Return to Menu

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

May 23, 1996

Office of
Prevention, Pesticides and Toxic Substances

MEMORANDUM

SUBJECT 2,4-DICHLOROPHENOXYACETIC ACID: Review of a Chronic toxicity/Carcinogenicity Study in **Rats**, a Carcinogenicity Study in **Mice**, and a Re-review of a Developmental Toxicity Study in **Rats**.

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THRU: Yiannakis loannou, Ph.D. Head
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DATA PACKAGE IDENTIFICATIONS:

Submission:	DP Barcode	MRID No.	STUDY
S487304	D215596	43612001	Rat
S487309	D215600	43597201	Mouse (Female)
S499298	D222295	43879801	Mouse (Male)

CHEMICAL: 2,4-Dichlorophenoxyacetic acid PC Code: 030001 Caswell No. 315

ACTION REQUESTED: Review the two year chronic toxicity/carcinogenicity study in rats and the two-year carcinogenicity study in mice submitted by the Industry Task force on 2,4-D Research in response to the Agency's 1989 Data Call-In Notice.

RESPONSE: Data Evaluation Records (DERs) for the chronic Toxicity/carcinogenicity Study in Fischer 344 Rats (MRID No. 43612001) and the carcinogenicity study in B6C3F1 mice (MRID Nos. 4359701 & 43879801) are attached. Also attached is a DER for a developmental Toxicity study in rats (Acc.No. 00251031) that was not included in this Data Package. This study was re-reviewed since the original review and the DER

(Memo: H. Spencer, HED, to R. Mountford, RD, 8/7/84 HED Document No. 003887) was determined to be inadequate. The Executive Summaries for these studies are provided below.

The chronic toxicity/carcinogenicity study in rats the carcinogenicity study in mice and the developmental toxicity study in rats are classified as Acceptable and satisfy the Subdivision F guideline requirements §83-5, §83-2(b) and §83-3(a), respectively.

Also attached to this Memorandum is a Toxicology Profile for 2,4-dichlorophenoxy acetic acid; the toxicology data base is complete and there are no data-gaps.

1. §83-5; COMBINED CHRONIC TOXICITY/CARCINOGENICITY STUDY IN RATS

CITATION: Jeffries, TK, Yano, BL, Ormand. JR and Battjes, JE. "2,4-DlCHLOROPHENOXYACETIC AClD: CHRONlC TOXlClTY/ ONCO-GENlCITY STUDY lN FISCHER 344 RATS-FlNAL" The Toxicology Research Laboratory, Dow Chemical Co., Midland, Michigan. Study ID: K-002372-064. 3/28/95. MRID No. 43612001.

EXECUTIVE SUMMARY: 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 approximately 6,12,18 and 24 months, and organ weights and histopathology at 12 and 24 months.

Treatment had no adverse effect on survival and there were not treatment-related clinical signs of toxicity. At termination, body weights were lower than respective controls in females at 75 mg/kg/day (-14%) and in males (-8%) and females (-26%) at 150 mg/kg/day. Body weight gains were lower than respective controls in females at 75 mg/kg/day and (-24%) and in males (-17%) and females (-48%) at 150 mg/kg/day. A corresponding depression in average food consumption occurred in females at 75 mg/kg/day (-4%) and in males (-5%) and females (-12%) at 150 mg/kg/day.

Statistically significant ($p \leq 0.05$) decreases in red blood cell and platelet counts were seen in females at 75 mg/kg/day and in both sexes at 150 mg/kg/day at different time points. These decreases, however, were not considered to be treatment related due to lack of dose- and/or time-response and corroborative histopathological lesions in the hematopoietic system. Decreased hematopoiesis of the bone marrow was seen only in females at 150 mg/kg/day at the 12 month sacrifice but not at the terminal sacrifice.

Statistically significant ($p \leq 0.05$) increases in plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), and/or cholesterol were seen in females at 75 mg/kg/day and in males and females at 150 mg/kg/day at various time periods. These increases may be attributed to treatment due to the hepatic lesions observed at the interim sacrifice in females at 75 mg/kg/day and at terminal sacrifice in males and females at 150 mg/kg/day. It should be noted, however, that the hepatic lesions were limited to altered tinctorial properties involving all hepatocytes within the hepatic nodules and were not associated with hepatocellular degeneration or necrosis. Although the thyroxin (T_4) levels were decreased in both sexes at 75 and 150 mg/kg/day at all intervals, increases in absolute and relative thyroid weights were seen only in females at 75 mg/kg/day and in males and females at 150 mg/kg/day at both the interim and terminal sacrifices while histopathological lesions of the thyroid glands were seen only in females at 150 mg/kg/day at the interim sacrifice.

