UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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Office of
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Toxic Substances

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

TXR# **0051360**

April 11, 2006

MEMORANDUM

SUBJECT: **2,4-D** (P. C. Code: 030001) [DP Barcode D287657]. Transmittal of Amended Data Evaluation Records (DERs) [Executive Summaries] to Upgrade Original DERs on 2,4-D and Its Amine Salts [P. C. Codes 030016, 030019, 030025, 030035] and Esters [P. C. Codes 030053, 030063, 030066] and New DERs on the Isopropyl Ester of 2,4-D.

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The original Data Evaluation Records (DERs) of the toxicology studies performed on 2,4-D and its amine salts and esters were upgraded [Executive Summaries] as part of the toxicology review for the risk assessment conducted in association with the Reregistration Eligibility Decision for 2,4-D. Additionally, ## studies not previously reviewed were evaluated, and DERs for these were generated. The new Executive Summaries, and in some cases data tables, and the new DERs are appended.

FEB 6 REC'D 2008

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MRID 41735201. Lochry, E. A. (1990). Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-D Dimethylamine Salt (2,4-D-DMA) Administered Orally *via* Gavage to Crl:CD®BR VAF/Plus® Presumed Pregnant Rats..
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MRID 42021402. Yano, B. L.; Cosse, P. F.; Atkin, L.; *et al.* (1991). 2,4-D Triisopropanolamine Salt [2,4-D TIPA]: A 13-Week Dietary Toxicity Study in Fischer 344 Rats.

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MRID 41920905. Siglin, J.C. (1991). 21-Day Dermal Toxicity Study in Rabbits with Diethanolamine Salt of 2,4-D.

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[2,4-DICHLOROPHENOXYACETIC ACID] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Supplement to DER for ACCESSION NO. ~~00251031~~ - Developmental Toxicity Study - Rat [2,4-Dichlorophenoxyacetic Acid] MRID 00130407

This supplement provides an EXECUTIVE SUMMARY and data tables to upgrade the re-review [Document Nos. 011934] of the original DER [Document No. 003887].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 11/19/02
Reregistration Branch I, Health Effects Division (7509C)
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Branch Senior Scientist, Reregistration Branch I, Health Effects Division (7509C)
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AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rat § 83-3(a)/OPPTS 870.3700
DP BARCODE: N/A SUBMISSION NO.:
P.C. CODE: 030001
CASWELL NO.:315 CAS NO.: 94-75-7
TEST MATERIAL (PURITY) : 2,4-Dichlorophenoxyacetic acid [%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force on 2,4-D Research Data

CITATION: Nemec, M. D.; Tasker, E. J.; Werchowski, K. M.; *et al.*. (1983). A Teratology Study in Fischer 344 Rats With 2,4-Dicholorphenoxyacetic Acid. WIL Research Laboratories, Inc.; Study No. WIL-81135. Accession No. 00251031. Document No. 011934. Unpublished. MRID 00130407

[MRID 00130407]

EXECUTIVE SUMMARY: In a developmental toxicity study [Accession No. 00251031], pregnant Fischer 344 rats [35/group] were administered 2,4-dichlorophenoxyacetic acid [97.5%] *via* gavage at dose levels of 0 [corn oil], 8 mg/kg/day, 25 mg/kg/day, and 75 mg/kg/day from gestation day [GD] 6 through gestation day 15.

There were no treatment-related deaths. Two [one control and one low-dose] dams delivered prematurely on gestation day 19, and in both instances the offspring produced were of similar size and development as those from full-term delivery. Clinical signs were comparable among the groups. Body weights were comparable among the groups throughout the study, but dams at the high-dose level displayed a decrease in body-weight gain during the dosing period [79% of control for GD 6-15; 57% of control for GD 6-10], although statistical significance was not attained. The corrected body-weight gain was comparable among the groups. Food consumption data were not reported.

The was a slight decrease in pregnancy rate with increasing dose [85%, 85%, 80% and 77%]. The numbers of corpora lutea, implantations, and live fetuses were comparable among the groups, and there were no dead fetuses. The number of resorptions, as well as pre- and post-implantation losses, were not adversely affected by treatment. The number of the dams with 100% resorptions was 2, 0, 1, and 1 [control, low-, mid-, and high-dose groups,

respectively]. One control, 2 low-, 4 mid-, and 2 high-dose dams had late resorptions. Fetal body weight and crown-rump length were comparable among the groups, as was the sex ratio.

There were no statistically-significant or treatment-related differences in the incidence of fetal external or visceral malformations. There was an increased incidence of skeletal malformations/variations at the high-dose level that included the presence of 7th cervical ribs [4 fetuses in 3 litters *vs* none in the control], presence of 14th rudimentary ribs [4 fetuses in 3 litters *vs* none in the control]; malaligned sternebrae [15 fetuses in 10 litters *vs* 7 fetuses in 7 litters of the control]; reduced ossification of the vertebral arches [6 fetuses in 5 litters *vs*2 fetuses in 1 litter of the control]; and unossified sternebrae # 5 or #6 [73 fetuses in 22 litters (3.32 fetuses/litter) *vs* 62 fetuses in 24 litters (2.58 fetuses/litter)]. Although none of the increases attained statistical significance, they were attributed to treatment since some of the findings [maligned sternebrae, 14th rudimentary ribs, and reduced ossification of vertebral arches] were also observed in the F1b 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 rat. Additionally, skeletal findings [2nd wavy ribs, lumbar ribs] and missing sternebrae were observed in another developmental toxicity study using a different strain of rat [Sprague-Dawley] at a comparable dose of 87.5 mg/kg/day [2,4-D].

The maternal toxicity NOAEL is 25 mg/kg/day, and the maternal toxicity LOAEL is 75 mg/kg/day, based on decreased body-weight gain.

The NOAEL for developmental toxicity is 25 mg/kg/day, and the developmental toxicity LOAEL is 75 mg/kg/day, based on increased incidence of skeletal abnormalities.

This developmental toxicity study is classified **Acceptable/Guideline**, and it satisfies the guideline requirement [OPPTS 870.3700; §83-3(a)] for a developmental toxicity study in the rodent. NOTE: In 1996, it was determined that the original DER [Document No. 003887] was inadequate, and the study was re-evaluated [Document No. 011934]. The NOEL/LOEL were the same in both reviews. The current EXECUTIVE SUMMARY does not alter the no-effect and effect levels but reflects current terminology for the no-observed-adverse-effect level [NOAEL] and lowest-observed-adverse-effect level [LOAEL]. Additionally, the presence of 7th cervical ribs and 14th rudimentary ribs are considered skeletal malformations; previously listed as skeletal variations.

[2,4-DICHLOROPHENOXYACETIC ACID] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Gravid Uterine Weight/Corrected Body Weight/Gain [grams]				
Dose✦/Day	0 mg/kg/day	8 mg/kg/day	25 mg/kg/day	75 mg/kg/day
body weight				
0	198±11	196±12	193±10	201±13
6	207±14	208±15	203±12	210±13
10	214±16	213±15	210±12	214±13
12	219±18	217±16	214±12	218±13
15	225±13	226±17	223±13	226±15
20	261±22	262±26	260±20	262±25
body-weight gain				
0-6	9±5	11±5	9±8	9±4
6-10	7±5	5±4	7±6	4±4 [57]√
10-12	5±6	5±3	4±3	4±2
12-15	7±14	8±4	9±3	7±4
15-20	34±14	36±12	37±12	37±14
6-15	19±8	18±6	20±8	15±7 [79]
0-20	62±21	66±17	67±17	61±19
gravid uterine weight	42.1±17.9	45.5±16.4	44.5±13.5	42.6±16.1
corrected body weight	217.1±12.8	217.1±15.6	214.9±12.5	219.5±14.9
corrected BWG [0-20]	18.3±7.9	20.7±6.3	21.5±7.6	18.5±6.9

√[% of control]; data from Tables 9-11, pages 32-52 of the report

Table 2. Resorptions				
Parameter/dose	0 mg/kg/day	8 mg/kg/day	25 mg/kg/day	75 mg/kg/day
# dams w/ resorptions				
early	9	8	8	8
late	1	2	4	2
# dams w/out viable fetuses	2	0	1	1

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SECONDARY REVIEWER: Stephen C. Dapson, Ph.D., Senior Pharmacologist
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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.**

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)].

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.

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.

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.

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 gains 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.

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.

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.

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

I. INTRODUCTION

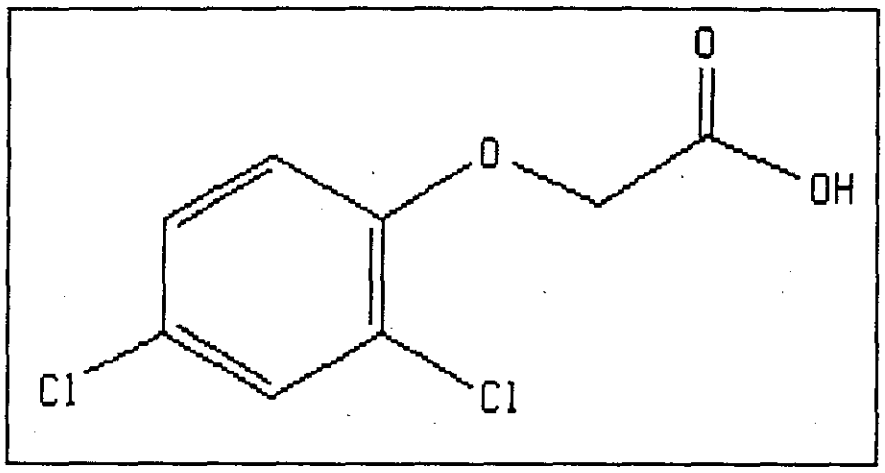
This study is re-reviewed due to the inadequacy of an earlier review (HED Doc. # 003887). The objective of this study was to assess the effects of 2,4-dichlorophenoxyacetic acid on the embryonic and fetal development following oral administration to rats during the period of organogenesis.

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

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.**

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)].

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

003887

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 3,C,2,b data call-in on 2,4-D. Teratology of 2,4-D and 2,4 Dichlorophenol with respect to PP# 3E2876 related action.
CASWELL No. 315.

TO: Richard Mountford, PM #23
Herbicide Branch/RD (TS-767)

Lois Rossi, PM #61
SRB/RD (TS-767)

THRU: Robert B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

FROM: Henry W. Spencer, Ph.D.
Review Section #1
Toxicology Branch/HED (TS-769)

Conclusions and Recommendations:

1. The range-finding and teratology studies are sufficient to indicate that 2,4-D and 2,4-Dichlorophenol are not teratogenic at up to 75 mg/kg and 750 mg/kg, respectively.

2. Toxicology Branch considers the studies adequate to indicate a fetotoxic effect for 2,4-D at 75 mg/kg (LEL) and a NOEL of 25 mg/kg. Delayed ossification is the fetotoxic effect.

3. Toxicology Branch considers the 2,4-Dichlorophenol teratology studies adequate to indicate an LEL of 750 mg/kg and a NOEL of 350 mg/kg for delayed ossification. A teratogenic effect is not demonstrated in the study.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 Page 21 of 250

4. Toxicology Branch recommends that these studies be added to the data base to support the registrations of 2,4-D.

5. It is noted that generally a maternally toxic dose should be attained in a teratology study. However, in these cases 2,4-D has been previously tested by a registrant, Dow Chemical Co. which demonstrated a slightly maternal toxic effect at 87.5 mg/kg in rodents. These data were run in a manner to indicate a NOEL for only fetotoxicity.

As a result of previous studies in other species and strains of rodents, the Toxicology Branch continues to consider 2,4-D to be a teratogenic agent. These previous studies are in no way negated by the lack of a teratogenic finding in these current studies since they were run at much higher dosage levels.

Summary of Toxicity Studies Reviewed

I. 2,4-D acid

a. Range finding study of 2,4-D for maternal toxicity:
(HDT) = 250 mg/kg
LEL for maternal toxicity = 150 mg/kg
NOEL for maternal toxicity = 100 mg/kg for reduction in feed consumption and body weight loss.

b. Teratology study of 2,4-D

LEL for maternal toxicity NOT found
NOEL for maternal toxicity = 75 mg/kg = (HDT)
LEL for fetotoxicity (delayed ossif.) = 75 mg/kg
NOEL for fetoxicity = 25 mg/kg
NOT teratogenic at up to 75 mg/kg (HDT)

II. 2,4-Dichorophenol

a. Range finding study with 2,4-Dichlorophenol:

Phase III LEL = 750 mg/kg (LDT)

Phase II LEL = 400 mg/kg (500 mg/kg = HDT)
NOEL = 300 mg/kg for mucous membrane effects (maternal)

Phase I LEL = 75 mg/kg (HDT = 150 mg/kg)
NOEL = 25 mg/kg for mucous membrane effects (maternal)

b. Teratology Study with 2,4-Dichlorophenol Maternal Toxicity LEL = 200 mg/kg (LDT) equivocal wt. gain effects.

Fetotoxicity LEL = 750 mg/kg
NOEL = 350 mg/kg as delayed ossification.
Not teratogenic at up to 750 mg/kg (HDT)

003887

Study:

Range-finding teratology study in Fischer 344 rats with 2,4-Dichlorophenoxy acetic acid by WIL Research Labs. Inc. dated May 17, 1983. WIL - 22002., Acc. No. 251032.

Material Tested:

2,4-Dichlorophenoxyacetic acid (Technical) from ITT Research Institute, Chicago, Ill. on March 15, 1982, 97.5% purity.

Animal Tested:

60 virgin, sexually mature female, Fischer 344 rats from Charles River Labs, Portage Michigan were used.

Methods:

After a 25 day quarantine, the females were bred if body wts. were greater than 170 g. Evidence of gestation day 0 was the finding of sperm from a vaginal smear. 10 rats were assigned to each dosage level. Dosages of 0, 75, 100, 150, 200 and 250 mg/kg were tested. The test material was mixed in corn oil and dosed at a volume of 4 ml/kg by gavage on days 6-15 of gestation.

Clinical observations were made daily on days 0-16 of gestation. Body wts. were also recorded on days 0-16 of gestation.

On day 16 of gestation the dams were sacrificed. The usual parameters were noted and included: number of corpora lutea formed, determination of viable and non viable fetuses and implantation status.

Statistical significance of changes was tested by using programs on a Digital Computer. Methods were obtained from: BMPD-79 Biomedical Computer Programs, Univ. of Calif. Press, Berkely, Calif. 1979. PP. 612, 780, 781.

Results:

A statistically significant reduction in body wt. gain was noted for groups at (1) 150 mg/kg on days 15 and 16 of gestation and (2) 200 mg/kg and 250 mg/kg on days 10 through 16 of gestation. Mean body wt. changes were not statistically different from controls at 75 and 100 mg/kg throughout the study while significant changes were noted at 150 mg/kg and above.

Feed consumed on a mean group basis was found to be significantly reduced at 150 mg/kg and above.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 23 of 959

003387

Dams treated in the study did not abort. However, 1/10 dam in the 200 mg/kg group and 3/10 dams in the 250 mg/kg group died during treatment. Total resorptions occurred in 2/8 pregnant females at 150 mg/kg and in 8/8 and 7/7 pregnant dams at 200 mg/kg and 250 mg/kg respectively.

A significant reduction in viable fetuses is seen at 150 mg/kg where 6.4 fetuses per litter were produced when compared to 9.1/litter in controls. No viable fetuses were found at 200 and 250 mg/kg. No effect on the number of viable fetuses at 100 mg/kg and below was seen. The LEL for resorptions, increased post implantation losses and implantation site effects is 150 mg/kg. A threshold NOEL is 100 mg/kg for fetotoxic and or lethal effects noted. It is based on the slight increase in litters displaying early resorptions even though no increase in numbers of litters effected was seen.

Liver-to-body wt. ratios and kidney wts./body wt. ratios in dams were increased above 150 mg/kg.

	Ratio Liver/bwt.	Right/Ratio Kidney/bwt.	Left/Ratio Kidney/bwt.
Control	.0436	347	359
75 mg/kg	.0412	361	370
100	.0414	372	367
150	.0440	374	369
200	.0473	384	386
250	.0524	394	399

Histopathological findings were reported as negative.

Conclusions:

This reviewer considers an LEL for maternal toxicity based on a reduction in feed consumption as 150 mg/kg. A NOEL is 100 mg/kg. The study is to determine a NOEL for maternal toxicity but is of limited value because the evaluation only lasted for 16 days and not the 21 days as would be the case in the teratology study.

Core:

Supplementary data.

Study:

A range finding Teratology Study/s in Fischer 344 rats with 2,4-Dichlorophenol. Phase I, II, III by WIL Research Labs. Inc. WIL-81133 dated March 31, 1983 for the Industry Task Force on 2,4-D Research Data. Acc. No. 251029.

Material Tested:

2,4-Dichlorophenol purity - approximately 99.2%. I.D. No.: AGR 182992.

Animal Tested:

Fischer 344 virgin female rats from Charles River Labs, Portage, Mich.

Methods:

After a quarantine period and an acceptable body wt. of 170 g or greater, animals were bred. Groups of ten animals were gavaged with 0, 750, or 1000 mg/kg in Phase III; 0, 200, 300, 400, or 500 mg/kg in Phase II; 0, 25, 75, or 50 mg/kg in Phase I. The test material was suspended in corn oil and dosed in a volume or 4 ml/kg.

Dosages were given on days 6 through 15 of gestation. Gestation day 0 was determined with the finding of sperm by a vaginal smear. Feed, Purina® rodent chow, and tap water were provided ad libitum. Temperature, humidity, and photoperiods were 72 ± 3° F, 40% and 12 hr. respectively throughtout the study.

Clinical observations were made daily on days 0-16 of the study. Body wts. were recorded daily and calculated for 3 day intervals. Food and water intake values were recorded for the above intervals. Uterine and ovarian examination allowed for counting corpora lutea, implantations and locations, number of viable fetuses, and resorptions. Organ wts. of livers and kidneys were recorded.

Statistical evaluation was made using appropriate programs for a Digital Computer with significance for 2-tailed tests set at $p < .05$. The Mann-Whitney U-test was used to compare early and late resorptions, dead fetuses and post implantation losses. Other parameters were analyzed by a one way ANOVA and Dunnett's test.

Results:

Phase III, (0, 750, 1000 mg/kg). Body wts. of treated dams were statistically reduced only on the last few days at 750 mg/kg and by day 9 at 1000 mg/kg. Food intake was significantly depressed at 750 mg/kg and markedly depressed at 1000 mg/kg after day 6 of study. No females aborted. However, 7/10 females and 1/10 at 1000 mg/kg and 750 mg/kg respectively died during the study. All dams dying were pregnant. One of 2 pregnant dams at 1000 mg/kg totally resorbed its embryos. Viable fetuses were reduced only at the highest dosage when compared to controls because one of two dams completely resorbed.

Implantation sites were not reduced in treated dams. However, early resorptions were increased at the HDT. No significant changes in liver and kidney were noted at 750 mg/kg wher compared to controls. A slight increase in kidney wt./body wt. ratios was seen, ($p < .05$), but so few (3) dams were available for analysis, that the signficance of this value is questionable. Red staining of the urogenital area was noted at 1000 mg/kg. Mucous membrane effects were also noted at 750 mg/kg.

Phase II: (0, 200, 300, 400, 500 mg/kg). All treated females exhibited a slight but non-significant reduction in weight gain values. Feed consumption was also only slightly reduced when compared to controls.

No females aborted in the study and none died prior to completion of the study. Total resorption of fetuses did not occur in treated dams. Only at 300 mg/kg was the number of viable fetuses reduced, but is considered to be only a spurious non-treatment effect since the effect was not seen at other dosage levels. Slight, nonsignificant increases in early resorptions and post implant loses in both groups treated with 400 and 500 mg/kg are noted. Only a relative organ wt. increase is noted in the liver of dams at the HDT (500 mg/kg).

Dried red discharges (LEL = 400 mg/kg, NOEL = 300 mg/kg) were increased over controls at 400 and 500 mg/kg. Some occurrances of these dried discharges appeared to be the result of cleaning other areas of the body. An LEL of 400 mg/kg is set for the dams using mucous membrane red discharges with a NOEL of 300 mg/kg.

Phase I (0, 25, 75, 150 mg/kg)

Body wt. gains of treated dams were not different from controls during gestation. Values for feed intake per animal in each group were similar. No females died or aborted in the study. Group pregnancy rates were not significantly different within the study. Controls exhibited an unusually low number of implantation sites, and a subsequent lower number of viable fetuses than seen in the other treatment groups. Relative organ wts. were not different when comparing groups.

Clinical observations included urogenital staining in 3 and 4 30% and 50% of treated dams in groups respectively, exhibiting the effect. Red vaginal discharge was occassionally observed in groups at 25, 75, and 150 mg/kg with 20%, 30% and 10%. females involved respectively.

There were no teratogenic or fetotoxic effects at doses up to and including 150 mg/kg (HDT). However, effects on the mucous membranes in the dams were evident at 75 mg/kg with no observable effects at 25 mg/kg.

The dried red discharge on the nares was noted in all groups with substantial increase in response seen at 75 and 150 mg/kg compared to controls.

These data (e.g. range-finding studies) are considered Core: Supplementary.

Study:

Teratology study in Fischer 344 rats with 2,4-Dichlorophenoxy acetic acid by WIL Research Labs. Inc. dated March 3, 1983. Lab No. WIL - 81135, for the Industry Task Force on 2,4-D Research Data. Acc. No. 251031.

Material Tested:

2,4-D acid, Technical grade from ITT Research Institute, Chicago, Ill. 97.5% 2,4-D acid and related chlorophenoxy compounds, 1.41% water, and low ppb levels of the 2, 7 dichlor; monochloro; 1, 3, 8 and 1, 3, 6, 8 chlorinated-p-dioxins as contaminants.

Animal Tested:

Virgin, Fischer 344 rats from Charles River, Labs., Portage, Michigan.

Methods:

After acclimation and quarantine for a period of 1 week, test animals were individually caged, given Purina® rodent chow #5002 and municipal water ad libitum. Fresh air was at 72° ± 3° F with 40% relative humidity and a 12 hr. light-darkness cycle. At breeding each female must have weighed greater than 170 g. Cohabitation with males was recorded and evidence of sperm by vaginal smear represented day 0 of gestation. Thirty five females were placed in each test group by randomized block determination.

The test material was suspended in corn oil and given in a dosage volume of 4 ml/kg by gavage so that groups were given either corn oil, 8, 25, or 75 mg/kg of the 2,4-D acid on days 6-15 of gestation. Clinical observations were recorded on days 0-20 of gestation. Observations were made 2X daily. Body wts. of dams were recorded on days 0, 6, 10, 12, 15 and 20 of gestation. At termination of the study, corpora lutea were enumerated, and fetuses were recorded as viable or nonviable. Resorptions were reported as early or late, and implantation sites were enumerated. Ammonium sulfide staining was performed on nongravid uteri.

Fetal Observations:

Individual body wts. and crown to rump lengths were determined for each fetus. External examination findings were recorded. Approximately one-half the number of fetuses born were fixed in Bouins solution for Wilson sectioning. The other 50% of fetuses were fixed in 95% alcohol and prepared for skeletal examination by the Alizarin Red S dye method of Dawson.

Statistical Examination: Excerpted from study report.

Two-tailed tests were limited at the 5% p-value. "All statistical tests were performed by a Digital Computer with appropriate programming as referenced below.

1. The fetal sex ratios were compared by the Chi-square test[7] with Yates correction factor.

2. The number of litters with malformations were compared by Fisher's Exact Test[7].

3. The number of early and late resorptions, dead fetuses and post-implantation losses were compared by the Mann-Whitney U-test[7].

4. Mean number of corpora lutea, total implantations, viable fetuses, mean fetal and maternal body weight at each interval and maternal body weight gain were analyzed by a one-way analysis of variance, and Dunnett's test.[8]

References for statistical tests above:

7. BMPD - 79 Biomedical Computer Programs, Univ. of California Press, Berkeley, California, 1979. pp. 612, 780-781.

8. Steel, R.G.D. and Torrie, J.H., Principles and Procedures of Statistics, McGraw Hill, New York, New York, 1960 pp. 106, 107, 114."

Results:

Pregnancy status of rats treated with 2,4-D:

	Number	Nongravid	Pregnant	% Pregnant
Controls	34	5	30	85
8 mg/kg	34	5	30	85
25 mg/kg	35	7	28	80
75 mg/kg	35	8	27	77

One control and one group 1 (8 mg/kg) female delivered early. Treated animal pregnancy rates were not significantly different from controls.

Mean weight gains in the dams were slowed in the 6-10 day period and only slightly in the 10-12 day period in the group four (75 mg/kg) females. These changes are only equivocal.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 29 of 959

003887

Data presented in the report indicate that no significant differences were apparent between treated and control groups when comparing the mean number of viable fetuses per litter for controls (9.0) vs. 9.3 in 75 mg/kg group litters (HDT). No increased numbers of born-dead fetuses are noted.

Fetal Parameters:

Fetal weights were not significantly different in the 75 mg/kg (HDT) group when compared to controls. In addition, crown-rump lengths were similar in all tested groups. Group 4 (75 mg/kg) early and later resorptions were not increased over control values.

External malformations occurred in a sporadic fashion. Cleft palate was seen in only 1 fetus of 1 litter at the HDT. One fetus in one litter of control exhibited exencephaly. One fetus in one litter at the HDT exhibited incomplete twining. Micropthalmia and/or anopthalmia occurred in 1 fetus in each of two litters in control and 8 mg/kg groups, respectively; while 1 fetus in 1 litter at HDT dose was also found with opthalmic abnormality.

Visceral abnormalites in the 75 mg/kg group were limited to one fetus in one litter as kidney and/or ureter missing, one fetus with hydrocephaly, and one fetus with ovary and/or uterus absent. No significance to these incidences is noted.

Skeletal anomalies were limited to occurrances of severely malaligned sternebrae with one fetus in one litter at 25 mg/kg, one fetus in each of two litters at 75 mg/kg. In addition, 1 vertebral anomally was noted in the control litters.

Developmental variations (feto toxicity) were seen to be slightly increased only at the HDT. These variations at the HDT included an increase in: 7th cervical rib to 4 occurrances in 3 litters, vs 0 in control; 14th rudimentary rib to 4 in 3 litters, vs 0 in controls; reduced ossification increased to 6 in 5 litters vs 2 in 1 control litter and; 15 fetuses in 10 litters with malaligned sternebrae vs 7 in 7 litters in controls. A slight increase in unossified sternebrae #5 and #6 was noted in 73 fetuses/22 litters (3.31 litter) at the HDT while 62 fetuses in 24 litters (2.58 aver/litter) exhibited the effects in controls.

Conclusion:

Toxicology Branch considers the study to exhibit slight fetotoxicity expressed as delayed ossification: LEL = 75 mg/kg
NOEL = 25 mg/kg

The study does not indicate a teratogenic effect at the 75 mg/kg (HDT). Core: Minimum.

003887

Study:

A teratology study in Fischer 344 Rats with 2,4-Dichlorophenol by WIL Research Labs Inc. Project No. WIL - 81134 dated March 31, 1983, for Industry Task Force on 2,4-D Research Data., Acc. No. 251030.

Material Tested:

AGR 182992, 99.2% of 2,4-Dichlorophenol.

Animal Tested:

Fischer 344 sexually mature, virgin, female rats from Charles River Labs, Portage Michigan.

Methods:

After a quarantine period of approximately 2 months 203 females were bred. Positive sperm findings on daily vaginal smears determined day 0 of gestation. Females with positive smears were randomly placed into 4 groups of 34 animals each. The groups were gavaged on days 6-15 of gestation with either 0, 200, 375 or 750 mg/kg of the test material suspended in corn oil at 4 ml/kg. Feed and water were supplied ad lib throughout the study. Photoperiods were 12 hrs., temperature was maintained at 72 ± 3° F with a relative humidity of 40%. Clinical observations of the individual dams were recorded daily through day 20 of gestation. Body wts. were recorded on days 0, 6, 10, 12, 15 and 20 of the study period. Uterine examination provided implantation sites, resorptions, post-implantation losses, and numbers of viable fetuses. Examination of the fetuses provided numbers of external malformations, anomalies and, crown-rump measurements. Visceral examination of approximately half the fetuses was carried out and the remaining fetuses were fixed in alcohol, stained and cleared for skeletal examination using Alizarin Red S.

Statistical evaluation of the parameters were carried out by the Laboratory as presented below: excerpted from study report.

"All statistical tests have been performed by a Digital Computer with appropriate programming as referenced below.

1. The fetal sex ratios were compared by the Chi-square test[6] with Yates' correction factor.

2. The number of litters with malformations were compared by Fisher's Exact Test[6].

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 31 of 959

003887

3. The number of early and late resorptions, dead fetuses and post-implantation losses were compared by the Mann-Whitney U-test[6].

4. Mean number of corpora lutea, total implantations, viable fetuses, mean fetal and maternal body weight at each interval and maternal body weight gain were analyzed by a one-way analysis of variance, and Dunnett's test[7].

References to Statistical Tests in Submission:

6. BMPD - 79 Biomedical Computer Programs, University of California Press, Berkeley, California, 1979. pp. 612, 780, 781.

7. Steel, R. G. D. and Torrie, J. H., Principles and Procedures of Statistics, McGraw Hill, New York, New York, 1960. pp. 106, 107, 114."

Results:

Mean body wts. of only group 4 (750 mg/kg) were significantly reduced throughout the study following day 6 of gestation. However, mean body wt. changes within the 3 day-time intervals of 12-15 days were significantly reduced in all treated groups compared to controls ($p < .01$). Only 4/34 dams at the (HDT), 750 mg/kg, died during the study. These 4 dead females were also gravid. Pregnancy rates were similiar in all groups. Total resorptions of litters were not increased compared to controls. Sex ratios of pups were similar in all groups. Mean numbers of viable pups per litter were reduced in all treated groups compared to controls but were not significantly reduceds. Early resorptions were increased on a litter basis in group 4 to 1.2 $\pm$ 2.7 S.D. compared to 0.8 $\pm$ 0.9 S.D. in controls. Postimplantation losses were also slightly increased to 1.4 $\pm$ 0.9 S.D. and late resorptions were increased to 0.2 $\pm$ 0.4 S.D. compared to none in control. The values taken together suggest an embryotoxic effect at 750 mg/kg but were all nonsignificant. Developmental effects such as exencephaly, micro/or/anopthalmia or cleft palate, occurred in test groups at rates which were not dissimilar to those occurances noted in controls. Skeletal effects such as malaligned sternebrae were not significantly different from controls. Development variations, with the exception of occurances of unossified sternebrae #1 - #4, and reduced ossification of vertebral arches, were not signficantly increased when compared to controls.

51

003887

Conclusion:

Fetotoxicity expressed as delayed or unossified bones exhibited an LEL of 750 mg/kg and a NOEL of 375 mg/kg. The test material was not teratogenic under the conditions of the study.

Maternal toxicity expressed as reduced body wt. gain was equivocal in all test groups except the HDT, which produced death in 4/34 as well as significant reductions in body wt. gains.

Core: Minimum.

TS-769L:SPENCER:sll:X73710:4/6/84: card

Supplement to DER for MRID [Accession No. ~~259442-259446~~]- 2-Generation Reproduction Study - Rat [2,4-D] MRID 00150557/00163996

This supplement provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document Nos. 005446, 005684, 005754].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 11/19/02
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Whang Phang, Ph.D. Whang Phang 11/19/02
Branch Senior Scientist, Reregistration Branch I, Health Effects Division (7509C)
TXR# 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 2-generation reproduction - rat § 83-4/OPPTS 870.3800
P.C. CODE: 030001
CASWELL NO.: 315 CAS #: 94-75-7
TEST MATERIAL (PURITY): 2,4-D [97.5% a.i.]
SYNONYMS: 2,4-dichlorophenoxyacetic acid
SPONSOR: Industry Task Force on 2,4-D Research Data

CITATION: Kopp, S., Leist, P. L., Mercieca, M. D., *et al.*. (1985). A Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxyacetic Acid. Study No. WIL-81137, July 26, 1985. Accession Nos. ~~259442-259446~~]. Document No. 005446 and Addendum: Accession No. 265489, Document Nos. 005754 and 005684. Unpublished. MRID 00150557 and MRID 00163996

MRID 00150557 & 00163996

EXECUTIVE SUMMARY: In a 2-generation reproduction study [MRID (Accession No. 259442-259446,265489)], 30 male/30 female F0 Fischer 344 rats/sex/group were administered 2,4-D [97.5% a.i.] *via* the diet for 105 days prior to mating and through gestation and lactation of two litters and for 30 days after weaning the last litter at target dose levels of 0, 5, 20, and 80 mg/kg/day. Rats were mated, one male with one female. The resulting F1a litters were weaned at day 28 *post partum*. After a 2-week rest period, the F0 parental rats were re-bred using different male/female combinations to produce the F1b litters, from which 30 males/30 females/group were selected to become the F1 parents. The F1 generation [30 rats/sex/group] was administered the test material at target dose levels of 0, 5, and 20 mg/kg/day [**high-dose level dropped due to excess toxicity**; there were an insufficient number of F1b pups] *in utero* and continuously *via* the milk or feed for 125 days postnatally and prior to mating and through gestation and lactation of two litters [F2a and F2b] and for 30 days after weaning the last litter.

There were no apparent treatment-related deaths, and clinical signs were comparable among the groups throughout the study. During the *pre-mating dosing period*, body weights of the **F0 parental animals** were slightly lower [males 95%-97% (by week 6)/females 95%-96% (by week 13) of control] at the high-dose level for both sexes. Body-weight gains of the F0 high-dose males were decreased initially [weeks 2-3 (86% of control] and overall [weeks 0-13 and weeks 0-40 (93% of control], as were those of the high-dose females [weeks 0-1 (79% of control); weeks 0-13 (92% of control) and weeks 0-40 (94% of control)] compared to the controls.

The high-dose **F0 dams** displayed a significantly lower body weight throughout [F1A litter] gestation (94%-95% of control) and by gestation day 20 during F1b pregnancy [90% of control]. The high-dose F0 dams displayed significantly reduced body-weight gains compared to the controls during both gestation periods, with the greater

deficit being observed during the second gestation period [F1a litters: days 0-7 (67%* of control); days 13-20 (95% of control; days 0-20 (87% of control); F1b litters: days 0-7 (70%* of control); days 13-20 (59%** of control); days 0-20 (67%** of control)]. The high-dose F0 dams displayed decreased body weight on day 7 of lactation [both litters; 92%-93% of control], but body weights were significantly increased compared to the controls at day 28 of lactation [F1a (108%/F1b 111% of control]. Body-weight gains were significantly reduced during lactation days 1-7 for both litters [F1a (40% of control); F1b (6% of control)]. Overall, however, the high-dose dams displayed positive body-weight gain during lactation days 1-28 compared to negative body-weight gains in the control and other dose groups.

Food consumption [g/rat/day] during the pre-mating period was slightly lower [94%-95% of control] in the high-dose females during a few weeks, but on a g/kg/day basis, both sexes at the high-dose level displayed a slight increase [104% of control] in food consumption compared to the controls. During the first week of the two-week rest period following the weaning of the first litter, the F0 dams displayed a significant decrease in food consumption [83%-84% of control]. Food consumption was decreased at the high-dose level during both gestation periods [F1a during first 2 weeks (91%-93% of control); F1b during third week (82% of control)]. A significant decrease in food consumption was observed throughout lactation [both litters] at the high-dose level [F1a litter (58% of control for days 1-28); F1b litter (71%-83% of control)]. At necropsy, no treatment-related adverse effects were observed at any dose level, although the F0 females displayed increased kidney weights at all dose levels but there was no dose response.

There were no apparent, treatment-related, adverse effects on body weights or body-weight gains of the **F1 parental animals** during the pre-mating dosing period at the two remaining dose levels, although the mid-dose [20 mg/kg/day; the highest dose in the F1 generation] males displayed an initial decrease in body-weight gain [weeks 35-36 (91%** of control) and weeks 36-37 (89%** of control)]. At 20 mg/kg/day, there were no significant differences in body weights in the **F1 dams** during gestation [F2a litters 95%-99%; F2b litters 95%-96% of control] or body-weight gains F2a litters 85% (days 7-13); F2b litters 83% (days 0-7); 86% (days 13-20); 90% (days 0-20) of control], and comparable body weights/gains were observed during lactation [both litters]. Food consumption was comparable among the groups [both sexes] throughout the study. At necropsy, no treatment-related adverse effects were observed at either dose level, although the F1 males and females displayed slightly increased kidney weights at the 20 mg/kg/day dose level, and the females at this dose level displayed a slight increase in liver weight.

F0 Generation. No apparent adverse effect was observed on fertility. Pre-coital intervals were comparable among the groups. The duration of gestation was significantly increased in the high-dose [80 mg/kg/day] F0 females producing the F1b pups [22.5 days *vs* 21.9 days]. The gestation survival index was comparable among the groups for the F1a pups but significantly decreased for the F1b litters [31.7% *vs* 97.8%]. There was a significant decrease in the number of F1a female fetuses at the high-dose level [39% *vs*54%]. The number of F1b pups born dead/dying by day 1 [110] was significantly increased at the high-dose level compared to the control [5]. F1a litter size was slightly lower at the high-dose level compared to the control [9.0 *vs* 10.1], but F1b litter size was significantly lower than the control [5.1** *vs* 9.5]. F1a pup viability was comparable throughout weaning, but F1b pup viability was significantly lower throughout the weaning period. There was a significant decrease in F1b pup survival to lactation day 4 at the high-dose level [86.3%] compared to the control [100%] and other dose levels [98% and 99.6%], as well as survival to lactation day 28 [71.4% *vs* 100% (control) and other dose groups 99.4% and 100%]. Decreased pup body weight [F1a males 89%/females 90% of control (day 1), 75%/81% of control (day 28); F1b males 78%/females 85% of control (day 1), 73%/76% of control (day 28)] and body-weight gains [F1a males 68%/females 70% of control (days 1-4), 75%/80% of control (days 4-28); F1b males 26%/females 43% of control (days 1-4), 76%/78% of control (days 4-28)] were observed at the high-dose level, with the F1b litters displaying the greater

effect. At the mid-dose level, there was a slight decrease in body weight [F1a males93%/females 94% of control (day 28); F1b males 84%/females 87% of control (day 28)] and body-weight gains [F1a males 92%/females 93% of control (days 4-28); F1b males 83%/females 85% of control (days 4-28)], with the deficits being greater in the F1b litters.

Skeletal anomalies and reduced ossification were observed in the high-dose F1b pups [80 mg/kg/day] that were dead at birth [only dose level examined].

F1 Generation. No apparent adverse effect was observed on fertility at either dose level. Pre-coital intervals and gestation lengths were comparable among the groups. The gestation survival index and the viability index were comparable among the groups for both the F2a and F2b litters. Litter size, body weights, and the sex ratio were comparable among the groups in both the F2a and F2b litters.

Degenerative changes in the tubules of the cortical region [high-dose F0 males] and outer medullary regions [mid- and high-dose F0 males, mid-dose F1 males (highest dose tested in this generation)] of the kidneys were found in a subsequent histopathological examination. The original reviewer noted that these effects on the kidney were not found originally but during a subsequent re-examination of the tissues, casting doubt on the quality of the histopathological examination of the reproductive organs. However, the RfD/QA Peer Review Committee determined that, based on the lack of effects on reproductive organs in the chronic and subchronic studies at similar or higher dose levels, reevaluation of these tissues [testes and ovaries] is not necessary [HED Document No. 011908, dated 5/9/96].

The NOAEL for parental toxicity is 5 mg/kg/day (target dose; actual dose range 3.8-13.5 mg/kg/day) and the parental LOAEL is 20 mg/kg/day (target dose; actual dose range 14-48 mg/kg/day), based on decreased female body weight/body- weight gain (F1) and male renal tubule alteration (F0 and F1).

The NOAEL for reproductive toxicity is 20 mg/kg/day (target dose; actual dose range 18-35 mg/kg/day), and the LOAEL for reproductive toxicity is 80 mg/kg/day (target dose; actual dose range 69-114 mg/kg/day), based on an increase in gestation length.

The NOAEL for offspring toxicity is 5 mg/kg/day (target dose; actual dose range 7.2-13.5 mg/kg/day), and the LOAEL for offspring toxicity is 20 mg/kg/day (target dose; actual dose range 26-48 mg/kg/day), based on decreased pup body weight [F1b]. At 80 mg/kg/day (target dose; actual dose range 76.1-133 mg/kg/day), there was an increase in pup deaths.

This 2-generation reproduction study is classified Acceptable/guideline,. This study satisfies the guideline requirement (OPPTS 870.3800; §83-4) for a 2-generation reproduction study.

Table 1. Body-Weight/Gains [grams] During Premating Period				
Generation/Sex/Week/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F0 MALES [body weight (g)]				
week 0	113.4±7.6	114.2±7.4	112.3±6.0	111.2±6.4
week 1	149.9±7.5	151.4±10.1	148.3±7.8	146.0±8.4
week6	261.0±9.3	262.5±12.3	258.9±11.2	253.7±10.4* [97]♪
week 10	299.4±10.3	299.7±12.5	292.6±12.9	286.2±10.8*
week 13	316.3±11.7	313.8±14.7	310.1±14.5	299.0±12.7** [95]
week 40	372.4±15.4	373.2±17.8	368.0±18.3	353.1±19.5** [95]
F0 FEMALES [body weight (g)]				
week 0	90.4±4.7	91.2±4.6	91.3±4.5	90.8±4.7
week 1	110.2±5.4	110.5±5.2	108.8±4.8	106.4±5.2
week6	158.5±7.6	161.8±7.9	159.3±7.1	155.4±7.8
week 10	173.3±8.3	175.3±8.6	174.0±7.7	168.1±9.2
week 13	179.0±9.1	179.6±9.5	180.2±8.3	172.7±9.7** [96]
week 40	216.2±10.9	219.3±8.6	214.8±7.7	206.2±12.1** [95]
F1 MALES [body weight (g)]				
week 35	112.5±18.8	123.5±19.4	122.2±14.6 [109]	-
week 36	151.4±18.3	158.6±21.5	157.4±15.0 [104]	-
week 37	191.2±17.0	192.6±21.0	192.7±14.2 [101]	-
week 49	331.0±12.6	326.1±22.9	323.8±18.2 [98]	-
week 65	374.2±13.2	370.1±24.9	364.4±19.5 [97]	-
week 77	394.2±13.7	398.1±26.2	384.4±22.5 [98]	-
F1 FEMALES [body weight (g)]				
week 35	99.6±16.5	100.8±10.7	94.1±11.2 [94]	-
week 36	117.4±16.4	117.8±9.1	115.1±9.6	-
week 37	131.9±17.1	130.8±8.0	130.6±9.9	-
week 53	204.1±9.0	200.7±9.3	198.1±10.4	-
week 77	237.9±9.5	233.1±8.2	231.7±10.2* [97]	-
F0 MALES [body-weight gain (g)]☹				
weeks 0-1	36.5	37.2	36.0	34.8 [95]
weeks 1-2	33.6	35.8*	36.0*	36.2**
weeks 2-3	26.5	24.5* [93]	25.5	22.9** [86]
weeks 0-13	202.9	199.6 [98]	197.8 [97]	187.8** [93]
weeks 0-40	259.0	259.0	255.7	242.0** [93]
F0 FEMALES [body-weight gain (g)]☹				
weeks 0-1	19.8	19.3	17.4** [88]	15.6** [79]
weeks 2-3	10.6	11.2	10.9	9.7 [92]
weeks 0-13	88.6	88.4	88.9	81.9** [92]
weeks 0-40	122.8	128.1	123.5	115.4** [94]
F1 MALES [body-weight gain (g)]				
weeks 35-36♬	38.9±5.1	35.1±4.7** [90]	35.2±4.3** [91]	-
weeks 36-37♬	39.7±5.0	34.0±5.6** [86]	35.3±3.9** [89]	-
weeks 35-75 ✖	274.5	258.5 [94]	254.6 [93]	-
weeks 35-77 ✖	281.7	274.6 [97]	262.2 [93]	-
F1 FEMALES [body-weight gain (g)]				
weeks 35-36♬	17.9±4.6	16.9±3.1	21.0±5.5* [117]	-
weeks 36-37♬	14.4±3.1	13.1±3.2	15.5±2.8 [108]	-

♪ [% of control]; ☹ data from Tables 2-4 (pages 61-70 of Volume 1; study report did not provide s.d.); ✖ calculated by this reviewer (no statistics performed); data from volume 1, pages 61-72, Appendix B, pages 162-227; from Tables 2 & 3, pages 737-767 of Volume 4 of study report]; ♬ from Table 3, pages 35 and 44 of Volume 4]; * $p<0.05$; ** $p<0.01$;

34

Table 2. Food Consumption ☹ During Mating Period, Gestation, and Lactation - F0 Generation

Generation/Sex/Week/Dose	0 mg/kg/day		5 mg/kg/day		20 mg/kg/day		80 mg/kg/day	
F0 MALES [g/rat/day]								
week 1	14.5		14.7		14.8		14.2	
week 3	17.6		17.6		17.8		17.2	
week 12	16.5		16.8		16.7		16.0 [97]♪	
week 15	15.7		16.1		16.9**		15.3	
week 24	16.5		16.5		16.5		15.9* [96]	
week 25	16.3		16.6		16.5		16.2	
F0 FEMALES [g/rat/day]								
week 1	11.2		12.0*		11.7		11.5	
week 3	12.5		12.6		12.2		11.8 [94]	
week 12	11.3		11.7		11.7		10.7** [95]	
week 15	10.7		10.9		11.2		10.7	
week 24	16.7		16.9		15.8 [95]		13.9** [83]	
week 25	14.6		15.0		14.4		14.0 [96]	
F0 MALES [g/kg/day]								
week 1	110.5		110.5		113.3*		110.6	
week 3	89.2		88.4		90.4		88.8	
week 12	53.6		54.9*		55.7**		54.8	
week 15	50.2		51.8*		54.9**		51.5	
week 24	48.7		48.7		49.3		49.3	
week 25	47.7		48.8		49.0*		49.8**	
F0 FEMALES [g/kg/day]								
week 1	112.1		118.8**		116.8		116.5	
week 3	96.3		96.1		93.3		92.6	
week 12	64.4		65.6		66.2		63.0	
week 15	60.6		68.4**		62.9		63.1	
week 24	86.0		61.6		80.6		72.6** [84]	
week 25	72.7		74.2		71.7		72.2	
F0 FEMALES	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day
gestation F1A mating								
days 0-7	11	61	11	61	11	60	10* [91]	59
days 7-13	14	68	13	66	13	68	13** [93]	68
days 13-20	14	63	13	65	13	65	14	65
gestation F1B mating								
days 0-7	13		13		13		12	
days 7-13	14		15		16*		14	
days 13-20	17		17		17		14** [82]	
F0 FEMALES	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day
lactation F1A mating								
days 1-7	23	118	24	118	22	115	18** [78]	96** [81]
days 7-14	36	172	36	172	34	167	29** [81]	144** [84]
days 14-21	44	204	43	204	44	205	39* [89]	186 [91]
days 21-28	59	291	54	273	55	273	45** [76]	213** [73]
days 1-28		203		199		197		169** [58]
lactation F1B mating								
days 1-7	24	109	26	116	25	116	16** [67]	77** [71]
days 7-14	36	160	38	162	38	168	27** [75]	124** [78]
days 14-21	44	191	45	189	45	193	36* [82]	158* [83]
days 21-28	55	256	52	239	51	238	45** [82]	196** [77]

♪ [% of control]; ☹ data from Tables 13-22 (pages 81-97 of the report; study report did not provide s.d.); * p<0.05; ** p<0.01

Table 3. Food Consumption ☹ During Gestation and Lactation - F1 Generation

Generation/Sex/Week/Dose	0 mg/kg/day		5 mg/kg/day		20 mg/kg/day		80 mg/kg/day	
F♀ FEMALES	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day
gestation F2A mating								
days 0-7	10	48	10	49	11*	56*	-	-
days 7-13	13	60	13	62	14	64	-	-
days 13-20	16	64	16	66	16	65	-	-
days 0-20	13	57	13	59	14	61**	-	-
gestation F2B mating								
days 0-7	12	51	12	53	11	52	-	-
days 7-13	15	62	16	65	15	66	-	-
days 13-20	18	65	17	64	16	61	-	-
days 0-20	15	59	15	60	14	59	-	-
F♀ FEMALES	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day
lactation F2A mating								
days 1-7	25	111	23	106	24	111	-	-
days 7-14	37	161	34	152	37	161	-	-
days 14-21	45	194	41	177	43	184	-	-
days 21-28	62	273	61	271	65	285	-	-
days 1-28	43	189	41	182	43	191	-	-
lactation F2B mating								
days 1-7	24	99	25	103	24	104	-	-
days 7-14	37	147	37	153	36	150	-	-
days 14-21	47	181	49	196	48	192	-	-
days 21-28	64	267	64	270	65	276	-	-
days 1-28	44	179	44	185	44	186	-	-

♪ [% of control]; ☹ data from Tables 12-21 (pages 779-804 of the report; study report did not provide s.d.); * p<0.05; ** p<0.01

Table 4. Female Body Weight [grams]✖ During Gestation

Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F0 [F1a litter] Gestation days				
0	178±7	179±9	178±8	173±9
7	190±9	191±10	191±9	181±10** [95]
13	208±9	208±10	206±8	196±10** [94]
20	246±21	252±18	249±11	232±16* [94]
F0 [F1b litter] Gestation days				
0	200±11	205±10	202±9	197±11
7	210±11	214±9	210±9	204±12
13	226±12	232±11	230±9	218±13 [96]
20	270±20	277±21	274±18	244±17** [90]
F1 [F2a litter] Gestation days				
0	201±7	198±8	198±12	-
7	221±17	208±12	211±13	-
13	234±9	227±9	228±14	-
20	271±24	271±17	270±17	-
F1 [F2b litter] Gestation days				
0	222±10	221±10	214±11	-
7	234±11	229±8	224±12	-
13	250±13	248±10	241±14 [96]	-
20	293±18	290±18	278±26 [95]	-

♪ [% of control]; ✖ data from Appendices C (pages186-192) and E (pages 200-205) of Volume 1 and Appendices C (pages 930-934) and E (pages 940-944) of Volume 4 of the study report; * p<0.05; ** p<0.01;

Table 5. Female Body-Weight Gain [grams]✖ During Gestation

Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F0 [F1a litter] Gestation days				
0-7	12±4	12±4	13±4	8±5* [67]
7-13	18±5	18±6	15±5 [83]♪	15±4 [83]
13-20	38±19	44±13	44±9	35±11 [95]
0-20	68±20	74±16	71±9	59±13 [87]
F0 [F1b litter] Gestation days				
0-7	10±5	9±4	8±5	6±4* [70]
7-13	16±4	18±5	20±4	14±6 [88]
13-20	43±16	45±15	44±14	27±9** [59]
0-20	70±15	72±14	72±13	47±10** [67]
F1 [F2a litter] Gestation days				
0-7	10±15	10±13	14±5	-
7-13	20±5	19±5	17±6	-
13-20	38±20	44±13	42±10	-
0-20	70±22	73±16	72±12	-
F1 [F2b litter] Gestation days				
0-7	12±6	8±5	10±7	-
7-13	16±5	19±6	17	-
13-20	43±14	42±13	37±20 [86]	-
0-20	70±16	69±15	64±20 [90]	-

♪ [% of control]; * p<0.05; ** p<0.01; ✖ data from Appendices D (pages 193-199) and F (pages 206-211) of Volume 1 and Appendices D (pages 935-939) and F (pages 945-949) of Volume 4 of the study report

Table 6. Female Body Weight [grams]✖ During Lactation

Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F0 [F1a litter] lactation days				
1	189±12	191±9	191±9	184±10
7	205±10	207±11	201±9	189±10** [92]♪
14	212±12	212±17	207±17	208±10
21	216±14	213±16	219±13	212±11
28	189±14	184±14	185±18	204±15** [108]
F0 [F1b litter] lactation days				
1	210±11	215±9	208±10	205±13
7	226±15	233±12	225±11	211±14* [93]
14	228±16	237±10	233±16	224±18
21	229±19	239±15	234±19	231±12
28	203±22	197±14	193±16	226±13* [111]
F1 [F2a litter] lactation days				
1	216±8	211±6	211±13	-
7	228±11	221±9	222±16	-
14	234±23	232±10	233±16	-
21	232±16	233±7	236±16	-
28	220±16	221±12	223±18	-
F1 [F2b litter] lactation days				
1	236±11	234±9	227±13	-
7	248±14	245±8	237±18	-
14	260±13	245±16	248±17	-
21	255±8	252±12	250±15	-
28	228±16	221±11	222±12	-

♪ [% of control]; * p<0.05; ** p<0.01 ✖ data from Appendices G (pages 212-215) and I (pages 220-223) of Volume 1and Appendices G (pages 950-952) and I (pages 956-958) of Volume 4 of the study report

Table 7. Female Body-Weight Gain [grams]✖ During Lactation				
Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F0 [F1a litter] lactation days				
1-7	15±7	16±7	11±5	6±6** [40]♪
7-14	8±10	5±14	6±15	19±5** [238]
14-21	4±12	1±19	11±18	4±4
21-28	-27±19	-29±26	-33±20	-9±16*
1-28	-1±15	-7±18	-5±17	20±15 **
F0 [F1b litter] lactation days				
1-7	16±7	17±8	17±7	1±5** [6]
7-14	2±14	4±14	8±13	14±5
14-21	1±19	2±12	0±25	8±8
21-28	-26±19	-41±20*	-40±18*	-5±9
1-28	-7±18	-18±16	-15±17	23±4**
F1 [F2a litter] lactation days				
1-7	12±7	10±9	11±8	-
7-14	6±19	11±9	11±9	-
14-21	-2±13	1±9	3±8	-
21-28	-12±17	-12±11	-13±11	-
1-28	4±15	10±10	12±10	-
F1 [F2b litter] lactation days				
1-7	12±10	11±6	10±9	-
7-14	12±17	0±13	11±20	-
14-21	-5±16	7±17	3±14	-
21-28	-27±13	-31±13	-29±15	-
1-28	-9±10	-14±11	-5±16	-

♪ [% of control]; ✖ data from Appendices H (pages 216-219) and J (pages 224-227) of Volume 1
Appendices H (pages 953-955) and J (pages 959-961) of Volume 4 of the study report

Table 8. F1a and F1b Pup Body-Weight Gain (grams)✖

Generation/Sex/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F1a male pups				
Lactation days				
1-4[a]	2.2	2.3	2.3	1.5 [68]♪
4[b]-7	3.9	3.9	3.9	2.1 [54]
7-14	10.6	10.3	9.5	8.7 [82]
14-21	10.1	9.6	9.6	9.5 [94]
21-28	19.2	17.1 [89]	17.1 [89]	12.4 [65]
4-28	43.8	40.9 [93]	40.1 [92]	32.7 [75]
F1a female pups				
Lactation days				
1-4	2.3	2.3	2.3	1.6 [70]
4-7	3.7	3.8	3.8	2.2 [59]
7-14	10.3	10.0	9.2	8.5 [83]
14-21	9.4	9.0	9.3	9.5
21-28	17.7	15.5	16.0	12.8 [72]
4-28	41.1	38.3 [93]	38.3 [93]	33.0 [80]
F1b male pups				
Lactation days				
1-4	2.7	2.8	2.5 [93]	0.7 [26]
4-7	3.9	4.1	3.9	2.0 [51]
7-14	11.5	11.4	10.9 [95]	8.7 [76]
14-21	10.7	10.4	9.9 [93]	10.4
21-28	21.4	16.3 [76]	14.6 [68]	14.8 [69]
4-28	47.5	42.2 [89]	39.3 [83]	35.9 [76]
F1b female pups				
Lactation				
1-4	2.8	2.7	2.4 [86]	1.2 [43]
4-7	3.6	3.9	3.6	1.7 [47]
7-14	10.8	11.0	10.5	7.9 [73]
14-21	9.8	9.7	9.2	9.9
21-28	18.7	14.8 [79]	13.3 [71]	14 [75]
4-28	42.9	39.4 [92]	36.6 [85]	33.5 [78]

4[a] day 4 pre-cull; 4[b] day 4 post-cull; ♪ [% of control]; ✖ calculated by this reviewer using data from Tables 7-8, pages 20-21 of original DER [no statistics performed]

41

Table 9. F1a and F1b Pup Body Weights [grams]✖

Generation/Sex/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F1a male pups Lactation days				
1	5.5±0.8 (21)	5.6±0.7 (25)	5.6±0.6 (23)	4.9±0.5 (19)[89]♪
4	7.7±1.5	7.9±1.2	7.9±0.7	6.4±0.7
4	8.0±1.1	7.9±1.1	7.9±0.7	6.4±0.7
7	11.9±1.1	11.8±1.8	11.8±0.8	8.5±1.3
14	22.5±1.7	22.1±2.7	21.3±2.3	17.2±2.1
21	32.6±2.8	31.7±3.1	30.9±2.8	26.7±2.2
28	51.8±6.3 (20)	48.8±6.4 (25)	48.0±5.6 (23)[93]	39.1±3.2 (19)[75]
F1a female pups Lactation days				
1	5.2±0.7 (21)	5.4±0.7 (25)	5.4±0.8 (24)	4.7±0.4 (20)[90]
4	7.5±1.5	7.7±1.2	7.7±0.6	6.3±0.9
4	7.7±0.9	7.7±1.3	7.7±0.6	6.3±0.9
7	11.4±1.0	11.5±1.8	11.5±0.6	8.5±1.5
14	21.7±1.9	21.5±2.6	20.7±2.3	17.0±2.6
21	31.1±2.9	30.5±2.7	30.0±2.8	26.5±3.1
28	48.8±5.3 (20)	46.0±5.5 (25)	46.0±5.3 (24)[94]	39.3±6.3 (20)[81]
F1b male pups Lactation days				
1	5.8±0.4 (23)	5.6±0.6 (25)	5.4±0.5 (23)	4.5±0.4 (8)[78]
4	8.5±0.8	8.4±0.9	7.9±0.6	5.2±1.1
4	8.5±0.8	8.4±0.9	7.9±0.6	5.2±1.2
7	12.4±1.2	12.5±1.3	11.8±0.9	7.2±1.8
14	23.9±2.3	23.9±2.2	22.7±1.2	15.9±3.6
21	34.6±3.6	34.3±3.3	32.6±2.3	26.3±4.2
28	56.0±8.9 (23)	50.6±5.2 (24)	47.2±7.3 (23)	41.1±6.6 (5)[73]
F1b female pups Lactation day				
1	5.3±0.5 (23)	5.3±0.6 (24)	5.2±0.5 (23)	4.4±0.5 (8)[85]
4	8.1±0.8	8.0±0.8	7.6±0.6	5.6±0.8
4	8.1±0.8	8.0±0.8	7.6±0.6	5.5±0.8
7	11.7±1.1	11.9±1.2	11.2±0.7	7.2±1.2
14	22.5±1.9	22.9±1.8	21.7±1.0	15.1±1.0
21	32.3±3.0	32.6±2.6	30.9±2.1	25.0±0.9
28	51.0±7.5 (23)	47.4±4.6 (24)	44.2±6.8 (23)	39.0±1.5 (5)[76]

(# litters); ♪ [% of control]; ✖ data from appendices Y and Z, pages 294-301 of the report

Table 10. F2a and F2b Pup Body Weights [grams]				
Generation/Sex/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F2a male pups Lactation days				
1	5.9±0.6	5.8±0.4	5.7±0.3	-
4	8.7±0.9	8.6±1.0	8.5±0.6	-
4	8.7±0.9	8.6±1.0	8.5±0.6	-
7	12.6±1.0	12.3±1.2	12.4±1.0	-
14	22.7±2.0	22.5±1.9	23.3±1.7	-
21	32.0±3.0	32.0±3.7	32.6±2.9	-
28	55.9±7.6	57.9±6.3	58.2±5.1	-
F2a female pups Lactation days				
1	5.7±0.6	5.4±0.5	5.4±0.3	
4	8.3±0.9	8.1±1.1	8.1±0.6	-
4	8.3±0.9	8.1±1.2	8.1±0.6	-
7	12.1±1.1	11.7±1.3	11.8±0.9	-
14	21.9±1.9	21.7±2.1	22.0±1.6	-
21	30.8±3.1	30.7±3.8	30.7±3.0	-
28	52.5±7.1	53.5±5.8	53.2±4.8	-
F2b male pups Lactation days				
1	5.8±0.4	5.8±0.5	5.8±0.6	
4	8.7±0.8	8.4±1.0	8.6±1.2	-
4	8.8±0.8	8.4±1.0	8.7±1.2	-
7	12.4±1.0	12.5±1.2	12.8±1.9	-
14	22.5±1.4	22.3±1.7	23.2±2.5	-
21	33.2±2.4	33.3±1.9	34.4±4.1	-
28	55.1±5.6	53.2±4.2	56.1±7.9	-
F2b female pups Lactation day				
1	5.5±0.4	5.6±0.4	5.5±0.5	
4	8.5±1.0	8.3±0.5	8.3±1.2	-
4	8.6±0.9	8.3±0.5	8.2±1.1	-
7	12.1±1.5	12.0±0.9	12.1±1.7	-
14	22.0±2.2	21.6±1.1	22.0±2.5	-
21	32.0±2.9	31.8±1.3	32.1±3.4	-
28	51.8±5.7	51.3±6.5	51.2±6.4	-

♪ [% of control]; data from Tables 35-36, pages 838-841 of the report

Table 11. F2a and F2b Pup Body-Weight Gain [grams]✖				
Generation/Sex/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F2a male pups Lactation days				
1-4	2.8	2.8	2.8	-
4-7	3.9	3.7	3.9	-
7-14	10.1	10.2	10.9	-
14-21	9.3	9.5	9.3	-
21-28	23.9	25.9	25.6	-
4-28	47.2	49.3	49.7	-
F2a female pups Lactation days				
1-4	2.6	2.7	2.7	-
4-7	3.8	3.6	3.7	-
7-14	9.8	10.0	10.2	-
14-21	8.9	9.0	8.7	-
21-28	21.7	22.8	22.5	-
4-28	44.2	45.4	45.1	-
F2b male pups Lactation days				
1-4	2.9	2.6	2.8	-
4-7	3.6	4.1	4.1	-
7-14	10.1	9.8	10.4	-
14-21	10.7	11.0	11.2	-
21-28	21.9	20.0	21.7	-
4-28	46.3	44.9	47.4	-
F2b female pups Lactation				
1-4	3.0	2.7	2.8	-
4-7	3.5	3.7	3.9	-
7-14	9.9	9.6	9.9	-
14-21	10.0	10.2	10.1	-
21-28	19.8	19.5	19.1	-
4-28	43.2	43.0	43.0	-

✖ calculated by this reviewer using data from Tables 35-36, pages 838-841 of the report [no statistics performed]

Table 12. Litter Data [# litters with dead pups and # dead pups on lactation day 0]				
Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F1a litters # litters # pups	2 [21]✔ 2 {1/sex}	5 [26] 13 {3 ♂/10♀}	0 [24] 0	5 [21] 6 {3/sex}
F1b litters # litters # pups	5 [23] 5 {3 ♂/2♀}	3 [26] 4 {3 ♂/1♀}	3 [23] 3 {2 ♂/1♀}	15 [20] 59 {23 ♂/36♀}
F2a litters # litters # pups	1 [20] 5 {1 ♂/4♀}	1 [24] 1 {1♀}	2 [23] 2 {1/sex}	- -
F2b litters # litters # pups	3 [18] 3 {2 ♂/1♀}	3 [20] 3 {1 ♂/2♀}	2 [19] 2 {1/sex}	- -

✔ total # litters; data from Appendices W & X [pages 286-293] and [pages 1012-1017] of the report;

Table 13 . Gestation Length				
Generation/Dose/Days	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F1a mating	22.0±0.4 [2]✔	22.2±0.7 [5 (24)♪]	21.9±0.4 [1]	22.1±0.5 [4]
F1b mating	21.9±0.3 [0]	22.0±0.4 [1]	21.7±0.4 [0]	22.5±0.5** [10]
F2a mating	22.3±0.4 [5]	22.0±0.5 [3]	22.0±0.2 [1]	-
F2b mating	22.0±0.3 [1]	22.1±0.5 [0 (24)]	21.9±0.5 [0]	-

✔ [# 23 days] (♪ 24 days for one low dose dam); Data from Tables 32 [pages 116 and 833] and 33 [pages 117 and 834]; Appendices U & V [pages 278-285] and [pages 1006-1011] of Volumes 1 and 4, respectively, of the report; ** $p<0.01$

4. **Reproductive function:** There was no assessment of reproductive function.

a. Estrous cycle length and periodicity: Results from the evaluation of vaginal smears were not provided. The study [1985] was performed according to the guidelines proposed in 1978, which did not include these parameters.

b. Sperm measures: Results from the evaluation of sperm parameters were not provided. The study [1985] was performed according to the guidelines proposed in 1978, which did not include these parameters.

5. **Reproductive performance:** There was no apparent adverse effect on reproductive performance. A second mating by a proven male was conducted when females demonstrated no evidence of sperm. The number of second matings producing the F1a, F1b, F2a, and F2b pups is shown in Table 13.

Table 14. Number of Second Matings				
Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F1a mating	0	5	1	0
F1b mating	6	6	2	2
F2a mating	3	2	4	-
F2b mating	4	1	4	-

Data from page 14 of the original DER [Document No. 005446]

Reproductive Performance

Observation/Dose Group	Table 15. F0 Generation - Litter F1a			
	Control	LDT	MDT	HDT
Mean (±SD) precoital interval (days)	5.05±2.46√ [4.0]✖	5.8±2.9 [5.7]	4.8±2.1 [4.8]	5.2±2.8 [4.8]
MALES				
Number mated	30	30	30	30
Number fertile	21 [70%]	25 [83.3]	24 [80]	21 [70]
Fertility not determined	na	na	na	na
Intercurrent deaths	na	na	na	na
FEMALES				
Number mated	30	30	30	30
Number fertile	21 [70]	26 [86.7]	24 [80]	21 [70]
Fertility not determined	na	na	na	na
Intercurrent deaths	1 {day 3}	0	0	0
Mean (±SD) gestation interval (days)	22.0±0.4	22.2±0.7	21.9±0.4	22.1±0.5
Number of litters	21	24	24	21

√ mean calculated by reviewer using data from Appendix S (pages 270-273); ✖mean from Table 30 page 114 in the study report]. Data also from Tables 28 and 32, pages 112 and116; na not available

Observation/Dose Group	Table 16. F0 Generation - Litter F1b			
	Control	LDT	MDT	HDT
Mean (±SD) precoital interval (days)	5.0±2.2√ [4.6]✖	5.36±3.67 [5.2]	4.5±2.1 [4.8]	4.1±2.4 [4.3]
MALES				
Number mated	29	30	30	30
Number fertile	23	25	23	21
Fertility not determined	na	na	na	na
Intercurrent deaths	na	na	na	na
FEMALES				
Number mated	29	30	30	30
Number fertile	23	27	23	21
Fertility not determined	na	na	na	na
Intercurrent deaths	0	1 {day 23}	0	1 {day 20}
Mean (±SD) gestation interval (days)	21.9±0.3	22.0±0.4	21.7±0.4	22.5±0.5**
Number of litters	23	25	23	10

√ mean calculated by reviewer using data from Appendix T [pages 274-277]; ✖mean from Table 30 page 114 in the study report] Data from Tables 29, 31 and 33, pages 113, 115 and 117 of study report. * p<0.01.

Observation/Dose Group	Table 17. F1 Generation - Litter F2a			
	Control	LDT	MDT	HDT
Mean (±SD) precoital interval (days)	4.8±2.3√ [4.6]✖	4.0±2.3 [4.1]	5.0±3.0 [4.3]	-
MALES				
Number mated	30	30	30	-
Number fertile	21	24	22	-
Fertility not determined	na	na	na	-
Intercurrent deaths	na	na	na	-
FEMALES				
Number mated	29	30	30	-
Number fertile	21	24	23	-
Fertility not determined	na	na	na	-
Intercurrent deaths				
Mean (±SD) gestation interval (days)	22.3±0.4	22.0±0.5	22.0±0.2	-
Number of litters	20	24	22	-

✖ Data from Table 29 [page 830] of study report. √Calculated by reviewer using data in Appendix S, pages 994-999

Observation/Dose Group	Table 18. F1 Generation - Litter F2b			
	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
Mean (±SD) precoital interval (days)	3.3±2.2 √ [3.2]✖	3.1±1.7 √ [3.4]	4.1±3.3 √ [4.0]	-
MALES				
Number mated	30	30	30	0
Number fertile	18	20	20	-
Fertility not determined	na	na	na	-
Intercurrent deaths	na	na	na	-
FEMALES				
Number mated	28	30	30	0
Number fertile	18	20	20♪	-
Fertility not determined	na	na	na	-
Intercurrent deaths	na	na	na	-
Mean (±SD) gestation interval (days)	22.0±0.3	22.1±0.5	21.9±0.2	-
Number of litters	18	20	20♪	-

√ mean calculated by reviewer using data from Appendix T (pages 1000-1005); ✖mean from Table 30 [page 831] in the study report]. Pre-coital interval ≥5 days [control 28%; low 40%; mid 55%]; ♪{data are listed for 20 in Appendix T (pages 1004-1005) and Table 30 (page 831); for 19 on pages 833 and 1011

Observation/Dose Group	Table 19. Litter parameters for F_1 generation			
	0 mg/kg/day	5 mg/kg/day	2 0 mg/kg/day	80 mg/kg/day
F_{1a} Generation				
Mean implantation sites	na	na	na	na
Number born live	214	258 ✗✗	240 ♬	183
Number born dead	3	20	3	9
Sex Ratio Day 0 (% ♂)	47	51	50	57
# Deaths Days 0-4 (%)	5	11	3	8
# Deaths Days 4-21 (%)	4	1	0	4
Mean litter size Day 0	10.2	9.9	10.0	9.2 [90]✔
Day 4 [b]	9.9	9.9	9.9	8.8 [89]
Day 4 [c]	8.0	7.6	7.6	7.4 [93]
Day 7	7.8	7.6	7.6	7.3 [94]
Day 14	7.8	7.6	7.6	7.2 [92]
Day 21	7.8	7.6	7.6	7.2 [92]
Live birth index	98.6	92.6**	98.8	95.2
Viability index	98.6	95.2	100	96.8
Lactation index	97.5	99.5	100	97.3
F_{1b} Generation				
Mean implantation sites	na	na	na	na
Number born live	224	266	241	161
Number born dead/dying by day 1	5	4/11	3	59/51
Sex Ratio Day 0 (% ♂)	51	48	46	47
# Deaths Days 0-4 (%)	5	20	4	117✦
# Deaths Days 4-21 (%)	0	1	0	12 (68%)
Mean litter size Day 0	9.5	10.0	10.4	5.1**
Day 4 [b]	9.5	9.8	10.3	4.4
Day 4 [c]	7.1	7.4	7.6	5.3
Day 7	7.1	7.4	7.6	5.7
Day 14	7.1	7.4	7.6	6.0
Day 21	7.1	7.3	7.6	6.0
Live birth index	97.8	94.4	98.8	31.7**
Viability index	100	98.0	99.6	86.3**
Lactation index	100	99.4	100	71.4**

a *Data obtained from Appendices W & X*, pages 286-293 and Tables 34-35 [pages 118-121]of study report.
✗ listed as 25 in Table 32 (page 118); ✗✗ listed as 238 in Appendix W, page 287 (day 0) and 251in Table 34 on page 118 [live litter size]; ♬ listed as 226 in Appendix W, page 288 (day 0) and 237 in Table 34 [live litter size];
✔ total # litters; data from Appendices W & X [pages 286-293] and [pages 1012-1017] of the report;
b Before standardization (culling)
c After standardization (culling)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$
✦ 12/20 total litter loss

Observation/Dose Group	Table 20. Litter parameters for the F_2 generation			
	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	-
F_{2a} Generation				
Mean implantation sites	na	na	na	-
Number born live	187	220	207	-
Number born dead	5	3	3	-
Sex Ratio Day 0 (% ♂)	51.9	48.2	53.6	-
# Deaths Days 0-4 (%)	2 (1)	2 (0.9)	0	-
# Deaths Days 4-21 (%)	0	1 (0.6)	0	-
Mean litter size Day 0	9.8	9.2	9.0	-
Day 4 [b]	9.7	9.1	9.0	-
Day 4 [c]	7.4	7.0	7.3	-
Day 7	7.4	7.0	7.3	-
Day 14	7.4	7.0	7.3	-
Day 21	7.4	7.0	7.3	-
Day 28	7.4	7.0	7.3	-
Live birth index	97.4	98.7	98.6	-
Viability index	98.9	99.1	100	-
Lactation index	100	99.4	100	-
F_{2b} Generation				
Mean implantation sites	na	na	na	-
Number born live✖	156	186	165	-
Number born dead	3	3	2	-
Sex Ratio Day 0 (% ♂)	46	47	49	-
# Deaths Days 0-4 (%)	4	1	0	-
# Deaths Days 4-21 (%)	0	3	0	-
Mean litter size Day 0	8.6	9.3	8.7	-
Day 4 [b]	8.9	9.3	8.7	-
Day 4 [c]	7.3	7.3	7.2	-
Day 7	7.3	7.2	7.2	-
Day 14	7.3	7.2	7.2	-
Day 21	7.3	7.2	7.2	-
Live birth index	97.5	98.4	98.8	-
Viability index	97.4	99.5	100	-
Lactation index	100	98.6	100	-

Data obtained from Appendices W & X, pages 1012-1017 and Tables 34 and 36 [pages 836-7, 840-841] of study report.
✔ total # litters;
b Before standardization (culling)
c After standardization (culling)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$
✖ the number of pups on day 0 was less than the number on day 1

3. <u>Sexual maturation</u> (F_1): Sexual maturation was not assessed.

<u>Discrepancies</u>: The data presentation is confusing. For example, there are several instances where the total number of pups increases between day of birth [day 0] and day 1. In Appendix W [page 286], the number dead on day 0 in the control group [F1a litters] is listed as 2 [1 male, 1 female]; the number surviving [born live] on day 0 is listed as 214 out of 216. For survival on day 1, it is not clear why the total number of pups is listed as 213 out of 216 instead of 213 out of 214 [survival on day one should be of those that were alive on day 0]. For the 5 mg/kg/day

dose group, the number of pups on day 0 for two litters is less than the number listed on day 1 [dam #5506 had 1 pup on day 0 and 12 listed for day 1; dam # 5534 had 4 pups on day 0 and 13 on day 1]. Survival on day 0 is listed as 238 out of 251. The denominator for the total alive on day 1 is 271, which is the number apparently born; survival on day 1 is listed as 251 out of 271. However, if the extra 20 pups not listed on day 0 are added to the total live on day 0 [238], the total is 258, which suggests there were 7 deaths between day 0 and 1. For dam #5531, there were 7 out of 8 pups alive on day 0 and 7 out of 8 alive on day 1, but the % survival on day 1 is listed as 88% [should read 100%].

