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

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MAY 23 1996

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.**

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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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. 43597201 & 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-DICHLOROPHENOXYACETIC ACID: CHRONIC TOXICITY/ONCOGENICITY STUDY IN FISCHER 344 RATS-FINAL"* 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 (80%) 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 females, 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 ≈ 75 mg/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 14th rudimentary ribs (4 fetuses of 3 litters vs. 0 in the controls); malaligned 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

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§ GUIDELINE #	STUDY	MRID No.	RESULTS
81-1	Acute Oral - Rat	00101605	LD_{50}: 699 mg/kg *TOX. CAT - III*
81-2	Acute Dermal - Rat	00101596	LD_{50}= > 2000 mg/kg *TOX. CAT - III*
81-3	Acute Inhalation - Rat.	00161660	LC_{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 Feeding - Mouse	41991502	NOEL= 15 mg/kg/day; 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 = 1 mg/kg/day LOEL = 5 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 (♂) 43597201 (♀)	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); 5 mg/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 41409807	Non-mutagenic *in vivo/in vitro*
85-1	Metabolism	41409807	Adequate study

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2,4-D ACID §83-2(b) Carcinogenicity - Mouse

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DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity Study - Mice. **GUIDELINE:** §83-2(b)

DP BARCODE(S): D215600 & D222295 **SUBMISSION(S):** S487309 & S499298

PC CODE: 030001 **TOX.CHEM.No: 315**

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid (2,4-D)

CITATION(S): 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.** Unpublished.

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.** Unpublished.

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. Neither 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 (80%) 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 females, 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.

This carcinogenicity study in mice is classified as **Acceptable** and satisfies the Subdivision F guideline requirement for a carcinogenicity study in mice (§ 83-2b).

I. INTRODUCTION

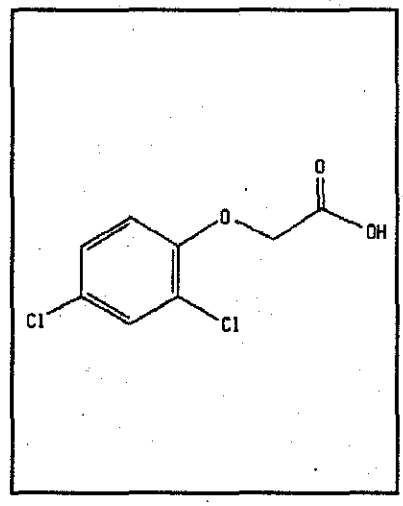
In 1988, the Agency required that rodent carcinogenicity testing with 2,4-dichlorophenoxyacetic acid (2,4-D) be repeated because a Maximum Tolerated Dose (MTD) had not been achieved in the Industry-sponsored studies. In the Data Call-In notice of 1989, the Agency formally requested that the carcinogenicity testing in rats and mice be repeated at higher doses. This Data Evaluation Report summarizes the results of a carcinogenicity study in mice.

II. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: 2,4-Dichlorophenoxyacetic acid
   Description: Solid
   Lot/Batch No.: 909
   Purity: Technical, 96.4%
   Stability of the compound: Concentrations of the active ingredient varied less than 1% between stability analyses conducted every 6 months over 2 years.
   CAS No.: 94-75-7

   Structural Formula:



2. **Vehicle Control:** A basal diet of Purina Certified Chow #5002.

3. **Test Animals:**

Species:	Mouse
Strain:	B6C3F1
Sex:	Males & females
Age at Initiation:	7-8 weeks
Weight at Initiation:	22 g ♂ & 15 g ♀
Identification:	S.C implanted transponder correlated to a unique i.d.number.
Acclimation:	14 days
Housing:	1/cage in suspended stainless steel cages.
Food:	Purina Certified Rodent Chow #5002
Water:	Tap water ad libitum
Environmental Conditions:	Temperature, 18.8-24.7°C; Humidity, 30-94%; Light cycle, 12 hr.on/off; Air flow, 10-12 air changes/hour.

B. **Study Design**

1. **In Life Dates:** Start: Males - 5/19/93; Females - 2/3/92
End: - 5/23/95 - 2/8/94

2. **Animal Assignment**

Test Group	Dose Level (mg/kg/day)		Main Study[a]		Interim Sacrifice (12 Months)	
	Males[b]	Females	Males	Females	Males	Females
Control	0	0	50	50	10	10
Low Dose	5.0	5.0	50	50	10	10
Mid Dose	62.5	150.0	50	50	10	10
High Dose	125.0	300.0	50	50	10	10

a = Hematology performed at 12, 18 and 24 months.

b = Initially, both sexes received the same doses of 2,4-D (i.e., 5, 150 or 300 mg/kg/day); however, due to severe body weight decrements in males at 150 and 300 mg/kg/day, a new study (MRID # 43879801) was conducted in males at lower doses (See 3. Dose Selection Rationale, below).

3. **Dose Selection Rationale:** In a subchronic toxicity study (MRID No. 41991502), B6C3F1 (10/sex/dose) were fed diets containing 2,4-D acid at 1, 15, 100 or 300 mg/kg/day for 90 days. At 100 mg/kg/day, treatment-related effects were decreases in glucose and thyroxine levels and increases in absolute and relative kidney weights. At 300 mg/kg/day, treatment-related effects were: transient decreases in food consumption; decreases in glucose and thyroxine levels; decreases in kidney-to-brain weight ratios; and histopathological lesions in the liver and kidneys. The NOEL was 15 mg/kg/day and the LOEL was 100 mg/kg/day.

   Based on the results of the 90-day study, a carcinogenicity study was initiated at 0, 5, 150 or 300 mg/kg/day. During the course of the first year of the study, female mice at all dose levels had normal weight gain. However male mice at 150 and 300 mg/kg/day had significantly lower mean body weights and body weight gains when compared to controls; decrements in body weight gain were -7 to -11% at 150 mg/kg/day and -20 to -28% at 300 mg/kg/day ($\leq$ 0.05). Although there were no mortalities, body weight gain data indicated that for males, the 300 mg/kg/day was certainly too high and 150 mg/kg/day conceivably may lead to excessive toxicity as the study progressed. Therefore, in concurrence with the Agency, all male mice were terminated and a new study with males (MRID # 43879801) was initiated at lower doses (0, 62.5 or 125 mg/kg/day).

4. **Diet Preparation and Analysis:** The test material was air milled prior to mixing the diets. Test diets were prepared by serially diluting a premix (test material-feed concentrate). Test diets were prepared weekly during the first 13 weeks and at least once every two weeks for the remainder of the study. Concentration analyses of each dose level was determined for the first four weeks, and at least quarterly, thereafter. Homogeneity was initiated prior to the start of the study and validated analytically concurrently with the conduct of the study. Stability analysis was initiated with the start of the study.

   Results: Concentration analysis indicated that the actual concentrations of 2,4-D in the low- mid- and high-dose test diets were within 83-127%, 88-106% and 92-114%, respectively, of the target for males, and 88-135%, 86-119% and 85-118%, respectively, in females. Homogeneity analyses showed that the diet mixes were homogeneously distributed with relative standard deviations of 2.65 to 14.52% for males and 2.9 to 38.17% for females. Stability analysis indicated the test material to be stable in the test diet for at least 49 days (91% of Day 0 value).

5. **Treatment:** Males were fed diets containing 2,4-D acid at 5, 62.5 or 125 mg/kg/day and females at 5, 75 or 150 mg/kg/day for a period of up to 24 months (732 to 734 days). Control animals received standard laboratory diet on the same schedule. The most recent group mean body weight and feed consumption data for each sex were used to adjust the concentration of the test material in the diet to maintain the targeted dose levels.

6. **Experimental Procedures:** Mortality/moribundity checks and cage-side observations for clinical signs of toxicity were performed twice daily. A detailed physical examination for signs of local or systemic toxicity, pharmacologic effects and palpation for tissue masses were conduced prior to initiation and weekly thereafter. Examination of central nervous system and behavior pattern of each animal included looking for signs of tremors, convulsions, salivation and diarrhea. Individual body weights and amount of feed consumed were recorded prior to initiation, weekly for the first 13 weeks, and at approximately monthly intervals, thereafter. Ophthalmologic examinations were conducted on all animals once prior to initiation and at scheduled necropsies. Blood was collected from 10 mice/sex/dose after approximately 12 and from 10 and 20 mice/sex/dose at 18 and 24 months, respectively for the following hematologic determinations: hematocrit; hemoglobin; erythrocyte count; total leukocyte count and platelet count. Blood smear examinations consisted of differential leukocyte counts on 100 leukocytes, as well as an assessment of erythrocyte, leukocyte and platelet morphology.

7. **Termination:** For the interim sacrifice 10 mice/sex/dose were sacrificed after 370 and 371 days of treatment for females and males, respectively. The surviving male and female mice were sacrificed between test days 732 and 734 (terminal sacrifice). Complete gross postmortem examination was performed on these animals as well as on animals dying spontaneously, accidentally, and sacrificed in a moribund condition. Postmortem procedures included: examination of the external surface; all orifices; the cranial cavity; carcass; the external and sectioned surfaces of the brain and spinal cord; nasal cavity and paranasal sinuses; the thoracic; abdominal and pelvic cavities and their viscera and the cervical tissue. Weights of brain, heart, kidneys and liver were recorded and the organ to final body weight and organ weight to brain weight ratios calculated for all animals.

8. **Histopathology:** The checked (x) tissues were trimmed and processed for histopathological examination of all mice in the control and high-dose groups and all mice from the intermediate and low dose groups terminated early. Microscopic examination of tissues from mice at the low-and mid-dose groups that survived until their scheduled termination of the study was limited to the liver, kidneys, lungs, gross lesions, and tissues routinely prepared with these protocol-designated tissues (i.e., gall bladder with liver, urinary bladder with the reproductive duct). In addition, due to observations made at necropsy (i.e., ovarian masses and cystic endometrial hyperplasia), the entire reproductive tract (cervix, vagina and oviducts in addition to ovaries and uterus) of these animals was examined histopathologically. When histopathology of protocol-defined organs at the low and mid-dose level mice from the terminal necropsy suggested lymphosarcoma, the spleen, thymus, mesenteric and mediastinal lymph nodes were also prepared and examined to establish a diagnosis.

Digestive System	Respiratory System
x Salivary glands[a]	x Trachea[a]
x Esophagus[a]	x Lung[a]
x Stomach	Pharynx[e]
x Duodenum[a]	x Larynx[e]
x Jejunum[a]	Nose[e]
x Cecum[a]	x Nasal Tissues
x Colon[a]	
x Ileum[a]	Cardiovascular/Hematopoietic System
x Rectum[a]	
x Liver[ac]	x Aorta (thoracic)[a]
x Pancreas[a]	x Heart[a]
	x Bone marrow[a]
Nervous System	x Lymph nodes[a]
	x Spleen[a]
x Brain [cerebrum, brain stem, cerebellum][ac]	x Thymus[a]
x Pituitary[a]	Urogenital System
x Peripheral nerve[ab]	
x Spinal cord (3 levels)[ab]	x Kidneys[ac]
x Eyes[ab]	x Urinary bladder[a]
	x Testes[ac]
Glandular System	x Epididymides
	x Prostate
x Adrenals[a]	x Seminal vesicles
x Lacrimal glands[b]	x Uterus[a]
x Parathyroids[ad]	x Ovaries[ac]
x Thyroids[ad]	x Vagina
	x Cervix
	x Oviducts
	Other
	x Skin
	x Mammary glands
	x All gross lesions and masses
	x Skeletal muscle[a]
	x Mesenteric tissues
	x Mediastinal tissues
	x Oral tissue
	x Coagulating glands
	x Auditory sebaceous glands

a. Required for subchronic and chronic studies.
b. In subchronic studies examined only if indicated by toxicity or target organ involvement.
c. Organ weights required in subchronic and chronic studies.
d. Organ weights required for nonrodent studies.
e. Required for chronic inhalation study.

9. **Statistical Analyses:** Differences in mortality patterns were tested by the Gehran-Wilcoxon procedure. Body weights, appropriate hematology parameter, and organ weight data were evaluated by Bartlett's test for equality of variance. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric ANOVA, followed respectively by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons. Statistical analysis of gross and histopathological lesions consisted of pair-wise comparisons of control and treated groups using the pair-wise chi-square test with Yate's continuity correction.

10. **Regulatory Compliances:** Signed and dated No Data Confidentiality Claim, Flagging, Good Laboratory Practices, and Quality Assurance statements were provided.

III. RESULTS

A. **Survival:** There was no treatment-related mortality in either sex throughout the study. As shown in **Table 1**, survival at 18 and 24 months exceeded the guideline requirement of not less than 50% and 25%, respectively, at these intervals.

Table 1. Survival Rate in B6C3F1 Mice Fed 2,4-D Acid for 2-years[a].

Percent Survival								
Interval	Males (mg/kg/day)				Females (mg/kg/day)			
	0	5	62.5	125	0	5	150	300
12-Months	100	98	96	98	96	96	96	96
18-Months	94	92	92	94	90	92	92	94
24-Months	76	86	84	86	78	84	84	70

a = Data obtained from Study Report Pages. 48 (♂), 47 & 48 (♀) .