Gross pathology revealed opacity of the lens and a general decrease in fat in females at 150 mg/kg/day, pale foci in the lungs of males and females at 150 mg/kg/day, and thyroid masses in males at 75 and 150 mg/kg/day and in females at all dose levels. Except for the increases in thyroid weights as noted above, no treatment-related effects were seen in any of the organ weight parameters.

After 12 months of treatment (Interim Sacrifice) treatment-related non-neoplastic lesions were: decreased hematopoiesis of the bone marrow of females at 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; bilateral retinal degeneration of the eyes of females at 150 mg/kg/day; multifocal alveolar histiocytosis in the lungs of females at 75 mg/kg/day and both sexes at 150 mg/kg/day; degeneration of the descending portion of the proximal convoluted tubules of the kidneys in both sexes at 75 mg/kg/day and 150 mg/kg/day; atrophy of the adipose tissue of females at 75 and 150 mg/kg/day; atrophy of the testes in males at 150 mg/kg/day; and decreased secretory material in the thyroid follicles of females at 150 mg/kg/day. No treatment-related neoplastic lesions were seen at any dose level.

After 24 months of treatment (Terminal Sacrifice) treatment-related non-neoplastic lesions were limited to the eyes, liver, lung, and the mesenteric fat. Eye lesions were characterized as slight to severe bilateral retinal degeneration and lenticular cataracts in both sexes at 150 mg/kg/day. Liver lesions manifested as increases in the size of hepatocytes, often accompanied by altered tinctorial properties that involved all hepatocytes within the hepatic lobule of both sexes at 150 mg/kg/day. Lesions of the respiratory system included subacute to chronic inflammation of the lungs in females at 75 mg/kg/day and both sexes at 150 mg/kg/day. Atrophy of the adipose tissue was increased in both sexes at 150 mg/kg/day. It is interesting to note that lesions seen in the spleen, kidneys, testes, and thyroid glands in rats sacrificed at 12 months were not seen in those sacrificed at 24 months. No treatment-related neoplastic lesions were seen in either sex at any dose level.

In this study, the highest dose tested (150 mg/kg/day) did not alter survival or induce any clinical signs, but did induce systemic toxicity in both sexes. Therefore, it is concluded that the doses used in this study were adequate to assess the chronic toxicity and the carcinogenic potential of 2,4-D acid.

Under the conditions of this study, for chronic toxicity, the NOEL is 75 mg/kg/day in males and 5 mg/kg/day in females. The LOEL is 150 mg/kg/day in males and 75 mg/kg/day in females. In males, the LOEL is based on decreases in body weight, body weight gain and food consumption, increases in liver enzymes, decreases in T_4, concentration, increases in absolute/relative thyroid weights, and histopathological lesions in the eyes, liver, lungs, and mesenteric fat (adipose tissue). In females, the LOEL is based on decreases in body weight, body weight gain and food consumption, increases in liver enzymes, decreases in T_4, concentration, increases in absolute/relative thyroid weights, and histopathological lesions in the liver, kidneys and lungs. 2,4-D acid was not carcinogenic in male or female Fischer 344 rats.

CORE CLASSIFICATION: This study is classified as **Acceptable** and satisfies the Subdivision F guideline requirement for a combined chronic toxicity/ carcinogenicity study in rats (§83-5).

2. §83-2(b) CARCINOGENICITY STUDY IN MICE

CITATIONS: Study in 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., Midland, Michigan.
Study ID: K-002372-063M. 11/16/95. **MRID No. 43879801**.

Study in 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., Midland, Michigan. Study ID: K-002372-063F. 03/10/95.
MRID No. 43597201.

EXECUTIVE SUMMARY: In a carcinogenicity study, 2,4-dichlorophenoxyacetic acid (96.4%) was administered in the diet for 104 weeks to male B6C3F1 mice 50/dose) at 0, 5, 62.5 or 125 mg/kg/day (MRID No. 43879801) and to female B6C3F1 mice (50/dose) at 0, 5, 150 or 300 mg/kg/day (MRID No. 43597201). 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.

In males, no treatment-related effects were seen on survival, body weight, body weight gain, clinical signs, hematology parameters, or gross pathology at any dose level. Females at 300 mg/kg/day exhibited 14% decreases in body weight gain at 3 months into the study but by study termination (24 months), body weight gains of these mice were similar to that of the controls. Treatment did not affect survival, induce clinical signs, alter hematology parameters, or cause gross pathological changes at any dose level in females. Kidney was identified as the target organ for both sexes; dose-related increases in kidney weights and renal lesions were seen in males at 62.5 and 125 mg/kg/day and in females at 150 and 300 mg/kg/day.