Additionally, there is confusing information on the number of dead pups born. For example, Appendix X [page 293] shows the number of dead pups on lactation day zero to be 23 males and 36 females [59] in the high-dose F1b group, which is different from the number [110] listed in Table 35 [page 120] as the number of dead pups [time frame not stated]. The number of pups dying between day 0 and day 1 was not found in the report, but a comparison of the number of pups listed on day 0 to the number listed for day 1 in the individual data in Appendix X [page 293] gives the additional 51 dead pups listed in Table 35.

Pup Deaths			
# dead on lactation day 0	Appendix W or X	Table 34 or 35	apparent # dying before day 1
F1a control	2	3	1
F1a 5 mg/kg/day	13	20	7
F1a 20 mg/kg/day	0	3	3
F1a 80 mg/kg/day	6	9	3
F1b control	5	5	0
F1b 5 mg/kg/day	4	15	11
F1b 20 mg/kg/day	3	3	0
F1b 80 mg/kg/day	59	110	51

In Appendix S [pages 994-999], the individual F1 data presented are for the F2a mating; however, page 998 has **F2b** mating listed.

CASWELL FILE

3,15

005446

SEP - 8 1986

Subject: The Effects of 2,4-D in a Two-Generation Study on Reproduction in Rats.

From: David G Anderson, PhD.
Toxicology Branch
Section VII
Hazard Evaluation Division (TS-769C)

David G. Anderson 9/5/86

To: Ms. Lois Rossi PM #61
Special Review Branch
Registration Division (TS-767C)

Thru: Albin B Kocialski, PhD.
Supervisory Pharacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ABK 9/8/86
9/8/86

The 2-generation rat reproduction feeding study on 2,4-D has been reviewed and classified as Core-minimum data. The calulated and nominal NOEL's and the LEL's with their respective effects are as follows.

F0 parental toxicity.
NOEL - 15(20) mg/kg/day.*
LEL - 58(80) mg/kg/day, reduced male body weight.

F1 parental toxicity.
NOEL - 4(5) mg/kg/day.
LEL - 14(20) mg/kg/day, reduced female body weight.

Developmental toxicity, dose level to dams.
NOEL - 7(5) mg/kg/day.
LEL - 26(20) mg/kg/day, reduced weight in F1b pups.

Nominal dose levels administered 0, 5, 20, or 80 mg/kg/day.

* Calculated lowest dose level within the range consumed by the animals at the nominal dose level administered (nominal dose level administered).

51

005446

Reviewed by: David G Anderson David G Anderson 9/5/86
Section VII, Tox. Branch (TS-769C)
Secondary reviewer: Albin B Kocialski
Section VII, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Effects of 2,4-D on Two-Generations of Reproduction in Rats

TEST SUBSTANCE: 2,4-Dichlorophenoxyacetic Acid (2,4-D)

SYNONYMS: 2,4-D TOX. CHEM. NO. 315

ACCESSION NO.: 259442-6 (Study in 5 Volumes)

SPONSOR: Industry Task Force on 2,4-D Research Data (ITF)

TESTING FACILITY: Wil Research Laboratories, Inc. (WIL)
Ashland, OH 44805-9281

TITLE OF REPORT: A Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxy-acetic Acid.

AUTHORS: Stanley Kopp, Patricia L Leist, Michael D Mercieca, Elaine J Tasker, Gabriela P Adam, Mark D Nemec, Dean E Rodwell.

STUDY NO.: WIL-81137

TESTING PERIOD: November 16, 1982 to May 15, 1984

REPORT ISSUED: July 26, 1985

PURITY OF TEST SUBSTANCE: ITF analysis 97.5%
WIL analysis 95.8%

CORE GRADE: Minimum.

A. CONCLUSIONS ON THE EFFECT AND NO EFFECT LEVELS:

The effect levels and no effect levels are expressed as the lowest dose level consumed within a measured dose level range. The target or nominal dose levels administered, for reference purposes only, are enclosed in parentheses(Discussed more fully in the section on Study Design and Conduct). Dose levels are given in mg/kg/day.

LEL and NOEL is expressed in mg/kg/day

F0 parental toxicity
LEL- 58(80), reduced body weight.
NOEL- 15(20)
F1 parental toxicity
LEL- 14(20), reduced body weight.
NOEL- 3.8(5)
Developmental toxicity
LEL- 26(20), F1b pup weight reduction.
NOEL- 7.2(5)

Target or nominal dose levels administered in the study are 0, 5, 20, or 80 mg/kg/day.

In designating the LEL and the NOEL, several considerations were applied. The lowest dose level in a range was used. Although it might be expected that the highest dose level within a range would initiate the toxicity, in a study on reproduction, where effects may be development stage or age specific as well as dose dependent, the highest dose level is not totally appropriate. In the study under consideration, the dose levels consumed varied widely during the study, and it was not always possible to determine adequately the dose level or the animal state at which the toxicity was initiated. Thus, it seems appropriate to select, for the LEL, the lowest dose level possibly resulting in the effect.

The NOEL is also designated as the lowest dose level in the range where no effects were observed. The upper dose level of the range was rejected because the animals did not continuously consume these levels. If they had, effects may have been demonstrated. Thus, for safety considerations, the lowest dose level within the range where no effects were observed is designated the NOEL.

The appropriate dose level range for the NOEL for the F1 female body weight reduction includes; a) the gestation and lactation for the F1b pups(the F1 females were selected from these pups), b) and the growth and develpment of the F1 females, c) and the gestation and lactation for the F2a and F2b litters, e) and for the 4 weeks of dosing after weaning the F2b litters. The lowest dose level consumed during these periods is considered to be the NOEL.

Similarly, the NOEL for the F1b pups is the lowest dose level consumed by F0 dams during the gestation and lactation for the F1b litters.

The effect and no effect levels from this study are also presented as the target dose levels and the range in the amount of test substance consumed. The target dose levels are the dose levels which were designed for the study and which the testing laboratory attempted to deliver to the animals. The amounts of test substance consumed are the actual dose-levels delivered to the animals, at least as best could be determined from the concentration of the test substance in the feed, food consumptions, and the animal weight for the week concerned. Since the dose levels are calculated for 1 week before they are delivered, the actual delivered dose varied somewhat from these anticipated dose-levels during the study.

Effect and No Effect Levels, with ranges

F0 parental LEL and NOEL in mg/kg/day
- LEL- 80(58-94)([a])([b]), F0 male body weight reduction.
- 80(71-86)([b]), F0 female body weight reduction.
- 80(69-114)([c]), F0 increase in length of gestation.
- NONE, F0 and F1 fertility.
- NOEL- 20(15-22)([b]), No F0 male body weight reduction occurred.
- 20(18-21)([b]), No F0 female body weight reduction,
- 20(18-35)([c]) or no increased length of gestation occurred.

F1 parental LEL and NOEL in mg/kg/day
- LEL- 20(14-48)([d]), F1 female body weight reduction.
- NOEL- 5(3.8-13.5)([d]), No F1 female body weight reduction occurred.

Developmental toxicity in mg/kg/day to dams
- LEL- 80(69-112, gestation and lactation for the F1a litters)([e]), F1a pup death.
- 80(103-133, gestation and lactation for the F1b litters)([e]), F1b pup death.
- 80(69-112, gestation and lactation for the F1a litters)([e]), F1a reduced pup weight.
- 20(26-48, gestation and lactation for the F1b litters)([e]), F1b reduced pup weight.
- 80(103-114, gestation producing the F1b litters)([e]), F1b skeletal anomalies, and reduced ossification, the only dose level studied.
- NOEL- 5(7.2-13.5, gestation and lactation for the F1b litters)([e]), for all developmental effects.

Discounted effects and toxicity

F0 male liver and liver/body weight ratio reduction at all dose levels.

F0 female kidney and kidney/body weight ratio increase at all dose levels.

The following dose levels were administered.

F0 males[f]	F0 females[f]
5(3.7-6.1)	5(4.2-8.6)
20(14.9-24.5)	20(17.8-29.5)
80(57.7-103.7)	80(70.7-124.5)

F1 males[f]	F1 females[f]
5(4.7-5.6)	5(4.5-6.0)
20(18-23)	20(19-24)
80[g]	

([a]) Target dose level(range in amount of test substance consumed, except during gestation and/or lactation, unless noted)
([b]) Includes test substance consumption prior to mating only.
([c]) Includes test substance consumption prior to mating, through gestation and lactation for the F1a litters and the gestation producing the F1b litters, the only gestation for which the effect was noted.
([d]) Includes test substance consumption throughout life time of the the F1 generation, which includes the F1b, F2a, and F2b litters.
([e]) Includes test substance consumption only during the period/s indicated.
([f]) Target dose levels administrated(range in test substance consumption for the test animals indicated, except for gestation and lacation) in mg/kg/day.
([g]) Due to excess toxicity the F1b litters, the highest dose level was not continued beyond weaning.

The dose levels were set at 50% of the premating dose during the second week of lactation and 33% of the premating dose during the third and forth week of lactation. This somewhat arbitrary setting of dose-levels during midlactation and end lactation, has merit but needs evaluation for its impact on Agency assessment of reproductive effects. Also, the consequence of the reduced dosing to young animals when the study was initiated and just after weaning needs evaluation. Animals eat approxmately twice as much food as they do as adults during the first 2-3 weeks post-weaning. Thus, they consumed less test substance in this study than would have if the concentration of the test substance in the feed had not been adjusted for body weight.

B. Conclusions

Toxicity was expressed in the F1b pups and in F1 females at dose levels lower than those administered to the F0 parents. Pup death occurred at birth and before lactation day 4 in F1a and F1b litters at the highest dose level which caused slight but statistically significant reduced weight gain in the F0 parents. Because of the toxicity to the pups at this target dose level of 80 mg/kg/day, this dose level was dropped from the study after weaning the remaining F1b pups. Reduced weight gain occurred in F1b pups during lactation at the middle dose level. At this same dose level, the F1b female pups, which became the F1 female generation, demonstrated a reduced body weight compared with controls during the last 4 weeks before sacrifice, but after weaning the F2b pups No significant effects occurred in any pups or any animals at the lowest target dose level. No reduced food consumption occurred to explain any of these effects on weights.

At all dose levels, absolute and relative liver weights were statistically significantly less than controls in F0 males and absolute and relative kidney weights at all dose levels were statistically significantly greater than controls in F0 females. These statistically significant effects did not demonstrate "smooth" dose response curves, and the effects were not confirmed in the F1 generation or in the histological examination of these organs. The report did not consider them to be biologically significant.

The toxicological significance of these effects are discounted. The liver weight reduction was not seen in 90 day subchronic and chronic studies conducted in this species and strain of rats. The increased kidney weights are also discounted because the kidney weights of 5 female controls were lower than the kidney weights of the remaining control animals of the F0 generation by approximately 3 standard deviations. If these animals are excluded from the average, then the kidney weights of dosed animals are comparable to the kidney weights of the remaining animals in the control group.

The LEL for development is reduced pup weight compared to controls during gestation and lactation of F0 dams at a target dose level of 20 mg/kg/day or a dose level range of

26-48 mg/kg/day. At the highest dose level, pup viability was reduced in the Fla and Flb litters. The NOEL for the reduced pup weight in the Flb litters compared to controls is a dose range of 7.2-13.5 mg/kg/day during gestation and lactation of F0 females.

Since the liver weight decrease in males,and the kidney weight increase in females is not considered biologically significant, the LEL in adults is in F1 females at the target dose level of 20 mg/kg/day, where statistically significant weight depression compared to controls occurred during the last 4 weeks before sacrifice,but after weaning the F2b pups. The NOEL for F1 adults then would be the target dose level of 5 mg/kg/day, the same target dose level as the NOEL for the Flb pup weight reduction. However, the range of dose levels consumed differed(see LEL and NOEL above).

No effects were seen on fertility in the F0 or the F1 males or females.

C. Study Design and Conduct

The study was conducted essentially according to the OPP guidelines proposed August 22, 1978, for a two-generation, two litters per generation study of reproduction. The quality assurance statment was signed the director of quality assurance, Ralfh Anderson, on 7/26/85.

About 140 Fischer 344 rats per sex were obtained from Charles River Breeding Laboratories, Inc, Kinston, NY on November 3, 1982, and quarantined for 13 days. Assignment of 30 rats per group were based on random selection of rats in a block design for body weight stratistication. Animals were housed individually under recommended conditions.

The F0 generation was placed on diets designed to deliver dose levels of 0, 5, 20, or 80 mg/kg/day, respectively, to each group, each of 30 rats per sex, for 105 days prior to mating. Subsequently, the animals were dosed in an analogous manner during each mating, each gestation, and each lactation. The total dosing and continuous dosing period for F0 animals was 40 weeks which included 2 weeks rest between the end of lactation for the Fla litters to the beginning of mating for the Flb litters and 30 days after weaning these latter litters.

The F1 generation, selected from the Flb pups, was exposed to the test substance in utero, and continuously via the

milk or the feed for 125 days postnatally and prior to mating and through mating, gestation and lactation for the F2a litters. Dosing continued through a 2 week rest period and mating, gestation, lactation for the F2b litters and for at least 30 days after weaning the F2b litters.

The total period of continuous administration of the test substance, from initial dosing of the F0 generation to the end of the F1 generation, was 77 weeks. During this period, the test substance was administered to the F0 generation, F1a and F1b litters, the F1 generation(selected from F1b litters), including the F2a and F2b litters and for 30 days after weaning the F2b litters.

The test substance was administered in the feed at target dose levels of 5, 20, or 80 mg/kg/day. The concentration in the feed was adjusted weekly according to the food consumption during the previous week and the average body weight for that week. This regimen was followed in the F0 generation up to week 15 (105 days) or just prior to mating to produce the F1a litters. Except as indicated below, monthly adjustments were made after mating. During mating, males and females were exposed to the diet prepared for the females which was based on the concentration prepared for the week prior to mating (week 15 for the F0 matings). The same dietary concentration was used throughout mating, gestation, and the first week of lactation. During the second week of lactation, the dietary concentration was reduced by 50 percent and during the third and fourth weeks of lactation, the dietary concentration was reduced by 67 percent of diet concentrations used during the first week of gestation (a concentration based on week 15). A similar dosing regimen was followed in producing the F1b litters, except the dosing regimen was based on body weights for week 24 and food consumption for week 15. The actual dose level consumed during gestation and lactation are given in tables 1 and 2.

The report claimed that the food consumption for week 15 was actually for 6 days instead of 7, but that the average daily food consumption used for week 24 was incorrectly based on a 7 day week. Thus, the average daily food consumption for week 15 was calculated to be 86%(6/7=.86) of the actual daily average. This would result in the intended concentration of test substance in the feed during production of the F1b litters to be 86% of the actual feed concentration used during this period. The report did not make it clear whether or not this same error was made in the test substance concentration in the feed used during production of the F1a litters.

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Table 1.

Test substance consumed during gestation in F0 and F1 dams producing F1a, F1b, F2a, and F2b litters.

		Target dose levels in mg/kg/day			
		0	5	20	80
		Test substance consumed, in mg/kg/day, was calculated from the concentration, food consumption, and body weight.			
F0 dams during the gestation producing the F1a	days 0-7	--	4.6	18.1	69.0
	days 7-13	--	5.0	20.5	79.6
	days 13-20	--	4.9	19.6	76.1
F0 dams during the gestation producing the F1b	days 0-7	--	7.2	26.4	103.4
	days 7-13	--	8.0	29.4	113.8
	days 13-20	--	7.5	28.4	106.9
F1 dams during the gestation producing the F2a	days 0-7	--	3.8	17.1	NC
	days 7-13	--	4.8	19.6	NC
	days 13-20	--	5.1	19.9	NC
F1 dams during the gestation producing the F2b	days 0-7	--	3.9	14.2	NC
	days 7-13	--	4.8	18.1	NC
	days 13-20	--	4.7	16.7	NC

NC-Testing of the F1 generation was not continued at this dose level after weaning.

59

Table 2

Test substance consumed during lactation in F0 and F1 dams for F1a, F1b, F2a, and F2b litters.

		Target dose levels in mg/kg/day			
		0	5	20	80
		Test substance consumption in mg/kg/day calculated from concentration, food consumption, and body weight. ([a])			
F0 dams during lactation for F1a	days 1-7	--	8.9	34.7	112.3
	days 7-14	--	6.5	25.2	84.0
	days 13-20	--	4.9	19.6	76.1
	days 14-21	--	5.1	20.7	72.4
	days 21-28	--	6.9	27.6	82.9
F0 dams during lactation for F1b	days 1-7	--	13.5	47.8	132.7
	days 7-14	--	9.4	34.6	106.9
	days 14-21	--	7.3	26.4	90.9
	days 21-28	--	9.3	32.6	112.7
F1 dams during lactation for F2a	days 1-7	--	8.2	34.0	NC
	days 7-14	--	5.9	24.6	NC
	days 14-21	--	4.6	18.8	NC
	days 21-28	--	7.0	29.1	NC
F1 dams during lactation for F2b	days 1-7	--	7.6	28.5	NC
	days 7-14	--	5.6	20.6	NC
	days 14-21	--	4.8	17.5	NC
	days 21-28	--	6.6	25.2	NC

NC-Testing of the F1 generation was not continued at this dose level after weaning.

([a]) Included in these values is the 50% reduction in the concentration of the test substance during the second week and the 67% reduction during the third and fourth week of lactation.

The F1b litters for the F1 generation remained on the reduced diet of lactation week 3 and 4 after weaning from the F0 dams. After selection for the F1 generation, pups were placed on test diets at target dose levels of 5 and 20 mg/kg/day, with weekly adjustments to week 53, the week prior to mating to produce the F2a litters. At this time, F1 males received the same diet as F1 females. Monthly adjustments were made to the diets after mating, except during the second week of lactation for the F2a and F2b litter when the concentration of the test substance was reduced by 50 percent and during the third and fourth weeks of lactation for the same litters when the concentrations of the test substance was reduced by 67 percent.

The study report did not specifically state how the doses were adjusted for the F1 generation but their dosing regimen can be calculated from the amounts of test substance consumed, and the body weights during the F1 generation for gestation and lactation for the F2a and F2b litters. The study report stated, "After selection, the F1 pups were placed on test diets at dose levels of 5 and 20 mg/kg/day." The study report also stated that adjustments were made to the diet based on food consumption and body weight.

These dosing regimens resulted in generally higher dose levels in the lactating F0 and F1 dams than for the premating target dose levels. The higher dose levels were greatest in F0 dams lactating for the F1b litters. Table 1 and 2 gives the amount of test substance consumed during gestation and lactation, respectively for F1a, F1b, F2a, and F2b litters. Consumption of test substance during gestation is included in table 1 because test substance consumption differed among these litters. The downward adjustment of the concentration in the feed during lactation did not occur during gestation. At other times the actual dose levels consumed were very close to the target dose levels.

The usual parameters were evaluated such as, fertility, duration of gestation, viability of pups at parturition and during lactation, the amount of food consumption, body weight, pup anomalies, and variations, in addition to histopathology on the testes, ovaries, kidneys, and livers. Organ weights were determined on the kidney, liver at necropsy and on testes after fixing in 10 percent formalin.

The following organs and tissues were taken at sacrifice and preserved, but histopathology was conducted only as previously indicated.

1. Adipose tissue
2. Adrenals
3. Aorta
4. Bladder
5. Bone marrow
6. Brain
7. Cecum, colon
8. Spinal cord
9. Epididymis
10. Esophagus
11. Eyes
12. Ovaries/Testes
13. Heart
14. Intestines
15. Kidneys
16. Liver
17. Lung/bronchi
18. Lymph node-thoracic and mesenteric
19. Mammary Gland and Skin
20. Nasal turbinates
21. Pancreas
22. Parathyroids
23. Pituitary
24. Prostate
25. Salivary Glands
26. Sciatic Nerve
27. Seminal Vesicle
28. Skeletal Muscle
29. Spinal Cord
30. Spleen
31. Sterum
32. Stomach
33. Thymus
34. Thyroid
35. Trachea
36. Uterus/cervix
37. Vagina

Statistical Methods

All analyses were conducted using two-tailed tests (unless otherwise specified).

1. Histopathological findings and incidence by sex were compared to control groups by Kalmogorov-Smirnov one-tailed test.

2. F0 and F1 male and female fertility indexes, F1a, F1b, F2a, and F2b pup sex ratios on lactation day 1, and F1a, F1b, F2a, and F2b pup survival indexes on on lactation day 4, 7, 14, 21, and 28 for the control groups were compared to each treated group by the Chi-square test with Yates correction factor.

3. Other effects in treated groups were compared to controls by analysis of variance followed by Dunnett's test.

Summary of Study Conduct

1. Test substance administered continuously throughout all phases of the study.

2. F0 dosed continuously from approx. 5 to 6 weeks of age for 105 days prior to first mating (i.e., approx. 20 weeks of age).

3. F0 mated 1:1 for 10 days and if no evidence of sperm, second matings were allowed with a proven male for 5 days.

4. F0 continued for 3 weeks of gestation and 4 weeks to weaning of F1a litters. Pups reduced to 8 per dam on day 4 of lactation.

5. All F1a litters necropsied and discarded after weaning.

6. F0 rested 2 weeks between weaning F1a and mating for production of the F1b as in #3.

7. F0 continued for 3 weeks gestation and 4 weeks to weaning of F1b litters. Pups reduced to 8 per dam on day 4 of lactation.

8. Ten F1b pups per sex per dose level randomly selected for necropsy, after weaning.

9. One pup per sex per dam per dose level randomly selected from F1b litters for the F1 generation. Because of excess toxicity at the target dose level of 80 mg/kg, only controls, and the target dose level groups of 5 and 20 mg/kg were continued on study. All F1b pups at 80 mg/kg/day were sacrified at the end of weaning.

10. All F0 animals were sacrificed on week 40 of the study.

11. Selected F1 pups were dosed via milk and in the feed for 125 days prior to mating to produce the F2a litters.

12. Dosing, mating, gestation, and weaning in the F1 generation producing the F2a and F2b litters followed procedures, including necropsy, similar to those followed for the F0 generation in producing the F1a and F1b litters.

13. All F1 animals were sacrificed on week 77 of the study.

14. All F1a, F1b, F2a, and F2b dying prior to weaning were studied for malformations and variations.

D. Test Chemical Identity and Concentration in the Feed

The study report, presented an analysis conducted by Wil Research Laboratories, and the Industry Task Force analysis on 2,4-D. According to a Wil Research analysis, the test substance was 95.8 percent pure 2,4-D. The report presented the following analysis of the test substance by the task force, but no further analysis or explanation of the differences between the Task Force analysis of 97.5 percent, and the Wil Research analysis of 95.8 percent, was presented.

2,4-D	97.5%	monochlorodibenzo-p-dioxin	0.003 ppm
2,6-D	0.91%	2,7-dichlorodi-benzo-p-dioxin	0.003 ppm
2,4,6-D	0.44%	1,3,8-trichlorodi-benzo-p-dioxin	0.010 ppm
0-chlorophenoxyacetic acid	0.31%	1,3,6,8-trichlorodi-benzo-p-dioxin	0.002 ppm
p-chlorophenoxyacetic acid	0.23%	dioxin	0.005 ppm
2,4-dichlorophenol	ND (0.03)	2,3,7,8-tetrachloro dibenzo-p-dioxin	ND (0.001)
water	0.18%		

ND = Not detected, (lowest level detectable).

Samples of the diets containing 2,4-D were collected for study weeks 0, 1, 2, 3, 4, 8, 13, 26, 39, 52, 65, 77. None of the sample diets were collected during weeks of gestation or lactation. The analyses after recovery of 2,4-D from the diets with the highest concentration were within 10 percent of the measured concentration. Analyses of 2,4-D in the diets at the middle dose level and the lowest dose level were always within 15 percent to 20 percent of the measured concentrations, except for three of the lowest dose levels which were 77 percent, 61 percent, and 55 percent of the measured dose levels. One was in a diet mixed on the 4th week of the study and two were for a diet mixed on the 13th week of the study. The 55 percent of the measured level was apparently a repeat analysis on a sample of the diet yielding the 61 percent of measured dose level.

E. Results

1. Fertility in F0 and F1 Males and Females.

No reduced fertility was expressed in males or females of the F0 generation in producing either the F1a or the F1b litters. However, a nonstatistically significant apparent reduction in male fertility occurred in producing the F1b litters (table 3). No reduced fertility was expressed in males or females of the F1 generation in producing the F2a and F2b litters. A second mating by a proven male was conducted when females demonstrated no evidence of sperm. The number of second matings producing the F1a/F1b pups were 0/6, 5/6, 1/2 and 0/2 for controls and the target dose levels of 5, 20, or 80 mg/kg/day, respectively. Second matings to produce F2a/F2b pups were 3/4, 2/1, and 4/4 for control and the target dose levels of 5, or 20 mg/kg/day.

The fertility index for production of the F1a and F1b litters is 70 to 79 percent in control F0 males and 70 to 79 percent in control F0 females (see table 3). The fertility index for males and females, respectively is the number of gravid females divided by the number males or females mated, respectively, adjusted to percent. These indexes ranged from 70 to 83 percent in treated males and 70 to 90 percent in treated females producing the F1a and F1b litters. Similarly, the fertility index for production of F2a and F2b litters is 60 to 70 percent in control F1 males and 64 to 72% in control F1 females (table 4.). These indexes range from 67 to 80 percent in treated F1 males and 64 to 80 percent in treated F1 females producing F2a and F2b litters. None were statistically significantly different from controls. The number of days required for mating ranged from 4.0 to 5.7 days of cohabitation to produce the F1a and F1b litters and 3.2 to 4.6 days of cohabitation to produce the F2a and F2b litters. These were no different from control values.

This failure to detect an effect on fertility is consistent with the lack of histopathological findings in the testes or epididymides of males and with the lack of histopathological findings in the ovaries or uteri of females from the F0 or F1 generation at terminal sacrifice. However, since the highest dose level was dropped from the study, fertility in the F1 generation was not evaluated at this dose level. Thus, the mid target dose level of 20 mg/kg/day should be considered the NOEL for fertility.

15

Table 3

Fertility indexes for F0 male and females producing F1a and F1b litters.

Fertility Index (no. gravid/no. males or females mated) x 100

	Producing F1a				Producing F1b			
Target dose	No. of males	%	No. of females	%	No. of males	%	No. of females	%
0	21/30	70	21/30	70	23/29	79	23/29	79
5	25/30	83	26/30	87	25/30	83	27/30	90
20	24/30	80	24/30	80	23/30	77	23/30	77
80	21/30	70	21/30	70	21/30	70	21/30	70

Table 4

Fertility indexes for F1 male and females producing F2a and F2b litters.

Fertility Index (no. gravid/no. males or females mated) x 100

	Producing F2a				Producing F2b			
Target dose	No. of males	%	No. of females	%	No. of males	%	No. of females	%
0	21/30	70	21/30	72	18/30	60	18/28	64
5	24/30	80	24/30	80	20/30	67	20/30	67
20	22/30	73	23/30	77	20/30	67	20/30	67

2. Length of Gestation in F0 and F1 Females

The lengths of gestation was statistically significantly prolonged in F0 females producing the F1b pups only and only at the highest target dose level of 80 mg/kg/day. This increase in gestational lengths was due to a gestation length of 23 days in approximately one half of the dams from this group instead of the usual 22 days of gestation demonstrated by most F0 and F1 dams in all groups. The LEL is between 103 and 114 mg/kg/day and NOEL is between 18 and 35 mg/kg/day.

The effect could result from delayed implantation, hormonal imbalance, or parturition problems. The effect is considered biologically significant and undesirable.

3. Body Weights of the F0 and F1 Generations.

The mean body weights of F0 males and female rats were statistically significantly less than controls in the high dose group only. In F0 males, the reduced body weight (97 percent of controls) was consistent after the sixth week of test substance consumption and in F0 females the body weight was consistently reduced (96 percent of controls) by the twelfth week of test substance consumption. The failure to gain as much weight as controls could not be attributed to reduced food consumption. The food consumption, and the food consumption per gram body weight gain was slightly increased. Body weights of the F0 generation in the target dose groups of 5 or 20 mg/kg were similar to control weights throughout this study, but food consumption appeared to be slightly elevated(not satistically significant).

F0 dams producing F1a and F1b litters had statistically significantly lower body weights than control weights on day 20 of the gestation producing the F1a and F1b litters in the highest dose group (table 5). At this dose level, body weights of dams were reduced on day 7, 13, and 20 of the gestation producing F1a litters, but the body weights of dams producing F1b litters were statistically significantly reduced only on day 20. Thus, toxicity was expressed in F0 dams during gestation of the F1a and F1b litters.

On lactation day 7, F0 dams lactating for F1a litters, express significantly reduced body weights in the highest dose group (table 5). For these dams, the body weight per gram of food consumed was about one half the value when compared to other dose groups and controls(data not shown). Dams demonstrated toxicity during lactation for the F1a, and for the F1b litters. At the end of lactation for the F1a and F1b litters, the body weights were satistically significantly elevated.

005446

Table 5

F0 Female Body Weight (g) during gestation and lactation for F1a and F1b litters.

	Target Dose Levels (mg/kg/day)			
	0	5	20	80
	Body wt. of F0 during gestation producing F1a			
Day 0	178	179	178	173
7	190	191	191	181**
13	208	208	206	196**
20	246	252	249	232*
	Body wt. of F0 during lactation for F1a			
Day 0	189	191	191	184
7	205	207	201	189**
14	212	212	207	208
21	216	213	219	212
28	189	184	185	204**
	Body wt. of F0 during gestation producing F1b			
Day 0	200	205	202	197
7	210	214	210	204
13	226	232	230	218
20	270	277	274	244**
	Body wt. of F0 during lactation for F1b			
Day 0	210	215	208	205
7	226	233	225	211*
14	228	237	233	224
21	229	239	234	231
28	203	197	193	226*

*p < 0.005, Dunnett's Test.
**p < 0.01, Dunnett's Test.

Table 6

F1 Female Body Weight (g) during gestation and lactation for F2a and F2b litters.

	Target Dose Levels (mg/kg/day)		
	0	5	20
	F1 during the gestation producing F2a		
Day 0	201	198	198
7	211	208	211
13	234	227	228
20	271	271	270
	F1 during lactation for F2a		
Day 0	216	211	211
7	228	221	222
14	234	232	233
21	232	233	236
28	220	221	223
	F1 during gestation producing F2b		
Day 0	222	221	214*
7	234	229	224*
13	250	248	241
20	293	290	278
	F1 during lactation for F2b		
Day 0	236	234	227*
7	248	245	237
14	260	245*	248
21	255	252	250
28	228	221	222

*$p < 0.05$, Dunnett's Test.

The body weights of the F1 generation, after selection, was comparable to control body weights, except in females at the target dose level of 20 mg/kg during weeks 74 to 77 where they were statistically significantly less than controls (97 percent of controls). The report stated that these body weight reductions in females were not biologically significant. No explanation was presented.

The body weights of F1 females during gestation and lactation demonstrated no consistently significant patterns during production or lactation for the F2a or F2b litters (table 6), however they were statistically significantly reduced on day 0 and 7 of the gestation producing the F2b litters, and on day 0 of lactation for the the F2b litters.

4. Pup Weights from F1a, F1b, F2a, and F2b Litters

Pup weights were significantly reduced over control weights in the F1a (table 7) and F1b (table 8) pups only. Both male and female F1a and F1b pup weights were less than control weights from birth to lactation day 28 in the 80 mg/kg target dose group. At the next lower dose level, both F1a and F1b male and female pup weights tended to be apparently lower than control weights toward the end of lactation. By day 20 of lactation, both male and female pups in the F1b litters only demonstrated a statistically significant decrease in body weight over control weights. The male pup weight in F1b litters in the lowest dose group which were statistically significantly reduced on lactation day 28 may not be biologically significant, since there were no apparent differences from control weights throughout the previous weeks of lactation.

None of the F2a or F2b pup weights were found to be different from control weights.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 71 of 959

005446

Table 7

Summary of Fla litter weights (g)
males and females

Group No.	Dose Level (mg/kg/day)	Males Mean S.D.	Lactation Days 1	4 Before Selection	4 After Selection	7	14	21	28
1	0	Mean	5.5	7.7	8.0	11.9	22.5	32.6	51.8
		S.D.	0.83	1.50	1.06	1.15	1.72	2.81	6.33
2	5	Mean	5.6	7.9	7.9	11.8	22.1	31.7	48.8
		S.D.	0.71	1.20	1.22	1.83	2.67	3.06	6.37
3	20	Mean	5.6	7.9	7.9	11.8	21.3	30.9	48.0
		S.D.	0.61	0.71	0.71	0.80	2.26	2.59	5.62
4	80	Mean	4.9*	6.4**	6.4**	8.5**	17.2**	26.7**	39.1**
		S.D.	0.46	0.71	7.22	1.30	2.10	2.22	5.24
Group No.		Females							
1	0	Mean	5.2	7.5	7.7	11.4	21.7	31.1	48.8
		S.D.	0.72	1.41	0.94	1.03	1.91	2.87	5.25
2	5	Mean	5.4	7.7	7.7	11.5	21.5	30.5	46.0
		S.D.	0.73	1.24	1.25	1.76	2.55	2.74	5.47
3	20	Mean	5.4	7.7	7.7	11.5	20.7	30.0	46.0
		S.D.	0.75	0.58	0.59	0.66	2.27	2.79	5.28
4	80	Mean	4.7	6.3**	6.3**	8.5**	17.0**	26.5**	39.3**
		S.D.	0.39	0.85	0.85	1.46	2.57	3.10	6.30

* = Significantly different from control group at .05 level using Dunnett's test.

** = Significantly different from control group at .01 level using Dunnett's test.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 72 of 959

Table 8

Summary of F1b litter weights (g)
Males and females on
lactation days

Group No.	Dose Level (mg/kg/day)	Males Mean S.D.	1	4 Before Selection	4 After Selection	7	14	21	28
1	0	Mean	5.8	8.5	8.5	12.4	23.9	34.6	56.0
		S.D.	0.43	0.77	0.77	1.17	2.31	3.58	8.87
2	5	Mean	5.6	8.4	8.4	12.5	23.9	34.3	50.6*
		S.D.	0.58	0.92	0.92	1.32	2.19	3.34	5.17
3	20	Mean	5.4	7.9*	7.9*	11.8	22.7	32.6	47.2**
		S.D.	0.50	0.63	0.63	0.86	1.18	2.34	7.26
4	80	Mean	4.5**	5.2**	5.2**	7.2**	15.9**	26.3**	41.1**
		S.D.	0.44	1.14	1.16	1.80	3.57	4.23	6.58
Group No.		Females							
1	0	Mean	5.3	8.1	8.1	11.7	22.5	32.3	51.0
		S.D.	0.46	0.83	0.83	1.11	1.89	2.95	7.52
2	5	Mean	5.3	8.0	8.0	11.9	22.9	32.6	47.4
		S.D.	0.59	0.83	0.84	1.19	1.79	2.59	4.56
3	20	Mean	5.2	7.6	7.6	11.2	21.7	30.9	44.2**
		S.D.	0.46	0.57	0.62	0.68	1.01	2.06	6.77
4	80	Mean	4.4**	5.6**	5.5**	7.2**	15.1**	25.0**	39.0**
		S.D.	0.54	0.83	0.80	1.23	1.00	0.91	1.47

* = Significantly different from control group at .05 level using Dunnett's test.
** = Significantly different from control group at .01 level using Dunnett's test.

The reduced F1b pup body weights in the mid dose level occurred from lactating dams demonstrating no statistically significant toxic signs at the time, although their body weights were apparently reduced from controls on lactation day 28. This may indicate that a change in the metabolism of 2,4-D occurred in F0 dams from production of the F1a to production of the F1b litters. Thus, dams exhibiting apparently no toxicity at the time, resulted in a reduction in pup weight over control weights.

5. Viability of F1a, F1b, F2a, and F2b Litters

The study demonstrated a statistically significantly reduced pup viability over controls only at the highest target dose level of 80 mg/kg (tables 9 and 10). The greatest reduction occurred in F1b pups at birth, with the mean litter size being about one half the control value due to deaths of portions and of entire litters. The mean litter size was reduced from five to three by day 14 of lactation, with no more deaths by lactation day 28 (table 10).

Some indication of reduced litter size was apparent in F1a litters of the target dose of 80 mg/kg, but the apparent decrease was not statistically significant (table 9). At birth however, there was a difference in the sex ratio of pups which was significant at the $p < 0.01$ level. From day 1 to day 28 of lactation, no further significant number of pup deaths occurred.

The study report stated that the decrease in female pups at births in F1a litters was not dose-related. I believe that it may be dose related, since at the highest test substance consumed by mothers producing F1b pups, where test substance consumption was higher than in dams producing the F1a litters, both male and female pup survival at birth were less in these F1b pups than the corresponding pup survival in the F1a pups. Thus, there appeared to be a dose response relationship.

Viability of the F2a and F2b pups was not affected.

6. Malformations and Variations

F1b pups which died before lactation day 28 were studied for malformation and variation. As can be seen from table 11, bent ribs, 14 the rudimentary ribs, malaligned sternebrae and unossitied sternebrae were seen in the F1b pups. Since most of these pups died at birth or were dead by day 1 of lactation, the effects were seen primarily just after birth at the highest dose only and in the F1b pups only. This was the only group for which there were sufficient deaths, and animals could be necropsied. Only pups which died were available for necropsy except at weaning. These effects are

Table 9
Summary of Fla viability indexes

Group No.	No. Dead Pups	Sex Ratios Day 1 M:F	Live Litter Size No.	Live Litter Size MEAN	Gestation Survival Index No.	Gestation Survival Index %	Day 4 Before Selection No.	Day 4 Before Selection %	Day 4 After Selection No.	Day 4 After Selection %
1	3	99:114	213/21	10.1	213/216	98.6	208/213	97.7	160/160	100.0
2	20	133:118	251/25	10.1	251/271**	92.6	247/251	98.4	191/191	100.0
3	3	121:116	237/24	9.9	237/240	98.8	237/237	100.0	183/183	100.0
4	9	109:71**	180/20	9.0	180/189	95.2	175/180	97.2	147/147	100.0

Group No.	Day 7 No.	Day 7 %	Day 14 No.	Day 14 %	Day 21 No.	Day 21 %	Day 28 No.	Day 28 %
1	156/160	97.5	156/160	97.5	156/160	97.5	156/160	97.5
2	190/191	99.5	190/191	99.5	190/191	99.5	190/191	99.5
3	183/183	100.0	183/183	100.0	183/183	100.0	183/183	100.0
4	146/147	99.3	143/147	97.3	143/147	97.3	143/147	97.3

1 - 0 mg/kg/day 2 - 5 mg/kg/day 3 - 20 mg/kg/day 4 - 80 mg/kg/day

Survival ratios and sex ratios compared using chi-square test.
Mean number of viable pups compared using analysis of variance.
** = Significantly different form control at .01 level.
Live litter size = No. pup alive on day 1 of lactation/no. litters.
Gestation index = No. pups alive on day 1 of lactation/total no. pups born.
Viability indexes = No. pups alive on day 4 before selection/no. pups alive day 1.
= No. pups alive day n/no. pups alive day 4 after selection.

005446

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 74 of 959

Table 10
Summary of F1b viability indexes

Group No.	No. Dead Pups	Sex Ratios Day 1 M:F	Live Litter Size No.	Live Litter Size MEAN	Gestation Survival Index No.	Gestation Survival Index %	Day 4 Before Selection No.	Day 4 Before Selection %	Day 4 After Selection No.	Day 4 After Selection %
1	5	112:107	219/23	9.5	219/224	97.8	219/219	100.0	164/164	100.0
2	15	120:131	251/25	10.0	251/266	94.4	246/251	98.0	177/177	100.0
3	3	110:128	238/23	10.4	238/241	98.8	237/238	99.6	174/174	100.0
4	110**	23:28	180/20	5.1**	51/161**	31.7	44/51**	86.3	42/42	100.0

Group No.	Day 7 No.	Day 7 %	Day 14 No.	Day 14 %	Day 21 No.	Day 21 %	Day 28 No.	Day 28 %
1	164/164	100.0	164/164	100.0	164/164	100.0	164/164	100.0
2	177/177	100.0	177/177	100.0	176/177	99.4	176/177	99.4
3	174/174	100.0	174/174	100.0	174/174	100.0	174/174	100.0
4	34/42**	81.0	30/42**	71.4	30/42**	71.4	30/42**	71.4

1 - 0 mg/kg/day 2 - 5 mg/kg/day 3 - 20 mg/kg/day 4 - 80 mg/kg/day

Survival and sex ratios compared using chi-square test.
Mean number of viable pups compared using analysis of variance.
** = Significantly different from control at .01 level.
Live litter size , gestation index and viability indexes = see legend table 9.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 75 of 959

sometimes seen at dose levels causing maternal toxicity, but administration of many compounds do not cause these effects at maternally toxic dose levels.

The number of malformations and variations in these F1b pup dying prior to weaning were apparently not sufficient for statistical significance by the Fischer exact test. As can be seen from Table 11, 50 percent of the litters which died in the high dose group had, for example, malaligned sternebrae compared with 20 percent in controls. The adequacy of these statistical evaluations appear questionable and perhaps should be reevaluated by OPP. However, even if the number of anomalies and variations were significant in the high dose group, the failure to find significant numbers of these effects in five litters examined in each of the controls and the lowest dose group may indicate that these effects did not occur below the highest dose level.

If comparable examinations were conducted in all F1a pups, a dose relationship may have been apparent in the anomalies and variations. There is no indication that this was done. A detailed study on developmental effects on the F1a pups which died during lactation was conducted but these numbers were insufficient to establish a NOEL. If the F1a pups were preserved, it may have been useful to have examined them for a dose related response in developmental effects. However, by day 28 of lactation, all of the apparent effects anologus to those seen in the F1b pups shortly after birth may have disappeared.

Dose levels consumed by dams around the perinatal period were greater for the F1b litters than for the F1a litters. The week immediately before parturition, gestational day 13-20, the dams of the F1a pups consumed the test substance at a daily rate of 76.1 mg/kg, while the dams of the F1b pup, during the corresponding time period consumed 107 mg/kg. The daily consumption of test substance by dams during the first week of lactation for the F1a and F1b pups was 112 and 133 mg/kg, respectively, in the 80 mg/kg target dose level group.

Table 11.

Total Number of Pups and Litters with Developmental and Genetic Variations - Only F1b Pups Found Dead Lactation Days 0-28

	Pups				Litters			
Dose Group	1	2	3	4	1	2	3	4
Number Examined Externally	5	15	3	103	5	5	3	18
Findings	----None--------				----None------			
Number Examined Viscerally	5	15	3	103	5	5	3	18
Findings	----None--------				----None------			
Number Examined Skeletally	5	14	3	98	5	5	3	18
Sternebra #5 and/or #6 Unossified	0	0	0	7	0	0	0	5
Sternebrae #1, #2, #3 and/or #4 Unossified	0	0	0	1	0	0	0	1
Sternebrae Malaligned (slight or moderate)	1	0	0	23	1	0	0	9
14th Rudimentary Rib(s)	0	0	0	12	0	0	0	6
Bent Rib(s)	0	0	0	30	0	0	0	6
Reduced Ossification of the Vertebral Arches	0	0	0	2	0	0	0	2

None significantly different from control group using Fisher's Exact Test.

7. Organ Weights and Histological Studies

The absolute and relative liver weights were reduced at all dose levels in the F0 males (table 12). Only the liver/body weight ratios are presented. The absolute and relative kidney weights were increased at all dose levels in F0 females (table 12). The report did not consider the effects on organ weights to be dose-related in either sex. No explanation for this opinion or for these possible test substance related effects was presented. However, neither effect exhibited a smooth dose-related decrease or increase, respectively.

In the F1 generation relative kidney weight of the left but not the right kidney was significantly elevated in males at the 20 mg/kg target dose level only (table 13). The relative liver weights in males of this group were apparently elevated but not statistically. The relative liver weights were increased in F1 females of this dose group but the apparently slightly elevated kidney weights, probably, are not dose related (table 13). Thus, the possible oragn weight effects in F1 generation failed to confirm the statistically significant organ weight effects seen in the F0 generation.

No organ weight effects or histopathology was seen in the testes from any dose level from any generation. No dose-related histological effects were seen in the ovary. Thyroids may have been saved but no histology was conducted on them. All the histological studies conducted failed to find any dose-related pathology in any of these organs in the F0 generation and the F1a, F1b, and F1 generation and F2a and F2b pups.

Two histological studies on the livers of the F0 animals were reported. One study was conducted by the testing facility (table 14), and the other was conducted by W. Ray Brown of Research Pathology Services,Inc., New Britain, P.A.(table 15).

When the livers from F0 males were examined histologically numbers of small foci of necrosis were found in all groups. This was initially diagnosed as Tyzzer's desease (table 14). This diagnosis was rejected because females were not affected, diarrhea was not detected, and survival was normal. Research Pathology Services found that small basophilic alterations in hepatocytes occurred at a slightly higher incidence in dosed animals(table 15). In females, these alterations occurred at a slightly higher incidence in controls. None of these histological findings were considered to be dose related by either pathologist.

Table 12.

F0 Terminal Body Weights and Relative Organ Weights

	Target Dose Levels mg/kg/day			
	0	5	20	80
F0 male bwt.	372.	373	368	354**
SD	15.4	17.8	18.3	19.5
F0 female bwt.	217	220	216	209**
SD	10.5	9.8	8.4	12.2
F0 male organ wt. per 100 g bwt.				
Lt Kidney	0.417	0.356**	0.421	0.435
SD	0.18	0.06	0.05	0.04
Rt Kidney	0.469	0.357**	0.420	0.429
SD	0.18	0.06	0.05	0.05
Liver	3.474	3.242**	3.337*	3.226**
SD	0.22	0.18	0.17	0.25
Testes	0.830	0.835	0.821	0.854
SD	0.04	0.04	0.05	0.08
F0 female organ wt. per 100 g bwt.				
Lt Kidney	0.351	0.471**	0.410*	0.425**
SD	0.14	0.12	0.05	0.04
Rt Kidney	0.361	0.476**	0.398	0.424
SD	0.15	0.11	0.06	0.04
Liver	3.477	3.663	3.608	3.627
SD	0.21	0.50	0.27	0.20

SD = Standard deviation; * = $p < 0.05$; ** = $p < 0.01$

Table 13.

F1 Terminal Body Weights and Organ Weight Ratios

	Target Dose Levels[a] mg/kg/day		
	0	5	20
F1 male bwt.	394.	388	386
SD	13.8	28.8	22.9
F1 female bwt.	238	231	231*
SD	9.8	9.0	11.1
F1 male organ wt. per 100 g bwt.			
Lt Kidney	0.394	0.381	0.411*
SD	0.025	0.03	0.02
Rt Kidney	0.390	0.378	0.402
SD	0.02	0.03	0.02
Liver	3.315	3.345	3.439
SD	0.25	0.25	0.17
Testes	0.865	0.857	0.861
SD	0.06	0.11	0.08
F1 female organ wt. per 100 g bwt.			
Lt Kidney	0.398	0.406	0.419
SD	0.03	0.04	0.03
Rt Kidney	0.402	0.400	0.415
SD	0.03	0.05	0.03
Liver	3.568	3.566	3.808**
SD	0.27	0.33	0.25

SD = Standard deviation; * = $p < 0.05$; ** = $p < 0.01$
[a]F1 at 80 mg/kg/day target dose level not dosed beyond weaning.

Table 15.

F0 histomorphological summary incidence for liver, at terminal sacrifice. Summary from Research Pathology Services

Sex	Male				Female			
Dose group	1	2	3	4	1	2	3	4
Number examined	30	30	30	30	29	29	30	29
Number normal	1	29	23	24	9	7	12	8
Multifocal bile duct proliferation	25	29	23	24	9	7	7	11
Focal necrosis	11	2	1	3	1	1	1	0
Multifocal necrosis	13	19	15	11	0	0	0	0
Focal cellular alteration								
Basophilic-cell focus/foci	0	4	3	6	8	9	3	1
Clear-cell focus/foci	1	0	0	2	0	0	0	0
Eosinophilic-cell focus/foci	0	0	0	0	0	0	1	0
Microgranuloma/s	2	5	4	2	6	3	7	9
Multifocal mononuclear cellular infiltration	6	2	4	3	6	10	6	8
Accessory lobe	1	1	0	3	2	1	0	3
Centrilobular hepato-celular vacuolation	0	0	0	1	0	0	0	0
Focal hepatocellular vacuolation	1	0	0	0	0	0	0	0
Congestion	0	0	0	1	0	0	0	0
Congenital anomaly	0	0	0	0	1	0	0	0

Table 14.

F0 histomorphological at terminal sacrifice.
Summary incidence for the live.
Testing laboratory summary.

Sex	Male				Female			
Dose group	1	2	3	4	1	2	3	4
Number of animals studied	30	30	30	30	29	29	30	29
Liver								
Total examined	30	30	30	30	29	29	30	29
Examined, unremarkable	6	3	9	13	20	22	20	17
Not examined	0	0	0	0	0	0	0	0
Cholangiofibrosis	21	20	19	14	5	3	5	5
Accessory lobe	1	1	0	3	2	1	0	3
Tyzzer's disease	4	18*	10	1	0	0	0	0
Nonspecific Kupffer cell granuloma	0	0	0	0	4	4	6	5

1= 0 mg/kg/day 2= 5 mg/kg/day 3= 20 mg/kg/day 4= 80 mg/kg/day

* Significantly different from control at 0.05 level, using Kolmogorov-Smirnov, one-tailed test.

8. Summary and Discussion

1) The study reviewed is a 2-generation, 2 litter per generation study of the effects of 2,4-dichlorophenoxy-acetic acid (2,4-D) on reproduction in Fischer 344 rats.

2) The test substance, (97.5% 2,4-D by an I.T.F analysis; and 95.8% 2,4-D by a WIL analysis) was administered in the feed, ad libitum, to 30 rats per sex per group. The concentration of the test substance was adjusted in the feed weekly or monthly according to food consumption and body weight in an attempt to meet target dose levels of 0, 5, 20 or 80 mg/kg/day. During gestation and lactation the actual dose level administered was generally higher, see table 1 and 2, even with 50 percent reduction in concentration during week 2 and 67 percent reduction in concentration during week 3 and 4 of lactation.

3) No significant effects on fertility of males or females at any dose or in any generation was evident. This conclusion is supported by the failure to find dose related effects on the testes weight or on histological examination of the testes. No dose related histological effects were seen in ovaries. There was no dose related differences in the number of second matings or in the time required for cohabitation. The fertility of Fischer 344 rats is not high, 60-79 percent in controls, and the variability of the fertility probably would allow detection of only severe reductions in fertility.

4) The lengths of gestation was prolonged by 1 day in approximately one half the F0 dams producing F1b litters only in the highest dose group. This effect could result from delayed implantation, hormonal imbalances, or parturition problems.

5) The mean body weights of the F0 generation were statistically significantly reduced compared to controls prior to mating, at the highest dose level. Since body weight gain per gram of food consumed was apparently nearly always less in the high dose group than in the other treatment groups or the controls, the body weight decrease cannot be explained by decreases in food consumption. At this dose level, food consumption was frequently satistically significantly

increased over control values. At the two lowest dose levels, food consumption was generally apparently increased, but it was seldom statistically significant. Thus, the weight reduction probably is real.

6) During lactation, the body weights of F0 dams in the high dose group were not consistently reduced and in the middle dose group in the F0 dams lactating for the F1b litters, there were no statistically significant reductions in body weight compared to controls. Note: It was in the mid dose group and during lactation for F1b litters, that the LEL for pup weight depression occurred.

7) The body weights of F1 females during gestation and lactation for F2a and F2b litters were infrequently significantly different from control weights(see table 6). After weaning of the F2b litters from week 44-77 were adult F1 female body weights significantly less than control weights for the target dose level of 20 mg/kg. The body weights of male F1 rats were not different from control weights at any time after weaning.

8) Pup body weights were significantly reduced over control weights in the F1a and F1b pups only. These reduced pup weights occurred at the highest dose throughout lactation and in the mid dose only toward the end of lactation, and only in the F1b pups. The NOEL was the lowest target dose level administered.

9) Pup viability was reduced at parturition and during the first day of lactation in F1a and F1b pups at the target dose level of 80 mg/kg (actual 76.1 to 133 mg/kg/day) only. A reduction in litter size probably also occurred in the highest dose group in the F1a litters. The apparent reduction probably was dominantly due to a decrease in number of female pups born, causing a significant difference in the sex ratio at birth.

Pup viability was more severely and significantly reduced in the F1b litters than in F1a litters at birth and between birth and lactation day 1 in addition to the period between lactation day 1 and lactation day 4. The sex ratio in these F1b pups was normal, probably because male, in addition to female pup viability, was less than in the F1a litters.

10) Anomalies and variations occurred in F1b litters of the high dose which died during lactation. This was the only group for which those effects could be determined because it was the only group apparently for which skeletal examinations were conducted. In addition, it was the only group in which a large number of nonscheduled pup deaths occurred.

These skeleltal anomalies and reductions in ossification are generally consistent with similar effects produced by 2,4-D in the teratogenicity study in Fischer 344 rats. The NOEL for developmental effects in that study is 25 mg/kg/day.

11) The absolute and relative liver weights of F0 males were statistically significantly reduced at all dose levels at terminal sacrifice. The absolute and relative kidney weights of F0 females were statistically elevated over control weights at all dose levels. There was not a "clean" dose-response relationship and the report did not consider the effect on either sex to be biologically significant.

The liver weight reductions seen in the males may not be toxicologically or pharmacologically significant, and could be an artifact of the study.
a) There was no "smooth" dose response relationship with the liver weight and the dose of the test chemical.
b) F1 males and females demonstrated no liver weight reductions.
c) No significant liver weight reductions occurred in a 90-day subchronic or a chronic study conducted at 1, 5, or 45 mg/kg/day in the Fischer 344 rat.
d) The reductions probably are not due to the slight thyroid effects analogus to the thyroid effects seen in the subchronic and chronic studies, because only higher elevations of T4 than those seen cause glycogen depletion in the liver.

e)The reductions are not due to an interaction of 2,4-D with the liver histological findings seen. The liver weights in control animals with and without focal necrosis, multifocal necrosis, or basophilic alterations were each not different from each other. Similar comparisions failed to detect diffences in the highest dose level group.
f) Food consumption apparently increased at all the higher dose levels, and in some cases the increase was satistically significant. Thus, the liver weight reduction is not due a reduction in food consumption.

The satistically significant kidney weight increase in females of the F0 generation probably are not correlated with the kidney histopathology seen the males and female of the subchronic and chronic studies. No kidney histopathology was seen in any animals in the reproduction study. In addition, the kidney weights of 5 females in control animals were an average of 0.18 g for the left or the right kidney, whereas the average kidney weights in the remaining control animals were 0.9 g for the left or the right kidney, approximately 3 standard deviations different. Thus, if these 5 animals are removed from controls, the kidney weights in dosed animals are comparable to controls.

It is concluded that the kidney weight increase is due to an anomaly in the kidney weights of 5 control females, and that it is not due to the test substance.

12) No significant dose-related histopathology occurred in any organ at any dose level in any generation.

005446

References:

1. Subchronic toxicity study in Fischer 344 rats conducted by Hazleton Laboratories, Report No. 2184-102, dated September 12, 1983, for the Industry Task Force on 2,4-D Research No. 251474.
Feeding study conducted 90 days at dose levels of 0, 1, 5, 15, or 45 mg/kg/day.

2. Interim 52-week report on 2,4-D chronic feeding/ oncogenicity study in Fischer 344 rats. Conducted by Hazleton Laboratories submitted by the Industry Task Force on 2,4-D Research. Accession No. 256019.

   Feeding study conducted at 0, 1, 5, 15, or 45 mg/kg/day.

3. Teratogenicity study of 2,4-D in Fischer 344 rats. Conducted at WIL Research Laboratories (WIL-81135) for the Industry Task Force on 2,4-D Research.

   Study conducted at 0, 8, 25, or 75 mg/kg/day by gavage.

4. Reproduction study of 2,4-D in Fischer 344 rats. Conducted by WIL Research Laboratories (WIL-81137) for the Industry Task Force on 2,4-D Research. Accession No's. 259442-6.

87

CASWELL FILE

MAR

005754

Subject: Addendum to the Effects of 2,4-D in a Two-Generation Study on Reproduction in Rats: Correction on the Histopathology of the Kidneys of Males

From: David G Anderson, PhD.
Toxicology Branch
Section VII
Hazard Evaluation Division (TS-769C)

To: Ms. Linda Vlier PM #61
Special Review Branch
Registration Division (TS-767C)

Thru: Albin B Kocialski, PhD.
Supervisory Pharacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ABK 1/14/87

An Addendum to the 2-generation rat reproduction feeding study on 2,4-D has been reviewed(accession # 265489). Only the LEL for the F0 generation changed. The LEL now includes kidney pathology as well as body weight gain reduction. The recalulated and nominal NOEL's and the LEL's with their respective effects are as follows.

F0 parental toxicity.
NOEL - 5(5) mg/kg/day.*
LEL - 20(20) mg/kg/day, male kidney pathology.

F1 parental toxicity.
NOEL - 4(5) mg/kg/day.
LEL - 14(20) mg/kg/day, male kidney pathology, and reduced female body weight.

Developmental toxicity, dose level to dams.
NOEL - 7(5) mg/kg/day.
LEL - 26(20) mg/kg/day, reduced weight in F1b pups.

Nominal dose levels administered 0, 5, 20, or 80 mg/kg/day.

* Calculated lowest dose level within the range consumed by the animals at the nominal dose level administered (nominal dose level administered). The values have been rounded off to the nearest whole number.

Reviewed by: David G Anderson David G Anderson 10/23/86
Section VII, Tox. Branch (TS-769C)
Secondary reviewer: Albin B Kocialski
Section VII, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Addendum to the study of 2,4-D on Two-Generations of Reproduction in Rats: Correction to histopathology of the kidneys.

TEST SUBSTANCE: 2,4-Dichlorophenoxyacetic Acid (2,4-D)

SYNONYMS: 2,4-D TOX. CHEM. NO. 315

ACCESSION NO.: 265489.

SPONSOR: Industry Task Force on 2,4-D Research Data (ITF)

TESTING FACILITY: Wil Research Laboratories, Inc. (WIL)
Ashland, OH 44805-9281

TITLE OF REPORT: A Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxyacetic Acid: Addendum to the final report.

AUTHORS: Dean E Rodwell, and W. Ray Brown.

STUDY NO.: WIL-81137, same study no. as accession number 265489.

TESTING PERIOD: November 16, 1982 to May 15, 1984.

REPORT ISSUED: September 30, 1986.

PURITY OF TEST SUBSTANCE: See original review of accession no. 259442-6.

CORE GRADE: Not applicable.

CONCLUSIONS ON THE EFFECT AND NO EFFECT LEVELS:

The effect levels for the F0 and F1 males were altered but the no effect levels described in the review of the original study are not altered by the results submitted in this addendum. The LEL and NOEL are restated on the following page. They include kidney histopathological findings reported in this addendum.

LEL and NOEL is expressed in mg/kg/day(Nominal dose level in mg/kg/day).

F0 parental toxicity
LEL- 19.9(20), degeneration of male kidney tubules.
NOEL- 5(5)
F1 parental toxicity
LEL- 14(20), kidney histopathology in males, and reduced body weight in females.
NOEL- 3.8(5)
Developmental toxicity
LEL- 26(20), F1b pup weight reduction.
NOEL- 7.2(5)

Target or nominal dose levels administered in the study are 0, 5, 20, or 80 mg/kg/day.

CONCLUSIONS:

The reexamination of the kidneys from the 2-generation study on reproduction indicated tubule degeneration in males of the F0 and F1 generations which apparently had not yet developed in 28 day old pups. Cortical tubule degeneration(observed mostly in the proximal convoluted tubules) was confined to the F0 males nominally dosed at 80 mg/kg/day, probably because no F1 animals were dosed at this level passed weaning. Most of these pups died prior to weaning; thus, the study was not continued past weaning. No test substance related kidney histopathology was observed in the remaining pups at any dose level. Both the F0 and the F1 male generations nominally dosed at 20 mg/kg/day demonstrated minimal degeneration of tubules in the outer medullary region of the kidney, but not in the cortical region. No test substance related effects occurred at the nominal dose level of 5 mg/kg/day.

These kidney findings on reexamination cast doubt on the quality of the histological examination conducted in the study on reproduction previously reviewed.

A. MATERIALS AND METHODS:

Kidney sections prepared on male rats from the F0 and F1 generations and the F1b pups dosed in the two-generation study of the effects of 2,4-D on reproduction in rats were reexamined.

Target or nominal dose levels(mg/kg/day)	Number of rats reexamined (Tissue sections from these animals) F0	F1	F1b
0 (Control)	30	29	10
5 (LDT)	29	30	10
20 (MDT)	30	29	10
80 (HDT)	30	0	14

F0 males were dosed approximately 40 weeks prior to sacrifice. F1 males were dosed approximately 47 weeks, including 3 weeks in utero and 4 weeks of lactation form the milk and from the mothers food supply especially during the last half of the lactaction period. F1b pups(from which the F1 generation was formed) were dosed for 7 weeks as indicated above, 3 weeks in utero and 4 weeks of lactation. The HDT F1 generation males were not reexamined because of poor survival at this dose level.

B. RESULTS:

The results of the histological reevaluation of the male kidneys are presented in Table 1. Tubules of outer medullary region were characterized in the report as demonstrating probable degenerative or atrophic changes of the epithelial cells in the mid and high dose groups. The involved segments were small and the appearance of increased nuclear density was the result of condensation of the effected portions of the tubule.

In addition to the medullary involvement the cortical tubules (mostly the proximal convoluted tubules) of the high dose F0 generation were large and demonstrated a dense, eosinophilic cytoplasm. The lumens of some of these tubules were indistinct when compared to controls. No F1 adult animals were studied at the highest dose level because of death due to excessive toxicity.

In the mid dose, the histology of the kidneys tubules from F0 and F1 males was less clear, but 7/30 F0 animals were reported to demonstrated the increased nuclear density and 4/29 F1 male rats demonstrated similar histopathology(Table 1).

Other sporatic effects occurred in the kidneys with no apparent dose related response.

C. DISCUSSION:

The kidneys of the F0 male rats from the high dose group in the study of reproduction demonsrated degenerative changes in the tubules of the cortical region and the outer medullary region. In the mid dose groups of the F0 generation and the F1 generation (the highest dose level studied in the F1 generation), less distinct changes occurred and they occurred only in the medullary tubules. No dose related kidney effects were seen in the F1b pups at any dose level.

Thus, no effect level for the study on reproduction did not change, however the effect level in adult rats must now include kidney histopathology and reduced weight gain. Prior to the addendum, the lowest effect level was characterized by only a reduced weight gain in adults and pups.

NOTE:

Since this reexamination of the kidney histology was conducted only after the sponsor identified these effects in a rat subchronic study, there is doubt about the quality of the original histological examination conducted in the reproduction study. The reexamination was conducted by Ray Brown of Research Pathology Associates but the original histological examination was conducted by the testing facility, Wil Research Laboratories. Other organs examined histologically by Wil Research but not by Ray Brown are the epidydimis testis, uterus, and ovary. The study on reproduction gave no indication that the kidneys nor any other organ required histological examination.

Table 1.

Incidence of findings reexamination of kidneys from males
from a 2-generation study of the effects of 2,4-D on reproduction.

Adults or pups	F0 adults				F1 adults			F1b pups			
Nominal dose group:	1	2	3	4	1	2	3	1	2	3	4
Number of rats/group:	30	29	30	30	29	30	29	10	10	10	14
Number examined:	30	29	30	30	29	30	29	10	10	10	14
Number normal:	18	21	16	1	12	16	12	9	9	9	11
Description:											
Increased cytoplasmic eosinophilia in cortical tubules.	0	0	0	28	0	0	4	0	0	0	0
Increased focal nuclear density in medullary tubules.											
minimal	0	0	7	17	0	0	4	0	0	0	0
slight	0	0	0	4	0	0	0	0	0	0	0
moderate	0	0	0	1	0	0	0	0	0	0	0
Total incidence	0	0	7	22	0	0	4	0	0	0	0
Multifocal tubular degeneration/basophilia											
minimal	10	8	8	3	13	8	10	1	0	1	3
slight	0	0	0	0	0	2	0	0	0	0	0
Total incidence	10	8	8	3	13	10	10	1	0	1	3
Microcalculi	1	1	1	1	2	4	1	0	1	0	0
Pelvic dilation/hydronephrosis, unilateral.	1	0	1	0	2	1	1	0	0	0	0
Focal/multifocal mononuclear cellular infiltation.	1	1	0	0	3	1	0	0	0	0	0
Focal tubular dilation.	0	0	0	0	3	2	6	0	0	0	0
Focal/multifocal chronic nephritis.	0	0	0	0	1	0	1	0	0	0	0
Pelvic mineralization	0	0	0	0	0	1	0	0	0	0	0
Focal papilllary edema	0	0	0	0	0	0	1	0	0	0	0

Nominal dose groups: 1 = 0 mg/kg/day, 2 = 5 mg/kg/day, 3 = 20 mg/kg/day, 4 = 80 mg/kg/day.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 93 of 959

005684

005754
(Same)

Subject: Addendum to the Effects of 2,4-D in a Two-Generation Study on Reproduction in Rats: Correction on the Histopathology of the Kidneys of Males.

Caswell # 315

From: David G Anderson, PhD.
Toxicology Branch
Section VII
Hazard Evaluation Division (TS-769C)

David G. Anderson 1/16/87

To: Ms. Linda Vlier, PM #61
Special Review Branch
Registration Division (TS-767C)

Thru: Albin B Kocialski, PhD.
Supervisory Pharacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ABK 1/20/87

An Addendum to the 2-generation rat reproduction feeding study on 2,4-D has been reviewed(accession # 265439). The LEL and the NOEL in the F0 generation changed. The LEL is now 20 mg/kg/day, and the NOEL is now 5 mg/kg/day for the F0 generation. The LEL now includes kidney pathology as well as body weight reduction. However, the NOEL inclusive of all endpoints in the review of the original study is not altered by the results of this addendum. The recalulated and nominal NOEL's and the LEL's with their respective effects are as follows.

F0 parental toxicity.
NOEL - 5(5) mg/kg/day.*
LEL - 20(20) mg/kg/day, male kidney tubule degeneration.

F1 parental toxicity.
NOEL - 4(5) mg/kg/day.
LEL - 14(20) mg/kg/day, male kidney tubule degeneration, and reduced female body weight.

Developmental toxicity, dose level to dams.
NOEL - 7(5) mg/kg/day.
LEL - 26(20) mg/kg/day, reduced weight in F1b pups.

Nominal dose levels administered 0, 5, 20, or 80 mg/kg/day.

* Calculated lowest dose level within the range consumed by the animals at the nominal dose level administered (nominal dose level administered). The values have been rounded off to the nearest whole number.

005684

Reviewed by: David G Anderson David G. Anderson 10/23/86
Section VII, Tox. Branch (TS-769C)
Secondary reviewer: Albin B Kocialski
Section VII, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Addendum to the study of 2,4-D on Two-Generations of Reproduction in Rats: Correction to histopathology of the kidneys.

TEST SUBSTANCE: 2,4-Dichlorophenoxyacetic Acid (2,4-D)

SYNONYMS: 2,4-D TOX. CHEM. NO. 315

ACCESSION NO.: 265489.

SPONSOR: Industry Task Force on 2,4-D Research Data (ITF)

TESTING FACILITY: Wil Research Laboratories, Inc. (WIL)
Ashland, OH 44805-9281

TITLE OF REPORT: A Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxyacetic Acid: Addendum to the final report.

AUTHORS: Dean E Rodwell, and W. Ray Brown.

STUDY NO.: WIL-81137, same study no. as accession number 265489.

TESTING PERIOD: November 16, 1982 to May 1, 1984.

REPORT ISSUED: September 30, 1986.

PURITY OF TEST SUBSTANCE: See original review of accession no. 259442-6.

CORE GRADE: Not applicable.

CONCLUSIONS ON THE EFFECT AND NO EFFECT LEVELS:

The effect levels for the F0 and F1 males were altered but the no effect levels described in the review of the original study are not altered by the results submitted in this addendum. The LEL and NOEL are restated on the following page. They include kidney histopathological findings reported in this addendum.

LEL and NOEL is expressed in mg/kg/day(Nominal dose level in mg/kg/day). The values have been rounded off to the nearest whole number.

F0 parental toxicity
LEL- 20(20), degeneration of male kidney tubules.
NOEL- 5(5)
F1 parental toxicity
LEL- 14(20), dengeration of male kidney tubules, and reduced body weight in females.
NOEL- 4(5)
Developmental toxicity
LEL- 26(20), F1b pup weight reduction.
NOEL- 7(5)

Target or nominal dose levels administered in the study are 0, 5, 20, or 80 mg/kg/day.

CONCLUSIONS:

The reexamination of the kidneys from the 2-generation study on reproduction indicated tubule degeneration in males of the F0 and F1 generations which apparently had not yet developed in 28 day old pups. Cortical tubule degeneration(observed mostly in the proximal convoluted tubules) was confined to the F0 males nominally dosed at 80 mg/kg/day, probably because no F1 animals were dosed at this level passed weaning. Most of these pups died prior to weaning; thus, the study was not continued past weaning. No test substance related kidney histopathology was observed in the remaining pups at any dose level. Both the F0 and the F1 male generations nominally dosed at 20 mg/kg/day demonstrated minimal degeneration of tubules in the outer medullary region of the kidney, but not in the cortical region. No test substance related effects occurred at the nominal dose level of 5 mg/kg/day.

These kidney findings on reexamination cast doubt on the quality of the histological examination conducted in the study on reproduction previously reviewed.

A. MATERIALS AND METHODS:

Kidney sections prepared on male rats from the F0 and F1 generations and the F1b pups dosed in the two-generation study of the effects of 2,4-D on reproduction in rats were reexamined.

Target or nominal dose levels(mg/kg/day)	Number of rats reexamined (Tissue sections from these animals) F0	F1	F1b
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5 (LDT)	29	30	10
20 (MDT)	30	29	10
80 (HDT)	30	0	14

F0 males were dosed approximately 40 weeks prior to sacrifice. F1 males were dosed approximately 47 weeks, including 3 weeks in utero and 4 weeks of lactation form the milk and from the mothers food supply especially during the last half of the lactaction period. F1b pups(from which the F1 generation was formed) were dosed for 7 weeks as indicated above, 3 weeks in utero and 4 weeks of lactation. The HDT F1 generation males were not reexamined because of poor survival at this dose level.

B. RESULTS:

The results of the histological reevaluation of the male kidneys are presented in Table 1. Tubules of outer medullary region were characterized in the report as demonstrating probable degenerative or atrophic changes of the epithelial cells in the mid and high dose groups. The involved segments were small and the appearance of increased nuclear density was the result of condensation of the effected portions of the tubule.

In addition to the medullary involvement the cortical tubules (mostly the proximal convoluted tubules) of the high dose F0 generation were large and demonstrated a dense, eosinophilic cytoplasm. The lumens of some of these tubules were indistinct when compared to controls. No F1 adult animals were studied at the highest dose level because of death due to excessive toxicity.

In the mid dose, the histology of the kidneys tubules from F0 and F1 males was less clear, but 7/30 F0 animals were reported to demonstrated the increased nuclear density and 4/29 F1 male rats demonstrated similar histopathology(Table 1).

Other sporatic effects occurred in the kidneys with no apparent dose related response.

C. DISCUSSION:

The kidneys of the F0 male rats from the high dose group in the study of reproduction demonsrated degenerative changes in the tubules of the cortical region and the outer medullary region. In the mid dose groups of the F0 generation and the F1 generation (the highest dose level studied in the F1 generation), less distinct changes occurred and they occurred only in the medullary tubules. No dose related kidney effects were seen in the F1b pups at any dose level.

Thus, no effect level for the study on reproduction did not change, however the effect level in adult rats must now include kidney histopathology and reduced weight gain. Prior to the addendum, the lowest effect level was characterized by only a reduced weight gain in adults and pups.

NOTE:

Since this reexamination of the kidney histology was conducted only after the sponsor identified these effects in a rat subchronic study, there is doubt about the quality of the original histological examination conducted in the reproduction study. The reexamination was conducted by Ray Brown of Research Pathology Associates but the original histological examination was conducted by the testing facility, Wil Research Laboratories. Other organs examined histologically by Wil Research but not by Ray Brown are the epidydimis testis, uterus, and ovary. The study on reproduction gave no indication that the kidneys nor any other organ required histological examination.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 99 of 959

005684

Table 1.

Incidence of findings reexamination of kidneys from males from a 2-generation study of the effects of 2,4-D on reproduction.

Adults or pups	F0 adults				F1 adults			F1b pups			
Nominal dose group:	1	2	3	4	1	2	3	1	2	3	4
Number of rats/group:	30	29	30	30	29	30	29	10	10	10	14
Number examined:	30	29	30	30	29	30	29	10	10	10	14
Number normal:	18	21	16	1	12	16	12	9	9	9	11
Description:											
Increased cytoplasmic eosinophilia in cortical tubules.	0	0	0	28	0	0	4	0	0	0	0
Increased focal nuclear density in medullary tubules.											
minimal	0	0	7	17	0	0	4	0	0	0	0
slight	0	0	0	4	0	0	0	0	0	0	0
moderate	0	0	0	1	0	0	0	0	0	0	0
Total incidence	0	0	7	22	0	0	4	0	0	0	0
Multifocal tubular degeneration/basophilia											
minimal	10	8	8	3	13	8	10	1	0	1	3
slight	0	0	0	0	0	2	0	0	0	0	0
Total incidence	10	8	8	3	13	10	10	1	0	1	3
Microcalculi	1	1	1	1	2	4	1	0	1	0	0
Pelvic dilation/hydronephrosis, unilateral.	1	0	1	0	2	1	1	0	0	0	0
Focal/multifocal mononuclear cellular infiltation.	1	1	0	0	3	1	0	0	0	0	0
Focal tubular dilation.	0	0	0	0	3	2	6	0	0	0	0
Focal/multifocal chronic nephritis.	0	0	0	0	1	0	1	0	0	0	0
Pelvic mineralization	0	0	0	0	0	1	0	0	0	0	0
Focal papilllary edema	0	0	0	0	0	0	1	0	0	0	0

Nominal dose groups: 1 = 0 mg/kg/day, 2 = 5 mg/kg/day, 3 = 20 mg/kg/day, 4 = 80 mg/kg/day.

[2,4-D DIETHANOLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Amendment to DER for MRID 41994001 - Subchronic Oral Toxicity Study [Rat] [2,4-D DIETHANOLAMINE SALT]

***This amendment provides an* EXECUTIVE SUMMARY and data tables to upgrade the original DER** [Document No. 009370].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/6/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 12/6/00
Reregistration Branch I, Health Effects Division (7509C)

TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: subchronic oral toxicity - rat § 82-1/OPPTS 870.3100
P.C. CODE: 030016 REREGISTRATION CASE NO.: 818713
CASWELL NO.: 315K CAS NO.: 5742-19-8
TEST MATERIAL (PURITY): 2,4-D diethanolamine salt [73.8%]
SYNONYMS: 2,4-D DEA; dichlorophenoxyacetic acid, diethanolamine salt
SPONSOR: PBI/Gordon Corporation, Kansas City, MO

CITATION: Serrone, D. M.; Killeen, J. C.; and Benz, G. (1991). A Subchronic Toxicity Study in Rats with Diethanolamine Salt of 2,4-Dichlorophenoxyacetic Acid. Ricerca, Inc., Painesville, OH. Study No. 90-0186, July 30, 1991. MRID 41994001. Document No. 009370. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [MRID 41994001], 10 Fischer 344 rats/sex/group were administered the diethanolamine salt of 2,4-dichlorophenoxyacetic acid [73.8%] *via* the diet for 13 weeks at concentrations of 0, 1.5 [acid equivalent, 1] mg/kg/day, 27.1 [acid equivalent, 18.4] mg/kg/day, 147.6 [acid equivalent, 100] mg/kg/day, and 442.8 [acid equivalent, 300] mg/kg/day. Achieved consumption values were 1.5, 27, 142, and 419 mg/kg/day for males and 1.6, 27, 139, and 412 mg/kg/day for females, respectively.