B. **Clinical Observations:** No treatment-related clinical signs of toxicity were seen in either sex at any dose level.

C. **Ophthalmology Examination:** No treatment-related ophthalmological findings were seen in either sex at any dose level.

D. **Body Weight/Body Weight Gain:** Mean body weight data are presented in **Table 2.** Mean body weights of male mice at all dose levels were comparable to those of the controls throughout the study. Mean body weights of female mice at 150 mg/kg/day were generally lower (approximately 2-6%) than controls over a majority of the dosing period with the decreases reaching statistical significance ($p < 0.05$) during Study Days 18 through 279. However, no significant differences were seen in body weights of treated females when compared to controls during Study Days 307 to 726.

Table 2. Mean Body Weights (g) in B6C3F1 Mice Fed 2,4-D Acid For 2-Years[a].

	Males (mg/kg/day)				Females (mg/kg/day)			
Interval	**0**	**5**	**62.5**	**125**	**0**	**5**	**5**	**300**
-3 Day	22.2	22.3	21.9	21.8	18.7	18.8	18.6	18.6
≈ 3-Months	29.2	28.9	29.3	29.5	25.8	**25.2***	25.3	**24.7***
≈ 6-Months	33.7	33.4	34.0	33.4	28.1	27.9	27.4	**27.1***
12-Months	36.3	35.7	36.1	36.1	31.5	32.1	30.8	30.2
18-Months	37.1	36.6	36.8	36.8	32.5	33.0	33.0	31.8
24-Months	34.5	34.6	34.5	33.5	30.6	32.1	31.9	31.3

a = Data obtained from Study Report Pages. 51-54 (♂) & 52-54 (♀)
* Significantly different from controls at $p < 0.05$.

Mean body weight gain data are presented in **Table 3.** Mean body weight gain of male mice at all dose levels were comparable to those of the controls throughout the study. Female mice at 300 mg/kg/day showed consistent reductions in body weight gain when compared to controls; the decreases amounted to approximately -20 to 23% during Study Days 0-49, appeared to stabilize around Study Day 50, and ranged between -5 to 14% until 20 months into the study. At termination, body weight gain of mice at all treated groups were comparable to that of the controls.

Table 3. Mean Body Weight Gain (G) in B6C3F1 Mice Fed 2,4-D Acid For 2-Years[a].

	Males (mg/kg/day)				Females (mg/kg/day)			
Interval	**0**	**5**	**62.5**	**125**	**0**	**5**	**150**	**300**
≈ 3-Months	7.0	6.7	7.3	7.6*	8.4	**7.8*** **(-7)[b]**	**7.9*** **(-6)**	**7.2*** **(-14)**
≈ 6-Months	11.5	11.2	12.1	11.5	10.6	10.5	10.0	**9.6*** **(-9)**
12-Months	14.1	13.5	14.2	14.3	14.1	14.7	13.4	**12.8*** **(-9)**
18-Months	14.9	14.5	14.9	14.9	15.1	15.5	15.5	14.4 (-5%)
24-Months	12.2	12.4	12.7	11.6	13.2	14.7	14.4	13.9

a = Data obtained from Study Report Pages. 57-60 (♂) & 56-60 (♀)
b = Values in parenthesis (% decrease vs. controls) were calculated by Reviewer.
* Significantly different from controls at $p < 0.05$.

E. **Food and Compound Consumption:** No consistent changes in food consumption of treated mice relative to controls was observed either in males or in females in spite of the decreased body weights in this sex at the high-dose. The average dosages received by male mice were 5.0, 61.9 or 128.6 mg/kg/day for the targeted doses of 5, 62.5 or 125 mg/kg/day, respectively and by the female mice were 5.01, 149.83 or 310.01 mg/kg/day for the targeted doses of 5, 150 or 300 mg/kg/day, respectively.

F. **Hematology:** No treatment-or statistically significant differences in any of the hematological parameters (RBC, HGB, HCT, WBC or platelet number) were observed in either sex at any interval (12, 18 or 24 month).

G. **Organ Weights:** Treatment related changes observed in kidney weights are presented in **Table 4**. In males at 62.5 and 125 mg/kg/day, dose-related increases in absolute and relative kidney weights were seen only after 24 months. In females at 150 and 300 mg/kg/day dose-related increases in absolute and relative kidney weights were seen after 12 and 24 months. The increases in kidney weights corroborated with histopathological lesions in the kidneys in both sexes and were thus determined to be treatment related. In contrast, the decreases (-5 to -10%) in absolute/relative heart weights and the increase (6%) in relative testes weights of males at 125 mg/kg/day and the increase (6%) in relative liver weights in females at 150 mg/kg/day were not attributed to treatment due to lack of dose- and/or time-response and corroborative histopathological lesions in these organs.

Table 4. Kidney Weights at the Interim & Terminal Sacrifices[a].

Males								
Dose (mg/kg/day)	**0**		**5**		**62.5**		**125**	
Sacrifice Interval (Months)	**12**	**24**	**12**	**24**	**12**	**24**	**12**	**24**
Absolute (g) Weights	0.710 -	0.744 -	0.729 -	0.721 -	0.687 -	0.778 (+5%)[b]	0.710	**0.797*** (+7%)
Relative (g/100) weights	1.915 -	2.123 -	1.970 -	2.085 -	1.921 -	**2.239*** (+6)	2.006	**2.343*** (+10)
Females								
Dose (mg/kg/day)	**0**		**5**		**150**		**300**	
Sacrifice Interval (Months)	**12**	**24**	**12**	**24**	**12**	**24**	**12**	**24**
Absolute (g) weights	0.428 -	0.486 -	0.421 -	0.478 -	**0.486*** (+14%)	**0.554*** (+14%)	**0.500*** (+17%)	**0.595*** (+22%)
Relative (g/100) weights	1.324 -	1.560 -	1.351 -	1.487 -	**1.616*** (+22%)	**1.740*** (+12%)	**1.719*** (+30%)	**1.872*** (+20%)

a = Data obtained from Study Report Pages. 82 and 83 (♂) & 81 and 83 (♀)
b = Values in parenthesis (% increase vs. controls) were calculated by the reviewer.

H. **Gross Pathology:** No treatment-related gross necropsy findings were seen in either sex at any dose level either at the interim or the terminal sacrifice. Necropsy findings in the control and treated groups occurred with comparable frequency and were similar to those commonly seen in this age/strain of mice .

I. **Histopathology - Interim Sacrifice (12-months)**

1. **Non-neoplastic Lesions:** Treatment-related non-neoplastic lesions observed at the 12-month interim sacrifice are presented in **Table 5.** Lesions were limited to the kidneys of both sexes of mice at the mid-and high doses and to the liver of females at the high dose.

2. **Neoplastic Lesions:** Neoplasms observed in males were: 2 undifferentiated subcutaneous sarcomas and 1 testicular gonadal stromal tumor in the control; a bronchioalveolar adenoma at 62.5 mg/kg/day; and a thyroid follicular cell adenoma and a lacrimal gland adenoma at 125 mg/kg/day. Neoplasms observed in females were: a lung adenoma and an abdominal hemangioma in the control and a lung adenoma and a pituitary adenoma at 150 mg/kg/day. These tumors were not attributed to treatment since the incidences were similar in number and frequency to those seen in this strain/age of mice.

Table 5. Treatment-Related Non-Neoplastic Lesions in B6C3F1 Mice at the INTERIM Sacrifice[a].

No. Examined: 10/Dose		Males (mg/kg/day)			
Tissue/Lesion		0	5	62.5	125
Kidneys:	- degeneration/regeneration, descending proximal tubule-very slight/slight	0	0	2 (20%)	10 (100%)
	- vacuolation - decreased, proximal tubule(s)	0	0	8 (80%)	10 (100%)
No. Examined: 10/Dose		**Females (mg/kg/day)**			
Tissue/Lesion		**0**	**5**	**150**	**300**
Kidneys: hypercellular, proximal tubule-descending part, slight		0	0	8 (80%)	10 (100%)
Liver: hyperchromic nuclei, midzonal, slight		1	0	2	5

a = Data obtained from Study Report Pages. 104 & 105 (♂) and 111 * 112 (♀)

In males, dose-related kidney lesions were degeneration with regeneration of the descending portion of the proximal tubule seen in 2/10 (20%) and 10/10 (100%) at 62.5 and 125 mg/kg/day, respectively and vacuolation of the proximal tubules seen in 8/10 (80%) and 10/10 (100%) at 62.5 and 125 mg/kg/day respectively, when compared to 0% for both lesions in control males.

In females, the dose-related kidney lesion was hypercellularity of the descending portion of the proximal tubule seen in 8/10 (80%) and 10/10 (100%) at 150 and 300 mg/kg/day, respectively. The lesion was of minimal degree and necrosis or indications of repair (i.e., mitotic figures) were not present. This renal lesion correlated with the increases in absolute/relative kidney weights observed at these dose levels. The other kidney lesion was the slightly increased incidence of minimal tubular degeneration with regeneration of the cortical tubules seen only at ≥150 mg/kg/day 4/10 (40%) compared to 1/10 (10%) in the controls.

In the liver of female mice, there was an increase in the incidence of hyperchromic nuclei at 300 mg/kg/day (5/10 (50%) compared to controls (1/10, 10%). This lesion/change may be considered to be equivocal, since there are normally some nuclear size variability and tinctorial changes (increased basophilic staining) of hepatocytes from the midzonal to centrilobular areas, particularly in sections from the right and caudate lobes, in mice of this strain and age.

The other non-neoplastic lesions observed at the interim sacrifice were similar in number and frequency of those seen in this strain//sex/age of mice.

Histopathology - Terminal Sacrifice (24-months)

1. **Non-neoplastic Lesions:** Treatment-related non-neoplastic lesions observed at the 24-month terminal sacrifice are presented in **Table 6.** Kidney lesions, similar to those seen in both sexes of mice at the mid- and high-dose groups at the interim sacrifice were also seen at the terminal sacrifice. These lesions were of minimal degree (very slight or slight) and considered unlikely to have an effect on renal function.

Table 6. Treatment-Related Non-Neoplastic Lesions in B6C3F1 Mice at the TERMINAL Sacrifice[a].

No. Examined: 50/Dose		Males (mg/kg/day)			
Tissue/Lesion		0	5	62.5	125
Kidneys	- degeneration/regeneration, descending proximal tubule- - any severity	0	0	**25*** (50%)	**48*** (96%)
	- vacuolation - decreased, proximal tubule(s)	0	0	**39*** (78%)	**48*** (96%)
	-mineralization, tubule(s), multifocal - very slight-	16 (32%)	19 (38%)	**29*** (58%)	**36*T** (72%)
	- cyst, cortex, focal or multifocal	9	9	**22*** (44%)	**20*T** (40%)
	-degeneration/regeneration, cortex, multifocal	39	43	43	45
Liver	-aggregates of RE cells frequently adjacent of degenerative or necrotic hepatocytes, multifocal, any severity	12 (24%)	16 (32%)	17 (34%)	**25*T** (50%)
No. Examined: 50/Dose		**Females (mg/kg/day)**			
Tissue/Lesion		**0**	**5**	**150**	**300**
Kidneys	-hypercellular, proximal tubule-descending part - slight	0	0	**32*** (64%)	**25*T** (50%)
	-degeneration/regeneration, tubule, multifocal - very slight	7	3	**35***	**26*T**
	- slight	0	0	3	**8***
	-mineralization, tubule(s), multifocal - very slight	0	0	1	**7*T**
Spleen	-extramedullary hematopoiesis, - very slight	2	0	3	**8***
	- slight	3	3	3	7
	- moderate	2	4	3	5
	- severe	1	0	0	0

a = Data obtained from Study Report Pages. 117-121 (♂) and 123, 124, 138 (♀)
* = Significantly different from controls; T = Trend

In males, the dose-related renal lesions, degeneration with regeneration of the descending portion of the proximal tubules and decreased vacuolation of the proximal tubules, observed at 62.5 and 125 mg/kg/day at the interim sacrifice were also dose-related at the terminal sacrifice. Also dose-related at 62.5 and 125 mg/kg/day was the increase in the multifocal, mineralization of scattered tubules (also known as microlithiasis). Multifocal cortical cysts were increased (not dose-related) at 62.5 and 125 mg/kg/day. Tubular degeneration/regeneration of the cortex was seen in all treated groups including the controls.

The degeneration/ regeneration changes were restricted to the outer stripe of the outer zone of the medulla but usually involved all the tubules in which this segment was present in the plane of section. When graded as slight, the change was manifested by increased number of epithelial cells in the straight descending portion of the proximal tubule with minimal nuclear pleomorphism and rare enlarge nuclei. The tubular lumens were more evident apparently due to loss of the brush border of the epithelium and there was basophilia of the cytoplasm of affected tubules. Necrosis or indications of repair (i.e., mitotic figures) were not present. When graded as very slight, the change was primarily hypercellularity of the descending portion of the proximal tubules with equivocal evidence of the other characteristics above.