Treatment-related organ weight changes were limited to kidney weights. In males, dose-related increases in absolute and relative kidney weights were seen only after 24 months; absolute weights were increased by 5% and 7% and relative weights by 6% and 10% at 62.5 and 125 mg/kg/day, respectively. In females, dose-related increases in absolute and relative kidney weights were seen after 12 and 24 months. After 12 months, absolute weights were increased by 14% and 17%, and relative weights by 22% and 30% at 150 and 300 mg/kg/day, respectively. After 24 months, absolute weights were increased by 14% and 22% and relative weights by 12% and 20% at 150 and 300 mg/kg/day, respectively. The increases in kidney weights were attributed to treatment due to corroborative dose-related renal lesions seen in both sexes after 12 and 24 months.

After 12 months of treatment (Interim-Sacrifice), dose-related renal lesions in male mice were degeneration with regeneration of the descending portion of the proximal tubule in 2/10 (20%) and 10/10 (100%) at 62.5 and 125 mg/kg/day, respectively and decreased vacuolation of the renal proximal tubule in 8/10 (80%) and 10/10 (100%) at 62.5 and 125 mg/kg/day, respectively. Either of these lesions were seen in the control or at 5 mg/kg/day. In females, renal lesion was limited to hypercellularity of the descending portion of the proximal tubules seen in 8/10 (8O%) and 10/10 (100%) mice at 150 and 300 mg/kg/day, respectively.

After 24 months of treatment (Terminal Sacrifice), dose-related renal lesions seen in males at 62.5 and 125 mg/kg/day comprised a constellation of changes that involved five different diagnoses. Degeneration with regeneration of the descending limb of the proximal tubule was seen in 25/50 (50%) and 48/50 (96%) at 62.5 and 125 mg/kg/day, respectively compared to none in the controls and at 5 mg/kg/day. Decreased vacuolation of the renal proximal tubule was seen in 39/50 (78%) and 48/50 (96%), respectively, at 62.5 and 125 mg/kg/day, compared to none in the controls and at 5 mg/kg/day. Both of these lesions were also seen in a dose-related manner at the interim (12-month) sacrifice. Also seen were, mineralization of the tubules in 29/50 (58%) and 36/50 (72%) and multifocal cortical cysts in 22/50 (44%) and 20/50 (40%) at 62.5 and 125 mg/kg/day, respectively. In fe-

males, renal lesions at 150 and 300 mg/kg/day were hypercellularity in 32/50 (64%) and 25/50 (50%) and degeneration with regeneration of the tubules in 38/50 (76%) and 34/50 (68%), respectively.

Under the conditions of this study, for chronic toxicity, the NOEL is 5 mg/kg/day in both sexes. The LOEL is 62.5 mg/kg/day in males and 150 mg/kg/day in females. In both sexes, the LOEL is based on increases in absolute and/or relative kidney weights and histopathological lesions in the kidneys. At the doses tested, 2,4-D acid was not carcinogenic in male or female B6C3F1 mice.

CORE CLASSIFICATION: This study is classified as acceptable and satisfies the Subdivision F guideline requirement for a carcinogenicity study in mice [§ 83-2(b)].

3. §83-3(a) DEVELOPMENTAL TOXICITY STUDY IN RATS.

CITATION: Nemec, M.D, Tasker, E.J. Werchowski, K.M, and Mercieca, M.D. "A TERATOLOGY STUDY IN FISCHER 344 RATS WITH 2,4-DICHLOROPHENXOY ACETIC ACID." WIL Research Laboratories, Inc. Study No. WIL-81135, 3/2/83.
Accession No. 000251031.

EXECUTIVE SUMMARY: In a developmental toxicity study (Acc. #000251031) pregnant Fischer-344 rats (35/group) were given oral administration (gavage) of 2,4-dichlorophenoxy acetic acid (technical, 97.5%) in corn oil at 0 (vehicle control), 8, 25, or 75 mg/kg/day during gestation Days 6 through 15, inclusive.

Treatment did not affect survival, induce clinical signs or maternal wastage, cause body weight changes, or alter reproductive parameters. Maternal toxicity was limited to decreases in body weight gain in dams at 75 mg/kg/day; when compared to the vehicle control, the decreases were -43%, -21% and -2% for gestation days 6-10, 6-15, and 0-20, respectively. Although these decreases were not statistically significant, they were considered to be treatment-related because decreases in body weight gain was also seen in a 2-generation reproduction toxicity study in the same strain (Fischer 344) of rats at a comparable dose of 80 mg/kg/day (actual dose = 75mg/kg/day). Based on these findings, for maternal toxicity, the NOEL was 25 mg/kg/day and the LOEL was 75 mg/kg/day.