There were 6 deaths during the study, and all were in high-dose rats [3 males during week 6, 2 females during week 7, and 1 female during week 9]. Treatment-related clinical signs of toxicity were limited to the high-dose level and consisted of pale eyes and ears, hunched posture, and some anogenital staining. There was a dose-related decrease in body weight throughout the study [weeks 1-13] in both sexes at the two highest dose levels [147.6 mg/kg/day: males 77%-85%/females 78%-80% of control; 442.8 mg/kg/day: males 45%-58%/females 50%-57% of control] and in males at 27.1 mg/kg/day during weeks 9-13 [89% of control], and the magnitude of the deficits increased with time on study for both sexes at the two highest dose levels. Terminal body weights were 44% and 49% of the control values for the high-dose males and females, respectively, and 76% and 80% of control values at the next highest dose level. Overall body-weight gain was decreased in both sexes at the three highest dose levels [males 85%, 64%, and 9% of control at 27.1, 147.6, and 442.8 mg/kg/day, respectively/females 86%, 54% of control at 27.1 and 147.6 mg/kg/day, respectively, and a negative gain [-6.7 grams] at the highest dose]. Food consumption [on a grams/kg basis] was decreased for both sexes at the three highest dose levels throughout the study, but when calculated on a g/kg/day basis, decreased food consumption was observed only during the first 6 weeks of the study at the highest dose level for both sexes.

There was a dose-related decrease in red blood cell, hemoglobin, and hematocrit [mid-high and high-dose levels

in both sexes], and white blood cell and platelet values at the high-dose level in both sexes at week 6 and study termination. Several clinical chemistry parameters [increased AST (mid-high and high-dose both sexes)/ALT (high dose both sexes) activity, BUN (mid-high males and high-dose both sexes); decreased T4 concentrations (mid-high and high-dose, both dose levels)] were altered by treatment and were consistent with changes observed in organ weights and/or histopathology.

At the highest dose level, both the testes weight and the ovarian weight were decreased compared to the respective control values on an absolute [males 24%/females 28% of control] and relative-to-body [males 55%/females 55% of control] and relative-to-brain weight [males 28%/females 32% of control] basis, and females at the next highest dose level displayed decreased absolute [72% of control] and relative-to-body weight [88% of control] ovarian weights. There was a dose-related decrease in thymus weights in both sexes, with the two highest dose levels [147.6 and 442.8 mg/kg/day] attaining statistical significance for the absolute [males 70% and 21%/females 80% and 19% of control] and relative-to-brain [males 71% and 23%/females 81% and 22% of control] thymus weights. At the highest dose level, both sexes displayed decreased absolute brain weight [males 89%/females 86% of control] and increased relative brain weight. Kidney weights were increased mainly in females at the 3 highest dose levels, but there was no dose response for absolute kidney weights in either sex. Females did display a dose-related increase in both the relative-to-body and relative-to-brain kidney weights.

Eye lesions were observed at the high-dose level and consisted of bilateral retinal degeneration [7 males and 8 females]. Liver lesions [hypertrophy (males 7/females 8) and individual cell necrosis (8/sex) of centrilobular hepatocytes] were observed at the highest dose levels in both sexes. Kidney lesions [diffuse regeneration of renal tubule epithelial ranging from minimal to moderate in severity with a tendency for increased severity with increased dose] were observed in all of the females in the 3 highest dose groups and in 2, 10, and 8 males of the 3 highest dose levels compared to none in the control or lowest dose level in either sex. Thyroid lesions consisted of hypertrophy of the follicular epithelium [all rats/both sexes] accompanied by decreased colloid [8 males and 10 females] in both sexes at the high-dose level. Atrophy of the ovaries and uterus was observed in the mid-high [4 and 4] and high-dose [9 and 10] females, and degeneration of the seminiferous epithelium in the testes was moderately severe in a majority of the 8 affected high-dose males and was diffuse and bilateral in distribution. The lesion was associated with decreased spermatozoa in the epididymis [all high-dose males] and decreased secretory content in the prostate [1 mid-high, 8 high-dose] and seminal vesicles [8 high-dose males].

The NOAEL is 1.5 mg/kg/day, and the LOAEL is 27.1 mg/kg/day, based on decreased mean body-weight gain, food consumption, increased relative kidney weight [females], and histopathological lesions in the kidneys in both sexes. At the highest dose level [442.8 mg/kg/day], three males and three females died between weeks 6 and 9 of the study. Additionally, effects were observed on the liver [increased weight (females), hypertrophy, individual cell necrosis], thyroid [follicular hypertrophy, decreased T4], testes [decreased weight, degeneration of seminiferous epithelium, decreased spermatozoa in epididymis, decreased secretory content (prostate and seminal vesicles] and ovaries [decreased weight, atrophy] at the two highest dose levels.

This subchronic oral toxicity study is classified **Acceptable/Guideline**, and the study satisfies the guideline requirement [OPPTS 870.3100; §82-1] for a subchronic oral toxicity study in the rodent.

NOTE: Although the original DER lists the **adrenals, thyroid, and pituitary** in the table of organs weighed, no data on these three organ weights were provided in the study report.

[2,4-D DIETHANOLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Table 1. Hematology Parameters

Parameter/Dose/Sex/Week	0 mg/kg/day	1.5 mg/kg/day	27.1 mg/kg/day	147.6 mg/kg/day	442.8 mg/kg/day
red blood cells [x 10^6/mm^3]					
week 6					
MALES	9.34±0.25	9.37±0.45	9.25±0.29	8.81±0.59*[94]	6.25±0.44**[67]
FEMALES	8.60±0.49	8.51±0.31	8.16±0.49	7.91±0.35**[92]	6.67±0.24**[77]
terminal					
MALES	8.18±0.25	8.58±0.50*	8.19±0.31	7.38±0.25**[90]	6.30±0.23**[77]
FEMALES	7.51±0.91♪	7.74±0.30	7.73±0.24	7.14±0.19*[95]	6.25±0.28**[83]
hemoglobin [g/dL]					
week 6					
MALES	17.44±0.38	17.35±0.41	17.23±0.49	15.90±0.82**	11.04±0.63**[63]
FEMALES	16.99±0.92	16.65±0.54	15.85±0.84**[93]√	15.14±0.68**[89]	11.56±0.52**[68]
terminal					
MALES	15.39±0.46	16.04±1.00	15.44±0.48	13.19±0.39**[86]	10.39±0.49**[68]
FEMALES	15.01±1.80	15.31±0.43	15.01±0.47	13.20±0.39**[88]	10.35±0.48**[69]
hematocrit [%]					
week 6					
MALES	49.35±1.22	49.36±1.88	48.59±1.42	45.46±2.56**[92]	33.55±2.42[68]
FEMALES	48.04±2.86	47.28±1.77	44.83±2.18**[93]	42.79±1.91**[89]	35.45±1.57**[74]
terminal					
MALES	41.90±1.20	43.94±2.53*	41.88±1.60	36.42±1.04**[87]	32.50±1.23**[78]
FEMALES	41.28±4.99	42.21±1.62	41.56±1.23	36.37±1.13**[88]	32.65±1.38**[79]
platelets [x 10^3/mm^3]					
week 6					
MALES	825.4±39.1	809.0±76.0	833.2±64.1	773.4±203.2	473.8±113.9**[57]
FEMALES	830.5±106.6	793.1±114.8	685.0±239.6	756.0±124.4	395.8±71.7**[48]
terminal					
MALES	739.7±76.4	784.7±30.0	811.6±56.3*	823.8±66.8**[111]	432.6±28.4**[58]
FEMALES	639.7±307.8	720.8±236.9	812.4±60.3	559.2±30.6	360.2±25.9*[56]
white blood cells [x 10^3/mm^3]					
week 6					
MALES	9.63±1.80	9.65±1.53	10.31±1.05	10.86±2.00	4.79±0.88**
FEMALES	8.76±1.10	8.03±0.95	8.32±1.87	9.38±0.91	2.49±0.62**[28]
terminal					
MALES	6.05±0.64	6.33±0.92	6.24±1.01	6.39±0.65	2.10±0.42**[35]
FEMALES	5.11±1.28	5.11±1.29	5.78±1.18	4.63±0.79	1.75±0.58**[34]

♪ data illegible in study report; √[% of control]; * $p<0.05$; ** $p<0.01$; N=10 unless ( ); data from Table 15-22, pages 93-104 of the report

Table 2. *Clinical Chemistry Parameters*

Parameter/Dose/Sex/Week	0 mg/kg/day	1.5 mg/kg/day	27.1 mg/kg/day	147.6 mg/kg/day	442.8 mg/kg/day
glucose [mg/dL]					
week 6					
MALES	128.6±25.9	121.9±13.9	124.4±14.0	117.6±9.3	100.5±10.8**[78]
FEMALES	122.4±16.2	116.4±10.0	109.5±8.2	109.5±11.8	89.9±12.3**[73]
terminal					
MALES	152.3±12.0	150.1±10.3	150.6±15.9	133.4±13.2**[88]	114.1±8.2**[75]
FEMALES	142.9±12.6	134.9±18.3	132.7±14.3	127.3±14.3	106.9±9.1**[75]
blood urea nitrogen [mg/dL]					
week 6					
MALES	16.2±1.5	16.8±1.7	16.4±2.2	20.8±1.5**[128]	22.7±3.1**[140]
FEMALES	19.7±1.7	18.1±2.8	19.9±2.5	22.7±1.7	27.1±4.6**[138]
terminal					
MALES	17.2±1.7	16.1±1.6	17.3±2.0	22.1±2.1**[128]	22.9±3.9**[133]
FEMALES	18.6±1.8	17.7±2.1	19.3±3.0	18.9±2.5	31.6±7.5**[170]
AST [U/L]					
week 6					
MALES	102.1±12.8	95.0±7.6	98.9±7.0	123.7±12.7	201.3±35.1**[197]
FEMALES	94.5±8.9	99.4±15.4	111.1±22.4	125.7±11.8**[133]	312.8±135.8**[328]
terminal					
MALES	103.1±18.1	110.2±17.4	102.5±9.9	144.5±17.5**[140]	201.3±28.4**[195]
FEMALES	88.1±13.5	94.0±13.1	97.0±13.4	149.8±30.2**[170]	280.7±87.6**[319]
ALT [U/L]					
week 6					
MALES	42.3±3.7	37.5±5.1	38.6±4.2	38.8±6.4	59.9±10.5**[142]
FEMALES	36.1±5.1	32.4±4.8	31.6±4.3	35.4±5.0	83.6±41.3*[232]
terminal					
MALES	51.4±8.7	56.2±9.4	41.4±7.4	49.3±8.4	59.7±10.5
FEMALES	41.3±5.7	36.7±2.8	33.4±6.7*[81]	47.2±18.5	95.0±15.9*[230]
total protein [g/dL]					
week 6					
MALES	7.16±0.26	7.26±0.23	7.02±0.27	7.56±0.18**[106]	6.98±0.38
FEMALES	6.67±0.18	6.49±0.26	6.80±0.21	7.27±0.19**[109]	6.49±0.39
terminal					
MALES	6.62±0.20	6.61±0.17	6.75±0.30	7.27±0.20**[110]	6.24±0.68
FEMALES	6.62±0.30	6.29±0.42	6.59±0.21	7.28±0.20**[110]	6.30±0.49
albumin [g/dL]					
week 6					
MALES	5.02±0.15	5.03±0.16	5.01±0.17	5.62±0.09**[112]	5.73±0.27**[114]
FEMALES	4.81±0.16	4.73±0.19	5.06±0.12*[105]	5.77±0.13**[120]	5.67±0.33**[118]
terminal					
MALES	4.36±0.21	4.31±0.14	4.60±0.53	5.22±0.13**[120]	4.94±0.46
FEMALES	4.50±0.22	4.42±0.10	4.87±0.31	5.70±0.31**[127]	5.24±0.45*[116]
globulin [g/dL]					
week 6					
MALES	2.14±0.16	2.23±0.15	2.01±0.16	1.94±0.20	1.13±0.29**[53]
FEMALES	1.86±0.10	1.76±0.18	1.74±0.16	1.50±0.12**[81]	0.74±0.16**[40]
terminal					
MALES	2.26±0.11	2.30±0.14	2.15±0.12	2.05±0.19*[91]	1.30±0.29**[58]
FEMALES	2.12±0.12	1.87±0.35	1.72±0.33*[81]	1.61±0.16**[76]	1.06±0.08**[50]

[2,4-D DIETHANOLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Table 2. Clinical Chemistry Parameters					
Parameter/Dose/Sex/Week	0 mg/kg/day	1.5 mg/kg/day	27.1 mg/kg/day	147.6 mg/kg/day	442.8 mg/kg/day
T3 [ng/dL]					
terminal					
MALES	89.96±18.52	90.68±10.03	94.30±15.26	100.10±19.34	101.16±5.36
FEMALES	94.96±12.02	86.88±8.69	97.42±12.87	91.48±15.91	83.36±25.55
T4 [µg/dL]					
terminal					
MALES	4.27±0.88	4.15±0.38	4.44±0.61	3.53±0.63*[83]♪	1.67±0.29**[39]
FEMALES	2.67±0.55	2.95±0.72	2.87±0.45	1.27±0.29**[48]	1.24±0.30**[46]

♪ [% of control]; * $p<0.05$; ** $p<0.01$; N=10; data from Table 23, pages 106-119 of the report

Table 3. Organ Weights [grams]

Parameter/Dose/Sex/Week	0 mg/kg/day	1.5 mg/kg/day	27.1 mg/kg/day	147.6 mg/kg/day	442.8 mg/kg/day
LIVER					
MALES					
absolute	6.61±0.73	6.61±0.77	6.13±0.70	6.03±0.66	6.78±0.61
relative/body	2.46±0.07	2.47±0.09	2.54±0.11	2.94±0.08**[120]♪	5.77±0.32**[235]
relative/brain	3.53±0.37	3.55±0.28	3.35±0.33	3.28±0.28	4.08±0.32**[116]
FEMALES					
absolute	3.99±0.26	3.76±0.46	3.87±0.34	4.30±0.34	5.10±0.67**[128]
relative/body	2.59±0.11	2.55±0.16	2.72±0.22	3.50±0.13**[135]	6.77±0.24**[261]
relative/brain	2.32±0.13	2.18±0.23	2.26±0.17	2.53±0.18	3.44±0.43**[149]
KIDNEY					
MALES					
absolute	1.83±0.18	1.87±0.16	1.96±0.16	1.93±0.14	1.67±0.15
relative/body	0.68±0.04	0.70±0.03	0.81±0.02*	0.94±0.03**[138]	1.42±0.07**[208]
relative/brain	0.98±0.08	1.01±0.05	1.07±0.07*[109]	1.05±0.05	1.00±0.07
FEMALES					
absolute	1.12±0.05	1.14±0.11	1.31±0.08**[117]	1.33±0.13**[119]	1.19±0.16
relative/body	0.73±0.05	0.77±0.05	0.92±0.06**[127]	1.08±0.05**[149]	1.58±0.09**[218]
relative/brain	0.65±0.03	0.66±0.05	0.77±0.04**[118]	0.78±0.07**[121]	0.80±0.11**[124]
BRAIN					
MALES					
absolute	1.87±0.06	1.86±0.09	1.83±0.06	1.84±0.07	1.66±0.04**[89]
relative/body	0.70±0.07	0.70±0.04	0.76±0.05	0.90±0.06**[128]	1.423±0.15**[202]
FEMALES					
absolute	1.72±0.03	1.72±0.10	1.71±0.05	1.70±0.06	1.48±0.04**[86]
relative/body	1.12±0.06	1.18±0.11	1.21±0.10	1.39±0.09**[124]	1.99±0.22**[177]
HEART					
MALES					
absolute	0.87±0.10	0.85±0.08	0.79±0.04*[90]	0.68±0.05**[78]	0.59±0.05**[67]
relative/body	0.32±0.02	0.32±0.02	0.33±0.03	0.33±0.02	0.50±0.04**[154]
relative/brain	0.47±0.05	0.46±0.03	0.43±0.02	0.37±0.02**[79]	0.35±0.03**[76]
FEMALES					
absolute	0.59±0.04	0.59±0.05	0.54±0.03	0.49±0.05**[84]	0.47±0.04**[81]
relative/body	0.38±0.03	0.40±0.03	0.38±0.02	0.40±0.02	0.63±0.08**[166]
relative/brain	0.34±0.02	0.34±0.02	0.32±0.02	0.29±0.02**[85]	0.32±0.02
THYMUS					
MALES					
absolute	0.30±0.05	0.26±0.05	0.26±0.04	0.21±0.03**[70]	0.06±0.03**[21]
relative/body	0.11±0.02	0.10±0.01	0.11±0.02	0.10±0.01	0.05±0.03**[47]
relative/brain	0.16±0.03	0.14±0.02	0.14±0.02	0.11±0.02**[71]	0.04±0.02**[23]
FEMALES					
absolute	0.22±0.02	0.21±0.03	0.22±0.03	0.17±0.01**[80]	0.04±0.02**[19]
relative/body	0.14±0.01	0.14±0.01	0.15±0.01	0.14±0.01	0.06±0.02**[39]
relative/brain	0.13±0.01	0.12±0.02	0.13±0.02	0.10±0.01**[81]	0.03±0.01**[22]
TESTES					
MALES					
absolute	2.91±0.17	2.92±0.18	2.78±0.11	2.75±0.18	0.71±0.15**[24]
relative/body	1.09±0.08	1.10±0.08	1.16±0.05	1.35±0.06**[124]	0.60±0.06**[55]
relative/brain	1.56±0.08	1.57±0.10	1.52±0.05	1.49±0.07	0.43±0.08**[28]

[2,4-D DIETHANOLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Table 3. Organ Weights [grams]					
Parameter/Dose/Sex/Week	0 mg/kg/day	1.5 mg/kg/day	27.1 mg/kg/day	147.6 mg/kg/day	442.8 mg/kg/day
OVARIES					
FEMALES					
absolute	0.06±0.01	0.06±0.01	0.06±0.01	0.05±0.01**[72]	0.02±0.01**[28]
relative/body	0.04±0.01	0.04±0.00	0.05±0.01	0.04±0.01	0.02±0.01**[55]
relative/brain	0.04±0.01	0.04±0.01	0.04±0.01	0.03±0.01**[88]	0.01±0.00**[32]
TERMINAL BODY WEIGHT					
MALES	268.9±27.3	267.3±23.3	240.9±18.7*[90]	204.6±18.3**[76]	118.1±16.0**[44]
FEMALES	153.8±9.3	147.0±15.6	142.7±13.4 [93]	122.7±8.0**[80]	75.4±9.9**[49]

N=10 except HDT (7 for both sexes); ♪ [% of control]; * $p<0.05$; ** $p<0.01$; data from Tables 33-38, pages 124-131 of the report

009370

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist Jess Rowland 3/12/92
Section II, Toxicology Branch II (H7509C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 3/13/92
Section II, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding-Rodent **GUIDELINE:** 82-1(a)

TOX.CHEM. No.: 315-K MRID No.: 419940-01

HED Project No. 1-2379 **Registrant:** PBI/Gordon Corp.

TEST MATERIAL: Diethanolamine salt of 2,4-D **(2,4-D DEA)**

TESTING LABORATORY: Ricerca, Inc. Painesville, OH.

STUDY IDENTIFICATION: Ricera Study # 90-0186

TITLE OF REPORT: A Subchronic Toxicity Study in Rats with the Diethanolamine Salt of 2,4-Dichlorophenoxyacetic Acid.

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REPORT DATE: July 30, 1991. **DOCUMENT NUMBER:** 3579-90-0186-TX-003

SUMMARY: Male and female Fischer-344 rats were fed diets containing the Diethanolamine salt of 2,4-Dichlorophenoxyacetic acid at 0, 1.5, 27.1, 147.6, and 442.8 mg/kg/day for 13 weeks. Treatment did not cause any adverse effects at 1.5 mg/kg/day. Treatment-related effects observed at higher dietary levels of 27.1, 147.6 and 442.8 mg/kg/day, included: mortality; decreases in mean body weight gain and mean food consumption; alterations in hematology and clinical chemistry parameters; changes in organ weights and gross pathology; and histopathological alterations in the kidneys [27.1, 147.6 and 442.8 mg/kg/day], liver [442.8 mg/kg/day], eyes [442.8 mg/kg/day], lungs [147.6 and 442.8 mg/kg/day], thyroid glands [442.8 mg/kg/day], stomach [147.6 and 442.8 mg/kg/day], sternal bone marrow [147.6 and 442.8 mg/kg/day], cervical lymph node, spleen, thymus, testes, epididymis, prostate, seminal vesicles [442.8 mg/kg/day], and ovaries and uterus [147.6 and 442.8 mg/kg/day].

Under the conditions of this study, a No-Observable-Effect Level (NOEL) of 1.5 mg/kg/day and a Lowest-Observable-Effect Level (LOEL) of 27.1 mg/kg/day are established. The LOEL is based on decreases in mean body weight gain and food consumption, increases in relative kidney weights, and histopathological changes in the kidneys of male and female rats at 27.1 mg/kg/day.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the subchronic toxicity of **Diethanolamine salt of 2,4-Dichlorophenoxyacetic acid** following dietary administration to rats.

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: Diethanolamine Salt of 2,4-Dichlorophenoxyacetic acid.
Purity: 73.8%
Lot No.: 0017
Description: Clear amber liquid.
Vehicle: Acetone [pesticide grade]

2. Test Animals

Species: Rats
Strain: CDF (F-344)/CrLBR VAF/Plus Fischer 344
Sex: Males and females
Age at Initiation: Approximately 42 days
Weight at Initiation: 98-120 g (M); 86-97.2 g (F)
Identification: Ear tags.
Acclimation: Approximately 2 weeks
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Chow #5002.
Water: Tap water ad libitum
Environment: Temperature- 67 - 75° F; Humidity- 40 - 70 %; 13+ air changes/hour.
Light/dark cycles: 12 hr light/12 dark cycle

3. Study Design

Group No.	Treatment	No. of Animals		Dose level [mg/kg/day]	Acid Equivalent [mg/kg/day]
		Males	Females		
1	Control	10	10	0	0
2	Low	10	10	1.5	1.0
3	Mid-1	10	10	27.1	18.4
4	Mid-2	10	10	147.6	100.0
5	High	10	10	442.8	300.0

4. Test Article Formulation and Analyses

The 73.8% [w/w] 2,4 D DEA was corrected to 100% active ingredient in the diets.

For each dose level, a premix was prepared by weighing the appropriate amount of the test article to which an appropriate volume of acetone was added, and the test article/acetone mixture was then slowly stirred until an apparently homogeneous mixture was achieved. The premix was placed in a mortar and ground with a pestle until the test material was uniformly mixed with the meal. The premix and the remainder of the meal were placed in a twin shell blender with an intensifier bar mixed for 10 minutes. The prepared diets were stored in the dark at room temperatures. The control diet was prepared in the same manner as the test diets using acetone only at the same level utilized in the test diets. Fresh diets were prepared weekly and reserve samples of each mixed batch were taken on the day of preparation and stored in a freezer.

During the in-life phase, concentration analyses was performed during Weeks 1 thru 4 and at Weeks 8 and 12. Homogeneity of the diet mix and the 14-day stability of the test material in the diets were evaluated prior to the initiation of the study.

5. Treatment

Rats were fed the control and test diets 7 days per week for at least 13 weeks. The oral route of administration was chosen because it is the route of potential human exposure.

6. Experimental Procedures

Mortality and moribundity checks were performed twice daily. Body weights were measured prior to initiation and once weekly. Food consumption was recorded weekly and physical examinations were performed weekly at the body weight intervals. Ophthalmologic examinations were conducted prior to initiation, during weeks 8, and at termination. Blood and urine were collected from all animals during Weeks 6 and at termination for hematology, clinical chemistry and urinalysis. 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]
x **Mean corpuscular HGB (MCH)**	x **Mean corpuscular HGB Concentration (MCHC)**
x **Mean corpuscular volume (MCV)**	Blood clotting measurements
Corrected leukocyte count (COR WBC)	Cell morphology

Clinical Chemistry

Electrolytes:	Other
x **Calcium**[a] x **Chloride**[a] Magnesium[a] x **Phosphorus**[a] x **Potassium**[a] x **Sodium**	x **Albumin**[a] x **Blood creatinine**[a] x **Blood urea nitrogen**[a] Cholesterol[a] x **Globulins** x **Glucose**[a] x **Total bilirubin**[a] x **Total serum protein**[a] Triglycerides Serum protein electrophoresis x **Triiodothyronine [T_3]** x **Thyroxine [T_4]** x **A/G Ratio**
Enzymes:	
Alkaline phosphatase x **Alanine aminotransferase (SGPT)**[a] x **Aspartate aminotransferase (SGOT)**[a] Cholinesterase[b] Creatinine phosphatase[a] Lactic acid dehydrogenase Gama glutamyl transferase	

Urinalysis

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

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

110

7. Termination

After 13 weeks of treatment, all surviving animals were weighed, anesthetized with sodium pentobarbital and exsanguinated. Necropsies were performed on each animal, all gross pathological changes were recorded, and the following organs were weighed.

Adrenals ?	Brain	Heart	Kidneys	Liver
Ovaries	Thymus	Thyroid ?	Pituitary ?	Testes

8. Histopathology

The checked (X) tissues from all animals were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Salivary glands[a]	x Trachea[a]
x Esophagus[a]	x Lung[a]
x Duodenum[a]	Pharynx[e]
x Jejunum[a]	Larynx[e]
x Cecum[a]	Nose[e]
x Colon[a]	
x Rectum[a]	Cardiovascular/Hemo.System
x Liver[ac]	
x Pancreas[a]	x Aorta (thoracic)[a]
Gall bladder[ab]	x Heart[a]
	x Bone marrow[a]
Neurological System	x Lymph nodes[a]
	x Spleen[a]
x Brain[ac]	x Thymus[a]
x Pituitary[a]	
x Peripheral nerve[ac]	Urinogenital System
x Spinal cord (3 levels)[ac]	
x Eyes (optical nerve)[ac]	x Kidneys[ac]
	x Urinary bladder[a]
	x Testes[ac]
Glandular System	x Epididymides
	x Uterus[a]
	x Ovaries[ac]
x Adrenals[a]	Others
Lacrimal glands[c]	
x Parathyroids[ad]	x All gross lesions and masses
x Thyroids[ad]	x Skeletal muscle[a]

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

Mean absolute body weight, body weight change, mean weekly food consumption, total food consumption, clinical pathology data and organ weight data of the control group were compared statistically to the data from the compound-treated groups. Tests employed were Bartlett's test, Dunn's summed-rank test, and Bonferroni's test. When comparing the group means in both nonparametric and the parametric cases, significance was reported at the two-sided experiment-wise error rates of 1% and 5%.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Ricerca Labs, and the Study Protocol. A quality assurance statement was signed and dated July, 29, 1991.

III. RESULTS

1. Analysis of Diet Mix

Concentration analyses indicated that the diets were accurately prepared throughout the study. The mean assay values were 91 ± 4%, 101 ± 6%, 101 ± 4% and 101 ± 4% respectively, of the nominal concentrations for males at 1.5, 27.1, 147.6, and 442.8 mg/kg/day diets, and 87 ± 3%, 98 ± 7 %, 104 ± 2% and 101 ± 2% respectively, of the nominal concentrations for females at 1.5, 27.1, 147.6, and 442.8 mg/kg/day diets. Homogeneity analysis indicated the diet mix to be homogeneous with target levels of 95% ± 3% and 104% ± 2%, respectively of the 15 and 10,000 µg/g nominal concentrations. Stability analyses of samples stored at room temperature demonstrated the test material to be stable with recovery of 96% and 96% at the low and high dose, respectively after 14 days.

2. Survival

All rats at the control, 1.5, 27.1 and 147.6 mg/kg/day dose survived the study. Of the six rats [3 males and 3 females] that died at 442.8 mg/kg/day, three males died shortly after the Week 6 bleeding, 2 females died during Week 7, and the other female died during Week 9.

3. Clinical Observations

Treatment-related clinical signs of toxicity limited to the high-dose group [442.8 mg/kg/day] included pale eyes and ears, hunched posture, and some anogenital staining. Clinical signs observed in rats in the other treated groups were also seen in the control rats and therefore were not considered to be treatment related.

4. Body Weights and Body Weight Changes

Mean body weight data are summarized in Tables 1 and 2 for males and females, respectively. Statistically significant ($p < 0.01$ or 0.05) reductions in mean body weight were observed in:

- Males and females at 442.8 mg/kg/day from Weeks 1 - 13;
- Males at 147.6 mg/kg/day from Weeks 3 - 13;
- Females at 147.6 mg/kg/day from Weeks 1-13, and
- Males at 27.1 mg/kg/day from Weeks 9 - 13.

Mean body weights of males and females at 1.5 mg/kg/day were comparable to that of the control rats.

Table 1. Mean Body Weights and S.D. (G) for Male Rats.

Dose Level [mg/kg/day]	Week: 0	Week: 6	Week: 10	Week: 13[a]
0	112.3 ± 6.1	221.9 ± 25.3	267.7 ± 30.5	284.9 ± 30.2
1.5	111.4 ± 5.1	227.1 ± 24.2	267.9 ± 26.9	285.2 ± 26.5
27.1	107.9 ± 3.7	201.7 ± 19.4	**237.9** ** **± 22.6**	**254.6** ** **± 20.5**
147.6	108.3 ± 5.1	**188.3** * **± 16.6**	**211.7** * **± 21.6**	**218.8** * **± 19.3**
442.8	111.5 ± 2.6	**128.7** * **± 11.3**	**129.1** * **± 14.3**	**127.0 ± 16.0** *

* Significantly different from control value at $p \leq 0.01$.
** Significantly different from control value at $p \leq 0.05$

Table 2. Mean Body Weights and S.D. (G) for Female Rats.

Dose Level [mg/kg/day]	Week: 0	Week: 6	Week: 10	Week: 13
0	89.4 ± 2.2	139.7 ± 6.7	159.5 ± 8.3	165.5 ± 8.9
1.5	89.8 ± 4.9	133.0 ± 10.5	152.2 ± 15.1	157.9 ± 16.1
27.1	88.3 ± 4.3	130.0 ± 10.0	149.5 ± 13.4	153.5 ± 13.6
147.6	88.3 ± 3.7	**111.2** * **± 9.2**	**126.1** * **± 10.5**	**129.3** * **± 9.0**
442.8	89.0 ± 2.9	**80.0** * **± 6.5**	**85.9** * **± 7.7**	**83.1** * **± 8.9**

* Significantly different from control value at $p \leq 0.01$

Mean body weight change data are presented in Tables 3 and 4 for males and females, respectively. In male rats, statistically significant ($p < 0.01$) decreases in mean body weight gains were seen during Weeks 1-13 at 442.8 mg/kg/day, during Weeks 3-13 at 147.6 mg/kg/day and during Weeks 10-13 at 27.1 mg/kg/day. In females, decreases in body weight gain were noted during Weeks 1-13 at 147.6 and 442.8 mg/kg/day and during Weeks 6, 8, 12, and 13 at 27.1 mg/kg/day. No body weight loss was seen in males or females at 1.5 mg/kg/day.

Table 3. Mean Body Weight Change (G) in Male Rats.

Dose Level [mg/kg/day]	Weeks: 0 - 4	Weeks: 0 - 8	Weeks: 0 - 13
0	102.3 ± 21.4	136.7 ± 26.9	172.9 ± 27.5
1.5	106.4 ± 15.6	138.9 ± 21.1	173.8 ± 22.5
27.1	88.6 ± 16.3	113.2 ± 23.5	**146.7** ** **± 19.8**
147.6	**82.4** ** **± 11.3** 81	**94.8** * **± 15.5** 69	**110.5** * **± 15.3**
442.8	**25.9** * **± 9.6** 25	**19.1** * **± 10.2** 14	**14.9** * **± 15.9**

* Significantly different from control at $p \leq 0.01$
** Significantly different from control at $p \leq 0.05$.

Table 4. Mean Body Weight Change (G) in Female Rats.

Dose Level [mg/kg/day]	Weeks: 0 - 4	Weeks: 0 - 8	Weeks: 0 - 13
0	49.6 ± 4.7	64.7 ± 6.9	76.1 ± 8.3
1.5	44.9 ± 5.5	57.4 ± 10.6	68.1 ± 11.8
27.1	44.3 ± 6.1	54.7 ± 9.5	**65.2** ** **± 10.1**
147.6	**29.7** * **± 5.5** 60	**33.0** * **± 6.8** 51	**41.0** * ± 6.5
442.8	**4.3** * **± 4.6** 9	**-4.0** * **± 5.8**	**-6.7** * **± 7.3**

* Significantly different from control at $p \leq 0.01$.
** Significantly different from control at $p \leq 0.05$.

5. Food Consumption

The mean absolute [g/day] food consumption values were reduced for males and females from the 27.1 mg/kg/day group and at higher dosage levels throughout the study, with the difference reaching statistical significance [p <0.01] at 147.6 and 442.8 mg/kg/day groups. The mean relative [g/kg/day] food consumption values were also reduced in both sexes from the 27.1 mg/kg/day group and at higher dosage levels, with the difference reaching statistical significance only at 442.8 mg/kg/day. The decrease in food consumption was related to the reduced body weights. The average daily compound consumption for Weeks 1-13 was 1.5, 27, 142, and 419 mg/kg/day for males and 1.6, 27, 139, and 412 mg/kg/day for females.

6. Ophthalmology Examination

Pallor was observed in the retinal vasculature of rat at 442.8 mg/kg/day during Weeks 8 [6 males and 8 females] and 12 [7 males and 7 females], and in rats at 147.6 mg/kg/day at Weeks 8 [1 female] and 12 [6 females]. No pallor was seen in males and female rats in the lower doses or in the control group.

7. Hematology and Clinical Chemistry

Treatment-related, statistically significant ($p < 0.05$, <0.01) decreases observed in hematology parameters are tabulated below:

Table 5. Changes in Hematology Parameters.

Dose Level [mg/kg/day] Sex	Parameter Affected	Effect	Interval [Week]
27.1 / ♂	Platelet	Increased	14
27.1 / ♀	MCH	Decreased	14
	MCV	Decreased	14
	HGB	Decreased	6
	HCT	Decreased	6
147.6 / ♂	RBC	Decreased	6,14
	HGB	Decreased	6,14
	HCT	Decreased	6,14
	MCV	Decreased	6,14
	MCH	Decreased	14
	Platelet	Increased	14
147.6 / ♀	RBC	Decreased	6,14
	HGB	Decreased	6,14
	HCT	Decreased	6,14
	MCV	Decreased	6,14
	MCH	Decreased	6,14
442.6 / ♂	WBC	Decreased	6,14
	RBC	Decreased	6,14
	HGB	Decreased	6,14
	HCT	Decreased	6,14
	MCV	Increased	6
	MCH	Decreased	6,14
	MCHC	Decreased	6,14
	Platelet	Decreased	6,14
442.6 / ♀	WBC	Decreased	6,14
	RBC	Decreased	6,14
	HGB	Decreased	6,14
	HCT	Decreased	6,14
	MCV	Decreased	6,14
	MCH	Decreased	6,14
	MCHC	Decreased	6,14
	Platelet	Decreased	6,14

Treatment-related, statistically significant ($p < 0.05 < 0.01$) differences observed in clinical chemistry parameters are tabulated below:

Table 6. Changes in Clinical Chemistry Parameters

Dose Level/Sex [mg/kg/day]	Parameter Affected	Effect	Interval [Week]
27.1 / ♀	ALT	Decreased	14
	Albumin	Increased	6
	Globulin	Decreased	14
	A/G Ratio	Increased	14
147.6 / ♂	BUN	Increased	6,14
	AST	Increased	14
	Tot.Prot.	Increased	6,14
	Albumin	Increased	6,14
	Globulin	Decreased	14
	A/G Ratio	Increased	6,14
	Glucose	Decreased	14
	Sodium	Increased	6
	Calcium	Increased	6,14
	T_4	Decreased	14
147.6 / ♀	Creatinine	Increased	6
	AST	Increased	6,14
	Tot.Prot.	Increased	6,14
	Albumin	Increased	6,14
	Globulin	Decreased	6,14
	A/G Ratio	Increased	6,14
	Sodium	Increased	6
	Potassium	Increased	6
	Calcium	Increased	6,14
	T_4	Decreased	14
442.6 / ♂	BUN	Increased	6,14
	ALT	Increased	6
	AST	Increased	6,14
	Tot.Bili.	Decreased	6,14
	Albumin	Increased	6
	Globulin	Decreased	6,14
	A/G Ratio	Increased	6,14
	Glucose	Decreased	6,14
	Sodium	Decreased	6
	Chloride	Increased	6
	Calcium	Increased	6
	T_4	Decreased	14
442.8 / ♀	BUN	Increased	6,14
	ALT	Increased	14
	AST	Increased	6,14
	Tot.Bili.	Decreased	6
	Albumin	Increased	6,14
	Globulin	Decreased	6,14
	A/G Ratio	Increased	6,14
	Glucose	Decreased	6,14
	Sodium	Increased	6,14
	Chloride	Increased	6
	T_4	Decreased	14

8. Urinalysis

Urinalysis results showed a significant [p <0.05] decrease in urine volume in males and females at 442.8 mg/kg/day at termination, in males at this dose during Week 6. Urinary ketone patterns of males at 147.6 and 442.8 mg/kg/day showed a decline in the incidence and grade. The urinary ketone patterns of females were not affected.

9. Gross Pathology

Gross necropsy revealed pinpoint white foci in the lobes of lungs of 5 males and 6 females at 442.8 mg/kg/day at termination; this lesion was not seen on the lungs of the high dose rats that died during the study. Kidneys were reported to have a green color in 4 males and 2 females at 147.6 mg/kg/day and in 3 males and 3 females at 442.8 mg/kg/day at termination; green-colored kidneys were not seen in rats that died during the study. Small thymus was seen in 3 males and 7 females at 442.8 mg/kg/day. Gonads were reported as small in rats at the high dose in comparison to controls. Other gross necropsy findings were considered incidental in nature.

10. Organ Weights

Only the increases in relative liver and brain [males and females at 442.8 mg/kg/day] and kidney weights [males and females at 27.1, 147.6, and 442.8 mg/kg/day] showed a dose-related trend. Changes in the weights of other organs did not show a trend. Statistically significant [p < 0.01, < 0.05] differences observed in the absolute and relative organ weight are summarized below:

Absolute Organ Weights			
Organ	Dose Level [mg/kg/day]	Sex	Effect
Brain	442.8	M, F	Decreased
Liver	442.8	F	Increased
Kidney	21.1 and 147.6	F	Increased
Heart	27.1, 147.6 and 442.8	M	Decreased
	147.6 and 442.8	F	Decreased
Thymus	147.6 and 442.8	M, F	Decreased
Testes	442.8	M	Decreased
Ovaries	147.6 and 442.8	F	Decreased

Relative Organ Weights			
Organ	Dose Level [mg/kg/day]	Sex	Effect
Brain	147.6 and 442.8	M, F	Increased
Liver	147.6 and 442.8	M, F	Increased
Kidney	**27.1, 147.6 and 442.8**	**M, F**	**Increased**
Heart	442.8	M, F	Increased
Thymus	442.8	M, F	Decreased
Testes	147.6	M	Increased
	442.6	M	Decreased
Ovaries	442.8	F	Decreased

11. Histopathology

No treatment-related histopathological changes were seen in male or female rats at 1.5 mg/kg/day. Treatment-related histopathological changes observed in the higher dose group animals are tabulated in Tables 7 and 8 and are summarized below:

Dose Level [mg/kg/day]	Sex	Organs with Lesions
27.1	M	Kidneys
	F	Kidneys
147.6	M	Kidneys, Lung, Prostate
	F	Kidneys, Lung, Stomach, Bone marrow, Ovary and Uterus
442.8	M	Kidneys, Lung, Stomach, Bone Marrow, Eye, Liver, Spleen, Thymus, Thyroid, Testes, Epididymis, Seminal Vesicle, and Prostate
	F	Kidneys, Lung, Stomach, Bone Marrow, Eye, Liver, Spleen, Thymus, Thyroid, Cervical Lymph nodes, Ovary and Uterus

Eyes: Bilateral retinal degeneration varied from moderate to moderately severe which was characterized by a decrease in retinal thickness which was most apparent in the inner and outer nuclear layers.

Kidneys: Diffuse regeneration of renal tubule epithelium ranged from minimal to moderate in severity with a tendency for increased severity with increased dose.

Liver: Hypertrophy and individual cell necrosis of centrilobular hepatocytes were of only minimal or mild severity.

Lungs: Accumulation of foamy macrophages ranged from minimal to mild in severity with an increase in incidence and severity with dose.

Spleen/Cervical Lymph Nodes: Lymphoid atrophy in the spleen and cervical lymph nodes ranged from mild to moderately severe and was indicated by a reduction in the normal cellularity of the lymphoid follicles in the spleen and of the lymphoid cortex in the cervical lymph node.

Sternal Bone Marrow: The cellularity of the sternal bone marrow was slightly reduced by one grade out of five.

Stomach: The diffuse necrosis and regeneration of the foveolar epithelium ranged from mild to moderate in severity while the submucosal edema ranged from minimal to moderate.

Thymus: Atrophy was moderately severe in a majority of affected rats and was characterized by a dramatic reduction in the overall size of the organ with loss of lymphoid cellularity.

Thyroid: Hypertrophy of the follicular epithelium accompanied by decreased colloid ranged from minimal to moderate in severity and the incidence and severity of follicular hypertrophy increased with dose.

Testes et al.: Degeneration of the seminiferous epithelium in the testes was moderately severe in a majority of the affected males and was diffuse and bilateral in distribution. This lesion was associated with decreased spermatozoa in the epididymis and decreased secretory content in the prostate and seminal vesicles.

Ovaries/Uterus: Atrophy of the ovaries and uterus ranged from mild to moderately severe.

Other microscopical changes were considered incidental and unrelated to treatment.

Table 7. Histopathological Findings in Male Rats.

Organ / Lesion	Dose Level [mg/kg/day]				
No.of animals= 10 / dose levels	0	1.5	27.1	147.6	442.8
Bone marrow [sternal]					
-Cellularity, Slight/Mild	1	0	4	1	9
Moderate	9	10	6	9	1
Epididymis					
-Decreased Spermatozoa	0	0	0	0	10
Eye					
- Retinal degeneration, Bilateral	0	0	0	0	7
Kidney					
-Renal tubule Regeneration, Diffuse	0	0	2	10	8
Liver, Centrilobular Hepatocyte					
-Hypertrophy	0	0	0	0	7
-Individual Cell Necrosis	0	0	0	0	8
Lung					
-Foamy Macrophage Accumulation	0	0	0	3	9
Prostate					
-Decreased Secretory Content	0	0	0	1	8
Seminal Vesicle					
-Decreased Secretory Content	0	0	0	0	8
Spleen					
-Lymphoid Atrophy	0	0	0	0	2
Stomach					
-Foveolar Epithelium Necrosis	0	0	0	0	6
-Foveolar Epithelium Regeneration	0	0	0	0	6
-Submucosal Edema	0	0	0	0	6
Testes					
-Seminiferous Epithelium					
-Degeneration	0	0	0	0	8
Thymus					
-Atrophy	0	0	0	0	9
Thyroid					
-Decreased Colloid	0	0	0	0	8
-Follicular Cell Hypertrophy	0	0	0	0	10

Table 8. Histopathological Findings in Female Rats.

Organ / Lesion	Dose Level [mg/kg/day]				
No.of animals= 10 / dose levels	0	1.5	27.1	147.6	442.8
Bone marrow					
-Cellularity, Slight/Mild	1	1	1	10	10
Moderate	9	9	9	-	-
Eye					
- Retinal degeneration, Bilateral	0	0	0	0	8
Kidney					
-Renal tubule Regeneration, Diffuse	0	0	10	10	10
Liver, Centrilobular Hepatocyte					
-Hypertrophy	0	0	0	0	8
-Individual Cell Necrosis	0	0	0	0	8
Lung					
-Foamy Macrophage Accumulation	0	0	0	7	8
Lymph Node, Cervical					
-Lymphoid Atrophy	0	0	0	0	5
Ovary					
-Atrophy	0	0	0	4	9
Spleen					
-Lymphoid Atrophy	0	0	0	0	6
Stomach					
-Foveolar Epithelium Necrosis	0	0	0	1	7
-Foveolar Epithelium Regeneration	0	0	0	1	7
-Submucosal Edema	0	0	0	0	3
Thymus					
-Atrophy	0	0	0	0	8
Thyroid					
-Decreased Colloid	0	0	0	0	10
-Follicular Cell Hypertrophy	0	0	0	0	10
Uterus					
Atrophy	0	0	0	4	10

IV. DISCUSSION

Male and female Fischer-344 rats were fed diets containing the Diethanolamine salt of 2,4-D at concentrations of 0, 1.5, 27.1, 147.6, or 442.8 mg/kg/day for 13 weeks.

Analytical data showed that the diet mixes were homogeneous, stable at room temperature for up to 14 days and the mean concentrations were within 10% of the target level.

No treatment-related effects were seen at 1.5 mg/kg/day on survival, mean body weight, mean body weight gain, mean food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, organ weights, gross pathology or histopathology.

Treatment-related effects observed at higher dietary levels of 27.1, 147.6 and 442.8 mg/kg/day, included: mortality; decreases in body weight gain and food consumption; alterations in hematology, clinical chemistry, and urinalysis; changes in organ weights, gross pathology; and histopathological changes in the bone marrow [sternal], eyes, kidneys, liver, lungs, lymphoid tissues, stomach, spleen, thyroid, thymus, testes, epididymis, seminal vessicle, prostate, ovaries and uterus.

Except for the three males and three females that died at the high dose, no mortality occured during the study. Clinical signs of toxicity limited to the high dose animals were pale eyes and ears. Both mean body weight gain and mean absolute food consumption values were reduced at 27.1, 147.6 and 442.8 mg/kg/day groups. Ophthalmological examination revealed pallor in the retinal vasculature in both sexes of rats only at 442.8 mg/kg/day.

Alterations in hematology parameters included: decreases in HGB, HCT, MCV, and MCH at 27.1 mg/kg/day; reduced values of RBC, HCB, HCT, MCV, and MCH at 147.6 mg/kg/day; and lower levels of WBC, RBC, HGB, HCT, MCV, MCH, MCHC, and platelet counts at 442.8 mg/kg/day. The observations of pale eyes and ears and the pallor in the retinal vasculature of rats at 442.8 mg/kg/day were indications of the reduction of red blood cell counts seen in rats at this dose level.

Treatment-related alterations in clinical chemistry parameters were limited to increases in BUN, ALT, and AST and decreases in T_4 primarily seen at 147.6 and 442.8 mg/kg/day groups. These changes correlated with histopathological lesions of the kidneys, liver, and thyroids. Remaining differences in clinical chemistry values were considered to be related to the effect on body weight and therefore, were not considered to be treatment related.

Differences in absolute and relative weights were seen in a number of organs at higher dose levels. However, only the dose-related increases in relative kidney and liver weights were considered to be treatment related due to collaborative clinical chemistry alterations and histopathological changes in these tissues. Differences seen in other organ weights were not considered to be treatment related since no histopathological alterations were seen in any of these tissues and the changes were considered secondary to the decrease in body weight gain.

Gross pathology showed lesions in the kidneys, lungs, thymus and gonads of both sexes of rats at doses of 27.1 mg/kg/day and above. Kidneys were green in color, the lungs had pinpoint white foci in all the lobes, while the thymus and the gonads were small when compared to controls.

Histopathology revealed that the DEA salt of 2,4-D induced alterations in several organs including the bone marrow, epididymis, eyes, kidneys, liver, lung, lymph node [cervical], ovaries, prostate seminal vesicles, spleen, stomach, testes, thymus, thyroid, and uterus.

In most cases, the histopathological changes correlated well with alterations observed in hematology and clinical chemistry parameters and organ weight data. The reduced cellularity seen in the sternal bone marrow may be correlated with the changes in hematology parameters [decreases in WBC, RBC, HGB, HCT, platelet counts]. The elevated BUN values and the increase in relative kidney weights may be related to the diffuse regeneration of the renal tubule epithelium of the kidneys. Increases in relative liver weight, ALT, and AST were associated with centrilobular hepatocellular hypertrophy of the liver. The follicular cell hypertrophy of the thyroid gland may be related to the lower levels of the thyroid hormone [T_4] noted at termination.

The bilateral retinal degeneration in the eye observed during histopathology was not evident during the ocular examination. The poor contrast due to the pallor in the retinal vasculature may have prevented the observation of the retinal degeneration during the in-life ocular examination. The white foci on surface of lungs observed during gross necropsy was confirmed by histopathology as evidenced by an increase in accumulation of foamy macrophages in the alveolar spaces. The lymphoid atrophy and atrophy of the thymus may be related to the effects on body weight. Degeneration of seminiferous epithelium in the testes was associated with decreased spermatozoa in the epididymis and decreased secretory content in the prostate and seminal vesicles. Atrophy of the ovaries and uterus was probably associated with treatment-induced weight loss.

V. CONCLUSION

Under the conditions of this study, a NOEL of 1.5 mg/kg/day and a LOEL of 27.1 mg/kg/day is established for the 90-day oral toxicity of the Diethanolamine Salt of 2,4-Dichlorophenoxyacetic acid to male and female rats. The LOEL is based on decreases in mean body weight gain and mean absolute food consumption, increase in relative kidney weights, and histopathological alterations in the kidneys of male and female rats.

VI. **CORE CLASSIFICATION:** Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

[2,4-D-2-BUTOXYETHYL ESTER] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Amendment to DER for MRID 41928101 - Subchronic Oral Toxicity Study [Rat] [2,4-D-2-BUTOXYETHYL ESTER]

This amendment provides an EXECUTIVE SUMMARY to upgrade the original DER [Document No. 008614].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/6/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P.H. Virginia A. Dobozy 12/8/00
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: subchronic oral toxicity - rat § 82-1/OPPTS 870.3100
P.C. CODE: 030053 REREGISTRATION CASE NO.: 818726
CASWELL NO.: 315AI CAS NO.: 1929-73-4
TEST MATERIAL (PURITY): 2,4-D butoxyethyl ester [94.6% (acid equivalent 65.8%)]
SYNONYMS: 2,4-D BEE; dichlorophenoxyacetic acid, 2-butoxyethyl ester
SPONSOR: DowElanco

CITATION: Szabo, J. R, and Rachunek, B. L. (1991). 2,4-D, Butoxyethyl Ester: 13-Week Dietary Toxicity Study in Fischer 344 Rats. Lake Jackson Research Center, Freeport, TX; Study No. DECO-TXT: K-007722-015, January 31, 1991. MRID 41928101. Document No. 008614. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [MRID 41928101], 10 Fischer 344 rats/sex/group were administered the butoxyethyl ester of 2,4-dichlorophenoxyacetic acid [94.6%] *via* the diet for 13 weeks at concentrations of 0, 1.5 [acid equivalent, 1] mg/kg/day, 22 [acid equivalent, 15] mg/kg/day, 145 [acid equivalent, 100] mg/kg/day, and 436 [acid equivalent, 300] mg/kg/day.

There were no spontaneous deaths during the study, but two high-dose rats [male on day 70 and female on day 56] were sacrificed moribund, and each displayed alterations/lesions in kidney, liver, and thyroid. Treatment-related clinical signs of toxicity were limited to a unilateral cataract in one high-dose female and bilateral cataracts in two high-dose females, which resulted in a decrease in the visual placing reflex in the Functional Observational Battery. Decreased body weight [males 70%-84%/females 63%-78% of control] was observed in the high-dose [436 mg/kg/day] rats throughout the study, with the magnitude of the deficit increasing with time. Females at the next highest dose level [145 mg/kg/day] also displayed a slight [91%-92% of control] decrease in body weight throughout the study. Overall body-weight gain was decreased in males at the highest dose level [34% of control] and in females at the next to the highest dose level [91% of control]. Females at the highest dose level displayed a negative body-weight gain [-6.7 grams]. Food consumption for both sexes at the two highest dose levels was decreased throughout the study, and the consumption values during the first week were 5% lower for the high-dose males and 21% lower for the high-dose females compared to their pre-test values and 12% and 25% of the control values, respectively, suggesting a possible palatability problem.

There was a dose-related decrease in red blood cell, hemoglobin, hematocrit, and platelet values at the mid-high and high-dose levels in both sexes at study termination, and white blood cell values in females at 145 mg/kg/day and both sexes at the high-dose level were decreased also. Several of the clinical chemistry parameters [increased

AP activity, total cholesterol, triglycerides, albumin, AST/ALT activity, phosphorus, and BUN; decreased glucose, total protein, globulin, sodium, and calcium concentrations, and creatinine values] were altered at the mid-high and/or high-dose levels in one or both sexes, but many of these changes can be attributed to the severe debilitation of both sexes as evidenced by the lack of food consumption and decreased body weight. Males at the high-dose level displayed an elevated T3 concentration [3-fold] and a decrease in T4 concentrations [49% of control]. Females at the two highest dose levels displayed decreases in T4 concentrations, but there was no dose response [45%/53% of control at 145 and 436 mg/kg/day, respectively]. Treatment-related effects [unilateral cataract in one male and 2 females and bilateral cataract in 2 females were observed at the high-dose level during the ophthalmoscopy examination at study termination.

Increased absolute and relative thyroid and liver weights were observed at the high-dose level in both sexes. Decreased absolute and increased relative adrenal, brain, and heart weights were observed in both sexes at the high-dose level also. Additionally decreased absolute and relative testes and ovarian weights were observed at the high-dose level in the respective sexes, and increased relative kidney weights were noted in both sexes at the mid-high and high-dose levels. Eye lesions were observed at the high-dose level and consisted of unilateral or bilateral cataracts in one male and 3 females, moderate degenerative lesions of the rods, cones, and the outer nuclear layer regions of the retina in 3 males and 8 females. Liver lesions [slight to moderate increase in eosinophilia of hypertrophic centrilobular hepatocytes] were observed at the two highest dose levels in both sexes, with the males displaying the greater incidence. Kidney lesions [loss of brush border and a decrease in cytoplasmic hyaline droplet accumulation in the apical portion of the cytoplasm of the proximal convoluted tubule epithelial cells] were observed in all of the high-dose males and in one high-dose female. Thyroid lesions consisted of hypertrophy and vacuolation of the follicular epithelium in males at the mid-high [slight in 9/10] and high-dose [moderate in 10/10] levels and in females at the high-dose level [slight in 10/10]. Decreased hematopoiesis [diffuse] of the bone marrow was observed at the high-dose level [both sexes].

The NOAEL is 22 mg/kg/day, and the LOAEL is 145 mg/kg/day, based on slightly decreased body weight, body-weight gain, and food consumption, and alterations in some hematological and clinical chemistry parameters, changes in thyroid hormone concentrations [females], and histopathological lesions [males] in the thyroids. At the highest dose level [436 mg/kg/day], one male and one female were sacrificed moribund, the females lost weight during thesecond and third month of the study, and both exhibited lesions in the thyroid, liver and kidney.

This subchronic oral toxicity study is classified **Acceptable/Guideline**, and the study satisfies the guideline requirement [OPPTS 870.3100; §82-1] for a subchronic oral toxicity study in the rodent.

Table 1. Hematology Parameters

Dose/Sex/Parameter	0 mg/kg/day	1.5 mg/kg/day	22 mg/kg/day	145 mg/kg/day	436 mg/kg/day
MALES					
RBC [x 10^6]	8.91±0.13	8.94±0.32	9.01±0.24	8.67±0.14*	7.43±0.72*
HGB [mg/dL]	14.3±0.3	14.3±0.6	14.3±0.4	13.8±0.3*	12.9±1.4*
PCV [%]	41.9±0.7	41.4±1.3	41.6±1.0	40.0±0.8*	34.6±3.7*
platelets [x 10^3]	577±25	559±25	555±32	517±21*	391±47*
WBC	6.88±2.56	5.98±1.04	7.12±0.92	7.06±1.98	3.62±0.71*
FEMALES					
RBC [x 10^6]	7.90±0.21	8.04±0.21	7.82±0.17	7.25±0.14*	6.59±0.21*
HGB [mg/dL]	13.6±0.3	13.9±0.3	13.4±0.3	13.0±0.3*	12.1±0.4*
PCV [%]	40.0±0.9	40.5±0.9	39.7±0.9	37.7±0.7*	33.3±1.0*
platelets [x 10^3]	609±45	594±29	577±50	455±27*	382±31*
WBC [x 10^3]	4.63±1.79	4.23±1.06	4.35±0.61	2.79±0.58*	2.18±0.32*

Data from Tables 13-16, pages 53-56 of the report; n=10 for groups 145 mg/kg/day and lower; 9 for high dose; * $p<0.05$

Table 2. Clinical Chemistry Parameters

Dose/Sex/Parameter	0 mg/kg/day	1.5 mg/kg/day	22 mg/kg/day	145 mg/kg/day	436 mg/kg/day
MALES					
alkaline phosphatase [U/L]	67±6	62±8	61±4	72±6	79±8*
ALT [U/L]	42±6	37±6	42±9	73±21*	59±38
AST [U/L]	87±9	69±11*	81±19	116±29	158±142*
glucose [mg/dL]	91.9±12.5	100.1±7.7	96.4±11.9	92.8±4.7	59.6±17.0*
BUN [mg/dL]	14.0±0.8	13.0±0.7	13.2±0.6	13.5±1.6	16.1±2.9
total protein [g/dL]	6.4±0.2	6.4±0.2	6.3±0.2	6.0±0.3*	5.8±0.4*
albumin [g/dL]	3.8±0.1	4.0±0.1*	3.9±0.1	3.9±0.1	4.2±0.3*
globulin [g/dL]	2.5±0.2	2.4±0.2	2.4±0.2	2.1±0.2*	1.6±0.2*
cholesterol [mg/dL]	26.4±4.2	28.3±4.3	27.4±7.5	26.1±5.1	55.4±27.1*
triglycerides [mg/dL]	38.3±12.6	40.6±8.3	41.6±10.4	45.4±8.2	54.3±20.5*
creatinine [mg/dL]	0.85±0.22	0.82±0.18	0.74±0.14	0.66±0.06*	0.70±0.12
sodium [μg/L]	145.7±0.9	145.1±0.8	144.7±1.0	144.2±1.5*	143.8±1.2*
calcium [μg/L]	9.48±0.18	9.55±0.20	9.66±0.26	9.26±0.20	9.16±0.12*
FEMALES					
alkaline phosphatase [U/L]	51±6	48±9	48±4	59±11	110±25*
ALT [U/L]	52±21	48±18	59±25	45±15	93±55
AST [U/L]	104±30	86±32	97±32	79±13	142±63
glucose [mg/dL]	81.6±8.8	89.8±9.7	91.7±8.1*	76.0±5.3	49.8±7.2*
BUN [mg/dL]	16.6±1.4	16.3±1.3	18.2±1.7	16.1±2.0	25.5±6.8*
total protein [g/dL]	6.6±0.3	6.4±0.1	6.5±0.3	6.2±0.2*	6.1±0.3*
albumin [g/dL]	4.1±0.2	4.1±0.2	4.2±0.3	4.2±0.2	4.5±0.2*
globulin [g/dL]	2.6±0.2	2.3±0.2	2.3±0.2*	2.1±0.2*	1.6±0.3*
cholesterol [mg/dL]	59.7±6.8	60.3±5.1	58.4±5.3	66.1±9.2*	70.6±10.4*
triglycerides [mg/dL]	36.9±6.1	35.1±4.9	38.7±10.3	37.0±29.5	58.8±16.1*
creatinine [mg/dL]	0.87±0.14	0.82±0.11	0.72±0.09*	0.80±0.13	0.61±0.09*
sodium [μg/L]	144.7±0.7	144.4±1.0	144.3±0.2	144.5±0.7	143.1±2.6
calcium [μg/L]	10.24±0.19	10.14±0.13	10.29±0.24	9.89±0.16*	9.62±0.37*

Data from Tables 19-20, pages 59-60 of the report; n=10 for groups 145 mg/kg/day and lower; 9 for high dose; * $p<0.05$

[2,4-D-2-BUTOXYETHYL ESTER] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Table 3. Thyroid Parameters

Dose/Sex/Parameter	0 mg/kg/day	1.5 mg/kg/day	22 mg/kg/day	145 mg/kg/day	436 mg/kg/day
MALES					
thyroid weight					
grams	0.01825±0.00276	0.02040±0.00192	0.01987±0.00310	0.01946±0.00199	0.02778±0.00245*
g/100 g	0.00609±0.00091	0.00664±0.00057	0.00673±0.00119	0.00681±0.00069	0.01369±0.00227*
T3 [ng/mL]	0.41±0.27	0.77±0.17*	0.75±0.15*	0.68±0.12	1.25±0.40*
T4 [μg/dL]	5.10±0.94	5.62±0.47	5.49±0.85	4.92±0.89	2.50±0.47*
FEMALES					
thyroid weight					
grams	0.01526±0.00140	0.01296±0.00097*	0.01416±0.00152	0.01495±0.00201	0.01936±0.00317*
g/100 g	0.00875±0.00091	0.00739±0.00069*	0.00830±0.00082	0.00941±0.00120	0.01785±0.00328*
T3 [ng/mL]	0.45±0.30	0.56±0.19	0.51±0.19	0.53±0.13	0.65±0.29
T4 [μg/dL]	3.93±0.82	4.07±0.55	4.03±0.49	1.75±0.46*	2.08±0.48*

Data from Tables 21-22, pages 61-62 of the report; n=10 for groups 145 mg/kg/day and lower; 9 for high dose; * $p<0.05$

Table 4. Organ-Weight Data

Dose/Sex/Parameter	0 mg/kg/day	1.5 mg/kg/day	22 mg/kg/day	145 mg/kg/day	436 mg/kg/day
MALES					
ADRENAL					
grams	0.0411±0.0041	0.0405±0.0045	0.0404±0.0036	0.0378±0.0042	0.0369±0.0052
g/100 g	0.0138±0.0016	0.0132±0.0020	0.0136±0.0008	0.0133±0.0018	0.0180±0.0024*
BRAIN					
grams	1.833±0.043	1.829±0.053	1.823±0.075	1.830±0.052	1.698±0.053*
g/100 g	0.613±0.031	0.596±0.031	0.616±0.034	0.641±0.032	0.834±0.093*
HEART					
grams	0.888±0.041	0.918±0.065	0.901±0.063	0.870±0.041	0.722±0.089*
g/100 g	0.297±0.012	0.296±0.014	0.304±0.023	0.300±0.012	0.351±0.021*
KIDNEYS					
grams	1.770±0.092	1.860±0.099	1.881±0.176	2.002±0.095*	1.615±0.198
g/100 g	0.591±0.018	0.605±0.019	0.633±0.032*	0.701±0.036*	0.786±0.061*
LIVER					
grams	7.391±0.613	7.586±0.532	7.407±0.693	7.364±0.280	8.086±0.656*
g/100 g	2.464±0.113	2.465±0.060	2.492±0.056	2.576±0.090	3.949±0.289*
TESTES					
grams	2.978±0.097	3.000±0.121	2.976±0.283	2.948±0.152	1.437±0.628*
g/100 g	0.994±0.062	0.977±0.051	1.003±0.078	1.031±0.051	0.684±0.255*
FEMALES					
ADRENAL					
grams	0.0470±0.0069	0.0446±0.0056	0.0453±0.0041	0.0420±0.0046	0.0293±0.0043*
g/100 g	0.0269±0.0041	0.0254±0.0031	0.0265±0.0023	0.264±0.0025	0.0271±0.0044
BRAIN					
grams	1.696±0.046	1.685±0.051	1.687±0.053	1.686±0.041	1.488±0.040*
g/100 g	0.972±0.031	0.960±0.036	0.989±0.042	1.063±0.032*	1.373±0.114*
HEART					
grams	0.571±0.033	0.578±0.026	0.572±0.042	0.536±0.030	0.490±0.036*
g/100 g	0.327±0.018	0.329±0.014	0.335±0.022	0.338±0.010	0.451±0.024*
KIDNEYS					
grams	1.128±0.058	1.079±0.022	1.091±0.065	1.109±0.054	0.911±0.039*
g/100 g	0.646±0.036	0.615±0.019	0.639±0.018	0.699±0.031*	0.840±0.061*
LIVER					
grams	4.402±0.244	4.301±0.205	4.318±0.282	4.139±0.187	4.998±0.268*
g/100 g	2.521±0.122	2.449±0.087	2.528±0.080	2.607±0.093	4.615±0.470*
OVARIES					
grams	0.100±0.017	0.092±0.010	0.101±0.035	0.083±0.015	0.045±0.006*
g/100 g	0.057±0.010	0.052±0.005	0.059±0.020	0.052±0.008	0.042±0.009*
Terminal fasted body weight [g]					
males	299.9±19.2	307.6±17.9	297.1±24.8	286.2±16.3	206.3±27.3
females	174.7±7.3	175.6±6.5	170.9±10.2	158.8±5.3*	109.2±10.9 [63%]√

Data from Tables 23-24, pages 63-64 of the report; n=10 for groups 145 mg/kg/day and lower; 9 for high dose; √ [% of control]; * $p<0.05$

008614

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist Jess Rowland 9/20/91
Section II, Toxicology Branch II (H7509C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 9/23/91
Section II, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding-Rodent **GUIDELINE:** 82-1(a)

TOX.CHEM. No.: 315 AS **MRID No.:** 419281-01

HED Project No. 1-1801 **Registrant:** DowElanco

TEST MATERIAL: Butoxyethyl Ester of 2,4-Dichlorophenoxyacetic acid (2,4-D BEE)

STUDY IDENTIFICATION: DECO-TXT: K-007722-015

TESTING LABORATORY: Lake Jackson Research Center, Freeport, TX.

TITLE OF REPORT: 2,4-D, Butoxyethyl Ester: 13-Week Dietary Toxicity Study in Fischer 344 Rats.

REPORT AUTHORS: J.R Szabo and B.L Rachunek

REPORT DATE: January 31, 1991

SUMMARY: Male and female Fischer-344 rats were fed diets containing the Butoxyethyl ester of 2,4-Dichlorophenoxyacetic acid at 0, 1.5, 22, 145.0, or 436 mg/kg/day for 13 weeks. Treatment did not cause any adverse effects at 1.5 or 22 mg/kg/day, but did induce decreases in mean body weight, body weight gain, and food consumption, alterations in some of the hematology and clinical chemistry parameters, changes in thyroid hormone concentrations, and histopathological lesions in the thyroids at 145 mg/kg/day. The highest dietary dose level of 436.0 mg/kg/day was associated with weight loss, reduced food consumption, alterations in hematology and clinical chemistry parameters, changes in various organ weights, changes in thyroid hormone concentrations, and histopathological lesions in the eye, liver, kidneys, and thyroids.

Under the conditions of this study, a No-Observable-Effect Level (NOEL) of 22 mg/kg/day and a Lowest-Observable-Effect Level (LOEL) of 145 mg/kg/day are established. The LOEL is based on decreases in mean body weight, body weight gain and food consumption, alterations in hematology and clinical chemistry parameters, changes in thyroid hormone concentrations, and histopathological lesions of the thyroid glands at 145 mg/kg/day.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the subchronic toxicity of **Butoxyethyl Ester of 2,4-Dichlorophenoxyacetic acid (2,4-D BEE)** following dietary administration to rats.

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: Butoxyethyl ester of 2,4-dichlorophenoxyacetic acid.
Purity: 94.6%
Lot No.: AGR-276426
Description: Dark brown liquid.

2. Test Animals

Species: Rats
Strain: Fischer-344
Sex: Males and females
Age: Approximately 6 weeks at initiation
Weight at initiation: 168.4-174.8 g (M); 120.2-122.7 g (F)
Identification: Eartags.
Acclimation: Approximately 2 weeks
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Chow #5002.
Water: Tap water ad libitum
Environment: Temperature and humidity controlled; 13 air changes/hr; Light/dark cycles: 12 hour photocycle.

3. Study Design

Group No.	Treatment	No. of Animals Males	No. of Animals Females	Dose Level (mg/kg/day)	Acid Equivalent (mg/kg/day)
1	Control	10	10	0	0
2	Low	10	10	1.5	1.0
3	Mid-1	10	10	22	15
4	Mid-2	10	10	145	100
5	High	10	10	436	300

4. Test Article Formulation and Analyses

Premixes and diets were prepared fresh weekly. The diets were prepared by serial dilution of premix according to written instructions. The concentrations of test material in the diets were based on group mean body weights and feed consumption.

Samples of premix, diets and control feed taken on Study Days 1, 43, and 85 were analyzed to confirm targeted concentrations, stability analysis were made on Day 1 and 85, and homogeneity was determined on Study Day 1.

5. Treatment

Rats were fed the control and test diets 7 days per week for at least 13 weeks. The oral route of administration was chosen because it is the route of potential human exposure.

6. Experimental Procedures

Mortality and moribundity checks were performed twice daily. Cageside observations for clinical signs of toxicity were performed once daily. Body weights and food consumption were measured prior to initiation (Study Days -8 and -1) and once weekly thereafter. Rats were observed once weekly for physical abnormalities and changes in somatomotor activity and behavior. Additionally, a functional observation battery was conducted on all rats on Study Days -5, 31, 58, and 86 which included: (i) any unusual responses with respect to body position, activity level, coordination of movement, and gait; (ii) any unusual or bizarre behavior including but not limited to head flicking, head searching, compulsive biting or licking, self-mutilation, circling, or walking backwards; (iii) the presence of convulsions, tremors, increased levels of salivation, piloerection, pupillary dilation or construction, unusual respiration and/or mouth breathing, diarrhea, excessive or diminished urination, vocalization; and (iv) hindlimb grip strength; (v) sensory function (audition, pain perception, and visual placing).

Blood and urine were collected from all animals at termination for hematology, clinical chemistry and urinalysis. 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]
x Mean corpuscular HGB (MCH)	x Mean corpuscular HGB Concentration (MCHC)
x Mean corpuscular volume(MCV)	x Erythrocyte fragility
x Corrected leukocyte count (COR WBC)	x Cell morphology

Clinical Chemistry

Electrolytes:	Other
x Calcium[a] x Chloride[a] Magnesium[a] x Phosphorus[a] x Potassium[a] x Sodium	x Albumin[a] x Blood creatinine[a] x Blood urea nitrogen[a] Cholesterol[a] x Globulins x Glucose[a] x Total bilirubin[a] x Total serum protein[a] Triglycerides Serum protein electrophoresis x Triiodothyronine (T_3 x Thyroxine (T_4)
Enzymes:	
x Alkaline phosphatase x Alanine aminotransferase (SGPT)[a] x Aspartate aminotransferase (SGOT)[a] Cholinesterase[b] x Creatinine phosphatase[a] Lactic acid dehydrogenase Gama glutamyl transferase	

Urinalysis

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

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

7. Termination

All surviving animals were fasted, weighed, anesthetized with methoxyflurane and exsanguinated. A gross necropsy was performed on Study Day 92 (males) and Study Day 93 (females), all gross pathological changes were recorded, and organs listed below were weighed.

Adrenal glands	Thyroid glands	Brain	Heart	Kidneys	Liver	Testes / Ovaries

8. Histopathology

The checked (X) tissues from control and high-dose group rats were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Salivary glands[a]	Trachea[a]
x Esophagus[a]	x Lung[a]
x Duodenum[a]	Pharynx[e]
x Jejunum[a]	Larynx[e]
x Cecum[a]	Nose[e]
x Colon[a]	
x Rectum[a]	Cardiovascular/Hemo.System
x Liver[ac]	
x Pancreas[a]	x Aorta (thoracic)[a]
Gall bladder[ab]	x Heart[a]
	x Bone marrow[a]
Neurological System	x Lymph nodes[a]
	x Spleen[a]
x Brain[ac]	x Thymus[a]
x Pituitary[a]	
Peripheral nerve[ab]	Urinogenital System
Spinal cord (3 levels)[ab]	
x Eyes (optical nerve)[ab]	x Kidneys[ac]
	x Urinary bladder[a]
	x Testes[ac]
Glandular System	x Epididymides
	x Uterus[a]
x Adrenals[a]	x Ovaries[ac]
Lacrimal glands[b]	Others
x Parathyroids[ad]	
x Thyroids[ad]	x All gross lesions and masses
	x Skeletal muscle[a]

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.

In addition, adrenal glands, kidneys, liver, lungs, and thyroid glands, eyes, and gross lesions were also examined histologically from animals at 1.5, 22, or 145 mg/kg/day groups. Also due to the presence of microscopic changes in the testes of male rats and bone marrow of male and female rats at 436 mg/kg/day, testes from male rats and bone/bone marrow from both sexes at 145 mg/kg/day group were examined histologically.

9. Statistical Analyses

In-life body weights, hematologic and clinical chemistry parameters, organ weights, thyroid hormones, and urinary specific gravities were compared for differences of statistical significance using Bartlett's test for equality of variance, followed by analysis of variance (ANOVA) for parametric data or non parametric data. If differences were observed among the groups, Dunnett's t-test or Wilcoxon's signed Rank-Sum test with Bonferroni's correction was used to test for differences between each treatment group and control group.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of the testing laboratory, and the Study Protocol. A quality assurance statement was signed and dated January 31, 1991.

III. RESULTS

1. Analysis of Diet Mix

Concentration analyses of samples of premix and test diets taken on Study Days 1, 43, and 85 revealed that the observed levels were sufficiently comparable to target level as shown below:

Study Day 1

Group No.	Dose (mg/kg/day)	Sex	Targeted Level (ppm)	Observed Level (ppm)
-	Premix	M, F	10000	9100
2	1.5	M	17.39	17.90
		F	18.13	19.20
3	22	M	245.3	233.0
		F	261.7	257.0
4	145	M	1580	1390
		F	1712	1580
5	436	M	5030	4680
		F	5219	4850

Study Day 43

Group No.	Dose (mg/kg/day)	Sex	Targeted Level (ppm)	Observed Level (ppm)
-	Premix	M, F	10000	10000
2	1.5	M	24.69	29.80
		F	22.88	24.70
3	22	M	363.2	388.0
		F	325.8	334.0
4	145	M	2355	2540
		F	2335	2510
5	436	M	6653	6830
		F	6667	6800

Study Day 85

Group No.	Dose (mg/kg/day)	Sex	Targeted Level (ppm)	Observed Level (ppm)
-	Premix	M, F	10000	9910
2	1.5	M	28.73	31.20
		F	24.92	24.60
3	22	M	423.0	433.0
		F	349.2	348.0
4	145	M	2733	3140
		F	2394	2360
5	436	M	7314	8230
		F	3501	3940

Homogeneity analysis of the 1.5 mg/kg/day female diet mixed on Study Day 1 showed a homogeneous dispersion of the test material in the diet as shown below:

Sample Site	Targeted Level (ppm)	Observed Level Mean ± SD (ppm)
Center Top	18.13	19.2 ± 2.8
Center Bottom	18.13	19.60 ± 0.04
Side Top	18.13	19.4 ± 1.0
Side Bottom	18.13	18.9 ± 0.06

As shown below stability analyses of a diet (1.5 mg/kg/day) mixed on Study Day 85 demonstrated the test material to be stable at room temperature for up to 10 days.

