. Clinical chemistry analyses indicated decreases in SGOT, SGPT, AP, BUN in both sexes at 15 and 45 mg/kg/day as well as an increase in T_4 values in males at 5 and 15 mg/kg/day. No treatment-related gross pathology was seen. Both the absolute and relative kidney weights were significantly ($p \leq 0.05$) increased in both sexes at 45 mg/kg/day. Absolute and relative thyroid weights were significantly increased in males at all dose levels and in females at 5, 15, and 45 mg/kg/day; however, there were no corroborative thyroid lesions.
Histopathology revealed renal lesions in both sexes at 5, 15 and 45 mg/kg/day. These alterations were characterized by increased homogeneity, altered tinctorial properties and fine

vacuolization of the cytoplasm in the renal cortex and were generally diffuse, most frequent and more severe at the high dose (45 mg/kg/day) male and female rats with more of a multifocal and less severe pattern at the lower doses. Renal lesions were seen in only one female at 1 mg/kg/day; none of the males at this dose exhibited this lesion. **A NOEL was not established and the LOEL was an equivocal 1 mg/kg/day.** Based on these findings the dose levels selected for the 2-year carcinogenicity study in rats were 0, 1, 5, or 45 mg/kg/day.

Reference: Schultze, GE. *"Subchronic toxicity study in rats with 2,4-Dichlorophenoxyacetic acid"*. Hazleton Laboratories America, Inc. Report No.2184-116. 8/7/91. MRID No. 41991501. HED Document No. 008754

In a subchronic toxicity study conducted to select the dose levels for the repeat chronic toxicity/carcinogenicity study, male and female Fischer 344 rats (20/sex/dose) received technical 2,4-D (96.1%) in their diets at 0, 1, 15, 100 or 300 mg/kg/day for 13 weeks. Treatment did not cause any adverse effects at 1 or 15 mg/kg/day, but it did induce toxicity in both sexes at 100 and 300 mg/kg/day. At termination, significant ($p < 0.05$) reductions in body weight gain were observed in males at 100 and 300 mg/kg/day and in females at 300 mg/kg/day. Both sexes of rats at 100 and 300 mg/kg/day exhibited alterations in some of the hematology and clinical chemistry parameters, as well as changes in various organ weights. Histopathological lesions in the liver, adrenals and kidneys were seen at 100 mg/kg/day and in the eye, liver, testes, adrenals, kidneys, thymus, bone marrow, spleen, thyroid, and lungs at 300 mg/kg/day. In some instances, the histopathological changes correlated well with alterations observed in hematology, clinical chemistry parameters, and organ weight data. Increases in liver weight, ALT, and AST were associated with centrilobular hepatocellular hypertrophy of the liver in both sexes at 100 and 300 mg/kg/day. Decreased thyroxine levels can be correlated with follicular cell hypertrophy of the thyroid gland in females at 300 mg/kg/day. An increase in adrenal weight may be correlated with hypertrophy of cells of the zona glomerulosa of the adrenal glands in both sexes at 100 and 300 mg/kg/day. Decreased mean thymic weight may be correlated with atrophy seen in males and females at 300 mg/kg/day. Atrophy of mesenteric adipose tissue in the peritoneal cavity can be correlated with mean body weight decreases noted in both sexes at 300 mg/kg/day. Additional treatment-related histological lesions seen were bilateral cataracts only in females at 300 mg/kg/day, brush border loss in proximal tubular cells of the

kidneys in both sexes at 100 and 300 mg/kg/day, and alveolar macrophage accumulation of the lungs and hypocellularity of the bone marrow in both sexes at 300 mg/kg/day. **The NOEL was 15 mg/kg/day and based on the findings described above, the LOEL was 100 mg/kg/day.** The dose levels selected for the 2-year chronic toxicity/carcinogenicity study were 0, 5, 75, or 150 mg/kg/day.

(iii) Dogs

Reference: Schultze, GE. *"Subchronic toxicity study in dogs with 2,4-dichlorophenoxyacetic acid"*. Hazleton Laboratories America, Inc. Report No.2184-115. 12/14/90. MRID No. 41737301. HED Document No. 008400.

Groups of beagle dogs (5/sex/dose) were given gelatin capsules containing 2,4-D (96.1%) at 0, 0.3, 1.0, 3.0, or 10 mg/kg/day for 13 weeks. No treatment-related effects were observed at 0.3 or 1.0 mg/kg/day. At 3.0 mg/kg/day 2,4-D acid caused significant ($p \leq 0.05$) increases in BUN and creatinine levels as well as renal lesions, characterized as cellular alterations in the proximal convoluted tubules in 3 of 5 male dogs (not in females). Treatment-related changes at 10 mg/kg/day were: morbidity (2 males & 1 female); clinical signs (thin and languid appearance, anorexia, emesis, and swollen testes); decreases in mean body weights (-8% in males and -14% in females) and body weight gains (-50% in males and -83% in females); decreases in HGB, HCT, and platelet counts and increases in BUN and creatinine levels in both sexes; decreases in absolute testicular weights in males; increase in relative kidney weights in females; and renal lesions in both sexes. Kidney lesions characterized as cellular alterations in the proximal convoluted tubules were seen in 3/3 males and 1/4 females. **The NOEL was 1 mg/kg/day and based on the alterations in clinical chemistry parameters (increases in BUN and creatinine) along with corroborative renal lesions, the LOEL was 3 mg/kg/day.**

Reference: Dalgard, DW. *"13-Week Dietary Toxicity study of 2,4-D in Dogs"*. Hazleton Laboratories America, Inc. Report No.2184-125. 5/6/93. MRID No. 42780001. HED Document No. 010938.

In a feeding study, male and female beagle dogs (4/sex/dose) received 2,4-D (96.7%) in their diet at 0, 0.5, 1.0, 3.75, or 7.5 mg/kg/day for 13 weeks. No treatment-related effects were observed at 0.5 or 1.0 mg/kg/day. No mortality, clinical signs of toxicity, ophthalmologic changes, alterations in hematology or urinalysis parameters, gross pathology or organ weights were seen at 3.75 and 7.5 mg/kg/day. Body weight gains were decreased in males (-50%) and females (-47%) at

3.75 mg/kg/day and in males (-39%) and females (-42%) at 7.5 mg/kg/day. Food consumption was decreased (approximately 15%) in both sexes at 3.75 and 7.5 mg/kg/day. Clinical chemistry showed significant ($p \leq 0.05$) increases in BUN, creatinine, and ALT levels at the 4- and 13-week intervals for both at 3.75 and 7.5 mg/kg/day. The toxicological significance of these increases is not known due to lack of alterations in organ weights or corroborative histopathological renal lesions. Except for a moderate chronic active perivascular inflammation of the liver seen in 1 male and 1 female at 7.5 mg/kg/day, no treatment-related histopathological lesions were seen. There was no correlation between the severity of liver lesions and the increased ALT activity. It is interesting to note the renal lesions observed when administered in gelatin capsule in the previous study were not seen when the same doses were administered in the diet in this study. **The NOEL was 1 mg/kg/day and based on decreases in body weight gains and food consumption, the LOEL was 3.75 mg/kg/day.**

c. Chronic Toxicity

(i) Mice & Rats

The two combined chronic toxicity/carcinogenicity studies in B6C3F1 mice and the two combined chronic toxicity/carcinogenicity studies in Fischer 344 rats are discussed earlier on Pages 6-12 of this document.