No treatment-related fetal gross external, visceral or skeletal malformations were seen at any dose level. Skeletal variation observed at 75 mg/kg/day included: the presence of 7th cervical ribs (4 fetuses of 3 litters vs. none in the controls); presence of l4th rudimentary ribs (4 fetuses of 3 litters vs. 0 in the controls); mala-

ligned sternebrae (15 fetuses of 10 litters vs. 7 fetuses of 7 litters in the controls); reduced ossification of the vertebral aches (6 fetuses of 5 litters vs. 2 fetuses of 1 litter in the controls; and unossified sternebrae #5 or #6 (73 fetuses of 22 litters; 3.32/litter vs. 62 fetuses of 24 litters; 2.58/litter in the controls). Although these increases were not statistically significant, they were attributed to treatment since some of the variations (malaligned sternebrae,14th rudimentary ribs and reduced ossification of vertebral arches) seen in this study were also seen in the F_{1b} pups of dams fed 2,4-D at 80 mg/kg/day (actual dose, = 75 mg/kg/day) in the 2-generation reproduction study in the same strain of rats (Fischer 344). In addition, skeletal variations of the ribs (2nd wavy ribs, lumbar ribs and missing sternebrae were also seen in an another teratology study using a different strain (Sprague-Dawley) of rats at a comparable dose of 87.5 mg/kg/day.

Thus, based upon a weight-of-evidence from the reproduction and developmental toxicity studies in Sprague-Dawley and Fischer 344 rats, it is concluded that developmental toxicity did occur at the high dose (75 mg/kg/day) in this study. Based on these findings, for developmental toxicity, the NOEL was 25 mg/kg/day and the LOEL was 75 mg/kg/day.

CORE CLASSIFICATION: This study is classified as Acceptable and satisfies the Subdivision F Guideline requirement for a developmental toxicity study in rats [83-3(a)].

TOXICOLOGY PROFILE FOR 2,4-DICHLOROPHENOXYACETIC ACID

§GUIDE-LINE#	STUDY	MRID NO.	RESULTS
81-1	Acute Oral - Rat	00101605	LD_{50}: 699mg/kg TOX.CAT-III
81-2	Acute Dermal - Rat	00101596	LD_{50} => 2000 mg/kg TOX.CAT-III
81-3	Acute Inhalation -Rat	001616650	LD_{50} = 1.79 mg/L TOX.CAT-III
81-4	Primary Eye Irritation	41125302	Severe irritant TOX.CAT-I
81-5	Primary Skin Irritation	42232701	Non irritant TOX.CAT-IV
81-6	Dermal Sensitization	00161659	Non sensitizer
81-8	Acute Neurotoxicity - Rat	43115201	Systemic Toxicity NOEL: 227 mg/kg LOEL: > 227 mg/kg Neurobehavioral NOEL: 67 mg/kg LOEL: 227 mg/kg
82-1 (a)	90 - Day	41991502	NOEL = 15 mg/kg/day;

	Feeding - Mouse		LOEL = 100 mg/kg/day
82-1 (a)	90 Day Feeding - Rat	41991501	NOEL = 15 mg/kg/day LOEL = 100 mg/kg/day
82-1 (b)	90 Day Feeding (capsule) - Dog	41737301	NOEL = 1 mg/kg/day LOEL = 3 mg/kg/day
82-1 (b)	90 Day Feeding (dietary) - Dog	42780001	NOEL = 1 mg/kg/day LOEL = 3.75 mg/kg/day
82-2	21-Day Dermal - Rat	41735304	Dermal & Systemic NOEL = 1000 mg/kg/day LOEL = > 1000 mg/kg/day
82-7	1-year Neurotoxicity - Rat	43293901	NOEL = 75 mg/kg/day LOEL = 150 mg/kg/day
83-1	Chronic Toxicity - Dog	43049001	NOEL = 1 mg/kg/day LOEL = 5 mg/kg/day
83-2 (b)	Carcinogenicity - Mouse	43879801-(Male) 43597201-(Female)	Non Carcinogenic; Systemic NOEL = 5 mg/kg/day (M,F) Systemic LOEL = 62.5 mg/kg/day (M) 150 mg/kg/day (F)
83-5	Chronic Toxicity/Carcinogenicity - Rat	43612001	Non Carcinogenic; Systemic NOEL = 75 mg/kg/day (M); 5mg/kg/day (F) Systemic LOEL = 150 mg/kg/day (M) 75 mg/kg/day (F)
83-3 (a)	Developmental Toxicity - Rat	000251031	Maternal & Developmental: NOEL = 25 mg/kg/day LOEL = 75 mg/kg/day
83-3 (b)	Developmental Toxicity - Rabbit	41747601	Maternal NOEL = 30 mg/kg/day LOEL = 90 mg/kg/day Developmental NOEL = 90 mg/kg/day LOEL = > 90 mg/kg/day
83-4	2-Generation Reproduction	25944206	Parental/Reproductive/systemic NOEL = 5 mg/kg/day LOEL = 20 mg/kg/day
84-2	Mutagenicity	41409801 41870101 41400807	Non-mutagenic *in vivo/in vitro*
85-1	Metabolism	41409807	Adequate Study