Assay Day	Sample Site	Observed Level Mean ± SD (ppm)	% of Initial Observed Level
0	-	24.6 ± 1.7	--
7	Top	22.3 ± 0.3	91
	Bottom	23.7 ± 0.4	96
10	Top	22.8 ± 0.1	93
	Bottom	22.4 ± 0.1	91

2. Survival

Except for the one female and one male that were sacrificed moribund on Study Days 56 and 70, respectively, at the high dose (436 mg/kg/day), no mortality occurred during the study. The cause of morbidity in the male rat (Day 70) appeared to be a combination of hepatic, kidney, and thyroid lesions in a cachectic animal with overgrown incisors inhibiting alimentation. The cause of morbidity in the female rat (Day 56) was primarily due to renal alterations, however, liver and thyroid gland lesions were also seen in this rat.

3. Functional Observational Battery

Treatment-related clinical sign of toxicity was limited to a unilateral cataract in one female and bilateral cataracts in two females at the high dose. The cataracts resulted in a decrease in visual placing reflex. Urine stains staining of the peritoneum noted in both sexes of rats at the high dose were not considered to be of toxicologic or biologic significance.

4. Body Weights and Body Weight Changes

Mean body weight data are summarized in Tables 1 and 2 for males and females, respectively. Mean body weight values for rats at 1.5 and 22 mg/kg/day were comparable to that of control rats. Statistically significant ($p < 0.05$) reduction in mean body weight was observed for males and females at 436 mg/kg/day throughout the study beginning on Study Day 14 for males and Study Day 7 for females. Similarly, reductions in mean body weight were also seen in both sexes at
145 mg/kg/day throughout the study, but reached statistical significance only in females beginning on Study Day 28.

Table 1. Mean Body Weights and S.D (G) for Male Rats.

Dose Level (mg/kg/day)	Day: -1	Day: 28	Day: 63	Day: 91
0	173.2±12.9	255.8±18.0	300.3±18.6	315.3±20.6
1.5	174.8±10.7	260.5±13.0	307.8±16.8	323.9±18.6
22.0	171.5±15.2	256.1±19.1	301.3±22.4	313.1±23.7
145.0	168.4±16.0	247.2±15.4	288.4±18.5	302.4±16.1
436.0	172.8±11.9	**214.9*± 24.6** 74	**228.5*±29.5** 76	**221.44±30.8*** 70

* Significantly different from control value at $p \leq 0.05$.

Table 2. Mean Body Weights and S.D (G) for Female Rats.

Dose Level (mg/kg/day)	Day: -1	Day: 28	Day: 63	Day: 91
0	120.2 ± 6.4	160.2 ± 5.7	179.8 ± 7.6	185.4 ± 8.1
1.5	121.1 ± 6.8	160.3 ± 6.4	178.7 ± 6.6	185.6 ± 6.8
22.0	120.3 ± 7.8	155.9 ± 11.4	174.6 ± 12.9	182.2 ± 11.0
145.0	122.7 ± 5.7	**148.0* ± 7.6**	**165.5* ± 8.4**	**169.6* ± 6.7**
436.0	122.7 ± 5.7	**125.6* ± 8.4**	**114.2* ± 15.8**	**116.0 ± 11.7***

* Significantly different from control value at $p \leq 0.05$

Reductions in body weight gain were observed in both sexes of rats at 145 and 436 mg/kg/day groups. The decreases were approximately 4% and 8% in males and females, respectively at 145 mg/kg/day, and 30% and 37% in males and females, respectively at 436 mg/kg/day. No reductions in body weight gain were seen in rats at 1.5 or 22 mg/kg/day groups.

BW HDT lost wt.

5. Food Consumption

The mean total food consumption was decreased in males and females at 145 and 436 mg/kg/day throughout the study but the decrease was not statistically significant when compared to controls. The magnitude of reductions in body weight gain in male and female rats at
436 mg/kg/day reflected the marked decrease in food consumption values. During Study Week 1, food consumption values at 436 mg/kg/day, were approximately 5% lower in males and 21% lower in females compared to pretest values. At 145 mg/kg/day, food consumption among females was consistently lower than the female control whereas the reductions were sporadically lower among males.

6. Hematology and Clinical Chemistry

Hematology data showed statistically significant ($p \leq 0.05$) decreases in erythrocyte, hemoglobin, hematocrit, and platelet values in male and female at 145 and 436 mg/kg/day, and in white blood cell values for males at 436 mg/kg/day and females at 145 and 436 mg/kg/day when compared to controls.

Clinical chemistry data exhibited statistically significant ($p \leq 0.5$) differences at 436 mg/kg/day which included: increases in ALP activity, total cholesterol, triglycerides, and albumin concentrations (both sexes); increase in AST activity (males); elevation in phosphorus and BUN levels (females); decreases in glucose, total protein, globulin, sodium, and calcium concentrations (both sexes); and a decrease in creatinine values in females. Significant differences observed at 145 mg/kg/day, included: decreases in total protein (both sexes), globulin (both sexes), sodium (males), and calcium (both sexes), creatinine (males) concentrations and an increase in ALT activity in males. All other differences were considered to be incidental due to lack of dose-response or inconsistency between sexes.

Thyroid hormone assay showed significantly ($p < 0.05$) elevated triiodothyronine (T_3) concentrations in males at 436 mg/kg/day and reduced thyroxine (T_4) concentrations in both sexes at 436 mg/kg/day, and in females at 145 mg/kg/day.

Dose (mg/kg/day	Sex	T_3 (ng/mL)	T_4 (µg/dL)
0	M	0.41 ± 0.27	5.10 ± 0.94
0	F	0.45 ± 0.30	3.93 ± 0.82
145	F	0.53 ± 0.13	1.75 ± 0.46
436	M	1.25 ± 0.40	2.50 ± 0.47
436	F	0.65 ± 0.29	2.08 ± 0.48

Except for a slight but significant lower specific gravity (1.061 ± 0.02 at 436 mg/kg/day vs. 1.08 ± 0.0 in controls), urinalysis data indicated no treatment-related changes.

7. Gross Pathology

Treatment-related gross necropsy findings were limited to the high dose group which consisted of unilateral cataract (1 male and 2 females, bilateral cataract (2 females), small testes (6 males), and decreased fat in the abdominal cavity (1 male and 9 females).

142

8. Organ Weights

Statistically significant differences in absolute and relative organ weight were primarily seen in male and/or female rats at the high dose which included elevated absolute and relative thyroid weights (boths sexes), elevated absolute and relative liver weights (both sexes), lower absolute (males) and elevated relative (females) adrenal gland weights, lower absolute and elevated relative brain weights (both sexes), lower absolute and elevated heart weights (both sexes), lower absolute and relative testes weights, lower absolute and relative ovarian weights, and elevated relative kidney weights (both sexes at 145 and 436 mg/kg/day). Differences observed in organ weights at the other dose levels were not considered biologically or toxicologically significant.

9. Histopathology

Treatment-related histopathological changes were seen in the eyes, liver, kidney, and thyroid. Secondary effects attributable to debilitation and/or toxicity included alterations in bone marrow and testes.

Eye lesions were manifested by unilateral or bilateral cataracts in 1 male and 3 female rats at 436 mg/kg/day. In addition, 3 males and 8 females showed moderate degenerative lesions of the rods and cones and the outer nuclear layer regions of the retina.

Liver lesions were characterized by a slight to moderate increase in eosinophilia of hypertrophic centrilobular hepatocytes with the incidence being higher in males than females at 145 and 436 mg/kg/day.

Kidney lesions consisted of a loss of the brush border and a decrease in cytoplasmic hyaline droplet accumulation in the apical portion of the cytoplasm of proximal convoluted tubule epithelial cells.

Thyroid lesions consisted of hypertrophy and vacuolation of the follicular epithelium in male rats at 145 mg/kg/day (slight, 9/10) and in male (moderate, 10/10) and female (slight, 10/10) rats at 436 mg/kg/day. This lesions was associated with elevated T_3 and lower T_4 concentrations in these groups of rats.

Decreased bone marrow cellularity (both sexes) and moderate to severe degeneration of the testes (males) were observed at the high dose.

Table 7. Histopathological Findings in Male Rats At Sacrifice.

Organ / Lesion	Dose Level (mg/kg/day)				
No.of animals= 10/dose levels	0	1.5	22.0	145	436
Eye					
-Cataract, unilateral	0	0	0	0	1
-Cataract, bilateral	0	0	0	0	1
-Retinal degeneration, moderate	0	0	0	0	3
Kidneys					
-Decreased cytoplasmic hyaline droplet accumulation					
----------very slight	0	0	0	0	1
----------slight	0	0	0	0	8
----------moderate	0	0	0	0	1
Liver					
-Centrilobular, diffuse,					
----------slight	0	0	0	3	0
----------moderate	0	0	0	0	10
----------severe	0	0	0	0	0
Thyroid					
-Hypertrophy and vacuolation of follicular epithelium,diffuse,					
----------slight	0	0	0	9	0
----------moderate	0	0	0	0	10
Marrow, bone,					
-Decreased hematopoiesis,diffuse	0	0	0	0	9
Testes					
-Degeneration, seminiferous tubules, diffuse, bilateral					
----------moderate	0	0	0	0	4
----------severe	0	0	0	0	6

Table 8. Histopathological Findings in Female Rats At Sacrifice.

Organ / Lesion	Dose Level (mg/kg/day)				
No.of animals= 10/dose levels	0	1.5	22.0	145	436
Eye					
-Cataract, unilateral	0	0	0	0	3
-Cataract, bilateral	0	0	0	0	3
-Retinal degeneration, moderate	0	0	0	0	8
Kidneys					
-Decreased cytoplasmic hyaline droplet accumulation					
----------very slight	0	0	0	0	0
----------slight	0	0	0	0	1
----------moderate	0	0	0	0	0
Liver					
-Centrilobular, diffuse,					
----------slight	0	0	0	0	0
----------moderate	0	0	0	0	9
----------severe	0	0	0	0	1
Thyroid					
-Hypertrophy and vacuolation of follicular epithelium,diffuse,					
----------slight	0	0	0	0	10
----------moderate	0	0	0	0	0
Marrow, bone,					
-Decreased hematopoiesis,diffuse	0	0	0	0	10

IV. DISCUSSION

Male and female Fischer-344 rats were fed diets containing Butoxyethyl ester of 2,4-D at concentrations of 0, 1.5, 22, 145.0, or 436 mg/kg/day for 13 weeks.

Analytical data showed that the diet mixes were homogeneous, stable at room temperature for up to 10 days and the mean concentrations were within 10% of the target level.

Treatment had no effect on survival in both sexes at 1.5, 22, or 145 mg/kg/day; 1 male and 1 female rat at 436 mg/kg/day were sacrificed moribund. No treatment-related effects were seen in mean body weight, body weight gain, or in total food consumption at 1.5 or 22 mg/kg/day. Decreases in mean body weight, body weight gain, and reduction in food consumption were observed in rats fed 145 and 436 mg/kg/day diets.

Treatment-related alterations in hematology parameters were confined to the 145 mg/kg/day and 436 mg/kg/day groups which included: decreased erythrocyte, hemoglobin, hematocrit and platelet counts.

Alterations in clinical chemistry parameters were primarily seen at 145 and 436 mg/kg/day groups which included: elevation in ALP and AST activities, and increases in total cholesterol, triglycerides BUN and phosphorus concentrations, and decreases in glucose, calcium, sodium, and total protein concentrations. Howver, except for the elevations in ALS and AST activities which can be correlated to the centrilobular hypertrophy of the liver, in most cases, the changes observed in hematology and clinical chemistry were not correlated to histopathological changes in the affected organ systems. Consequently, the alterations observed in some of the hematology and clinical chemistry parameters at the high dose were attributed to the severe debilitation of both sexes of rats at this dose and were considered to be a secondary effect to diet refusal (i.e., they reflect the marked decrease in body weight and food consumption).

The elevated T_3 and lower T_4 concentrations observed at 145 and 436 mg/kg/day was associated with histopathologic lesions in the thyroid.

Treatment-related gross pathological changes were limited to unilateral/bilateral cataract (both sexes), small testes (males) and decreased fat in the abdominal cavity (both sexes) at the high dose. Compound-induced differences in absolute and relative organ weights were generally limited to the high-dose which included thyroid, brain, heart, liver, kidney and testes weights.

Treatment-related histopathological changes which were limited to the high-dose group included unilateral/bilateral cataracts, retinal degeneration and cataract formation (both sexes), centrilobular hepatocellular hypertrophy (both sexes), decrease in cytoplasmic protein droplet accumulation and decrease in apical cytoplasm of proximal tubule epithelial cells of the kidneys (both sexes), and hypertrophy and vacuolation of the follicular epithelium of the thyroids (both sexes). The decreased bone marrow cellularity and degeneration of the testes were attributed to the debilitated physical condition of the rats at the high dose, undernutrition, and deleterious effects of altered thyroid hormone(s) on the maturation, development, and maintenance of the reproductive tract.

V. CONCLUSION

Under the conditions of this study, a NOEL of 22 mg/kg/day and a LOEL of 145.0 mg/kg/day is established for the 90-day oral toxicity of the Butoxyethyl ester of 2,4-Dichlorophenoxyacetic acid to male and female rats. The LOEL is based on decreases in mean body weight, body weight gain and food consumption, and alterations in some of the hematology and clinical chemistry parameters, changes in the thyroid hormone concentrations, and histopathological lesions of the thyroid glands observed at 145 mg/kg/day.

VI. **CORE CLASSIFICATION:** Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

Amendment to DER for MRID 41735306/ 21-Day Dermal Toxicity Study [Rabbit][DIMETHYL AMINE SALT OF 2,4-D] 41735303

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 008462]. The NOAEL/LOAEL for systemic toxicity has been added.

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 1/31/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A. Dobozy 1/31/01
Reregistration Branch I, Health Effects Division (7509C)

TXR #0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 21-day dermal toxicity - rabbit § 82-2/OPPTS 870.3200
P.C. CODE: 030019 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315O CAS #: 2008-39-1
TEST MATERIAL (PURITY): Dimethylamine salt of 2,4-D [66.18%; 55.5% acid equivalents]
SYNONYMS: 2,4-D DMA; dichlorophenoxyacetic acid, dimethylamine salt
SPONSOR: Industry Task Force II on 2,4-D Research Data

CITATION: Schulze, G. E. (1990). 21-Day Dermal Toxicity Study in Rabbits with Dimethylamine Salt of 2,4-D. Hazleton Laboratories America, Inc.; Study No. HLA 2184-111, August 9, 1990. MRID 41735306, Document No. 008462. Unpublished. 41735303

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study [MRID 41735306], 5 Hra: New Zealand White rabbits/sex/group were administered the dimethylamine salt of 2,4-dichlorophenoxyaceticacid [66.18% a.i.; 55.5% acid equivalents] *via* dermal application in sterile distilled water [once a day for 6 hours, 7 days/week] for 21 days at nominal doses of 0, 18, 180.1, and 540.5 mg/kg/day [actual: 11.9, 119.2, 357.7 mg/kg/day]. Doses [actual] equate to 10, 100, and 300 mg/kg/day acid equivalents; calculated by this reviewer].

There were two deaths at the high-dose level. One high-dose female, sacrificed moribund on day 10, was thin and languid in appearance on days 8 and 9 and prostate on day 10. Another high-dose female who experienced a lower back injury/trauma was sacrificed on day 14, but this latter death is not considered treatment-related. Treatment had no adverse effect on survival of the remaining rabbits, and clinical signs, body weight/gain, food consumption, hematology, urinalysis, and gross pathology findings were comparable among the groups for both sexes. Although overall body-weight gain was decreased in males at the mid- [15% of control] and high-dose [72% of control] levels compared to the control, there was no dose response. All groups lost weight during the first week of the study, which was attributed to acclimation to wearing the plastic collars. Food consumption was comparable among the groups for both sexes.

There was a dose-related increase in the incidence and severity of the signs of dermal irritation [erythema (slight, well-defined, moderate-to-severe), edema (very slight), thickening, epidermal scaling, fissuring (with and without bleeding), sloughing (females), necrosis] and gross pathology findings [dark areas, thickening, fissuring] in both sexes. At the mid- and high-dose levels, 2,4-D DMA induced dermal lesions, which included the increased incidence and/or severity of acanthosis, hyperkeratosis, and edema.

Centrilobular hepatocyte hypertrophy was observed in one high-dose female only. Other microscopic lesions observed were comparable among the groups for both sexes.

The dermal irritation NOAEL is 18 mg/kg/day, based on microscopic dermal lesions at the dermal irritation LOAEL of 180.1 mg/kg/day. The NOAEL for systemic toxicity is 180.1 mg/kg/day, based on one female death at the systemic toxicity LOAEL of 540.5 mg/kg/day.

This 21-day dermal toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [§82-2; 870.3200] for a 21-day dermal toxicity study.

NOTE: The original DER did not set a NOAEL or LOAEL for systemic toxicity.

[DIMETHYLAMINE SALT OF 2,4-D] 21-Day Dermal Toxicity Study - Rabbit [§82-2/OPPTS 870.3200]

Table 1.Body Weight/Body-Weight/Gain [grams]				
Dose/Day	0 mg/kg/day	18.0 mg/kg/day	180.1 mg/kg/day	540.5 mg/kg/day
MALE body weight				
0	2206±46	2219±94	2260±31	2338±69* [106]√
7	2109±63	2169±85	2135±116	2176±101
14	2155±114	2304±83	2168±223	2258±130
21	2315±19	2391±83	2276±337	2417±126 [104]
MALE body-weight gain				
0-7	-98±46	-50±104	-125±100	-162±50
0-14	-52±98	85±95	-92±228	-80±88
0-21	109±34	172±114	16±330 [15]	79±72 [72]
7-14	46±119	135±37	33±172	82±94
14-21	160±114	87±27	109±201	158±43
FEMALE body weight				
0	2281±44	2333±57	2370±76	2368±81 [104]
7	2107±147	2192±133	2257±141	2210±122
14	2224±171	2282±183	2429±114	2395±78
21	2401±105	2416±180	2563±93	2582±68 [108]
FEMALE body-weight gain				
0-7	-174±150	-141±89	113±85	-158±101
0-14	-57±183	-51±151	59±64	40±18
0-21	120±118	83±150	192±44	208±30 [173]
7-14	117±97	90±106	172±32	156±52
14-21	177±96	134±36	133±37	165±24 [93]

* $p<0.05$; √[% of control]; n=5; data from Tables 3A-3B, pages 51-54 of the report

Table 2. Food Consumption

Dose/Day	0 mg/kg/day	18.0 mg/kg/day	180.1 mg/kg/day	540.5 mg/kg/day
MALES grams/rat/day				
0-2	154±27	161±12	146±59	144±23 [94]√
2-4	178±24	176±17	156±86	147±43 [83]
4-6	162±10	171±15	153±40	140±51 [86]
13-14	164±73	223±18	178±59	223±16 [136]
16-18	179±7	191±14	162±66	203±20 [113]
20-21	205±17	197±12	195±42	214±21
FEMALES grams/rat/day				
0-2	147±57	142±24	154±51	151±46
2-4	146±70	161±23	175±21	155±32
4-6	188±8	188±9	180±39	171±20 [91]
13-14	190±15	198±90	224±8	214±22 [113]
16-18	188±20	189±3	204±6	205±23
20-21	191±22	195±18	230±47	225±21 [118]

√[% of control]; data from Table 4, pages 55-58 of the report

Table 3. Organ Weights

Dose/Day	0 mg/kg/day	18.0 mg/kg/day	180.1 mg/kg/day	540.5 mg/kg/day
MALES				
THYROID				
absolute	0.213±0.034	0.206±0.028	0.177±0.019 [83]√	0.226±0.033 [106]
relative	0.0097±0.0016	0.0091±0.0013	0.0082±0.0008	0.0100±0.0017
FEMALES				
THYROID				
absolute	0.257±0.085	0.182±0.063 [71]	0.213±0.056 [83]	0.337±0.182 [131]
relative	0.0115±0.0042	0.0079±0.0025 [69]	0.0088±0.0024 [77]	0.0141±0.0081 [123]
MALES				
LIVER				
absolute	53.7±4.7	53.0±5.6	52.9±4.3	56.4±5.6
relative	2.43±0.21	2.34±0.18	2.45±0.29	2.50±0.26
FEMALES				
LIVER				
absolute	55.9±4.7	59.0±1.9	59.4±6.8	58.1±1.2
relative	2.50±0.19	2.59±0.24	2.44±0.21	2.41±0.12
MALES				
KIDNEY				
absolute	14.8±1.6	15.3±0.9	17.6±1.2* [119]	15.3±1.3
relative	0.67±0.08	0.68±0.03	0.82±0.14 [122]	0.68±0.08
FEMALES				
KIDNEY				
absolute	15.7±1.8	15.2±0.8	15.7±1.1	15.4±0.9
relative	0.70±0.08	0.67±0.09	0.65±0.06	0.64±0.05
TERMINAL BODY WEIGHT				
MALES	2207±30	2261±91	2178±239	2257±121
FEMALES	2234±69	2292±178	2434±105	2419±109

√[% of control]; n=5; data from Table 8-9, pages 68-69 of the report

Table 4. Gross Pathology

Lesion/Dose/Sex	0 mg/kg/day	18.0 mg/kg/day	180.1 mg/kg/day	540.5 mg/kg/day
treated skin				
dark area	0/0♪	0/0	3/2	3/3
thickened	0/0	0/0	0/3	5/3
fissured	0/0	0/0	1/1	5/3
residue	0/0	0/0	4/5	5/3
sore	0/0	0/0	0/0	2/1
desquamated	0/0	0/0	1/0	1/1
eschar	0/0	0/0	0/0	0/1

♪ *male incidence/female incidence; n=5; data from Table 7, page 66 of the report*

Table 5. Skin Irritation Data

	MALES				FEMALES			
Lesion/Sex/Dose [mg/kg/day]	0	18.0	180.1	540.5	0	18.0	180.1	540.5
very slight erythema	0	1 [2-6]	1 [8-10, 12-13, 21] 2 [2, 7, 11, 20], 3 [1, 3-4, 18-19] 4 [5-6]	1-2 [1-4, 6-11] 3 [5]	1 [1-4]	1 [2-11, 21]	1-3 [1-21]	2-3 [1-3]
well-defined erythema	0	0	1 [2-6], 2 [14], 3-4 [7-13, 20-21] 5 [14-17]	2 [5], 3 [6-11, 14-21] 4 [12-13]	0	0	2-4 [3-21]	2 [1-3], 5 [4-6], 1 [7] 0 [8-16], 2 [17-21]
moderate-to-severe erythema	0	0	0	1 [9-13] 2 [14-21]	0	0	0	4-5 [7-14], 3 [15-16] 1 [7-21]
very slight edema	0	0	1 [6-7]	0	0	0	0	1 [1-3]
thickening	0	0	1-2 [6-7, 11, 16-19] 3 [8, 12-15], 4 [9-10]	2 [4] 5 [6-21]	0	0	1 [10-13] 1 [15-21]	1 [4], 2 [5], 3 [6, 15-21] 4 [7-8, 11-14], 5 [9-10]
epidermal scaling	0	0	1 [21], 2 [16-17], 3 [6, 15], 4 [7, 14] 5 [8-13]	1-3 [6-21]	0	0	1 [4, 19-20], 2 [6-7] 3-4 [8-13]	1 [10-14, 17-21], 2 [7-9]
fissuring	0	0	1 [7-9, 16-17, 21] 2 [9] , 3 [8, 10] 4 [11-14]	1-3 [6-10] 4-5 [11-21]	0	0	1-3 [7-9, 14-21] 4-5 [10-13]	1 [6], 2 [8], 3 [6, 9, 15-21] 4 [11-13], 5 [10]
fissuring with bleeding	0	0	0	1-2 [8-15]	0	0	0	1-2 [7-9]
necrosis	0	0	0	1 [5], 2 [6-8], 3 [9-19] 2 [20-21]	0	0	0	1 [2-3, 20-21], 2 [4-6, 19] 3 [15-18], 4 [7, 11-14] 5 [8-10]
sloughing	0	0	0	0	0	0	0	1 [15-21]

number of rabbits displaying lesion; [days lesion observed]; data from tables 2-3, pages 37-38 of the report

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 153 of 959

3. 21-Day Dermal Irritation and Dermal **Toxicity Study** in Rabbits with **the Dimethylamine Salt of 2,4-Dichlorophenoxyacetic Acid(MRID No. 417353-06).**

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a 21-day dermal toxicity study in rabbits with **2,4-D DMA.**

II. MATERIALS AND METHODS

1. Test Material

Chemical Name: **Dimethylamine salt of 2,4-Dichlorophenoxyacetic Acid**
Purity: 66.18% pure
Acid equivalent: 55.5%
Lot No.: 04FD31349
Description: Brown liquid
Vehicle: Distilled water
Lot No.: 1023913

2. Test Animals

Species: Rabbit
Strain: Hra:(NZW)SPF
Sex: Males and females
Age at Initiation: Adult
Weight at Initiation: males - 2.1 to 2.4 kg;
females - 2.2 to 2.5 kg
Identification: Individual numbered ear tags and cage cards.
Acclimation: Approximately two weeks
Health Status: Good
Housing: Individually in suspended wire mesh cages
Food:Certified High Fiber Rabbit Chow #5325ad libitum Water:
Water: Tap water ad libitum
Environment: Temperature- 61 to 76°F; Humidity- 24 to 66%
Light / dark cycles: 12 hr. light/dark cycle

3. **Study Design**

Group No.	No. of Animals Males	Females	Theoretical Dose Level (mg/kg/day)[a]	Actual Test Material (mg/kg/day)[b]
1 (control)	5	5	0.0	0
2 (Low-dose)	5	5	18.0	11.9
3 (Mid-dose)	5	5	180.1	119.2
4 (High-dose)	5	5	540.5	357.7

[a] Based on the results of a 21-day range-finding study, it was concluded that the doses for the full scale study would be a dose less than or equal to 18.0 mg/kg/day as the low dose, with an additional dose of 54.1 (mid-dose) and a high dose of 180.2 mg/kg/day. However, the mid- and high doses employed in the full scale study were higher than what was determined in the range finding study and no exaplanation was given for this divergence.

[b] Actual dosage levels are based on the purity of the test material (66.18%).

4. **Test Material Formulation**

The test material/vehicle suspension was prepared fresh weekly. The test material was shaken well (approximately 1 hour) prior to mixing to ensure homogeneity. The required amount of the compound was weighed, sufficient of distilled water was added, and the suspension was stirred on a magnetic stirrer for 3-4 minutes.

5. **Treatment**

Approximately 24 hours prior to dosing, the fur was clipped from dorsally and laterally from shoulder to rump to cover an area of approximately 10 % of the total body surface. The appropriate volume (dosing volume 1 mL/kg) of test material suspension was applied uniformly over the exposed area and covered with a 4 x 4 gauze patch and that was secured with a Coban® wrap, which was secured with an nonirritating adhesive tape. The animals were exposed for a period of 6 hours. To prevent possible ingestion of the test material, during the course of the 6 hour exposure period all animals wore a flexible plastic restraint collars. At the end of the exposure period, the dressings and collars were removed and the treated area was gently wiped (but not washed) with dry gauze to remove any remaining test material. Rabbits were treated for 6 hours/day, 7 days/week for 21 days.

6. Experimental Procedures

Parameter	Time measured
Mortality and Moribundity	Twice daily
Dermal irritation	Daily prior to treatment
Clinical signs	Days 0, 7, 14, and 21.
Body weight	Prior to initiation and on Days 7, 14, and 21.
Food consumption	Days 2,4,6,7,9,11,13,14,16, 18,20, and 21.
Hematology & Clinical Chemistry	Prior to initiation and at termination
Urinalysis	Termination

Hematology

Hematocrit (HCT)	Leukocyte count (WBC)
Hemoglobin (HGB)	Platelet count
Erythrocyte count (RBC)	Leukocyte differential
Mean cell volume (MCV)	Cell morphology
Mean cell hemoglobin (MCH)	Corrected leukocyte count
Mean cell hemoglobin concentration (MCHC)	

Clinical Chemistry

Calcium	Albumin
Inorganic phosphorus	Globulin
Chloride	Total protein
Sodium	Creatinine
Potassium	Total bilirubin
Glucose	Triglycerides
Alanine aminotransferase (ALT)	Total cholesterol
Aspartate aminotransferase (AST)	Alkaline phosphatase
Blood urea nitrogen	

Urinalysis

Appearance	Bilirubin
Specific gravity	Occult blood
pH	Urobilinogen
Protein	Glucose
Ketone	Microscopic examination of sediment

7. **Termination**

At termination, animals were weighed, anesthetized with sodium pentobarbital, and sacrificed by exsanguination and were subjected to a complete necropsy. At necropsy the kidneys, thyroid/parathyroid, testes with epididymides, and liver with drained gall bladder were weighed and organ-to-body weight ratios were calculated. Skin (treated and untreated), liver, kidneys, eyes, and target organs (those organs showing gross pathological changes) from all animals were fixed in 10% neutral-buffered formalin.

8. **Histopathology**

The tissues listed above from all animals were trimmed and processed for histopathological evaluation.

9. **Statistical Analyses**

Appropriate statistical methods were employed for the analyses of body weights, body weight changes, food consumption, clinical pathology parameters and absolute and relative organ weight values.

10. **Quality Assurance**

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the standard Operating Procedures of Hazleton Laboratories America, and the study Protocol.

III. RESULTS

1. Analytical Chemistry Data

As shown in Table 1 results of anlayses for stability (Day 0,3, 7, and 14), and routine analysis of Week 1 indicated adequate stability under the considtions of use.

2. Survival

Except for the two female rabbits at the high-dose that were sacrificed moribund on days 10 and 14, respectively, all animals survived until study termination.

3. Clinical Signs

The female rabbit at the high dose (Animal No. E50096) that was sacrificed moribund on day 10 was thin and languid in appearance on days 8 and 9 and prostrate on day 10. Another female rabbit at this dose (E50100) experienced immobility of the hindlegs due to injury/trauma to the lower back; necropsy of this animal confirmed lumbar spinal column fracture. Other clinical signs observed in control and treated animals included thin appearance, lacrimation, reddish mucus-like substance around hind legs, and diarrhea; none were considered to be treatment-related.

4. Dermal Observations

The incidences of dermal irritation are presented in Tables 3 and 4 for males and females, respectively. In the control group, one female exhibited very slight erythema on Days 1-4. In the low dose (18 mg/kg/day), very slight erythema was seen in one male from Days 2-6, and in one female from Days 2-11 and again on Day 21.

At the mid dose (180.1 mg/kg/day), very slight to well-defined erythema was seen on most males and females starting on Days 6 and 7. Other dermal effects observed in one or more males included thickening (Days 6-19), fissuring (Days 7-21), epidermal scaling (Days 6-17 and 21), and compound-like material at the treatment site (Days 4-21). Dermal observations in one or more females were thickening (Days 10-13) and (Days 15-21), epidermal scaling during Days (4-20), fissuring (Days 7-21), atonia (Days 7-11 and 17), and compound-like material at the treatment site during Days 3-21.

At the high dose (540.5 mg/kg/day), very slight to well-defined erythema was seen in all males and ~~during Days~~ 1-21 and the erythema increased to moderate to severe in one or two males from Day 9-21. Other dermal effects seen in one or more males included: thickening (Days 5-21), epidermal scaling (Days 6-21), fissuring (Days 6-21), fissuring with bleeding (Days 8-15), necrosis (Days 5-21), and compound-like material at the application site (Days 1-21). Among females, very slight to well-defined erythema was seen in all animals (Days 1-7) and again in a few animals from Days 17-21. Moderate to severe erythema was seen in a number of females from Days 7-21. Except for the very slight edema seen in one female during Days 1-3, no other edema was seen.Other dermal effects seen in one or more females were: thickening (Days 4-21), epidermal scaling (Days 7-14 and 17-21), fissuring (Days 6-21), fissuring with bleeding (Days 7-9), necrosis (Days 1-21), sloughing (Days 15-21), and compound-like material at the application site (Days 1-21).

5. **Body Weight**

No statistically significant differences were seen in mean body weight changes among treatment animals when compared to controls. The weight loss seen during the first few weeks of the study was attributed to the acclimation of the rabbits to wearing plastic collars.

6. **Food Consumption**

Food consumption was generally comparable between the treated and control animals.

7. **Hematology, Clinical Chemistry, and Urinalysis**

No treatment-related changes were seen in hematology, clinical chemistry, and urinalyses parameters.

8. **Gross Pathology**

Treatment-related gross pathological findings observed primarily in the treated skin of male and female rabbits included dark areas, desquamation, thickening, sores, eschar, compound residue, and/or fissuring. Gross pathological changes were also observed in the kidneys, stomach, bone, testes, urinary bladder and/or muscle; these findings were sporadic and not considered to be treatment related.

9. **Organ Weight**

A statistically significant ($p \leq 0.05$) increase was seen in the mean absolute kidney weights of males at 180.1 mg/kg/day group (17.6 g vs 14.8 g in controls); this increase was not considered to be treatment related since no significant changes in relative kidney weight and no corroborative histopathological changes were seen in the kidneys.

10. **Histopathology**

Histopathological lesions of the treated and untreated skins are summarized below. Treatment-related histopathological changes were limited to the treated skin of the mid and high-dose group animals. The treated skin sections of the low-dose were comparable to that of the controls.

No. of Animals with **Lesions in the Treated Skin**								
Sex	Males				Females			
Dose(mg/kg/day)	0.0	18.0	180.1	540.5	0.0	18.0	180.1	540.5
Acanthosis	0	0	5	5	0	0	5	4
Hyperkeratosis	0	0	5	5	0	0	5	4
Edema	0	0	3	2	0	0	1	4
Superficial crusting	0	0	1	5	0	0	1	4
Ulcer	0	0	2	0	0	0	0	0
Inflammation, cronic active	0	1	5	5	1	1	5	4

No. of Animals with **Lesions in the Untreated Skin**								
Sex	Males				Females			
Dose (mg/kg/day)	0.0	18.0	180.1	540.5	0.0	18.0	180.1	540.5
Acanthosis	0	0	1	0	0	0	0	1
Hyperkeratosis	0	0	1	0	0	0	0	1
Superficial crusting	0	0	0	0	0	0	0	1
Inflammation, subacute	2	1	1	2	5	2	1	2
Inflammation, Chronic active	0	0	0	0	0	0	0	1

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160

IV. DISCUSSION

Repeated dermal application of Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid to male and female rabbits at doses 0, 18, 180.1, or 540.5 mg/kg, 6 hours/day, 7 days/week for 21 days did not result in severe systemic toxicity. Treatment-related dermal irritation consisted of various degrees of erythema and edema; the incidence and severity being dose dependent and generally greater in females than males. Additional dermal changes, also dose dependent, included skin thickening, epidermal scaling, fissuring (some with bleeding), necrosis, sloughing, compound-like material at treatment site, and atonia. Remarkable dermal changes were seen at 180.1 and 540.4 mg/kg/day dose groups, while only occasional, very slight erythema was seen at 18 mg/kg/day group; the latter finding was not considered to be toxicologically significant. No treatment-related effects were seen in survival, clinical signs, body weight, body weight gain, food consumption, or clinical pathology. The statistically significant increase in absolute kidney weight of male rabbits at 180.1 mg/kg/day group was not considered to be biologically significant since no changes were observed in relative kidney weight or in histopathology. Histopathological changes observed in treated skin at 180.1 and 540.5 mg/kg/day groups included acanthosis, hyperkeratosis, edema, superficial crusting (inspissated serum, necrotic cells and debris on epidermal surface) and chronic active inflammation. The treated skin lesions of the low-dose animals were comparable to controls.

V. CONCLUSION

Based on the results of this study, the no-observable-effect level (NOEL) was 18 mg/kg/day for dermal irritation and 540.5 mg/kg/day for systemic toxicity. The LOEL for dermal irritation was 180.1 mg/kg/day.

VI. CORE CLASSIFICATION

Guideline; this study satisfies the requirements for a 21-day dermal toxicity study (82-3) in rabbits.

Table 1. Analysis of Dosing Solution.

Group	Dosage Level (mg/kg)		Corrected[a] Assayed Value (mg/ml)	Corrected[a] Percent Target %
			Stability - Day 0	
1	0		ND	
2	18.0	A	11.44	96.1
		B	11.27	94.6
4	540.5	A	335.2	93.7
		B	329.6	92.2
			Stability - Day 3	
1	0		ND	-
2	18.0	A	16.18	89.9
		B	15.76	87.6
4	540.5	A	474.1	87.7
		B	491.9	91.0
			Stability - Day 7	
1	0		ND	-
2	18.0	A	17.12	95.1
		B	16.71	92.8
4	540.5	A	505.3	93.5
		B	491.8	91.0
			Stability - Day 14	
1	0		ND	-
2	18.0	A	15.56	86.4
		B	15.74	87.4
4	540.5	A	467.1	86.4
		B	459.7	85.1
			Routine Analysis - Week 1	
1	0		ND	-
2	18.0	A	17.48	97.1
		B	17.48	97.1
3	180.1	A	172.8	95.9
		B	169.8	94.3
4	540.5	A	509.6	94.3
		B	500.7	92.6

[a] The formulation was not adjusted for the given percent purity (66.18%); therefore, this value was used to determine the corrected assayed value.

- = Not applicable
ND = None detected

Table 2. Dermal Irritation Scores In Male Rabbits Following Repeated Dermal Application of Dimethylamine Salt of 2,4-Dichlorophenoxyacetic acid.

SEX: MALES — SUMMARY INCIDENCE OF DERMAL IRRITATION SCORES

	DAY	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NUMBER OF ANIMALS EXAMINED																							
GROUP 1 0 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 2 16.0 MG/KG/DAY		5	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 3 180.1 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 4 540.5 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
SKIN/FUR																							
NO ERYTHEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	4	4	4	4	4	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5
	3	5	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	5	4	4	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VERY SLIGHT ERYTHEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	3	2	3	3	4	4	2	1	1	1	2	1	1	0	0	0	0	3	3	2	1
	4	0	1	1	2	2	3	2	2	2	1	1	1	0	0	0	0	0	0	0	0	0	0
WELL DEFINED ERYTHEMA = 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	1	1	1	1	1	3	4	4	4	3	4	4	5	5	5	5	2	2	3	4
	4	0	0	0	0	0	2	3	3	3	3	3	3	4	4	3	3	3	3	3	3	3	3
MODERATE TO SEVERE ERYTHEMA = 3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2	2	2	2	2	2	2	2
NO EDEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
VERY SLIGHT EDEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
THICKENING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	1	2	3	4	4	2	3	3	3	3	2	1	2	1	0	0
	4	0	0	0	0	0	2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
EPIDERMAL SCALING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	3	4	5	5	5	5	5	5	4	3	2	2	0	0	0	1
	4	0	0	0	0	0	0	3	3	3	2	2	2	2	2	2	1	1	1	2	2	2	1
FISSURING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	1	1	1	4	4	4	4	4	2	1	1	2	2	2	1
	4	0	0	0	0	0	0	1	1	3	2	3	4	4	4	4	4	5	5	5	5	5	5
FISSURING WITH BLEEDING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	1	2	2	1	1	1	1	1	0	0	0	0	0	0
NECROSIS	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	1	2	2	2	3	3	3	3	3	3	3	3	3	3	3	2	2
COMPOUND-LIKE MATERIAL ON APPLICATION SITE	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	2	3	5	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	4	0	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 3. Dermal Irritation Scores In Female Rabbits Following Repeated Dermal Application of Dimethylamine Salt of 2,4-Dichlorophenoxyacetic acid.

SEX: FEMALES — SUMMARY INCIDENCE OF DERMAL IRRITATION SCORES

	DAY	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NUMBER OF ANIMALS EXAMINED																							
GROUP 1 0 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 2 18.0 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 3 180.1 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	3	5	5	5	5	5	5	5
GROUP 4 540.5 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	3	3	3	3	3	3	3
SKIN/FUR																							
NO ERYTHEMA = 0	1	5	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	4	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	4
	3	5	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VERY SLIGHT ERYTHEMA = 1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1
	3	0	3	3	2	2	2	2	1	1	2	2	1	1	1	1	2	2	3	3	3	2	2
	4	0	2	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WELL DEFINED ERYTHEMA = 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	2	3	3	3	4	4	3	3	4	4	4	2	3	3	2	2	2	3	3
	4	0	2	2	2	5	5	5	1	0	0	0	0	0	0	0	0	0	2	2	2	2	2
MODERATE TO SEVERE ERYTHEMA = 3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	4	5	5	5	4	4	4	4	3	3	1	1	1	1	1
NO EDEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	3	5	5	5	5	5	5	5
	4	5	4	4	4	5	5	5	5	5	5	5	4	4	4	4	3	3	3	3	3	3	3
VERY SLIGHT EDEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
THICKENING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1
	4	0	0	0	0	1	2	3	4	4	5	5	4	4	4	4	3	3	3	3	3	3	3
EPIDERMAL SCALING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	1	2	2	2	3	3	4	4	4	4	2	3	3	3	2	1	1	0
	4	0	0	0	0	0	0	0	2	2	2	1	1	1	1	1	0	0	1	1	1	1	1
FISSURING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	1	1	2	4	4	4	5	3	3	3	3	2	1	1	1
	4	0	0	0	0	0	0	1	3	2	3	5	4	4	4	4	3	3	3	3	3	3	3
FISSURING WITH BLEEDING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0
ATONIA	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	1	1	1	2	2	0	0	0	0	0	1	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NECROSIS	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	1	1	1	2	2	2	4	5	5	5	4	4	4	4	3	3	3	3	2	1	1
SLOUGHING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1
COMPOUND-LIKE MATERIAL ON APPLICATION SITE	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	5	5	5	5	5	5	5	5	5	5	5	3	5	5	5	5	5	5	5
	4	0	3	4	5	5	5	5	5	5	5	5	4	4	4	4	3	3	3	3	3	3	3

164

3. **21-Day** Dermal Irritation and **Dermal Range Finding Study** in Rabbits with **Dimethylamine Salt of 2,4-Dichlorophenoxy-acetic acid (MRID No.417353-03).**

I. INTRODUCTION

A **range-finding study** was conducted **with 2,4-D DMA** in rabbits to select dose levels for a full scale 21-day dermal irritation study.

II. MATERIALS & METHODS

Test Material:	**2,4-D DMA** (lot No.04FD31349; Purity: 66.18%; acid equivalent-55.5%).
Test Animals:	Male and female Hra:(NZW)SPF rabbits.
Groups Size:	1 male and 1 female.
Dose Levels:	18, 54.1, 180.2, 540.5, and 1801.8 mg/kg/day
Dose Volume:	0.02, 0.04, 0.15, 0.44 and 1.50 mL/kg
Treatment:	Test material (liquid) was applied (neat) topically on the dorsal shaved area of each animal and the test site was covered with gauze/binder/wrap.
Exposure Time:	6 hours/day, 7 days/week for 21-days
Observations:	Mortality, moribundity, clinical signs, body weights (Days 0, 7, 14, and 21), and gross pathology.

III. RESULTS

No severe treatment-related effects were seen in rabbits receiving 2,4-D DMA at doses 18, 44.1, 180.2, or 540.5 mg/kg/day. The two high-dose (1801.8 mg/kg/day) animals found prostrate and exhibiting tremors were sacrificed on Day 2 of the study. No gross pathological changes were seen at necropsy. Dermal irritation and skin changes occurred in all treatment groups in a dose-related manner and consisted of various degrees of erythema and edema. Additional dermal changes included skin thickening, epidermal scaling, atonia, fissuring (some with bleeding), sloughing, necrosis, necrotic raised areas and brown staining of the application area.

IV. CONCLUSION

Based on these findings, the dose levels selected for the full scale 21-day dermal toxicity study with **2,4-D DMA** were: 18, 54.1, and 180.2 mg/kg/day.

V. CORE CLASSIFICATION

Not applicable; range finding study to determine dose levels for a full scale study.

Amendment to DER for MRID 41735305/41735302 - 21-Day Dermal Toxicity Study [Rabbit] [2,4-Dichlorophenoxyacetic acid-2-ethylhexyl ester]

This amendment provides an EXECUTIVE SUMMARY to upgrade the original DER [Document No. 008462].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 3/27/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P. H. Virginia A Dobozy 3/16/01
Reregistration Branch I, Health Effects Division (7509C)

TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 21-day dermal toxicity - rabbit § 82-2/OPPTS 870.3200
P.C. CODE: 0300063
CASWELL NO.: 315AS CAS #: 1928-43-4
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid-2-ethylhexyl ester [98%; acid equivalent 62.8%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force II on 2,4-D Research Data

CITATION: Schulze, G. E. (1990). 21-Day Dermal Irritation and Dermal Toxicity Study in Rabbits with 2,4-Dichlorophenoxyacetic Acid-2-EthylhexylEster. Hazleton Laboratories America, Inc.; Study No. HLA 2184-110, July 30, 1990. MRID 41735305. Document No. 008462. Unpublished. Range Finding Study MRID41735302.

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study [MRID 41735305], 5 Hra: (NZW) SPF rabbits/sex/group were administered the 2,4-dichlorophenoxyacetic acid-2-ethylhexyl ester [98%; 62.8% acid equivalent] *via* dermal application [neat material] for 6 hours/day, 7 days/week for 21 days at concentrations of 0, 16.3, 162.8, and 1627.5 mg/kg/day.

Treatment had no adverse effect on survival, clinical signs, hematology, clinical chemistry, urinalysis, gross pathology, or histopathology. Decreased and/or negative body-weight gains were observed in both sexes at the mid- and high-dose levels and in males at the low-dose level compared to controls [overall, days 0-21], but there was no dose response. During the first week of exposure, all rabbits in all groups [both sexes], including controls, displayed negative body-weight gains [no dose response], with the exception of two low-dose females. During the last week of exposure, all treatment groups [both sexes] displayed lower or negative body-weight gains compared to the controls, but again there was no dose response and the differences in body-weight gain are not considered toxicologically significance. At the high-dose level, increased kidney weights [absolute and relative] were observed in both sexes, but statistical significance was attained only for relative weight in the males, and there were no corroborating changes in clinical chemistry parameters or histopathology findings. Histopathological changes observed in the skin included acanthosis, hyperkeratosis, and necrotic cellular debris on the epidermal surface, and the incidence and severity of these lesions were increased in both sexes at the mid- and high-dose levels.

The NOAEL for dermal irritation is 16.3 mg/kg/day and the LOAEL for dermal irritation is 162.5 mg/kg/day, based on histopathologicallesions of the skin. The systemic NOAEL is 1627.5 mg/kg/day, the highest dose tested.

This 21-day dermal toxicity study is classified ACCEPTABLE/Guidelineand satisfies the guideline requirement [§82-2; 870.3200] for a 21-day dermal toxicity study.

2. 21-Day Dermal Irritation and Dermal **Toxicity Study** in Rabbits with **2,4-Dichlorophenoxyacetic Acid-2-ethylhexyl Ester (MRID No. 417353-05).**

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a 21-day dermal toxicity study in rabbits with **2,4-D acid-2-ethylhexyl ester (2,4-D EH Ester).**

II. MATERIALS AND METHODS

1. Test Material

Chemical Name: **2,4-Dichlorophenoxyacetic Acid-2-ethylhexyl Ester**
Purity: 98% pure
Acid equivalent: 62.8%
Lot No.: 04KF54479
Description: Pale yellow liquid

2. Test Animals

Species: Rabbit
Strain: Hra:(NZW)SPF
Sex: Males and females
Age at Initiation: Adult
Weight at Initiation:males - 2.4 to 2.7 kg;
females - 2.1 to 2.6 kg
Identification: Individual numbered ear tags and cage cards.
Acclimation: Approximately three weeks
Health Status: Good
Housing: Individually in suspended wire mesh cages
Food:Certified High Fiber Rabbit Chow #5325 ad libitum
Water: Tap water ad libitum
Environment: Temperature- 64 to 76F; Humidity- 34 to 79%
Light / dark cycles: 12 hr. light/dark cycle

3. Study Design

Group No.	No. of Animals Males	Females	Test Material (mg/kg/day)[a]	Dose Volume (ml/kg/day)
1 (control)	5	5	0.0	0.00
2 (Low-dose)	5	5	16.3	0.01
3 (Mid-dose)	5	5	162.8	0.14
4 (High-dose)	5	5	1627.5	1.40

[a] Based on results of a 21-day pilot range-finding study.

167

4. Test Material Formulation

The test material was administered neat on a mg/kg/day basis. The appropriate volume of test material was applied to the shave area of the animals. Since test material was applied neat analyses were not required.

5. Treatment

Approximately 18 hours prior to dosing, the fur was clipped from dorsally and laterally from shoulder to rump to cover an area of approximately 10 % of the total body surface. The appropriate volume of the test material was applied to the shaved area and covered with a 4 x 4" gauze patch and a Coban® wrap with a 4 x 4" plastic wrap secured in place with 1/2" two-sided sticky tape. To prevent possible ingestion of the test material, during the course of the 6 hour exposure period all animals wore a flexible plastic restraint collars. At the end of the exposure period, the dressings and collars were removed and the treated area was wiped with dry gauze to remove any remaining test material. Rabbits were treated for 6 hours/day, 7 days/week for 21 days.

6. Experimental Procedures

Parameter	Time measured
Mortality and Moribundity	Twice daily
Dermal irritation	Daily prior to treatment
Clinical signs	Days 0, 7, 14, and 21.
Body weight	Prior to initiation and on Days 7, 14, and 21.
Food consumption	Days 2,4,6,7,9,11,13,14,16, 18,20, and 21.
Hematology & Clinical Chemistry	Prior to initiation and at termination
Urinalysis	Termination

Hematology

Hematocrit (HCT)	Leukocyte count (WBC)
Hemoglobin (HGB)	Platelet count
Erythrocyte count (RBC)	Leukocyte differential
Mean cell volume (MCV)	Cell morphology
Mean cell hemoglobin (MCH)	Corrected leukocyte count
Mean cell hemoglobin concentration (MCHC)	

Clinical Chemistry

Calcium	Albumin
Inorganic phosphorus	Globulin
Chloride	Total protein
Sodium	Creatinine
Potassium	Total bilirubin
Glucose	Triglycerides
Alanine aminotransferase (ALT)	Total cholesterol
Aspartate aminotransferase (AST)	Alkaline phosphatase
Blood urea nitrogen	

Urinalysis

Appearance	Bilirubin
Specific gravity	Occult blood
pH	Urobilinogen
Protein	Glucose
Ketone	Microscopic examination of sediment

7. Termination

At termination, animals were weighed, anesthetized with sodium pentobarbital, and sacrificed by exsanguination and were subjected to a complete necropsy. At necropsy the kidneys, thyroid/parathyroid, testes with epididymides, and liver with drained gall bladder were weighed and organ-to-body weight ratios were calculated. Skin (treated and untreated), liver, kidneys, eyes, and target organs (those organs showing gross pathological changes) from all animals were fixed in 10% neutral-buffered formalin.

8. Histopathology

The tissues listed above from all animals were trimmed and processed for histopathological evaluation.

9. Statistical Analyses

Appropriate statistical methods were employed for the analyses of body weights, body weight changes, food consumption, clinical pathology parameters and absolute and relative organ weight values.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Laboratories America, and the study protocol.

170

III. RESULTS

1. Survival

No mortality occurred during the study.

2. Clinical Signs

No treatment-related clinical signs of toxicity were observed.

3. Dermal Observations

The dermal irritation scores are summarized in Tables 1 and 2 for males and females, respectively. Very slight erythema, epidermal scaling and, atonia were observed at the low-dose (16.3 mg/kg/day) while notable dermal irritation was observed at the mid-(162.8 mg/kg/day) and high-(1627.5 mg/kg/day) dose groups only. In general, edema was limited to the high-dose (1627.5 mg/kg/day) and its incidence and severity was greater for males than females. Edema was usually graded as very slight in severity (except for one male at the high dose where moderate edema was seen on Days 16 and 21). Additional dermal changes included skin thickening, epidermal scaling, fissuring (some with bleeding at the high dose) and atonia.

4. Body Weight

A reduction in mean body weight gain was observed in males at the low and mid dose from Days 0 to 21; the decrease reached statistical significance ($p < 0.01$) only at the mid dose. Males at all dose levels showed decreases in mean body weight gain from Days 14 to 21; however, the change was not statistically significant when compared to controls. Significantly ($p < 0.05$ or 0.01) lower mean body weight gain was observed in females at all dose levels from Days 14 to 21.

5. Food Consumption

Food consumption was generally comparable for all treated and control groups during the study.

Changes in Mean Body Weight (g) Changes in Rabbitss

Sex	Days	0 mg/kg/day	16.3 mg/kg/day	162.8 mg/kg/day	1627.5 mg/kg/day
Males	0 to 21	111.0 ± 103.8	-11.0 ± 64.3	-78.6** ±91.2	4.6 ± 72.9
	14 to 21	23.8 ± 51.3	-14.8 ± 41.2	-73.8 ± 52.3	-8.6 ± 60.7
Females	0 to 21	83.0 ± 156.9	92.0 ± 157.9	-2.6 ± 150.4	18.8 ± 69.1
	14 to 21	86.2 ± 32.6	-4.2* ±61.9	-37.6** ± 52.3	9.0* ± 32.0

* = $p \leq 0.05$; ** $p \leq 0.01$.

6. **Hematology, Clinical Chemistry, and Urinalysis**

No biologically significant changes were seen in mean hematology, clinical chemistry, or urinalysis in treated groups when compared to appropriate corresponding control group values. The statistically significant ($p < 0.05$) differences seen in a few hematology and clinical chemistry data considered to be spurious and/or incidental are listed below:

- Increase in mean cell hemoglobin concentration in males at 162.8 mg/kg/day group at Week 4.
- Decrease in creatinine level in females at all treated groups at Week 4; however, there was a slight increase in the mean value of the control group.

7. **Gross Pathology**

Gross pathology findings at the time of necropsy were noted primarily in the treated skin (dark, desquamated, edematous, and/or fissured) of the treated males and females.

8. **Organ Weight**

Mean absolute and relative kidney weights values were somewhat higher in males and females at the high dose; the difference, however, reached statistical significance ($p < 0.05$) only for relative weight in males. Other organ weight data were comparable between the treated and control groups.

9. Histopathology

Treatment-related histopathologic lesions of selected tissues was limited to treated skin; no compound related lesions were seen in the liver and kidneys. Lesions observed in the **treated and untreated skin** are summarized below:

No. of Animals with **Lesions in the Treated Skin**								
Sex	Males				Females			
Dose (mg/kg/day)	0	16.3	162.8	1627.5	0	16.3	162.8	1627.5
Lesion								
Acanthosis	0	0	3	5	0	0	3	4
Hyperkeratosis	0	0	2	4	0	1	3	5
Necrotic cellular debris on epidermal surface	0	0	1	5	0	0	1	2
Subepidermal edema	0	0	1	0	0	0	0	1
Inflammation, Chronic	1	1	3	5	2	1	3	5
Hemorrhage	0	0	2	0	0	0	0	2

No. of Animals with **Lesions in the Untreated Skin**								
Sex	Males				Females			
Dose (mg/kg/day)	0	16.3	162.8	1627.5	0	16.3	162.8	1627.5
Lesion								
Acanthosis	0	3	0	1	0	0	0	2
Inflammation, chronic	0	2	0	1	1	2	2	2
Subepidermal edema	0	0	1	0	0	0	0	0
Hemorrhage	0	2	0	0	1	0	0	0

IV. DISCUSSION

Repeated dermal application 2,4-Dichlorophenoxyacetic acid-2-ethylhexyl ester to male and female rabbits at doses 0, 16.3, 162.8, 1627.5 mg/kg, 6 hours/day, 7 days/week for 21 days did not result in severe systemic toxicity. 2,4-D-2-ethylhexyl ester induced dermal irritating consisted of various degrees of erythema and edema whose incidence and severity were dose dependent. Additional dermal changes included skin thickening, epidermal scaling, fissuring (some with bleeding), and atonia. No treatment-related effects were seen in survival, clinical signs, food consumption or clinical pathology. Statistically significant depressions of body weight change were seen in treated females and nonsignificant depressions were seen in treated males during study week 3. A significant increase in mean relative kidney weights was observed for males at 1627.5 mg/kg/day group; however, the biological significance of this increase is not clear due to a lack of corroborating changes in clinical chemistry parameter or histopathological changes in the kidneys. Histopathologic changes observed in the treated skin included acanthosis, hyperkeratosis, and necrotic cellular debris on the epidermal surface; the incidence and severity of these lesions were increased in both males and females at the 162.8 and 1627.5 mg/kg/day groups. No histopathological lesions were seen in either sex at the low dose (16.3 mg/kg/day).

V. CONCLUSION

Based on the results of this study, the no-observable-effect level (NOEL) was 16.3 mg/kg/day for dermal irritation and 1627.5 mg/kg/day for systemic toxicity. The lowest-observable-effect level (LOEL) for dermal irritation was 162.5 mg/kg/day.

VI. CORE CLASSIFICATION

Guideline; this study satisfies the requirements for a 21-day dermal toxicity study (82-3) in rabbits.

Table 1. Dermal Irritation Scores In Male Rabbits Following Repeated Dermal Application of 2,4-D-ethylhexyl Ester.

SUMMARY INCIDENCE OF DERMAL IRRITATION SCORES

	DAY :	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NUMBER OF ANIMALS EXAMINED																							
GROUP 1 0 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 2 16.3 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 3 162.8 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 4 1627.5 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
SKIN/FUR																							
NO ERYTHEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	5	4	3	3	3	3	3	3	2	3	3	3	2	1	2	0	0	0	0	0
	3	5	5	3	3	3	3	3	3	3	1	0	0	0	0	1	0	0	0	0	0	0	0
	4	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VERY SLIGHT ERYTHEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	1	2	2	2	2	2	2	3	2	2	2	3	4	3	5	5	5	5	5
	3	0	0	2	2	2	2	2	2	2	4	5	5	5	5	4	5	4	4	5	5	5	4
	4	0	0	5	5	0	1	1	0	0	1	1	3	2	2	5	3	2	2	5	4	5	4
WELL-DEFINED ERYTHEMA = 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1
	4	0	0	0	0	5	4	4	5	5	4	4	2	3	3	0	2	3	3	0	1	0	1
NO EDEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	4	5	5	5	5	3	3	3	5	5	5	5	5	5	5	4	4	3	1	2	2	2	3
VERY SLIGHT EDEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	2	2	2	0	0	0	0	0	0	0	1	1	1	3	2	2	3	1
SLIGHT EDEMA = 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
MODERATE EDEMA = 3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
THICKENING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	4	4	5	5	4	0	0	0	0	0	1	1	0	0	0	0	0
EPIDERMAL SCALING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	3	4
	4	0	0	0	0	0	0	0	0	0	0	1	3	3	3	1	2	0	0	0	0	0	0
FISSURING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	4	0	0	0	0	0	[illegible]	2	3	3	4	3	5	5	5	3	4	5	5	4	5	5	5
FISSURING WITH BLEEDING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	2	2	1	2	0	0	0	0	1	0	0	0	0	0	0
ATONIA	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	2	3	3	3	1	5	5	5	5	5	1	0
	4	0	0	0	0	0	0	0	4	4	4	5	4	4	4	5	5	5	5	5	5	5	5

Table 2. Dermal Irritation Scores In Female Rabbits Following Repeated Dermal Application of 2,4-D-ethylhexyl Ester.

SUMMARY INCIDENCE OF DERMAL IRRITATION SCORES

		DAY : 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NUMBER OF ANIMALS EXAMINED																							
GROUP 1 0 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 2 15.3 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 3 162.8 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 4 1627.5 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
SKIN/FUR																							
NO ERYTHEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	5	1	1	1	3	3	1	1	1	1	1	3	0	0	0	0	0	0	0	0
	3	5	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VERY SLIGHT ERYTHEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	4	4	4	2	2	4	4	4	4	4	2	5	5	5	5	5	5	5	5
	3	0	4	5	5	5	5	5	5	5	4	5	5	5	5	5	4	5	5	5	5	5	4
	4	0	5	4	2	0	0	0	0	1	5	5	5	5	5	5	5	5	5	5	5	5	5
WELL-DEFINED ERYTHEMA = 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	4	0	0	1	3	3	2	3	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0
MODERATE TO SEVERE ERYTHEMA = 3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	2	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SEVERE ERYTHEMA = 4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NO EDEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4
	4	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5
VERY SLIGHT EDEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
THICKENING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	5	5	5	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0
EPIDERMAL SCALING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	3	3	0	2	2	5
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3	5	5	5	5	2	4	5	5
	4	0	0	0	0	0	0	0	1	2	0	0	0	0	3	1	1	1	1	0	0	0	0
FISSURING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	4	0	0	0	0	0	1	2	3	3	5	5	5	5	2	5	5	5	5	5	5	5	5
FISSURING WITH BLEEDING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
ATONIA	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	3	4	4	2
	4	0	0	0	0	1	2	3	3	4	5	5	5	5	1	4	5	5	5	5	5	5	5

2. **21-Day** Dermal Irritation and **Dermal Range Finding Study** in Rabbits with **2,4-Dichlorphenoxyacetic Acid-2-Ethylhexyl Ester (MRID No.417353-02).**

I. INTRODUCTION:

A **range-finding study** was conducted **with 2,4-D EH Ester** in rabbits to select dose levels for a full scale 21-day dermal irritation study.

II. MATERIALS & METHODS

Test Material:	**2,4-D acid-2-ethylhexyl ester** (lot No.04KF54479; Purity: 98%; acid equivalent-62.8%).
Test Animals:	Male and female Hra:(NZW)SPF rabbits.
Groups Size:	1 male and 1 female.
Dose Levels:	16.3, 48.8, 162.8, 488.3, and 1627.5 mg/kg/day
Dose Volume:	0.01, 0.04, 0.14, 0.42, and 1.40 mL/kg
Treatment:	Test material (yellow liquid) was applied (neat) topically on the dorsal shaved area and the test site was covered with gauze/binder/wrap.
Exposure Time:	6 hours/day, 7 days/week for 21-days
Observations:	Mortality, moribundity, clinical signs, body weights (Days 0, 7, 14, and 21), and gross pathology.

III. RESULTS

One female rabbit at 48.8 mg/kg/day group was sacrificed moribund on Day 10 due to enteritis, a common condition seen in rabbits in laboratory housing. No treatment-related effects were seen in body weight gain, clinical signs or gross pathology at any dose level. No dermal irritation was observed at 16.3 mg/kg/day dose. Dose-related dermal irritation at 48.8, 162.8, 488.3 and 1627.5 mg/kg/day included erythema, slight edema, atonia, epidermal scaling, skin fissuring, and transient thickening. Generally, erythema scores were more severe during the first 3 days of the study and the incidence and severity of the scores appeared to be dose related. Epidermal scaling, atonia and skin fissuring were first seen at 1627.5 mg/kg/day group animals after 5 to 9 days.

IV. CONCLUSION

Based on these findings, the dose levels selected for the full scale 21-day dermal toxicity study with **2,4-D acid-2 EH Ester** were:16.3, 162,8, and 1627.5 mg/kg/day (limit dose).

V. CORE CLASSIFICATION

Not applicable; range finding study to determine dose levels for a full scale study.

Amendment to DER for MRID 41735304/41735301 - 21-Day Dermal Toxicity Study [Rabbit] [2,4-Dichlorophenoxyacetic acid]

This amendment provides an EXECUTIVE SUMMARY to upgrade the original DER [Document No. 008462].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 3/27/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P. H. Virginia A Dobozy 3/16/01
Reregistration Branch I, Health Effects Division (7509C)
TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 21-day dermal toxicity - rabbit § 82-2/OPPTS 870.3200
P.C. CODE: 030001
CASWELL NO.: 315 CAS #: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [96.1%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force II on 2,4-D Research Data

CITATION: Schulze, G. E. (1990). 21-Day Dermal Irritation and Dermal Toxicity Study in Rabbits with 2,4-DichlorophenoxyaceticAcid. Hazleton Laboratories America, Inc.; Study No. HLA 2184-109, October 16, 1990. MRID 41735304. Document No. 008462. Unpublished. Range-Finding Study MRID 41735301.

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study [MRID 41735304], 5 Hra: (NZW) SPF rabbits/sex/group were administered the 2,4-dichlorophenoxyacetic acid [96.1%] *via* dermal application [neat material (powder) spread evenly on 4" x 4" gauze pad and moistened with 0.5mL distilled water [6 hours/day, 7 days/week for 21 days at concentrations of 0 [distilled water], 10, 100, and 1000 mg/kg/day.

Treatment had no adverse effect on survival, clinical signs, mean body weight, hematology, clinical chemistry, urinalysis, gross pathology, or histopathology. At the high-dose level, increased kidney weights [absolute and relative] were observed in both sexes, but statistical significance was attained in the females only, and there were no corroborating changes in clinical chemistry parameters or histopathology in the kidneys. 2,4-D was only slightly irritating to the skin of the rabbits, inducing very slight erythema and epidermal scaling, with the females displaying a higher incidence than males.

The NOAEL for dermal and systemic toxicity is 1000 mg/kg/day, the highest dose tested.

This 21-day dermal toxicity study is classified ACCEPTABLE/Guideline and satisfies the guideline requirement [§82-2; 870.3200] for a 21-day dermal toxicity study.

1. 21-Day Dermal Irritation and Dermal **Toxicity Study** in Rabbits with **2,4-Dichlorophenoxyacetic Acid (MRID No. 417353-04).**

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a 21-day dermal toxicity study in rabbits with **2,4-D acid.**

II. MATERIALS AND METHODS

1. Test Material

Chemical Name: **2,4-Dichlorophenoxyacetic Acid**
Purity: 96.1% pure
Lot No.: 909
Description: Beige-colored powder

2. Test Animals

Species: Rabbit
Strain: Hra:(NZW)SPF
Sex: Males and females
Age at Initiation: Adult
Weight at Initiation: males - 2.1 to 2.6 kg;
females - 2.2 to 2.6 kg
Identification: Individual numbered ear tags and cage cards.
Acclimation: Approximately two weeks
Health Status: Good
Housing: Individually in suspended wire mesh cages
Food: Certified High Fiber Rabbit Chow #5325 ad libitum
Water: Tap water ad libitum
Environment: Temperature- 70 to 79°F; Humidity- 24 to 62%
Light / dark cycles: 12 hr. light/dark cycle

3. Study Design

Group No.	No. of Animals Males	Females	Actual Test Material (mg/kg/day)[a]
1 (vehicle control)	5	5	0[b]
2 (Low-dose)	5	5	10
3 (Mid-dose)	5	5	100
4 (High-dose)	5	5	1000

[a] Based on results of a 21-day pilot range-finding study.
[b] The vehicle control group received distilled water.

4. Test Material Formulation

The test material was administered neat on a mg/kg/day basis. The test material was weighed, and subsequently sprinkled evenly on a 4- x 4-inch gauze pad and moistened with 0.5 mL of distilled water to ensure good contact with the skin.

5. Treatment

Approximately 24 hours prior to dosing, the fur was clipped from dorsally and laterally from shoulder to rump to cover an area of approximately 10 % of the total body surface. The appropriate amount of the test material paste was applied uniformly over the exposure area; the control group received distilled water. The test material was held in contact with the skin for a 6-hour period each day by a gauze dressing that was secured with a Coban® wrap, and the wrap was secured with an nonirritating adhesive tape. To prevent possible ingestion of the test material, during the course of the 6 hour exposure period all animals wore a flexible plastic restraint collars. At the end of the exposure period, the dressings and collars were removed and the treated area was gently wiped (but not washed) with dry gauze to remove any remaining test material. Rabbits were treated for 6 hours/day, 7 days/week for 21 days.

6. Experimental Procedures

Parameter	Time measured
Mortality and Moribundity	Twice daily
Dermal irritation	Daily prior to treatment
Clinical signs	Days 0, 7, 14, and 21.
Body weight	Prior to initiation and on Days 7, 14, and 21.
Food consumption	Days 2,4,6,7,9,11,13,14,16, 18,20, and 21.
Hematology & Clinical Chemistry	Prior to initiation and at termination
Urinalysis	Termination

Hematology

Hematocrit (HCT)	Leukocyte count (WBC)
Hemoglobin (HGB)	Platelet count
Erythrocyte count (RBC)	Leukocyte differential
Mean cell volume (MCV)	Cell morphology
Mean cell hemoglobin (MCH)	Corrected leukocyte count
Mean cell hemoglobin concentration (MCHC)	

Clinical Chemistry

Calcium	Albumin
Inorganic phosphorus	Globulin
Chloride	Total protein
Sodium	Creatinine
Potassium	Total bilirubin
Glucose	Triglycerides
Alanine aminotransferase (ALT)	Total cholesterol
Aspartate aminotransferase (AST)	Alkaline phosphatase
Blood urea nitrogen	

Urinalysis

Appearance	Bilirubin
Specific gravity	Occult blood
pH	Urobilinogen
Protein	Glucose
Ketone	Microscopic examination of sediment

7. Termination

At termination, animals were weighed, anesthetized with sodium pentobarbital, and sacrificed by exsanguination and were subjected to a complete necropsy. At necropsy the kidneys, thyroid/parathyroid, testes with epididymides, and liver with drained gall bladder were weighed and organ-to-body weight ratios were calculated. Skin (treated and untreated), liver, kidneys, eyes, and target organs (those organs showing gross pathological changes) from all animals were fixed in 10% neutral-buffered formalin.

8. Histopathology

The tissues listed above from all animals were trimmed and processed for histopathological evaluation.

9. Statistical Analyses

Appropriate statistical methods were employed for the analyses of body weights, body weight changes, food consumption, clinical pathology parameters and absolute and relative organ weight values.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Laboratories America, and the study Protocol.

III. RESULTS

1. Survival

One male rabbit at the high-dose (1000 mg/kg/day) was found dead on Day 5; death was not attributed to treatment.

2. Clinical Signs

Except for pale/languid appearance and wheezing of the male that died, no treatment-related clinical signs of toxicity were observed during the study.

3. Dermal Observations

Vehicle controls: one female showed very slight erythema on Days 17 and 18, and another female had well-defined erythema on Day 16; no dermal irritation was seen in the males.

Low dose (10 mg/kg/day): one male had very slight erythema on Day 21, and 4 of 5 females developed very slight erythema during the dosing period. Females exhibited these dermal reactions during Days 6 to 12 and 19 to 21. Epidermal scaling was observed in one female on Days 6 and 7, and in two females on Day 12; no epidermal scaling was seen in males. One female had very slight edema on Day 21; no edema was seen among males.

Mid-dose (100 mg/kg/day): Among males, very slight erythema was seen beginning on Day 6: in 1 animal during Days 6 to 9 and on Day 15; in 2 animals on Days 10, 11, 14, 16, and from Days 18 to 20; and in 4 rabbits on Day 21. No erythema was seen on Days 12, 13, and 17. Among females, very slight erythema was seen beginning on Day 1; no erythema was seen on Days 2, 3, 13, 14, and 15. This lesion was observed in 2 rabbits on Days 1, during Days 6 to 12, and during Days 16 to 21; and in 3 animals on Days 4 and 5. No edema was seen in this group. One female had epidermal scaling during Days 7 to 10 and on Day 12; no epidermal scaling was seen in males.

High-dose (1000 mg/kg/day): Among males very slight erythema was seen beginning on Day 3: in 1 animal on Days 3,4, 6,7, 14, 15, 16, 21; in 2 animals on Day 8; in 3 rabbits during Days 10-13; and in 4 animals on Day 9. No erythema was seen in males on Days 5, 17, 18, 19, and 20. Among females, very slight erythema was seen beginning on Day 1. This reaction was seen: in 1 female on Days 13, 14, 20, and 21; in 2 on Days 1, 4, and 5; in 3 during Days 6 to 11; and in 4 on Day 12. Epidermal scaling was noted in one male during Days 5 to 12 and on Day 17, and in one female on during Days 5, 6, 7 and 12, and in 2 on Days 8,9, and 10. No edema was seen in this group.

4. **Body Weight**

There were no biologically significant differences in body weight changes for males or females between treated and control groups. The transient weight loses observed in both sexes at all dose levels including the controls during the first week of treatment were attributed to acclimation of animals to wearing plastic collars. Consequently the statistically significant ($p < 0.05$) lower mean body weight change observed from Days 0 to 7 in males at the low-dose (-143.2 g) compared to controls (-13.6 g) was considered to be not toxicologically significant. Mean body weight an body weight gain were comparable between the treated and control groups from Days 7 to 14 and Days 14 to 21.

5. **Food Consumption**

Food consumption was generally comparable for all treated and control groups during the study.

6. **Hematology, Clinical Chemistry, and Urinalysis**

No biologically significant changes were seen in mean hematology, clinical chemistry, or urinalysis in treated groups when compared to appropriate corresponding control group values. The statistically significant differences seen in a few hematology and clinical chemistry data listed below:

- Decreases in leukocyte count and corrected leukocyte count in females at 10 mg/kg/day.
- Decreased absolute segmented neutrophil count in females at all treated groups at the pretreatment interval.
- Increases in creatinine values in males at 10 and 100 mg/kg/day groups.
- Decreases in cholesterol and triglyceride in females at 100 mg/kg/day.
- Increases in calcium values in males at 10 and 1000 mg/kg/day.
- Increases in total protein in males at 100 mg/kg/day.

These differences were not considered to be treatment-related due to the low magnitude of the change, presence of similar differences at the pretreatment intervals, and/or lack of dose response.

7. **Gross Pathology**

Gross pathological changes observed at necropsy were dark and/or edematous/swollen areas in the treated skin. The incidences were as follows:

	No. of males affected				No.of females affected			
Dose (mg/kg/day)	0	10	100	1000	0	10	100	1000
Dark areas	0	1	3	1	0	1	1	0
Edematous/swollen	0	0	0	0	0	1	0	0

Other gross pathological changes included one male in the control with a ruptured globe prior to terminal sacrifice; 1 female at the high dose with a ruptured globe after the terminal sacrifice; 1 male at the high-dose with dark areas on the glandular mucosa of the stomach; and 1 female at the mid-dose with dark areas of the lung.

8. **Organ Weight**

The mean absolute and relative kidney weights values were higher for males and females at the high dose; the difference, however, reached statistical significant ($p < 0.05$) only in the females. Other organ weight data were comparable between the treated and control groups.

9. **Histopathology**

There were no treatment-related histopathological changes were seen at any dose level. A similar frequency and severity of commonly seen spontaneous changes were seen in control and treated rabbits of both sexes.