(ii) Dogs

Reference: Dalgard, DW. *"52-Week Dietary Toxicity Study with 2,4-D in Dogs"*. Hazleton Washington, Inc. Report No. HWA 2184-124. 12/2/93. MRID No. 43049001. HED Document No. 011271.

In a chronic toxicity study, beagle dogs (5/sex/dose) were fed diets containing 2,4-D (96.5%) at 0, 1, 5, or 7.5 m/kg/day for 52 weeks. No treatment-related effects were seen on survival, clinical signs, ophthalmology, hematology, urinalysis, organ weights or gross pathology at any dose level. Body weight gains of dogs at 1 mg/kg/day were comparable to those of the controls. Body weight gains were decreased in both sexes at 5 and 7.5 mg/kg/day, with the effect being more pronounced in females at the high dose. The increases in BUN, creatinine, total cholesterol and ALT activity in dogs at 5 and 7.5 mg/kg/day were corroborated with histopathological changes in the liver and kidneys of these dogs. The increases in BUN and creatinine are compatible with either dehydration or mild

renal tubular epithelial compromise while the elevations in ALT activity are indicative of hepatocellular injury. The increases in total cholesterol are nonspecific but are typically seen with alterations in lipid metabolism by the liver. Histopathology revealed: a minimal increase in the frequency and average severity of sinusoidal lining cells of the liver of females only at 5 and 7.5 mg/kg/day; minimal increases in the frequency as well as average severity of perivascular, chronic active inflammation of the liver; and an increase in pigment in the tubular epithelium of the kidneys in both sexes at 5 and 7.5 mg/kg/day. **The NOEL was 1 mg/kg/day and based on alterations in clinical chemistry parameters (BUN, creatinine, total cholesterol) and histopathology (liver and kidneys), the LOEL was 5 mg/kg/day.**

5. Neurotoxicity

Reference: Mattsson, JL, Jeffries, TK and Yano, BL. *"2,4-Dichlorophenoxyacetic acid: Neurotoxicity Study in Fischer 344 Rats - Final"* The Toxicology Research Laboratory, Dow Chemical Co. Study ID: K-002372-064N. 6/28/94. MRID No. 43293901. HED Document # 011614.

The chronic neurotoxic potential of 2,4-D was evaluated in a subset of 15 rats/sex/dose from the chronic/carcinogenicity study discussed earlier on Pages 9-12 of this document. Functional observational battery (FOB) and motor activity evaluations were conducted at 3, 6, 9, or 12 months. No treatment-related FOB handheld observations were seen at any interval. Relative forelimb grip strength (g of grip/g BW) of both sexes of rats at 150 mg/kg/day were significantly increased; no treatment-related changes were seen in absolute grip strength. No treatment-related differences were seen in either the total motor activity or activity within each epoch. Motor activity at 3-, 6-, and 12-month evaluations were comparable to the pre-study values. Neuropathological examination at 12 months revealed an increased incidence of bilateral retinal degeneration in females at 150 mg/kg/day; males were not affected. **For neurotoxicity, the NOEL was 75 mg/kg/day and based on increased relative forelimb grip strength, the LOEL was 150 mg/kg/day.**

II. EVIDENCE FROM EPIDEMIOLOGIC STUDIES

1. Evaluation of Studies

The available epidemiological studies of persons potentially exposed to 2,4-D include case control and cohort studies. In case control studies, individuals with specific diseases are identified and comparable controls and their past exposures ascertained, usually by means of interviews. In cohort studies, the experience of exposed individuals is followed in comparison with an unexposed group often drawn from the general population. States where major epidemiologic studies were conducted (Kansas, Nebraska, Iowa, Minnesota and Washington) were all among the top 11 users of 2,4-D according to a 1974 EPA survey.

Epidemiological studies have suggested an association between exposure to chlorophenoxy herbicides, including 2,4-D, and two forms of cancer in humans; specifically, soft-tissue sarcomas (STS) and non-Hodgkin's lymphoma (NHL). These studies, however, are not consistent, the associations found are weak, and conflicting conclusions have been reached by the investigators. In addition, most of these studies did not provide information on exposure specific to 2,4-D and related the risk to the general category of phenoxy herbicides -- a group that might include, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and substances contaminated with dioxins, specifically, TCDD. There are different results from many of the studies. While some have indeed shown a relationship between 2,4-D and NHL, others have produced inconsistent results (even of the positive studies) which raise doubts as to whether the relationship is causal. One of the issues is the inconsistency of some of the findings: Some of the confidence intervals for the odds ratio for 2,4-D use do not overlap. Various explanations have been put forth for these inconsistencies.

(i). Soft-Tissue Sarcoma

Case-Control Studies: An overview of the six case-control studies that examined the association between exposure to phenoxy herbicides and chlorophenols and the development of STS are presented in **Table 4.** A positive association was reported in patients with exposure to phenoxy herbicides or chlorophenols in Sweden (Hardell and Sandstrom, 1979; Eriksson *et al.*, 1981) and in female rice weeders in northern Italy (Vineis *et al.*, 1987). None of these studies, however, reported an odds ratio (OR) for exposure to 2,4-D. On the

other hand, a number of case-control studies in New Zealand and the U.S.A. have failed to find an association between phenoxy herbicide use and the development of STS (Smith *et al.*, 1983; 1984; Hoar *et al.*, 1986; and Woods *et al.*, 1987).

Hardell and Sandstrom (1979) studied 52 male patients (21 living and 31 deceased) with STS in northern Sweden with previous exposure to phenoxyacids or chlorophenols. There were 220 controls from the general population. The STS cases were identified from records of the Department of Oncology of the University Hospital of Umea between 1970 and 1977. With information on use patterns of herbicides and chlorophenols obtained from questionnaires, the authors observed that 36.5% of the cases and 9.2% of the controls recalled exposure to these compounds. There was a significant ($p < 0.001$) six-fold increase in risk for STS (OR = 5.3; 95% CI: 2.4-11.5) with 13 cases of exposure to phenoxyacids. Of the 13 cases exposed, 9 had been exposed to 2,4-D and 2,4,5-T combined, 2 to 2,4,5-T alone, 1 to MCPA alone, and 1 possibly to 2,4-D only. The authors cautioned that a specific evaluation of the effect of separate chemical substances was not possible, as nearly all exposed subjects were also exposed to chlorinated dioxins, including 2,3,7,8-TCDD.