The decreased vacuolation of the renal proximal tubules seen after 12 and 24 months may represent a lesion of uncertain significance and etiology. Male mice normally have large, clearly demarcated vacuoles in some of the proximal tubular epithelial cells. The vacuoles normally occur in the proximal convoluted portion of the tubules and are noted histologically in clusters in the cortex. Therefore, this lesions may not have been related to tubular degeneration.

The mineralization of scattered tubule and the multifocal cortical cysts, although attributed to treatment, can be considered to be of minimal functional significance.

The tubular degeneration with regeneration of the renal tubules in the cortex seen in both the control and treated groups are an exacerbation of a geriatric change commonly seen in untreated aged mice and is not attributed to treatment.

In the liver, there was an increase in the aggregates of RE cells frequently adjacent to degenerative or necrotic hepatocytes at all dose levels with the increase reaching statistical significance only at 125 mg/kg/day.

In females, the dose-related renal lesion, hypercellularity of the descending portion of the proximal tubules, observed at 150 and 300 mg/kg/day at the interim sacrifice was also dose-related at these doses at the terminal sacrifice. The other treatment-related but not dose-related renal changes were the degeneration with regeneration of the cortical tubules seen at 150 and 300 mg/kg/day and mineralization of scattered tubules seen only at 300 mg/kg/day. These renal changes may correlate with the increases in absolute and relative kidney weights observed at the terminal sacrifice in mice at these dose levels.

Hypercellularity of the descending portion of the proximal tubule was characterized by increased numbers of epithelial cells in the straight descending portion of the proximal tubules. These cells appeared to be somewhat shorter than normal and may have contributed to the hypercellular appearance. Necrosis or indications of repair (i.e., mitotic figures) were not present. Mineralization of scattered tubules (also known as microlithiasis) was seen only at 300 mg/kg/day.

In the spleen, the increase in extramedullary hematopoiesis showed statistical significance only for the very slight grade with no statistical significance for the slight, moderate or severe grades. Since no statistically-identified anemia or other blood cell differences were seen at the 12, 18 or 24 months, the splenic hematopoiesis was considered to be equivocal and may be attributed to a secondary effect to treatment.

2. **Neoplastic Lesions:** Histopathology revealed a variety of benign and malignant tumors at different sites in both treated and control animals, but none showed statistical significance in individual tumor types in any treated group of either sex. Neoplastic lesions observed were similar in number and type commonly seen in this strain/age mice. A summary of the neoplastic lesions presented in Tables 34 for males (Study Report Pages 142 to 145) and 35 for females (Study Report Pages 151 to 156) are appended to this DER (Appendix 1).

IV. DISCUSSION

The purpose of this discussion that follows is to compare the findings of the 1987 (Hazleton) and the 1995 (Dow) carcinogenicity studies conducted with 2,4-D acid in B6C3F1 mice. In the 1987 study, 50/sex/dose were fed diets containing 2,4-D acid technical (97.5%) at 0, 1, 15 or 45 mg/kg/day for 104 weeks, and in the 1995 study, 2,4-D acid (96.45%) was administered in the diet at 0, 5, 62.5 or 125 mg/kg/day to males and at 0, 5, 150 or 300 mg/kg/day to females for 104 weeks.

A. **Survival:** A shown below in **Table 7**, no treatment-related effect was seen on survival in either study.

Table 7. Survival (%) in B6C3F1 Mice In The Two Studies[a].

Sex	Months	Dose (mg/kg/day)									
		0 (H)	0 (D)	1 (H)	5 (D)	15 (H)	45 (H)	62.5 (D)	125 (D)	150 (D)	300 (D)
Males	12	98	100	100	98	96	90	96	98	NOT APPLICABLE	
	24	80	94	88	86	80	76	84	86		
Females	12	97	96	93	96	92	97	NOT APPLICABLE		96	96
	24	76	78	78	84	72	70			84	70

a = Data obtained from 1987 Study Report Pages. 94-102 & Table 1 of this DER
H = 1987 Hazleton Study. D = 1995 Dow Study

B. **Body Weights:** Cumulative body weight gain data for both studies are presented in **Table 8.** In the 1987 decreases (-9% to 16%) in mean cumulative (0-104 weeks) body weight gain was seen in males at all dose levels; a similar effect was not seen in females. In the 1995 study, no treatment-related effects on body weight gain were seen in males at much higher doses. Decrements in body weight gain (-5% to -15%) were seen in females at 300 mg/kg/day until 20 months into the study. Terminal body weights at this dose were comparable to those of the controls.

Table 8. Cumulative Body Weight Gain (g) in B6CF1 Mice In The Two Studies[a].

Sex	Months	Dose (mg/kg/day)									
		0 (H)	0 (D)	1 (H)	5 (D)	15 (H)	45 (H)	62.5 (D)	125 (D)	150 (D)	300 (D)
Males	12	14.3	14.1	13.6	14.5	14.2	13.8	14.2	14.3	NOT DOSED	
	24	14.1	12.2	12.8* (-9%)	12.4	12.6* (-11%)	11.8* (-16%)	12.7	11.6		
Females	12	13.3	14.1	12.5	14.7	12.4	12.8	NOT DOSED		15.5	14.4
	24	12.9	13.2	11.9	14.7	12.4	12.5			14.4	13.9

a = Data obtained from the 1987 Study Report Pages. 94-104 & Table 3.
* = $p \geq 0.05$. H = 1987 Hazleton Study D = 1995 Dow Study

C. **Food Consumption:** No treatment-related changes associated with food consumption were seen in either study.

D. **Ophthalmology:** No ocular toxicity was seen in either study.

E. **Hematology:** No treatment-related effects were observed in any of the hematological parameters in either study.

F. **Organ Weights:** In both studies, treatment-related changes were limited to kidney weights in both sexes. In the 1987 study, relative kidney weights were increased in females at 15 mg/kg/day, absolute kidney weights were increased in males at 45 mg/kg/day, and the relative kidney weights were increased in males and females at 45 mg/kg/day. In the 1995 study, dose-related increases in absolute and relative kidney weights were seen in males at 62.5 and 125 mg/kg/day only after 24 months. Whereas dose-related increases in absolute and relative kidney weights were seen in females at 150 and 300 mg/kg/day after 12 and 24 month. In both studies, the increases in kidney weights corroborated with histopathological lesions in the kidneys in both sexes and were thus determined to be treatment related.

G. **Gross Pathology:** No treatment-related gross pathological lesions were seen in either study.

H. **Histopathology**

1. **Non-neoplastic Lesions:** In both studies, kidneys were the target organ with renal lesions seen at the interim and terminal sacrifices.

   In the 1987 study, renal lesions, characterized as a cytoplasmic homogeneity seen in the renal tubule epithelium, were seen only in males at the mid- and high-dose groups; 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.

   In the 1995 study, renal lesions were seen in both sexes at 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, renal lesions seen at 150 and 300 mg/kg/day comprised of hypercellularity of the descending portion of the proximal tubules and degeneration with regeneration of cortical tubules.

2. **Neoplastic Lesions:** No treatment-related neoplastic lesions were seen in either study.

VI. 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. Consequently, 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.

Under the conditions of this study, for chronic toxicity, the NOEL is 5 mg/kg/day for both sexes and 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/relative kidney weights and histopathological lesions in the kidneys.

In this study, at the dose levels tested, **2,4-D acid was not carcinogenic in male or female B6C3F1 mice.**

APPENDIX - 1

NEOPLASTIC LESIONS OBSERVED IN B6C3F1 MICE FED 2,4-D ACID FOR 24 MONTHS

THE DOW CHEMICAL COMPANY

STUDY ID; K-002372-063MF

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011934

2,4-D ACID §83-5 Combined Chronic Toxicity/Carcinogenicity - Rat

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DATA EVALUATION RECORD

STUDY TYPE: Combined Chronic Toxicity/Carcinogenicity Study. **GUIDELINE:** §83-5

DP BARCODE: D215596 **SUBMISSION:** S487304

PC CODE: 030001 **TOX.CHEM.No: 315**

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid [2,4-D]

CITATION: 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., Midland, Michigan. Study ID: K-002372-064. 3/28/95. **MRID No. 43612001.** Unpublished.

EXECUTIVE SUMMARY: In a combined chronic toxicity/carcinogenicity study (MRID No. 43612001), 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 as described above. 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, decrease 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, decrease in T_4 concentration, increases in absolute/relative thyroid weights, and histopathological lesions in the liver, kidneys and lungs. At the doses tested, 2,4-D acid was not carcinogenic in male or female Fischer 344 rats.

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

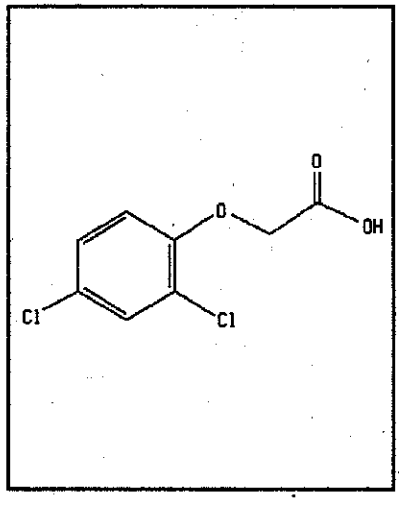
I. INTRODUCTION

In 1988, the Agency required that rodent carcinogenicity testing with 2,4-dichlorophenoxy acetic acid (2,4-D) be repeated because a Maximum Tolerated Dose (MTD) had not been achieved in the Industry-sponsored studies. In the Data Call-In notice of 1989, the Agency formally requested that the carcinogenicity testing in rats and mice be repeated at higher doses. This Data Evaluation Report summarizes the results of a combined chronic toxicity/carcinogenicity study in rats.

II. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: 2,4-Dichlorophenoxyacetic acid
   Description: Solid
   Lot/Batch No.: 909
   Purity: Technical, 96.45%
   Stability of the compound: Concentrations of the active ingredient varied less than 1% between stability analyses conducted every 6 months over 2 years.
   CAS No.: 94-75-7
   Structure:



2. Vehicle Control: A basal diet of Purina Certified Chow #5002.

3. Test Animals: Species: Rat
   Strain: Fischer 344
   Sex: Males & females
   Age at Initiation: 7-8 weeks
   Weight at Initiation: 172-222 g ♂ and 114-140 g ♀
   Identification: S.C implanted transponder correlated to a unique i.d.number.
   Acclimation: 14 days
   Housing: Males, 1/cage & females 2/cage; suspended stainless steel cages.
   Food: Purina Certified Rodent Chow #5002
   Water: Tap water ad libitum
   Environmental Conditions: Temperature, 20-25°C; Humidity, 40-69%; Light cycle, 12 hr.on/off; Air flow, 10-12 air changes/hour.

B. **Study Design**

1. In Life Dates: Start: 3/392 End: 3/28/95

2. Animal Assignment

Test Group	Dose Level (mg/kg/day)	Main Study[a]		Interim Sacrifice (12 Months)[b]	
		Males	Females	Males	Females
Control	0	50	50	15	15
Low Dose	5	50	50	15	15
Mid Dose	75	50	50	15	15
High Dose	150	50	50	15	15

a = Hematology, clinical chemistry and urinalyses performed at 6, 12, 18 and 24 months.
b = Of these 15 rats, 5/sex/dose were used for assessment of chronic neurotoxicity. Results of the neurotoxicity findings are discussed in a separate DER (HED Doc. No. 011614).

3. **Dose Selection Rationale:** In a subchronic toxicity study (MRID No. 41991501), Fischer 344 rats (10/sex/dose) were fed diets containing 2,4-D acid at 1, 15, 100 or 300 mg/kg/day for 90 days. At 100 and 300 mg/kg/day, treatment-related effects were decreases in body weight, body weight gain and food consumption, alterations in clinical pathology, changes in organ weights, and histopathological lesions in the eyes, liver, kidneys, and thyroid. The LOEL was 100 mg/kg/day and the NOEL was 15 mg/kg/day.

4. **Diet Preparation and Analysis:** The test material was air milled prior to mixing the diets. Test diets were prepared by serially diluting a premix (test material-feed concentrate). Test diets were prepared weekly during the first 13 weeks and at least once every two weeks for the remainder of the study. Concentration analyses of each dose level was determined for the first four weeks, and at least quarterly, thereafter. Homogeneity was initiated prior to the start of the study and validated analytically concurrently with the conduct of the study. Stability analysis was initiated with the start of the study.

   Results: Concentration analysis indicated that the actual concentrations of 2,4-D in the low- mid- and high-dose test diets were within 79-124%, 88-103% and 85-108%, respectively, of the target for males, and 81-119%, 83-104% and 70-109%, respectively, in females. Homogeneity analyses showed that except for one "non-typical" aliquot, the diet mixes were homogeneously distributed with relative standard deviations of 2.91, 3.67 and 6.05% at pre-study, Week 7 and Week 38, respectively. Stability analysis indicated the test material to be stable in the test diet for at least 48 days (91% of Day 0 value).

5. **Treatment:** Male and female rats were fed diets containing 2,4-D acid at 5, 75 or 150 mg/kg/day for a period of up to 24 months (732 to 735 days). Control animals received standard laboratory diet on the same schedule. The most recent group mean body weight and feed consumption data for each sex were used to adjust the concentration of the test material in the diet to maintain the targeted dose levels.