IV. DISCUSSION

Repeated dermal application 2,4-Dichlorophenoxyacetic acid to male and female rabbits at doses 0, 10, 100, or 1000 mg/kg, 6 hours/day, 7 days/week for 21 days did not result in severe systemic toxicity. 2,4-D acid was mildly irritating to the skin of rabbits inducing very slight erythema and epidermal scaling; in general, females were noted to have a higher incidence of dermal findings than males. No treatment-related effects were seen in survival, clinical signs, body weight gain, clinical, gross, or histopathology. A significant increase in mean absolute and relative kidney weights was observed for females at 1000 mg/kg/day group; however, the biological significance of this increase is not clear due to a lack of corroborating changes in clinical chemistry parameter or histopathological changes in the kidneys.

V. CONCLUSION

Based on the results of this study, the no-observable-effect level (NOEL) for dermal irritation and systemic toxicity was 1000 mg/kg/day, the highest dose tested.

VI. CORE CLASSIFICATION

Guideline; this study satisfies the requirements for a 21-day dermal toxicity study (82-3) in rabbits.

1. **21-Day** Dermal Irritation and **Dermal Range Finding Study** in Rabbits with **2,4-Dichlorophenoxyacetic acid (MRID No.417353-01).**

I. INTRODUCTION

A **range-finding study** was conducted **with 2,4-D acid** in rabbits to select dose levels for a full scale 21-day dermal irritation study.

II. MATERIALS & METHODS

Test Material:	**2,4-D acid** (lot No.909; Purity: 96.1%).
Test Animals:	Male and female Hra:(NZW)SPF rabbits.
Groups Size:	1 male and 1 female.
Dose Levels:	10, 30, 100, 300, and 1000 mg/kg/day
Treatment:	Test material (beige powder) was applied topically on the dorsal shaved area (approximately 10% of the total body surface) of each animal and 0.5 ml of distilled water was dispersed over the compound. The test site was covered with gauze/binder/wrap.
Exposure Time:	6 hours/day, 7 days/week for 21-days
Observations:	Mortality, moribundity, clinical signs, body weights (Days 0, 7, 14, and 21), and gross pathology.

III. RESULTS

No treatment-related effects were seen on survival, clinical signs, body weight, body weight gain, and gross pathology. Except for a slight erythema in one male rabbit at the 10 mg/kg/day on days 2 and 3, no compound-related dermal irritation was observed at the dosage levels tested.

IV. CONCLUSION

Based on these findings, the dose levels selected for the full scale 21-day dermal toxicity study with **2,4-D acid** were: 10, 100, and 1000 mg/kg/day (limit dose).

V. CORE CLASSIFICATION

Not applicable; range finding study to determine dose levels for a full scale study.

Amendment to DER for MRID 40061801 - Carcinogenicity Study [Mouse] [2,4-DICHLORO-PHENOXYACETIC ACID]

This amendment provides an EXECUTIVE SUMMARY and a data table to upgrade the original DER [Document No. 005794]. The study classification has been changed.

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 11/19/02
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Whang Phang, Ph.D. Whang Phang 11/21/02
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: carcinogenicity - mouse § 83-2/OPPTS 870.4200
P.C. CODE: 030001 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315 CAS NO.: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [97.5%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force for 2,4-D Research Data

CITATION: Serota, D. G. (1987). Oncogenicity Study in Mice With 2,4-Dichlorophenoxyacetic acid (2,4-D). Hazleton Laboratories America Inc.; Study No. 2184-101, not dated [Compliance Statement signed by Study Author, dated 1/15/87]. MRID 40061801. Document No. 005794. Unpublished.

EXECUTIVE SUMMARY: In a carcinogenicitystudy [MRID 40061801], 50 $B_6C_3F_1$ CRL BR mice/sex/group were administered 2,4-dichlorophenoxyacetic acid [97.5%] *via* the diet for 104 weeks at concentrations of 0, 1 mg/kg/day, 15 mg/kg/day, and 45 mg/kg/day. Additionally, 10 mice/sex/group were sacrificed at 52 weeks [interim sacrifice].

No treatment-related effect was observed on survival in either sex, and there were no treatment-related clinical signs of toxicity. Males at the high-dose level displayed a slight decrease [94% of control] in body weight at study termination, but body weight was comparable among the female groups. Body-weight gain during the first week of exposure was decreased at the high-dose level in both sexes [males 50%/females 12%]. Thereafter, the females displayed comparable body-weight gains among the groups. The high-dose males displayed decreased body-weight gains during the 0-13 week [91% of control] and 0-104 week [84% of control, $p<0.05$] intervals, and the low- [91% of control, $p<0.05$] and mid- [89% of control, 0.05] dose males displayed lower body-weight gains overall [weeks 0-104] also. There was no apparent adverse effect of treatment on food consumption in either sex.

Hematology parameters were comparable among the groups [both sexes]. There was no ophthalmoscopic assessment, and thyroid toxicity was not assessed in this study.

Increased kidney weights were observed in the high-dose females at both the 52-week and terminal sacrifices. Kidney weights of the high-dose males were slightly increased at the terminal sacrifice only. Adrenal weights were decreased at all dose levels in the males at the 52-week sacrifice, but there was no dose response. At study termination, the mid- and high-dose males displayed a significant increase in adrenal weight.

Gross pathology findings were comparable among the groups [both sexes]. Microscopically, there was an increased incidence of lesions in the kidney of males at the mid- and high-dose levels [histomorphologic alteration in the renal tubule epithelium, characterized as a cytoplasmic homogeneity due to the reduction of cytoplasmic vacuoles normally present] in unscheduled deaths and at both the interim and terminal sacrifices. This kidney lesion was not observed in females. No microscopic lesions were observed in the adrenal. There was no treatment-related increase in any tumor type in either sex.

The NOAEL is 1 mg/kg/day. The LOAEL of 15 mg/kg/day is based on treatment-related increase in kidney weights in both sexes and microscopic renal lesions in males. There was no treatment-related increase in the incidence of any tumor type.

This carcinogenicity study is classified Unacceptable because the high dose [45 mg/kg/day] is considered inadequate to assess the carcinogenic potential of 2,4-D. It was previously classified Core Minimum in the original DER; however, the HED Carcinogenicity Peer Review Committee [HED TXR No. 0050017] concluded that a repeat carcinogenicity study in mice be performed at higher dose levels to adequately assess the carcinogenic potential of 2,4-D. The current study does not satisfy the guideline requirement [OPPTS 870. 4200; §83-2] for a carcinogenicity study in the mouse. The repeated study [MRIDs 43879801 and 43597201] employed dosages from 5-120 mg/kg/day for males and 5-300 mg/kg/day for females. The study has been reviewed under a separate DER [TXR No. 011934], and the results indicated no treatment-related increase in any tumor incidence relative to controls.

Table 1. Body-Weight/Body-Weight Gain [g]

Parameter/Week/Sex/Dose	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	45 mg/kg/day
BODY WEIGHT [g]				
MALES				
0	21.0±1.4	21.0±1.4	20.8±1.2	21.2±1.4
1	22.6±1.3	22.1±1.3	22.0±1.1	22.0±1.2
2	23.0±1.4	22.7±1.3	22.9±1.2	22.9±1.2
2	23.9±1.4	23.6±1.4	24.1±1.2	24.2±1.7
13	27.7±1.7	26.9±2.0	27.7±1.3	27.3±1.6
52	35.2±4.0	34.6±4.0	34.9±2.8	34.9±3.1
104	35.1±4.0	33.5±4.3	33.1±2.6	33.1±2.6 [94]√
FEMALES				
0	17.9±1.2	18.0±1.0	17.7±1.2	18.1±1.2
1	19.6±1.2	19.6±1.0	19.3±1.2	19.5±1.3
2	20.4±1.0	20.3±1.0	20.5±1.0	20.4±1.2
2	21.3±1.1	21.1±1.0	21.3±1.0	21.6±1.2
13	24.9±1.4	25.0±1.5	24.8±1.2	24.9±1.7
52	31.2±3.2	30.5±3.2	30.1±2.8	30.8±3.1
104	30.8±2.0	30.0±2.1	30.0±2.2	30.5±2.7
BODY-WEIGHT GAIN [g]				
MALES				
0-1	1.6±0.8	1.1±0.6	1.2±0.7	0.8±0.6 [50]
1-2	0.4±0.5	0.6±0.5	0.9±0.6	0.9±0.6
2-3	1.0±0.7	1.0±0.6	1.2±0.6	1.3±1.0
0-3 ✔	2.9	2.6	3.3	3.0
0-13 ✔	6.7	5.9	6.9	6.1 [91]
0-52 ✔	14.2	13.6	14.1	13.7 [96]
0-104	14.1±3.0	12.8* [91]	12.6* [89]	11.8±2.8* [84]
FEMALES				
0-1	1.7±0.6	1.4±0.7 [82]	1.6±0.8	1.5±0.7 [88]
1-2	0.8±0.6	0.9±0.6	1.2±0.8	0.8±0.7
2-3	0.8±0.6	0.8±0.7	0.9±0.6	1.2±0.6
0-3 ✔	3.4	3.1	3.6	3.5
0-13 ✔	7.0	7.0	7.1	6.8
0-52 ✔	13.3	12.1 [91]	12.4 [93]	12.7 [95]
0-104	12.9±1.7	11.9±2.2 [92]	12.4±2.2	12.5±2.6

* $p<0.05$; ✔ calculated by reviewer; √ [% of control]; data from Tables 3-4 [pages 85-102] of the report

CASWELL FILE

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

MAR 27 1987

005794

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Review of the Mouse Oncogenicity Study on 2,4-Dichlorophenoxyacetic acid

From: Marcia van Gemert, Ph.D. M. van Gemert 3/23/87
Head, Section III
Toxicolpgy Branch, HED (TS-769C)

To: Lin Vlier
Registration Division (TS-767C)

Thru: Theodore M. Farber, Ph.D. Theodore M. Farber 3/27/87
Chief, Toxicology Branch, HED

Compound: 2,4-Dichlorophenoxyacetic acid

Tox Chem No: 315

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

Accession No: 400618-01

Action Requested: The Toxicology Branch has been requested to review the recently submitted mouse oncogenicity study on 2,4-Dichlorophenoxyacetic acid (2,4-D). This review, along with the chronic/oncogenicity study in rats will be presented before the Peer Review Committee April 21, 1987. The conclusions reached from the review of this study are presented below.

Conclusions: 2,4-D at doses of 0, 1, 15 and 45 mg/kg were fed to $B_6C_3F_1$ mice for 104 weeks with a 52 week interim sacrifice. Effects were seen in absolute and relative kidney and adrenal weights at 15 and 45 mg/kg. Histopathology revealed an increase in the mid and high dose groups in cytoplasmic homogeneity of the renal tubular epithelium due to a reduction of cytoplasmic vacuoles.
NOEL = 1 mg/kg
LEL = 15 mg/kg based on treatment-related kidney and adrenal effects
Classification: core minimum; batch number and dietary stability and homogeneity data were not provided.
Oncogenic NOEL = 45 mg/kg, (HDT)

Reviewed by: Marcia van Gemert, Ph.D. M. van Gemert 3/20/87
Head, Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D. Theodore M. Farber 3/27/87
Chief, Tox. Branch (TS-769C)

005794

DATA EVALUATION REPORT

STUDY TYPE: Mouse Oncogenicity study

TOX. CHEM. NO.: 315

ACCESSION NUMBER: 400618-01

MRID NO.: 40061801

TEST MATERIAL: 2,4-dichlorophenoxyacetic acid

SYNONYMS: 2,4-D

STUDY NUMBER(S): 2184-101

SPONSOR: Industry Task Force for 2,4-D Research Data

TESTING FACILITY: Hazleton Laboratories America Inc.
9200 Leesburg Turnpike, Vienna Va. 22180

TITLE OF REPORT: Oncogenicity Study in Mice with 2,4-dichlorophenoxy-acetic acid (2,4-D)

AUTHOR(S): D.G. Serota

REPORT ISSUED: Not dated, but compliance statement was signed and dated by study director 1/15/87

CONCLUSIONS: 2,4-D at doses of 0, 1, 15, and 45 mg/kg were fed to $B_6C_3F_1$ mice for 104 weeks with a 52 week interim sacrifice. Effects were seen in absolute and relative kidney and adrenal weights at 15 and 45 mg/kg. Histopathology revealed an increase in the mid and high dose groups in cytoplasmic homogeneity of the renal tubular epithelium due to a reduction of cytoplasmic vacuoles.
NOEL = 1 mg/kg
LEL = 15 mg/kg based on treatment-related kidney and adrenal effects.

Classification: core-Minimum, batch number and dietary stability and homogeneity data were not provided.

Special Review Criteria (40 CFR 154.7)

005794

A. MATERIALS:

1. Test compound: 2,4-dichlorophenoxyacetic acid (2,4-D)
Description: beige powder
Batch # not given, Purity 97.5%, contaminants: listed in CBI appendix

2. Test animals: Species: mice, Strain: B_6 C_3F_1Crl Br
Age: 7 weeks at initiation of study
Weight: males: 16.4-24.2 gms, females: 14.8-20.9 gms.
Source: Charles River Breeding Laboratories Inc. Kingston, N.Y.

B. STUDY DESIGN:

1. Animal assignment

240 animals/sex were assigned randomly to the following test groups:

Test Group	Dose in diet mg/kg	Main Study 24 months male	Main Study 24 months female	Interim Sac. 52 weeks male	Interim Sac. 52 weeks female
1 Cont.	0	50	50	10	10
2 Low (LDT)	1	50	50	10	10
3 Mid (MDT)	15	50	50	10	10
4 High(HDT)	45	50	50	10	10

2. Diet preparation

Diet was prepared weekly. The concentration of test material in the diet was adjusted weekly through the first 15 weeks and every 4th week thereafter. Reserve samples were taken from the initial batch received and from each mixed batch of test diet and sent to the sponsor. Additional reserve samples were taken from each mixed batch of diet and retained under refrigeration.

Results - Stability data were not provided. A statement in the study text was made that "the sponsor provided information that 2,4-D was stable in the diet for at least one month. Homogeneity of 2,4-D in the diet was established in previous studies conducted at Hazleton Biotechnologies Corp."

Routine analysis conducted during the test on 2,4-D mouse study gave the following results on table I.

005794

TABLE I

group	sex	Percent of target range low	Percent of target range high	percent of target mean + S.D.
2	male	73.99	136.1	96.48 ± 14.824
2	female	77.92	121.2	97.57 ± 10.677
3	male	85.17	111.5	97.56 ± 6.490
3	female	80.91	110.0	95.43 ± 8.234
4	male	80.24	103.4	95.61 ± 4.966
4	female	78.79	101.9	94.26 ± 5.341

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics - The procedures utilized in analyzing the numerical data are on appended pages 3-7.

5. Quality assurance statement was signed by Frederick G Snyder and dated 1/7/87.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. Detailed physical examinations for unusual appearance and behavior, and palpation of tissue masses and abdominal distension were performed weekly for weeks 1-14 and biweekly thereafter.

Mortality (survival) There were no statistically significant differences between treated and controls concerning mortality. Appended pages 8 and 9 graphically represent survival data. The numbers of animals dying on test are tabulated in table II.

TABLE II
Animals which died or were sacrificed in extremis prior to week 104

Group	males	females[c]
1	10	12
2	6	8[b]
3	10	17
4	11[a]	15

[a] One found out of cage at week 30 and was removed from study.
[b] One was found dead during week 105 prior to scheduled sacrifice
[c] 3 animals (2 group 2, one group 3) were missexed and discovered at week 2 and removed from the study

Toxicity: No treatment-related effects were seen.

2. Body weight

Animals were weighed at initiation of study and weekly for weeks 1-14, and biweekly thereafter.

Results: There were no treatment-related effects on body weight or body weight gains. Appended pages 10 and 11 graphically represent body weight data.

3. Food consumption and compound intake

Consumption was determined weekly for weeks 1-14 and biweekly thereafter. Data on food efficiency and compound intake were not given.

Results:
There did not appear to be any treatment-related changes associated with food consumption. There was a significant increase in food consumption in males at weeks 1-52 and 1-104 weeks in groups 2 and 4. Data are presented in Table IV below. Females in group 2 at 1-104 weeks showed an increase in food consumption. Females for weeks 1-52 showed a positive trend in food consumption.

TABLE IV

weeks	males				females			
	1	2	3	4	1	2	3	4
1-52	1361.8	1419.1*	1368.0	1418.9*	1485.0	1536.3	1532.2	1523.9
SD	91.97	87.51	84.19	104.6	91.79	119.66	109.61	109.74
N	56	59	54	50	53	47	50	56
1-104	2313.8	2439.0*	2349.6	2437.6*	2542.6	2668.2*	2568.3	2607.6
SD	137.41	139.7	134.81	170.54	142.91	146.68	178.84	178.09
N	31	35	35	32	30	29	27	33

* Significantly different from controls $p < 0.05$.

4. Ophthalmological examinations were not performed.

5. Blood was collected for hematology from the last surviving 10 mice/sex/group following 52 weeks of treatment and from the first 10/sex/group following 104 weeks of treatment. Clinical Chemistry analyses were not performed. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpuscular HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood Clotting Measurements	X	Cell morphology

* Required for subchronic and chronic studies

Results: No compound-related changes in hematological parameters were seen.

6. Urinalysis was not performed.

7. Sacrifice and Pathology -
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue		.Aorta*	XX	.Brain*† at least 3 levels
X	.Salivary glands*	XX	.Heart*	X	Periph. nerve*#
X	.Esophagus*	X	.Bone marrow*	X	Spinal cord (3 levels)*#
X	.Stomach*	X	.Lymph nodes*[6]	XX	.Pituitary*[5]
X	.Duodenum*	X	.Spleen*	X	Eyes (optic n.)*#[7]
X	.Jejunum*	X	.Thymus* (when presnt)		Glandular
X	.Ileum*		Urogenital	XX	.Adrenals*[5]
X	.Cecum*	XX	.Kidneys*†[3]		Lacrimal gland#
X	.Colon*	X	.Urinary bladder*	X	Mammary gland*#
	.Rectum*	XX	.Testes*†[2]		.Parathyroids*††
XX	.Liver*†[1]		Epididymides	XX	.Thyroids*††[4]
	Gall bladder*#	X	Prostate		Other
X	.Pancreas*		Seminal vesicle	X	Bone*# Sternum w marrow
	Respiratory	XX	Ovaries*†[5]	X	Skeletal muscle*#
X	.Trachea*	X	.Uterus*	X	Skin*#
X	.Lung*			X	All gross lesions
				X	3 coronal sections[8]

Footnotes are on the next page

005794

* Required for subchronic and chronic studies
° Required for chronic inhalation
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

[1] weighed with gall bladder
[2] weighed with epididymides
[3] left and right (weighed separately, combined weight was calculated
[4] weighed with parathyroids post fixation, and sectioned together
[5] weighed post fixation
[6] all grossly enlarged or otherwise abnormal lymph nodes, or nodes draining known or suspected tumor sites.
[7] with contiguous harderian gland
[8] coronal sections through head to include nasal cavity, paranasal sinuses, oral cavity, nasopharynx and middle ear

Gross examination included examination of :
external surface, all orifices, cranial cavity, carcass, nasal cavity and paranasal sinuses (not opened at necropsy but opened and examined post fixation, except for 10 animals/sex/group selected for histopathology exam of the spinal cord and coronal head sections), cervical tissues and organs, thoracic, abdominal and pelvic cavities and their viscera, cut surface of brain and external and cut surfaces of the spinal cord (examined post fixation during tissue trimming) according to the study text. All preserved tissues (except spinal cord, skeletal muscle and coronal sections of the head) from all mice were embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined microscopically. The spinal cord (cervical and thoracic) and coronal sections through the head were examined microscopically from the last 10 animals/sex/group surviving to study termination.

a. Organ weight: Both kidneys and adrenals appeared to be affected by treatment.

Kidneys: absolute kidney weights, both left and right as well as combined, were significantly increased in group 4 females, and all revealed a significant positive trend. There were significantly increased kidney/brain weight ratios in group 4 females for left right and combined kidneys. At 104 weeks all absolute and relative kidney weight parameters were significantly increased weight in group 4 females with most significant positive trends seen, except kidney/brain weights for combined kidneys. Kidney/body weight ratios in group 3 females for left, right and combined kidneys were significantly elevated. Group 4 males also showed an increase in kidney/body weight ratios, possibly due to the drop in body weight seen at high dose. Data for the 52 week sacrifice are in table V, and data for the terminal sacrifice are in table VI.

Adrenals: Absolute and all relative male adrenal weights were decreased at all doses tested at week 54, with significant trends seen for all parameters. At week 104, all parameters in the mid and high dose groups were increased (rather than decreased at 54 weeks) with significant positive trends seen in all parameters for males. Data are given in table V and VI.

TABLE V

005794

ORGAN WEIGHTS 52 WEEK SACRIFICE

left kidney male	#	Abs Wts Mean	SD	Organ-to body wts. mean	S.D.	Organ-to Brain wts. Mean	S.D.
1	10	0.28	0.03	0.975	0.068	0.622	0.067
2	10	0.29	0.03	1.006	0.073	0.632	0.056
3	10	0.28	0.03	0.899	0.061	0.627	0.062
4	10	0.28	0.02	0.873*[a]	0.091	0.617	0.041
left kidney female							
1	10	0.20	0.02	0.763	0.065	0.436	0.033
2	10	0.20	0.02	0.742	0.092	0.420	0.036
3	10	0.20	0.01	0.749	0.076	0.435	0.029
4	10	0.22*[a]	0.02	0.825	0.116	0.471*	0.032
right kidney male							
1	10	0.29	0.02	1.014	0.048	0.647	0.064
2	10	0.31	0.02	1.074	0.093	0.673	0.039
3	10	0.30	0.03	0.952*	0.051	0.665	0.065
4	10	0.30	0.02	0.923[a]	0.118	0.651	0.049
right kidney female							
1	10	0.21	0.02	0.794	0.063	0.455	0.045
2	10	0.22	0.02	0.801	0.079	0.454	0.031
3	10	0.22	0.01	0.802	0.083	0.466	0.027
4	10	0.24*[a]	0.02	0.893*	0.099	0.512*[a]	0.040

Combined kidneys males	X	Abs wts mean	S.D.	Organ-to body wts mean	S.D.	Organ-to Brain wts Mean	S.D.
1	10	0.58	0.05	1.991	0.111	1.270	0.125
2	10	0.60	0.05	2.085	0.169	1.307	0.084
3	10	0.58	0.05	1.845*	0.100	1.288	0.123
4	10	0.58	0.04	1.793	0.206	1.266	0.086
Combined kidneys females							
1	10	0.42	0.04	1.558	0.119	0.892	0.079
2	10	0.42	0.04	1.552	0.169	0.879	0.063
3	10	0.42	0.02	1.546	0.153	0.899	0.055
4	10	0.46*[a]	0.03	1.716	0.207	0.982*	0.061
Adrenal males							
1	10	0.007	0.002	0.0258	0.0068	0.0163	0.0039
2	10	0.005*	0.001	0.0189*	0.0047	0.0117*	0.0026
3	10	0.005*	0.001	0.0164*	0.0023	0.0114*	0.0016
4	10	0.006*[a]	0.001	0.0177*[a]	0.0047	0.0125*[a]	0.0030
Adrenal females							
1	10	0.008	0.001	0.0306	0.0041	0.0175	0.0023
2	10	0.008	0.001	0.0290	0.0036	0.0164	0.0009
3	10	0.008	0.001	0.0299	0.0060	0.0173	0.0027
4	10	0.008	0.003	0.0304	0.0137	0.0170	0.0065

* Significantly different from controls ($p \leq 0.05$)
[a] Significant trend ($p \leq 0.05$)

199

005794

TABLE VI

Organ Weights for the Terminal Sacrifice

Left Kidney		Abs Wts		Organ-to Body Wts.		Organ-to Brain Wts	
males	X	Mean	S.D.	Mean	S.D.	Mean	S.D.
1	40	0.32	0.04	1.049	0.104	0.707	0.068
2	44	0.32	0.03	1.093	0.115	0.712	0.058
3	40	0.32	0.03	1.095	0.098	0.712	0.064
4	38	0.33	0.03	1.135*[a]	0.112	0.729	0.065
left kidney							
females							
1	38	0.22	0.02	0.819	0.090	0.469	0.044
2	38	0.23	0.02	0.857	0.091	0.486	0.051
3	31	0.23	0.02	0.880*	0.079	0.487	0.055
4	34	0.27*[a]	0.02	1.003*[a]	0.092	0.559*[a]	0.049
Right kidney							
males							
1	40	0.34	0.04	1.106	0.102	0.745	0.069
2	44	0.34	0.03	1.142	0.112	0.744	0.060
3	40	0.34	0.03	1.139	0.106	0.740	0.068
4	38	0.35[a]	0.03	1.185*[a]	0.106	0.762[a]	0.069
Right kidney							
females							
1	38	0.24	0.02	0.883	0.091	0.505	0.043
2	38	0.24	0.03	0.903	0.082	0.510	0.048
3	31	0.25	0.03	0.952*	0.122	0.527	0.079
4	33	0.28*	0.03	1.042*[a]	0.101	0.581*	0.048
Combined kidneys							
males							
1	40	0.67	0.07	2.153	0.180	1.450	0.122
2	44	0.66	0.06	2.236	0.216	1.457	0.109
3	40	0.66	0.05	2.234	0.194	1.453	0.123
4	38	0.68	0.06	2.322*[a]	0.208	1.492	0.129
Combined kidneys							
females							
1	38	0.46	0.04	1.701	0.178	0.973	0.084
2	38	0.47	0.04	1.762	0.167	0.998	0.094
3	31	0.48	0.05	1.832*	0.191	1.014	0.128
4	33	0.55*[a]	0.05	2.045*[a]	0.182	1.141*	0.092
Adrenals							
male							
1	40	0.005	0.002	0.0157	0.005	0.0107	0.0040
2	43	0.005	0.002	0.0182	0.0063	0.0118	0.0042
3	40	0.006*	0.002	0.0214*	0.0078	0.0138*	0.0047
4	37	0.006*[a]	0.002	0.0213*[a]	0.006	0.0135*[a]	0.0035
Adrenals							
female							
1	37	0.008	0.002	0.0283	0.0083	0.0164	0.0052
2	38	0.007	0.002	0.0280	0.006	0.0158	0.0037
3	31	0.007	0.002	0.0277	0.0075	0.0153	0.0043
4	35	0.008	0.002	0.0288	0.0073	0.0162	0.0044

* Significantly different from controls $p \leq 0.05$
[a] Significant trend $p \leq 0.05$

b. Gross pathology: No treatment-related effects were noted in the unscheduled deaths, interim sacrifice and terminal sacrifice.

c. Microscopic pathology

1) Non-neoplastic:
Kidney: There was a compound related increase in histomorphologic alteration in the renal tubule epithelium of males. This was characterized as a cytoplasmic homogeneity, and was due to the reduction of cytoplasmic vacuoles that are normally present in the renal tubular epithelium. These effects are presented in Table VII. Other lesions seen were not treatment-related and were the result of normal spontaneous disease processes associated with the B_6 C_3F_1 mouse.

TABLE VII

Incidence of Cytoplasmic Homogeneity- Increased Tubular Epithelium

Group/sex	1m	2m	3m	4m	1f	2f	3f	4f
Unscheduled deaths								
N	10	6	10	11	0	0	0	0
Increased Tubular Epi.	3	5*	10***	10***	0	0	0	0
52-week sacrifice								
N	10	10	10	10	10	10	10	10
Increased Tubular Epi.	0	1	4*	10***	0	0	0	0
Terminal sacrifice								
N	40	44	40	38	38	38	31	35
Increased Tubular Epi.	8	9	34***	38***	0	0	0	0
Combined deaths								
N	60	60	60	59	60	58	59	60
Increased Tubular Epi.	11	15	48***	58***	0	0	0	0

* Significantly different from Controls $p < 0.05$
** Significantly different from Controls $p < 0.01$
*** Significantly different from Controls $p < 0.001$
Fishers Exact Test used by reviewer

Adrenal: No histopathological findings were noted in the adrenal at the interim sacrifice or terminal sacrifice, or in unscheduled deaths.

2) Neoplastic: No treatment-related increased incidence in tumors was seen at the 52 week sacrifice, in unscheduled deaths, at terminal sacrifice, or in combined scheduled and unscheduled deaths.

Discussion:

005794

There were no significant treatment-related findings in toxicity, mortality, body weights, food consumption, hematology and gross pathology. Kidney and adrenal weights were effected by 2,4-D treatment. At 52 weeks there was an increase in high dose females in combined absolute kidney and kidney/brain weight ratios with a significant trend seen in absolute weights. All male adrenal weight parameters were elevated at all doses at 52 weeks with significant trends seen in all parameters. At 104 weeks, female and male kidney weights were elevated at the high dose with significant trends in female absolute kidney and organ/brain weight ratios and organ/body weight in males. Female organ/body weight ratios were elevated at the mid dose also. All adrenal organ weight parameters were elevated for males in both the mid and high dose groups at 104 weeks with significant trends seen in all parameters. Histopathology revealed a treatment-related histomorphologic alteration of the renal tubular epithelium of males seen mainly in the mid and high dose groups. The unscheduled deaths showed a marginal increase in tubular epithelium alteration at the low dose. No other treatment-related effects were evident and no increase in treatment-related tumors was seen.
The adrenal weight data indicated significant increases in weight at all doses at the 52 week sacrifice. However, at terminal sacrifice the low dose animals appeared similar to controls. No histopathologic signs were evident either at 52 weeks or at terminal sacrifice, so the henomen may have been stress induced. Since the low dose showed no effects at terminal sacrifice, the 1 mg/kg was considered the NOEL.

NOEL = 1 mg/kg
LEL = 15 mg/kg based on treatment-related kidney and adrenal effects

Amendment to DER for MRID 43612001 - Combined Chronic Toxicity/Carcinogenicity Study [Rat] [2,4-DICHLOROPHENOXYACETIC ACID]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 011934].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 11/19/02
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Whang Phang, Ph.D. Whang Phang 11/20/02
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: combined chronic toxicity/carcinogenicity - rat § 83-5/OPPTS 870.4300
P.C. CODE: 030001 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315 CAS NO.: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [96.7%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force II on 2,4-D Research Data

CITATION: Jeffries, T. K., Yano, B. L., Ormand, J. R. and Battjes, J. E. (1995). 2,4-Dichlorophenoxyacetic acid: Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats. The Toxicology Research Laboratory, Dow Chemical Co.; Study No. K-002372-064, March 28, 1995. MRID 43612001. Document No. 011934. Unpublished.

EXECUTIVE SUMMARY: In a combined chronic toxicity/carcinogenicity study [MRID 43612001], 50 Fischer 344 rats/sex/group were administered 2,4-dichlorophenoxyacetic acid [96.4%] *via* the diet for up to 24 months at concentrations of 0, 5 mg/kg/day, 75 mg/kg/day, and 150 mg/kg/day. The achieved doses were 4.77, 73.15, and 144.98 mg/kg/day [males] and 4.89, 73.11, and 143.52 mg/kg/day [females], respectively. Additionally, 10 rats/sex/group were sacrificed at 12 months [interim sacrifice]. NOTE: The interim sacrifice data were reported in MRID 43293901, HED Document No. 011614, along with the chronic neurotoxicity screening battery substudy.

There were no treatment-related deaths or clinical signs of toxicity. Decreased body weight was observed throughout the study at the high-dose level in both sexes [males 92%-96%/females 74%-90% of control] and at the mid-dose level in females [86%-90% of control]. At week 13, decreased body weight was very slight in the high-dose males [96% of control] and somewhat greater in the high-dose females [90% of control]. At study termination, both sexes displayed decreased body weight at the high-dose level [males 92%/females 74% of control] with the females displaying a greater effect than males. The mid-dose females also displayed a decrease in body weight at study termination [86% of control]. Body-weight gains were decreased throughout the study in females [3-month interval (86% and 74% of control); 6-month interval (88% and 71% of control), and overall (77% and 52% of control)] at the mid- and high-dose levels, respectively. Similarly, high-dose males displayed decreased body-weight gains throughout the study [83%-87% of control]. Consistent with the decreased body-weight gains was a decrease in food consumption, which was observed at the mid-dose level in females [-3.9%] and in both sexes at the high-dose level [males (-4.7%)/females (-11.6%)].

Ophthalmology findings at study termination consisted of increased incidences of constricted blood vessels, fundus and hyper-reflective, fundus in the high-dose males and an increased incidence of lens opacity in the high-dose

females compared to the control and lower dose groups. Decreased RBC, HCT, and HGB values were observed at various time points in the mid- and high-dose females, and platelet counts were decreased at various time points in both sexes at the mid- and high-dose levels. Elevations in creatinine were observed in both sexes at the mid- and high-dose levels throughout the study, except at termination when comparable levels were observed among the male groups. Increased aspartate aminotransferase [mid- and high-dose males], alanine aminotransferase [mid- and high-dose males], and alkaline phosphatase [mid- and high-dose, both sexes], and decreased glucose levels [mid-dose females, high-dose both sexes], cholesterol [mid- and high-dose, both sexes], and triglycerides [mid-dose females and high-dose both sexes] were observed throughout the study, although a dose response was not always apparent. There was a dose-related decrease in T4 values throughout the study in both sexes at the mid- and high-dose levels, and the females displayed the greater effect except at study termination.

Thyroid weights were increased in the mid-dose females and in both sexes at the high-dose level at the 12-month interim sacrifice. At study termination, thyroid weights were increased in both sexes at the mid- and high-dose levels, although the mid-dose males did not attain statistical significance and the increase at the mid-dose level [both sexes] was greater than at the high-dose level. Decreased testes weights were observed at the high-dose level at both the interim and terminal sacrifices and at the mid-dose level at study termination, although statistical significance was not attained at the mid dose. Decreased ovarian weights were observed at the high-dose level at both sacrifice times and in the mid-dose females at study termination. The decreases in testes and ovarian weights are consistent findings in other studies on 2,4-D and its salts/esters. Kidney weights were increased only at the interim sacrifice in males at the mid- and high-dose levels [dose-related].

Gross pathology findings included decreased fat in high-dose females at both sacrifice times, multifocal pale foci in the lungs [interim: 1 mid-dose, 10 high-dose females; terminal: 4 high-dose males, one control, 4 mid-, 40 high-dose females], and lens opacity in high-dose females at termination. Microscopically, there were increased incidences of lesions in the bone marrow [decreased hematopoiesis in high-dose females at the interim sacrifice], eyes [retina degeneration in 1 male and 9 females at high dose], kidney [proximal tubule degeneration in mid- and high-dose males and females], liver [altered tinctorial properties in mid-dose females and both sexes at high dose], lungs [multifocal alveolar histiocytosis in mid-dose females and both sexes at high dose], adipose tissue [atrophy in mid-dose female, both sexes at high dose], testes [atrophy at high dose], and thyroid [hyperplasia-high-dose males; hypertrophy and epithelial cells-high-dose females] at the interim sacrifice. At study termination, there were increased incidences of cataracts and retina degeneration of the eyes in both sexes at the high-dose level, and the severity of the retina degeneration was increased also. In the liver, there was an increased incidence of increased size of the hepatocytes with altered tinctorial properties in both sexes at the high-dose level. In the lungs, both the incidence and severity of inflammation were increased at the high-dose level in both sexes, and the incidence of atrophy of the adipose tissue was increased at the high-dose level in both sexes at termination. Tumor incidence was not affected by treatment.

The NOAEL is 5 mg/kg/day. The LOAEL of 75 mg/kg/day is based on decreased body-weight gain (females) and food consumption (females), alterations in hematology [decreased RBC (females), HGB (females), platelets (both sexes)] and clinical chemistry parameters [increased creatinine (both sexes), alanine and aspartate aminotransferases (males), alkaline phosphatase (both sexes), decreased T4 (both sexes), glucose (females), cholesterol (both sexes), and triglycerides (females)], increased thyroid weights (both sexes at study termination), decreased testes and ovarian weights, and microscopic lesions in the lungs (females). At the high-dose level, there were microscopic lesions in the eyes, liver, adipose tissue, and lungs. There was no treatment-related increase in the incidence of any tumor.

[2,4-D] Combined Chronic Toxicity/Carcinogenicity Study - Rat [§83-5/OPPTS 870.4300]

This chronic toxicity/carcinogenicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [OPPTS 870. 4300; §83-5] for a chronic toxicity/carcinogenicity study in the rat.

NOTE: The current study was performed to address whether the finding of an increased incidence of astrocytomas of the brain found in the 1986 rat study [MRID 00160876; Accession Nos. 263112-263114] was attributable to 2,4-D. The HED Carcinogenicity Peer Review Committee [CPRC; TXR No. 0050017] concluded that the doses used in the 1986 rat study were not adequate to assess the carcinogenic potential of 2,4-D. In the 1986 study, there was a significant trend for astrocytomas in male rats but no pair-wise significance. The incidence in both the treated and control males exceeded the historical control incidence for this tumor. Additionally, the CPRC concluded that the high-dose level in the 1986 study was not adequate. The current repeat chronic toxicity/carcinogenicity study in rats was performed at higher dose levels. There was one astrocytoma in the brain of males at the high dose *vs* none in the control. There was one tumor each in the control and high-dose female group. However, all of the brains of the rats in the low- and mid-dose groups were not examined. The HED Carcinogenicity Peer Review Committee [CPRC; TXR No. 0050017] requested that the slides of the low- and mid-dose brains of the males be evaluated. These additional data were submitted [MRID 44284501], and no additional tumors were observed. The additional data do not alter the findings of the study.

Table 1. Body-Weight and Food Consumption Data

Parameter/Day/Sex/Dose	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
BODY WEIGHT [g]				
MALES				
-2	197.6±11.5	197.6±9.9	197.1±9.2	197.8±10.6
6	217.5±13.1	216.8±12.0	214.4±11.1	213.5±11.9
27	246.0±15.9	247.7±13.9	243.3±14.6	239.5±14.6*[97]
90	308.3±22.1	311.8±20.0	305.3±20.4	295.2±19.4*[96]
365	409.8±28.5	415.5±23.5	400.0±30.2	372.5±26.6*[91]
729	344.2±33.8	350.2±24.5	347.2±30.1	317.9±33.6*[92]
FEMALES				
-2	129.2±4.9	127.9±5.6	126.6±5.0*[98]✓	126.2±5.2*[98]
6	139.2±5.6	138.4±5.6	134.1±5.7*[96]	130.7±5.1*[94]
27	160.5±7.4	160.3±7.6	151.1±6.3*[94]	145.8±6.1*[91]
90	187.0±9.1	186.3±8.6	176.8±8.0*[95]	169.3±7.3*[91]
365	217.5±10.9	217.6±12.4	205.6±11.8*[95]	190.7±9.1*[88]
729	271.0±27.7	265.5±24.1	234.4±20.1*[86]	200.2±13.0*[74]
BODY-WEIGHT GAIN [g]				
MALES				
-2-6	19.9±4.1	19.2±5.8	17.3±3.5*[87]	15.7±3.6*[79]
-2-13	33.0±5.7	33.3±5.0	29.8±6.4*[90]	28.0±5.6*[85]
-2-90	110.7±15.0	114.3±14.7	108.3±14.6	97.4±12.9*[88]
-2-365	212.2±20.4	217.9±19.2	202.4±25.8*[95]	175.0±20.5*[82]
-2-729	144.030.0	151.6±26.3	148.7±29.8	120.0±28.8*[83]
FEMALES				
-2-6	10.0±2.7	10.5±2.8	7.5±3.5*[75]	4.5±3.7*[45]
-2-13	17.5±3.4	19.1±3.1*	14.2±3.3*[81]	11.0±3.5*[63]
-2-90	57.8±7.6	58.4±6.5	50.2±6.6*[87]	43.1±5.9*[75]
-2-365	88.3±10.4	89.7±10.7	79.0±10.6*[89]	64.5±8.0*[73]
-2-729	140.8±27.7	137.7±23.8	108.0±20.2*[77]	73.4±11.8*[52]

✓ [% of control]; * $p<0.05$; data from Tables 11-14 [pages 74-91] of the report

Table 2. Clinical Pathology Data - MALES

Dose/Month/Parameter	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
platelets [x10E3/cumm]				
6	615±41	613±32	604±36	529±25*[86]
12	586±65	598±25	572±33	474±45*[81]
18	623±66	589±134[95]✓	568±74 [91]	495±40*[79]
24	760±237	668±163	620±117*[82]	592±141*[78]
RBC[x10E6/cumm]				
6	9.82±0.35	9.85±0.30	10.11±0.26	9.60±0.34
12	9.31±0.28	9.20±0.22	9.36±0.19	8.55±0.35*[92]
18	9.06±0.49	8.55±2.23	9.60±1.80	8.92±0.74
24	7.89±1.31	8.15±1.64	8.65±0.90	7.74±1.11
HGB [g/dL]				
6	17.2±0.5	17.2±0.6	17.5±0.5	17.1±0.4
12	15.0±0.5	15.1±0.3	15.1±0.3	14.9±0.7
18	16.0±0.8	15.1±3.2	16.5±2.8	16.0±0.8
24	13.7±1.9	14.2±2.3	14.3±1.7	13.7±2.6

Table 2. Clinical Pathology Data - MALES				
Dose/Month/Parameter	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
HCT (%)				
6	61.9±2.2	61.9±2.1	62.6±1.7	59.3±1.7*[86]
12	58.5±1.8	58.1±1.5	58.2±1.2	55.3±2.7*[95]
18	48.1±2.0	45.9±8.5	49.8±7.3	47.7±2.4
24	41.4±5.3	42.5±6.3	42.5±4.4	41.0±6.2
alanine aminotransferase [U/L]				
6	65±8	63±14	120±61*[185]	122±26*[188]
12	66±18	61±15	91±21*[138]	68±15
18	41±8	47±26	72±20*[176]	53±8*[129]
24	38±23	38±12	68±30*[180]	67±50*[176]
aspartate aminotransferase [U/L]				
6	116±16	111±21	162±64*[140]	172±27*[148]
12	106±21	95±20	132±39 [125]	103±15
18	79±16	108±106 [137]	114±37 [144]	92±12 [116]
24	78±27	92±44	115±56*[147]	129±157 [165]
blood urea nitrogen [mg/dL]				
6	17±1	17±1	18±1	18±3
12	15±2	13±1 [87]	14±2	13±1
18	19±1	18±1	17±5	18±1
24	23±5	21±3	18±2*[78]	18±4 [78]
creatinine [mg/dL]				
6	0.7±0.0	0.8±0.1	0.8±0.1	0.9±0.1*[129]
12	0.6±0.0	0.6±0.0	0.7±0.1*[117]	0.7±0.1*[117]
18	0.7±0.0	0.8±0.1	0.9±0.1*[129]	0.9±0.1*[129]
24	0.9±0.2	0.9±0.1	0.9±0.1	0.9±0.1
alkaline phosphatase [U/L]				
6	73±7	75±7	85±11*[116]	92±9*[126]
12	57±4	57±5	58±6	55±7
18	58±8	77±53	73±38	74±22
24	53±20	58±40	74±24*[140]	98±47*[185]
glucose [mg/dL]				
6	112±16	116±17	108±15	102±15
12	117±9	124±4	111±7	100±10*[85]
18	98±5	100±7	92±12	74±22*[76]
24	75±13	79±15	77±14	72±9
cholesterol [mg/dL]				
6	86±10	80±3	84±10	71±8*[83]
12	87±17	83±11	69±11*[79]	53±6*[61]
18	154±23	142±19 [92]	120±33*[78]	83±17*[54]
24	194±76	169±65 [87]	124±30*[64]	128±52*[66]
triglycerides [mg/dL]				
6	140±27	139±36	171±38	129±12
12	101±29	105±29	89±21 [88]	70±9*[69]
18	186±26	173±53	151±39 [81]	94±18*[51]
24	207±171	nl	nl	110±47*[53]

√[% of control]; n=10 [6, 12, 18 months]/n=20 [24 months]; nl not legible; * $p<0.05$;
data from Tables 18-25 [pages 100-107] and 58-73 [pages 161-176] of the report

Table 3. Clinical Pathology Data - FEMALES				
Dose/Month/Parameter	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
platelets [x10E3/cumm]]				
6	599±39	609±44	461±30*[77]√	403±30*[67]
12	583±23	548±120 [94]	471±48*[81]	486±44*[84]
18	591±40	613±47	496±33*[84]	491±33*[83]
24	562±92	549±139	551±130	523±135 [93]
RBC[x10E6/cumm]				
6	9.11±0.21	9.05±0.22	8.71±0.16*[96]	8.22±0.28
12	8.37±0.29	8.46±0.50	7.78±0.48*[93]	7.59±0.25
18	8.61±0.18	8.41±0.21	7.83±0.93*[91]	7.61±0.25*[88]
24	8.28±1.60	7.91±1.53 [96]	7.44±1.49*[90]	7.01±1.77*[85]
HGB [g/dL]				
6	17.2±0.3	17.1±0.3	16.9±0.4	16.6±0.4*[97]
12	14.8±0.4	15.0±0.9	14.4±0.9	14.5±0.6
18	16.1±0.3	15.8±0.3*[98]	14.8±2.1*[92]	14.9±0.3*[93]
24	14.7±2.6	14.6±1.9	13.9±2.3[95]	13.3±3.0 [90]
HCT [&]				
6	60.6±1.2	60.3±2.0	58.4±1.2*[96]	55.9±2.3*[92]
12	57.3±1.9	57.7±3.4	52.6±3.1*[92]	51.8±2.2*[90]
18	46.8±1.2	xx.5±0.9✖	44.0±4.4	43.5±1.4*[93]
24	43.6±7.6	43.6±4.3	41.6±6.4 [95]	40.9±6.5 [94]
alanine aminotransferase [U/L]				
6	51±8	50±19	56±16	40±6*
12	37±10	42±20	37±8	33±10
18	42±8	40±5	34±12	36±4
24	48±28	50±28	61±78	55±62
aspartate aminotransferase [U/L]				
6	96±11	101±35	103±14	98±13
12	71±15	80±34	63±10	68±16
18	80±15	70±12	72±8	81±9
24	92±60	105±110	136±291 [148]	134±216 [146]
blood urea nitrogen [mg/dL]				
6	20±3	20±3	21±3	21±2
12	14±1	14±1	15±2	13±2
18	20±1	18±2	21±3	19±2
24	20±10	16±2	20±6	25±28
creatinine [mg/dL]				
6	0.8±0.1	0.8±0.1	0.9±0.1*[113]	0.9±0.1*[113]
12	0.7±0.1	0.7±0.1	0.8±0.1*[114]	0.7±0.0*
18	0.7±0.0	0.7±0.0*	0.9±0.0*[129]	0.9±0.1*[129]
24	0.7±0.1	0.7±0.0	0.8±0.1*[114]	0.8±0.1*[114]
alkaline phosphatase [U/L]				
6	47±9	46±4	59±7*[126]	73±16*[155]
12	32±3	32±6	47±8*[147]	54±10*[169]
18	37±5	40±10	69±48*[186]	76±18*[205]
24	47±28	63±50	84±77*[179]	99±52*[211]
glucose [mg/dL]				
6	96±12	96±10	88±9 [92]	72±8[75]
12	109±8	114±10	112±9	113±11
18	97±6	103±10	86±11*[89]	79±6*[81]
24	94±18	94±11	82±18*[87]	77±11*[82]

Table 3. Clinical Pathology Data - FEMALES				
Dose/Month/Parameter	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
cholesterol [mg/dL]				
6	116±11	123±5	81±6*[70]	76±8*[66]
12	105±13	110±16	54±7*[51]	57±7*[54]
18	155±9	148±14	76±11*[49]	81±4*[52]
24	169±38	166±29	104±57*[62]	109±36*[64]
triglycerides [mg/dL]				
6	77±14	88±22	88±16	83±6
12	52±8	56±18	54±6	56±4
18	136±46	129±34	93±14*[68]	88±14*[65]
24	183±85	180±98	126±52*[69]	105±21*[57]

√[% of control]; n=10 [6, 12, 18 months]/n=20 [24 months]; * p<0.05; ✖ illegible
data from Tables 22-25 [pages 104-107] and 62-65 [pages 165-168] of the report

Table 4. T4 Results [µg/dL]				
Dose/Sex/Month	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
MALES				
6	3.2±0.5	3.4±0.6	2.7±0.2 [84]	2.1±0.9*[66]
12	3.0±0.4	3.1±0.3	2.6±0.3*[87]	0.9±0.2*[30]
18	3.0±0.2	3.4±0.4	2.6±0.6 [87]	1.1±0.5*[37]
24	2.2±0.6	2.2±0.4	1.5±0.7*[68]	0.8±0.4*[36]
FE MALES				
6	2.6±0.5	2.7±0.4	1.1±0.3*[42]	0.9±0.2*[35]
12	2.3±0.4	2.2±0.4	0.8±0.2*[35]	0.7±0.2*[30]
18	1.9±0.4	2.1±0.3	0.8±0.2*[42]	0.5±0.2*[26]
24	1.9±0.6	2.2±0.6	1.3±0.6*[68]	1.1±0.7*[58]

√[% of control]; n=10 [6, 12, 18 months]/n=20 [24 months]; * p<0.05; data from Tables 66-73 [pages 169-176] of the report

Table 5a. Organ Weight Data - MALES [12 months]				
Dose/Organ	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
brain				
absolute [g]	2.039±0.105	2.000±0.066	2.025±0.062	1.988±0.095 [97]√
relative-to-body [g/100]	0.536±0.016	0.515±0.017	0.557±0.040	0.056±0.031
liver				
absolute [g]	9.508±0.662	9.630±0.661	8.543±0.677*[90]	8.942±0.370
relative-to-body [g/100]	2.496±0.062	2.476±0.111	2.338±0.080*[94]	2.520±0.125
relative-to-brain [g/100]	466.26±19.50	481.45±30.38	421.69±28.47*[90]	450.74±30.15
kidney				
absolute [g]	2.231±0.105	2.255±0.111	2.391±0.150*[107]	2.409±0.125*[108]
relative-to-body [g/100]	0.586±0.019	0.580±0.017	0.655±0.026*[112]	0.678±0.013*[116]
relative-to-brain [g/100]	109.47±3.49	112.75±4.24	118.09±6.45*[108]	121.32±6.73*[111]
testes				
absolute [g]	3.158±0.293	3.247±0.128	3.198±0.193	2.669±0.540*[85]
relative-to-body [g/100]	0.832±0.092	0.836±0.032	0.877±0.052	0.748±0.139 [90]
relative-to-brain [g/100]	155.10±14.49	162.35±5.67	157.96±8.63	134.09±26.32*[86]
thyroid				
absolute [g]	0.027±0.002	0.025±0.002	0.026±0.003	0.032±0.003*[119]
relative-to-body [g/100]	0.0071±0.0006	0.0064±0.0006	0.0070±0.0006	0.0091±0.0009*[128]
relative-to-brain [g/100]	1.3178±0.1091	1.2517±0.1068	1.2582±0.1198	1.6308±0.1805*[124]
terminal body weight [g]	380.8±21.9	388.6±14.8	365.5±28.3	355.4±18.8*[93]

√[% of control]; n=10; * p<0.05; data from Tables 74-75 [pages 177-179] of the report

Table 5b. Organ Weight Data - MALES [24 months]				
Dose/Organ	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
brain				
absolute [g]	2.035±0.054	2.050±0.068	2.029±0.059	2.014±0.072
relative-to-body [g/100]	0.619±0.074	0.607±0.047	0.606±0.059	0.660±0.079*[107]√
liver				
absolute [g]	11.036±2.018	11.179±2.497	9.492±1.610*[86]	9.490±1.343*[86]
relative-to-body [g/100]	3.338±0.629	3.294±0.702	2.848±0.661*[85]	3.117 [93]
relative-to-brain [g/100]	542.37±98.77	545.62±121.04	468.47±81.71*[86]	472.12±72.01*[87]
kidney				
absolute [g]	2.851±0.296	2.939±0.325	2.982±0.326	2.804±0.357
relative-to-body [g/100]	0.865±0.121	0.868±0.098	0.889±0.115	0.917±0.143
relative-to-brain [g/100]	140.07±14.12	143.46±15.93	147.00±15.42	139.05±15.35
testes				
absolute [g]	6.877±3.256	7.121±1.887	5.776±3.045 [84]	3.277±1.871*[48]
relative-to-body [g/100]	2.044±0.898	2.096±0.531	1.713±0.949 [84]	1.047±0.609*[51]
relative-to-brain [g/100]	335.85±153.71	347.68±93.03	283.97±148.77 [85]	161.48±90.33*[48]
thyroid				
absolute [g]	0.038±0.010	0.041±0.021	0.060±0.119 [158]	0.050±0.035*[132]
relative-to-body [g/100]	0.0115±0.0032	0.0120±0.0062	0.0178±0.0357 [154]	0.0161±0.0107*[140]
relative-to-brain [g/100]	1.8615±0.4975	1.977±1.0032	2.9194±5.6871 [157]	2.4678±1.7013*[131]
terminal body weight [g]	333.1±36.6	339.5±24.8	337.5±30.0	309.1±36.5*[93]

√[% of control];* p<0.05; n=28, 25, 33, 36 [control, low, mid, high, respectively]; data from Tables 76-77, pages 180-182 of the report

210

Table 6a. Organ Weight Data - FEMALES [12 months]				
Dose/Organ	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
brain				
absolute [g]	1.833±0.058	1.852±0.051	1.837±0.049	1.796±0.056
relative-to-body [g/100]	0.926±0.037	0.887±0.045	0.942±0.060	1.013±0.056*[109] √
liver				
absolute [g]	5.294±0.350	5.560±0.418	5.354±0.445	5.499±0.422
relative-to-body [g/100]	2.673±0.154	2.655±0.125	2.735±0.118	3.094±0.171*[116]
relative-to-brain [g/100]	288.80±15.35	300.34±23.66	291.59±24.39	305.97±19.45 [106]
kidney				
absolute [g]	1.440±0.069	1.481±0.112	1.558±0.092	1.544±0.148 [107]
relative-to-body [g/100]	0.727±0.029	0.708±0.041	0.797±0.036*[110]	0.868±0.061*[119]
relative-to-brain [g/100]	78.55±2.53	80.05±6.75	84.89±5.60 [108]	85.94±7.63*[109]
ovaries				
absolute [g]	0.057±0.013	0.056±0.012	0.058±0.005	0.052±0.009 [91]
relative-to-body [g/100]	0.029±0.006	0.027±0.005	0.029±0.003	0.029±0.005
relative-to-brain [g/100]	3.11±0.70	3.03±0.64	3.13±0.24	2.87±0.44 [92]
thyroid				
absolute [g]	0.018±0.001	0.019±0.002	0.022±0.001*[122]	0.022±0.003*[122]
relative-to-body [g/100]	0.0093±0.0008	0.0091±0.0010	0.0111±0.0006*[119]	0.0126±0.0012*[135]
relative-to-brain [g/100]	1.0052±0.0872	1.0276±0.1280	1.1816±0.0659*[118]	1.2463±0.1265*[124]
terminal body weight [g]	198.2±10.5	209.3±10.5	195.7±13.1	177.7±9.8*[90]

√[% of control];* p<0.05; n=10; data from Tables 78-79 [pages 183-185] of the report

Table 6b. Organ Weight Data - FEMALES [24 months]				
Dose/Organ	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
brain				
absolute [g]	1.885±0.039	1.889±0.063	1.889±0.044	1.859±0.047
relative-to-body [g/100]	0.713±0.052	0.752±0.079*[105] √	0.842±0.065*[118]	0.959±0.057*[135]
liver				
absolute [g]	6.913±0.949	6.708±0.952	6.064±1.128*[88]	6.331±0.651*[92]
relative-to-body [g/100]	2.596±0.260	2.660±0.407	2.689±0.425	3.272±0.444*[126]
relative-to-brain [g/100]	366.87±51.14	355.30±50.11	321.33±62.14*[88]	341.06±38.58 [93]
kidney				
absolute [g]	1.966±0.121	1.973±0.173	1.996±0.198	1.857±0.130*[94]
relative-to-body [g/100]	0.741±0.040	0.784±0.089	0.886±0.070*[120]	0.957±0.064*[129]
relative-to-brain [g/100]	104.34±6.49	104.51±8.89	105.68±10.80	99.89±6.03*[96]
ovaries				
absolute [g]	0.063±0.089	0.056±0.059	0.041±0.010*[65]	0.037±0.033
relative-to-body [g/100]	0.024±0.034	0.022±0.021	0.018±0.005 [75]	0.019±0.016*[79]
relative-to-brain [g/100]	3.389±0.889	3.014±3.299	2.184±0.544*[64]	1.989±1.813*[59]
thyroid				
absolute [g]	0.025±0.005	0.033±0.033 [132]	0.035±0.029*[140]	0.033±0.018*[132]
relative-to-body [g/100]	0.0094±0.0020	0.0133±0.0145	0.0156±0.0134*[166]	0.0169±0.0096*[180]
relative-to-brain [g/100]	1.3224±0.2493	1.7350±1.6717	1.8541±1.5513*[140]	1.7608±0.9598*[133]
terminal body weight [g]	265.8±19.5	253.5±23.7	225.5±17.0*[85]	194.5±12.8*[73]

√[% of control]; n=35, 39, 40, 34 [control, low, mid, high, respectively]; * p<0.05; data from Tables 80-81 [pages 186-188]] of the report

011934

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

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist 5/16/96
Section I, Toxicology Branch II (7509C)

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Section I, Toxicology Branch II (7509C)

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

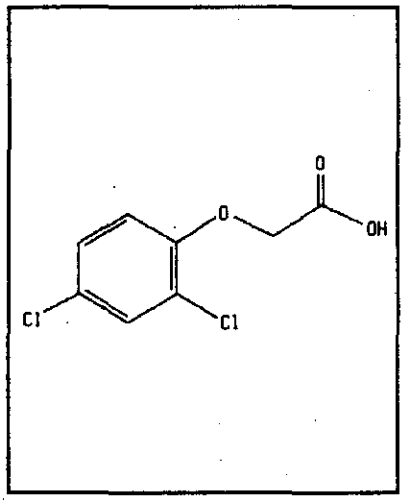
In 1988, the Agency required that rodent carcinogenicity testing with 2,4-dichlorophenoxy acetic acid (2,4-D) be repeated because a Maximum Tolerated Dose (MTD) had not been achieved in the Industry-sponsored studies. In the Data Call-In notice of 1989, the Agency formally requested that the carcinogenicity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

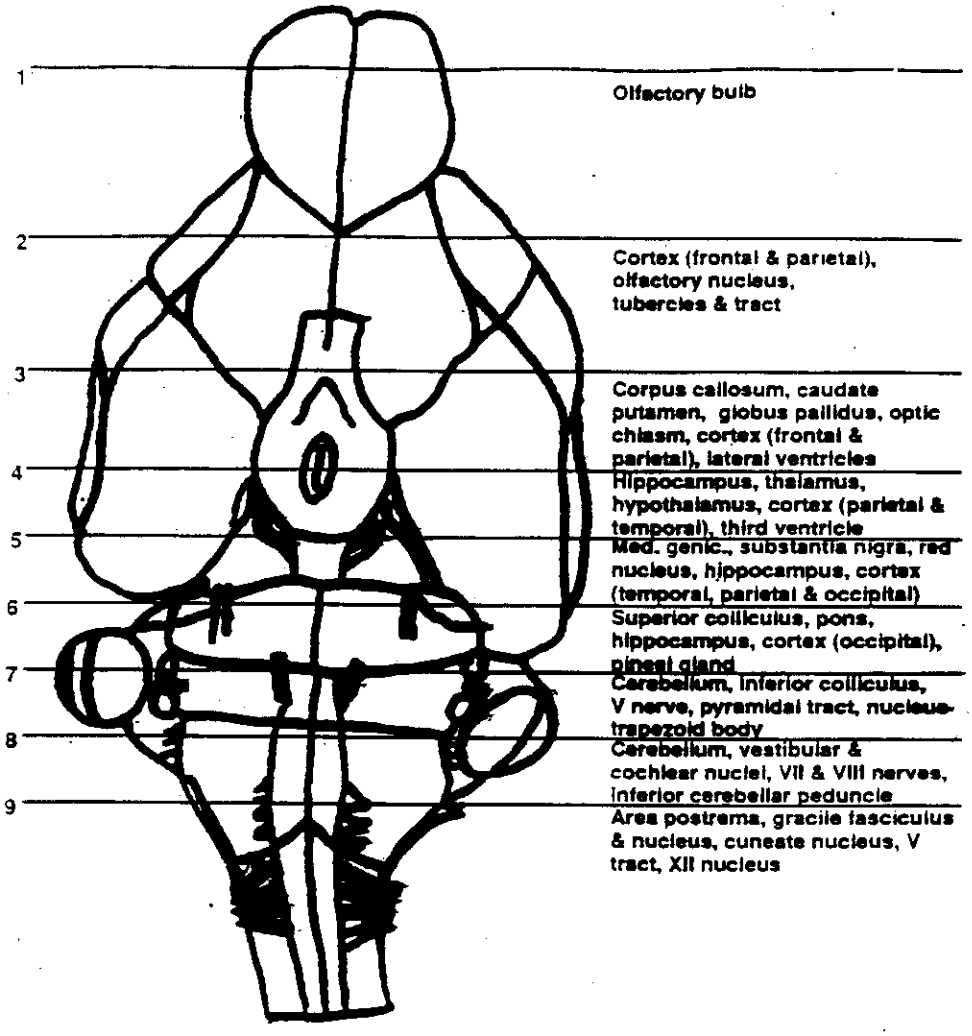


Figure 1. Diagramatic representation of the ventral surface of the rat brain. Numbered lines indicate the approximate topographic location of nine transverse sections and include a partial listing of expected microscopic structures.

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. <u>Urinalysis:</u> 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. <u>Organ Weights:</u> 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

TABLE 91

2,4-DICHLOROPHENOXYACETIC ACID: CHRONIC TOXICITY/ONCOGENICITY STUDY IN FISCHER 344 RATS - FINAL REPORT

TUMOR INCIDENCE SUMMARY[@] - ALL MODES OF DEATH - 24 MONTHS

SEX	MALES					FEMALES			
DOSE IN MG/KG/DAY	0	5	75	150		0	5	75	150
NUMBER OF RATS EXAMINED	50	50	50	50		50	50	50	50
ADRENALS (NO. OF TISSUES EXAMINED)	50	26	18	50		50	12	11	50
ADENOMA, CORTEX, BENIGN, PRIMARY:	0	0	0	0		5	1	0	0
PHEOCHROMOCYTOMA, MEDULLA, BENIGN, PRIMARY:	11	4	1	17		0	1	0	2
PHEOCHROMOCYTOMA, MEDULLA, BENIGN, PRIMARY: (TWO)	4	0	0	3		0	0	1	0
PHEOCHROMOCYTOMA, MEDULLA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	2	0		0	0	0	1
PHEOCHROMOCYTOMA, MEDULLA, MALIGNANT, PRIMARY, METASTASIS:	0	0	0	2		1	1	0	0
PHEOCHROMOCYTOMA, MEDULLA, BENIGN OR MALIGNANT, PRIMARY, METASTASIS OR NO METASTASIS:	15	4	3	22	**	1	2	1	3
BONE (NO. OF TISSUES EXAMINED)	50	50	50	50		50	50	50	50
OSTEOGENIC SARCOMA, HEAD, MALIGNANT, PRIMARY, METASTASIS:	0	0	0	1		0	0	0	0
BRAIN (NO. OF TISSUES EXAMINED)	50	26	18	50		50	14	11	50
CARCINOMA, PITUITARY, MALIGNANT, SECONDARY:	0	1	0	0		0	0	0	0
SQUAMOUS CELL CARCINOMA, MENINGES, MALIGNANT, SECONDARY:	0	0	0	1		0	0	0	0
ASTROCYTOMA, BENIGN, PRIMARY:	0	0	0	0		0	0	0	1
ASTROCYTOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	1		1	0	0	0
ASTROCYTOMA, BENIGN OR MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	1	**	1	0	0	1
OLIGODENDROGLIOMA, BENIGN, PRIMARY:	1	0	0	0		0	0	0	0
SCHWANNOMA, OLFACTORY LOBE, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	0		1	0	0	0
CERVIX (NO. OF TISSUES EXAMINED)	-	-	-	-		48	11	10	50
STROMAL CELL SARCOMA, MALIGNANT, PRIMARY, METASTASIS:	-	-	-	-		0	0	2	0

@ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
** THIS LINE REPRESENTS THE COMBINATION OF TWO OR MORE PRECEDING LINES WITH SIMILAR OBSERVATIONS AND LOCATORS.
- INDICATES NOT APPLICABLE.

30

APPENDIX - 1

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 241 of 959

TABLE 91 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: CHRONIC TOXICITY/ONCOGENICITY STUDY IN FISCHER 344 RATS - FINAL REPORT

TUMOR INCIDENCE SUMMARY@ - ALL MODES OF DEATH - 24 MONTHS

SEX	MALES				FEMALES			
DOSE IN MG/KG/DAY	0	5	75	150	0	5	75	150
NUMBER OF RATS EXAMINED	50	50	50	50	50	50	50	50
COLON (NO. OF TISSUES EXAMINED)	50	25	17	50	50	11	10	50
LEIOMYOMA, BENIGN, PRIMARY:	0	1	0	0	0	0	0	0
HEART (NO. OF TISSUES EXAMINED)	50	50	50	50	50	11	12	50
SQUAMOUS CELL CARCINOMA, MALIGNANT, SECONDARY:	0	0	1	0	0	0	0	0
SCHWANNOMA, BENIGN, PRIMARY:	0	0	0	0	0	0	0	1
KIDNEYS (NO. OF TISSUES EXAMINED)	50	50	50	50	50	50	50	50
ADENOMA, TUBULE(S), BENIGN, PRIMARY:	0	1	0	0	0	0	0	0
LIVER (NO. OF TISSUES EXAMINED)	50	50	50	50	50	50	50	50
ADENOMA, HEPATOCELLULAR, BENIGN, PRIMARY:	2	1	1	2	0	0	2	0
CARCINOMA, HEPATOCELLULAR, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	0	1	0	0	0
LUNGS (NO. OF TISSUES EXAMINED)	50	50	50	50	50	50	50	50
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY:	0	1	0	0	1	0	0	1
PHEOCHROMOCYTOMA, MEDULLA, MALIGNANT, SECONDARY:	0	0	0	1	1	1	0	0
CHONDROSARCOMA, MALIGNANT, SECONDARY, METASTASIS:	0	1	0	0	0	0	0	0
OSTEOGENIC SARCOMA, MALIGNANT, SECONDARY:	1	0	0	0	0	0	0	0
LYMPH NODE - MISCELLANEOUS (NO. OF TISSUES EXAMINED)	8	7	6	7	15	16	9	28
CARCINOMA, UTERUS, MALIGNANT, SECONDARY:	0	0	0	0	0	0	0	1
MAMMARY GLAND (NO. OF TISSUES EXAMINED)	49	25	24	48	50	35	25	50
FIBROADENOMA, BENIGN, PRIMARY:	1	0	3	0	6	3	4	2

@ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
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- INDICATES NOT APPLICABLE.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 242 of 959

TABLE 91 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: CHRONIC TOXICITY/ONCOGENICITY STUDY IN FISCHER 344 RATS - FINAL REPORT

TUMOR INCIDENCE SUMMARY[@] - ALL MODES OF DEATH - 24 MONTHS

SEX	MALES					FEMALES			
DOSE IN MG/KG/DAY	0	5	75	150		0	5	75	150
NUMBER OF RATS EXAMINED	50	50	50	50		50	50	50	50
MESENTERIC TISSUES (NO. OF TISSUES EXAMINED)	50	48	48	50		50	47	48	50
FIBROMA, BENIGN, PRIMARY:	0	1	0	0		0	0	0	0
LEIOMYOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	0		0	0	1	0
UNDIFFERENTIATED SARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	0		0	1	0	0
MULTIPLE ORGANS (NO. OF TISSUES EXAMINED)	50	50	49	49		50	37	36	50
PHEOCHROMOCYTOMA, MALIGNANT, SECONDARY:	0	0	0	1		0	0	0	0
SQUAMOUS CELL CARCINOMA, MALIGNANT, SECONDARY:	0	0	0	0		0	0	0	1
UNDIFFERENTIATED CARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	1	0		0	0	0	0
UNDIFFERENTIATED CARCINOMA, MALIGNANT, PRIMARY, METASTASIS:	0	0	0	0		1	0	0	0
UNDIFFERENTIATED CARCINOMA, MALIGNANT, PRIMARY, METASTASIS OR NO METASTASIS:	0	0	1	0	**	1	0	0	0
MESOTHELIOMA, MALIGNANT, PRIMARY:	1	0	1	2		0	0	0	0
OSTEOGENIC SARCOMA, MALIGNANT, SECONDARY:	0	0	0	1		0	0	0	0
STROMAL CELL SARCOMA, MALIGNANT, SECONDARY:	0	0	0	0		0	0	2	0
HISTIOCYTIC SARCOMA, MALIGNANT, PRIMARY:	0	0	0	0		0	0	1	1
LEUKEMIA - LARGE GRANULAR LYMPHOCYTE (FISCHER RAT), MALIGNANT, PRIMARY:	16	21	14	8		10	11	8	12
ORAL TISSUES (NO. OF TISSUES EXAMINED)	50	25	17	50		50	11	10	50
SQUAMOUS CELL CARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	1	1	0	0		0	0	0	0
SQUAMOUS CELL CARCINOMA, MALIGNANT, PRIMARY, METASTASIS:	0	0	0	0		0	0	0	1
SQUAMOUS CELL CARCINOMA, MALIGNANT, PRIMARY, METASTASIS OR NO METASTASIS:	1	1	0	0	**	0	0	0	1
OVARIES (NO. OF TISSUES EXAMINED)	-	-	-	-		49	11	10	50
GRANULOSA - THECAL CELL TUMOR, BENIGN, PRIMARY:	-	-	-	-		1	0	0	1

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EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 243 of 959

TABLE 91 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: CHRONIC TOXICITY/ONCOGENICITY STUDY IN FISCHER 344 RATS - FINAL REPORT

TUMOR INCIDENCE SUMMARY@ - ALL MODES OF DEATH - 24 MONTHS

SEX	MALES				FEMALES			
DOSE IN MG/KG/DAY	0	5	75	150	0	5	75	150
NUMBER OF RATS EXAMINED	50	50	50	50	50	50	50	50
PANCREAS (NO. OF TISSUES EXAMINED)	50	25	17	50	50	12	11	50
ADENOMA, ISLETS, BENIGN, PRIMARY:	4	1	0	6	0	0	0	0
CARCINOMA, ISLETS, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	2	0	0	0	0	0
PITUITARY (NO. OF TISSUES EXAMINED)	50	32	28	49	50	29	21	50
ADENOMA, ANTERIOR (PARS DISTALIS), BENIGN, PRIMARY:	19	15	12	9	21	15	9	1
ADENOMA, PARS INTERMEDIA, BENIGN, PRIMARY:	1	1	1	0	1	0	0	0
CARCINOMA, ANTERIOR (PARS DISTALIS), MALIGNANT, PRIMARY, NO METASTASIS:	1	1	0	1	1	2	1	0
PREPUTIAL OR CLITORAL GLANDS (NO. OF TISSUES EXAMINED)	4	1	4	7	5	2	5	4
ADENOCARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	1	0	0	0	0
CARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	1	1	0	0	0
SQUAMOUS CELL CARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	2	0	0	1	1	0
RECTUM (NO. OF TISSUES EXAMINED)	50	25	17	50	50	11	10	50
ADENOMA, BENIGN, PRIMARY:	0	1	0	0	0	0	0	0
SKIN AND SUBCUTIS (NO. OF TISSUES EXAMINED)	50	33	32	50	50	34	23	50
ADENOMA, SEBACEOUS GLANDS, BENIGN, PRIMARY:	0	0	0	1	0	0	0	0
BASAL CELL ADENOMA, BENIGN, PRIMARY:	0	2	0	0	0	0	0	0
BASAL CELL CARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	1	0	0	0	0	0	0
KERATOACANTHOMA, BENIGN, PRIMARY:	2	1	0	2	0	0	0	0

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33

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 244 of 959

TABLE 91 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: CHRONIC TOXICITY/ONCOGENICITY STUDY IN FISCHER 344 RATS - FINAL REPORT

TUMOR INCIDENCE SUMMARY[@] - ALL MODES OF DEATH - 24 MONTHS

SEX	MALES					FEMALES			
DOSE IN MG/KG/DAY	0	5	75	150		0	5	75	150
NUMBER OF RATS EXAMINED	50	50	50	50		50	50	50	50
SKIN AND SUBCUTIS (CONTINUED)									
SQUAMOUS CELL CARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	1	0		0	0	0	0
SQUAMOUS CELL CARCINOMA, MALIGNANT, PRIMARY, METASTASIS:	0	0	0	1		0	0	0	0
SQUAMOUS CELL CARCINOMA, MALIGNANT, PRIMARY, METASTASIS OR NO METASTASIS:	0	0	1	1	**	0	0	0	0
SQUAMOUS PAPILLOMA, BENIGN, PRIMARY:	1	1	2	0		0	0	0	0
FIBROMA, BENIGN, PRIMARY:	4	2	4	3		0	1	0	0
FIBROMA, BENIGN, PRIMARY: (TWO)	1	0	0	0		0	0	0	0
FIBROMA, BENIGN, PRIMARY:	5	2	4	3	**	0	1	0	0
FIBROSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	1	0		0	0	0	0
FIBROUS HISTIOCYTOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	1	0		0	0	0	0
LIPOMA, BENIGN, PRIMARY:	0	0	1	0		0	0	0	0
HISTIOCYTIC SARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	1	0		0	0	0	0
SPINAL CORD (CERV, THOR & LUM) (NO. OF TISSUES EXAMINED)	49	25	17	50		50	11	10	50
ASTROCYTOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	1	0	0		0	0	0	0
SPLEEN (NO. OF TISSUES EXAMINED)	50	33	23	50		50	13	16	50
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	1	0	0	0		0	0	1	0
LEUKEMIA - LARGE GRANULAR LYMPHOCYTE (FISCHER RAT), MALIGNANT, PRIMARY:	1	0	0	0		1	0	1	0
STOMACH (NO. OF TISSUES EXAMINED)	50	50	50	50		50	49	50	50
ADENOMA, GLANDULAR MUCOSA, BENIGN, PRIMARY:	0	0	0	1		0	0	0	0

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34

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 245 of 959

TABLE 91 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: CHRONIC TOXICITY/ONCOGENICITY STUDY IN FISCHER 344 RATS - FINAL REPORT

TUMOR INCIDENCE SUMMARY@ - ALL MODES OF DEATH - 24 MONTHS

SEX	MALES					FEMALES			
DOSE IN MG/KG/DAY	0	5	75	150		0	5	75	150
NUMBER OF RATS EXAMINED	50	50	50	50		50	50	50	50
TESTES (NO. OF TISSUES EXAMINED)	50	50	50	50		-	-	-	-
LEYDIG CELL TUMOR, BENIGN, PRIMARY:	4	2	2	10		-	-	-	-
LEYDIG CELL TUMOR, BENIGN, PRIMARY: (TWO)	44	45	45	36		-	-	-	-
LEYDIG CELL TUMOR, BENIGN, PRIMARY:	48	47	47	46	**	-	-	-	-
THYROID GLAND (NO. OF TISSUES EXAMINED)	50	49	50	50		42	41	41	40
ADENOCARCINOMA, FOLLICLE(S), MALIGNANT, PRIMARY:	0	1	0	1		0	0	1	0
ADENOMA, FOLLICLE(S), BENIGN, PRIMARY:	2	1	1	1		0	0	0	0
ADENOMA, FOLLICLE(S), BENIGN, PRIMARY: (TWO)	0	0	0	1		0	0	0	0
ADENOMA, FOLLICLE(S), BENIGN, PRIMARY:	2	1	1	2	**	0	0	0	0
ADENOMA, PARAFOLLICULAR CELLS, BENIGN, PRIMARY:	12	11	10	10		11	8	5	8
ADENOMA, PARAFOLLICULAR CELLS, BENIGN, PRIMARY: (TWO)	0	0	1	0		0	1	1	0
ADENOMA, PARAFOLLICULAR CELLS, BENIGN, PRIMARY:	12	11	11	10	**	11	9	6	8
CARCINOMA, PARAFOLLICULAR CELLS, MALIGNANT, PRIMARY:	0	1	3	0		2	3	0	1
TONGUE (NO. OF TISSUES EXAMINED)	50	25	17	50		50	11	10	50
SQUAMOUS PAPILLOMA, BENIGN, PRIMARY:	0	0	0	0		1	1	0	0
TRIGEMINAL GANGLIA (NO. OF TISSUES EXAMINED)	0	1	0	0		0	1	0	0
SCHWANNOMA, MALIGNANT, PRIMARY, NO METASTASIS:	-	1	-	-		-	0	-	-
URINARY BLADDER (NO. OF TISSUES EXAMINED)	50	25	18	50		49	10	10	50
LEIOMYOMA, BENIGN, PRIMARY:	0	0	0	1		0	0	0	0

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35

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 246 of 959

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

MEMORANDUM

SUBJECT: 2,4 Dichlorophenoxyacetic acid (2,4-D) - Review of Additional Histopathology Data from Mouse Carcinogenicity Study and Rat Combined Chronic Toxicity/Carcinogenicity Study

DP Barcode: D236685; D239909
PC Code: 030001
Case: 838294; 818706
Submission: S525309; S531709

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer
Reregistration Branch I, Health Effects Division (7509C) Virginia A. Dobozy 2/9/99

TO: Richard Dumas/Jill Bloom
Special Review and Reregistration Division

THRU: Whang Phang, Ph.D., Branch Senior Scientist
Reregistration Branch I, Health Effects Division (7509C) Whang Phang 2/9/99

Action Requested: Review additional data from Rat Combined Chronic Toxicity/Carcinogenicity Study (MRID 44284501) and Mouse Carcinogenicity Study (MRID 44284502)

Recommendation: The additional data do not alter the conclusions of either study concerning the carcinogenic potential of 2,4-D. The chemical should continue to be classified as a Group D carcinogen pending receipt of the additional epidemiological data referred to in the July 17. 1996 Cancer Peer Review Committee report.