Eriksson *et al.* (1981) confirmed the study by Hardell and Sandstrom by examining the association of STS and phenoxy acetic acid in southern Sweden, where MCPA and 2,4-D have been widely used in agriculture. The study involved 110 STS cases reported in 1974-1978 and 220 controls from the general population. The OR was 6.8 (95% CI: 2.6-17.3) for exposure to any phenoxy herbicide and 4.2 to chlorphenoxy herbicides other than 2,4,5-T.

Vineis *et al.* (1981) studied phenoxy herbicides and STS in female rice weeders in northern Italy, where phenoxy herbicides were used beginning in the 1950s. The study included interviews with 68 histologically confirmed cases of STS and 158 controls. Exposure included 2,4-D, MCPA and 2,4,5,-T. Among living women who were exposed to phenoxy herbicides in any period of their lives, the OR was 2.7 (90% CI: 0.6-12.4).

Smith *et al.* (1983 and 1984) investigated the association between STS and exposure to phenoxy herbicides in New Zealand. The authors selected 82 subjects (cases) with STS and 92 controls with other types of cancer from the National Cancer Registry for the years 1976-1980. The study failed to show any statistically significant association between phenoxy

herbicide use and STS (OR= 1.3; 90% CI: 0.7-2.5).

Hoar *et al.* (1986) conducted a population-based case-control study in Kansas, where 2,4-D had been the most commonly used herbicide; 2,4,5-T was also used "along with myriad other chemicals." The study included 113 STS cases identified through the University of Kansas Cancer Data Service for the years 1976 - 1982 and 948 controls from the general population of Kansas. No consistent patterns of excess risk of STS were seen for farmers when compared to non-farmers (OR= 1.0; 95% CI: 0.7-1.6) for herbicide use (OR = 0.9; 95% CI:0.5-1.6) or for duration and frequency of herbicide use (OR= 1.1; 95% CI: 0.7-1.7).

In a population-based case-control study, Woods *et al.* (1987) evaluated the relationship between occupational exposure of men in Washington state to phenoxyacetic acid herbicides and the risk of developing STS. The study included 128 STS cases and 694 randomly selected controls without cancer. No statistically significant association was seen between STS and phenoxy herbicide exposure (OR = 0.89; CI: 0.4-1.9).

Cohort-Studies: Results of the two cohort studies conducted among workers with occupational exposure to phenoxy herbicides that did not show an association between 2,4-D and STS are presented in **Table 5.**

Coggon *et al.* (1991) examined the mortality and cancer incidences at four factories in England that produced phenoxy herbicides. The four British cohorts, comprised of 2239 men employed during 1964 - 1985, were exposed not only to 2,4-D, but also to MCPA, 2,4,5-T, and other phenoxy herbicides. The subjects were traced through the National Health Service Central Registrar and the National Insurance Index, and their mortality was compared with that in the national population. No cases of STS were identified.

Saracci *et al.* (1991) surveyed a population of 18,390 (16,863 males and 1527 females) production workers or sprayers from 10 countries from the International Registry of Workers Exposed to Phenoxy Herbicides and their Contaminants established by the International Agency for Research on Cancer and the U.S. National Institute of Environmental Health Sciences. These workers were exposed to 2,4-D, 2,4-DP, 2,4,5-T, MCPA, other phenoxy acids, and/or a number of chlorinated phenols. There was no overall increase in mortality nor mortality from cancer. There were 4 deaths due to STS compared to 2.04 expected (RR= 1.96; 95% CI:0.53-5.02). But three of the four cases of STS were found in sprayers (RR= 8.82; 95% CI:1.82-25.79), were restricted to 10 -19 years after first exposure, and two of the four cases arose after exposure of less than

one year. Since the exposure was to a number of chlorophenoxy herbicides, chlorinated phenols, or both, it could not be determined which, if any, of these chemicals caused the reported increase in STS.

(ii). Non-Hodgkin's Lymphoma

Case-Control Studies: An overview of the case-control studies are presented in **Table 6.** These studies, taken as a whole, although they suggest an association between phenoxy herbicide use and NHL, the evidence is not entirely consistent. Less clear, but still suggestive, was the evidence for a specific association between NHL and exposure to 2,4-D. These studies must be interpreted with caution, because it is difficult for such studies to isolate which specific herbicide (or other factors) is responsible for the association; the association may be explained by other chemicals that farmers mix with 2,4-D or with impurities in the 2,4-D that was sold commercially; the association with 2,4-D specifically has not been replicated; and use of 2,4-D may serve as a surrogate in these studies for some other unknown confounding factors.

Hardell *et al.* (1981) examined the association between exposure to phenoxyacids or chlorophenols and malignant lymphomas in Sweden. The study included 60 Hodgkin's disease (HD) and 109 NHL hospital-based cases and 338 controls from the general population. Methodology (questionnaire) used was similar to Hardell and Sandstrom (1979). There was a significantly increased risk for exposure to phenoxy herbicides; OR = 4.8 (95% CI: 2.9-8.1). Although risk estimates were not reported separately for HD and NHL, the authors indicated that no meaningful differences existed.

Hoar *et al.* (1986) conducted a population-based case-control study in Kansas that included 121 HD and 170 NHL cases identified through the University of Kansas Cancer Data Service for the years 1976 - 1982 and 948 controls from the general population of Kansas. Results are summarized in **Table 6.**

In another population-based case-control study, Woods *et al.* (1987) evaluated the relationship between occupational exposure of men in western Washington state to phenoxyacetic acid herbicides and chlorinated phenols and the risk of developing NHL. The study included 576 NHL cases and 694 randomly selected controls with cancer. There was an association between NHL and forestry herbicide applicators (OR

= 4.8; 95% CI: 1.2-19.4); however, it should be noted that these forestry sprayers reported the combined use of 2,4-D and 2,4,5-T, as well as commercial preparations containing other chemicals.

Pearce *et al.* (1986, 1987, 1989) studied NHL and exposure to phenoxy herbicides in New Zealand. In contrast to the U.S, where the phenoxy herbicide evaluated was 2,4-D, the predominant phenoxy herbicide used in New Zealand during the 1950 - 1980 period was 2,4,5-T. These studies included 183 male cases of NHL and 338 controls obtained from the New Zealand Cancer Registry for the years 1977 - 1981. No excess risk was found (OR = 1.0; 90% CI: 0.7-1.5).