6. **Experimental Procedures:** Mortality/moribundity checks and cage-side observations for clinical signs of toxicity were performed twice daily. A detailed physical examination for signs of local or systemic toxicity, pharmacologic effects and palpation for tissue masses were conduced prior to initiation and weekly thereafter. Examination of central nervous system and behavior pattern of each animal included looking for signs of tremors, convulsions, salivation and diarrhea. Individual body weights and amount of feed consumed were recorded prior to initiation, weekly for the first 13 weeks, and at approximately monthly intervals, thereafter. Ophthalmologic examinations were conducted on all animals once prior to initiation and at scheduled necropsies. Blood was collected from 10 rats/sex/dose after approximately 6, 12, 18 months and from 20 rat/sex/dose at termination for hematology and clinical chemistry determinations. The checked **(x) parameters** were determined:

Hematology

x Hematocrit (HCT)[a]	*x Leukocyte count (WBC)*[a]
x Hemoglobin (HGB)[a]	*x Platelet count*[a]
x Erythrocyte count (RBC)[a]	*x Leukocyte differential*[a]
Mean corpuscular HGB (MCH)	Mean corpuscular HGB Concentration (MCHC)
Mean corpuscular volume (MCV)	Blood clotting measurements
Cell morphology	

Clinical Chemistry

Electrolytes:	Other
x Calcium[a]	*x Albumin*[a]
x Chloride[a]	*x Blood Creatinine*[a]
Magnesium[a]	*x Blood Urea Nitrogen*[a]
x Phosphorus[a]	*x Total Cholesterol*[a]
x Potassium[a]	*x Globulin*
x Sodium	*x Glucose*[a]
	x Total Bilirubin[a]
Enzymes:	*x Total Protein*[a]
	x Triglycerides
x Alkaline phosphatase	Serum Protein Electrophoresis
x Alanine aminotransferase (SGPT)[a]	Triiodothyronine (T_3
x Aspartate aminotransferase (SGOT)[a]	*x Thyroxine (T_4)*
Cholinesterase[b]	A/G Ratio
x Creatinine phosphatase[a]	
Lactic acid dehydrogenase	
τ-Glutamyl transpeptidase [GGPT]	

[a] Required for subchronic and chronic studies.
[b] Required only for organophosphates and carbamates.

Urinalysis

x Appearance[c]	*x Bilirubin*[c]
x Specific gravity[c]	*x Occult blood*[c]
x pH[c]	*x Urobilinogen*
x Protein[c]	*x Glucose*[c]
x Ketones[c]	*x Microscopic examination of sediment*[c]

[c] Required for chronic studies.

7. **Termination:** For the interim sacrifice 10 rats/sex/dose were sacrificed after 369 days of treatment. The surviving male and female rats were sacrificed between test days 732 and 735 [Terminal sacrifice]. Complete gross postmortem examination was performed on these animals as well as on animals dying spontaneously, accidentally, and sacrificed in a moribund condition. Postmortem procedures included: examination of the external surface; all orifices; the cranial cavity; carcass; the external and sectioned surfaces of the brain and spinal cord; nasal cavity and paranasal sinuses; the thoracic; abdominal and pelvic cavities and their viscera and the cervical tissue. Organs weighed in animals sacrificed at 12 and 24 months were:

Adrenals	Brain	Heart	Kidneys	Liver	Thyroid/parathyroid	Testes	Ovaries

8. **Histopathology:** The checked (x) tissues from control and high-dose group animals of the interim and final sacrifice groups were trimmed and processed for histopathological evaluation. Histopathological examination of specific tissues from the low-and mid-dose group rats from the interim and terminal sacrifices were conducted since they were considered to be target organs in a previous study in this species. Tissues examined from rats at the low-and mid-dose groups at the interim sacrifice were: lungs, liver, kidneys, and eyes from both sexes, bone marrow/bone, mesenteric tissues/lymph node, and thyroid/parathyroid glands from females only, and testes and eight cross sections of brain (excluding the olfactory lob) from males only. Tissues examined from rats at the low-and mid-dose groups at the terminal sacrifice were: lungs, liver, kidneys, eyes, bone marrow/bone, mesenteric tissues/lymph node, and thyroid/parathyroid gland (both sexes), stomach and oral tissues (females), heart and testes (males) and gross lesions (both sexes). In addition, the kidneys from two control rats and three high-dose female rats were also stained with periodic acid-Schiff to characterize a treatment-related kidney effect. The approximate regions from which 9 sections of brain were prepared for histologic evaluation are shown **Figure 1.**

Digestive System	Respiratory System
x Salivary glands[a]	x Trachea[a]
x Esophagus[a]	x Lung[a]
x Stomach	Pharynx[e]
x Duodenum[a]	x Larynx[e]
x Jejunum[a]	Nose[e]
x Cecum[a]	x Nasal Tissues
x Colon[a]	
x Ileum[a]	Cardiovascular/Hematopoietic System
x Rectum[a]	
x Liver[ac]	x Aorta (thoracic)[a]
x Pancreas[a]	x Heart[a]
	x Bone marrow[a]
Nervous System	x Lymph nodes[a]
	x Spleen[a]
x Brain [cerebrum, brain stem, cerebellum][ac]	x Thymus[a]
x Pituitary[a]	Urogenital System
x Peripheral nerve[ab]	
x Spinal cord (3 levels)[ab]	x Kidneys[ac]
x Eyes[ab]	x Urinary bladder[a]
	x Testes[ac]
Glandular System	x Epididymides
	x Prostate
x Adrenals[a]	x Seminal vesicles
x Lacrimal glands[b]	x Uterus[a]
x Parathyroids[ad]	x Ovaries[ac]
x Thyroids[ad]	x Vagina
	x Cervix
	x Oviducts
	Other
	x Skin
	x Mammary glands
	x All gross lesions and masses
	x Skeletal muscle[a]
	x Mesenteric tissues
	x Mediastinal tissues
	x Oral tissue
	x Coagulating glands
	x Auditory sebaceous glands

a. Required for subchronic and chronic studies.
b. In subchronic studies examined only if indicated by toxicity or target organ involvement.
c. Organ weights required in subchronic and chronic studies.
d. Organ weights required for nonrodent studies.
e. Required for chronic inhalation study.

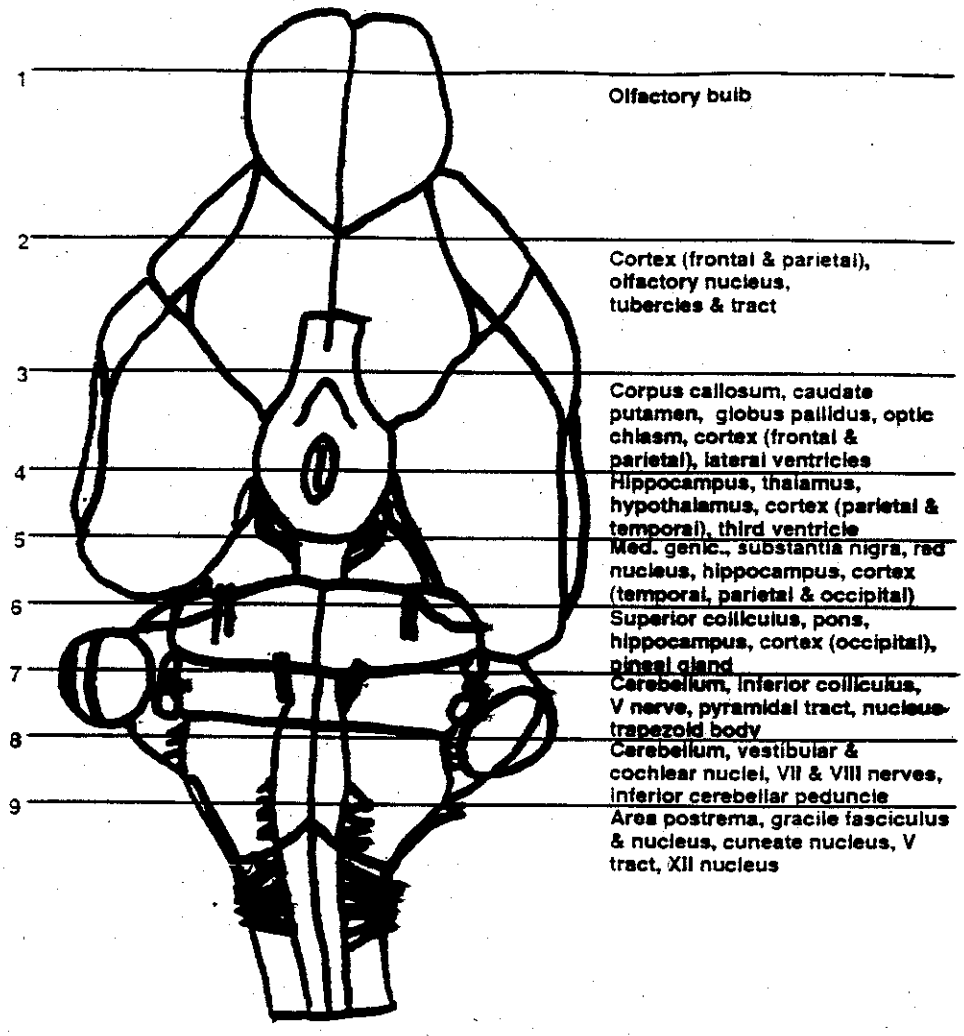


Figure 1. Diagramatic representation of the ventral surface of the rat brain. Numbered lines indicate the approximate topographic location of nine transverse sections and include a partial listing of expected microscopic structures.

9. **Statistical Analyses:** Differences in mortality patterns were tested by the Gehran-Wilcoxon procedure. Body weights, appropriate hematology data, clinical chemistry, urine specific gravity, and organ weight data were evaluated by Bartlett's test for equality of=variance. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric ANOVA, followed respectively by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons. Statistical analysis of gross and histopathological lesions consisted of pair-wise comparisons of control and treated groups using the pair-wise chi-square test with Yate's continuity correction.

10. **Regulatory Compliances:** Signed and dated No Data Confidentiality Claim, Flagging, Good Laboratory Practices, and Quality Assurance statements were provided.

III. RESULTS

A. **Survival:** There was no treatment-related mortality in either sex throughout the study. As shown in **Table 1**, survival at 18 and 24 months exceeded the guideline requirement of not less than 50% and 25%, respectively, at these intervals.

Table 1. Survival Rate in Rats Fed 2,4-D Acid for 2-years[a].

Percent Survival								
Interval	Males (mg/kg/day)				Females (mg/kg/day)			
	0	5	75	150	0	5	75	150
12-Months	100	100	96	98	100	100	100	100
18-Months	92	94	92	92	100	98	96	94
24-Months	56	50	66	72	70	78	80	70

a = Data obtained from Study Report Pg. 65 and 66.

B. **Clinical Observations:** No treatment-related clinical signs of toxicity were seen in either sex at any dose level.

C. **Ophthalmology Examination:** Noteworthy ophthalmological findings are summarized in **Table 2.** No treatment-related ophthalmologic effects were seen in rats at the interim sacrifice. At the terminal sacrifice, changes were observed in the eyes of male and female rats at 150 mg/kg/day. Lens opacity was seen in females at 150 mg/kg/day.

Table 2. Ophthalmological Observations in Rats Sacrificed at 24 Months[a].

	Males				Females			
(mg/kg/day)	0	5	75	150	0	5	75	150
Constricted, Blood Vessels, Fundus	2	4	2	18	2	2	7	3
Hyper-Reflective, Fundus	0	4	1	14	0	0	1	2
Opacity, Lens	0	2	1	1	3	2	3	34

a = Data obtained from Study Report Pg. 67

D. **Body Weight/Body Weight Gain:** Mean body weight data are presented in **Table 3.** Mean body weights of both sexes of rats at 5 and males at 75 mg/kg/day were comparable to respective controls. Mean body weights of females at 75 mg/kg/day and males and females at 150 mg/kg/day were significantly ($p < 0.05$) lower than controls throughout the study. At termination, when compared to controls, mean body weights were 8% lower in males at 150 mg/kg/day, 14% in females at 75 mg/kg/day, 26% in females at 150 mg/kg/day.

Table 3. Mean Body Weights (G) in Rats Fed 2,4-D Acid For 2-Years[a].

	Males (mg/kg/day)				Females (mg/kg/day)			
Interval	**0**	**5**	**75**	**150**	**0**	**5**	**75**	**150**
-2	198	198	197	198	129	128	127	126
90 (3-Months)	308	312	305	**295***	187	186	**177***	**169***
200 (6-Months	365	368	360	**344***	204	203	**193***	**180***
365 (12-Months)	410	416	400	**373***	218	218	**206***	**191***
533 (18-Month)	393	397	388	**359***	242	244	**219***	**201***
729 (24-Months)	344	350	347	**318***	271	266	**234***	**200***

a = Data obtained from Study Report Pages. 74-79
* Significantly different from controls at $p < 0.05$.