TABLE 91 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: CHRONIC TOXICITY/ONCOGENICITY STUDY IN FISCHER 344 RATS - FINAL REPORT

TUMOR INCIDENCE SUMMARY@ - ALL MODES OF DEATH - 24 MONTHS

SEX	MALES					FEMALES			
DOSE IN MG/KG/DAY	0	5	75	150		0	5	75	150
NUMBER OF RATS EXAMINED	50	50	50	50		50	50	50	50
UTERUS (NO. OF TISSUES EXAMINED)	-	-	-	-		49	22	30	50
ADENOCARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	-	-	-	-		4	0	1	0
ADENOMA, BENIGN, PRIMARY:	-	-	-	-		0	0	1	0
CARCINOMA, MALIGNANT, PRIMARY, METASTASIS:	-	-	-	-		0	0	0	1
ENDOMETRIAL STROMAL POLYP, BENIGN, PRIMARY:	-	-	-	-		16	10	13	17
ENDOMETRIAL STROMAL POLYP, BENIGN, PRIMARY: (TWO)	-	-	-	-		2	2	2	1
ENDOMETRIAL STROMAL POLYP, BENIGN, PRIMARY: (THREE)	-	-	-	-		1	0	0	0
ENDOMETRIAL STROMAL POLYP, BENIGN, PRIMARY:	-	-	-	-	**	19	12	15	18
SQUAMOUS CELL CARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	-	-	-	-		0	0	1	0
FIBROMA, BENIGN, PRIMARY:	-	-	-	-		0	0	1	0
LEIOMYOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	-	-	-	-		1	0	0	0
UNDIFFERENTIATED SARCOMA, MALIGNANT, PRIMARY, METASTASIS:	-	-	-	-		1	0	0	0

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36

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EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 248 of 959

BACKGROUND

Additional data from the mouse carcinogenicity study and rat combined chronic toxicity/carcinogenicity study were requested in the HED Carcinogenicity Peer Review Committee (CPRC) report of the July 17, 1998 meeting. In the mouse carcinogenicity study, there was an increase in hemangiosarcomas of the spleen in male mice at the low and mid doses, which was not sustained at the highest dose, however the slides from all animals at the low and mid doses were not evaluated. There were two rat combined chronic toxicity/carcinogenicity studies reviewed by the CPRC. In a 1986 study, there was a statistically significant trend for astrocytomas in male rats, but there was no pair-wise significance. The number of tumors in the control, 1, 5, 15 and 45 mg/kg/day groups was 1/60, 0/60, 0/60, 2/60 and 6/60, respectively. The incidence in both the treated and control animals exceeded the historical control incidences for this tumor in studies conducted by the National Toxicology Program. In addition, the CPRC concluded that the high dose was not adequate for testing the chemical's carcinogenicity potential. Therefore, a 1995 study was conducted at higher doses. In this study, there was one astrocytoma in the brain of males at the high dose vs. none in the control males. There was one tumor each in the control group and the high dose group for females. However, all the brains of animals in the low- and mid-dose groups were not examined. The CPRC concluded that these tissues from male animals should be examined. In addition to requesting the additional tissue examinations, the report also stated that a better characterization of the impurities in the test materials used in the original and new study might be helpful in explaining the conflicting results (i.e., astrocytomas in male rats).

DATA REVIEW

1) Rat Brains - MRID 44284501

The brains of 24 male rats dosed at 5 mg/kg/day and 32 males dosed at 75 mg/kg/day were examined by standard light microscopy. No additional neoplasms were observed. The incidence of astrocytomas can be found on page 7 of the July 17, 1996 CPRC report. The findings were consistent with rats of this strain (Fischer 344) and age (> 2 years). When the data from all the rats at the 24-month sacrifice were compared, the only observations which were statistically significantly increased were swollen axon, very slight, in the mid-dose group and swollen axon, gracile nucleus, very slight, in the low- and mid-dose groups.

2) Mice Spleens - MRID 44284502

The spleens of 35 male mice in the 5 mg/kg/day group and 33 in the 62.5 mg/kg/day group were examined by light microscopy. No neoplasms were observed. When all the data on the 50 animals/group were combined, there was no dose-related increase in hemangiosarcomas or in possible preneoplastic lesions, such as splenic extramedullary hematopoiesis, as presented in the following Table 1.

Table 1: Incidence of Splenic Lesions in Male Mice[a,b]

	Dosage Levels (ppm)			
	0	5	62.5	125
Increased extramedullary hematopoiesis, slight	10	4	5	4
Increased extramedullary hematopoiesis, moderate	3	4	11	3
Increased extramedullary hematopoiesis, any severity**	13	8	16	7
Hemangiosarcoma, malignant, primary, no metastasis	0	5#	3	1
Hemangiosarcoma, malignant, primary, metastasis	0	1#	1	1
Hemangiosarcoma, malignant, primary, metastasis or no metastasis**	0	6	4	2
Hemangiosarcoma (liver), malignant, secondary	1	0	0	0
Lymphosarcoma, malignant, primary, no metastasis	0	0	0	2

a Extracted from Table 2 (page 10) of MRID 44284502
b Fifty mice were examined in all dose groups.
** This line represents the combination of two or more preceding lines with similar observations and locators.
# Change from previously reported data. One of the hemangiosarcomas from the 5 mg/kg/day dose group had a metastasis to the liver; the total remains six for this dose group, five primary splenic hemangiosarcomas without metastasis and one with metastasis.

The registrant argues that the splenic hemangiosarcomas should not be considered treatment-related for several reasons:

1) There was no dose response.

2) A similar pattern of vascular tumors was not present in any other organ. Since neoplasms of blood vessels may arise in any organ, it is misleading to tabulate and analyze hemangiosarcomas by an individual organ. It is preferable to examine the incidence from all sites. In this study, when the incidence of hemangiosarcomas from all sites, or of total vascular tumors (hemangiomas and hemangiosarcomas) from all sites, is examined, there is no pattern of dose responsiveness. These data are presented in the Table 2 below.

Table 2: Incidence of Vascular Tumors[a,b]

	Dose Levels (ppm)			
	0	5	62.5	125
Total number of mice with hemangioma, benign, any site	2	2	2	3
Number of mice with hemangiosarcoma				
Primary site in heart	1	0	0	0
Primary site in liver	1	0	1	1
Primary site in skin	0	1	0	0
Primary site in spleen	0	6	4	2
Total number of mice with hemangiosarcoma, malignant, any site	2	7	5	3
Total number of mice with hemangioma and/or hemangiosarcoma, any site	4	7	7	5

a Extracted from Table 3 (page 11) of MRID 44284502
b Fifty animals were examined in all dose groups.

3) Data from all dose groups fall within the historical control range. Historical control data were submitted from 5 dietary studies conducted from 7/90 to 8/95. Using only those studies which are within two to three years of the study in question in accordance with the proposed *Guidelines for Carcinogen Risk Assessment*, the following data are applicable.

Study & Date Reported	B (8/95)	C (5/95)	D (2/95)
Total Number of Mice with Hemangioma, Benign, Any Site	N.R.	0/50	N.R.
Total Number of Mice with Hemangiosarcoma, Malignant, Any Site	N.R.	8/50	N.R.
Total Number of Mice with Hemangioma and/or Hemangiosarcoma, Any Site	6/50	8/50	5/50

N.R. Data not reported for this category in final report (data reported for benign or malignant vascular neoplasms for each organ separately).

4) The incidence of splenic hemangiosarcomas was not statistically significantly increased.

CONCLUSIONS

The additional data do not alter the findings of the study. The data were discussed with the present Chairman of the Cancer Assessment Review Committee and it was concluded that another meeting of the Committee is not required. The chemical should remain a Group D carcinogen, as classified at July 17, 1996 CPRC meeting.

4) The incidence of splenic hemangiosarcomas was not statistically significantly increased.

CONCLUSIONS

The additional data do not alter the findings of the study. The data were discussed with the present Chairman of the Cancer Assessment Review Committee and it was concluded that another meeting of the Committee is not required. The chemical should continue to be classified as a Group D carcinogen pending receipt of the additional epidemiological data referred to in the July 17, 1996 Cancer Peer Review Committee report.

cc: William Burnam, SAB
Cancer File, SAB

[2,4-D-2-BUTOXYETHYL ESTER] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Amendment to DER for MRID 41527101 - Developmental Toxicity Study - Rat [2,4-D-2-BUTOXYETHYL ESTER]

This Amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 008186]. The NOAEL/LOAEL have been modified from those in the original DER

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/4/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P. H. Virginia A Dobozy 12/8/00
Reregistration Branch I, Health Effects Division (7509C)
TXR #005 1360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rat § 83-3(a)/OPPTS 870.3700
P.C. CODE: 030053 REREGISTRATION CASE NO/CASE NO.: 818726
CASWELL NO.:315AI CAS NO.: 1929-73-3
TEST MATERIAL (PURITY) :2,4-D butoxyethyl ester [95.6%; acid equivalent 65.8%]
SYNONYMS: 2,4-D BEE; dichlorophenoxyacetic acid, 2-butoxyethyl ester
SPONSOR: The Dow Chemical Company

CITATION: Schroeder, R. E. (1990). A Teratogenicity Study in Rats with 2-Butoxyethyl Ester of 2,4-D. Bio/dynamics, Inc. Study No. HET K-007722-017, May 9, 1990. MRID 41527101. Document No. 008186. Unpublished.

EXECUTIVE SUMMARY: In developmental toxicity studies [MRID 41527101], female CD® [Sprague-Dawley derived] rats [30/group] were administered butoxyethyl ester of 2,4-dichlorophenoxyaceticacid [95.6%] *via* gavage at dose levels of 0 [corn oil], 25 mg/kg/day, 75 mg/kg/day, and 185 mg/kg/day from gestation day 6 through gestation day 15.

There were no deaths. One high-dose female that was not pregnant displayed decreased food consumption, along with ataxia, emaciation, and negative activity by day 11, and poor condition by day 14 of gestation. There was a dose-related increase in the incidence of excessive salivation at the mid- and high-dose levels during the dosing period [0%, 7%, 33%, 57%]. Decreased body-weight gain [88% of control overall] was observed at the high-dose level during the dosing period only. Corrected body-weight gain for the entire gestation period was comparable among the groups, with the high-dose dams gaining 7% less than the controls. A slightly lower food consumption was noted at the high-dose level during the first week of dosing [92% of control]. There was a statistically-significant decrease in RBC and increase in reticulocyte count in females dosed at 185 mg/kg/day.

There were no abortions, and the pregnancy rate was comparable among the groups. The numbers of corpora lutea, implantation, live fetuses, and resorptions [both total and per dam], as well as pre- and post-implantation losses were

comparable among the groups. Fetal body weight was not adversely affected, and the sex ratio was comparable among the groups. There was a statistically-significant increase in the fetal incidence of total skeletal variations at the high-dose level compared to the control incidence, but the litter incidence was comparable among the groups. There was an increased incidence [litter and fetal basis] with increasing dose of incomplete ossification of the following bones: supraoccipital, squamosal, maxilla, and the 4th sternebra. Additionally, there was an increase in the incidence of rudimentary rib(s), short ribs, unilateral 14th rib(s), and the presence of 14 rib pairs at the high-dose level compared to the control. There was no apparent effect on the incidence of any malformation, although skeletal malformations occurred only in the mid- [2 fetuses in 2 litters] and high-[2 fetuses in 2 litters] dose groups, and the external malformation of one high-dose fetus [filamentous tail] is reported in the historical control at an incidence rate of 1 fetus in one litter out of 7193 fetuses in 527 litters.

The maternal toxicity NOAEL is 75 mg/kg/day, and the maternal toxicity LOAEL is 185 mg/kg/day, based on decreased body-weight gain on days 6-16, decreased food consumption, decreased red blood cells, and increased reticulocytes. The NOAEL for developmental toxicity is 75 mg/kg/day, and the developmental toxicity LOAEL is 185 mg/kg/day, based on an increase in the incidence of skeletal malformations/variations [incompletely ossified supraoccipital, squamosal(s), maxilla, and 4th sternebra, and 14 rib pairs, short ribs, rudimentary first lumbar rib(s), and unilateral 14th rib(s)].

This guideline developmental toxicity study is classified Acceptable, and it satisfies the guideline [OPPTS 870.3700; §83-3(a)] for a developmental toxicity study in the rodent.

NOTE: The developmental toxicity NOAEL/LOAEL differ from the original DER. In this subsequent review of the data, the LOAEL was determined to be 185 mg/kg/day [high dose] instead of 75 mg/kg/day [original DER], the dose level where the endpoint [total skeletal malformations/variations] was statistically-significantly increased.

[2,4-D-2-BUTOXYETHYL ESTER] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Gravid Uterine Weight/Corrected Body Weight/Gain [grams]				
Dose/Day	0 mg/kg/day	25 mg/kg/day	75 mg/kg/day	185 mg/kg/day
body weight				
0	223±20	217±21	218±21	217±19
6	260±23	259±25	254±26	253±21
9	271±24	268±22	265±25	261±21
12	292±25	287±25	285±26	278±24
16	324±27	319±22	317±27	309±24
20	392±32	391±25	387±32	377±28
gravid uterine weight	78.2±18.6	79.9±10.9	80.5±10.4	75.7±11.1
corrected body weight	313.8±25.1	311.2±22.8	306.2±27.5	301.5±24.0
corrected BWG	54.3±11.7	51.9±10.7	52.0±10.2	48.4±10.7 [89]√

√[% of control]; data from Appendix C page 56 and Appendix E, page 67 of the report

Table 2. Skeletal Variations				
Dose/Variation/%	0 mg/kg/day	25 mg/kg/day	75 mg/kg/day	185 mg/kg/day
incompletely ossified:	fetuses/litters✖	fetuses/litters	fetuses/litters	fetuses/litters
interparietal	15%/48%	22%/67%	24%/60%	22%/70%
supraoccipital	8%/24%	11%/37%	13%/37%	16%/44%
squamosal(s)	4%/28%	5%/22%	9%/37%	14%/52%
malar(s)	2%/12%	3%/15%	8%/27%	5%/22%
maxilla	0	1%/7%	3%/13%	3%/15%
nasal(s)	1%/8%	2%/15%	5%/17%	3%/15%
parietal(s)	3%/16%	3%/15%	4%/17%	4%/26%
4th sternebrae	7%/24%	5%/26%	5%/30%	11%/41%
not ossified	fetuses/litters	fetuses/litters	fetuses/litters	fetuses/litters
hyoid	22%/60%	20%/63%	23%/63%	28%/78%
5th sternebrae	31%/80%	18%/63%	23%/60%	48%/85%
rib(s)	fetuses/litters	fetuses/litters	fetuses/litters	fetuses/litters
presence of 14th rib pairs	0.5%/4%	0.5%/4%	0	2%/11%
rib(s) - short	2%/4%	1%/7%	2%/10%	6%/19%
rib(s) - rudimentary	1%/4%	0.5%/4%	2%/7%	4%/19%
rib(s) - 1st lumbar rudimentary	10%/40%	9%/37%	21%/70%	25%/67%
ribs(s) - 14th unilateral	1%/4%	0.5%/4%	2%/10%	5%/22%
# evaluated				
fetuses	174	183	205	181
litters	25	27	30	27

✖[% fetuses/% litters], calculated by reviewer; data from Appendix O, pages 323-328 of the report

GUIDELINE: 83-3

Primary Review by: Karen E. Whitby, Ph.D.
Toxicologist, Review Section II, Toxicology Branch II/HED
(H7509C)

Secondary Review by: K. Clark Swentzel
Section Head, Review Section II, Toxicology Branch II/HED
(H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3

EPA Identification No.s: EPA MRID (Accession) No. 415271-01
EPA ID No. 030025-1
EPA Record No. 268,213
Caswell No. 315AI
HED Project No. 0-1707

Test Material: 2-Butoxyethyl Ester of 2,4-D

Synonyms: 2,4-D BEE

Sponsor: The Dow Chemical Co., The Toxicology Research Lab., 1803 Building, Midland MI

Study Number(s): HET K-007722-017 (The Dow Chemical Co.)
Project No. 89-3467 (Bio/dynamics)

Testing Facility: Bio/dynamics, Inc., East Millstone, New Jersey 08875

Title of Report: A Teratogenicity Study in Rats With 2-Butoxyethyl Ester of 2,4-D

Author(s): Raymond E. Schroeder

Report Issued: May 9, 1990

Dates of In-Life Phase: December 6, 1989 to January 11, 1990

Conclusions: Maternal toxicity was evidenced at 185 mg/kg by significant effects on body weight gain, food consumption, and hematology. Developmental toxicity was observed at 75 mg/kg and above as skeletal variations.

Maternal NOEL = 75 mg/kg
Maternal LOEL = 185 mg/kg
Developmental Toxicity NOEL = 25 mg/kg
Developmental Toxicity LOEL = 75 mg/kg

Core Classification: Minimum

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: 2,4-D Butoxyethyl Ester (2,4-D BEE), AGR276426
Purity: 95.6%
Description: dark brown liquid
Lot No.: AGR 276426
Contaminant: none provided

Vehicle(s): Mazola® Corn Oil
Purity: 100%
Description: clear yellow liquid
Lot No. SEP 26 90B
Contaminant: none provided

Test Animal(s): Species: Rat
Strain: CD® (Sprague-Dawley derived)
Source: Charles River Laboratories, Inc.
Portage, MI 49081
Age at Initiation of Mating: ♀ 57 days/♂ 48 weeks
♀ Weight: Range on Day 0, 169-275 g (mean = 218 g)

B. Study Design

This study was designed to assess the developmental toxicity potential of 2,4-D Butoxyethyl Ester when administered by gavage to rats on gestation days 6 through 15, inclusive.

Mating

Females were mated with males nightly in a 1:1 ratio. Animals not utilized in the study that remained from the original population were sacrificed and discarded after all study groups had been filled and the last mated females within study groups had initiated dosing.

Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	30
Low Dose	25	30
Mid Dose	75	30
High Dose	185	30

Dosing:

The control group received the vehicle (corn oil) by gavage. All doses were administered in a volume of 5 ml/kg, and were prepared once prior to initiation of treatment with sufficient quantity prepared to accommodate the entire dosing regimen. The volume of test article used for the preparation of the dosing solutions was adjusted to compensate for the purity. The dosing solutions were analyzed for concentration, homogeneity, and stability. Initial dose volumes were derived from day 6 body weights and were adjusted for each animal during the remaining portion of the treatment period to the most recent body weights.

Observations

Animals were checked twice daily for signs of obvious pharmacologic or toxicologic effects and mortality/morbidity. Females were given an additional detailed exam on gestation days 0, 6-15, and 20. Dams were sacrificed on day 20 of gestation. Animals were weighed on days 0, 6, 9, 12, 16, and 20. Food consumption was recorded days 0-6, 6-11, 11-16, and 16-20. Bodyweight was used to calculate the grams of feed consumed/kg of bodyweight/day.

A gross post mortem exam was performed. Examinations at sacrifice consisted of: the external surface, all orifices, the cranial cavity, carcass, the external surface of the spinal cord, and sectioned surfaces of the brain, nasal cavity and paranasal sinuses; thoracic, abdominal and pelvic cavities, viscera, cervical tissues and organs, and kidney and liver weights. The intact uterus and ovaries were removed from the abdominal cavity, weighed and then number and location of live fetuses, dead fetuses, late resorptions, early resorptions, corpora lutea, and implantation sites were recorded. In the event implants were not evident, the uterus was stained with ammonium sulfide. Females found to have implants when observed in this manner were included as pregnant; the number of foci noted for these females were not included in the calculation of uterine implantation data.

The fetuses were examined in the following manner. Each fetus was individually identified. weighed, sexed, and examined for external malformations/variations (including palatal defect).

Approximately one half of the fetuses in each litter were examined for visceral defects using a technique similar to that of Staples (Staples, 1974), shortly after removal from the uterus. The fetuses were decapitated, and the heads were fixed in Bouin's for subsequent exam by razor blade sectioning. Upon completion of the exam of fetal tissues in the thoracic and abdominal cavities under a dissecting scope, the viscera were removed and placed in 70% ethanol for fixation.

The other half of the fetuses were euthanized with ether, eviscerated (internally sexed), and processed for staining by Alizarin Red S (Crary, 1962) via a method modified by Bio/dynamics.

Historical control data were provided to allow comparison with concurrent controls.

Statistical Analysis

Mean maternal bodyweight, bodyweight change, gravid uterus weight, day 20 corrected weights, net maternal bodyweight change, mean food consumption, mean organ weight data (absolute and relative to corrected day 20 bodyweight), and reproduction data were statistically evaluated as follows. First, Bartlett's test was performed to determine if the groups had equal variance. If the variances were equal, parametric tests (standard one way ANOVA using the F distribution) were used; if not, nonparametric procedures were used. Finally, Dunnett's test was used to determine which means (when appropriate) were significantly different from the control. The Kruskal-Wallis test was used to determine which treatments differed from control for testing the equality of means by a nonparametric procedure.

The incidence of litters with resorption sites, mortality rates, pregnancy rates, and incidences of malformations/variations was first analyzed by a standard chi-square to determine if the proportion of incidences differed between the groups tested. Next, each treatment group was compared to the control group using a 2X2 Fisher Exact test; the significance level was corrected via the Bonferroni inequality to assure an overall test of the stated significance level. Finally, Armitage's test for linear trend in the dosage groups was performed. If any one cell had an expected value less than 5, the chi-square and Armitage's tests were not reported. In this instance, only the Fisher Exact test (corrected via Bonferroni inequality) was performed and reported.

Compliance

A signed Statement of No Confidentiality Claims was provided.

A signed Good Laboratory Practice Statement (EPA GLP's) was provided.

A signed Flagging Statement was provided.

A signed Quality Assurance Statement was provided.

C. Results

Maternal Toxicity

Mortality

There was no mortality in the control or treated groups.

Clinical Observations

Excessive salivation was seen with increased incidence, particularly among the animals at the mid- and high-dose groups. The incidences of females with excessive salivation noted at one or more intervals during the treatment period for the control, low-, mid-, and high-dose groups were 0%, 6.7% (2/30), 33.3% (10/30), and 56.7% (17/30), respectively.

One non-pregnant female in the high dose group was noted to exhibit ataxia, emaciation, decreased food consumption, and poor condition on day 11 of gestation (6 days after initiation of treatment). Gross postmortem observation did not reveal any lesions that explained the deterioration of the animal.

Bodyweight

The investigators supplied the following data:

Table 1: Bodyweight Gains (grams)[a]

Group mg/kg:	Days 0-6	Days 6-16	Days 16-20	Days 0-20	Corrected Body Weight Gains Dosing P.[1]	Corrected Body Weight Gains Entire[2]
0	37	64	60	169	-14.0	91.2
25	42	60	72	174	-20.2	93.3
75	37	62	70	169	-18.0	88.7
185	36	56* 88	68	160 95	-19.5	84.6 93%

[1] = corrected bodyweight gain for dosing period = bodyweight gain days 6-16 minus gravid uterus weight.
[2] = corrected bodyweight gain for entire gestation period = body weight gain days 0-20 minus gravid uterus weight.
a = Data extracted from Report HET K-007722-017 Appendices C, D, and E (pp. 57-71)
* Significantly different from control ($p \leq 0.05$).
[Some of the above values were calculated by the reviewer from individual animal data]

Food Consumption

The investigators supplied the following data:

Table 2: Mean Maternal Food Consumption (g/kg/day)[a]

Group mg/kg:	Days 0-6	Days 6-11	Days 11-16	Days 16-20	Days 6-20	Days 0-20
0	101	87	94	92	273	374
25	108	82	90	98	271	378
75	106	83	92	94	269	375
185	106	80**	90	92	261	367

[a] Data extracted from Report HET K-007722-017 Appendix F (pp.72-76).

** Significantly different from control ($p \leq 0.01$).

Hematology Data

The majority of the hematological parameters (Table 3) were comparable within the groups, except for the RBC count, which was significantly lower than control (-4.4%). This was also observed in the dose-range finding study. In addition, an increase in the reticulocyte count (+47.4%) was noted in the high dose group relative to the control, which was statistically significant.

Table 3 Mean Hematology Values[a]

mg/kg	0	25	75	185
Hgb (g/dl)	13.0	12.9	12.7	12.7
Hct (%)	35	35	35	35
RBC (mil/ul)	5.72	5.65	5.55	5.47* 4%
Plat (100 T/ul)	13.29	11.94	12.73	12.41
WBC (thous/ul)	12.1	12.1	13.3	12.4
Retic (% RBC)	1.9	2.2	2.1	2.8** 47%

[a] Data taken from Report HET K-007722-017 Appendix J (p.100)

* Significantly different from control ($p \leq 0.05$)
** Significantly different from control ($p \leq 0.01$).

Organ Weight Data

Absolute and relative organ bodyweights (corrected to day 20 gestation bodyweight) were similar among treated and control

Gross Pathological Observations

Gross pathological findings did not indicate an adverse treatment related effect.

Cesarean Section Observations

Table 4: Cesarean Section Observations[a]

Dose (mg/kg):	0	25	75	185
#Animals Assigned	30	30	30	30
#Animals Mated/Inseminated	26	28	30	27
Pregnancy Rate (%)	86.7	93.3[b]	100	90.0
Maternal Wastage				
#Died	0	0	0	0
#Died/pregnant	0	0	0	0
#Non pregnant	4	2	0	3
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	420	435	483	424
Corpora Lutea/dam	16.2 ±2.8	16.1 ±2.0	16.1 ±2.0	15.7 ±2.0
Total Implantation	388	399	451	397
Implantations/Dam	14.9 ±3.4	14.8 ±1.8	15.0 ±2.0	14.7 ±2.1
Total Live Fetuses	362	379	431	375
Live Fetuses/Dam	13.9 ±3.4	14.0 ±1.9	14.4 ±1.9	13.9 ±2.0
Total Resorptions	26	20	20	22
Resorptions/Dam	1.0 ±1.0	0.7 ±0.9	0.7 ±1.0	0.8 0.9
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm)	3.53 ±0.24	3.66 ±0.19	3.62 ±0.23	3.44 ±0.23
Males	3.68 ±0.21	3.75 ±0.18	3.72 ±0.20	3.55 ±0.26
Females	3.43 ±0.23	3.53 ±0.20	3.53 ±0.24	3.33 ±0.22
Preimplantation Loss(%)	9.0	7.8	6.4	6.0
Postimplantation Loss(%)	7.6	5.0	4.3	5.4
Sex Ratio (total ♂/total ♀)	0.9	1.1	0.9	1.0

[a] Data extracted from Report HET K-007722-017 Appendix G (pp.

77-81)

[b] One female was noted to have one focus upon staining of the uterus with ammonium sulfide and is included in calculation of pregnancy rate, but the data for this female was excluded from calculation of uterine implantation data.
<<Some of the above values were calculated by the reviewer from the individual animal data.>>

There was no significant treatment related effect on the parameters investigated.

2. Developmental Toxicity

External Malformations

Table 5: External Examinations[a]

Observations[+]	0 mg/kg	25 mg/kg	75 mg/kg	185 mg/kg
#pups(litters) examined	362(26)	379(27)	431(30)	375(27)
malformations #pups(litters) affected	0	1(1)	0	1(1)
variations #pups(litters) affected	1(1)	0	0	1(1)

[a] Data extracted from Report HET K-007722-017 Appendix G (pp. 77-81)

The incidences noted in the above table did not differ statistically between the control and treated groups. The fetus in the low-dose group that was malformed did not have normal sized eye bulges [upon visceral exam, right-sided microphthalmia and left sided anophthalmia was observed (see visceral examinations below)]. The malformations noted in the fetus in the high-dose group included: filamentous tail; abnormal curvature of the spine; misshapen jaw; and defect of the urethral/anal opening (upon visceral exam this same fetus was noted to have fused and ectopic kidneys).

A fetus in the control group had a shiny appearance, was quite small for the litter (1.5 g), and had a constriction on the genital tubercle. The fetus in the high dose group with multiple external malformations also had a shiny appearance, and was quite small for the litter (1.6 g). The shiny appearance noted in these fetuses was attributed to the thin translucent skin of these developmentally retarded fetuses.

Visceral Examination

Table 6: Visceral Examinations[a,b]

Observations	0 mg/kg	25 mg/kg	75 mg/kg	185 mg/kg
Visceral Malformations				
#pups(litters) examined	188(26)	196(27)	226(30)	195(27)
Retina(s) - folded	0	1(1)	0	0
Microphthalmia	0	1(1)	0	1(1)
Anophthalmia	0	1(1)	0	0
Distension of the Lateral Cerebral Ventricle(s)	0	0	0	1(1)
Distension of the Third Cerebral Ventricle	0	0	0	1(1)
Abn. Pattern of Aortic Arch Vessels	0	0	1(1)	0
Kidney - fused	0	0	0	1(1)
Kidney - ectopic	0	0	0	1(1)
Total Visceral Malform.				
fetal incidence N(%)	0	2(1.0)	1(0.4)	3(1.5)
litter incidence N(%)	0	2(7.4)	1(3.3)	3(11.1)
Visceral Variations				
#pups(litters) examined	188(26)	196(27)	226(30)	195(27)
Kidney(s) - Distended Renal Pelvis (Papilla Present)	0	2(2)	1(1)	1(1)
Ureter(s) - Tortuous	2(1)	2(2)	1(1)	4(4)
Ureter(s) - Distended	2(1)	2(2)	2(1)	1(1)
Total Visceral Variations				
fetal incidence N(%)	4(2.1)	3(1.5)	3(1.3)	5(2.6)
litter incidence N(%)	2(7.7)	3(11.1)	1(3.3)	4(14.8)

[a] fetal [litter] incidence

[b] Data from Report HET K-007722-017 Appendix N (pp. 293-294)

The incidences of the visceral malformations for the treated groups did not differ statistically from the controls.

With the exception of the ocular malformations (anopthalmia and micropthalmia) discussed above in the section on external malformations, the only other malformation noted in the low-dose group was a folded retina. This was observed in a fetus of a

different litter, than the one exhibiting the other ocular defects.

In the high dose group, the 3 fetuses exhibiting visceral malformations are of different litters.

There were no statistically significant treatment related differences found for the visceral variations noted in the above table. Distended and/or tortuous ureters were the most commonly seen visceral variation in this study. Renal variations noted in the above table found by this laboratory in CD rats during 12/30-1/15/87, were such that: 0/163 fetuses (from 22 litters) exhibited distended renal pelvis only, 3/163 fetuses (in 2/22 litters) exhibited distended renal pelvis and distended ureter, and 3/163 fetuses (in 1/22 litters) exhibited distended or tortuous ureter. The historical control data has been appended.

Skeletal Examinations

The incidences of skeletal malformations in the treated groups did not differ statistically from the control when the analyses were performed on the litter or fetal basis. In the mid-dose group, one fetus was observed to have a cervical rib, another fetus in a different litter was found to have wavy ribs. These findings were not noted in the high-dose group. With the exception of the fused cervical transverse process, all skeletal malformations noted in the high-dose group occurred in one fetus. This was the same fetus observed to have numerous malformations during the external exam (shiny appearance, filamentous tail, misshapen lower jaw, and a defect of the urethral/anal openings). Data for this fetus appears in the results of both the visceral and skeletal findings (although not stated, it is apparent that the fetus was not decapitated, and the head fixed in Bouin's and subsequently sectioned, as was standard procedure following the visceral exam). The historical control data for the CD rat, from Bio/dynamics during 1982-1987 Appendix S (p. 448) indicates that of 4,470 fetuses (527 litters) in 23 study groups, indicates that 27 fetuses in 21 litters with malformed fetuses were observed to have wavy ribs. Similarly, the historical incidence for cervical rib was 1 fetus.

Because of the numerous skeletal variations which were observed in this study, only those that occurred with notable frequency are reported in the table.

Table 7: Skeletal Examinations

Observations+	0 mg/kg	25 mg/kg	75 mg/kg	185 mg/kg
Skeletal Malformations				
#pups(litters) examined	174(25)	183(27)	205(30)	181(27)
Mandible - fused	0	0	0	1(1)
Cervical Rib(s)	0	0	1(1)	0
Cervical Transverse Process - fused	0	0	0	1(1)
Thoracic Transverse Process - fused	0	0	0	1(1)
Thoracic Vert. & Corresp. Ribs - absent	0	0	0	1(1)
Lumbar Vert. - absent	0	0	0	1(1)
Sacral Vert. - absent	0	0	0	1(1)
Caudal Vert. - absent	0	0	0	1(1)
Ribs - Wavy	0	0	1(1)	0
Ilia - misaligned	0	0	0	1(1)
total skeletal malformations				
fetal incidence N(%)	0	0	2(1.0)	2(1.1)
litter incidence N(%)	0	0	2(6.7)	2(7.4)
Skeletal Variations				
#pups(litters) examined	174(25)	183(27)	205(30)	181(27)
Incompletely Ossified				
Interparietal	26(12)	40(18)	49(18)	40(19)
Supraoccipital	14(6)	20(10)	27(11)	29(12)
Squamosal(s)	7(7)	9(6)	19(11)	26(14)
Malar(s)	4(3)	5(4)	17(8)	9(6)
Maxilla	0	2(2)	6(4)	6(4)
Nasal(s)	2(2)	4(4)	10(5)	5(4)
Parietal(s)	5(4)	6(4)	9(5)	8(7)
4th Sternebra	12(6)	9(7)	11(9)	20(11)
Not Ossified				
Hyoid	39(15)	36(17)	48(19)	51(21)
5th Sternebra	53(20)	32(17)	47(18)	87(23)
Presence of 14th rib pairs	1(1)	1(1)	0	4(3)
Rib(s) - short	3(1)	2(2)	4(3)	10(5)
Rib(s) - rudimentary	2(1)	1(1)	3(2)	8(5)
Rib(s) - 1st lumbar rudimentary	18(10)	16(10)	42(21)	45(18)
Rib(s) - 14th unilateral	2(1)	1(1)	4(3)	9(6)

Table 7: Skeletal Examinations - cont'd

Observations[+]	0 mg/kg	25 mg/kg	75 mg/kg	185 mg/kg
total skeletal variations				
fetal incidence N(%)	138(79.3)	133(72.7)	167(81.5)	162(89.5)**
litter incidence N(%)	25(100)	27(100)	29(96.7)	27(100)

[+] some observation may be grouped together
[a] fetal [litter] incidence
* fetuses with at least one ossification variation
** Significantly different from control ($p \leq 0.05$).
[b] Data from Report HET K-007722-017 Appendix O (pp. 321-401)

D. Discussion/Conclusions

a. Maternal Toxicity:

There was no mortality as a result of the administration of 2,4-D BEE. The daily observation of the animals found an increased incidence of excessive salivation among the mid- and high-dose animals. In the high-dose group the mean weight gains for the day 6-9 and 9-12 intervals were -33.3% and -19.0% respectively. Furthermore, during days 6-16 of gestation the mean weight gain for the high-dose group was lower than control (-12.5%). This difference was statistically significant. Using the corrected day 20 body weights for the 185 mg/kg dose group, the mean weight gain for the interval during days 6-20 was lower than control (-10.9%). However this difference was not statistically significant. Days 6-11 of gestation, the mean food consumption for the high-dose group was significantly lower than control (-8.0%). Otherwise there were no significant effects on food consumption. The statistical evaluation of the hematology data indicated that there was a significant reduction (-4.4%) in the RBC count and a significant increase in the reticulocytes in the 185 mg/kg group when compared to the control.

NOTE:
Significant treatment related effects were found in the dose range finding study (MRID # 415271-04) at 145 and 218 mg/kg, on maternal body weight gain, red blood cell counts, hemaglobin, and hematocrit.

b. Developmental Toxicity:

i. Deaths/Resorptions:
The mean number of uterine implantations, viable fetuses, and resorptions were similar among control and treated groups.

ii. Altered Growth:
No treatment related effects were noted

iii. Developmental Variations:
There was a statistically significant increase in the fetal incidence of total skeletal variations at the 185 mg/kg level, as compared to the control. Dose related trends were observed for the following incompletely ossified bones: supraoccipital, squamosal, maxilla, and the 4th sternebra. There was a dose related increase in the incidence of rudimentary rib(s), and unilateral 14th ribs.

iv. Malformations:
There were a few sporadic malformations noted in the visceral and skeletal exams. In the visceral exam, microphthalmia was seen in one low-dose and one high-dose fetus. A folded retina, and anophthalmia were each observed in one low-dose fetus. One fetus at the high dose group was noted to have fused and ectopic kidneys. Due to the sporadic nature of the malformations observed, they were not interpreted as being related to treatment.

E. Study Deficiencies:

No deficiencies were noted in this study. As this study was conducted in late 1989 to early 1990 it would be helpful to have historical control data more recent than 1982-1987.

F. Core Classification: Core Minimum Data.

This study satisfies the guideline requirements (83-3) for a developmental toxicity study.

Maternal NOEL = 75 mg/kg
Maternal LOEL = 185 mg/kg based upon the following significant effects: decreased bodyweight gain days 6-16, decreased food consumption days 6-11, decreased red blood cells, and increased reticulocytes.
Developmental Toxicity NOEL = 25 mg/kg
Developmental Toxicity LOEL = 75 mg/kg based upon dose related skeletal variations (incompletely ossified interparietal, supraoccipital, squamosal(s), malar(s), and 4th sternebra; the presence of 14th rib pairs, short rib(s), rudimentary first lumbar ribs(s), and unilateral 14th rib(s).

G. Risk Assessment:

None at this time.

[2,4-D TRIISOPROPANOLAMINE SALT] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Amendment to DER for MRID 41527102/41527105 Developmental Toxicity Study - Rat [2,4-D TRIISOPROPANOLAMINE SALT

This amendment provides an EXECUTIVE SUMMARY and a data table to upgrade the original DER [Document No. 008186].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/20/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia Dobozy, V.M.D., M.P.H. Virginia A. Dobozy 12/20/00
Reregistration Branch I, Health Effects Division (7509C)

TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rat § 83-3(a)/OPPTS 870.3700
P.C. CODE: 030035
CASWELL NO.: 315AE CAS NO.: 18584-79-7
TEST MATERIAL (PURITY) :2,4-D *triisopropanolamine salt* [72.2%; acid equivalent 38.7%]
SYNONYMS: 2,4-D IPA; dichlorophenoxyacetic acid, triisopropanolamine salt
SPONSOR: The Dow Chemical Company

CITATION: Schroeder, R. E. (1990). A Teratogenicity Study in Rats with 2,4-D Triisopropanolamine. Bio/dynamics, Inc. Study No. HET K-008866-012, May 25, 1990. MRID 41527102. Document No. 008186. Unpublished. A Range-Finding Study to Evaluate the Toxicity of 2,4-Triisopropanolamine Salt in the Pregnant Rat. Study No. HET K-008866-011, April 10, 1990. MRID 41527105.

EXECUTIVE SUMMARY: In a developmental toxicity studies [MRID 41527102], female CD® [Sprague-Dawley derived] rats [30/group] were administered the triisopropanolamine salt [72.2% a.i.] of 2,4-dichlorophenoxyacetic acid *via* gavage at dose levels of 0 [distilled, deionized water], 32.5 mg/kg/day, 100 mg/kg/day, and 325 mg/kg/day from gestation day 6 through gestation day 15.

At the high-dose level, there were two deaths [gestation days 9 and 11] and two dams were sacrificed moribund [gestation days 9 and 12]. All of these dams were pregnant, and each had lost weight during the dosing interval prior to death/sacrifice, and each had exhibited stiffness of the limbs at one or more intervals. During the dosing period, stiffness of the limbs was observed in the majority [29/30] of the high-dose dams at one or more intervals [observed on day 6 in 3 dams and in the others on day 8], one high-dose dam still exhibited stiffness of the limbs on day 20 prior to sacrifice. Additionally, there was an increased incidence [30%] of excessive salivation during the dosing period at the high-dose level. Negative body-weight gain [-3 grams] was observed at the high-dose level during the first few days of dosing [days 6-9 of gestation], and decreased body-weight gains were observed at this dose level throughout the study [53% of control during days 9-12; 76% of control during days 12-16]. Corrected body-weight gain for the entire gestation period was 68% of control at the high-dose level. Decreased food consumption was noted at the high-dose level throughout the dosing period [74%-87% of control].

There were no abortions or premature deliveries, and the pregnancy rate was comparable among the groups. The numbers of corpora lutea/dam and implantation/dam were comparable among the groups. There was a slight decrease in the number of live fetuses/dam at the high-dose level [11.3] compared to the control [14.1] and other treatment groups [13.0 and 13.6], and the number of resorptions [both total (62) and per dam (2.6)] was increased compared to the control [24 (0.8)] and other treatment groups [22 (0.8) and 29 (1.0) with increasing dose]. The majority of the resorptions [40] can be attributed to 3 high-dose dams; 28.6% of the high-dose dams displayed greater than 5 resorptions compared to 0%-3.7% in the control and other dose groups. Additionally, both pre- and post-implantation losses were increased at the high-dose level compared to the control and other treatment groups. One high-dose dam totally resorbed a litter of 18. There was a statistically-significant decrease in the mean number of males per litter [5.1] at the high-dose level compared to the control [7.9]; the sex ratio was 1.3, 1.0, 1.0, and 0.8 in the control, low-, mid-, and high-dose groups. Fetal body weights were decreased at the high-dose level [males 85%/females 83% of control] compared to the control.

External malformations were observed in 2/352 fetuses in 2/27 litters at the low-dose [edematous, exencephaly, protruding tongue, absent eye bulge] and in 5/272 fetuses in 5/23 litters at the high dose [filamentous tail, absent eye bulge, small eye bulge] compared to none in the control and mid dose; the incidence at the high-dose level attained statistical significance. Visceral malformations were observed in 1/183 fetuses in 1/27 litters at the low dose [anophthalmia] and in 7/144 fetuses in 6/23 litters at the high-dose [anophthalmia, microphthalmia, folded retina, aortic arch malformation,distended lateral cerebral ventricle, cardiac interventricular septal defect] levels compared to none in the control and mid dose; the incidence at the high-dose level attained statistical significance. There was a dose-related increase in skeletal malformations [wavy and fused ribs, reduction in # of lumbar and cervical vertebrae, presence of cervical ribs, sacral and caudal vertebrae absent, scapulae, femur, and humerus bent, fused vertebral process], both on a fetal [1.2%, 3.8%, and 12.2%; statistically significant at mid- and high-dose] and litter [7.4%, 17.9%, 39.1%; statistically significant at high dose] basis; no skeletal malformations were observed in the control. Additionally, there was a significant increase in the **fetal** [88.2%, 81.8%, 83.1%, and 96.9%; statistically significant at high dose] incidences of total skeletal variations at the high-dose level compared to the control. The skeletal variations appear with some frequency in the vertebral transverse processes, sternebrae, and ribs. These effects for the most part are dose-related; the lowest dose at which the incidence begins to increase is 100 mg/kg/day, the mid-dose level. The litter incidence of skeletal variations was 100% for all groups.

The maternal toxicity NOAEL was not determined; the maternal toxicity LOAEL is 32.5 mg/kg/day, based on decreased body-weight gain on days 6-9. The NOAEL for developmental toxicity is 32.5 mg/kg/day, and the developmental toxicity LOAEL is 100 mg/kg/day, based on a significant increase in the incidence of skeletal malformations and fetal incidence of total skeletal variations.

This developmental toxicity study is classified Acceptable/Guideline and satisfies the guideline requirement [OPPTS 870.3700; §83-3(a)] for a developmental toxicity study in the rodent.

[2,4-D TRIISOPROPANOLAMINE SALT] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Gravid Uterine Weight/Corrected Body Weight/Gain [grams]

Dose/Day	0 mg/kg/day	32.5 mg/kg/day	100 mg/kg/day	325 mg/kg/day
body weight				
0	225±16	220±17	225±16	223±17
6	253±17	229±18✖	245±17	250±18
9	263±19	235±16	252±17	248±22* [94]✖
12	278±19	269±17	267±18	259±21** 93]
16	303±22	293±18	289*±20✖	289±19** [79]✖
20	366±27	353±21	349*±25	334±24** [91]
body weight gain				
0-6	29±8	29±6	30±7	27±6
6-9	10±7	6±5* [60]	7±5* [70]	-3±13**
9-12	15±6	15±4	15±3	8±11 [53]
12-16	25±6	24±6	23±6	19±9 76]
16-20	63±8	59±9 [94]	60±9 [95]	52±16 [83]
6-16	50±11	45±9 [90]	44±8 [88]	29±14** [58]
gravid uterine weight	75.9±11.4	70.4±13.3	73.0±10.6	55.2±24.1** [73]
corrected body weight	290.2±23.0	282.3±19.8	276.1±19.1*	277.0±15.3 [95]
corrected BWG	37.0±9.9	33.4±12.6	31.0±10.2	25.2±10.7** [68]

√[% of control]; * $p<0.05$; ** $p<0.01$; ✖ [many of the numbers were illegible]
data from Appendix C, D, E [pages 64-75] of the report

Table 2. Fetal Observations

Observation/Dose	0 mg/kg/day	32.5 mg/kg/day	100 mg/kg/day	325 mg/kg/day	historical control
filamentous tail # fetuses/# litters affected # fetuses/# litters examined	0/0 422/30	0/0 352/27	0/0 382/28	2/2 272/23	1/1 7193/527
folded retina # fetuses/# litters affected # fetuses/# litters examined	0/0 219/30	0/0 183/27	0/0 199/28	2/1 144/23	1/1 3757/527
anophthalmia # fetuses/# litters affected # fetuses/# litters examined	0/0 219/30	1/1 183/27	0/0 199/28	2/2 144/23	2/2 3757/527
microphthalmia # fetuses/# litters affected # fetuses/# litters examined	0/0 219/30	0/0 183/27	0/0 199/28	2/2 144/23	1/1 3757/527
distended lateral cerebral ventricle # fetuses/# litters affected # fetuses/# litters examined	0/0 219/30	0/0 183/27	0/0 199/28	1 [0.7%]/1 [4.3%] 144/23	9 [0.2%]/9 [1.7%] 3757/527

Data from pages 12-16 of DER; historical control data from Appendix Q of report [appended to DER]; also, the incidence of fetuses and litters with *fetuses with fused ribs, the presence of cervical ribs, reduction in # of lumbar vertebrae, etc.*, were not listed in the available historical control data from the testing facility.

CASWELL FILE

GUIDELINE: 83-3

Primary Review by: Karen E. Whitby, Ph.D. 12/4/90
Toxicologist, Review Section II, Toxicology Branch II/HED
(H7509C)

Secondary Review by: K. Clark Swentzel 12/4/90
Section Head, Review Section II, Toxicology Branch II/HED
(H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3

EPA Identification Nos.: EPA MRID (Accession) No. 415271-02/-05
EPA ID No. 030025-1
EPA Record No. 268,213
Caswell No. 315AE
HED Project No. 0-1707

Test Material: 2,4-D Triisopropanolamine Salt

Synonyms: (2,4-D-TIPA)

Sponsor: The Dow Chemical Co., The Toxicology Research Laboratory
1803 Building, Midland, MI 48674

Study Number(s): HET K-008866-012 (The Dow Chemical Co.,)
Project No. 89-3463 (Bio/dynamics, Inc.)

Testing Facility: Bio/dynamics, Inc., East Millstone, New Jersey
08875

Title of Report: A Teratogenicity Study in Rats with 2,4-D
Triisopropanolamine

Author(s): Raymond E. Schroeder

Report Issued: May 25, 1990

Conclusions:

2,4-D TIPA was evaluated for maternal and developmental toxicity at 0, 32.5, 100, and 325 mg/kg. Maternal toxicity was evident at 32.5 mg/kg by statistically significant decreased bodyweight gain days 6-9 of gestation. A maternal NOEL was not achieved. Developmental toxicity was observed at 100 mg/kg and above by skeletal malformations/variations. At 325 mg/kg postimplantation loss was three times that of control.

Maternal NOEL = not determined
Maternal LOEL = 32.5 mg/kg

Developmental Toxicity NOEL = 32.5 mg/kg
Developmental Toxicity LOEL = 100 mg/kg

Core Classification: Core Minimum Data

RANGE-FINDING STUDY

The main developmental toxicity study was preceded by a pilot study, which was performed to determine the maternal and embryotoxic potential of 2,4-D Triisopropanolamine Salt in the pregnant rat. Evaluation of fetal toxicity consisted of recording the number of live fetuses on day 16 at the time of cesareans. The study was conducted in two phases. The first phase evaluated 0, 95, 190, and 280 mg/kg with 10 mated rats/group, and did not establish embryo/fetal toxicity. The second phase, utilizing a second shipment of animals, evaluated 10 mated animals/group receiving 0, 280, 375, and 465 mg/kg. Both of these studies were conducted by Bio/dynamics.

Study Title: A Range-Finding Study to Evaluate the Toxicity of 2,4-D Triisopropanolamine Salt in the Pregnant Rat

Study No.: MRID No. 415271-05
Study ID: HET K-008866-011 (Dow Chemical Co.)
Project No. 89-3464 (Bio/dynamics)
89-3464A

Date of Report: April 10, 1990

Date of Study: (Live Portion of 89-3464) July 10 - 29,1989
(Live Portion of 89-3464A) August 26 - September 15, 1989)

Author: Raymond E. Schroeder

Materials and Methods:

2,4-D Triisopropanolamine (TIPA) Salt [Lot No. AGR 276428, purity 72.3% (the amount of test article added to the vehicle was corrected for purity)] was administered to mated CD® (Sprague-Dawley derived) ♀ rats (10/group) by gavage at dose levels of 0 (vehicle: distilled, deionized water), 95, 190, 280 mg/kg (study 89-3464); and 0, 280, 375, and 465 mg/kg (study 89-3464A). Test article was administered on gestation days 6-15. Dams were sacrificed on day 16 of gestation; postmortem examination included weighing of the gravid uterus, kidneys, and liver; recording the number of corpora lutea and uterine implants (no. live, dead, and resorbed).

Results:

89-3464
There was no mortality in this study. In the 280 mg/kg group there was a significant reduction in bodyweight gain (-58.3%) relative to the control, days 6-9. Days 6-16 bodyweight gain for this group was -11.8% compared to control. The corrected bodyweight gain (days 6-16) was -16.4% relative to the control

for the group receiving 280 mg/kg (not statistically significant). Mean food consumption days 6-11 of gestation was -10.5% for the 280 mg/kg group relative to the control. The incidence of excessive salivation was 0, 50, 90, and 100% among the 0, 95, 190, and 280 mg/kg levels, respectively. The corpora lutea and implantation data did not reveal any treatment related differences.

89-3464A
The 465 mg/kg group had 30% mortality (1 ♀ died after three days of treatment, 2 ♀ died after four days of treatment). At necropsy these females were found to have yellow staining of the skin/fur in the ano-genital region, and 2 had a slight dilatation of the renal pelvis. Mean weight gain was reduced in a dose related manner during the dosing period. Bodyweight gain was significantly reduced at 375 and 465 mg/kg days 6-9, 12-16, and 6-16. There was a dose-related decrease in uterine weight which was significant only at 465 mg/kg. Corrected bodyweight gain was significantly reduced at 375 and 465 mg/kg. Food consumption was significantly depressed in the 375 and 465 mg/kg groups days 6-11 and 11-16. Daily observation of the animals found an increased incidence of salivation and stiffness of the limbs in all treated groups. Corpora lutea and implantation data revealed a nonsignificant decrease in the mean number of viable fetuses, an increase in the number of resorption sites, and a higher mean resorption/implant ratio at 375 and 465 mg/kg.

Conclusions

The dosage levels of 2,4-D TIPA chosen for the main developmental toxicity study were 0, 32.5, 100, and 325 mg/kg. Based upon the treatment related effects observed in this study, the dosages selected for the main study appeared to be appropriate.

Core Classification: Supplementary Data (Range-Finding Study)

MAIN STUDY

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: Purity: 72.2%
Description: Black Liquid
Lot No.: AGR 276428

Vehicle(s): Identity: Distilled, deionized water

Test Animal(s): Species: Rat
Strain: CD® (Sprague-Dawley derived)
Source: Charles River Laboratories, Inc., Portage, MI 49081
Age at Initiation of Mating: ♀ approx. 11 weeks ♂ approx. 34 weeks
Weight of ♀ on day 0: (mean) 220.6 g (range) 183 - 271 g

B. Study Design

This study was designed to assess the developmental toxicity potential of 2,4-D Triisopropanolamine Salt when administered by gavage to rats on gestation days 6 through 15, inclusive.

Mating

Females were placed with males nightly in a 1:1 ratio. Animals not utilized in the study that remained from the original population were sacrificed and discarded after all study groups had been filled and the last mated females within study groups had initiated dosing.

Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	30
Low Dose	32.5	30
Mid Dose	100.0	30
High Dose	325.0	30

Dosing:

All doses were in a volume of 5 ml/kg of body weight/day prepared once prior to initiation of treatment in a volume adequate for the duration of the study. When the dosing solutions were

prepared, the amount of test article added to the vehicle was adjusted to correct for the purity of the test article. The dosing solutions were analyzed for concentration, homogeneity, and stability. Concentrations of the test chemical ranged from 110.2 to 114.9% of the target concentration at the beginning of dosing. Upon the conclusion of the dosing period, the range of concentration was 108.1 to 110.4%. The coefficient of variation was 1.2% or less, indicating homogeneity. The dosing solution was found to be stable for at least 34 days (± 10% of the day 0 concentration). Initial dose volumes were based on gestation day 6 bodyweights and were adjusted for each animal during the remaining portion of treatment to the most recent bodyweight.

Observations

Maternal

The animals were checked for mortality or abnormal condition twice daily. In addition, each female was given a detailed physical examination on days 0, 6-15, and 20 of gestation. Dams were sacrificed on day 20 of gestation by overdose of carbon dioxide inhalation. Examinations at sacrifice consisted of: the external surface, all orifices, the cranial cavity, carcass, external surface of the spinal cord and sectioned surfaces of the brain, nasal cavity and paranasal sinuses, the thoracic, abdominal, and pelvic cavities, viscera, kidney and liver weights cervical tissues and organs.

Maternal bodyweights were recorded on days 0, 6, 9, 12, 16, and 20 of gestation. Feed consumption was recorded on days 0-6, 6-11, 11-16, and 16-20.

Reproductive

The intact uterus including ovaries was removed and weighed. The number and location of the following was recorded for each uterine horn: live fetuses, dead fetuses, late resorptions, early resorptions, and implantation sites. The ovaries were dissected free and the number of corpora lutea were recorded. If uterine implantations were not evident, the uterus was stained with ammonium sulfide,according to the method of Salewski, 1964.

Fetal

The fetuses were examined in the following manner: each fetus was individually identified, weighed, sexed externally, and examined for gross external malformations/variations. Approximately one-half of the fetuses of each litter (every other fetus within the litter) were evaluated for visceral malformations/variations via a modified Staples, 1974 technique. Fetuses designated to undergo the visceral exam were decapitated and the heads were fixed in Bouin's for subsequent examination. The fetuses were

secured under a dissecting scope, and the thoracic and abdominal cavities were examined. Upon completion of this, the fetuses were eviscerated and individually fixed in 70% ethanol. The heads were serially transverse sectioned using a razor blade after fixation was complete. The sections were observed for malformations of the palate, eyes, and brain via dissecting scope.

The remaining fetuses in each litter were euthanized via inhalation of ether. The fetuses were eviscerated (internally sexed), and stained with Alizarin Red S via a procedure (Crary, 1962) modified by Bio/dynamics. Skeletal specimens were evaluated under a dissecting scope for ossification malformations and variations.

Historical control data were provided to allow comparison with concurrent controls.

Statistical Analysis

The interval data (mean bodyweights, mean bodyweight change, gravid uterus weight, day 20 corrected bodyweight, mean food consumption, mean organ weight, and reproduction data) were analysed by the following procedures. Bartlett's test was performed to determine if groups had equal variance. If so, a standard one way ANOVA using the F distribution to assess significance was performed. If warranted, Dunnett's test was used to determine which means were significantly different from the control. When necessary, the Kruskal-Wallis test was used as a nonparametric test of equality of means. If differences were found, a summed rank test (Dunn) was used to determine which groups differed from the control. A test for trend in the dose levels was also performed. For parametric data, standard regression techniques with a test for trend and lack of fit were used. For nonparametric data, Jonckheere's test for monotonic trend was used. All ratios were transformed via the arc sine transformation before analysis.

The incidence data (litters with resorptions, mortality rates, pregnancy rates, fetuses with malformations/variations, and litters with fetuses with malformations/variations) were analysed as follows. First, a standard chi-square analysis was performed to determine if the proportion of incidences differed between the groups tested. Each treatment group was compared to the control using a 2X2 Fisher Exact Test. The significance level was corrected via the Bonferroni inequality. Armitage's test for linear trend in the dose groups was performed. If any one cell had an expected value less than 5, the chi-square and Armitage's tests were not reported. When this occurred, only the Fisher Exact test (corrected via Bonferroni inequality) was performed and reported.

Compliance

A signed Statement of No Data Confidentiality Claims was provided.

A signed Statement of compliance with EPA GLP's was provided.

A signed Flagging Statement was provided.

A signed Quality Assurance Statement was provided.

C. Results

Mortality

In the 0, 32.5, and 100.0 mg/kg groups no mortality occurred. In the group receiving 325 mg/kg, four females died (13.3% mortality) or were sacrificed in the moribund condition during the treatment period. All of the animals that died or were sacrificed in the moribund condition were pregnant and were noted with stiffness of the limbs at least once.

Clinical Observations

"Stiffness of the limbs" was seen at one or more intervals during the treatment period in 96.7% of the animals (29/30) in the 325 mg/kg group. This was first observed in three animals on day 6 of treatment. The majority of animals exhibiting this finding did not do so until day 8. One female was still noticed to have "stiffness of the limbs" on day 20 prior to sacrifice. This finding was not observed in the 0, 32.5, and 100 mg/kg groups.

Excessive salivation was also seen among the 325 mg/kg group. Ten animals were noted with this finding during the treatment period. This finding was not observed in the 0, 32.5, and 100 mg/kg groups.

Bodyweight

The investigators supplied the following data:

Table 1 Bodyweight Gains (grams)[a]

Group (mg/kg):	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gains Dosing P.[1]	Entire[2]
0	29	50	63	141.4	-25.6	65.5
32.5	29	45	59	132.5	-25.8	61.0
100.0	30	44	60	134.0	-28.9	61.0
325.0	27	29**	52	109.9	-26.5	52.6

[1] corrected bodyweight gain for dosing period = bodyweight gain for dosing period minus gravid uterus weight.
[2] corrected bodyweight gain for entire gestation period = bodyweight gain for entire gestation period minus gravid uterus weight.
** Significantly different form control ($p \leq 0.01$)
[Some of the above values were calculated by the reviewer from individual animal data]

[a] Data extracted from (study number HET K-008866-012 Appendices C, D, and E pp. 64-79)

There were significant reductions in bodyweight gain days 6-9 in the low-, mid-, and high-dose groups -40, -30, and -130%, respectively, relative to the control. When maternal bodyweight gain was calculated over the entire gestation period, there was a -6.3, -5.2, and -22.3% change relative to the control for the 32.5, 100, and 325 mg/kg groups respectively (these differences were not analysed statistically). The corrected maternal bodyweight gain for the entire gestation period indicated changes of -6.9, -6.9, and -19.7% relative to the control, for the 32.5, 100, and 325 mg/kg groups respectively (these differences were not analysed statistically).

On day 9 of gestation, after receiving three days of treatment, a female in the high-dose group was sacrificed moribund. This female lost 50 g days 6-9. Another female in the same group who underwent convulsions on day 7 subsequently died on day 9, after having lost 35 g days 6-9. A female in the high-dose group was sacrificed moribund on day 12 of gestation; this female lost 36 g during days 6-12. Also in the 325 mg/kg group, a female lost 25 g days 6-9, then died on day 11.

Food Consumption

The investigators supplied the following data:

Table 2 Food Consumption Data (g/kg/day)[a]

Group: (mg/kg)	Days 0-6	Days 6-11	Days 11-16	Days 16-20	Days 0-20
0	101	90	92	89	368
32.5	98	89	92	88	368
100.0	104	91	93	90	378
325.0	101	67**	80*	91	343

[a] Data extracted from (study number HET K-008866-012 appendix F pp. 80-84)
* Significantly different from control ($p \leq 0.05$)
** Significantly different from control ($p \leq 0.01$)

[Some of the above values were calculated by the reviewer from individual animal data]

There were no statistically significant differences in food consumption between the 0, 32.5, and the 100 mg/kg groups. During the treatment period, mean food consumption for the 325 mg/kg group was significantly less than the control group (-25.6 and -13.0%, days 6-11 and 11-16, respectively).

Organ Weight Data

Mean absolute kidney weights were comparable between the control and treated groups. Mean relative kidney weights were slightly increased in a dose-related manner. Mean absolute liver weights were comparable between the 0 and 32.5 mg/kg groups. At the 100 and 325 mg/kg levels, a significant decrease in the mean absolute liver weight was seen relative to the control. However, since the mean relative liver to bodyweight ratio for these groups were comparable to the control data, the differences were probably due to the lower final bodyweight.

Table 3 Terminal Organ and Bodyweights and Organ/Bodyweight Ratios

mg/kg	0	32.5	100	325
Terminal Bodyweight (g)	290.2	282.3	276.2*	277.0
Kidney Wt. Absolute (g)	1.926	1.885	1.852	1.885
Relative (X 1000)	6.66	6.69	6.72	6.81
Liver Wt. Absolute (g)	15.359	14.726	14.408*	14.507*
Relative (X 1000)	5.31	5.22	5.22	5.26

* Signifcantly different from control ($p \leq 0.05$)
[a] Data taken from Report HET K-008866-012 Appendix J (p. 110)

Gross Pathological Observations

There were no significant adverse pathological effects as a result of treatment. Ten percent of the animals in the high dose group were found to have abnormal contents in their uterine cavities [i.e. both horns filled with a black fluid (2 animals)

or one horn filled with a red material (1 animal)]. This observation was not made in the control or mid-dose animals, but was made once in the low-dose animals. Dilated renal pelvis was an observation made once in each treatment group.

Cesarean Section Observations

Table 4 Cesarean Section Observations[a]

Dose (mg/kg):	0	32.2	100	325
#Animals Assigned	30	30	30	30
#Animals Mated/Inseminated	30	27	28	28
Pregnancy Rate (%)	100	90	93	93
Maternal Wastage				
#Died	0	0	0	4
#Died/pregnant	0	0	0	4/28
#Non pregnant	0	3	2	2
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	472	406	441	370
Corpora Lutea/dam	15.7 ±1.3	15.0 ±1.6	15.8 ±2.5	15.4 ±1.4
Total Implantations	446	374	411	334
Implantations/Dam	14.9 ±1.9	13.9 ±2.5	14.7 ±1.4	13.9 ±3.5
Total Live Fetuses	422	352	382	272
Live Fetuses/Dam	14.1 ±2.1	13.0 ±2.7	13.6 ±1.6	11.3 ±5.0
Mean no. ♂/litter	7.9	6.6	6.7	5.1**
Mean no. ♀/litter	6.2	6.4	7.0	6.2
Total Resorptions	24	22	29	62
Resorptions/Dam	0.8 ±1.2	0.8 ±1.1	1.0 ±1.0	2.6 ±4.6
Total Dead Fetuses	0	0	0	0
Mean Fetal Weight (gm)	3.42	3.40	3.35	2.81
♂ fetuses	3.48 ±0.2	3.52 ±0.2	3.45 ±0.4	2.95** ±0.5
♀ fetuses	3.32 ±0.2	3.31 ±0.2	3.27 ±0.4	2.75** ±0.5
Preimplantation Loss(%)	5.4	3.3	5.5	9.6
Postimplantation Loss(%)	5.3	6.0	6.9	16.8
Sex Ratio (Total ♂/Total ♀)	1.3	1.0	1.0	0.8

[a] Data extracted from (study number HET K-008866-012 appendix G pp. 85-89)

** Significantly different from control ($p \leq 0.01$)

Although the number of viable fetuses in the 325 mg/kg group was lower than the study control (-19.9%) the difference was not statistically significant (the decrease was -20.4% relative to the historical control). Although the preimplantation loss for the 325 mg/kg group is increased (77.8%) relative to the study control, the value is not as vastly different when compared to the historical control (18.5%). The mean number of resorption sites per dam was also increased for the 325 mg/kg group (225% relative to the study control, and 271% relative to the historical control). One female in the 325 mg/kg group totally resorbed a litter of 18. Another female in this group resorbed 13 of 14 implantations, while another dam resorbed 9 of 15 implants. These findings apparently account for the dose-related increase in post-implantation loss (13.2, 30.2, and 217% were the increases for the 32.5, 100, and 325 mg/kg groups respectively, relative to the study control, and 33.3, 53.3, and 273.3% relative to the historical control). The mean number of males per litter statistically indicated a linear response related to the dose levels (the number of males/litter was -16.5, -15.2, and -35.4% respectively for the 32.5, 100, and 325 mg/kg levels relative to the control). Mean fetal weight at 325 mg/kg was -17.8% of the study control and -19.9% of the historical control for litters; -15.2% of the study control and -18.1% of the historical control for males; and -17.2% of the study control and -19.6% of the historical control for females.

2. Developmental Toxicity

Table 5 External Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
External Malformations[a]				
#pups(litters) examined	422(30)	352(27)	382(28)	272(23)
Edematous	0(0)	1(1)	0(0)	1(1)
Filamentous tail	0(0)	0(0)	0(0)	2(2)
Exencephaly	0(0)	1(1)	0(0)	0(0)
Protruding tongue	0(0)	1(1)	0(0)	0(0)
Eye bulge(s) - absent	0(0)	2(2)	0(0)	2(2)
Eye bulge(s) - small	0(0)	0(0)	0(0)	1(1)
Total External Malformations				
Fetal Incidence N(%)	0(0)	2(0.6)	0(0)	5(1.8)*
Litter Incidence N(%)	0(0)	2(7.4)	0(0)	5(21.7)*
External Variations[a]				
#pups(litters) examined	422(30)	352(27)	382(28)	272(23)
Shiny (glassy) appearance	0(0)	3(1)	4(3)	2(1)

Total External Variations				
Fetal Incidence N(%)	0(0)	3(0.9)	4(1.0)	2(0.7)
Litter Incidence N(%)	0(0)	1(3.7)	3(10.7)	1(4.3)

+ some observations may be grouped together
[a] fetal (litter) incidence
* Significantly different from control ($p \leq 0.05$)
Data taken from study number HET K-008866-012 appendix L pp. 250-251

The incidence of external malformations at the 325 mg/kg level was significantly different from control when evaluated on the fetal and litter basis. The edema, bulging eyes, and protruding tongue were observed in one fetus of the low-dose group.

The only external variation noted was shiny skin. This is attributed to the thin translucent skin of developmentally retarded fetuses. The finding was not dose-related and therefore, not attributed to treatment.

Table 6 Visceral Examinations

Observations+	Control	Low Dose	Mid Dose	High Dose
Visceral Malformations[a]				
#pups(litters) examined	219(30)	183(27)	199(28)	144(23)
Anophthalmia	0(0)	1(1)	0(0)	2(2)
Microphthalmia	0(0)	0(0)	0(0)	2(2)
Retina(s) - folded	0(0)	0(0)	0(0)	2(1)
Lateral Cerebral Ventricle(s) - distended	0(0)	0(0)	0(0)	1(1)
Aortic Arch Malformation	0(0)	0(0)	0(0)	1(1)
Cardiac Interventricular Septal Defect	0(0)	0(0)	0(0)	1(1)
Total Visceral Malformations				
Fetal incidence N(%)	0(0)	1(0.5)	0(0)	7(4.9)*
Litter incidence N(%)	0(0)	1(3.7)	0(0)	6(26.1)*
Visceral Variations[a]				
#pups(litters) examined	219(30)	183(27)	199(28)	144(23)
Distended Renal Pelvis (papilla present)	2(2)	3(3)	0(0)	1(1)
Ureter(s) - distended	4(4)	1(1)	2(2)	4(3)
Ureter(s) - tortuous	7(4)	11(9)	3(2)	6(6)
Total Visceral Variations				
Fetal incidence N(%)	10(4.6)	12(6.6)	5(2.5)	9(6.3)
Litter incidence N(%	7(23.3)	9(33.3)	3(10.7)	8(34.8)

[+] some observations may be grouped together
[a] fetal (litter) incidence
[*] Significantly different from control ($p \leq 0.05$)
Data taken from study number HET K-008866-012 appendix M pp. 290-314

There were no visceral malformations in the 0, and 100 mg/kg group. The only visceral malformation in the 32.5 mg/kg group was anophthalmia. Malformations of the eye (micro- and anophthalmia) were seen in four 325 mg/kg fetuses of four different litters. The cardiac defects were observed in the high-dose fetus found to have edema of the cervical and thoracic regions during external examinations.

Tortuous and/or distended ureters were the most commonly observed visceral variations. The incidence of this and the other visceral variations were relatively comparable to the control.

Table 7 Skeletal Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
Skeletal Malformations[a]				
#pups(litters) examined	203(30)	170(27)	183(28)	131(23)
Hole in Cranium	0(0)	1(1)	0(0)	0(0)
Premaxilla(e) - fused	0(0)	1(1)	0(0)	0(0)
Exoccipital(s) - misshapen	0(0)	1(1)	0(0)	0(0)
Cervical Transverse Process(es) - misshapen	0(0)	1(1)	0(0)	0(0)
Reduction in # of Cervical Vertebrae	0(0)	0(0)	0(0)	1(1)
Presence of Cervical rib(s)	0(0)	0(0)	0(0)	3(1)
Thoracic Transverse Process(es) - fused	0(0)	0(0)	1(1)	1(1)
Transverse Process(es) - Small and misshapen	0(0)	0(0)	1(1)	0(0)
Thoracic Centrum(a) - Split & misshapen	0(0)	0(0)	1(1)	0(0)
Thoracic Transverse Process(es) - absent	0(0)	0(0)	0(0)	1(1)
Lumbar Centra - fused	0(0)	1(1)	0(0)	0(0)
Lumbar Centrum(a) - Split & misshapen	0(0)	0(0)	1(1)	0(0)
Reduction in # of Lumbar Vertebra(e)	0(0)	0(0)	0(0)	4(2)
Sacral Vertebra(e) - absent	0(0)	0(0)	0(0)	1(1)
Caudal Vertebra(e) - absent	0(0)	0(0)	0(0)	1(1)
Rib(s) - wavy	0(0)	0(0)	5(4)	5(4)
Rib(s) - fused	0(0)	0(0)	0(0)	5(4)
Scapula(e) - bent	0(0)	0(0)	0(0)	3(3)
Humerus(i) - bent	0(0)	0(0)	0(0)	2(2)
Ulna - thickened and misshapen	0(0)	0(0)	0(0)	1(1)
Femur - bent	0(0)	0(0)	0(0)	2(1)
Ilium - misaligned	0(0)	0(0)	0(0)	1(1)
Total Skeletal Malformations				
Fetal incidence N(%)	0(0)	2(1.2)	7(3.8)*	16(12.2)*
Litter Incidence N(%)	0(0)	2(7.4)	5(17.9)	9(39.1)*
Skeletal Variations[a]				
#pups(litters) examined	203(30)	170(27)	183(28)	131(23)
Nasal(s) - inc. oss.	9(5)	1(1)	10(7)	13(7)
Hyoid - inc. oss.	3(3)	1(1)	5(4)	5(5)
Premaxilla - inc. oss.	0(0)	0(0)	2(2)	6(3)
Cervical Transverse Process(es) - inc. oss.	9(7)	17(12)	21(12)	50(16)

Table 7 Skeletal Examinations - cont'd

Observations+	Control	Low Dose	Mid Dose	High Dose
Sacral Transverse Process(es) - inc. oss.	5(3)	9(5)	7(6)	10(6)
Oss. Adj. to 7th Cervical Vertebra(ae)	2(1)	1(1)	1(1)	10(6)
Thoracic Centrum(a) - not oss.	2(2)	1(1)	4(3)	6(4)
Thoracic Centrum(a) - split	0(0)	0(0)	0(0)	3(3)
Sacral Centrum(a) - not oss.	0(0)	0(0)	0(0)	2(2)
Sacral Centrum(a) - inc. oss.	0(0)	1(1)	1(1)	3(1)
Caudal Centrum(a) - not oss.	2(1)	1(1)	6(4)	14(8)
2nd Sternebra - not oss.	4(3)	1(1)	3(2)	5(4)
3rd Sternebra - inc. oss.	4(2)	1(1)	8(6)	7(6)
3rd Sternebra - not oss.	1(1)	0(0)	0(0)	3(3)
4th Sternebra - inc. oss.	18(11)	9(8)	25(15)	40(17)
4th Sternebra - not oss	1(1)	0(0)	3(2)	6(3)
5th Sternebra - not oss.	60(23)	46(19)	68(24)	104(22)
6th Sternebra - not oss.	24(12)	10(6)	29(14)	49(16)
Sternebra(ae) - misshapen	0(0)	0(0)	0(0)	2(2)
Rib(s) - 1st Lumbar Rudimentary	3(2)	4(4)	7(6)	11(6)
Ribs - thickened	0(0)	0(0)	5(4)	12(7)
Metacarpal(s) - inc.oss.	4(4)	1(1)	6(6)	11(6)
Metatarsal(s) - inc.oss.	1(1)	2(1)	6(4)	11(7)
Scapula(e) - inc. oss.	1(1)	1(1)	1(1)	5(4)
Total Skeletal Variations				
Fetal incidence N(%)	179(88.2)	139(81.8)	152(83.1)	127(96.9)**
Litter incidence N(%)	30(100)	27(100)	28(100)	23(100)

+ some observations may be grouped together
a fetal (litter) incidence
* Significantly different from control ($p \leq 0.05$)
** Significantly different from control ($p \leq 0.01$)
Data taken from study number HET K-008866-012 appendix N pp. 315-418

The evaluation of the fetal specimens revealed sporadic malformations and numerous skeletal variations. Therefore, for the most part, only those that occur in a treatment related manner are included in table 7. The incidence of litters with at least one skeletal variation was 100% in each of the groups. Skeletal variations appear with some frequency in the vertebral transverse processes, sternebrae, and ribs. These effects for the most part are dose related, the lowest dose at which the incidence begins to increase is 100 mg/kg.