Zahm *et al.* (1990) examined the association between exposure to 2,4-D and the development of NHL in eastern Nebraska in a population-based case-control study that included 201 white men with NHL and 725 controls. The distinctive feature of this study was the collection of specific information regarding the duration and frequency of 2,4-D use. Results are summarized in **Table 6.**

Weisenberger *et al.*(1991) combined the data from Nebraska (Zham *et al.* 1990) and Kansas (Hoar *et al.* 1986) to show that the odds ratio for 2,4-D is significantly elevated; this was not the case for Nebraska alone and was not initially measured in the Kansas study.

Cantor *et al.* (1992) studied pesticides and other agricultural risk factors for NHL among men in Iowa and Minnesota. The study included 622 white men with NHL and 1245 white controls. Small risks (OR=1.2) were seen for 2,4-D use, but the risk did not increase with latency or failure to use protective equipment.

Zahm *et al.* (1993) in a follow-up to the above study conducted on men in eastern Nebraska (Zaham et al., 1990), examined the associations for 119 women with NHL and 471 controls. OR for 2,4-D use on farms where the women resided and NHL were 0.8 (95% confidence interval (CI) 0.4-1.6). For those women that personally handled 2.4-D the OR was 1.0 (95% CI 0.1-5.2). The authors note that the number of women handling 2,4-D was too small, 10 overall among cases and controls. They concluded that the "risk of NHL from pesticide exposures among women who live and work on farms, if real, is smaller than for men. This may reflect lower levels of exposure, unstable risk estimates that result from the small number of exposed women, or failure to collect information on the activities most likely to result in exposure of women."

Kogevinas *et al.* (1995), conducted a follow-up study to the Saracci et al. (1991), which examined a cohort of production workers in 10 countries. The earlier study found no excess of NHL but a non-significant excess of STS. The current study involves two nested case-control studies (STS and NHL), includes four new production cohorts from Germany, and includes both morbidity and mortality cases. The odds ratios for 2,4-D (which includes 2,4-D, 2,4-DP and 2,4-DB) were as follows: STS and 2,4-D odds ratio = 5.72 (95% CI 1.1-28.6) NHL and 2,4-D odds ratio = 1.11 (95% CI 0.5-2.6). When the STS cases were divided into 3 exposure categories (low, medium and high) there was a significantly increasing risk with exposure. This study also found significant risks for STS with exposure to MCPA/MCPP/MCPB (OR = 11.3, 95% CI 1.3-98) and any exposure to dioxin or furan (OR = 5.56, 95% CI 1.1-28). The authors note "an evaluation of the independent effect of each herbicide or contaminant on cancer risk is complicated, since few subjects in this study were exposed to only a single herbicide or chlorophenol, and exposures to many of the chemicals examined was highly correlated."
Commenting on the lack of an excess between 2,4-D and NHL they stated "Frequent use of phenoxy herbicides, in particular 2,4-D, has been associated with increased risk for non-Hodgkin's lymphoma in case-control studies conducted in Sweden, Kansas, Nebraska, and Saskatchewan, Canada, and in a U.S. cohort of 2,4-D manufacturing workers. Low or no excess risks have been found in other cohort studies of workers manufacturing phenoxy herbicides, including cohorts in the international register, and in case-control studies in New Zealand, Washington State, and Iowa and Minnesota. These contrasting findings may partially be due to the concomitant exposure of farmers to other potential risk factors for non-Hodgkin's lymphoma, such as other agricultural chemicals, zoonotic viruses, and solvents."

The authors concluded that workers with substantial exposure to phenoxy herbicides and their contaminants were at higher risk of soft tissue sarcoma and that the risk was not specifically associated with those herbicides contaminated with TCDD. No conclusion was drawn specifically for 2,4-D.

In a hospital based case-control study, Hayes *et al.*, 1991 examined, as a model for human non-Hodgkin's lymphoma, the association between 2,4-D exposure and the development of malignant lymphoma in companion dogs. Dogs with histopathologically confirmed malignant lymphoma were identified. Two types of controls were selected from the registry: dogs diagnosed with other malignancies and dogs in a veterinary hospital for other reasons. Subjects were matched by age, year of hospital visits and hospital. Exposure and confounder data were solicited by mail questionnaire.

Information from self-administered owner questionnaire and/or a telephone interview of about 491 cases, 466 nontumor controls, and 479 tumor controls were employed. Results are summarized in **Table 6**. The major weakness of this study was that there were no precise exposure data for herbicides. Frequency of application was the primary measure of exposure used in this study .

Cohort Studies: An overview of cohort studies conducted among workers with occupational exposure to phenoxy herbicides that have not confirmed the initial hypothesis of an association between 2,4-D and NHL are presented in **Table 7**.

Bond *et al.* (1988) investigated the mortality incidence of 878 workers potentially exposed to 2,4-D and its derivatives during their manufacture, formulation, or packaging between 1945 and 1983. Exposure was estimated by using an 8 hour Time Weighted Average for each task, and the workers were split into three exposure categories: <0.5, 0.5 - 4.9, and >5.0 mg/m^3/year. Special attention was given to deaths from brain neoplasms in the cohort because of the brain astrocytomas seen in male rats fed 2,4-D in the diet. No deaths from brain neoplasms were observed among the 111 deaths in this cohort. There were two deaths from NHL (one with generalized lymphosarcoma and the other with reticulum cell sarcoma) among a subset of workers with the potential for additional exposure to TCDD or H/OCDD (2 observed vs. 0.5 expected; RR = 3.9; 95% CI:0.4 -14.1). Overall the authors concluded that the study did not support a cause-effect relationship between 2,4-D exposure and mortality from all causes or from any specific cancer.

Wigle *et al.* (1990) conducted a mortality study of almost 70,000 male farmers in Saskatchewan, Canada identified on the 1971 Census of Agriculture. There was no excess mortality for any cause of death including NHL, but there was a correlation between NHL and acres sprayed with herbicides. Results are presented in Table 7. The authors reported that the chlorophenoxy compound in general use in the area was 2,4-D (90% and 75% by weight throughout the 1960s and 1970s, respectively), but the exposure was not directly related to cases of the disease.

Coggon *et al.* (1991) examined the mortality and cancer incidences at four factories in England that produced phenoxy herbicides. The four British cohorts, comprised of 2239 men employed during 1964 - 1985, were exposed not only to 2,4-D, but also to MCPA, 2,4,5-T, and other phenoxy herbicides. The subjects were traced through the National Health Service Central Registrar and the National Insurance Index, and their

mortality was compared with that in the national population. No cases of STS or HD were identified, but there were 2 deaths from NHL compared with 0.87 expected (RR= 2.3; 95% CI:0.3-8.3), and both deaths occurred more than 10 years after first exposure to phenoxy compounds.