Mean body weight gain data are presented in **Table 4.** Mean body weight gain of males at 5 and 75 mg/kg/day were similar to or slightly higher than the controls throughout the study. In contrast males at 150 mg/kg/day had a statistically significantly ($p \leq 0.05$) lower body weight gain throughout the study. While no differences from control values were seen in female rats at 5 mg/kg/day, females at 75 and 150 mg/kg/day gained significantly ($p \leq 0.05$) lower body weight gain throughout the study. At termination, when compared to controls, body weight gain was 17% lower in males at 150 mg/kg/day, and in females, 24% lower at 75 mg/kg/day and 48% lower at 150 mg/kg/day.

Table 4. Mean Body Weight Gain (g) in Rats Fed 2,4-D Acid For 2-Years[a].

	Males (mg/kg/day)				Females (mg/kg/day)			
Interval	**0**	**5**	**75**	**150**	**0**	**5**	**75**	**150**
90 (3-Month)	111	114	108 (-3)[b]	**97*** (-13)	58	58	**50*** (-14)	**43*** (-26)
200 (6-Month)	168	171	164 (-2)	**147*** (-12)	75	75	**66*** (-12)	**53*** (-29)
365 (12-Month)	212	218	**202*** (-5)	**175*** (-17)	88	90	**79*** (-10)	**65*** (-26)
533 (18-Months)	194	199	190 (-5)	**162*** (-16)	112	116	**93*** (-14)	**75*** (-33)
729 (24-Months)	144	152	149	**120*** (-17)	141	138	**108*** (-24)	**73*** (-48)

a = Data obtained from Study Report Pg. 80-91;
b = Values in paranthesis (% decrease vs. controls) were calculated by Reviewer.
* Significantly different from controls at $p < 0.05$.

E. **Food and Compound Consumption:** Mean food consumption data are presented in **Table 5.** The decreases in body weights of male rats at 150 mg/kg/day and female rats at 75 and 150 mg/kg/day were accompanied with concomitant decreases in food consumption in these rats. The average dosages received by male rats were 4.77, 73.15 or 144.98 mg/kg/day; the corresponding number for female rats were 4.89, 73.11 or 143.52 mg/kg/day for the targeted doses of 5, 75 or 150 mg/kg/day, respectively.

Table 5. Mean Food Consumption Values in Rats fed 2,4-D Acid for 2-Years[a].

Sex	**Males**			**Females**		
Dose (mg/kg/day)	**5**	**75**	**150**	**5**	**75**	**150**
% Difference from Control Feed Consumption						
Mean	2.01	0.43	**-4.72**	0.75	**-3.93**	**-11.59**
S.D	1.75	1.51	2.51	2.35	3.67	4.88
Minimum	-1.2	-3.0	-11.1	-4.9	-12.8	-23.5
Maximum	5.2	2.9	-.06	5.3	1.7	0.0

F. Clinical Pathology

1. Hematology: Statistically significant ($p \leq 0.05$) differences in specific hematological parameters observed in both sexes of rats at 75 and 150 mg/kg/day, are presented in **Table 6.**

RBC were decreased in males at 150 mg/kg/day at 12 months (-8%), in females at 75 mg/kg/day at 6 (-4%), 12 (-7%) and 18 (-9%) months, and in females at 150 mg/kg/day at 6 (-10%), 12 (-10%), 18 (-12%) and 24 (-16%) months. Platelet coutns were decreased in females at 75 mg/kg/day at 6 (-4%), 12 (-19%) and 18 (-16%) months, in males at 150 mg/kg/day at 6 (-14%), 12 (-19%), 18 (-21%) and 24 (-22%) months, and in females at 6(-33%), 12 (-17%) and 18 (-17%) months. Decreases in HCT were seen at 150 mg/kg/day in males at 6 (-5%) and 12 (-5%) months and in females at 6 (-8%), 12 (-10%) and 18 (-7%) months. The decreases in RBC and possibly the platelets may be correlated with the decreased hematopoiesis of the bone marrow seen in females at 150 mg/kg/day at the 12 month sacrifice; however, decreased hematopoiesis was not seen at termination inspite of a decrease in RBC in females at termination. Consequently the decrease in RBC and platelet counts were not considered to be treatment-related. Other statistical differences were not considered to be toxicologically significant due to lack of dose- and/or time-response and corroborative histopathology.

2. Clinical Chemistry: Statistically significant ($p \leq 0.05$) differences observed in clinical chemistry parameters in both sexes of rats at 75 and 150 mg/kg/day are presented in **Table 7.**

ALT activity was increased in males at 75 mg/kg/day at 6 (+85%), 12 (+38%), 18 (+76%) and 24 (+79%) months and in males at 150 mg/kg/day 6 (+88%), 18 (+29%) and 24 (+76%) months. AP activity was increased in males at 75 mg/kg/day at 6 (+16%) and 24 (+40%) months, in females at 75 mg/kg/day at 6 (+26%), 12 (+47%), 18 (+86%) and 24 (+79%) months, in males at 150 mg/kg/day at 6 (+26%) and 24 (+85%) months, and in females at 150 mg/kg/day at 6 (+55%), 12 (+69%), 18 (+105%) and 24 (+110%) months. AST activity was increased in males at 75 mg/kg/day at 6 (+40%) and 24 (+47%) months and in males at 150 mg/kg/day at 6 (+48%) months only. Decreases in cholesterol levels were seen in males at 75 mg/kg/day at 12 (-21%), 18 (-22%) and 24 (-36%) months, in females at 75 mg/kg/day at 6 (-30%), 12 (-59%), 18 (49%) and 24 (-38%) months, in males at 150 mg/kg/day at 6 (-17%), 12 (-39%), 18 (-46%) and 24 (-34%) months and in females at 150 mg/kg/day at 6 (-34%), 12 (-46%), 18 (-48%) and 24 (-36%) months. Triglyceride was decreased in females at 75 mg/kg/day at 18 (-32%) and 24 (-31%) months, in males at 150 mg/kg/day at 12 (-31%), 18 (-49%) and 24 (-47%) months, and in females at 150 mg/kg/day at 18 (-35%) and 24 (-43%) months. Alterations in ALT, AP, AST and cholesterol may be attributed to treatment due to histopathological lesions in the liver of both sexes at 150 mg/kg/day. Other statistical differences were not considered to be toxicologically significant due to lack of dose- and/or time-response and corroborative histopathology.

Table 6. Statistically Significant Differences in Hematology Parameters[a].

DOSE / SEX	INTERVAL (Months)	RBC ($x10^6/mm^3$)	HGB (g/dl)	HCT (%)	PLATELET ($x10^6/mm^3$)
0 mg/kg/day (M)	6	9.8	17.2	61.9	615
	12	9.3	15.0	58.5	586
	18	9.1	16.0	48.1	623
	24	7.9	13.7	41.4	760
0 mg/kg/day (F)	6	9.1	17.2	60.6	599
	12	8.4	14.8	57.3	583
	18	8.6	16.1	46.8	591
	24	8.3	14.7	43.6	562
75 mg/kg/day (M)	24	8.7	14.3	42.5	**620***
75 mg/kg/day (F)	6	**8.7***	16.9	**58.4***	**461***
	12	**7.8***	14.4	**52.6***	**471***
	18	**7.8***	**14.8***	44.0	**496***
	24	7.4	13.9	41.6	551
150 mg/kg/day (M)	6	9.6	17.1	**59.3***	**529***
	12	**8.6***	14.9	**55.3***	**474***
	18	8.9	16.0	47.7	**495***
=	24	7.7	13.7	41.0	**592***
150 mg/kg/day (F)	6	**8.2***	**16.6***	**55.9***	**403***
	12	**7.6***	**14.5**	**51.8***	**486***
	18	**7.6***	**14.9***	**43.5***	**491***
	24	**7.0***	13.3	40.9	523

a = Data obtained from Study Report Pages. 100-107.

Table 7. Statistically Significant Differences in Clinical Chemistry Parameters[a].

DOSE / SEX	INTERVAL (Months)	ALT (mu/ml)	AP (mu/ml)	AST (mu/ml)	CHOL (mg/dl)	TRIG (mg/dl)
0 mg/kg/day (M)	6	65	73	116	86	140
	12	66	57	106	87	101
	18	41	58	79	154	186
	24	38	53	78	194	207
0 mg/kg/day (F)	6	51	47	96	116	77
	12	37	32	71	105	52
	18	42	37	80	155	136
	24	48	47	92	169	183
75 mg/kg/day (M)	6	**120***	**85***	**162***	84	171
	12	**91***	58	132	**69***	89
	18	**72***	73	114	**120***	151
	24	**68***	**74***	**115***	**124***	119
75 mg/kg/day (F)	6	56	**59***	103	**81***	88
	12	37	**47***	63	**54***	54
	18	34	**69***	72	**76***	**93***
	24	61	**84***	136	**104***	**126***
150 mg/kg/day (M)	6	**122***	**92***	**172***	**71***	129
	12	**68**	55	103	**53***	**70***
	18	**53***	74	92	**83***	**94***
	24	**67***	**98***	129	**128***	**110***
150 mg/kg/day (F)	6	**40***	**73***	98	**76***	83
	12	33	**54***	68	**57***	56
	18	36	**76***	81	**81***	**88***
	24	55	**99***	134	**109***	**105***

a = Data obtained from Study Report Pages. 161-168.

Concentrations of thyroxin (T_4) levels are presented in **Table 8**. Decreases in T_4 levels were observed in both sexes at 75 and 150 mg/kg/day at all intervals; however, the decreases in males at 75 mg/kg/day showed statistical significance only at 12 and 24 months. These changes were attributed to treatment due to increases in absolute and relative weights and histopathological lesions in the thyroid glands.

Table 8. Thyroxin [T_4] Levels (μg/mL) in Rats Fed 2,4-D Acid.

	Males				Females			
(mg/kg/day)	0	5	75	150	0	5	75	150
At 6 Months	3.2	3.4	2.7 (-16%)[b]	2.1* (-34%)	2.6	2.7	1.1* (-58%)	0.9* (-65%)
At 12 Months	3.0	3.1	2.6* (-14%)	0.9* (-70%)	2.3	2.2	0.8* (-65%)	0.7* (-70%)
At 18 Months	3.0	3.4	2.6 (-14%)	1.1* (-63%)	1.9	2.1	0.8* (-58%)	0.5* (-73%)
At 24 Months	2.2	2.2	1.5* (-32%)	0.8* (-64%)	1.9	2.2	1.3* (-32%)	1.1* (-42%)

a = Data obtained from Study Report Pages. 169-176.
b = Values in paranthesis (% decrease vs. controls) were calculated by the reviewer.

3. Urinalysis: The statistically significant differences observed in urine specific gravity, urinary protein and ketones were not considered to be biologically significant due to lack of dose-and time-response as well as histopathological lesions in the kidneys.

F. **Organ Weights:** Thyroid weights with percent increase are presented in **Table 9**. Statistically significant [$p < 0.05$] increases were observed in both absolute and relative thyroid weights of female rats at 75 mg/kg/day and in male and females rats at 150 mg/kg/day at both sacrifice. Increases in thyroid weights correlated with decreases in T_4 levels seen in rats at these dose levels, however, histopathological lesions of the thyroid glands characterized as a decrease in the secretory material (thyroglobulin) within the thyroid follicles was seen only in females at 150 mg/kg/day at the interim sacrifice (12-months). These lesions, however, were not confirmed in rats sacrificed at the terminal sacrifice (24-months).

Table 9. Thyroid Weights at the Interim (12-Months) & Terminal (24-Months) Sacrifices[a].

	Dose Level [mg/kg/day]							
	0		5		75		150	
Males								
Sacrifice Interval[b] (Months)	12	24	12	24	12	24	12	24
Absolute (g) Weights	0.027 -	0.036 -	0.025 -	0.037 -	0.026 -	0.036 -	**0.032*** **(+19%)[c]**	**0.040*** **(+11%)**
Relative (g/100) weights	0.0071 -	0.0110 -	0.0064 -	0.0108 -	0.0070 -	0.0107 -	**0.0091*** **(+28%)**	**0.013*** **(+18%)**
Females								
Absolute (g) weights	0.018 -	0.025 -	0.019 -	0.025 -	**0.022*** **(+22%)**	**0.028*** **(+12%)**	**0.022*** **(+22%)**	**0.030*** **(+20%)**
Relative (g/100) weights	0.0093 -	0.0094 -	0.0091 -	0.0100 -	**0.0111*** **(+19%)**	**0.0127*** **(+35%)**	**0.026*** **(+35%)**	**0.015*** **(+60%)**

a = Data obtained from Study Report Pages. 37, 38, 178, 181, 184 and 187.
b = Thyroid weights of 12 ♂ and 7 ♀ with masses at 24 months were excluded from analysis.
c = Values in paranthesis (% increase vs. controls) were calculated by the reviewer.

G. **Gross Pathology:** Treatment-related gross necropsy findings are summarized in **Tables 10 and 11** for the 12 and 24 month sacrifices, respectively. Gross pathology observed at both sacrifices were a general decrease in fat in females at 150 mg/kg/day and pale foci in the lungs of females at 75 and 150 mg/kg/day (interim) and in males at 150 mg/kg/day and in females at 75 and 150 mg/kg/day (terminal). Bilateral flaccid testes seen in few males at the high dose at 12 months was not seen at 24 months. Gross pathology limited to the terminal sacrifices were opacity of the lens only in 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. Other findings in the control and treated groups occurred with comparable frequency and were similar to those commonly seen in this age/ strain of rats.