289

D. Discussion/Conclusions

a. Maternal Toxicity:

Mortality in this study was 13.3% at the high dose (325 mg/kg). "Stiffness of the limbs" was seen at one or more intervals during the treatment period in 96.7% of the animals (29/30) in the 325 mg/kg group. There was a significant decrease in terminal and corrected bodyweight of the 100 mg/kg females. There was a significant decrease in the absolute liver weight of the 100 and 325 mg/kg dams. Bodyweight gain was significantly reduced at 32.5 mg/kg and above, days 6-9. Bodyweight gain was significantly reduced through the dosing period at 325 mg/kg. Food consumption was also significantly reduced days 6-11 and 11-16 in this group. Food consumption days 0-20 of gestation in the 325 mg/kg group was -6.8% relative to the control.

b. Developmental Toxicity:

i. Deaths/Resorptions:
Preimplantation loss in the 325 mg/kg group was increased 77.8% relative to the control. There was a nonsignificant increase in the number of resorptions/dam at 325 mg/kg. Postimplantation loss was increased in a dose-related manner [13.2, 30.2, and 217%, respectively for the 32.5, 100, and 325 mg/kg groups. There was a significant decrease in the number of males per litter at 325 mg/kg. This effect was treatment related.

ii. Altered Growth:
Fetal weights at 325 mg/kg were significantly reduced for males and females (not for litters).

iii. Developmental Anomalies:
The incidence of fetuses with at least one ossification variation was significantly higher than control at 325 mg/kg. The incidence of litters containing at least one fetus with an ossification variation was 100% for the control and each treated group. At 100 mg/kg there was an increased incidence (dose-related) of incomplete ossification of the cervical and sacral vertebral transverse processes, metatarsals, and the 3rd and 4th sternebrae. In addition, at this dose, the incidence of unossified caudal centra, rudimentary first lumbar ribs, and thickened ribs was increased.

In general, the incidence of these effects increases at 325 mg/kg with additional findings (unossified caudal centra, unossified sacral and cervical vertebral transverse processes, unossified 4th, 5th, and/or 6th sternebra, and incompletely ossified metacarpals).

iv. Malformations:
There was a significant increase in the fetal incidence of skeletal malformations at 100 mg/kg. For the 325 mg/kg group there was a significant increase in the incidence of skeletal malformations on the fetal and the litter basis. The skeletal malformations seen most frequently in this study were wavy ribs (increased incidence at 100 and 325 mg/kg), fused ribs, and defects of the vertebrae. The registrant has supplied a reference to support their opinion that these malformations are frequently associated with maternal toxicity. There was a significant increase at the 325 mg/kg level in the fetal and litter incidence of visceral malformations.

E. Study Deficiencies:

A maternal NOEL was not attained in this study. More recent historical control data than 1982-1987 would be helpful as this study was conducted in late 1989.

F. Core Classification: Core Minimum Data.

This study satisfies the guideline requirements (83-3) for a developmental toxicity study.

Maternal NOEL = not determined
Maternal NOEL = 32.5 mg/kg based upon decreased bodyweight gain days 6-9

Developmental Toxicity NOEL = 32.5 mg/kg
Developmental Toxicity LOEL = 100 mg/kg based upon a significant increase in the fetal incidence of skeletal malformations (wavy ribs) and variations of select bones (vertebral transverse processes, caudal centra, sternebrae, rudimentary ribs, thickened ribs, and metatarsals)

G. **Risk Assessment**: None at this time.

[2,4-D ISOPROPYLAMINE SALT] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Amendment to DER for MRID 41527103 / Developmental Toxicity Study - Rat [2,4-D ISOPROPYLAMINE SALT 41527106

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 008186].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/7/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P.H. Virginia A Dobozy 12/11/00
Reregistration Branch I, Health Effects Division (7509C)
TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rat § 83-3(a)/OPPTS 870.3700
P.C. CODE: 030025
CASWELL NO.:315U CAS NO.: 5742-17-6
TEST MATERIAL (PURITY) :2,4-D isopropylamine salt [50.2%; acid equivalent 39.6%]
SYNONYMS: 2,4-D IPA; dichlorophenoxyacetic acid, isopropylamine salt
SPONSOR: The Dow Chemical Company

CITATION: Schroeder, R. E. (1990). A Teratogenicity Study in Rats with 2,4-D Isopropylamine. Bio/dynamics, Inc. Study No. HET M-004725-011, May 7, 1990 [10/24/89-11/27/89]. MRID 41527103. A Range-Finding Study to Evaluate the Toxicity of 2,4-D Isopropylamine Salt in the Pregnant Rat. Study No. HET M/K-004725-010, March 20, 1990. MRID 41527106. Document No. 008186. Unpublished.

EXECUTIVE SUMMARY: In developmental toxicity studies [MRID 41527103], female CD® [Sprague-Dawley derived] rats [30/group] were administered the isopropylamine salt of 2,4-dichlorophenoxyacetic acid [50.2%] *via* gavage at dose levels of 0 [distilled, deionized water], 22 mg/kg/day, 65 mg/kg/day, and 190 mg/kg/day from gestation day 6 through gestation day 15 [=17, 51, and 150 mg/kg/day of the 2,4-D acid].

There was one death; a high-dose dam died on day 7 after one day of treatment. There was a dose-related increase in the incidence of excessive salivation at the mid- and high-dose levels during the dosing period [0%, 0%, 17%, 50%], although the significance of this finding is unclear. Decreased body-weight gain [43% of control] was observed at the high-dose level during the first few days of dosing [days 6-9 of gestation]. Corrected body-weight gain for the entire gestation period was comparable among the groups. A slightly lower food consumption was noted at the high-dose level during the first week of dosing [91% of control].

There were no abortions, and the pregnancy rate was comparable among the groups, although the high-dose dams [86.7%] displayed the lowest rate [control (96.7%), low dose (90%), and mid dose (93.3%)]. The numbers of corpora lutea/dam, implantation/dam, live fetuses/dam, and resorptions [both total and per dam], as well as pre- and post-implantation losses were comparable among the groups. There were no dead fetuses. One high-dose dam totally

resorbed a litter of 4 [early resorptions]. Fetal body weights were slightly lower than the control [96% of control]. The sex ratios were comparable among the groups. External malformations [hindlimb flexure and constriction at base of tail; filamentous tail; defect of abdominal wall and protrusion of the viscera (omphalocele); and protrusion of brain tissue through opening in brain] were observed only at the two highest dose levels [1 fetus (0.3%) in 1 litter (3.6%) at the mid-dose and 3 fetuses (1%) in 2 litters (8.7%) at the high-dose levels]. Skeletal malformations were observed in 2 fetuses in 2 control litters, 1 fetus in 1 low-dose litter, 3 fetuses in 3 litters at both the mid- and high-dose levels. The one mid-dose fetus with constriction at the base of the tail displayed no ossified vertebral elements posterior to the third lumbar vertebral, the high-dose fetus with the filamentous tail displayed no ossified vertebral elements posterior to the second sacral vertebra, and the high-dose fetus with exencephaly displayed a hole in the cranium in the region of the frontals and parietals. The incidence of total skeletal variations was slightly higher at the mid- and high-dose levels compared to the control but the fetal incidence did not show a dose-response [fetal: 84.1%, 82.2%, 89.8%, and 86.5%] and litter [96.6% in control and 100% for all treatment groups].

The maternal toxicity NOAEL is 65 mg/kg/day, and the maternal toxicity LOAEL is 190 mg/kg/day, based on mortality [one death], decreased body-weight gain on gestation days 6-9, and decreased food consumption on days 6-11. The NOAEL for developmental toxicity is 65 mg/kg/day, and the developmental toxicity LOAEL is 190 mg/kg/day, based on a slight increase in the incidence of skeletal malformations/variations and external malformations.

This developmental toxicity study is classified Acceptable/Guideline, and it satisfies the guideline requirements [OPPTS 870.3700; §83-3(a)] for a developmental toxicity study in the rodent.

[2,4-D ISOPROPYLAMINE SALT] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Gravid Uterine Weight/Corrected Body Weight/Gain [grams]

Dose/Day	0 mg/kg/day	22 mg/kg/day	65 mg/kg/day	190 mg/kg/day
body weight				
0	201±17	204±20	201±17	198±16
6	233±19	237±22	234±20	229±20 [98]
9	247±19	250±23	244±22	236±20 [96]
12	262±18	266±23	261±21	252±18 [96]
16	286±19	292±24	284±22	278±19
20	344±22	352±27	342±26	333±30
body weight gain				
0-6	32±6	33±5	33±7	31±7
6-9	14±4	13±5	10±5	6±7 [43]**
9-12	15±5	16±5	17±7	17±6
12-16	25±6	26±7	23±6	25±8
16-20	58±8	59±11	58±10	56±15
6-16	53±10	55±10	51±10 [96]	48±11 [91]
gravid uterine weight	72.6±8.3	75.0±9.3	70.9±12.6	66.7±16.0 [92]
corrected body weight	271.5±19.2	276.6±23.0	271.0±23.4	266.7±19.7
corrected BWG	38.4±10.0	39.1±12.3	37.1±9.6	37.2±9.7

√[% of control]; n=24-29; ** p<0.01; data from Appendices C-1, D-1, and E-2 [pages 52, 57, and 63] of the report

[2,4-D ISOPROPYLAMINE SALT] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Table 2. Skeletal Malformations/Variations				
Dose/Finding/%	0 mg/kg/day	22 mg/kg/day	65 mg/kg/day	190 mg/kg/day
malformation	% fetuses/% litters	% fetuses/% litters	% fetuses/% litters	% fetuses/% litters
hole in cranium	0/0	0/0	0/0	0.7/4.3
presence of cervical ribs	0/0	0/0	0/0	0.7/4.3
absence of sacral vertebrae	0/0	0/0	1/1	0.7/4.3
presence of small discrete ossifications	0/0	0/0	0/0	0.7/4.3
absence of caudal vertebrae	0/0	0/0	1/1	0.7/4.3
total skeletal malformations	1.1/6.9	0.6/3.7	1.7/10.7	2.0/13.0
Variation - incompletely ossified:	% fetuses/% litters	% fetuses/% litters	% fetuses/% litters	% fetuses/% litters
frontal	0.5/3.4	0.6/3.7	1.7/10.7	2.7/8.7
supraoccipital	16.5/37.9	17.8/55.6	20.5/57.1	17.6/60.9
squamosal(s)	4.9/20.7	10.9/44.4	8.5/25.0	13.5/43.5
malar(s)	3.8/20.7	8.6/33.3	5.7/17.9	8.1/34.8
maxilla	2.2/13.8	4.6/22.2	3.4/7.1	5.4/21.7
nasal(s)	0.5/3.4	2.3/11.1	3.4/10.7	5.4/17.4
parietal(s)	1.6/6.9	2.9/11.1	6.3/21.4	5.5/17.4
hyoid	1.6/10.3	2.3/14.8	1.1/7.1	2.7/17.4
Variation - not ossified	% fetuses/% litters	% fetuses/% litters	% fetuses/% litters	% fetuses/% litters
hyoid	23.6/62.1	24.7/63.0	27.3/64.3	24.3/56.5
presphenoid	0/0	0/0	0/0	0.7/4.3
5th sternebrae	34.1/79.3	27.0/70.4	40.9/85.7	46.6/78.3
Variation - rib(s)	% fetuses/% litters	% fetuses/% litters	% fetuses/% litters	% fetuses/% litters
rib(s) - short	0	0	0	1.4/8.7
rib(s) - rudimentary	0	0.6/3.7	0	1.4/8.7
rib(s) - 1st lumbar rudimentary	3.8/17.2	2.9/14.8	4.5/25.0	10.1/26.1
total skeletal variations	84.1%/96.6%	82.2%/100%	89.8%/100%	86.5%/100%
# evaluated				
fetuses	182	174	176	148
litters	29	27	28	23

✖[% fetuses/% litters], calculated by reviewer; data from Appendices M-N, pages 293-300 of the report

CASWELL FILE

GUIDELINE: 83-3

Primary Review by: Karen E. Whitby, Ph.D. 11/8/90
Toxicologist, Review Section II, Toxicology Branch II/HED (H7509C)

Secondary Review by: K. Clark Swentzel 11/19/90
Section Head, Review Section II, Toxicology Branch II/HED (H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3

EPA Identification Nos.: EPA MRID (Accession) No. 415271-03 /-06
EPA ID No. 030025-1
EPA Record No. 268,213
Caswell No. 315U
HED Project No. 0-1707

Test Material: 2,4-D Isopropylamine Salt

Synonyms: (2,4-D IPA)

Sponsor: The Dow Chemical Co., The Toxicology Research Laboratory 1803 Building, Midland, MI 48674

Study Number(s): HET M-004725-011 (The Dow Chemical Co.,)
Project No. 89-3465 (Bio/dynamics, Inc.)

Testing Facility: Bio/dynamics, Inc., East Millstone, New Jersey 08875

Title of Report: A Teratogenicity Study in Rats with 2,4-D Isopropylamine

Author(s): Raymond E. Schroeder

Report Issued: May 7, 1990

Conclusions:

2,4-D IPA was evaluated for maternal and developmental toxicity at 0, 22, 65, and 190 mg/kg. Maternal toxicity was evident at 190 mg/kg by decreased bodyweight gain days 6-9, and decreased food consumption 6-11. There was mild developmental toxicity at 190 mg/kg indicated by skeletal variations. Although the incidence is not high in any of the studies, it is worthy of note that the 2,4-D TIPA (MRID No. 415271-02) 2,4-D BEE (MRID No. 415271-01) and the current study have detected ocular effects (anopthalmia, micropthalmia, and folded retina) that did

not occur at a high incidence. In addition, all studies found variations of the ureters (distended and/or tortuous) and dilated renal pelvises. In the current study dilated renal pelvis was noted in the mothers as well as the fetuses. It was also noted once in the mothers of each group in the 2,4-D TIPA main study (MRID No. 415271-02). It is not clear whether these effects are related to treatment or are a spontaneous occurrence.

Maternal NOEL = 65 mg/kg
Maternal LOEL = 190 mg/kg (HDT)

Developmental Toxicity NOEL = 65 mg/kg
Developmental Toxicity LOEL $\geq$ 190 mg/kg

Core Classification: Core Minimum Data

RANGE-FINDING STUDY

The main developmental toxicity study was preceded by a pilot study, which was performed to determine the maternal and embryotoxic potential of 2,4-D Isopropylamine Salt in the pregnant rat. Evaluation of developmental toxicity consisted of recording the number of live fetuses on day 16 at the time of cesareans. Both of these studies were performed by Bio/dynamics.

Study Title: A Range-Finding Study to Evaluate the Toxicity of 2,4-D Isopropylamine Salt in the Pregnant Rat

Study No.: MRID No.415271-06
Study ID: HET M-004725-010 and HET K-004725-010 (The Dow Chemical Co.) both appear on the title page
Project No. 89-3466 (Bio/dynamics, Inc.)

Date of Report: March 20, 1990

Date of Study: (Live Portion) July 18, 1989 - August 9, 1989

Author: Raymond E. Schroeder

Materials and Methods

2,4-D Isopropylamine Salt [Lot No. AGR 276461, purity 50.2% (the amount of test article added to the vehicle was corrected for purity)] was administered to mated CD® (Sprague-Dawley derived) ♀ rats (10/group) by gavage at dose levels of 0 (vehicle: distilled, deionized water), 63, 127, and 190 mg/kg. Test article was administered on days 6-15 of gestation. Dams were euthanized on day 16 of gestation; postmortem examination included weighing of the gravid uterus, kidneys, and liver; recording the number of corpora lutea and uterine implants (no. live, dead, and resorbed).

Results

There was no mortality in this study. Days 6-9 and 6-16 of gestation the mean bodyweight gain of the 190 mg/kg group was -58.3 and -28.3% relative to control, respectively. The difference was only significant days 6-16. The corrected maternal bodyweight gain days 6-16 of the 190 mg/kg group was significantly lower than control (-46.1%). The incidence of excessive salivation was 0, 70, 100, and 100% among the 0, 63, 127, and 190 mg/kg groups. Two low- and 3 mid-dose group animals were determined to be not pregnant. There was an increase (125%) in the number of resorptions in the high-dose group relative to the control that was reported to be not significant. As such, there was an increase (77.1%) in the postimplantation loss that was also reported to be not significant.

Conclusions

The dosage levels of 2,4-D IPA for the main developmental toxicity study were 0, 22, 65, and 190 mg/kg. Based upon the treatment related effects observed in this study, the dosages selected for the main study appeared to be appropriate.

Core Classification: Supplementary Data (Range-Finding Study)

Main Study

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: Purity: 50.2%
Description: Dark Brown Liquid
Lot No.: AGR 276461

Vehicle(s): Identity: Distilled, deionized water

Test Animal(s): Species: Rat
Strain: CD® (Sprague-Dawley derived)
Source: Charles River Laboratories, Inc., Portage, MI 49081
Age at Initiation of Mating: ♀ 63 days
♂ approx. 43 weeks
Weight of ♀ on day 0: (mean) 200 g
(range) 167 - 254 g

B. Study Design

This study was designed to assess the developmental toxicity potential of 2,4-D Isopropylamine Salt when administered by gavage to rats on gestation days 6 through 15, inclusive.

Mating

Females were placed with males nightly in a 1:1 ratio. Animals not utilized in the study that remained from the original population were sacrificed and discarded after all study groups had been filled and the last mated females within study groups had initiated dosing.

Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	30
Low Dose	22	30
Mid Dose	65	30
High Dose	190	30

Dosing:

All doses were in a volume of 5 ml/kg of body weight/day prepared once prior to initiation of treatment in a volume adequate for the duration of the study. When the dosing solutions were prepared, the amount of test article added to the vehicle was adjusted to correct for the

300

purity of the test article. The dosing solutions were analyzed for concentration, homogeneity, and stability. Concentrations of the test chemical ranged from 98.5 to 102.1% of the target concentration at the beginning of dosing. Upon the conclusion of the dosing period, the range of concentration was 108.1 to 110.4%. The coefficient of variation was within 3%, indicating homogeneity. The dosing solution was found to be stable for at least 51 days (± 10% of the day 0 concentration). Initial dose volumes were based on gestation day 6 bodyweights and were adjusted for each animal during the remaining portion of treatment to the most recent bodyweight.

Observations

Maternal

The animals were checked for mortality or abnormal condition twice daily. In addition, each female was given a detailed physical examination on days 0, 6-15, and 20 of gestation. Dams were sacrificed on day 20 of gestation by overdose of carbon dioxide inhalation. Examinations at sacrifice consisted of: the external surface, all orifices, the cranial cavity, carcass, external surface of the spinal cord and sectioned surfaces of the brain, nasal cavity and paranasal sinuses, the thoracic, abdominal, and pelvic cavities, viscera, kidney and liver weights cervical tissues and organs.

Maternal bodyweights were recorded on days 0, 6, 9, 12, 16, and 20 of gestation. Feed consumption was recorded on days 0-6, 6-11, 11-16, and 16-20.

Reproductive

The intact uterus including ovaries was removed and weighed. The number and location of the following was recorded for each uterine horn: live fetuses, dead fetuses, late resorptions, early resorptions, and implantation sites. The ovaries were dissected free and the number of corpora lutea were recorded. If uterine implantations were not evident, the uterus was stained with ammonium sulfide,according to the method of Salewski, 1964.

Fetal

The fetuses were examined in the following manner: each fetus was individually identified, weighed, sexed externally, and examined for gross external malformations/variations. Approximately one-half of the fetuses of each litter (every other fetus within the litter) were evaluated for visceral malformations/variations via a modified Staples, 1974 technique. Fetuses designated to undergo the visceral exam were decapitated and the heads were fixed in Bouin's for subsequent examination. The fetuses were secured under a dissecting scope, and the thoracic and abdominal cavities were examined. Upon completion of this, the fetuses were eviscerated and individually fixed in 70% ethanol. The heads were serially transverse sectioned using a razor

blade after fixation was complete. The sections were observed for malformations of the palate, eyes, and brain via dissecting scope.

The remaining fetuses in each litter were euthanized via inhalation of ether. The fetuses were eviscerated (internally sexed), and stained with Alizarin Red S via a procedure (Crary, 1962) modified by Bio/dynamics. Skeletal specimens were evaluated under a dissecting scope for ossification malformations and variations.

Historical control data were provided to allow comparison with concurrent controls.

Statistical Analysis

The interval data (mean bodyweights, mean weight change, gravid uterus weight, day 20 corrected bodyweight, mean food consumption, mean organ weight, and reproduction data) were analysed by the following procedures. Bartlett's test was performed to determine if groups had equal variance. If so, a standard one way ANOVA using the F distribution to assess significance was performed. If warranted, Dunnett's test was used to determine which means were significantly different from the control. When necessary, the Kruskal-Wallis test was used as a nonparametric test of equality of means. If differences were found, a summed rank test (Dunn) was used to determine which groups differed from the control. A test for trend in the dose levels was also performed. For parametric data, standard regression techniques with a test for trend and lack of fit were used. For nonparametric data, Jonckheere's test for monotonic trend was used. All ratios were transformed via the arc sine transformation before analysis.

The incidence data (litters with resorptions, mortality rates, pregnancy rates, fetuses with malformations/variations, and litters with fetuses with malformations/variations) were analysed as follows. First, a standard chi-square analysis was performed to determine if the proportion of incidences differed between the groups tested. Each treatment group was compared to the control using a 2X2 Fisher Exact Test. The significance level was corrected via the Bonferroni inequality. Armitage's test for linear trend in the dose groups was performed. If any one cell had an expected value less than 5, the chi-square and Armitage's tests were not reported. When this occurred, only the Fisher Exact test (corrected via Bonferroni inequality) was performed and reported.

Compliance

A signed Statement of No Data Confidentiality Claims was provided.

A signed Statement of compliance with EPA GLP's was provided.

A signed Flagging Statement was provided.

A signed Quality Assurance Statement was provided.

C. Results

Mortality

There was no mortality in the control, low-, or mid-dose groups. In the high dose group one female died on day 7 of gestation (after one day of treatment).

Clinical Observations

The incidence of excessive salivation was noted at least once during the treatment period for the 0, 22, 65, and 190 mg/kg groups were 0, 0, 16.7, and 50%, respectively. Transient alopecia was noted in 0, 10, 3.3, and 20% of the animals in the 0, 22, 65, and 190 mg/kg groups, respectively.

Bodyweight

The investigators supplied the following data:

Table 1 Bodyweight Gains (grams)[a]

Group (mg/kg):	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gains Dosing P.[1]	Entire[2]
0	32	53	58	143.0	-19.5	70.4
22	33	55	59	145.0	-20.1	69.9
65	33	51	58	140.4	-19.6	69.4
190	31	48	56	135.6	-18.7	68.9

[1] corrected bodyweight gain for dosing period = bodyweight gain for dosing period minus gravid uterus weight.
[2] corrected bodyweight gain for entire gestation period = bodyweight gain for entire gestation period minus gravid uterus weight.
[Some of the above values were calculated by the reviewer from individual animal data]

[a] Data extracted from (study number HET K-004725-011 Appendices C, D, and E pp. 52-67)

Mean bodyweight gain for the pre- and post-treatment periods of gestation were comparable between the treated and control groups. Days 6-9 of gestation the mean weight gain of the 190 mg/kg group was significantly lower than the control (-57.1%).

Food Consumption

The investigators supplied the following data:

Table 2 Food Consumption Data (g/kg/day)[a]

Group: (mg/kg)	Days 0-6	Days 6-11	Days 11-16	Days 16-20	Days 0-20
0	114	102	103	96	414
22	113	101	100	96	406
65	115	102	102	96	414
190	112	93**	103	99	408

[a] Data extracted from (study number HET K-004725-011 appendix F pp. 68-73)
** Significantly different from control ($p \leq 0.01$)
[Some of the above values were calculated by the reviewer from individual animal data]

The only significant finding for the food consumption data was in the 190 mg/kg group days 6-11, where the amount consumed was -8.8% relative to the control.

Organ Weight Data

There were no significant changes in the absolute and relative liver and kidney weights.

Gross Pathological Observations

Gross pathological examination did not reveal any treatment related effects. Most of the observations noted were similar among the groups. The incidence of dilated renal pelvis was 1/28, 2/28, 2/29, and 3/28 in the 0, 22, 65, and 190 mg/kg groups, respectively.

Cesarean Section Observations

Table 3 Cesarean Section Observations[a]

Dose (mg/kg):	0	22	65	190
#Animals Assigned	30	30	30	30
#Animals Mated/Inseminated	29	27	28	26
Pregnancy Rate (%)	96.7	90.0	93.3	86.7
Maternal Wastage				
#Died	0	0	0	1
#Died/pregnant	0	0	0	1/26
#Non pregnant	1	3	2	5
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	432	407	409	361
Corpora Lutea/dam	14.9 ±1.7	15.1 ±2.1	14.6 ±2.0	15.0 ±1.9
Total Implantations	403	383	391	324
Implantations/Dam	13.9 ±1.4	14.2 ±1.5	14.0 ±2.1	13.5 ±2.7
Total Live Fetuses	380	365	364	302
Live Fetuses/Dam	13.1 ±1.7	13.5 ±1.7	13.0 ±2.5	12.6 ±3.1
Mean no. ♂/litter	6.7	7.2	5.9	6.4
Mean no. ♀/litter	6.4	6.3	7.1	6.2
Total Resorptions	23	18	27	22
Resorptions/Dam	0.8 ±0.9	0.7 ±0.8	1.0 ±0.9	0.9 ±0.9
Total Dead Fetuses	0	0	0	0
Mean Fetal Weight (gm)	3.51	3.54	3.45	3.37
♂ fetuses	3.60 ±0.2	3.60 ±0.2	3.51 ±0.2	3.44 ±0.3
♀ fetuses	3.41 ±0.2	3.47 ±0.2	3.40 ±0.2	3.30 ±0.3
Preimplantation Loss(%)	6.3	5.3	4.4	9.9
Postimplantation Loss(%)	5.7	4.7	7.4	9.4
Sex Ratio (Total ♂/Total ♀)	1.1	1.1	0.8	1.0

[a] Data extracted from (study number HET K-004725-011 appendix G pp. 74-78)

One female in the high dose group was noted to have one focus upon staining of the uterus with ammonium sulfide and was included in calculation of the pregnancy rate; however, the data for this female were excluded from the calculation of uterine implantation data.

Statistical evaluation of the corpora lutea and uterine implantation data did not reveal any differences between the control and treated groups. In the high dose group there was one female that totally resorbed a litter of 4. Omission of this data point results in a postimplantation loss of 5.5%. There was a slight treatment related decrease (-4.4% for the 190 mg/kg group relative to the control) in male fetal bodyweights.

2. Developmental Toxicity

Four fetuses with external malformations in the high dose group were processed for both visceral and skeletal malformations. These fetuses were not decapitated and the visceral evaluation was restricted to microdissection and examination of the tissues in the thoracic, abdominal and pelvic cavities.

One fetus in the 65 mg/kg group had a hindlimb flexure and a constriction at the base of the tail. The 2 fetuses in the 190 mg/kg group with omphalocele and exencephaly were from the same litter. No external variations were noted in any of the groups, except for one fetus in the 65 mg/kg group that had shiny skin and was very small.

Table 4 External Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
External Malformations[a]				
#pups(litters) examined	380(29)	365(27)	364(28)	302(23)
Filamentous Tail	0(0)	0(0)	0(0)	1(1)
Constriction of Tail	0(0)	0(0)	1(1)	0(0)
Hindlimb Flexure	0(0)	0(0)	1(1)	0(0)
Omphalocele (abdominal viscera protruding)	0(0)	0(0)	0(0)	1(1)
Exencephaly (brain tissue protruding)	0(0)	0(0)	0(0)	1(1)
Total External Malformations				
fetal incidence N(%)	0(0)	0(0)	1(0.3)	3(1.0)
litter incidence N(%)	0(0)	0(0)	1(3.6)	2(8.7)

[+] some observations may be grouped together
[a] fetal (litter) incidence
Data taken from study number HET K-004725-011 appendix L p. 230

The most common visceral malformation observed was folded retina. This was observed only in one litter of the control group. Therefore, given the sporadic nature of the observed malformations which were not treatment related it would appear that treatment of pregnant rats with 22, 65, and 190 mg/kg of 2,4-D Isopropylamine salt did not result in visceral malformations.

Table 5 Visceral Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
Visceral Malformations[a]				
#pups(litters) examined	198(29)	191(27)	189(28)	157(23)
Retina(s) - folded	3(1)	1(1)	1(1)	0(0)
Anophthalmia	0(0)	0(0)	0(0)	1(1)
Total Visceral Variations				
fetal incidence N(%)	3(1.5)	1(0.5)	1(0.5)	1(0.6)
litter incidence N(%)	1(3.4)	1(3.7)	1(3.6)	1(4.3)
Visceral Variations[a]				
#pups(litters) examined	198(29)	191(27)	189(28)	157(23)
Kidney(s) - distended renal pelvis (papilla present)	0(0)	2(2)	3(2)	1(1)
Ureter(s) - tortuous	6(4)	11(6)	5(2)	1(1)
Ureter(s) - distended	3(3)	0(0)	6(2)	1(1)
Total Visceral Variations				
fetal incidence N(%)	8(4.0)	11(5.8)	12(6.3)	2(1.3)
litter incidence N(%)	5(17.2)	6(22.2)	4(14.3)	2(8.7)

[+] some observations may be grouped together
[a] fetal (litter) incidence
Data taken from study number HET K-004725-011 appendix M pp. 268-292

The most common visceral variation was distended and/or tortuous ureters. The fetal incidence for this variation did not indicate a treatment related effect.

Table 6 Skeletal Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
Skeletal Malformations[a]				
#pups(litters) examined	182(29)	174(27)	176(28)	148(23)
Hole in Cranium	0(0)	0(0)	0(0)	1(1)
Reduced Number of Cervical Vertebra(ae)	0(0)	0(0)	1(1)	0(0)
Cervical Transverse Process(es) - misshapen	0(0)	0(0)	1(1)	0(0)
Presence of Cervical Rib(s)	0(0)	0(0)	0(0)	1(1)
Skeletal Variations[a]				
#pups(litters) examined	182(29)	174(27)	176(28)	148(23)

Table 6 Skeletal Examinations - cont'd

Observations[+]	Control	Low Dose	Mid Dose	High Dose
Squamosal(s) - inc. oss.	9(6)	19(12)	15(7)	20(10)
Malar(s) -inc. oss.	7(6)	15(9)	10(5)	12(8)
Parietal(s) - inc. oss.	3(2)	5(3)	11(6)	8(4)
Frontal(s) - inc.oss.	1(1)	1(1)	3(3)	4(2)
Nasal(s) - inc. oss.	1(1)	4(3)	6(3)	8(4)
Cervical Transverse Process(es) - inc. oss.	4(3)	12(7)	10(7)	17(11)
Ossification Adj. to 7th Cervical Vertebra(ae)	3(2)	2(2)	2(2)	6(5)
Rib(s) - Rudimentary	0(0)	1(1)	0(0)	2(2)
Rib(s) - 1st Lumbar Rudimentary	7(5)	5(4)	8(7)	15(6)
3rd Sternebra - inc. oss.	11(5)	8(5)	4(3)	11(6)
Total Skeletal Variations				
fetal incidence N(%)	153(82.2)	143(82.2)	158(89.8)	138(86.5)
litter incidence N(%)	28(96.6)	27(100)	28(100)	23(100)

[+] some observations may be grouped together
[a] fetal (litter) incidence
Data taken from study number HET K-004725-011 appendix N pp. 293-381

Skeletal malformations were seen in the one mid-dose and one high-dose fetus noted externally with tail defects. The mid-dose fetus had a constriction at the base of the tail and did not have ossified vertebral elements posterior to the third lumbar vertebra. The high-dose fetus had a filamentous tail and no ossified vertebral elements posterior to the second sacral vertebra. A hole in the cranium in the area of the frontals and parietals was noted in the high-dose fetus with exencephaly. The incidence of fetuses with at least one skeletal variation was 100% in each of the treatment groups and 96.6% in the control. There was a slight treatment related increase in the incidence of incompletely ossified squamosal(s), nasal(s), and cervical transverse process(es). In addition, there was a slightly increased incidence of ossification adjacent to the 7th cervical vertebra(ae). These effects are most prominent at the high-dose.

D. **Discussion/Conclusions**

a. Maternal Toxicity:

In the 190 mg/kg group there was one death on day 7 of gestation. Other than this there were no deaths in this study. Days 6-9 of gestation there was a significant decrease in maternal bodyweight at 190 mg/kg. There was a significant decrease in food consumption for this group days 6-11 of gestation. Also at 190 mg/kg, excessive

salivation was noted in 50% of the animals.

b. Developmental Toxicity:

i. Deaths/Resorptions:
There were no fetal deaths. The number of resorptions per litter was comparable among the treatment groups.

ii. Altered Growth:
There was a slight reduction in the mean body weight of viable fetuses and of male fetuses at 190 mg/kg.

iii. Developmental Anomalies:
At 190 mg/kg, there was a slight increase in the incidence of incompletely ossified squamosal(s), nasal(s), and cervical transverse process(es). In addition, there was a slight increased incidence of ossification adjacent to the 7th cervical vertebra(ae).

iv. Malformations:
The distribution and incidence of the malformations noted in this study do not indicate a treatment related effect.

E. Study Deficiencies:

The title page for this report has two study ID #'s HET M-004725-011 and HET K-004725-011. More recent historical control data than 1982-1987 would be helpful as this study was conducted in late 1989.

F. Core Classification: Core Minimum Data.

This study satisfies the guideline requirements (83-3) for a developmental toxicity study.

Maternal NOEL = 65 mg/kg
Maternal LOEL = 190 mg/kg (HDT) based upon decreased bodyweight gain days 6-9 and decreased food consumption days 6-11.
Developmental Toxicity NOEL = 65 mg/kg
Developmental Toxicity LOEL $\geq$ 190 mg/kg based upon slight increases in skeletal variations.

G. **Risk Assessment**: None at this time.

[DIMETHYLAMINE SALT OF 2,4-D] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Amendment to DER for MRID 41735201 - Developmental Toxicity Study - Rat [DIMETHYL AMINE SALT OF 2,4-D]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document Nos. 008400 and 010273].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/6/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia Dobozy, V.M.D., M.P.H. Virginia Dobozy 12/8/00
Reregistration Branch I, Health Effects Division (7509C)
TXR# 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rat § 83-3(a)/OPPTS 870.3700
P.C. CODE: 030019 REREGISTRATION CASE NO/CASE NO.: 818706
CASWELL NO.:315O CAS NO.: 2008-39-1
TEST MATERIAL (PURITY) : dimethylamine salt of 2,4-D [66.18%; acid equivalent 55.5%]
SYNONYMS: 2,4-D DMA; dichlorophenoxyacetic acid, dimethylamine salt
SPONSOR: Industry Task Force II on 2,4-D Research Data

CITATION: Lochry, E. A. (1990). Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-D Dimethylamine Salt (2,4-D-DMA) Administered Orally *via* Gavage to Crl:CD®BR VAF/Plus® Presumed Pregnant Rats. Argus Research Laboratories, Inc., Perkasie, PA; Study No. 320-001. MRID 41735201. Document No. 008400. Unpublished.

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 41735201], female Crl:CD® BR VAF/Plus® rats [25/group] were administered the dimethylamine salt of 2,4-dichlorophenoxyacetic acid [66.18%] *via* gavage at dose levels of 0 [deionized water], 12.5 mg/kg/day, 50 mg/kg/day, and 100 mg/kg/day [acid equivalents] from gestation day 6 through gestation day 15.

There were no deaths. Decreased motor activity and ataxia were observed in 4 of the 25 high-dose rats [days 12-16 of gestation], and an additional high-dose dam displayed decreased motor activity on day 15 of gestation. Body weights were comparable among the groups throughout the study, but there was a statistically-significant decrease in body-weight gain during the dosing period in the mid- (91% of control) and high- (87% of control) dose groups. The corrected body-weight gain [mid-(93%) and high-(89%) of control] was also decreased in a dose-related manner, although statistical significance was not attained. There was a dose-related decrease in food consumption [g/kg/day] during the dosing period [96%, 94%, 90% of control with increasing dose] and for the entire gestation period [97%, 96%, 94% of control with increasing dose], with statistical significance being attained at all dose levels.

There were no abortions, and the pregnancy rate was comparable among the groups. One control dam had

resorptions only; all other dams had viable fetuses. The numbers of corpora lutea, implantations, and live fetuses were comparable among the groups. The number of resorptions, as well as pre- and post-implantation losses, were not adversely affected, although the number of dams with one or more resorptions was slightly increased at the high-dose level [16 (64%)] compared to the control [9 (42.8%)]. None of the dams in any group had 100% resorptions. One high-dose dam had 1 late resorption compared to no late resorptions in the control or other groups. There were no dead fetuses. Fetal body weight was significantly reduced [92%-93% of control] at the high-dose level compared to the control, and the sex ratio was comparable among the groups, although the high-dose groups displayed a greater number of males [56.7%] compared to the control [49.2%].

There were no statistically-significant differences in the incidence of fetal external, visceral, or skeletal malformations. One high-dose fetus had fused ribs, and one high-dose fetus had undescended testes. There was a significant increase in the incidence of wavy ribs and/or incompletely ossified ribs at the high-dose level [both on a fetal and litter basis]. There was an increased number of litters with fetuses with alterations [malformations and variations] present in the treated groups [7 (29.2%), 7 (28%), and 16 (64%) with increasing dose] compared to the control [4 (19%], and the number of fetuses with any alteration was increased significantly at the mid- [15 (3.5)] and high- [23 (5.3%] dose levels compared to the control [6 (1.7%)]. Delays in ossification of the ribs, sternum, and hyoid were observed at the high-dose level.

The maternal toxicity NOAEL is 12.5 mg/kg/day, and the maternal toxicity LOAEL is 50 mg/kg/day, based on decreased body-weight gain and decreased food consumption. The NOAEL for developmental toxicity is 50 mg/kg/day, and the developmental toxicity LOAEL is 100 mg/kg/day, based on decreased fetal body weight, increased incidence of wavy ribs and/or incompletely-ossified ribs.

This guideline developmental toxicity study is classified Acceptable, and it satisfies the guideline [OPPTS 870.3700; §83-3(a)] for a developmental toxicity study in the rodent.

NOTE: This study was classified as unacceptable previously due to the lack of purity information for the test material. Adequate data/information of the purity were submitted subsequently and the study was upgraded to acceptable [HED Document No. 010273].

[DIMETHYLAMINE SALT OF 2,4-D] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Gravid Uterine Weight/Corrected Body Weight/Gain [grams]

Dose✖/Day	0 mg/kg/day	12.5 mg/kg/day	50 mg/kg/day	100 mg/kg/day
body weight				
0	263.3±7.1	263.7±7.3	263.4±7.2	263.2±7.1
6	301.0±14.8	301.8±9.8	302.6±11.0	301.0±11.7
7	305.5±11.9	306.6±10.6	304.6±11.8	300.0±13.2 [98]√
9	315.8±13.4	313.7±12.2	313.8±10.2	307.4±12.4
12	336.5±14.5	332.8±15.3	332.0±10.3	324.0±14.1 [96]
16	376.2±20.2	374.3±14.8	371.3±14.3	366.4±14.9
20	453.5±30.3	447.8±23.2	447.3±22.1	441.0±20.5 [97]
corrected	354.9±22.2	350.5±15.4	348.6±14.5	344.9±17.4 [97]
body-weight gain				
0-6	37.6±11.4	38.0±5.8	39.2±10.5	37.8±10.2
6-7	4.6±5.7	4.9±4.3	2.0±4.2	-1.0±6.3**
6-9	14.9±6.0	11.9±5.4	11.3±6.5	6.4±7.1** [43]
9-12	20.7±5.0	19.1±8.0	18.2±3.0	16.5±6.3 [80]
12-16	39.7±8.2	41.5±7.9	39.2±4.7	42.4±7.0
16-20	77.3±13.1	73.4±11.9	76.0±12.6	74.6±9.9 [97]
6-16	75.3±10.6	72.5±9.8	68.7±7.2* [91]	65.4±10.2** [87]
0-20	190.2±29.0	184.0±21.2	183.9±20.1	177.8±18.9 [93]
6-20C♪	53.9±14.8	47.5±13.6	46.1±11.9	43.9±12.0 [81]
gravid uterine weight	98.1±17.8	97.3±19.4	98.6±12.9	96.1±9.3

✖ acid equivalents; √[% of control]; * p<0.05; ** p<0.01; data from Table 3, pages 42-44 of the report

Table 2. Number of Litters With Resorptions

Dose✖/Day	0 mg/kg/day	12.5 mg/kg/day	50 mg/kg/day	100 mg/kg/day
# dams w/ 1 or more resorptions	9/21 [42.8%]	9/24 [37.5%]	12/25 [48%]	16/25 [64%]
historical control	in 53 studies [performed during 1986-1988], 741/1301 [57%] of control dams had 1 or more resorptions [range 28.6%-92.8%]■			

✖ acid equivalents; ♪ corrected for uterine weight; ■ footnote [page 28]; * p<0.05; ** p<0.01;
data from Tables 5-6, pages 26-27 of the report

Table 3. Summary of Alterations Observed in Fetuses

Dose✖/Parameter	0 mg/kg/day	12.5 mg/kg/day	50 mg/kg/day	100 mg/kg/day
litters evaluated fetuses evaluated	21 352	24 388	25 425	25 436
litters w/ fetuses w/ any alteration	4 [19%]	7 [29.2%]	7 [28%]	16 [64%]**
fetuses w/ any alteration	6 [1.7%]	11 [2.8%]	15 [3.5%]*	23 [5.3%]**
% fetuses w/ any alteration/litter	1.55±3.54	4.28±10.71	3.27±6.13	5.43±5.02**

✖ acid equivalents; * p<0.05; ** p<0.01; data from Table 7, page 49 of the report

[DIMETHYLAMINE SALT OF 2,4-D] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Table 4. Summary of Fetal Skeletal Alterations				
Dose✖/Parameter	0 mg/kg/day	12.5 mg/kg/day	50 mg/kg/day	100 mg/kg/day
litters evaluated	21	24	25	25
fetuses evaluated	181	199	220	226
RIBS - wavy &/or incompletely ossified/ hypoplastic				
litter incidence n [%]	0	0	1 [4]	4 [16]**
fetal incidence n [%]	0	0	2 [0.9]	5 [2.2]**
RIBS - cervical rib at 7th cervical vertebra				
litter incidence n [%]	1 [4.8]	1 [4.2]	2 [8.0]	1 [4.0]
fetal incidence n [%]	1 [0.6]	1 [0.5]	2 [0.9]	1 [0.4]
RIBS - fused				
litter incidence n [%]	0	0	0	1 [4.0]
fetal incidence n [%]	0	0	0	1 [0.4]
sternebrae summary [includes incompletely/unossified]				
litter incidence n [%]	1 [4.8]	1 [4.2]	2 [8]	7 [28]**
fetal incidence n [%]	2 [1.1]	1 [0.5]	4 [1.8]	9 [4]
ossification sites per fetus per litter				
hyoid	0.97±≈0.06	0.93±0.09	0.95±0.10	0.87±0.16*
sternum - sternal centers	3.75±0.21	3.66±0.31	3.66±0.25	4.44±0.28**

✖ acid equivalents; * $p<0.05$; ** $p<0.01$; data from Tables 10-11, pages 54-55, 57 of the report

304

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GUIDELINE: 83-3

Primary Review by: Karen E. Whitby, Ph.D. KEW 5/13/91
Toxicologist, Review Section II, Toxicology Branch II/HED
(H7509C)

Secondary Review by: K. Clark Swentzel K. Clark Swentzel 5/17/91
Section Head, Review Section II, Toxicology Branch II/HED
(H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3

EPA Identification No.s: EPA MRID (Accession) No.:417352-01
Caswell No.: 315-O
HED Project No.: 1-0620

Test Material: 2,4-D Dimethylamine salt (2,4-D-DMA)

Sponsor: Industry Task Force II on 2,4-D Research Data

Study Number(s): 320-001 (Argus Research Laboratories)

Testing Facility: Argus Research Laboratories, Inc.
2025 Ridge Rd.
Perkasie, Pa. 18944

Title of Report: Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-D Dimethylamine Salt (2,4-D-DMA) Administered Orally via Gavage to Crl:CD®BR VAF/Plus® Presumed Pregnant Rats

Author(s): Elizabeth A. Lochry, Ph.D.

Report Issued: November 15, 1990 (Final Report)

Study Dates: January 22, 1990 (beginning of mating)

Bibliographic Citation: Lochry, E.A., (1990) Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-D Dimethylamine Salt (2,4-D-DMA) Administered Orally via Gavage to Crl:CD®BR VAF/Plus® Presumed Pregnant Rats. Study Number(s) 320-001. (Testing Facility) Argus Research Laboratories, Inc. 2025 Ridge Rd. Perkasie, Pa. 18944

Conclusions:

The test article, 2,4-D-DMA was administered by gavage to presumed pregnant rats at 0, 12.5, 50, and 100 mg/kg/day in Reverse Osmosis Membrane Processed Water (R.O. deionized water). Decreased motor activity and ataxia were noted to occur at a significantly higher incidence in the 100 mg/kg group, than in the control group. Maternal bodyweight was significantly reduced in the 50 and 100 mg/kg groups during the dosing period relative to the controls. In addition, during the dosing period, all treatment groups had a significant reduction in food consumption relative to the controls. There were significant reductions in male and female fetal bodyweight and litter weight at 100 mg/kg. There was a significant increase in the number of litters with fetuses with any alteration observed and the percentage of fetuses with any alteration/litter at 100 mg/kg. An evaluation of fetuses with any alteration observed was significantly increased at 50 and 100 mg/kg. There was a significant increase in the incidence of wavy and or incompletely ossified ribs at 100 mg/kg. This study is considered as supplementary data due to the lack of stability data on the test substance. In addition, clarification/justification of the use of such a low purity of the test substance is requested. If the chemistry information is provided, this study may be upgraded.

Maternal NOEL = 12.5 mg (acid equivalent)/kg/day
Maternal LOEL = 50 mg (acid equivalent)/kg/day
Developmental Toxicity NOEL = 50 mg (acid equivalent)/kg/day
Developmental Toxicity LOEL = 100 (acid equivalent)/kg/day

Core Classification: Supplementary Data.

RANGE-FINDING STUDY

The main teratogenicity study was preceded by a pilot study, which was performed to determine the dosage levels to be used in the main study. The study was sponsored by Industry Task Force II on 2,4-D Research Data, and was performed by Argus Research Laboratories, Inc..

Study Title: Dosage-Range Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-D Dimethylamine Salt (2,4-D-DMA) Administered Orally via Gavage to Crl:CD®BR VAF/Plus® Presumed Pregnant Rats (Pilot Study)

Study No.: Argus Research Laboratories, Inc., Protocol 320-001P

Date of Report: May 11, 1990

Date of Study: November 6, 1989 (beginning of cohabitation period)

Author: Elizabeth Lochry, Ph.D. (Study Director)

Materials and Methods

The test substance, lot # 04FD31349 (purity 66.18%) was administered by gavage days 6 through 15 of gestation at 0 (vehicle), 12.5, 25, 50, 100 and 200 mg(acid equivalent)/kg/day in a 10 ml volume of Reverse Osmosis Membrane Processed Water (R.O. deionized water). The volume was adjusted daily for bodyweight and the doses were corrected for the 55.5% acid equivalent of the test substance and expressed as milligrams of the acid. There were 8 animals per treatment group. On day 20 the animals were euthanized and necropsied. Corpora lutea were counted and the livers and kidneys weighed. The gravid uterus was excised and weighed. The number and location of implantation sites, early and late resorptions, and live and dead fetuses were recorded. Fetuses were weighed sexed, and examined for gross alterations.

Results

All animals were pregnant and survived until day 20. The highest dose tested (200 mg/kg) produced ataxia, decreased motor activity, impaired righting reflex, tip-toe walk, excessive salivation, and urine-stained abdominal fur. Mean maternal bodyweight gain and food consumption were reduced at 25 mg/kg and above. Gravid uterine weights were reduced at 50 mg/kg and above. There was no effect reported on maternal liver and kidney weights.

Fetal bodyweight was decreased at 100 and 200 mg/kg. At 200

mg/kg there was an increase in resorptions and a concomitant decrease in live litter size at 200 mg/kg. Fetal alterations were only observed at 200 mg/kg (small or absent eye bulges and kinked tail).

Conclusions:

The dosage levels selected for the main study were 0, 12.5, 50, and 100 mg/kg.

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound:

Information on the test substance was not provided. The report (page 191) indicates that information on identity, composition, strength and purity of the test substance is on file with the Sponsor. The report also indicates (page 10) that the test substance had an acid equivalent of 55.5% (w/w) and purity of 66.18%. All dosage calculations were adjusted using the 55.5% acid equivalency factor and are expressed in milligrams of the acid equivalent of 2,4-D contained in the 2,4-D-DMA.

Description: brown liquid
Lot No.: 04FD31349
Contaminants: Not Stated

Vehicle(s): Reverse Osmosis Membrane Processed Water (R.O. deionized water)

Test Animal(s):

Species:	Rat
Strain:	Crl:CD®BR VAF/Plus®
Source:	females: Charles River Breeding Laboratories, Inc. Raleigh, North Carolina males: Charles River Breeding Laboratories, Inc. Portage, MI
Age:	♀ 92 days at receipt ♂ 64 days at receipt
Weight:	♀ 184 to 236 g the day following arrival; 250 to 278 g day 0 of gestation. ♂ 224 to 306 g two days following arrival

B. Study Design

This study was designed to assess the developmental toxicity potential of 2,4-D-DMA when administered by gavage to rats on gestation days 6 through 15, inclusive.

Mating

After a two-week acclimation period, 150 healthy virgin females were united with 150 breeder males in a 1:1 ratio. There was a maximum four day cohabitation period. The day of a confirmed mating was considered as day 0 of gestation. Mated and unmated females that were not placed on the study were placed in the Test Facility's general population.

Group Arrangement:

Animals were assigned to the dose groups by a computer generated (weight-ordered) randomization procedure based upon day 0 gestation bodyweight.

Test Group	Dose Level (mg/kg)	Number Assigned
Vehicle Control	0	25
Low Dose	12.5	25
Mid Dose	50	25
High Dose	100	25

Animal Husbandry:

The study room had 144 sq. ft. of floor space and a minimum of 10 changes per hour of 100% fresh HEPA filtered (99.7%) air. The room temperature was 74 $\pm$ 4° F; humidity was 30 - 65%. The animals were fed Purina Certified Rodent Chow® # 5002 ad lib. Local water that had passed through a R.O. membrane was also available ad lib via an automatic watering system. Chlorine had been added to the water as a bacteriostat. The monthly water samples contained from 0.6 to 0.8 ppm of chlorine.

Dosing:

The doses used in this investigation were selected on the basis of a dose range finding study.

All doses were in a volume of 10 mL/kg of bodyweight/day (adjusted daily for bodyweight). Doses were prepared weekly during the dosing period. Triplicate 10 mL samples of each concentration were reserved from each fresh preparation of the dosing solution. Two of the samples were sent to Lancaster Laboratories, Inc. for analysis. The third sample was kept at the facility as a backup. Five mLs of the bulk test substance was also shipped to Lancaster Laboratories for analysis. In addition, on the last day of the dosing period triplicate ten mL samples were also taken of the dosing solution and sent to Lancaster Laboratories and a 5 mL sample of the bulk test substance was sent to the Sponsor for possible analysis.

The dosing solutions for the pilot and main study were analyzed for concentration by Lancaster Laboratories. According to the protocol, stability analysis was also performed by Lancaster Laboratories. Results of the stability analysis were not provided. Homogeneity analyses was not performed because the test substance is highly soluble in water. The detection limit was <0.01 mg/g 2,4-D Acid Equivalent. The results for both of these studies combined are as follows:

Theoretical Concentration of Dosing Solution:	Actual Range:
0 mg/mL	0
1.25 mg/mL	-2 to 2%
2.5 mg/mL	2 to 6%
5.0 mg/mL	-4 to 4%
10.0 mg/mL	-4 to 4%
20.0 mg/mL	-2 to 0%

Observations

The animals were checked for mortality at least twice daily. Clinical observations were performed on day 0, several times each day during the dosing period (6-15), and once a day during the post dosing period (16-20).

Bodyweight and food consumption were recorded on days 0, and 6 through 20 of gestation.

Dams were sacrificed on day 20 of gestation by carbon dioxide asphyxiation. A laparotomy was performed and the intact uterus was excised and weighed. Uteri that appeared nonpregnant were pressed between two glass plates to confirm pregnancy status. Abdominal and thoracic cavities were examined for gross lesions. In the event of gross lesions, the tissues were preserved in neutral buffered 10% formalin.

Corpora lutea were counted, the number and placement of implantations, early and late resorptions, and live and dead fetuses were recorded. Each fetus was removed from the uterus and individually identified with a tag, weighed, sexed, and observed for gross external alterations. Viable fetuses were sacrificed. One half of each litter was processed for soft tissue alterations, the other half was processed for skeletal alterations.

Historical control data were provided to allow comparison with concurrent controls.

Statistical Analysis

The section on statistical analyses is appended.

Compliance

A signed Statement of No Confidentiality Claim was provided dated December 5, 1990 (p 2).

A signed Statement of Compliance with EPA GLP's was provided that was dated December 5, 1990 (p 3).

A signed Quality Assurance Statement was provided dated November 15, 1990 (pp. 434 to 436).

A signed statement from the Chairman of the Technical Committee Industry Task Force II on 2,4-D Research Data, dated December 5, 1990 was provided, which indicated the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to this study. This study neither reportedly met or exceeded any of these criteria.

C. Results

1. Maternal Toxicity

Mortality

All females survived to their scheduled sacrifice.

Clinical Observations

The 100 mg/kg dose (HDT) caused a significant increase in the incidence of decreased motor activity and ataxia relative to the controls. (These effects were noted in the pilot study at 200 mg/kg but not at 100 mg/kg.) Other observations that were not attributed to treatment included alopecia, misaligned or broken incisors, chromodacryorrhea and chromorrhinorrhea.

Bodyweight

One female in the control group (17602) had a bodyweight loss of 55 g from days 0 to 6 of gestation (prior to the first dose). This female was excluded from the data shown below. There were no significant differences in the initial or pre-dosing bodyweights.

Table I: Bodyweight Gains (grams)[a]

Dose (mg/kg):	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gains Dosing P.[1]	Entire[2]
0	37.6	75.3	77.3	190.2	-38.5	91.5
12.5	38.0	72.5	73.4	184.0	-42.6	86.8
50.0	39.2	68.7*	76.0	183.9	-45.4	85.3
100.0	37.8	65.4**	74.6	177.8	-49.0	81.7

1 = corrected bodyweight gain for dosing period = bodyweight gain for dosing period minus gravid uterus weight.
2 = corrected bodyweight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight.
a = Data extracted from (study number 320-001 table 3 p. 44)
* Significantly different from control ($p \leq 0.05$)
** Significantly different from control ($p \leq 0.01$)
(Some of the above values were calculated by this reviewer.)

There were dose related decreases in maternal bodyweight gain during the dosing period. Significant reductions were observed in the 50 and 100 mg/kg groups during this period. Mean corrected bodyweight gain during the dosing period was reduced in a dose related fashion. However, this decrease was not significant.

Food Consumption

Prior to dosing there were no significant differences in relative (g/kg/day) maternal food consumption. Relative food consumption was significantly reduced for all treatment groups days 6 to 16, 12 to 16, 6 to 20 and 0 to 20 of gestation. Page 27 of the report indicates that days 0-20 of gestation, there was a significant reduction in food consumption at all three levels for absolute and or relative feed consumption. A significant reduction was not indicated on table 4 (p.45) for the absolute food consumption for the 12.5 mg/kg group.

Table II: Food Consumption Data (g/kg/day)[a]

Dose (mg/kg)	Prior to Dosing Period	Dosing Period	Post-Dosing Period	Entire Gestation Period
0	90.0	86.5	79.1	81.3
12.5	87.6	83.1*	75.7	78.6*
50.0	88.1	81.9**	74.9	78.0**
100.0	89.9	77.9**	74.3	76.8**

a = Data extracted from (study number 320-001 table 4 p.46)
* Significantly different from control ($p \leq 0.05$)
** Significantly different from control ($p \leq 0.01$)

Gross Pathological Observations

Upon necropsy only one dam in the high dose group was observed to have gross lesions. This female had enlarged adrenals, spleen and thymus, with pale areas present on the spleen and thymus. More than 30 black foci were observed on the liver. Due to the low incidence and the absence of a similar observation in the pilot study, this observation was not considered to be related to treatment.

Cesarean Section Observations

One animal in the control group (17602) had a bodyweight loss of 55 g days 0-6. Because of this unexplained weight loss, the data for this dam and her litter were not included in data tabulations and statistics. Significant increases were found at all treatment levels relative to the control. These increases could not have been related to treatment since treatment began after mating.

The report indicates a slight nonsignificant increase in the number of dams with one or more resorptions in the high dose group [16(64%)] as compared to the control group [9(42.8%)]. Comparison of postimplantation loss for these groups does not indicate a treatment related effect.

A significant reduction in fetal bodyweight was observed in the 100 mg/kg group relative to the control for males, females, and both sexes combined (the litter).

Page 26 of the report states that gravid uterine weight was comparable for all 4 groups. However in the pilot study, (p.154 of the report) 50 mg/kg and above produced reductions in gravid uterus wt.

Table III: Cesarean Section Observations[a]

Dose (mg/kg):	0	12.5	50	100
#Animals Assigned	25	25	25	25
#Animals Mated	25	25	25	25
# (%) Pregnant	22(88.0)	24(96.0)*	25(100)*	25(100)*
N	21[b]	24	25	25
Maternal Wastage				
#Died	0	0	0	0
#Non pregnant	3	1	0	0
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	385	425	470	476
Corpora Lutea/Dam	18.3	17.7	18.8	19.0
Total Implantation	368	403	451	461
Implantations/Dam	17.5	16.8	18.0	18.4
Total Live Fetuses	352	388	425	436
Live Fetuses/Dam	16.7	16.2	17.0	17.4
Total Resorptions	16	15	26	25
Early	16	15	26	24
Late	0	0	0	1
Resorptions/Dam	0.8	0.6	1.0	1.0
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (g)	3.53	3.66	3.50	3.28*
♂ fetuses	3.63	3.76	3.57	3.38*
♀ fetuses	3.44	3.55	3.43	3.15*
Preimplantation Loss(%)	5.1	5.3	4.0	3.0
Postimplantation Loss(%)	3.9	4.8	6.1	5.4
Sex Ratio (% Male)	49.2	49.2	51.8	56.7

a = Data extracted from (study number 320-001; tables 5, 6 pp. 47, 48 and table 15 pp.78-81)
b = Data from dam 17602 was excluded.
* Significantly different from vehicle control ($p \leq 0.01$)
(Some of the above values were calculated by this reviewer.)

2. Developmental Toxicity

Table IV: External Examinations

Dose (mg/kg):	0	12.5	50	100
Observations+				
#pups(litters) examined	352(21)	388(24)	425(25)	436(25)
Eye: left, bulge depressed	1(1)	0(0)	0(0)	0(0)
Hematoma:				
Snout	0(0)	1(1)	0(0)	0(0)
left hindlimb	0(0)	0(0)	0(0)	1(1)
Edema:				
cervical & thoracic	0(0)	0(0)	0(0)	1(1)
Tail: thread-like	0(0)	0(0)	1(1)	0(0)
Anus: small opening	0(0)	0(0)	1(1)	0(0)

Data extracted from study number 320-001; table 8 pp. 50-51.
+ = some observations may be grouped together
a = fetal (litter) incidence

Table IV: Visceral Examinations

Dose (mg/kg):	0	12.5	50	100
Observations+				
#pups(litters) examined	171(21)	189(24)	205(25)	210(25)
Microphthalmia, left	1(1)	0(0)	0(0)	0(0)
Testes, undescended	0(0)	0(0)	0(0)	1(1)

Data extracted from study number 320-001; table 9 p. 52.
+ = some observations may be grouped together
a = fetal (litter) incidence

Table IV: Skeletal Examinations

Dose (mg/kg):	0	12.5	50	100
Observations+				
#pups(litters) examined	181(21)	199(24)	220(25)	226(25)
VERTEBRAE				
Thoracic:				
Centrum:				
Bifid	1(1)	2(2)	1(1)	2(2)
Unilateral Ossification	0(0)	0(0)	1(1)	1(1)
Asymmetric	0(0)	0(0)	1(1)	0(0)
Lumbar:				
Arches inc. ossified	0(0)	1(1)	0(0)	0(0)
Two Present	0(0)	0(0)	1(1)	0(0)
Sacral:				
None Present	0(0)	0(0)	1(1)	0(0)
Caudal:				
None Present	0(0)	0(0)	1(1)	0(0)
RIBS				
Cervical Rib (C7)	1(1)	1(1)	2(2)	1(1)
Wavy &/or inc. oss.	0(0)	0(0)	2(1)	5(4)**
Fused	0(0)	0(0)	0(0)	1(1)
STERNEBRAE				
Inc. Oss.	2(1)	0(0)	3(2)	6(5)
Unossified	0(0)	1(1)	1(1)	4(3)
PELVIS				
Pubes				
Inc. Oss.	1(1)	7(4)	3(1)	3(3)
Ischia				
Inc. Oss.	0(0)	2(1)	3(2)	1(1)

Data extracted from study number 320-001; table 10 pp. 53-56.
+ = some observations may be grouped together
a = fetal (litter) incidence

There was a significant increase in the incidence of wavy and/or incompletely ossified ribs at 100 mg/kg on the fetal and litter basis.

In the 12.5 mg/kg group, fetus 17074-5 had incompletely ossified right lumbar arches 5 and 6, and the pubis and ischium on the left were incompletely ossified. Fetus no. 11 of the same litter had a cervical rib at the 7th cervical vertebrae on the right, and the pubis and ischium were incompletely ossified on the left.

At the 50 mg/kg level, asymmetric thoracic centra and unilateral ossification of the thoracic centrum, were noted in the same fetus. Also at this level, another fetus had only two lumbar

vertebrae present, and no sacral or caudal vertebrae present. In a third litter at 50 mg/kg, one fetus had an unossified first sternebral bone and incompletely ossified pubic bone. Another fetus in this litter had incomplete ossification of the pubic and first sternebral bones.

Fetus 18 of litter 17121, in the highest dose group, had incomplete ossification of the ischium (right) and first and second sternebrae. In the same group, a fetus in another litter had incomplete ossification of the third sternebrae, and no ossification of the first sternebrae.

There was a significant increase in the litter incidence of incompletely and unossified sternebrae in the highest dose tested (100 mg/kg) relative to the control group. Table 11 of the report on page 57 indicates that the mean number of ossification sites per fetus per litter was significantly reduced for the hyoid and sternebral centers, both at the 100 mg/kg level.

D. Discussion/Conclusions

a. Maternal Toxicity:

Decreased motor activity and ataxia were noted to occur at a significantly higher incidence in the 100 mg/kg group, than in the control group. Maternal bodyweight was significantly reduced in the 50 and 100 mg/kg groups during the dosing period relative to the controls. In addition, during the dosing period, all treatment groups had a significant reduction in food consumption relative to the controls.

b. Developmental Toxicity:

i. Deaths/Resorptions:

There were no significant increases in fetal deaths or resorptions in any of the treatment groups.

ii. Altered Growth:

There were significant reductions in male and female fetal bodyweight and litter weight at 100 mg/kg.

iii. Developmental Anomalies:

There was a significant increase in the number of litters with fetuses with any alteration observed and the percentage of fetuses with any alteration/litter at 100 mg/kg. An evaluation of fetuses with any alteration observed was significantly increased at 50 and 100 mg/kg. There was a significant increase in the litter incidence of incompletely and unossified sternebrae in the highest dose tested (100 mg/kg) relative to the control group. The mean number of ossification sites per fetus per litter was significantly reduced for the hyoid and sternebral

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WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT DIMETHYLAMINE SALT OF 2,4-D: Registrants Response to a Review of the Developmental Toxicity Study in Rats.

FROM: Jess Rowland, Toxicologist 5/18/93
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TO: Walter Waldrop/Judy Coombs
Product Manager 71
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THRU: K.Clark Swentzel, Section Head 5/18/93
Section II, Toxicology Branch II
Health Effects Division (H7509C)
and
Marcia van Gemert, Ph.D., Chief 5/20/93
Toxicology Branch II
Health Effects Division (H7509C)

TASK IDENTIFICATIONS: Submission: S435555 DP Barcode: D188294
PC Code: 030019 Caswell No: 315 O

ACTION REQUESTED: Review the 2,4-D Industry Task Force's response to Agency review of a developmental toxicity study [83-3a] in rats with the 2,4-Dimethylamine salt.

RESPONSE: The developmental toxicity study in rats [MRID No. 417352-01] was classified as Core supplementary due to the lack of stability data on the dosing solution and clarification or justification of the use of a low purity of test substance [HED Document No. 008400; Agency Letter of 09/11/91]].

In this submission the Task Force provided data on the stability of the dosing solution solution which indicated that the dosing solutions were stable for seven days at room temperature; concentrations deviated 0% from Day 0 for the low dose and no more than -2% for the high dose. The test article, lot No. 04FD31349 was the technical grade of the dimethylamine salt of 2,4-D containing 66.18% active ingredient and an acid equivalent of 55.5%. There are no added ingredients in the test article other than water as a solvent.

Based on these adequate information, the developmental toxicity study in rats [MRID No. 413752-01] classified Core supplementary is upgraded to minimum, satisfies the Guideline requirement [83-3a], and is acceptable for regulatory purposes.

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centers, both at the 100 mg/kg level. There was a significant increase in the incidence of wavy and or incompletely ossified ribs at 100 mg/kg.

iv. Malformations:

There was a significant increase in the incidence of wavy ribs at 100 mg/kg.

D. Study Deficiencies:

1. Clinical signs were not observed days 1-5 of gestation.

2. According to the footnote on page 10, the test substance had an acid equivalent of 55.5% (w/w) and purity of 66.18%. Was this the best available form of the test article?

3. Results of stability analyses were not provided.

E. Core Classification: Supplementary Data.

Maternal NOEL = 12.5 mg (acid equivalent)/kg/day
Maternal LOEL = 50 mg (acid equivalent)/kg/day
Developmental Toxicity NOEL = 50 mg (acid equivalent)/kg/day
Developmental Toxicity LOEL = 100 (acid equivalent)/kg/day

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Amendment to DER for MRID 41737301 - Subchronic Oral Toxicity Study [Dog] [2,4-DICHLOROPHENOXYACETIC ACID]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 008400].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 3/27/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 4/2/01
Reregistration Branch I, Health Effects Division (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: subchronic oral toxicity - dog — § 82-1(b)/OPPTS 870.3150
P.C. CODE: 030001 — REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315 — CAS NO.: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [96.1%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force on 2,4-D Research Data

CITATION: Schulze, G. E. (1990). Subchronic Toxicity Study in Dogs with 2,4-Dichlorophenoxyacetic Acid. Hazleton Laboratories America, Inc.; Study No. 2184-115, December 14, 1990. Document No. 008400. Unpublished. MRID 41737301

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [MRID 41737301], 5 beagle dogs/sex/group were administered 2,4-dichlorophenoxyacetic acid [96.1%] *via* capsules for 13 weeks at concentrations of 0, 0.3 mg/kg/day, 1.0 mg/kg/day, 3.0 mg/kg/day, and 10 mg/kg/day.

Two males [weeks 4, 13] and one female [week 9] at the high-dose level were sacrificed moribund. Treatment-related clinical signs [thin, languid appearance, anorexia, tremors] were observed at the high-dose level in the three dogs sacrificed *in extremis*. Decreased body weights were observed in males at the two highest dose levels [83% and 80% of control at 3 and 10 mg/kg/day, respectively] and in females at the high-dose level [86% of control] at week 13. Body-weight gains were decreased throughout the study in males at the two highest dose levels [weeks 0-13: 56% and 44%, respectively]. The mid- and high-dose females displayed decreased body-weight gains also [weeks 0-13: 84% and 52% of control, respectively]. Decreased food consumption was observed in both sexes at the highest dose level and in males at the next highest dose level throughout the study.

Decreased RBC, hemoglobin, hematocrit, and platelet count values were observed in both sexes at the highest dose level, although statistical significance was not always attained [n=4-5]. Treatment-related alterations in clinical chemistry parameters were observed mainly at the two-highest dose levels in both sexes at various times during the dosing period [increased blood urea nitrogen (both sexes), creatinine (both sexes), alanine aminotransferase (high-dose females; males not statistically significant); decreased T4 (both sexes at high-dose; not statistically significant), and glucose (high dose, both sexes]. High-dose females displayed slightly increased kidney and thyroid weights [absolute, relative to body and brain weights] compared to the control group. Decreased testis weight was observed at the two-highest dose levels, and two of the 3 surviving high-dose males and one of the 2 high-dose males sacrificed moribund displayed hypospermatogenesis and giant cell formation. Microscopic lesions [cellular

alterations, proximal tubule] were observed in the kidney of both sexes at the high-dose level [3 of 5 males at 3 mg/kg/day, 3/3 surviving males at 10 mg/kg/day, 1 of 4 surviving females at 10 mg/kg/day, and all dogs sacrificed moribund].

The NOAEL is 1.0 mg/kg/day. The LOAEL of 3.0 mg/kg/day is based on decreased body weight/body-weight gain and food consumption (males), alterations in clinical chemistry parameters [increased BUN (both sexes), creatinine (both sexes)], increased kidney and thyroid weights (females), and decreased testis weight (males).

This subchronic oral toxicity study is classified ACCEPTABLE/Guideline,and it satisfies the guideline requirement [OPPTS 870. 3150; §82-1(b)] for a subchronic non-rodent oral toxicity study.