Bolemen *et al.* (1993) reported the results of four years of additional mortality follow-up through 1986 for the previous study by Bond *et al.* No new deaths from NHL were observed in the extended follow-up period. It was concluded that the data did not suggest a causal relationship between exposure to 2,4-D and any particular cause of death, including cancer.

Persson *et al.* (1993) examined some occupational exposures as risk factors for malignant lymphoma cases diagnosed in 3 counties in Sweden between 1975 and 1984. Cases had to be between 20 and 80 years old, alive and able to respond to a questionnaire. There were 31 cases of Hodgkin's disease (HD), 93 NHL, and 204 controls. Logistic regression found exposure to phenoxy herbicides and fresh wood were significant risk factors for Hodgkin's disease. Welding, working as a lumberjack, and nursing were associated with significantly increased NHL. Odds ratios were as follows: HD and phenoxy herbicides OR = 7.4 (90% CI 1.4-40) NHL and phenoxy herbicides OR = 2.3 (90% CI 0.7-7.2). Note that the first odds ratio is based on 5 cases and 40 controls and the second on 10 cases and 14 controls. The authors suggest that misclassification between Hodgkin's disease and NHL may explain differences between their study and others. The study assessed exposure to chlorophenoxy herbicides but not 2.4-D specifically.

Morrison *et al.* (1994) in a follow-up to the Wigle et al. (1990) study added two additional years of data and two additional provinces of Canada. The earlier study had found a significant association with acres sprayed with herbicides and use of fuel oil. The follow-up study found no excess risk of NHL and herbicide spraying in Alberta or Manitoba. However, a significant risk was found for all three provinces combined between acres sprayed from the 1981 Census of Agriculture and NHL (rate ratio 2.11, 95% CI 1.1-3.9). The authors noted that farmers in Saskatchewan were more likely to apply the pesticides themselves and to have used 2,4-D than farmers in the other provinces. They concluded "any exposure-disease relationship in the present study between 2,4-D and non-Hodgkin's lymphoma must be inferred and must, at best, be considered tenuous; the census questionnaire asked about acres sprayed in 1970 with herbicides, not about exposure to 2,4-D."

Waterhouse *et al.* (1996) conducted a community-based longitudinal prospective study which has been ongoing since 1959. Among 6,702 participants who were eligible and had

responded to a follow-up questionnaire, there were 731 cancer cases. When compared to the Connecticut tumor registry, the standardized incidence ratio (SIR) for males and females was 0.91 and 0.95, respectively. These ratios were not significantly different from 1.0. Tecumseh community is located in Lenawee county, which, according to the U.S. Census of Agriculture, had the highest average annual levels of spraying or treatment with pesticides in the 1970s and 1980s. When lymphomas (non-Hodgkin's lymphoma and Hodgkin's disease combined) and chronic lymphocytic leukemia were combined, males and females had a significantly elevated SIR of 1.40 (95% confidence interval 1.03-1.86). In a nested case-control study, based on exposure information documented prior to diagnosis, the odds ratio for having worked on a farm and regular household use of pesticide sprays was elevated, but not significantly for lymphomas, leukemia and multiple myeloma. This study did not specifically tie any of these associations to use of 2,4-D or chlorophenoxy herbicides, but mention heavy use of atrazine, methoxymethyl-acetanilide herbicide, and organophosphate insecticides in this county.

2. Exposure Assessment

Exposure assessment is one of the principle difficulties when undertaking pesticide epidemiology studies. Only 2 of the 5 cohort studies used biological monitoring data to assess exposure. The other studies relied on surrogate measures, such as usage or sales data for 2,4-D. Some studies such as those conducted by the National Cancer Institute have used a number of different measures and then checked for consistency among the different types of indices. In lieu of direct exposure data, the issue arises as to how much weight should be placed on partial consistency among surrogate measures of exposure.

Sometimes surrogate measures are divided into 3 or more categories, permitting tests for trend. When such tests show significance, they may provide important evidence for dose-response relationships and some thought should be given to factoring in additional weight for such evidence, depending on the strength of the relationship.

Much concern has centered around the ability of respondents to recall what specific pesticides were used, the duration and frequency of use, types of application, and the use of protective equipment. This is especially a problem when next-of-kin are used in place of cases or controls that have died. Sales records have sometimes been used to supplement memory

and limit recall bias. More attention is warranted where attempts have been made to validate exposure measures, depending on the level of validation. Underreporting of all pesticide use is another concern, with some reviewers suggesting that individual pesticide use may be underreported by 20-50%.

Table 4. Overview of Case-Control Studies of 2,4-D and *SOFT TISSUE SARCOMAS*.

REFERENCE/ LOCATION	EXPOSURE TYPE/CHEMICAL	RISK ESTIMATES[a]	95% CI[b]	COMMENTS
Hardell & Sandstorm, 1979 **Sweden**	Phenoxy acids	**5.3***	2.4 - 11.5	Hospital-based; Exposure to 2,4-D, 2,4,5-T & MCPA; No quantitative data on 2,4-D; Bias in selection of cases and controls
Eriksson *et al.* 1981 **Sweden**	Phenoxy acids	**6.8***	2.6 - 17.3	Population-based; Subjective exposure information.
Vineis *et al.* 1981 **Italy**	Agricultural occupation (women)	2.7	0.6 - 12.4	Exposure to 2,4-D, 2,4,5-T & MCPA
Smith *et al.* 1983, 1984 **New Zealand**	Occupational; phenoxy herbicides	1.3	0.7 - 2.5	Population-based; Exposure based on questionnaire
Hoar *et al.* 1986 **Kansas**	Agricultural herbicide use	0.9	0.5 - 1.6	Exposure mainly to 2,4-D
Woods *et al.* 1987 **Washington**	Occupational; phenoxy herbicides	0.89	0.4 - 1.9	Exposure based on questionnaire
Eriksson *et al.* 1990 **Sweden**	Phenoxy herbicides (not 2,4,5-T)	0.6	0.2 - 2.1	-

Table 5. Overview of Cohort Studies of 2,4-D and *SOFT TISSUE SARCOMAS*.

REFERENCE/ LOCATION	EXPOSURE TYPE/CHEMICAL	RISK ESTIMATES[a]	95% CI[b]	COMMENTS
Coggon *et al.* 1991 England	Manufacturing phenoxy herbicides	0.0	0.0 - 20.9	S, Part of Saracci et al, 1991[c]
Sarraci *et al.* 1991	All occupations/phenoxy herbicides	2.0	0.5 - 5.0	Uncertain S, exposures
	Manufacturers of phenoxy herbicides	1.0	0.0 - 5.4	
10 countries	Sprayers of phenoxy herbicides	3.0	0.6 - 8.7	S

* = Statistically significant a = Odds Ratio b = Confidence Interval
S = Very small sample, less than 10 cases H = Relatively high estimate of risk

Table 6. Overview of Case-Control Studies of 2,4-D and *NON-HODGKIN,S LYMPHOMA*.

