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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 **MRID No. 43612001.** Unpublished.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

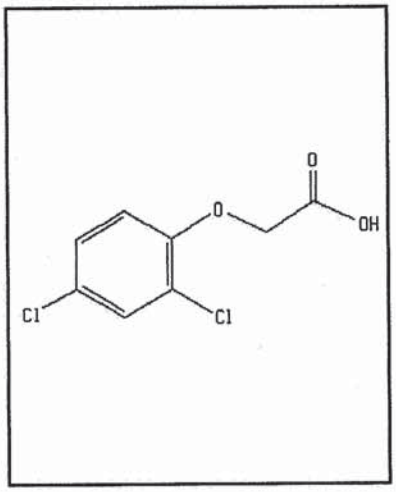
In 1988, the Agency required that rodent carcinogenicity testing with 2,4-dichlorophenoxy acetic acid (2,4-D) be repeated because a Maximum Tolerated Dose (MTD) had not been achieved in the Industry-sponsored studies. In the Data Call-In notice of 1989, the Agency formally requested that the carcinogenicity 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:



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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 Esophagus[a] x Stomach x Duodenum[a] x Jejunum[a] x Cecum[a] x Colon[a] x Ileum[a] x Rectum[a] x Liver[ac] x Pancreas[a] Nervous System x Brain [cerebrum, brain stem, cerebellum][ac] x Pituitary[a] x Peripheral nerve[ab] x Spinal cord (3 levels)[ab] x Eyes[ab] Glandular System x Adrenals[a] x Lacrimal glands[b] x Parathyroids[ad] x Thyroids[ad]	x Trachea[a] x Lung[a] Pharynx[e] x Larynx[e] Nose[e] x Nasal Tissues Cardiovascular/Hematopoietic System x Aorta (thoracic)[a] x Heart[a] x Bone marrow[a] x Lymph nodes[a] x Spleen[a] x Thymus[a] Urogenital System x Kidneys[ac] x Urinary bladder[a] x Testes[ac] x Epididymides x Prostate x Seminal vesicles x Uterus[a] x Ovaries[ac] 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 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.

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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	0	0	0	10	0	0	0	0
lobular and midzonal :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
		2	-	-	4	4	0	0	0
		0	-	-	0	0	0	0	1
hyperplasia, parafollicular cells, focal									
hypertrophy, epithelial cells, focal									

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

83-5 Combined Chronic Toxicity/Carcinogenicity - Rat

PC Code: 030001 Tox Chem No. 315 File Last Updated _____ Current Date _____

2,4-Dichlorophenoxy acetic acid

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA MRID NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
83-5 Combined Chronic Toxicity/Carcinogenicity Species: Rats Tox.Res.Labs K-002372-064 3/28/95	2,4-D acid 96.4%, Technical	436120-01	Dose levels: 0, 5, 75 or 150 mg/kg/day for up to 24 months. No treatment-related effects were seen in either sex at 5 mg/kg/day. Treatment-related effects observed at 75 mg/kg/day were decreases in body weight, body weight gain and food consumption (females), decreases in T_4 concentration (both sexes), increases in absolute/relative thyroid weights (femaels), degeneration of the proximal kidney tubules (both sexes), altered tinctorial properties in the liver (females) and sub acute to chronic inflammation of the lungs (females). Treatment-related effects at 150 mg/kg/day in both sexes included: decreases in body weight, body weight gain and food consumption; increases in ALT, AST, and AP acitivities; decreases in cholesterol level and T_4 concentration; increases in absolute and/or relative weights of thyroids; and histopathological lesions in the eyes, liver, lungs, and mesenteric tissue (adipose tissue). For chronic toxicity, the NOEL = 75 mg/kg/day in males and 5 mg/kg/day in females. The LOEL = 150 mg/kg/day in males and 75 in females. There was no evidence of carcinogenicity in either sex.	NA	Acceptable

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