Table 10. Gross Necropsy Findings in Rats At the Interim Sacrifice[a].

Sex	Males				Females			
Dose (mg/kg/day)	**0**	**5**	**75**	**150**	**0**	**5**	**75**	**150**
Fat: Decreased amount	0	0	0	1	0	0	0	4
Lungs: Foci-pale, multifocal	0	0	0	0	0	0	1	10

a = Data obtained from Study Report Pages. 189-191.

Table 11. Gross Necropsy Findings in Rats Sacrificed At the Terminal Sacrifice][a].

Sex	Males				Females			
Dose (mg/kg/day)	**0**	**5**	**75**	**150**	**0**	**5**	**75**	**150**
Eyes: Opacity, lens, unilateral	0	2	1	1	2	1	3	5
Opacity, lens, bilateral	0	0	0	0	0	0	0	30
Fat: Decreased amount	15	15	12	13	4	2	5	12
Lungs: Foci-pale, multifocal	0	0	0	4	1	0	4	40
Thyroid: Mass/Nodule	1	1	7	4	0	3	3	3

a = Data obtained from Study Report Pages. 197, 201 & 208.

H. Histopathology - Interim Sacrifice (12-months)

1. **Non-neoplastic Lesions:** Treatment-related non-neoplastic lesions 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 are presented in **Table 12**. Histopathologic examinations 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 the 12 months were similar to those frequently seen in this strain/age of rats.

Table 12. Treatment-Related Non-Neoplastic Lesions in Rats At the INTERIM Sacrifice[a].

No. Examined: 10/Sex/Dose	Males (mg/kg/day)				Females (mg/kg/day)			
Tissue/Lesion	**0**	**5**	**75**	**150**	**0**	**5**	**75**	**150**
Bone marrow: hematopoiesis, decreased	0	-	-	0	0	0	0	4
Eyes: retina, degeneration, bilateral	0	0	0	1	0	0	0	9
Kidneys: proximal tubule, degeneration	0	0	8	10	1	0	7	9
Liver: altered tinctorial properties - increased eosinophilia, central lobular and midzonal	0	0	0	10	0	0	0	0
:altered tinctorial properties, panlobular	0	0	0	0	0	0	8	10
Lungs: alveolar histiocytosis, multifocal	0	0	0	2	2	0	4	10
Mesenteric tissue: adipose tissue, atrophy	0	-	0	1	0	0	5	8
Testes: atrophy, tubules, bilateral, diffuse	0	0	0	2	-	-	-	-
Thyroid: decreased secretory material, epithelial cells	0	-	-	0	0	0	0	8
hyperplasia, parafollicular cells, focal	2	-	-	4	4	0	0	0
hypertrophy, epithelial cells, focal	0	-	-	0	0	0	0	1

a = Data obtained from Study Report Pages. 210-224,

2. **Neoplastic Lesions:** The 16 neoplastic lesions seen both in the control and treated groups are presented in **Table 13.** These tumors were not considered to be treatment related since the incidences were not statistically significantly different from the controls and the incidences were similar to those seen in this strain/age of rats.

Table 13. Neoplastic Lesions in Rats At the INTERIM Sacrifice[a].

No.Examined: 10/Sex/Dose	Males (mg/kg/day)				Females (mg/kg/day)			
Organ/Lesions	**0**	**5**	**75**	**150**	**0**	**5**	**75**	**150**
Liver: adenoma, hepatocellular, benign - primary	0	1	0	0	0	0	0	1
Pituitary: adenoma, anterior, benign - primary	1	-	1	0	1	2	-	0
Testes: leydig cell tumor, benign - primary	2	2	3	0	-	-	-	-
Uterus: endometrial stromal polyp,benign-primary	-	-	-	-	0	1	0	1

a = Data obtained from Study Report Page. 346.

Histopathology - Terminal Sacrifice (24-months)

1. **Non-neoplastic Lesions:** Treatment-related non-neoplastic lesions observed in the eyes, liver, lungs, and mesenteric fat (adipose tissue) at the 24-month terminal sacrifice are presented in **Table 14.** Eye lesions in both sexes of rats 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 in the male and female rats 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 of rats 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. In addition, parafollicular cell nodular hyperplasia the thyroid glands was increased (not significant) in females at 150 mg/kg/day (20%) compared to control females (7%); however, this was not attributed to treatment since there was no dose-response, were seen only in females, and the incidences were within the historical control range. Also in the thyroids, the decreases in secretory material (thyroglobulin) within the thyroid follicles seen only in females at 150 mg/kg/day at the interim sacrifice were not confirmed at termination. Other non-neoplastic lesions seen were similar to those occurring spontaneously in this strain/age of rats.

Table 14. Treatment-Related Non-Neoplastic Lesions in Rats At the TERMINAL Sacrifice[a].

No. Examined: 50/Sex/Dose	Males (mg/kg/day)				Females (mg/kg/day)			
Tissue/Lesion	**0**	**5**	**75**	**150**	**0**	**5**	**75**	**150**
Eyes: retina, degeneration, bilateral - very slight	23	19	21	**6***	23	26	30	**0***
- slight	0	1	1	**8***	0	0	1	0
- moderate	0	0	0	**6***	0	0	0	2
- severe	0	0	0	**15***	0	0	0	**42***
Eyes: cataract	1	3	3	**8***	3	2	4	**39***
Liver: increased size of hepatocytes with altered tinctorial properties, panlobular	0	0	0	**32***	3	0	3	**34***
Lungs: inflammation, subacute to chronic - very slight	5	6	5	**15***	16	11	**26***	**5***
- slight	2	4	3	2	1	1	2	**43***
- moderate	0	0	0	3	0	0	0	1
- severe	0	1	0	0	0	0	0	0
- any severity	7	11	8	**20***	17	12	**28***	**49***
Mesenteric tissue: adipose tissue, atrophy	31	26	24	**49***	6	5	12	**36***

a = Data obtained from Study Report Pages. 303-340.

2. **Neoplastic Lesions:** Histopathology revealed a variety of benign and malignant tumors at different sites in both treated and control animals, but none showed statistical significance in individual tumor types in any treated group of either sex. The tumor incidence and types were similar to those commonly seen in aging Fisher 344 rats. Astrocytomas, a rare tumor type in rats, were seen only at 150 mg/kg/day; a malignant tumor in 1 of 50 (2%) treated males compared to none in control males and a benign tumor each in 1 of 50 (2%) treated and control females. The incidence, however, was not statistically significant when compared to the concurrent controls and there was no dose-response (none were seen in either sex at the low- or the mid-doses). Other neoplastic lesions were comparable in number and frequency between the control and treated animals and were frequently seen in aging Fisher 344 rats. A summary of the neoplastic lesions presented in Table 91 of the Study Report Pages 358-364 are appended to this DER (Appendix 1).

IV. DISCUSSION

The purpose of this discussion that follows is to compare the findings of the 1986 (Hazelton) and the 1995 (Dow) chornic toxicity/carcinogenicity studies conducted with 2,4-D acid in Fischer 344 rats. In the 1986 study, (50/sex/dose) were fed diets containing 2,4-D acid technical (97.5%) at 0, 1, 5, 15 or 45 mg/kg/day for 104 weeks, and in the 1995 study, (50/sex/dose) received 2,4-D acid (96.45%) in the diet at 0, 5, 75 or 150 mg/kg/day for 104 weeks.

A. **Survival:** No treatment-related effects on mortality (survival) were seen in either study as shown below:

Table 15. Survival (%) in Rats In The Two Studies[a].

Sex	Months	Dose (mg/kg/day)								
		0 (H)	0 (D)	1 (H)	5 (H)	5 (D)	15 (H)	45 (H)	75 (D)	150 (D)
Males	12	98	100	100	100	100	97	100	96	98
	24	64	56	85	96	50	84	76	66	72
Females	12	97	100	100	100	100	97	100	100	100
	24	80	70	74	96	78	84	76	80	70

a = Data obtained from Study Report Page: 1754.
H = 1986 Hazleton Study; D = 1995 Dow Study

B. **Body Weight/Body Weight Gain:** Cumulative body weight gain data for both studies are summarized below. Body weight gains were lower in males at 150 mg/kg/day (-17%), in females at 45 mg/kg/day (-9%), 75 mg/kg/day (-24%) and 150 mg/kg/day (-48%) when compared to respective controls over the entire course of the study.

Table 16. Cumulative Body Weight Gain (g) in Rats In The Two Studies[a].

Sex	Months	Dose (mg/kg/day)								
		0 (H)	0 (D)	1 (H)	5 (H)	5 (D)	15 (H)	45 (H)	75 (D)	150 (D)
Males	0-12	230	212	225	227	218	232	228	**202***	**175***
	0-24	217	144	211	215	152	214	207	149	**120***
Females	0-12	113	88	114	117	90	114	**105***	**79***	**65***
	0-24	146	141	143	141	138	145	**133***	**108***	**73***

a = Data obtained from Study Report Pages: 81, 95 & 1755
H = 1986 Hazleton Study D = 1995 Dow Study * = $p \geq 0.05$.

C. **Food Consumption:** In the 1986 study, females at 45 mg/kg/day exhibited decreased feed consumption of approximately 2.4% through 52 weeks compared to female controls; no effect on feed consumption was seen in males. In the 1995 study, feed consumption was decreased by approximately 5% in males at 150 mg/kg/day, by 4% in females at 75 mg/kg/day, and by 12% in females at 150 mg/kg/day.

D. **Ophthalmology:** No ocular toxicity was seen in the 1986 study. In the 1995 study, constricted blood vessels, hyper-reflectivity of the fundus, and lens opacity were seen in 18, 14, and 34 females, respectively, at 150 mg/kg/day. These findings were confirmed during necropsy.

E. **Hematology:** No treatment-related effects on any of the hematological parameters were observed at any dose level in the 1986 study. In the 1995 study, statistically significant decreases in RBC and platelet counts were observed mainly in females at 75 and 150 mg/kg/day at one or more time periods with no dose- and/or time-response. Additionally, hematopoiesis of the bone marrow was seen only in females at 150 mg/kg/day sacrificed at 12 months but not in rats at any dose level sacrificed at 24 months. Consequently, alterations in hematology was not considered to be toxicologically significant.

F. **Clinical Chemistry:** In both studies, a number of clinical chemistry parameters were identified as statistically different from control values at one or more time points in males and/or females at 45, 75, or 150 mg/kg/day. ALT was increased in both sexes at these dose levels. Both studies exhibited decreases in T_4 levels; females at 45 in the 1986 study and both sexes at 75 and 150 mg/kg/day in the 1995 study. Decreases in T_4 concentrations corroborated with increases in thyroid weights in both studies. Also in the 1995 study, AST and AP activities were increased, cholesterol levels were decreased at 75 and 150 mg/kg/day dose groups. Alterations in liver enzyme activity were attributed to treatment due to corroborative histopathology in the liver of rats at 75 and 150 mg/kg/day.

G. **Organ Weights:** In both studies, thyroid weights were increased; the 1986 study presented a dose-related increase in both sexes at 15 and 45 mg/kg/day, and the 1995 study had increases in females at 75 mg/kg/day and in both sexes at 150 mg/kg/day. However, treatment-related histological lesions in the thyroids were seen only in the 1995 study and were limited to females at 150 mg/kg/day sacrificed at 12 months. In the 1986 study, increase in kidney weights was seen in males and females (statistically significant) at 45 mg/kg/day, while in the 1995 study, no clear effects were observed on kidney weights. No effects were noted for liver weights in either study.

H. **Gross Pathology:** No treatment-related gross pathological lesions were seen in the 1986 study. In the 1995 study, treatment cause opacity of the lens and a general decrease in fat of females at 150 mg/kg/day, pale foci of the lungs in males at 150 mg/kg/day and females at 75 and 150 mg/kg/day, and thyroid masses in males at 75 and 150 mg/kg/day and in females at all dose levels.

I. **Histopathology**

1. Non-neoplastic Lesions: In the 1986 study, kidneys were identified as the target organ based on the treatment-related non-neoplastic lesions which were characterized as increased frequency of a brown tubular epithelial cell pigment, pelvic microcalculi, and transitional epithelial cell hyperplasia secondary to microcalculi as shown in **Table 17**. In the 1995 study, kidney lesions characterized as degeneration of the descending portion of the proximal convoluted tubules of the kidneys were seen in both sexes of rats at 75 and 150 mg/kg/day only at 12 months; no treatment-related kidney lesions were seen at 24 months.

Table 17. Non-Neoplastic lesions of the Kidneys in the 1986 Study[a].