REFERENCE/ LOCATION	EXPOSURE TYPE/CHEMICAL	RISK ESTIMATES[a]	95% CI[b]	COMMENTS
Hoar et al.1986	Farm/herbicides	1.6	.9 - 2.6	Internally
	Herbicides adjusted for insecticides	1.4	0.8 - 2.4	consistent
Kansas	Farm/insecticides	1.5	0.9 - 2.4	
	Insecticides adjusted for herbicides	1.1	0.6 - 2.2	
	Fungicides	**2.1***	1.2 - 3.7	
	Fungicides with herbicide use	**2.3***	1.2 - 4.3	
	Fungicides without herbicide use	1.9	0.8 - 4.4	
	Phenoxy herbicides	**2.2***	1.2 - 4.1	
	Triazine herbicides	**2.5***	1.2 - 5.4	
	Triazines adjusted for phenoxys	1.9	0.4 - 8.0	
	Amides (no adjustment)	**2.9***	1.1 - 7.6	S
	Trifluralin (no adjustment)	**12.5***	1.6-116.1	S, H
	Non-specified herbicides (no adjustment)	**5.8***	1.9 -17.2	S, H
	<u>All herbicides use, not just 2,4-D:</u>			
	Mixed/applied by self	**1.9***	1.1 - 3.3	
	Mixed/applied by someone else	1.1	-	
	Mixed/applied by self/>20 days/year	**8.0***	2.3 - 27.9	S, H
	Protective equipment not used	**2.1***	1.0 - 4.2	
	Protective equipment used	1.5	0.7 - 3.1	
	Applied by backpack/hand	**2.3***	1.0 - 5.2	
	Applied by tractor/aerial	1.5	-	
	<u>Herbicide use - only among users of 2,4 D:</u>			
	2,4-D use only (no 2,4,5-T)	**2.6***	1.4 -5.0	
	<u>Duration of herbicide use:</u>	trend significant	-	dose-response
	Duration of herbicide use: > 25 years	2.3	0.7 - 6.8	S
	<u>Frequency of herbicide use:</u>	trend significant	-	dose-response
	Frequency of herbicide use: > 20 days/year	**7.6***	1.8 - 32.3	S, H
	<u>First year of herbicide use:</u>	trend significant	-	dose-response
	First year of use <1946	6.2	0.6 -65.3	S, H

* = Statistically significant a = Odds Ratio b = Confidence Interval
S = Very small sample, less than 10 cases H = Relatively high estimate of risk

Table 6. Overview of Case-Control Studies of 2,4-D and *NON-HODGKIN'S LYMPHOMA* (Continued).

REFERENCE/ LOCATION	EXPOSURE TYPE/CHEMICAL	RISK ESTIMATES[a]	95% CI[b]	COMMENTS
Zahm *et al.* 1990	Farm/carbamates	**1.8***	1.0 - 3.2	Other
	Farm/organophosphates	**1.9***	1.1 - 3.1	pesticides also
	Farm/herbicides	1.3	0.8 - 2.0	implicated
	Mixed or applied 2,4-D	1.5	0.9 - 2.5	
	2,4-D adjusted for OP use	1.1	-	
Nebraska	2,4-D adjusted for fungicides	**1.8***	1.1 - 3.0	
	<u>Duration of 2,4-D use</u>	trend Not significant	-	
	Duration of >20 years	1.3	0.6 - 2.7	
	<u>Frequency of 2,4-D use</u>	borderline significance	-	proxy risk 3X
	Frequency of >20 days/year	3.3	0.5 - 22.1	S - self responders
	First year of 2,4-D use	trend Not significant	-	
	First year of use <1946	1.4	0.8 - 2.6	S
	<u>Includes OP & fungicide use</u>			
	Use of 2,4-D >20 days/year	2.1	-	
	Use of organophosphates	2.4	-	
	<u>Method of application:</u>			
	2,4-D applied by hand	1.7	0.4 - 6.7	S
	2,4-D applied by tractor	1.4	0.8 - 2.6	
	<u>Among those who mixed/applied 2,4-D:</u>			
	Changed clothes less often	trend significant		Dose-response
	Changed clothes >1 day	**4.7***	1.1 - 21.5	S, H
	Changed at end of day	1.5	0.8 - 2.6	
	Changed after use	1.1	0.4 - 3.1	S
	Protective equipment not used	1.2	0.6 -2.4	
	Protective equipment used	1.7	0.9 - 3.1	

* = Statistically significant
a = Odds Ratio
b = Confidence Interval
S = Very small sample, less than 10 cases
H = Relatively high estimate of risk

Table 6. Overview of Case-Control Studies of 2,4-D and *NON-HODGKIN'S LYMPHOMA* (Continued).

REFERENCE/ LOCATION	EXPOSURE TYPE/CHEMICAL	RISK ESTIMATES[a]	95% CI[b]	COMMENTS
Weisenberger *et al.* 1991 **Kansas & Nebraska Combined**	Used 2,4-D Used 2,4-D >20 days/year	**1.9*** **4.5***	1.3 - 2.8 1.1 - 18.3	
Cantor *et al.* 1992 **Iowa & Minnesota**	Used 2,4-D 2,4-D handled prior to 1965 Protective equipment not used	1.2 1.3 1.2	0.9 - 1.6 0.9 - 1.8 0.9 - 1.7	
Woods *et al.* 1987, 1989 **Washington State**	Farm/phenoxy herbicides Farm/2,4-D All occupations/phenoxy herbicides All occupations/2,4-D Forest/phenoxy herbicides	0.7 0.7 0.9 0.7 **4.8***	0.3 - 1.5 0.3 - 1.4 0.5 - 1.5 0.4 - 1.3 1.2 - 19.4	 S
Pearce *et al.* 1986, 1987, Pearce, 1989 **New Zealand**	Farm/phenoxy herbicides > 5 days, >10 years ago Duration of use >14 years Frequency of use >20 days/year	1.0 0.0 1.2 1.1	0.7 - 1.5 0.6 - 1.5 0.6 - 2.3 1.1 - 4.1	90% CI
Hardell *et al.* 1981 **Sweden**	Farm/phenoxy herbicides	**4.8***	2.9 - 8.1	H, Recall bias
Olsson *et al.* 1988 **Sweden**	>1 day exposure to phenoxy herbicides	**10.0***	2.7 - 37.1	Localized skin NHL
Dalager *et al.* 1991 **Vietnam Vets**	Service in Vietnam	1.0	0.7 - 1.5	Maximum latency 20 Years
Hays *et al.* 1991 **DOG study** **Veterinary hospitals in Colorado, Indiana & Minnesota**	Owner used 2,4-D/lawn service Owner used 2,4-D Owner used lawn care service Used 2,4-D and lawn care service Frequency of 2,4-D use Frequency of 2,4-D >4/year Duration of 2,4-D use Duration of 2,4-D >4 years	**1.3*** 1.3 1.3 1.9 trend significant 2.0 trend Not sig. 1.5	1.04 - 1.7 0.9 - 1.8 0.96 - 1.7 0.9 - 4.1 - 0.9 - 4.2 - -	 Dose-response