	Males (mg/kg/day)					Females (mg/kg/day)				
Lesions	0	1	5	15	45	0	1	5	15	45
Brown tubular cell pigment	2/50 4%	1/50 2%	**9/50* 18%**	**18/50* 36%**	**19/59* 38%**	8/50 16%	10/50 20%	**23/50* 46%**	**20/50* 40%**	15/50 30%
Pelvic microcalculi	2/50 4%	2/50 4%	4/50 8%	8/50 16%	11/50 22%	19/50 38%	11/50 22%	15/50 30%	23/50 46%	**35/50* 70%**
Transitional epithelial cell hyperplasia	0/50 0%	1/50 2%	1/50 2%	1/50 2%	3/50 6%	1/50 2%	1/50 2%	3/50 6%	4/50 8%	11/50* 22%

a = Data obtained from Study Report Pages: 1985, 1998 & 2011

* Significantly different from control a t $p \leq 0.05$.

In subchronic studies with Fisher 344 rats, kidney lesions characterized as loss of epithelial cells in the proximal tubule brush border were seen only at doses ($\geq$ 100 mg/kg/day) in excess of the threshold for saturation of renal tubular secretion. Changes affected the tubules rather than the glomeruli. In the 1986 study kidney lesions were seen in both sexes at doses of 5, 15 or 45 mg/kg/day after 2-years (Table 19). In the 1995 study, at higher doses (75 or 150 mg/kg/day), kidneys lesions characterized as degeneration of the descending portion of the proximal convoluted tubules were seen only in those sacrificed after 1-year (Table 14) but not after 2-years. Pharmacokinetics studies have shown that 2,4-D acid is excreted through renal tubular secretion, therefore, it stands to reason that overloading the secretion mechanism may cause damage to renal tubules.

In the 1995 study, treatment-related non-neoplastic lesions were seen in the eyes (retinal degeneration and cataracts), liver (panlobular tinctorial properties), lungs (inflammation), and mesenteric fat (atrophy of the adipose tissue) of male and female rats at 150 mg/kg/day. These lesions were not seen in the 1986 study. While thyroid lesions were not seen in the 1986 study, parafollicular cell nodular hyperplasia was seen only in females at 150 mg/kg/day. This lesion, however was not attributed to treatment since the difference was not statistically significant and the incidences were within the historical control range.

2. Neoplastic Lesions: In the 1986 study, astrocytomas of the brain, a rare tumor in rats, were observed in both the treated and control animals and the incidence of astrocytomas are presented in **Table 18.** Although a positive trend ($p = 0.002$) was seen in males, when the incidence at the high dose (6/50) was compared with that of the controls (1/50), in a pair wise test, there was no statistical significance ($p = 0.0550$).

Table 18. Astrocytomas of the Brain in Rats Fed 2,4-D Acid (1986 Study) [a].

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	1	5	15	45	0	1	5	15	45
Brain Astrocytomas	**1/50*** 2%	0/50 0%	0/50 0%	2/48 4%	6/50 12%	0/50 0%	1/50 2%	2/50 4%	1/50 2%	1/50 2%

a = Data obtained from Study Report Pages: 1981 & 2007.
* *Significant trend (p = 0.002).*

The 1995 study was designed specifically to address this finding and the protocol required the evaluation of 8-9 sections of the brain histologically (See Figure 1). The incidence of astrocytomas in males was 0/50, 0/26, 0/18, and 1/50, and in females 1/50, 0/14, 0/11, and 1/50 for the 0, 5, 75, and 150 mg/kg/day, respectively. When compared to historical controls, the incidence (2%) of brain astrocytomas in both the control and treated rats were within the historical control ranges in males (0-4.4%) and females (0-3%).

The lack of this tumor type at higher doses (75 or 150 mg/kg/day) clearly indicate that the observance of this tumor in the 1986 study is an aberration and not treatment-related because characteristics generally attributed to a brain carcinogen were not seen in the 1986 study. 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, most, if not all known brain carcinogens show clear genotoxicity in mutational assay (Kleihues et al. 1982 and Ward and Rice, 1982), whereas 2,4-D is negative in most assays. Therefore, 2,4-D does not support a carcinogenic response in the brain of male or female Fisher 344 rats.

Kleihues, P., Patzschke, K. and Doerjer, G. 1982. *DNA Modification and Repair in the Experimental Induction of Nervous System Tumors by Chemical Carcinogens: In: Selikoff, I.J. and Hammond, E.C.(Eds) Brain Tumors in the Chemical Industry.* The New York Academy of Sciences, New York, New York, pp 290-319.

Ward, J.M. and Rice, J. M. 1982. *Naturally Occurring and chemically induced brain tumors or rats and mice in carcinogenesis bioassays.* Annals of the New York Academy of Sciences, 381: 304-319.

VI. STUDY DEFICIENCY

During the preparation of the tissues for histologic evaluation, the individual identification for 35 female thyroid/parathyroid gland was lost. This occurred during the dehydration process, due to the removal of the identifying ink from the tissue containers by alcohol and xylene. This resulted in 8, 9, 9, 9 thyroid glands missing from the 0, 5, 75 and 150 mg/kg/day groups, respectively. An independent forensic laboratory attempted a number of techniques, such as fluorescence microscopy to read residues of the identification numbers. However, the precision of the technique was insufficient.

Histologic findings in the 35 unidentified thyroids in females and those of the identified thyroids from females in all treated groups are compared in **Table 19.** No treatment-related effects were seen in either group; unidentified thyroids had lesions and incidence similar to identified tissues. Consequently, the loss of the 35 thyroid tissue did not alter the outcome of the study.

Table 19. Comparison of Histopathology of the Unidentified and Identified Thyroids in Females[a].

Histologic Observation	Unidentified Thyroids	Identified Thyroids			
		mg/kg/day			
		0	5	75	150
No. Examined	35	42	41	41	40
# Missing	--	8	9	9	10
Cyst(s) with keratinous debris, focal	2	0	0	1	1
Aggregate (s) of mononuclear cell (lymphoid), focal	1	1	1	1	2
Cystic dilatation, follicle(s), focal	8	2	4	8	8
Hyperplasia - nodular, parafollicular cells, focal	4	3	2	2	8
Hyperplasia - parafollicular cells, diffuse	30	27	33	31	21
Adenoma, parafollicualr cells, benign, primary (one)	4	11	8	5	8
Adenoma, parafollicular cells, benign, primary (two)	1	0	1	1	0
Carcinoma, parafollicular cells, malignant, primary	1	2	3	0	1
Adenoma, follicle(s), benign, primary	1	0	0	0	0
Adenocarcinoma, follicel(s), malignant, primary	1	0	0	1	0

a = Data obtained from Study Report Pages: 340-341.

VII. 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.

Under the conditions of this study, the following NOELs and LOELs are established for chronic toxicity:

Sex	NOEL (mg/kg/day)	LOEL (mg/kg/day)	BASIS FOR LOEL
Male	75	150	Decreases in mean body weight, body weight gain and food consumption, alterations in clinical chemistry parameters, decrease in T_4 concentration, increase in absolute/relative thyroid weights, and histopathological lesions in the eyes, liver, lungs, and mesenteric fat (adipose tissue).
Female	5	75	Decreases in mean body weight, body weight gain and food consumption, alterations in clinical chemistry parameters, decrease in T_4 concentration, increase in absolute/relative thyroid weights, and histopathological lesions in the kidneys, liver and lungs.

In this study, at the dose levels tested, there was no evidence of carcinogenicity in either sex. Brain astrocytomas observed in the 1986 study were not replicated at higher doses in the 1995 study, thereby indicating that **2,4-D acid is not carcinogenic in male or female Fischer-344 rats.**

APPENDIX-1

NEOPLASTIC LESIONS OBSERVED IN FISCHER-344 RATS FED 2,4-D ACID FOR 24 MONTHS

THE DOW CHEMICAL COMPANRY

STUDY ID: K-002372-064F

Page _____ is not included in this copy.

Pages __66__ through __72__ are not included in this copy.

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__ _ __ Identity of product inert ingredients.

__ _ __ Identity of product impurities.

__ _ __ Description of the product manufacturing process.

__ _ __ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

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_____ Attorney-Client work product.

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PRIMARY REVIEWER: Jess Rowland, M.S., Toxicologist Jess Rowland 5/16/96
Section I, Toxicology Branch II

SECONDARY REVIEWER: Stephen C. Dapson, Ph.D., Senior Pharmacologist
Section I, Toxicology Branch II Stephen C. Dapson 5/16/96

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rat **GUIDELINE:** 83-3(a)

P.C. CODE: 030001 **TOX. CHEM. No:** 315

TEST MATERIAL: **2,4-Dichlorophenoxy acetic acid (2,4-D)**

CITATION: Nemec, M.D, Tasker, E.J. Werchowski, K.M, and Mercieca, M.D . *" A TERATOLOGY STUDY IN FISCHER 344 RATS WITH 2,4-DICHLOROPHENXOYACETIC ACID"*. WIL Research Laboratories, Inc. Study No. WIL-81135, March 2, 1983. **Accession No. 000251031.** Unpublished.

REGISTRANT: Industry Task Force on 2,4-D Research Data.

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 ≈ 75 mg/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 14th rudimentary ribs (4 fetuses of 3 litters vs. 0 in the controls); malaligned 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, $\approx$ 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.**

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

I. INTRODUCTION

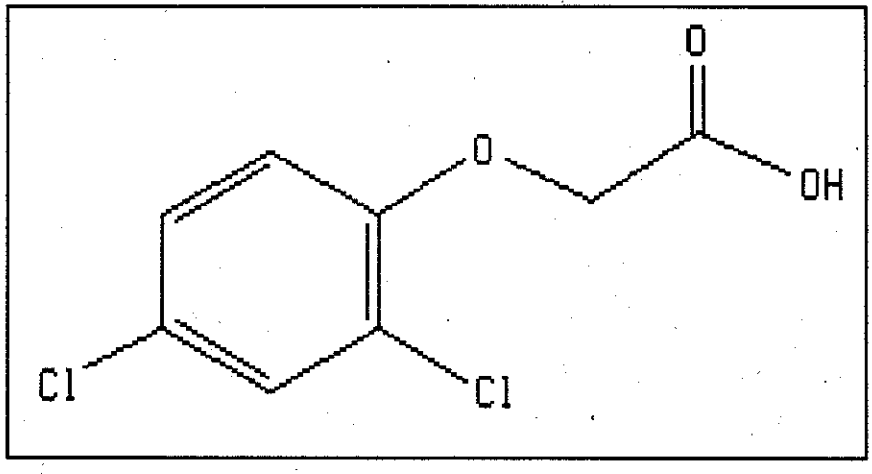
This study is re-reviewed due to the inadequacy of an earlier review (HED Doc. # 003887). The objective of this study was to assess the effects of 2,4-dichlorophenoxyacetic acid on the embryonic and fetal development following oral administration to rats during the period of organogenesis.

II. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: 2,4-Dichlorophenoxyacetic acid, Technical

Description: White phenolic powder
Lot No.: 3/82
Purity: 97.%, technical
CAS #: 94-75-7
Structure:



2. Vehicle: Mazola Corn oil

3. Test Animals: Rats

Strain: Fischer 344, Charles River, Portage, MI
Age: Approximately 20 weeks old at initiation.
Weight: 173 to 238 g on Day 0 of pregnancy
Identification: Ear tags/cage cards.
Acclimation Period: Seven (7) days
Housing: Individually in stainless steel cages

Food:	Purina Certified Rodent Chow #5002 ad libitum.
Water:	Tap water ad libitum
Environment:	Temperature, 72 ± 3°F; Humidity, 40% +; Light, 12 hr light/dark

B. PROCEDURES AND STUDY DESIGNS

1. In Life Dates - Start: 5/25/82; End: 7/22/82

2. Mating: Each female was paired with one male. Vaginal smears were taken daily during cohabitation, and the presence of copulatory plug or sperm in the vaginal smear was considered evidence of mating. The day this evidence was seen was designated as Day 0 of gestation and females were housed individually.

3. Animal Assignment: Rats were assigned, randomly, to dose groups as shown below:

Table 1. Study Design

Treatment	No. of Pregnant females	Dose Level
Control	35	0 mg/kg/day
Low Dose	35	8 mg/kg/day
Mid Dose	35	25 mg/kg/day
High Dose	35	75 mg/kg/day

4. Dose Selection Rationale: The rationale of dose level selection is based on the following: A "no effect" level of at least 25 mg/kg/day for embryo/fetotoxicity was suggested by the Pest Control Products Section, Health and Welfare Canada. In a study in Sprague-Dawley rats[a], following oral administration in corn oil at doses of 0, 12.5, 25, 50, 75 or 87.5 mg/kg/day during gestation days 6-15, inclusive, embryo/fetotoxicity was limited to the 87.5 mg/kg/day and included: decrease in fetal body weight, subcutaneous edema, delayed ossification of bone, lumber ribs and wavy ribs. Based on these data a low dose of 8 mg/kg/day was selected since it wa anticipated to be a "no effect" level. Conversely, the high dose of 75 mg/kg/day was expected to produce some degree of maternal and/or developmental toxicity.