S = Very small sample, less than 10 cases H = Relatively high estimate of risk

Table 7. Overview of Cohort Studies of 2,4-D and *NON-HODGKIN'S LYMPHOMA*.

REFERENCE/ LOCATION	EXPOSURE TYPE/CHEMICAL	RISK ESTIMATES[a]	95% CI[b]	COMMENTS
Bloeman *et al.* 1993 Bond *et al.* 1988 **U.S.A**	Manufacturing 2,4-D	3.03	0.78 - 11.85	S, 2 cases with 3 and 10 years latency
Coggon *et al.* 1991 **England**	Manufacturing phenoxy herbicides	2.3	0.3 - 8.3	S, Part of Saracci *et al.*, 1991[c]
Sarraci *et al.* 1991 **10 countries**	All occupations/phenoxy herbicides Manufacturers of phenoxy herbicides Sprayers of phenoxy herbicides	1.0 1.5 0.5	0.5 - 1.7 0.6 - 1.4 0.1 - 1.4	Uncertain S, exposures[c] S
Wigle *et al.* 1990 **Saskatchewan**	Acres sprayed with herbicides >250 acres <u>For farms of <1000 acres:</u> acres sprayed with herbicides >250 acres Dollars spent of fuel or oil >$900 (adjusted for herbicide acres)	trend Not significant 1.3 trend significant **2.2*** trend significant **2.3***	- 0.7 - 2.4 - 1.02 - 4.6 - 1.1 - 4.7	75% of herbicide use was 2,4-D. Fuel/oil possible confounders

* = Statistically significant
a = Odds Ratio
b = Confidence Interval
C = Exposure from job histories more uncertain than in other cohort studies using monitoring data.
H = Relatively high estimate of risk
S = Very small sample, less than 10 cases

F. WEIGHT OF EVIDENCE CONSIDERATIONS

The Committee was asked to consider the following factors in the Weight-of-the Evidence to assess the carcinogenic potential of 2,4-D.

1. Evidence of Carcinogenicity in Animals

The carcinogenic potential of 2,4-D has been evaluated in two independent studies with B6C3F1 mice at doses ranging from 1 to 300 mg/kg/day and in two independent studies with Fisher 344 rats at doses ranging from 1 to 150 mg/kg/day.

- When administered in the diet of male and female B6C3F1 mice for 104 weeks at 0, 1, 15 or 45 mg/kg/day, there were no increases in individual tumor types in any of the treated mice. The high dose was judged to be inadequate to assess the carcinogenic potential, therefore, another study was conducted at higher doses.

- In the second study, no increases in individual tumor types were seen when administered in the diet to male B6C3F1 mice at 0, 5, 62.5, or 125 mg/kg/day or to female B6C3F1 mice at 0, 5, 150, or 300 mg/kg/day for 104 weeks.

- Male and Female Fischer 344 rats were fed diets containing 2,4-D at 0, 1, 5, 15, or 45 mg/kg/day for 104 weeks. Astrocytomas of the brain were observed in both control and treated rats of both sexes. The incidences were: 1/60 (1.6%), 0/60, 0/60, 2/58 (3.4%) and 6/60 (10%) in males and 0/60, 1/60 (1.6%), 2/60 (3.3%), 1/60 (1.6%), and 1/60 (1.6%) in females at 0, 1, 5, 15, or 45 mg/kg/day, respectively.

- A positive significant trend ($p = 0.0026$) was seen in males. The incidence at the high dose (6/60; 10%), however, was not statistically significant ($p = 0.702$) when compared to controls (1/60; 1.6%). No statistical significance was seen in females.

- When compared to historical controls, the 10% incidence in males at the high dose slightly exceeded the historical control range (0 - 4.4%) of studies conducted at the National Toxicology Program. Incidences in females were within the historical control range.

- The dose levels tested were judged to be inadequate to

assess the carcinogenic potential, therefore, another study was conducted at higher doses.

- In the second study conducted to ascertain the astrocytomas seen in the first study, male and female Fischer 344 rats received 2,4-D in their diet at 0, 5, 75, or 150 mg/kg/day for 104 weeks.

- Astrocytomas of the brain were seen in 1/50 (2%) males and 1/50 (2%) females at 150 mg/kg/day compared to 0/50 in control males and 1/50 (2%) in control females. When compared to historical controls, the 2% incidence in both the control and treated rats were within the historical control range (0 - 4.4%).

- The lack of an increase in astrocytomas in the above study in which rats received 2,4-D at 150 mg/kg/day suggests that the astrocytomas reported in the earlier study were an aberration and not treatment-related.

- In addition, characteristics generally attributed to a brain carcinogen were not seen in the earlier study in which astrocytomas were seen. 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 rats, all tumors were solitary, and the tumors in treated rats were not larger or more anaplastic than generally seen in control rats. In fact, the largest and most lethal tumor was the one in the control male. Also, while most, if not all known brain carcinogens show clear genotoxicity in mutation assays, 2,4-D was negative both *in vitro* and *in vivo* in most assays.

- The mutagenicity of 2,4-D has been studied extensively in a wide variety of *in vitro* and *in vivo* assays. 2,4-D was non mutagenic in several strains of *Salmonella* with and without metabolic activation, in a mouse bone marrow micronucleus, and UDS assays in rat hepatocytes. Conflicting results were obtained in *Drosophila*; positive effects were seen in larvae, while negative results were seen in adults after feeding or injection. Conflicting results were also seen *in vitro* mammalian cell cytogenetic assays; 2,4-D was negative for structural chromosomal damage up to an insoluble level but positive in the presence of metabolic activation at high doses. The positive evidence, however, tends to be weak and generally not supported by the data from *in*

vivo cytogenetic assays. 2,4-D was non mutagenic in mammalian cell DNA repair assays. The lack of genotoxicity in *in vitro* bacterial and mammalian test systems with an exogenous source of metabolic activation provides evidence that 2,4-D is not metabolized to potentially reactive intermediates. Based on the overall pattern of responses observed both in *in vivo* and *in vitro* tests, 2,4-D was not mutagenic (although some cytogenetic effects were seen).