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a = Schwetz, BA et al. 1971. *"The effect of 2,4-Dichlorophenoxyacetic acid (2,4-D) and Esters of 2,4-D on Rat Embryonal, Foetal and Neonatal Growth and Development"*. Fd. Cosmet. Toxicol. 9:801-817

5. Dosage Preparation and Analysis of Dosing Solutions: The test material for each group was weighed and added to a tissue grinder vessel. The vehicle was then added and the mixture was spun at a moderate speed until homogenous. Dosing solutions were prepared fresh weekly and dispensed as required, and stored at room temperature. The test material/corn oil suspension was stirred on a magnetic stirrer. Dosing solution concentrations were 2, 6.25, 18.75 ml/kg, for 8, 25 and 75 mg/kg/day dose levels, respectively Except for the analysis of the bulk material for purity and identity, no analyses of the dosing solutions were made during the study.

6. Dosage Administration: 2,4-D in corn oil was administered daily orally via gavage at doses of 0, 8 25 or 75 mg/kg/day during gestation days 6 through 15, inclusive. The vehicle control group received corn oil only. All groups received a dosing volume of 4 ml/kg body weight and the individual dosages were adjusted at each weighing interval to provide the proper mg/kg/day dose. During dosing, the dosing solution was stirred continuously using a magnetic stir plate and bars to ensure proper mixture.

C. OBSERVATIONS

1. Maternal Observations and Evaluations: Dams were observed twice daily for general change in appearance and behavior prior to dosing, pharmacokinetics signs following dosing, and mortality/moribundity. Body weights were obtained on gestation Days 0, 6, 10, 12, 15 and 20. Maternal body weight changes during the gestation period were calculated (G-Day 20 weights minus G.Day 0 weights minus the gravid uterine weights). All surviving does were sacrificed on gestation day 20, obvious gross pathologic alterations were recorded and each gravid uterus was weighed. The thoracic, abdominal and pelvic cavities were examined for gross lesions, and in the event of gross lesions, the tissues were preserved in neutral buffered 10% formalin. The uterus was removed from the body, examined externally, weighed and then opened for internal examination. Uteri that appeared to be from nonpregnant rats were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantations, early/late resorptions, and live/dead fetuses were recorded.

2. Fetal Examinations: Each fetus was removed from the uterus, weighed and observed for gross external alterations. Every fetus was examined to determine sex and soft tissue alterations. Fetuses were then eviscerated, stained with Alizarin red-S, and examined for skeletal alterations. In addition, the Staples fresh dissection technique to include the heart and major vessels were used to examine the incomplete twinning observed in one fetus (#6) from dam #2371 at the high dose.

D. DATA ANALYSIS

1. Statistical Analysis: Maternal body weights, maternal body weight gain, mean number of corpora lutea, total implantations, viable fetuses, and fetal body weights were analyzed by ANOVA and Dunnett's test. The number of early and late resorptions, dead fetuses and post-implantation losses were compared by the Mann-Whittney U-test. The number of fetal sex ratios were compared by the Chi-square test with Yates correction factor. The number of litters with malformations were compared by Fisher's Exact Test.

E. REGULATORY COMPLIANCE: This study was performed in conformity with the FDA-GLP Regulations (21 CFR 58) which were in effect at the time the study was conducted (1981). A Quality Assurance Statement was dated 3/2/83.

III. RESULTS

A. Maternal Toxicity

1. Mortality and Clinical Observations: No dams died at 25 or 75 mg/kg/day dose groups. Two dams delivered prematurely on gestation Day 19; one each in the control and 8 mg/kg/day groups. In both incidences, the offspring produced were of similar size and development as those from a full-term delivery. No treatment-related clinical signs of toxicity were seen; hair loss of the extremities, dried red material around the eyes and/or nares, and lacrimation were seen with similar frequency between the control and treated dams.

2. Body Weight/Body Weight Gain: Mean body weight change data are presented in Table 2. Mean body weights and body weight gain of dams at 8 and 25 mg/kg/day were comparable to those of the controls. Maternal toxicity manifested as decreases in body weight gaind in dams at 75 mg/kg/day during the dosing period; -43% during Days 6 through 10, -21% during Days 6 -15, and -2% during Days 0-20. Following cessation of treatment, however, body weight gain of these dams were either comparable to controls or slightly exceeded the control group for the remainder of gestation. There were no differences in mean uterine weight, net body weight (corrected body weight after removal of the uterus and contents) or in the adjusted body weight change in any of treated groups.

Table 2. Mean Body Weight Gain (g) of Rats Given Oral Administration of 2,4- D[a].

	Gestation Day				
Dose (mg/kg/day)	**0- 6**	**6 - 10**	**6 - 15**	**15- 20**	**0-20**
Vehicle Control	9	7	19	34	62
8	11(+22%)	5 (-29%)	18 (-5%)	36 (+6%)	66 (+7%)
25	= 9 (0%)	7 (0%)	20 (+5%)	37 (+9%)	67 (+8%)
75	9 (0%)	**4 (-43%)**	**15 (-21%)**	37 (+9%)	**61 (-2%)**

a = Data from Study Report Page 12.
b = value in parenthesis % difference from control.

3. Gross Pathology: No treatment-related macroscopical changes were observed either in the two dams that died at the high dose or in those sacrificed at termination.

4. Cesarean Section Data: Reproductive data (cesarean-sectioning and litter observations) are presented in Table 3. No biologically or statistically significant effects were seen on pregnancy rate, number of implantations, litter sizes, live fetuses or dead fetuses per litter, early and rate resorptions, fetal sex ratio, crown-rump distance, or mean fetal body weights.

Table 3. Cesarean Section Findings in Pregnant Rats Given 2,4-D[a].

Observations	Dose Level [mg/kg/day]			
	0	8	25	75
# Assigned	35	35	35	35
Pregnancy Rate (%)	29(85%)	29 (85%)	28 (80%)	27 (77%)
# Nonpregnant	5 (15%)	5 (15%)	7 (20%)	8 (23%)
Maternal Wastage				
# Died	0	0	0	0
# Died pregnant	0	0	0	0
# Died nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	1	1	0	0
Total Corpora Lutea	373	351	347	337
Corpora Lutea/Dam	12.9	12.1	12.4	12.5
Total Implantations	302	286	286	272
Implantation/Dam	10.4	9.9	10.2	10.1
Total Live Fetuses	262	276	269	250
Live Fetuses/Dam	9.0	9.5	9.6	9.3
Total Dead Fetuses	0.0	0.0	0.0	0.0
Total Resorptions	40	10	17	22
Early/Dam	1.3	0.3	0.5	0.7
Late/Dam	0.0	0.1	0.1	0.1
Pre Implantation Loss (%)	19	19	18	19
Post Implantation Loss (%)	13	4	6	8
Sex Ratio (%) ♂ : ♀	58:42	53:47	57:43	57:43
Crown-Rump Length (cm)	3.6	3.6	3.6	3.6
Mean Fetal Body Wt.(g)	3.0	3.0	3.1	3.1

a = Data obtained from Study Report Pages 26, 27.

B. Developmental Toxicity

1. External Examination: No treatment-related external anomalies were seen in any of the 262, 276, 269 and 250 fetuses examined at 0, 8, 25 or 75 mg/kg/day, respectively.

2. Visceral Examination: No treatment-related visceral anomalies were seen in any of the 131, 140, 136 and 123 fetuses examined at 0, 8, 25 or 75 mg/kg/day, respectively.

3. Skeletal Examination: No treatment-related skeletal malformations were seen in any of the 131, 140, 136 and 123 fetuses examined at 0, 8, 25 or 75 mg/kg/day, respectively. As shown in Table 4, skeletal variation observed at 75 mg/kg/day included the presence of 7th cervical and 14th rudimentary ribs, malaligned sternebrae, reduced ossification of the vertebral aches, and unossified sternebrae #5 or #6.

Table 4. Fetal Skeletal Variation in Dams Following Oral Administration of 2,4-D.

	Dose (mg/kg/day)			
PARAMETER	**0**	**8**	**25**	**75**
Litters Evaluated	26	29	27	26
Fetuses Evaluated	131	136	133	127
7th Cervical Rib(s)				
Fetuses # (%)	0	0	1 (1)[b]	4 (3)
Litters # (%)	0	0	1 (4)	3 (12)
14th Rudimentary Rib(s)				
Fetuses # (%)	0	1 (1)	0	4 (3)
Litters # (%)	0	1 (3)	0	3 (12)
Sternebrae malaligned				
Fetuses # (%)	7 (5)	12 (9)	11 (8)	15 (12)
Litters # (%)	7 (27)	9 (31)	8 (30)	10 (38)
Unossified Sternebrae #5/6				
Fetuses # (%)	62 (47)	62 (46)	71 (53)	73 (57)
Litters # (%)	24 (92)	27 (93)	27 (100)	22 (85)
Reduced ossification of the vertebral arch(es)				
Fetuses # (%)	2 (2)	0	3 (2)	6 (5)
Litters # (%)	1 (4)	0	3 (11)	5 (19)

a = Data obtained from Study Report Page. 30.

IV. DISCUSSION

1. INVESTIGATOR'S CONCLUSIONS: The authors concluded that the inhibition of body weight gain (-21 to -43%) observed at the high-dose (75 mg/kg/day) during the dosing period (Days 6-15) as slight maternal toxicity since mean values never attained control values for the period corresponding to treatment. It was also concluded that treatment had no effect on any of the cesarean parameters and that 2,4-D was not a teratogen in rats.

2. REVIEWERS DISCUSSION

   A. Maternal Toxicity

   Body Weight: When compared to the vehicle control, maternal body weight gains of dams at 75 mg/kg/day were decreased by -43%, -21% and -2% for gestation days 6-10, 6-15, and 0-20, respectively. Although these decreases were not statistically significant, they were attributed treatment-related because decreases in body weight gain were also seen in a 2-generation reproduction toxicity study (MRID No. 25944206) in the same strain (Fischer 344) of rats at a comparable dose of 80 mg/kg/day (actual dose ≈ 75 mg/kg/day). Based on these findings, for maternal toxicity, the NOEL was 25 mg/kg/day and the LOEL was 75 mg/kg/day.

   B. Developmental Toxicity: The increases in skeletal variation observed at 75 mg/kg/day (presence of 7th cervical and 14th rudimentary ribs, malaligned sternebrae, reduced ossification of the vertebral aches, and unossified sternebrae) were not statistically significant when compared to the vehicle control nor did they show dose-response. However, they were considered to be treatment related 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, as shown below in Table 5, skeletal variations of the ribs (2nd wavy ribs, lumbar ribs) and missing sternebrae were also seen in a teratology study using a different strain (Sprague-Dawley) of rats at a comparable dose of 87.5 mg/kg/day. In that study, pregnant Sprague-Dawley rats were given oral administration of 2,4-D acid at 0, 12.5, 25, 50, 75 or 87.5 mg/kg/day during gestation days 6 through 15 (Schwetz *et al.*, 197).

   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.

Table 5. Embryotoxicity and Fetotoxicity Observed in Sprague-Dawley Rats.[a]

ANOMALIES	Vehicle Control		87.5 mg/kg/day	
INCIDENCE (%)	Fetus	Litter	Fetus	Litter
Subcutaneous edema	8/242 (3)	8/41 (20)	31/117 (26)*	15/19 (79)*
Delayed ossification of sternebrae	98/245 (40)	31/41 (76)	31/119 (26)	10/19 (53)
Sternebrae with split centers of ossification	35/245 ((14)	19/41 (46)	48/119 (40)*	11/19 (58)
2nd, Wavy ribs	0/245 (0)	0/41 (0)	6/119 (5)*	2/19 (11)
Lumbar ribs	5/245 (2)	3/41 (7)	10/119 (8)*	4/19 (21)
Missing sternebrae	0/245 (0)	0/35 (0)	8/119 (7)*	5/19 (26)*

a = Data obtained from Schwetz et al. 1971.

C. Developmental Toxicity in Rabbits: In a parallel study (MRID # 41747601; HED Doc.# 008462), artificially impregnated New Zealand rabbits (20/dose) were given oral administration of 2,4-D acid in 0.5% methylcellulose at 0, 10, 30 or 90 mg/kg/day during gestation days 6 through 18. Maternal toxicity was limited to the high dose and was based on ataxia, abortion, slight nonsignificant decreases in body weight gain during the dosing and post dosing period, and a nonsignificant reduction in corrected body weight gain during the entire period. No developmental toxicity was seen at any dose level. No external, visceral, or skeletal anomalies (malformations or variations) were seen in any of the fetuses. For maternal toxicity the NOEL was 30 mg/kg/day and the LOEL was 90 mg/kg/day, For developmental toxicity, the NOEL was 90 mg/kg/day and the LOEL was > 90 mg/kg/day.

V. CONCLUSIONS

Under the conditions of this study, the following NOELs and LOELs are established:

Maternal toxicity NOEL = 25 mg/kg/day
Maternal toxicity LOEL = 75 mg/kg/day
Developmental toxicity NOEL = 25 mg/kg/day
Developmental toxicity LOEL = 75 mg/kg/day

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

13544

001175

Chemical:	**2,4-Dichlorophenoxyacetic acid**
PC Code:	***030001***
HED File Code	**13000 Tox Reviews**
Memo Date:	**05/23/1996**
File ID:	**TX011934**
Accession Number:	**412-01-0082**

HED Records Reference Center
01/10/2001