- 2,4-D is structurally related to 2,4-DB, 2,4-DP, MCPA, MCPP, 2,4,5-T and 2,4,5-TP. No evidence of carcinogenicity was seen following dietary administration of 2,4-DB, 2,4-DP and MCPA to mice and rats or 2,4,5-T to rats. No studies are available to assess the carcinogenic potential of MCPP or 2,4,5-TP.

2. Epidemiological Considerations

Over the past 8 years, a number of scientific panels, convened under the auspices of various groups, have evaluated the epidemiological studies that attempted to link the use of phenoxy herbicides, 2,4-D in particular, to STS and NHL. Their findings are as follows:

- *1987 - International Agency for Research on Cancer (IARC):* The Working Group concluded that there was limited evidence that chlorophenoxy herbicides were carcinogenic to humans. There was no clear delineation of data related to 2,4-D as opposed to other chlorophenoxy herbicides, some of which contain dioxins (TCDD) (IARC, 1987).

- *1987 - Ontario Pesticide Advisory Committee of the Ontario Ministry of the Environment:* The panel, using IARC terminology, 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." (Anders *et al.*, 1987).

- *1991 - Harvard School of Public Health Panel:* The panel concluded: "Although a cause-effect relationship is far from being established, the epidemiological evidence for an association between exposure to 2,4-D and non-Hodgkin's lymphoma is suggestive and requires further investigation.

There is little evidence of an association between use of 2,4-D and soft-tissue sarcoma or Hodgkin's disease, and no evidence of an association between 2,4-D use and any other form of cancer" (Ibrahim *et al.*, 1991).

- *1992 - Munro et al.,:* The author concluded that "The case-control epidemiological studies that have been the source of the cancer risk hypothesis are inconclusive. Problems in assessing exposure based on patient's memories make these studies difficult to interpret. Cohort studies of exposed workers do not generally support the specific hypothesis that 2,4-D causes cancer. Taken together, the epidemiological studies provide, at best, only weak evidence of an association between 2,4-D and the risk of cancer."(Munro *et al.*, 1992).

- *1994 - Joint Committee of the Science Advisory Board/Scientific Advisory Panel:* concluded "...that while there is some evidence that NHL may occur in excess in populations which are likely to be exposed to 2,4-D, the data are not sufficient to conclude that there is a cause and effect relationship between exposure to 2,4-D and NHL. The data are, however, sufficient to require continued examination of the issue through further studies." (USEPA, 1994).

The Weight-of-Evidence considered by the panel at the Joint Meeting of the SAB/SAP is provided below:

" Epidemiologic studies of 2,4-D have included both case control studies of NHL in geographic areas where the numbers of farmers might be high and cohort studies of manufacturers of the chemical as well as applicators and farming populations. The case control studies have focused on the association of the general class, phenoxyherbicides, with NHL. Many studies did not specify the specific chemical so the exposure to 2,4-D had to be inferred from the usual uses in the area. In general the studies indicated an approximately 20% to 30% increased odds ratio associated with farming. If phenoxy acids were responsible for the observed excess of NHL in farmers, the odds ratio for the association between the chemicals and NHL should be much higher than the 1.2 to 1.3 seen for farmers because the etiologic exposure is now more specific. This is not the case.

In the NCI studies of NHL in Kansas and Nebraska (but not in Iowa) the odds ratio increased with the number of days per year of exposure to suggest a dose-response relationship. However, in these studies as well as some others there was not sign of increased risk with number of years of use. So, unlike many other carcinogens, there is no indication of a cumulative dose effect on risk - only an increasing risk with heavy exposure at some time during the life time. Thus the lack of an increase in the risk ratio when we move from a non-specific exposure (as with farming to a more specific exposure (as with 2,4-D) and the absence of a positive dose response where cumulative exposure by years is used is not consistent a causal relationship between the chemical and NHL".

"The cohort studies in general have not suggested an increased risk of NHL for individuals exposed to 2,4-D. However, many of these studies had a small exposed population and did not have sufficient follow-up to be expected to show a risk even if it did exist. Therefore, the negative results are relatively uninformative as to whether there is an effect from this chemical. As with most epidemiologic studies the retrospective assessment of exposure is suspect in all these studies. However, for most studies, the subjects may well have had many chemical exposures which were not taken into account in the analysis. Exposure to some of the chemicals are highly correlated, making individual assessment of the exposure to a single chemical difficult or impossible. Thus, while the epidemiologic studies suggest it is possible that 2,4-D may be carcinogenic in humans, the evidence is not strong enough to support a causal relationship to the specific phenoxy herbicide or any other farm exposure. However, there is suggestive evidence of an association between exposure to 2,4-D and NHL in some of the studies, and this observation required further investigation. Future studies must try to establish the exposure to 2,4-D and distinguish its effects from those of farming in general and from other specific chemicals and pesticides used in the same environment".

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The CPRC agree that 2,4-D should remain classified as a Group D (not classifiable as to human carcinogenicity). In two new adequate studies in rodents, which were conducted at doses high enough to assess the carcinogenic potential of 2,4-D, there were no compound related statistically significant increases in tumors in either rats or mice. There were no increases in astrocytomas in male rats (as were observed in the original study); however, the slides from all animals at the low and intermediate doses were not evaluated. There was an increase in hemangiosarcomas of the spleen in male mice at the low and mid-doses, which was not sustained at the highest dose; however, the slides from all animals at the low and intermediate doses were also not evaluated.

Further analysis of the epidemiology data and review of five recent studies of 2,4-D exposure and cancer were presented to the CPRC (details are given in section E.II). The CPRC concluded that these studies are not sufficient to change the conclusions drawn by the joint SAB/SAP that in humans, while there was some evidence that non-Hodgkin's lymphoma (NHL) may occur, the data were not sufficient to conclude a cause and effect relationship between exposure to 2,4-D and NHL.

A large prospective Agricultural Health Study has been undertaken to help determine which pesticide exposures, if any, are associated with NHL and other cancers in farming populations. This study which will continue for 7 more years, will overcome many of the deficiencies in earlier studies, and help to resolve the question of which risk factors are contributing to the excessive rates of NHL in farmers.

The CPRC agreed that the Registrant should be required to provide additional histopathology data to include all animals in the low and mid-doses in both the male rat brain and the male mouse spleen.

A better characterization of the impurities in the test materials used in both the original and the new studies, might also be helpful to explain the conflicting results (ie: astrocytomas in male rats).

APPENDIX

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