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 1. Clinical Observations

Dose/Sex/Finding	0 mg/kg/day	0.3 mg/kg/day	1.0 mg/kg/day	3.0 mg/kg/day	10 mg/kg/day
MALES					
thin	0	0	0	0	2
languid	0	0	0	0	1
anorexia	0	0	0	0	1
tremors	0	0	0	0	1
few or no feces	0	0	0	0	2
soft feces [mucoid]	1	0	0	3	5
soft feces [mucoid/sanguineous]	0	0	0	0	3
small testes/both	0	0	0	0	2
FEMALES					
thin	0	0	0	0	1
languid	0	0	0	0	1
anorexia	0	0	0	0	1
few or no feces	1	0	0	0	1
soft feces [mucoid]	0	1	3	4	4
soft feces [mucoid/sanguineous]	0	0	1	4	2

N=5; data from Tables 1A and 1B, pages 39, 41, and 42 of the report

Table 2. Hematology Parameters

Dose/Sex/Finding	0 mg/kg/day	0.3 mg/kg/day	1.0 mg/kg/day	3.0 mg/kg/day	10 mg/kg/day
MALES					
RBC [MI/UL]					
-1	5.75±0.34	6.49±0.58*	5.86±0.39	6.04±0.48	5.65±0.18 [98]√
4	5.71±0.43	6.43±0.27*	5.67±0.58	6.08±0.37	5.43±0.33 [95]
8	5.80±0.30	6.80±0.22*	6.22±0.60	6.27±0.41	5.41±0.13 [93]
13	6.28±0.45	7.02±0.52	6.78±0.73	6.77±0.57	5.49±0.18 [87]
HGB [G/dL]					
-1	12.8±0.7	14.4±1.1*	13.3±0.9	13.7±0.8	12.9±0.5
4	12.7±0.9	14.2±1.1	12.7±1.0	13.6±0.8	12.4±0.7 [98]
8	13.0±0.5	14.9±1.1*	13.8±1.5	13.6±0.8	11.9±0.3* [92]
13	14.5±0.9	15.8±1.1	15.4±1.7	15.0±1.2	11.9±0.5* [82]
HCT [%]					
-1	37.7±1.6	42.4±3.1*	38.8±2.3	39.8±2.1	38.2±1.5
4	38.0±2.5	42.2±2.9	37.8±3.2	40.1±2.2	36.1±1.9 [95]
8	38.5±2.1	44.2±2.8*	41.0±4.3	40.5±2.0	35.2±1.6 [91]
13	41.4±2.4	45.2±3.0	44.2±4.9	42.8±3.0	34.4±1.6* [83]
platelet [TH/UL]					
-1	471±36	388±123	426±62	407±32	403±85 [86]
4	389±12	325±74	334±67	339±23	243±91* [62]
8	384±30	339±100	335±93	314±42	227±127 [59]
13	354±6	311±96	310±74	351±60	252±96 [71]

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150

Table 2. Hematology Parameters

Dose/Sex/Finding	0 mg/kg/day	0.3 mg/kg/day	1.0 mg/kg/day	3.0 mg/kg/day	10 mg/kg/day
FEMALES					
RBC [MI/UL]					
-1	6.26±0.16	6.09±0.48	6.37±0.38	5.92±0.23	6.13±0.68 [98]√
4	5.78±0.31	6.08±0.29	6.30±0.43	5.82±0.47	5.74±0.72 [99]
8	6.26±0.51	6.25±0.19	6.60±0.45	6.14±0.68	5.18±2.11 [83]
13	6.43±0.36	6.51±0.29	6.97±0.69	6.59±0.91	6.19±0.45 [96]
HGB [G/dL]					
-1	14.3±0.3	13.9±1.6	14.4±0.9	13.6±0.6	14.0±1.6
4	13.0±0.5	13.8±1.1	14.2±1.0	13.1±0.9	12.9±1.6
8	14.1±1.3	14.2±0.9	14.8±1.0	13.6±1.2	11.2±4.4 [79]
13	14.9±0.9	15.0±1.1	15.9±1.6	14.9±2.0	13.3±0.8 [89]
HCT [%]					
-1	41.7±0.9	40.8±4.1	42.4±2.5	39.6±1.8	41.1±4.6 [99]
4	38.8±1.7	41.0±2.5	41.9±2.8	38.7±2.8	38.0±4.5 [98]
8	41.7±3.6	42.5±2.3	43.6±2.9	40.2±3.6	33.0±13.3 [79]
13	42.7±2.6	43.2±2.8	45.9±4.5	42.6±5.2	38.0±2.1 [89]
platelet [TH/UL]					
-1	452±85	446±80	452±60	455±94	427±87
4	374±83	365±49	370±49	340±54	261±143 [70]
8	348±46	352±52	379±60	342±43	211±138* [61]
13	330±61	357±30	335±51	318±41	281±83 [85]

N=5 , except high-dose (4); √ [% of control]; * p<0.05; data from Table 5, pages 56-59 of the report

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150

Table 3. Clinical Chemistry Parameters - MALES					
Dose/Week/Parameter	0 mg/kg/day	0.3 mg/kg/day	1.0 mg/kg/day	3.0 mg/kg/day	10 mg/kg/day
alanine aminotransferase [U/L]					
-1	43±28	34±14	32±7	34±16	29±12
4	36±15	33±9	37±12	79±65 [219] √	99±92 [275]
8	41±18	38±7	44±19	110±136 [268]	143±166 (4)[349]
13	39±15	38±12	43±16	156±230 [400]	133±142 (4)[341]
aspartate aminotransferase [U/L]					
-1	24±2	26±6	24±3	21±3	23±3
4	36±7	35±5	38±3	41±12	46±24
8	42±14	46±7	44±8	42±4	40±4
13	28±1	37±14	37±11	40±10	39±11
blood urea nitrogen [mg/dL]					
-1	11±3	13±3	13±3 [118]	10±1	11±2
4	14±3	15±2	20±5 [143]	19±4	26±6* [186]
8	12±2	15±2	18±4* [150]	19±5* [158]	35±6* [292]
13	12±2	13±3	18±5 [150]	23±5* [192]	30±9* [250]
creatinine [mg/dL]					
-1	0.6±0.05	0.7±0.08	0.6±0.05	0.6±0.05	0.7±0.09
4	0.7±0.08	0.8±0.04	0.8±0.04	0.9±0.13	1.0±0.38* [143]
8	0.8±0.09	1.0±0.08	1.0±0.09	1.1±0.12	1.4±0.34* [175]
13	0.8±0.07	0.9±0.09	1.0±0.13	1.2±0.13* [150]	1.2±0.47* [150]
T3 [mg/dL]					
-1	83±20	93±9	93±11	83±18	85±9
4	93±13	95±10	92±16	102±14	98±38
8	85±17	99±26	101±15	103±22	97±7
13	78±10	88±15	93±8	100±16	71±23 [91]
T4 [UG/dL]					
-1	3.0±1.0	2.8±0.3	3.0±0.5	3.1±0.6	2.9±0.4
4	3.0±0.7	2.?±?✖	2.8±0.6	3.2±0.5	2.3±0.9 [77]
8	2.7±0.8	2.8±0.8	3.4±0.3	3.2±1.0	2.3±0.2 [85]
13	2.6±0.3	2.7±0.6	2.9±0.7	3.2±0.7	1.7±0.8 [59]
total protein [g/dL]					
-1	5.5±0.2	5.7±0.4	5.8±0.2	5.6±0.3	5.7±0.5
4	5.7±0.2	5.8±0.3	5.8±0.2	6.1±0.4	5.9±0.2
8	5.9±0.2	6.2±0.3	6.1±0.4	6.4±0.6	6.2±0.2
13	6.0±0.3	6.2±0.3	6.3±0.3	6.3±0.6	5.7±0.8
glucose [mg/dL]					
-1	101±3	107±5	101±6	105±4	107±11
4	96±4	97±6	97±6	100±10	83±20
8	98±8	100±5	100±10	101±9	90±5
13	97±4	98±8	97±4	94±5	76±15* [78]

✖ illegible in report; N=5, except 4 for high-dose males for weeks 8 and 13; √ [% of control]; * $p<0.05$; data from Table 6, pages 69-80 of the report

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150

Table 4. Clinical Chemistry Parameters - FEMALES					
Dose/Week/Parameter	0 mg/kg/day	0.3 mg/kg/day	1.0 mg/kg/day	3.0 mg/kg/day	10 mg/kg/day
alanine aminotransferase [U/L]					
-1	27±6	28±4	26±3	23±5	29±7
4	31±9	31±9	32±3	33±9	235±397* [758]
8	33±8	33±8	37±5	38±9	114±156 [345]
13	34±8	32±9	37±2	43±17	186±170* [547]
aspartate aminotransferase [U/L]					
-1	23±5	26±5	20±2	22±5	21±?[a]
4	38±9	36±3	37±3	34±6	37±7
8	44±9	44±10	40±10	37±7	34±11
13	36±6	36±16	37±10	37±13	45±14 [125]
blood urea nitrogen [mg/dL]					
-1	19±3	15±3	15±4	20±9	15±5 [82]
4	17±3	17±3	16±3	21±3 [124]	31±11* [182]
8	17±3	16±3	18±4	23±5 [135]	33±24 [194]
13	17±3	15±2	18±3	23±4* [135]	26±3* [153]
creatinine [mg/dL]					
-1	0.6±0.08	0.6±0.04	0.6±0.07	0.6±0.04	0.6±0.00
4	0.7±0.07	0.7±0.05	0.8±0.05* [114]	0.9±0.00* [129]	0.9±0.13* [129]
8	0.9±0.04	0.9±0.11	1.1±0.09* [122]	1.2±0.04* [133]	1.0±0.21
13	0.9±0.10	0.9±0.09	1.0±0.05	1.2±0.04 [133]	1.1±0.12
T3 [mg/dL]					
-1	89±10	86±32	88±15	84±22	81±7
4	99±21	96±30	116±12	95±10	104±16
8	88±16	85±21	100±6	86±16	70±32
13	80±11	74±20	93±13	88±16	85±12
T4 [UG/dL]					
-1	2.8±0.3	2.6±1.0	2.4±0.5	2.8±0.7	2.7±0.4
4	3.0±0.7	2.9±0.9	3.1±0.5	2.8±0.4	2.6±0.4 [87]
8	3.0±0.6	3.0±1.1	3.0±0.5	2.9±0.8	2.1±0.4 [70]
13	2.9±0.2	2.4±0.8 [83]	2.6±0.4	3.2±0.7	2.5±0.4 [86]
total protein [g/dL]					
-1	5.8±0.3	5.7±0.4	5.6±0.1	5.8±0.5	5.7±0.3
4	5.6±0.4	5.7±0.2	5.6±0.2	5.8±0.5	5.7±0.3
8	5.9±0.2	5.9±0.2	6.0±0.3	6.0±0.4	6.1±0.5
13	5.9±0.3	6.0±0.3	6.0±0.3	6.3±0.6	5.9±0.4
glucose [mg/dL]					
-1	106±11	98±8	101±10	96±9	99±11
4	99±6	98±9	98±9	92±8	88±5 [83]
8	100±7	97±9	101±9	97±4	86±9 [86]
13	96±2	94±6	95±3	92±7	81±8* [82]

N=5, except 4 for high-dose females for week 13; [a] not legible; √[% of control]; ✖ illegible in report; * p<0.05;
data from Table 6, pages 69-80 of the report

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 5. Organ Weights

Dose/Organ	0 mg/kg/day	0.3 mg/kg/day	1.0 mg/kg/day	3.0 mg/kg/day	10 mg/kg/day
LIVER					
males					
absolute	250±44	256±37	276±45	236±26	241±12
relative to body	2.6±0.3	2.9±0.4	2.9±0.3	2.9±0.2	3.1±0.0
relative to brain	3.2±0.6	3.4±0.4	3.6±0.6	3.2±0.3	3.3±0.3
females					
absolute	186±23	194±22	202±36	187±12	194±23
relative to body	2.6±0.2	2.7±0.4	2.9±0.5	2.7±0.2	3.1±0.4
relative to brain	2.6±0.3	2.8±0.5	2.8±0.5	2.7±0.2	2.9±0.3
KIDNEY					
males					
absolute	47±4	51±10	51±5	44±3	47±4
relative to body	0.50±0.08	0.57±0.06	0.52±0.04	0.55±0.04	0.61±0.02
relative to brain	0.60±0.03	0.68±0.12	0.67±0.09	0.59±0.02	0.64±0.08
females					
absolute	34±4	38±4	36±4	33±3	38±2 [112]
relative to body	0.49±0.02	0.52±0.07	0.52±0.03	0.47±0.04	0.61±0.07* [124]
relative to brain	0.49±0.06	0.54±0.07	0.50±0.07	0.47±0.02	0.55±0.02 [112]
BRAIN					
males					
absolute	78.5±5.4	74.9±4.4	76.3±9.8	74.2±3.4	73.4±5.0 [94]
relative to body	0.83±0.14	0.84±0.12	0.78±0.10	0.93±0.07	0.96±0.08 [116]
females					
absolute	70.4±6.2	70.2±4.4	71.6±3.3	68.6±3.3	67.9±2.0 [96]
relative to body	0.99±0.13	0.96±0.12	1.04±0.12	1.00±0.06	1.10±0.15 [111]
TESTES					
males					
absolute	15.5±2.9	16.3±3.4	15.3±2.8	12.3±3.6 [79]	8.2±4.6* [53]
relative to body	0.16±0.03	0.18±0.04	0.16±0.02	0.15±0.04 [94]	0.11±0.06 [69]
relative to brain	0.20±0.03	0.22±0.05	0.20±0.04	0.16±0.04 [80]	0.11±0.07 [55]
THYROID					
males					
absolute	1.00±0.25	1.03±0.26	1.28±0.28	1.12±0.30	1.02±0.13
relative to body	0.010±0.002	0.012±0.003	0.013±0.003	0.014±0.003	0.013±0.001
relative to brain	0.013±0.006	0.014±0.003	0.017±0.005	0.015±0.004	0.014±0.002
females					
absolute	0.73±0.10	0.74±0.09	0.81±0.21	0.79±0.11	0.87±0.17 [119]
relative to body	0.010±0.002	0.010±0.002	0.012±0.004	0.011±0.002	0.014±0.004 [140]
relative to brain	0.010±0.001	0.011±0.001	0.011±0.003	0.011±0.001	0.013±0.002 [130]
TERMINAL BODY WEIGHT					
males	9640±1534	9020±1381	9880±1301	8040±874 [83]	7667±306 [80]
females	7200±1054	7360±984	6960±764	6860±372 [95]	6275±1050 [87]

N=5, except high-dose males (3)/females (4); √[% of control]; data from Tables 8, 9A, 9B [pages 99-106] of the report

327

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 6. Histopathology

Dose/Organ	0 mg/kg/day		0.3 mg/kg/day		1.0 mg/kg/day		3.0 mg/kg/day		10 mg/kg/day	
LIVER	**male**	**female**	**males**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
subacute inflammation, focal or multifocal	0	1	1	1	0	1	0	0	0	0
necrosis, centrilobular	0	0	0	0	0	0	0	0	0	1
congestion	0	0	0	0	0	0	0	0	2	0
KIDNEY										
cellular alteration, proximal tubule	0	0	0	0	0	0	3	0	5	2
cortical scarring	0	0	0	0	1	0	0	0	1	1
TESTES✖										
hypospermatogenesis	0		0		0		0		3	
giant cells	0		0		0		0		3	

✖ prostate not examined; n=5; data from Tables 10A and 10B , pages 108-121 of the report

CASWELL FILE
008400

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist 5/28/91
Section II, Toxicology Branch II (H7509C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head 6/3/91
Section II, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding-Nonrodent **GUIDELINE:** 82-1(b)

TOX.CHEM. No.: 315 **MRID No.:** 417373-01 **HED Project No.**1-0620

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

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

STUDY IDENTIFICATION: HLA Study No. 2184-115

TESTING LABORATORY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: Subchronic Toxicity Study in Dogs with 2,4-Dichlorophenoxyacetic acid.

REPORT AUTHOR: Gene E. Schulze, Ph.D

REPORT DATE: December 14, 1990

SUMMARY: Groups of five male and five female dogs were given oral administration (via capsules) of **2,4-Dichlorophenoxyacetic acid** at 0, 0.3, 1.0, 3.0, or 10 mg/kg/day for 13 weeks. No treatment-related effects were observed at 0.3 mg/kg/day. At 1 mg/kg/day, the only compound-induced effects were transient changes in a few clinical chemistry parameters. However, the values for these parameters were well within the reference range for beagle dogs and therefore were not considered to be adverse effects. The dose level of 3 mg/kg/day was associated with altered hematological and clinical chemistry parameters, and histopathological changes in the kidneys of male dogs. At a dose of 10 mg/kg/day, 2,4-D acid increased mortality, lowered body weight gain, altered hematological and clinical chemistry parameters, decreased testicular weights and increased kidney weights, and induced histopathological changes in the kidneys of both sexes and testes of male dogs.

Under the conditions of this study, a NOEL of 1 mg/kg/day and a LOEL of 3.0 mg/kg/day was established for the 90-day oral toxicity of 2,4-Dichlorophenoxyacetic acid in male and female beagle dogs.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1b) for a 90-day feeding study in nonrodents.

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a subchronic toxicity study in dogs with **2,4-Dichlorophenoxyacetic acid.**

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: 2,4-Dichlorophenoxyacetic acid.
Purity: 96.1%
Lot No.: 909
Description: Brown powder.
Control Material: Hard gelatin capsules, 1/4 oz.
Batch No.: 3403
Manufacturer: Topac Limited, Toronto, Canada.

2. Test Animals

Species: Dogs
Strain: Beagle
Sex: Males and females
Age: 4-6 months at initiation of study.
Weight: 5.6-8.2 kg (M), 4.5-6.1 kg,(F) at initiation.
Identification: Ear tags.
Acclimation: 14-days
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Canine Diet Meal #5007.
Water: Tap water ad libitum
Environment: Temperature- 70 - 77°F; Humidity- 40 - 85 %;
Light/dark cycles: 12 hr.light/12 dark cycle

3. Study Design

Group No.	Treatment	No. of Animals		Dose Level mg/kg/day[a]
		Males	Females	
1	Control	5	5	0
2	Low	5	5	0.3
3	Mid-1	5	5	1.0
4	Mid-2	5	5	3.0
5	High	5	5	10.0

[a] Adjusted based on most recent body weight.

4. Test Material Formulation

The amount of test material required for each dog was weighed out and then transferred into one 1/4 oz gelatin capsule for administration to the appropriate animal. The amount weighed for each dog was recorded with the project, week, and dog numbers, and the date.

5. Treatment

Dogs were given either one empty gelatin capsule (control, Group 1)) or the test material in hard gelatin capsule(s) (Groups 2, 3, 4, and 5) daily for 13 weeks.

6. Experimental Procedures

Mortality and moribundity checks were performed twice daily. Pharmacotoxic effects were monitored one-half hour post-dosing. Body weights and food consumption were measured once weekly. Ophthalmologic examinations were conducted prior to initiation and at termination. The following hematology, clinical chemistry, and urinalysis parameters were measured prior to initiation, and at weeks 4, 8 and 13.

The checked (x) parameters were measured

Hematology	Clinical Chemistry
x Hematocrit (HCT)*	Alkaline phosphatase
x Hemoglobin (HGB)*	Cholinesterase}
x Leukocyte count (WBC)*	x Creatinine phosphokinase‡
x Erythrocyte count (RBC)*	Lactic acid dehydrogenase
x Leukocyte diff. count*	x Serum alanine aminotransferase*
Mean corpuscular HGB (MCH)	x Serum aspartate aminotransferase*
Mean corpuscular conc.(MCHC)	Gama glutamyl transferase
Mean corpuscular volume (MCV)	Glutamate dehydrogenase
x Platelet count*	x Albumin*
Reticulocyte count	x Albumin/globulin ratio
Blood clotting time	x Blood urea nitrogen*
	x Creatinine*
	Cholesterol
	x Globulins
	x Glucose*
	x Total bilirubin*
	x Total protein*
	Serum protein electrophoresis
	x Triiodothyronine T_3
	x Thyroxine T_4

* Required for subchronic studies

* Required for subchronic studies
} Only for organophosphates

Urinalysis[a]

x Appearance*
x Glucose*
x Ketones*
Volume*
x Bilirubin*
x Specific gravity*
x pH
x Blood
x Protein*
x Sediment (microscopic)*
x Urobilinogen*
x Ova and parasites

@ Not required for subchronic studies.
* Required for chronic studies.

7. **Termination**

After 13 weeks of treatment, all surviving animals were weighed, anesthetized with sodium thiamylal, and exsanguinated. Necropsies were performed on each animal, all gross pathological changes were recorded, and the following organs were weighed.

Brain	Kidneys	Liver with drained gall bladder
Ovaries	Heart	Testes with epididymides
Thyroid/parathyroids		

8. **Histopathology**

The checked (X) tissues from all animals were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Tongue x Salivary gland* x Esophagus* x Duodenum* x Jejunum* x Cecum* x Colon* x Rectum* x Liver*} x Gall bladder* x Pancreas Neurological System x Brain*} x Pituitary* Peripheral nerve*# x Spinal cord (3 levels)*# Eyes (optical nerve)*# Glandular System x Adrenals* Lacrimal glands# x Parathyroids*‡ x Thyroids*‡	x Trachea* x Lung* Pharynx+ Larynx+ Nose+ Cardiovascular/Hemo.System x Aorta (thoracic)* x Heart* x Bone marrow* x Lymph nodes* x Spleen* x Thymus* Urinogenital System x Kidneys*‡ x Urinary bladder* x Tests*} Epididymides x Seminal vesicle x Uterus* x Ovaries*} Others x All gross lesions and masses x Skeletal muscle*

* Required for subchronic and chronic studies.
\+ Required for chronic inhalation study.
\# In subchronic studies examined only if indicated by toxicity or target organ involvement.
} Organ weights required in subchronic and chronic studies.
‡ Organ weights required for nonrodent studies.

9. Statistical Analyses

Mean body weights and body weight changes, mean weekly food consumption, total food consumption, clinical pathology data (with the exception of urinalyses and cell morphology), and organ weight data of treated groups were compared statistically against data of the control group of same sex by One Way Analysis of Variance (ANOVA) followed by Dunnett's test. All analyses were conducted using two-tailed tests with a minimum significance level of 5%.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Labs, and the Study Protocol.

III. RESULTS

1. Survival

No mortality occurred during the study. However, two males and one female at the 10 mg/kg/day group were sacrificed moribund during study Week 4, 13, and 9, respectively. Histopathology revealed inflammation in various areas of the skin and subcutis in the males and hepatic and splenic changes (which were compatible with severe anemia) in the female.

2. Body Weights and Body Weight Changes

Mean body weight data are summarized in Tables 1 and 2 for male and female dogs, respectively. A statistically significant ($p < 0.05$) reduction in mean body weight was observed for the high-dose (10 mg/kg/day) males at the Week 8 interval when compared with control males at this interval. Mean body weights of males in this group were consistently lower than the controls throughout the study. Mean body weights of the high-dose females were also lower than the controls throughout the study; however, the decreases were not statistically significant.

Table 1. Mean Body Weights and S.D (kg) for **Male Dogs.**

Dose Level (mg/kg/day)	Week: 0	Week: 4	Week: 8	Week: 13
0	6.7 ± 0.9	8.7 ± 1.1	9.7 ± 1.6	10.3± 1.7
0.3	7.0 ± 0.9	8.5 ± 1.2	9.3 ± 1.3	9.5 ± 1.5
1.0	7.1 ± 1.0	9.1 ± 1.2	10.0 ± 1.3	10.3± 1.2
3.0	6.6 ± 0.8	8.0 ± 0.6	8.5 ± 0.8	8.6 ± 0.8
10.0	6.6 ± 0.9	7.6 ± 0.8	**7.5* ± 0.8**	8.2 ± 0.3

* Significantly different from control value at $p < 0.05$.

Table 2. Mean Body Weights and S.D (kg) for **Female Dogs.**

Dose Level (mg/kg/day)	Week: 0	Week: 4	Week: 8	Week: 13
0	5.2 ± 0.4	6.7 ± 0.9	7.3 ± 1.0	7.7 ± 1.2
0.3	5.6 ± 0.5	7.0 ± 0.6	7.6 ± 0.7	8.0 ± 0.6
1.0	5.2 ± 0.4	6.5 ± 0.9	7.2 ± 0.8	7.6 ± 1.0
3.0	5.3 ± 0.3	6.7 ± 0.4	7.1 ± 0.4	7.4 ± 0.4
10.0	5.3 ± 0.4	6.2 ± 0.7	6.1 ± 1.3	6.6 ± 1.2

Body weight change data are presented in Tables 3 and 4 for male and female dogs, respectively. Statistically significant ($p < 0.05$) differences in body weight gains were seen in both males and females a the high-dose. Males exhibited lowered body weight gains during Weeks 0 through 4 interval and during the Weeks 4 through 8 interval and females during the Weeks 4 through 8 interval when compared to controls. In addition, the mean body weight changes for females at the high-dose were lower than control throughout the study.

Table 3. Body Weight Change (kg) in **Male Dogs.**

Dose Level (mg/kg/day	Weeks: 0 - 4	Weeks: 4 - 8	Weeks: 8 - 13
0	2.0 ± 0.38	1.0 ± 0.49	0.6 ± 0.28
0.3	1.5 ± 0.37	0.8 ± 0.30	0.2 ± 0.31
1.0	1.9 ± 0.48	0.9 ± 0.39	0.3 ± 0.37
3.0	1.3 ± 0.31	0.6 ± 0.26	0.1 ± 0.13
10.0	**0.9* ± 0.62**	**0.0* ± 0.52**	0.3 ± 0.21

* Significantly different from control at $p < 0.05$.

Table 4. Body Weight Change (kg) in **Female Dogs.**

Dose Level (mg/kg/day	Weeks: 0 - 4	Weeks: 4 - 8	Weeks: 8 - 13
0	1.5 ± 0.49	0.6 ± 0.13	0.4 ± 0.33
0.3	1.4 ± 0.54	0.6 ± 0.324	0.4 ± 0.20
1.0	1.4 ± 0.48	0.6± 0.29	0.5 ± 0.30
3.0	1.3 ± 0.26	0.4 ± 0.16	0.3 ± 0.15
10.0	0.9 ± 0.33	**0.1* ± 0.65**	0.0 ± 0.30

* Significantly different from control at $p < 0.05$.

3. Clinical Observations

Treatment-related clinical signs of toxicity observed in both sexes of treated dogs were thin appearance, languid appearance, anorexia, emesis, swollen paw, and swollen testes (males); these signs were most prevalent in the high-dose group and were especially prevalent in the three animals (2 males and 1 female) that were sacrificed moribund. Other signs were comparable to those seen in controls.

4. Ophthalmology Examination

No treatment-related ocular changes were seen at any dose level.

5. Food Consumption

Mean food consumption was significantly ($p < 0.05$) decreased in males at the 10 mg/kg/day during Weeks 4 (1.3 kg vs 2.0 kg in controls) and 8 (1.4 kg vs 2.0 kg). Total food consumption was also significantly ($p < 0.05$) reduced in the high-dose males throughout the study; 19.1 $\pm$ 1.9 kg vs 24.9 $\pm$ 3.27 in controls). Although no statistically significant differences were noted, mean and total food consumption values were also lower in the high-dose females.

6. Hematology and Clinical Chemistry

The significant, treatment-related hematological changes observed in dogs only at the 10 mg/kg/day are summarized Tables 5, 6, 7 and 8. Significant ($p < 0.05$) decreases in hemoglobin values were seen during Weeks 8 and 13 for male at the 10 mg/kg/day dose, and in hematocrit values in these dogs at Week 13. Male dogs at this dose also exhibited nonsignificant decreases in mean erythrocyte count, hemoglobin, and/or hematocrit values at most of the remaining treated intervals. Mean platelet counts were significantly ($p < 0.05$) decreased in the high-dose males and females at Week 4 and 8, respectively, with nonsignificant decreases at the remaining treated intervals.

The female dog (Animal # G27543) at the 10 mg/kg/day group that was sacrificed moribund showed severe anemia with a profound decline in erythrocyte count (1.39×10^{-6} μL), hemoglobin (3.3 g/DL), and hematocrit (9.3%), leucocyte count (2.2×10^{-3} μL), absolute segmented lymphocyte count (1.1×10^{-3} μL) at Week 8. This dog also had prominent decreases in platelet counts at Week 4 (56×10^{-3} μL) and Week 8 (11×10^{-3} μL). The male dog (Animal # G27538) that was sacrificed moribund at the high-dose did not exhibit anemia, however, decreases in platelet count were seen at Weeks 4 through 13 (127×10^{-3}, 41×10^{-3}, 125×10^{-3} μL, respectively).

Table 5. Mean Hemoglobin (G/DL) Values in **Male Dogs.**

Dose/ Week	-1	4	8	13
0 mg/kg/day	12.8 ± 0.7	12.7 ± 0.9	13.0 ± 0.5	14.5 ± 0.9
10 mg/kg/day	12.9 ± 0.5	12.4 ± 0.7	**11.9* ± 0.3**	**11.9* ± 0.5**

* Significantly different from control at p <0.05.

Table 6. Mean Hematocrit (%) Values in **Male Dogs.**

Dose/ Week	-1	4	8	13
0 mg/kg/day	37.7 ± 1.6	38.0 ± 2.0	38.5 ± 2.1	41.4 ± 2.4
10 mg/kg/day	38.2 ± 1.5	36.1 ± 1.9	35.2 ± 1.5	**34.4* ± 1.6**

* Significantly different from control at p <0.05.

Table 7. Mean Platelet (TH/UL) Values in **Male Dogs.**

Dose/ Week	-1	4	8	13
0 mg/kg/day	471 ± 36	389 ± 13	384 ± 30	354 ± 6
10 mg/kg/day	403 ± 85	**243* ± 91**	227 ± 127	252 ± 96

* Significantly different from control at p <0.05.

Table 8. Mean Platelet (TH/UL) values in **Female Dogs.**

Dose/ Week	-1	4	8	13
0 mg/kg/day	452 ± 85	374 ± 83	348 ± 46	330 ± 61
10 mg/kg/day	427 ± 86	261 ± 143	**211* ± 83**	281 ± 83

* Significantly different from control at p <0.05.

Treatment-related changes (significantly different from control value at p <0.05) observed in clinical chemistry parameters are summarized in Table 9.

Table 9. Significant Changes in Clinical Chemistry Parameters.

Parameter Evaluated	Dose Level mg/kg/day	Sex	Study Week	Effect(s)
GLUCOSE	10	Males	13	Decreased
GLUCOSE	10	Females	13	Decreased
BUN	10	Males	4	Increased
BUN	10	Females	4	Increased
BUN	1,3 & 10	Males	8	Increased
BUN	1,3,& 10	Males	13	Increased
BUN	3 & 10	Females	13	Increased
CREAT	3	Males	13	Increased
CREAT	10	Males	4,8,13	Increased
CREAT	3	Females	13	Increased
CREAT	1, 3 & 10	Females	4	Increased
CREAT	1 & 3	Females	8	Increased
IN PHOS	0.3,1,3,10	Males	4	Decreased
T.BILI	1	Females	13	Increased
ALT	10	Females	4,13	Increased
CALCIUM	1 & 3	Females	13	Increased

Urinalyses were generally comparable between control and treated groups except for the lower specific gravity values for males and female dogs at the high dose during Week 13. These differences may reflect a loss of renal concentrating ability.

7. **Gross Pathology**

Treatment-related gross pathological changes observed at terminal sacrifice were limited to small testes in male dogs in the 3 and 10 mg/kg/day groups.

8. **Organ Weights**

Evaluation of the organ weight data showed a significantly ($p < 0.05$) lower absolute testis/epididymis weight for male dogs at the high dose and a significantly elevated relative kidney weight for females at the high dose. No other significant differences were observed in group mean absolute and relative organ weight values between the treated and control groups.

9. **Histopathology**

Moribund Sacrifices: Of the two males sacrificed moribund at the high dose, one (Animal # G27534) showed inflammation in the subcutis of the cervical region, extending around and into the salivary gland and thyroid gland. The lesion was compatible with bacterial infection.The dog had thymic lymphoid depletion which was considered to be secondary to severe stress. The inflammatory lesion in the subcutis was considered to be the cause of debility.

The other dog (G27538) had multiple ulcered and inflammatory lesions in the skin as well as thymic lymphoid depletion. The thymic lymphoid depletion was considered to be related to the in-life observations rather than the dermatitis.

The female dog (G27543) which was sacrificed had severe centrilobular necrosis in the liver, moderate extramedullary hematopoiesis in the spleen, and thymic lymphoid depletion.
The combination of hepatic and splenic lesions was compatible with severe anemia, and the thymic lesion was considered to be secondary to severe stress.

All three dogs exhibited cellular alterations in the tubular epithelium of the kidneys.

Terminal Sacrifice: Treatment-related histopathological alterations observed in dogs sacrificed at the end of the study are were limited to kidneys and testes. No histopathological lesions were seen in the testes or kidneys of control males or in the kidneys of control females.The incidence are summarized in Table 10.

340

Table 10. Histopathological Lesions in Dogs Given 2,4-D acid.

Lesions	Dose Level (mg/kg/day)	Incidence Males	Incidence Females
Kidneys: Cellular alterations, proximal tubules	3.0	3/5	0/5
	10.0	3/3	1/4
Testes: Hypo-spermatogenesis	10	1/3	
Giant cell formation	10	1/3	

Kidney lesions were characterized as cellular alterations in the proximal convoluted tubules. This subtle change consisted of a reduction in the size and reduction in cytoplasmic eosinophilia of epithelial cell lining some proximal convoluted tubules. The pathogenesis was similar to the changes seen in regenerative tubular epithelium.

Testicular lesions were characterized by testicular hypospermatogenesis and giant cell formation in the testes. Hypospermatogenesis consisted of a reduction in spermatogenic activity and seminiferous tubules, sometimes to the degree that individual tubules were lined only by Sertoli cells. Giant cells consisted of intra-tubular aggregations of multinucleated and/or karyomeglic cells which appeared to the derived from spermatogenic elements.

The other histopathological alterations observed were considered agonal or typical findings in clinically normal dogs of this strain and age.

IV. DISCUSSION

Oral administration of 0.3 mg 2,4-D acid/kg/day was not associated with any treatment-related changes.

At 1.0 mg/kg/day, the only compound-induced effect was transient changes in a few clinical chemistry parameters. However, as shown below, the values for these parameters were well within the reference range for beagle dogs and therefore were not considered to be toxicologically significant.

Sex	Parameter	1.0 mg/kg/day	Concurrent Controls	Reference Range for Hazleton dogs[a]
Males	BUN (MG/DL)	18 ± 4 (week 8)	12 ± 1.6	9 - 20
	PHOS (MG/DL)	6.7 ± 0.7 (week 4)	7.7 ± 0.6	3.1 - 8.4
Females	CREAT (MG/DL)	1.1 ± 0.9 (week 13)	0.9 ± 0.04	0.6 - 1.1
	CALCIUM (MG/DL)	11.5 ± 0.3 (week 13)	11 ± 0.15	9.8 - 11.6
	T.BILI (MG/DL)	0.2 ± 0.04	0.1 ± 0.0	0.2 - 0.5

[a] Source: Historical Control Data, Hazleton Labs 1984.

The dose level of 3.0 mg/kg/day was associated with altered hematological and clinical chemistry parameters, and microscopic changes in the kidneys of male dogs.

At a dose of 10 mg/kg/day, 2,4-D acid increased mortality, produced body weight loss, altered hematological and clinical chemistry parameters, decreased testicular weights and increased kidney weights, and induced histopathological changes in the kidneys of both sexes and testes of male dogs. The changes in BUN and creatinine levels seen in dogs given the 3.0 and 10 mg/kg/day doses were correlated with histopathological changes in the kidneys of these dogs. Kidney lesions in these animals consisted of a reduction in cytoplasmic eosinophilia of epithelial cells lining some proximal convoluted tubules of the kidney. The reduction in testicular weight noted in the high dose males was associated with testicular hypospermatogenesis and giant cell formation.

No mortality occurred during the study; however, 2 males and 1 female dog were sacrificed moribund at the 10 mg/kg/day group. Of the 2 males, 1 exhibited severe bacterial infection while the other animal was thin, languid, anorexic and displayed tremors. Microscopic lesions observed in this dog were multiple ulcerative inflammatory lesions in the skin, thymic lymphoid depletion and reduction in cytoplasmic eosinophilia of epithelial cells lining some proximal convoluted tubules of the kidneys similar to those observed in males at this dose level. The female dog exhibited severe signs of anemia which was associated with severe centrilobular hepatocellular necrosis, moderate extramedullary hematopoiesis in the spleen and thymic lymphoid depletion.

V. CONCLUSION

Under the conditions of this study, a NOEL of 1 mg/kg/day and a LOEL of 3.0 mg/kg/day was established for the 90-day oral toxicity of 2,4-Dichlorophenoxyacetic acid to male and female dogs.

[2,4-DICHLOROPHENOXYACETIC ACID] Developmental Toxicity Study/Rabbit [§83-3(b)/OPPTS 870.3700]

Amendment to DER for MRID 41747601 - Developmental Toxicity Study - Rabbit [2,4-D]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 008462].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 11/19/02
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Whang Phang, Ph.D. Vithy Phang 11/21/02
Reregistration Branch I, Health Effects Division (7509C)
TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rabbit § 83-3(b)/OPPTS 870.3700
P.C. CODE: 030001 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315 CAS #: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [96.1%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force on 2,4-D Research Data

CITATION: Hoberman, A. M. (1990). Developmental Toxicity [Embryo-Fetal Toxicity and Teratogenic Potential] Study of 2,4-Dichlorophenoxyacetic Acid [2,4-D Acid] Administered Orally *via* Stomach Tube to New Zealand White Rabbits. Argus Research Laboratories, Inc. Study No. 320-003, December 12, 1990. MRID 41747601; Document No. 008462. Unpublished.

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 41747601], artificially-inseminated female New Zealand White rabbits [20/group] were administered 2,4-Dichlorophenoxyacetic acid [96.1%] at dose levels of 0 [aqueous 0.5% methylcellulose], 10 mg/kg/day, 30 mg/kg/day, and 90 mg/kg/day from gestation day 6 through gestation day 18. NOTE: All dose concentrations were corrected for the 96.1% purity of the test material.

There were no treatment-related deaths. Two high-dose does aborted [days 21 and 24]. Treatment-related clinical signs of toxicity were observed in the two does that aborted [days 21 and 24, after 13 doses each] and included ataxia in both [days 16-19 and after day 13], and decreased motor activity, loss of righting reflex, extremities that were cold to the touch, and dried feces in doe that aborted on day 21. Body weights were comparable among the groups throughout the study, but body-weight gains were decreased at the high-dose level [73% of control] during the dosing period [days 6-19; statistical significance was not attained]. During days 7-8, the low- and mid-dose groups showed no body-weight gain, and the high-dose group displayed a negative body-weight gain [-0.01 grams] compared to the control [+0.01 gram]. During days 15-19, the high-dose group displayed no body-weight gain, and corrected body-weight gain was decreased at the high-dose level [77% of control; statistical significance was not attained] also. Food consumption was comparable among the groups.

Pregnancy rates were comparable among the groups. Comparable numbers of corpora lutea, implantations, and live fetuses were observed among the groups, and there were no dead fetuses. One control doe had 100% resorptions. The number of resorption and pre- and post-implantation losses were comparable among the groups also. Gravid uterine weights were comparable among the groups.

Mean fetal body weight was comparable among the groups. At the high-dose level, there was a significant increase in the percent of live male fetuses [71.2%] compared to the control [52.8%] and other dose groups [low: 54.4%; mid: 59.4%]. At the high-dose level, the fetal incidence [3 fetuses of one litter; $p<0.01$] of hindlimbs turned inward was increased compared to the control (0) and other treatment groups (0), and the same fetuses displayed domed head [hydrocephaly]. This finding is not considered treatment-related. There were no apparent differences in the incidence of external, visceral, or skeletal variations, anomalies, retardations, or malformations among the groups.

The maternal toxicity NOAEL is 30 mg/kg/day, based on abortions, decreased body-weight gain, and clinical signs of toxicity [decreased motor activity, ataxia, loss of righting reflex, extremities cold to the touch] at the maternal toxicity LOAEL of 90 mg/kg/day.

The developmental toxicity NOAEL is 30 mg/kg/day, based on abortions at the developmental toxicity LOAEL of 90 mg/kg/day.

This developmental toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [**§83-3(b)/OPPTS 870.3700**] for a developmental toxicity study in rabbits.

[2,4-DICHLOROPHENOXYACETIC ACID] Developmental Toxicity Study/Rabbit [§83-3(b)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Body-Weight Gain [kilograms]				
Dose/Time	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
Body Weight [kg]				
gestation day 0	3.16±0.23	3.19±0.28	3.15±0.25	3.17±0.24
gestation day 7	3.36±0.25	3.38±0.29	3.34±0.29	3.38±0.25
gestation day 14	3.50±0.26	3.51±0.31	3.52±0.30	3.52±0.27
gestation day 20	3.59±0.27	3.58±0.30	3.56±0.30	3.54±0.32
gestation day 29	3.82±0.30	3.80±0.32	3.82±0.34	3.76±0.28√
# pregnant	17	18	16	18
Body-Weight Gain [kg]				
days 0-6	0.19±0.07	0.18±0.04	0.17±0.06	0.19±0.07
days 6-7	0.01±0.03	0.01±0.04	0.02±0.05	0.01±0.05
days 7-8	0.01±0.04	0.00±0.03	0.00±0.03	-0.01±0.05
days 9-12	0.05±0.04	0.04±0.05	0.09±0.04	0.06±0.04
days 15-19	0.05±0.04	0.05±0.03	0.05±0.06	0.00±0.15
days 6-19	0.22±0.08	0.22±0.07	0.24±0.08	0.16±0.21 [73]♪
days 6-29	0.47±0.12	0.43±0.12	0.49±0.12	0.42♪0.11√ [89]
days 0-29	0.66±0.14	0.61±0.13	0.66±0.14	0.62±0.12√
days 6-29C	0.03±0.16	0.02±0.16	0.06±0.11	-0.03±0.12√
days 0-29C	0.22±0.17	0.20±0.16	0.23±0.13	0.17±0.13√ [77]
Gravid Uterine Wt. [g]	439.89±129.43	405.72±98.12	430.98±106.06	448.66±122.63

√ excludes 2 does that aborted; ♪ [% of control]; C corrected for gravid uterine weight
Data from Tables 4-5, pages 47-50 of the report

Table 2. Food Consumption				
interval [days]	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
grams/day				
0-6	175.9±12.0	177.8±11.5	178.4±7.7	180.4±4.8
6-7	172.2±20.4	168.9±32.0	174.0±16.0	177.8±11.5
7-8	174.5±19.5	167.8±21.7	172.2±22.2	167.4±45.8
6-9	172.6±19.2	166.6±28.4	176.8±11.8	171.6±29.5
15-19	170.6±21.2	172.1±17.8	174.9±14.5	160.3±50.2
6-19	169.3±16.5	166.1±22.4	174.2±14.5	166.9±26.4
0-29	164.1±18.2	161.8±18.1	169.6±16.0	165.1±12.0√
grams/kg/day				
0-6	54.1±3.4	54.3±3.4	55.3±3.7	55.2±3.4
6-7	51.3±5.3	50.1±9.0	52.6±6.9	53.0±5.6
7-8	31.8±4.1	49.8±6.1	51.8±7.4	49.6±14.3
6-9	51.2±4.3	49.1±7.5	53.3±5.0	50.3±8.8
15-19	48.0±5.3	48.4±3.4	49.2±5.2	45.3±14.3
6-19	48.9±3.6	47.9±5.0	50.3±5.3	48.4±7.9
0-29	46.2±3.7	45.9±3.8	48.3±5.0	47.1±3.2

√ excluded 2 does that aborted; data from Tables 6-7, pages 51-54 of the report

008462

GUIDELINE: 83-3

Primary Review by: Karen E. Whitby, Ph.D. Karen Whitby 7/3/91
Toxicologist, Review Section II, Toxicology Branch II/HED (H7509C)

Secondary Review by: K. Clark Swentzel K. Clark Swentzel 7/3/91
Section Head, Review Section II, Toxicology Branch II/HED (H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rabbit
Guideline: 83-3

EPA Identification No.s: EPA MRID (Accession) No.:417476-01
Caswell No.: 315
HED Project No.: 1-0621

Test Material: 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid)

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

Study Number(s): 320-003 (Argus Research Laboratories)

Testing Facility: Argus Research Laboratories, Inc.
935 Horsham Road
Horsham, Pa. 19044

Title of Report: Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid) Administered Orally via Stomach Tube to New Zealand White Rabbits

Author(s): Alan M. Hoberman, Ph.D.

Report Issued: December 12, 1990 (Final Report)

Study Dates: April 2, 1990 (first insemination)
May 4, 1990 (last cesarean section)

Bibliographic Citation: Hoberman, A.M., (1990) Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid) Administered Orally via Stomach Tube to New Zealand White Rabbits. Study Number(s): 320-003 (Testing Facility) Argus Research Laboratories, Inc. 935 Horsham Road Horsham, Pa. 19044

Conclusions:

The test substance, 2,4-D Acid was administered by gavage to presumed pregnant rabbits at 0, 10, 30, and 90 mg/kg/day in 0.5% methylcellulose. Maternal toxicity was observed in two high dose does with ataxia. One high dose doe that aborted on day 21 had decreased bodyweight, food consumption, dried feces, ataxia, loss of righting reflex, decreased motor activity, extremities that were cold to the touch, and findings at necropsy. There was a slight nonsignificant reduction in bodyweight gain during the dosing and postdosing periods and a nonsignificant reduction in corrected bodyweight gain during the entire period for the high dose group. There were no significant or dose related alterations in fetal development that could be attributed to treatment. The fetal incidence [3(1)] of hindlimbs turned inward was significantly increased ($p \leq 0.01$) at 90 mg/kg. Also at 90 mg/kg, the fetal and litter incidence of domed head (and therefore, hydrocephaly) was equal to that for hindlimbs turned inward relative to the control.

Core Classification: Core-Minimum

Maternal NOEL = 30 mg/kg/day
Maternal LOEL = 90 mg/kg/day
Developmental Toxicity NOEL = 90 mg/kg/day
Developmental Toxicity LOEL = >90 mg/kg/day

RANGE-FINDING STUDY

The main teratogenicity study was preceded by a pilot study, which was performed to determine the dosage levels to be used in the main study. The study was sponsored by Industry Task Force on 2,4-D Research Data, and was performed by Argus Research Laboratories, Inc..

Study Title: Dosage-Range Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid) Administered Orally via Stomach Tube to New Zealand White Rabbits (Pilot Study)

Study No.: Argus Research Laboratories, Inc., Protocol 320-003P

Date of Report: May 11, 1990

Date of Study: November, 2, 1989 (Insemination)

Author: Alan Hoberman, Ph.D. (Study Director)

Materials and Methods

The test substance 2,4-D Acid was administered by gavage days 6 through 18 of gestation at 0 (vehicle), 12.5, 25, 50, 100, and 200 mg (4 ml/kg/day, adjusted daily for bodyweight). The vehicle was 0.5% (w/w) methylcellulose. The doses were corrected for the 96.1% acid equivalent of the test substance. There were 4 animals per treatment group. On day 29 the animals were euthanized and necropsied. Corpora lutea were counted and the livers and kidneys weighed. The gravid uterus was excised and weighed. The number and location of implantation sites, early and late resorptions, and live and dead fetuses were recorded. Fetuses were weighed sexed, and examined for gross alterations.

Results

One doe in the 100 mg/kg group aborted and was sacrificed on day 22. Another doe in this group was sacrificed in the moribund condition. At the 200 mg/kg level, three does died. The does that were found dead, sacrificed, or aborted were found to have weight loss and decreased food consumption, impaired or lost righting reflex, decreased motor activity, ataxia, dyspnea, coldness to the touch, and/or a red substance present in the anogenital area or cage pan. A red-brown viscous fluid or orange-red fluid was present in the urinary bladder of the does that were found dead or sacrificed. With the exception of the does that aborted, were sacrificed moribund, or found dead there were no alterations in clinical signs, bodyweight or food consumption. There was no effect on maternal kidney weights. Absolute and relative liver weight was increased for the one doe surviving to the end of the

study. The litter of the one surviving doe at 200 mg/kg consisted of one viable fetus and 8 early resorptions, and the litter of the 100 mg/kg doe that aborted consisted of three early resorptions. All fetuses appeared normal at gross external examination.

Conclusions:

The dosage levels selected for the main study were 0, 30, 60, and 90 mg/kg.

? 09/13, 30 [illegible]

p. 6

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound:

The test article was stored at room temperature. Suspensions of the test article were prepared daily [as 0 (vehicle), 2.5, 7.5, and 22.5 mg/mL) and administered in a volume of 4 mL/kg. All dose concentrations were corrected for the 96.1% activity (purity) of the test substance.

The report references the pilot study for the homogeneity, concentration, and stability analyses. In addition, the report indicates that two 5 g samples of the test substance were sent to the sponsor for possible analyses, one at the beginning and one at the end of the dosing period. The results of this analyses are available in the sponsor's records.

Description:	tan granular powder
Lot No.:	909

Vehicle(s):

The vehicle was aqueous 0.5% methylcellulose (Sigma Chemical Co., Lot 88F0051). The vehicle was received in the form of white powder and stored at room temperature. The vehicle was prepared in R.O. deionized water. The water was analyzed for chemical and bacterial contaminants. After preparation, the vehicle was stored under refrigeration.

Test Animal(s):	Species:	Rabbit
	Strain:	New Zealand White [Hra:(NZW)SPF]
	Source:	Hazleton Research Products, Inc. Denver, PA
	Age:	approx. 5 mo. (upon receipt)
	Weight:	2.21 - 3.73 kg the day after arrival

B. Study Design

This study was designed to assess the developmental toxicity potential of 2,4-D Acid when administered orally by stomach tube to female New Zealand White rabbits on gestation days 6 through 18, inclusive.

Mating

The females were artificially inseminated with semen from proven male breeders of the same strain, obtained from the same source. Each of these rabbits were given an i.v. injection of 20 USP Units of HCG (PREGNYL®, Organon, Inc., Lot 0130289315) approx. 3 hrs.

351

prior to insemination. Semen was diluted with normal saline (Abbott Laboratories, Inc., Lot 34-201-JT) to achieve a concentration of 6.0 X 10^6 spermatozoa/0.25 mL for insemination. The day of insemination was considered day 0 of gestation. One quarter of the rabbits assigned to each group were inseminated each day. Four proven male breeders inseminated each group of females.

Group Arrangement:

One hundred four virgin female rabbits were received from the supplier. One animal upon exam by the Staff Veterinarian was found to be unacceptable. Another rabbit had an apparent fracture of the right rear leg and was sacrificed prior to assignment to the study. Eighty of these animals were assigned to the study (20/group) using a computer-generated weight ordered randomization procedure, after a 3 week acclimation period. The remaining 22 were reassigned to the general population.

Test Group	Dose Level (mg/kg)	Number Assigned
Vehicle Control	0	20
Low Dose	10	20
Mid Dose	30	20
High Dose	90	20

NOTE: Animal 16888 (vehicle control) was not dosed on day 9 due to repeated difficulties in placing the stomach tube. The technician deemed it necessary to refrain from further attempts so as to not jeopardize the health and life of the animal.

Animal Husbandry:

The study room had 370 sq. ft. of floor space and a minimum of 10 changes per hour of 100% fresh HEPA filtered (99.97%) air. The room temperature was 64 - 74° F; humidity was 35 - 65% during the study period. The animals were maintained on a 12 hour light/dark cycle. The animals were fed 180 g Purina Certified Rabbit Chow® # 5322. Local water that had passed through a R.O. membrane was also available ad lib via glass water bottles. Chlorine had been added to the water as a bacteriostat. The water samples contained from 0.0 to 0.6 ppm of chlorine.

Dosing:

The doses used in this investigation were selected on the basis of a dose range finding study.

All doses were in a volume of 4 mL/kg of bodyweight/day (adjusted daily for bodyweight). Suspensions of the dosing solutions were prepared daily. Triplicate 10 mL samples of each concentration

were reserved from the first and last time the dosage solution were prepared. Two of the samples were frozen and sent to Lancaster Laboratories, Inc. for analysis. The third frozen sample was kept at the facility as a backup. Two 5 g samples of the bulk test substance were also shipped to Lancaster Laboratories for possible analysis.

The dosing solutions for the pilot and main study were analyzed by Lancaster Laboratories. The detection limit was <0.05 mg/g. The results for the main study are as follows:

Theoretical Concentration of Dosing Solution:	Actual Range:
0 mg/mL	0
2.5 mg/mL	3 to 6%
7.5 mg/mL	3 to 4%
22.5 mg/mL	5 to 8%

The protocol indicates that the bulk test substance is stable at room temperature (stability of the test substance is on file with the sponsor).

Observations

Animals were observed for clinical signs several times during the acclimation period and on day 0 of pregnancy. The animals were checked for mortality at least twice daily. Clinical observations were performed three times each day during the dosing period (days 6-18), and once a day during the post dosing period.

Bodyweight was recorded on day 0, and days 6 through 29 of gestation. Food consumption was recorded daily.

Does were sacrificed on day 29 of gestation by i.v. administration of T-61® Euthanasia Solution. Thoracic and abdominal cavities were examined for gross lesions. In the event of gross lesions (except for parovarian cysts which are common in rabbits) the tissues were preserved in neutral buffered 10% formalin. A laparotomy was performed and the intact uterus was excised and weighed. Uteri that appeared nonpregnant were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantation, early and late resorptions, and live and dead fetuses were recorded. Each fetus was removed from the uterus and individually identified with a tag, weighed, and observed for gross external alterations. Viable fetuses were sacrificed. Every fetus was examined to determine sex and soft tissue alterations. The brain was free-hand sectioned, and examined. Fetuses were then eviscerated, stained with Alizarin red S, and examined for skeletal changes. Skeletal specimens were stored in 80% glycerine with thymol crystals to retard fungal growth. Photographs were taken of all abnormal findings.

Historical control data were provided to allow comparison with concurrent controls.

Statistical Analysis

The section on statistical analyses is appended.

Compliance

A signed Statement of No Confidentiality Claim was provided dated December 15, 1990 (p 2).

A signed Statement of Compliance with EPA GLP's was provided that was dated December 15, 1990 (p 3).

A signed Quality Assurance Statement was provided dated December 12, 1990 (pp. 475 to 478).

A signed statement from the Chairman of the Technical Committee Industry Task Force II on 2,4-D Research Data, dated December 15, 1990 was provided, which indicated the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to this study. This study neither reportedly met or exceeded any of these criteria.

C. Results

1. Maternal Toxicity

Mortality

With the exception of two nonpregnant control does, all females survived to their scheduled sacrifice. One of the does was presumed to have died (on day 12) as a consequence of an intubation accident. Necropsy of the doe revealed hemorrhagic lungs. The cause of death for the other doe was not determined.

Clinical Observations

Alterations in clinical observations which were attributable to the test substance were observed in two high dose does. One doe (16937) had ataxia on days 16 to 19. Doe 16944 aborted on day 21. This doe presented the following clinical findings: dried feces, ataxia, decreased motor activity, loss of righting reflex, and extremities that were cold to the touch. Weight loss was observed in this animal after day 13. Feed consumption was decreased after day 5, decreases were more marked after day 12. Necropsy revealed a red substance in the anogenital area, a large gallbladder, and parovarian cysts.

Alopecia occurred in 2, 0, 3, and 2 does in the control, low, mid, and high dose groups. Rales occurred for 2, 3, 1, and 0 does in

the control, low, mid, and high dose groups. One control group doe had a red substance in the in the cage pan and was noted at necropsy to have a fluid filled uterus (the litter of this doe consisted of four early resorptions).

Bodyweight

Table I: Bodyweight Gains (kg)[a]

Dose (mg/kg):	Days 0-6	Days 6-19	Days 19-29	Days 0-29	Corrected Body Weight Gains Dosing P.[1]	Entire[2]
0	0.19	0.22	0.25	0.66	-0.22	0.22
10.0	0.18	0.22	0.22	0.61	-0.19	0.20
30.0	0.17	0.24	0.25	0.66	-0.19	0.23
90.0	0.19	0.16	0.21	0.62	-0.24	0.17

1 = bodyweight gain during dosing period minus gravid uterus weight.
2 = corrected bodyweight = day 29 gestation bodyweight minus gravid uterine weight
a = Data extracted from (study number 320-003 tables 5 and 17 pp. 49 and 78-89)
(Some of the above values were calculated by this reviewer.)

The only time during which a reduction in maternal bodyweight gain was noted was during the dosing period (most notably during the initial phase of dosing). The average maternal weight gain was 100, 109.1, and 72.7% of the control value for the low, mid, and high dose groups, respectively. During the post-dosing period, the average maternal bodyweight gains were 88, 100, and 84% of the control values for the low, mid, and high dose groups. For the same respective groups the corrected bodyweight gain during the dosing period was 86.4, 86.4, and 109.1% of the control values; and the corrected bodyweight gain during the entire gestation period was 90.9, 104.5, and 77.3% of the control value.

Food Consumption

There were no significant differences in absolute (g/day) or relative (g/kg/day) maternal food consumption. Food consumption data excludes values for wet or spilled feed. Values for the does that aborted at the highest dose group were also excluded from the data.

Table II: Food Consumption Data (g/kg/day)[a]

Dose (mg/kg)	Days 0-6	Days 6-19	Days 19-29	Days 0-29
0	54.1	48.9	40.4	46.2
10.0	54.3	47.9	39.2	45.8
30.0	55.3	50.3	43.4	48.3
90.0	55.2	48.4	40.4	47.1

a = Data extracted from (study number 320-003 table 7 p.53)

Gross Pathological Observations

None of the necropsy findings were considered to be related to treatment. Some of the findings noted at necropsy have been presented above. Doe 16936 (of the high dose group) that aborted on day 24 was found to have mottled lungs. Doe 16944 (of the high dose group) that aborted on day 21 and was found to have a red substance present in the anogenital area also was found to have a large gall bladder and a parovarian cyst. Another doe of the high dose group was found to have the abdominal and thoracic cavities filled with clear fluid. Additional necropsy findings included parovarian cysts among all treatment groups.

Cesarean Section Observations

The does that aborted in the HDT were excluded from cesarean section observations. Table 9 of the report (p.56) indicates that the % resorbed conceptuses/litter was 6.4 for the control group. Apparently the value for doe 16876 was omitted from this mean. This doe had a litter that consisted of four early resorptions (litter 100% resorbed), which the author states is a relatively common event in rabbits. Historical control data is provided to support this statement.

There was a significant (dose-related) increase in the average percentage of live male fetuses in the 90 mg/kg group. The author reports that this value was even greater than that observed historically. When the author excluded litters containing only one sex, this finding was not statistically significant or dose-dependent.

No other findings were significantly altered relative to the control.

Table III: Cesarean Section Observations[a]

Dose (mg/kg):	0	10	30	90
#Animals Assigned	20	20	20	20
#Animals Inseminated	20	20	20	20
# (%) Pregnant	17(85.0)	18(90.0)	16(80.0)	18(90.0)
N	17	18	16	16
Maternal Wastage				
#Died	2	0	0	0
#Non pregnant	3	2	4	2
#Aborted	0	0	0	2
#Premature Delivery	0	0	0	0
Total Corpora Lutea	171	178	158	163
Corpora Lutea/Dam	10.0	9.9	9.9	10.2
Total Implantation	132	124	125	129
Implantation/Dam	7.8	6.9	7.8	8.1
Total Live Fetuses	119	120	109	116
Live Fetuses/Dam	7.0	6.7	6.8	7.2
Total Resorptions	13	4	16	13
Early	9	3	11	13
Late	4	1	5	0
Resorptions/Dam	0.8	0.2	1.0	0.8
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (g)	45.13	46.14	45.05	44.43
♂ fetuses	45.32	44.62	45.42	44.57
♀ fetuses	44.21	46.34	43.82	43.81
Preimplantation Loss(%)	22.5	29.6	19.4	19.3
Postimplantation Loss(%)	11.9	3.8	11.5	10.4
Sex Ratio (% Male)	52.8	54.4	59.4	71.2*

a Data extracted from (study number 320-003; tables 8, 9 pp. 55, 56 and tables 19 and 20 pp.102-109)

* Significantly different from vehicle control ($p \leq 0.05$)

(Some of the above values were calculated by this reviewer.)

2. Developmental Toxicity

Table IV: External Examinations

Dose (mg/kg):	0	30	60	90
Observations+				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
Head:				
Domed	0(0)	1(1)	0(0)	3(1)
Rhinocephaly	0(0)	1(1)	0(0)	0(0)
Palate:				
Cleft, Medial	0(0)	0(0)	0(0)	1(1)
Body:				
Kyphosis	0(0)	0(0)	0(0)	1(1)
Umbilical Hernia	0(0)	1(1)	0(0)	1(1)
Pale	0(0)	0(0)	0(0)	1(1)
Forelimb(s):				
Turned Inward	0(0)	0(0)	0(0)	2(1)
Paw, Left, Clenched	0(0)	0(0)	1(1)	0(0)
Hindlimb(s):				
Turned Inward	0(0)	0(0)	0(0)	3*(1)

Data extracted from (study number 320-003; table 11 pp. 58-59, and table 22 pp.118-149)
+ = some observations may be grouped together
a = fetal (litter) incidence
* = significantly different from control ($p \leq 0.01$)

The fetal incidence of hindlimbs turned inward was significantly increased at 90 mg/kg. The fetal and litter incidence of domed head (and therefore, hydrocephaly) relative to the control was equal to that for hindlimbs turned inward also at 90 mg/kg. Fetus 16909-5 (of the 10 mg/kg group) had rhinocephaly, proboscis like nose above partially fused eyes and additional observations made upon soft tissue and skeletal exam. Fetus 16937-1 had a dome shaped head, a left turned fore- and hindlimb and other abnormalities observed with the soft tissue and skeletal exam. Fetus 16937-3 had a dome shaped head; the hindlimbs were bilaterally turned inward and other abnormalities observed with the soft tissue and skeletal exam. Fetus 16937-6 had a dome shaped head, medial cleft palate, kyphosis, fore- and hindlimbs were bilaterally turned inward and other abnormalities observed with the soft tissue and skeletal exam.

Table IV: Visceral Examinations

Dose (mg/kg):	0	10	30	90
Observations+				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
Brain:				
Extreme Dilation of Lateral Ventricles (Hydrocephalus)	0(0)	1(1)	0(0)	3(1)
Protrudes	0(0)	1(1)	0(0)	0(0)
Lungs:				
Intermediate Lobe, Agenesis	2(2)	7(5)	3(2)	5(2)
Gallbladder:				
Smaller than Normal	2(2)	0(0)	0(0)	3(3)
Larger than Normal	0(0)	0(0)	1(1)	1(1)
Liver:				
Protrudes	0(0)	1(1)	0(0)	1(1)

Data extracted from (study number 320-003; table 12 pp. 61-62 and table 22 pp.118-149)
+ = some observations may be grouped together
a = fetal (litter) incidence

Upon soft tissue exam, fetus 16891-7 of the 10 mg/kg group, was found to have agenesis of the intermediate lobe of the lung and protruding liver. This fetus also exhibited findings in the skeletal exam. The three fetuses of the same litter (16937) that were observed to have domed head in the external exam were found to have hydrocephalus in the soft tissue exam.

Table IV: Skeletal Examinations

Dose (mg/kg):	0	10	30	90
Observations+				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
SKULL:				
Irregular Ossification	45(14)	42(17)	40(13)	45(15)
Nasal(s), Irregular Ossification:				
Internasal	0(0)	2(1)	1(1)	1(1)
Intranasal	0(0)	2(2)	3(3)	2(1)
Irregular Suture	4(3)	0(0)	0(0)	2(2)
Midline Suture Displaced	20(10)	16(10)	11(9)	22(11)
Small &/or Irregularly Shaped	0(0)	1(1)	1(1)	0(0)
Nasal(s)-Frontal(s) Irregular Suture	1(1)	0(0)	3(2)	3(3)
Frontal(s), Irregular Ossification:				
Interfrontal	3(3)	5(3)	4(3)	3(3)
Intrafrontal	2(2)	2(2)	4(4)	2(2)
Irregular Suture	26(11)	22(13)	20(12)	17(9)
Fused	0(0)	1(1)	0(0)	0(0)
Small and Irregularly Shaped	0(0)	1(1)	0(0)	0(0)
Parietal, Intraparietal	0(0)	1(1)	1(1)	0(0)
Zygomatics, Appear Broad	0(0)	1(1)	0(0)	0(0)
Maxillas, Short & Close-Set	0(0)	1(1)	0(0)	0(0)
Premaxillas, Absent	0(0)	1(1)	0(0)	0(0)
Palate, Inc. Ossified	0(0)	0(0)	0(0)	1(1)
Eye Sockets, Formed as One Below the Nasal	0(0)	1(1)	0(0)	0(0)
Anterior & Posterior Fontanelles Enlarged (Moderate)	0(0)	0(0)	0(0)	2(1)
Anterior & Posterior Fontanelles Enlarged (Marked)	0(0)	1(1)	0(0)	1(1)
Anterior Fontanelle, Additional Ossif.	0(0)	1(1)	0(0)	0(0)
Parietals, Contain Small Holes	0(0)	1(1)	0(0)	2(1)
Frontals, Contain Small Holes	0(0)	1(1)	0(0)	0(0)
HYOID, Ala(e), Angulated	4(3)	3(3)	4(3)	5(3)

Table IV: Skeletal Examinations- cont'd

Dose (mg/kg):	0	10	30	90
Observations+				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
VERTEBRAE:				
Cervical:				
Centrum Unilateral Ossification	0(0)	1(1)	0(0)	0(0)
Arch, Absent	0(0)	1(1)	0(0)	0(0)
Thoracic:				
Hemivertebra	0(0)	1(1)	0(0)	1(1)
Centrum, Unilateral Ossification	0(0)	1(1)	0(0)	1(1)
Lumbar:				
Hemivertebra	0(0)	0(0)	1(1)	0(0)
Caudal:				
Misaligned	1(1)	1(1)	1(1)	1(1)
INTERRELATED VERTEBRAL/ RIB MALFORMATIONS	0(0)	1(1)	0(0)	2(1)
RIBS:				
Fused	0(0)	1(1)	0(0)	3(1)
Split	0(0)	2(1)	0(0)	2(1)
Thickened	3(3)	0(0)	1(1)	0(0)
Wavy	0(0)	0(0)	0(0)	1(1)
STERNEBRAE:				
Fused	0(0)	1(1)	1(1)	0(0)
SCAPULAE:				
Alae, Irregularly-Shaped	0(0)	1(1)	0(0)	0(0)

Data extracted from (study number 320-003; table 13 pp. 63-69 and table 22 pp.118-149)
+ = some observations may be grouped together
a = fetal (litter) incidence

Table 14, page 70 of the report indicates that there was a significant increase ($p \leq 0.01$) in the mean number of ossification sites in the lumbar vertebrae, and a significant decrease ($p \leq 0.01$) in the number of thoracic vertebrae at the 10 mg/kg level. Also at this level there was a significant reduction ($p \leq 0.05$) in the mean number of rib pairs. The report indicates that the author's facility identifies the number of thoracic vertebrae on the basis of the number of thoracic ribs present. Therefore, this observation was apparently related to the vertebral finding discussed above. Because these findings were not dose related and the incidences are within the historical control range, this observation is apparently unrelated to treatment.

The three fetuses in the high dose group of the same litter that were observed to have hydrocephalus and limbs that were turned inward were found to have enlarged anterior and posterior fontanelles. The parietals of two of these fetuses were found to contain small holes. The palate of the fetus found to have cleft palate was observed to be incompletely ossified. In addition, the ribs of this fetus bilaterally 1-12 were wavy and flat. Kyphosis in this fetus was confirmed upon the skeletal exam.

D. Discussion/Conclusions

a. Maternal Toxicity:

Two high dose does had ataxia. One high dose doe that aborted on day 21 had decreased bodyweight, food consumption, dried feces, ataxia, loss of righting reflex, decreased motor activity, extremities that were cold to the touch, and findings at necropsy. There was a slight nonsignificant reduction in bodyweight gain during the dosing and postdosing periods and a nonsignificant reduction in corrected bodyweight gain during the entire period for the high dose group.

b. Developmental Toxicity:

i. Deaths/Resorptions:

There were no significant increases in fetal deaths or resorptions in any of the treatment groups.

ii. Altered Growth:

There were no significant alterations in fetal growth observed.

iii. Developmental Anomalies:

There was no increased incidence of developmental anomalies observed in this study that may be attributed to treatment.

iv. Malformations:

The fetal incidence [3(1)] of hindlimbs turned inward was significantly increased ($p \leq 0.01$) at 90 mg/kg. Also at 90 mg/kg, the fetal and litter incidence of domed head (and therefore, hydrocephaly) was equal to that for hindlimbs turned inward relative to the control.

E. Core Classification: Core-Minimum

Maternal NOEL = 30 mg/kg/day
Maternal LOEL = 90 mg/kg/day
Developmental Toxicity NOEL = 90 mg/kg/day
Developmental Toxicity LOEL = >90 mg/kg/day

[DIETHANOLAMINE SALT OF 2,4-D] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Amendment to DER for MRID 41920906/41986602 Developmental Toxicity Study - Rat [DIETHANOLAMINE SALT OF 2,4-D]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 009262].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/12/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 12/13/00
Reregistration Branch I, Health Effects Division (7509C)
TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rat § 83-3(a)/OPPTS 870.3700
P.C. CODE: 030016 REREGISTRATION CASE NO/CASE NO.: 818713
CASWELL NO.:315K CAS NO.: 5742-19-8
TEST MATERIAL (PURITY) : diethanolamine salt of 2,4-D [73.09%]
SYNONYMS: 2,4-D DEA; dichlorophenoxyacetic acid, diethanolamine salt
SPONSOR: PBI/Gordon Corporation, Kansas City, MO

CITATION: Siglin, J. C.; Mercieca, M. D.; and Rodwell, D. E. (1990). Teratology Study in Rats with Diethanolamine Salt of 2,4-D. Springborn Laboratories, Inc., OH; Study No. SLS 3229.3. MRID 41920906/. Document No. 009262. Unpublished. Range-Finding Teratology Study in Rats with Diethanolamine Salt of 2,4-D. Study No. SLS 3229.2. August 31, 1990. MRID 41986602.

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 41920906], female Sprague-Dawley Crl:CD BR VAF/Plus rats [25/group] were administered the diethanolamine salt of 2,4-dichlorophenoxyacetic acid [73.09%] *via* gavage at dose levels of 0 [deionized distilled water], 15 mg/kg/day, 75 mg/kg/day, and 150 mg/kg/day from gestation day 6 through gestation day 15 [actual doses: 11, 55, and 110 mg/kg/day, respectively].

There were no deaths. The only possible clinical sign attributed to treatment was an increased incidence of urine staining and vaginal discharge at the high-dose level. Body weights were comparable among the groups throughout the study, but there was a dose-related decrease in body-weight gains, which was statistically significant at the mid- and high-dose levels after the start of dosing [days 6-9: 79%, 57%, and 29% of control with increasing dose]. Although there was a significant decrease in body-weight gain at the mid-dose level for the dosing period [81% of control] and overall [corrected for uterine weight: 85% of control], there was no dose-response. Corrected body-weight gains for the entire gestation period were lower in the treated groups compared to the control [91%, 85%, 90% of control (increasing dose)], but there was no dose-response. A lower food consumption was noted at the high-dose level during gestation days 6-9 and 6-16 [88% of control on a grams/rat/day basis].

There were no abortions, and the pregnancy rate was comparable among the groups. One control dam had 100%

litter resorptions; all other dams had viable fetuses. The numbers of corpora lutea, implantation, and live fetuses were comparable among the groups, and the number of resorptions, as well as pre- and post-implantation losses were not adversely affected; the low- and mid-dose groups displayed a statistically-significant decrease in early resorptions and post-implantation loss compared to the control. There was one dead fetus at the high-dose level. Fetal body weight was significantly reduced [92% of control] at the high-dose level compared to the control, and the sex ratio was comparable among the groups. There were no statistically-significant differences in the incidence of fetal malformations, although the total percent of litters with malformations was slightly greater [13%] at the high-dose level than the control [4.3%]. The types of malformations were generally dissimilar, and the incidence of individual malformations remained well within each respective historical control range. No visceral abnormalities were noted among the groups. An increased incidence in fetal skeletal malformations/variations was found at the mid- and high-dose levels compared to the concurrent and/or historical control incidences, which included a dose-related increase in the incidence of 14th rudimentary rib(s) and 7th cervical rib(s) and increases in the incidence of reduced ossification of the skull and bent ribs. The incidences of these latter two variations were greater at the mid-dose level than at the high-dose level, but the incidence at the high-dose level, although not statistically significant, was greater than both the concurrent incidence and the historical control range.

The maternal toxicity NOAEL is 15 mg/kg/day, and the maternal toxicity LOAEL is 75 mg/kg/day, based on decreased body-weight gain. The NOAEL for developmental toxicity is 15 mg/kg/day, and the developmental toxicity LOAEL is 75 mg/kg/day, based on an increase in the incidence of skeletal malformations/variations [reduced ossification of the skull, 14th rudimentary rib(s), 7th cervical rib(s), and bent rib(s)].

This developmental toxicity study is classified Acceptable/Guideline, and it satisfies the guideline requirement [OPPTS 870.3700; §83-3(a)] for a developmental toxicity study in the rodent.

[DIETHANOLAMINE SALT OF 2,4-D] Developmental Toxicity Study - Rat [§83-3(a)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Gravid Uterine Weight/Corrected Body Weight/Gain [grams]				
Dose/Day	0 mg/kg/day	15 mg/kg/day	75 mg/kg/day	150 mg/kg/day
body weight				
0	258±17	261±11	258±11	260±12
6	295±21	296±13	293±14	295±17
9	309±21	307±14	300±16	299±14
12	324±23	320±18	309±18* [95]√	312±15 [96]
16	353±29	353±23	339±23	345±20
20	421±42	426±23	415±31	412±26
body-weight gain				
0-6	37±9.9	35±6.5	35±8.3	36±12.4
6-9	14±6.5	11±10.3	8±9.5* [57]	4±10.2** [29]
9-12	15±6.2	13±11.0	9±12.5 [60]	13±8.0
12-16	30±13.5	33±12.0	30±15.7	33±10.5
16-20	68±18.0	73±8.8	75±11.8	68±8.6
6-16	58±16.4	57±14.8	47±15.8* [81]	50±12.7 [86]
0-20	163±31.6	164±20.1	157±25.0 [96]	153±19.8 [94]
gravid uterine weight	76.8±21.3	86.7±9.7	83.5±19.4	75.5±18.3
corrected body weight	344±25	339±21	331±18	337±20
corrected BWG	86±17.2	78±16.0 [91]	73±10.8* [85]	77±14.9 [90]

√[% of control]; * $p<0.05$; ** $p<0.01$; data from Tables 3-4, pages 24-25 of the report

Table 2. Maternal Food Consumption				
Dose/Day	0 mg/kg/day	15 mg/kg/day	75 mg/kg/day	150 mg/kg/day
grams/rat/day				
0-6	24±2.4	24±2.3	24±2.1	24±3.1
6-9	24±2.8	24±3.0	22±2.7	21±3.3** [80]√
9-12	24±2.5	24±2.8	23±10.9	20±3.0 [83]
12-16	26±2.5	26±4.0	25±3.5	25±2.8
16-20	29±3.5	30±2.3	30±3.4	30±3.5
6-16	25±2.0	25±2.4	24±4.2	22±1.8** [88]
0-20	25±2.0	26±2.1	25±2.8	24±1.8
grams/kg/day				
0-6	86±6.7	87±7.0	86±6.2	86±9.8
6-9	79±6.4	80±8.1	75±8.1	71±11.1** [90]
9-12	77±5.9	76±7.1	76±32.3	67±9.3 [87]
12-16	77±5.5	76±9.6	75±9.3	76±7.6
16-20	76±6.2	77±4.6	80±5.6	80±8.9
6-16	78±3.9	77±5.2	75±10.9	72±5.9 ** [92]
0-20	78±3.5	78±4.0	78±6.3	76±4.3

√[% of control]; * $p<0.05$; ** $p<0.01$; data from Tables 5-6, pages 26-27 of the report

009262

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II

DATA EVALUATION REPORT

5. RANGE-FINDING STUDY IN RATS

STUDY TYPE: Range-Finding **GUIDELINE: N/A**

Caswell No. 315 K MRID No. 419866-02 HED PROJECT No. 1-2159

TEST MATERIAL: Diethanolamine salt of 2,4-D Acid.

REGISTRANT: PBI Gordon Corporation, Kansas City, MO

TESTING LABORATORY: Springborn Laboratories, Inc. OH

STUDY IDENTIFICATION: SLS 3229.2

TITLE OF REPORT: Range-finding Teratology Study in Rats with Diethanolamine Salt of 2,4-D.

AUTHORS: J.C. Siglin, J.T. Liao and D. E. Rodwell

REPORT DATE: August 31, 1990

SUMMARY: Pregnant Sprague-Dawley rats were given oral administration of 2,4-D DEA [73.09%] at dose 0, 37, 75, 150 or 300 mg/kg/day [equivalent to 0, 27, 55, 110, or 219 mg/kg/day 2,4-D DEA] during gestation days 6-15. Dams were sacrificed on gestation day 20. 2,4-D DEA did not induce maternal or developmental toxicity at 37 or 75 mg/kg/day. Treatment did cause maternal toxicity at 150 and 300 mg/kg/day manifested by mortality, clinical signs, and decreases in body weight gain and food consumption. Minimal developmental toxicity seen at 150 mg/kg/day was characterized by a slight decrease in fetal weight. Developmental toxicity observed at 300 mg/kg/day included: increase in post-implantation loss, decrease in viable fetuses, and reduced fetal weights.

Based on this study the dose levels selected for the main study were 0, 15, 75 and 150 mg/kg/day [equivalent to 0, 11, 55, or 110 mg/kg/day 2,4-D DEA.

CORE CLASSIFICATION: Not applicable; range-finding study.

1. OBJECTIVE

The objective of this range-finding study, was to establish appropriate dose levels of the Diethanolamine salt of 2,4-D for the main study.

2. PROTOCOL

Groups of six mated female Sprague-Dawley Crl:CD BR VAF/Plus rats [80-120 days old] were given oral administrations of 2,4-D DEA (73.09% pure; Batch no.0017) at doses of 0, 37, 75, 150, or 300 mg/kg/day] equivalent to 0, 27, 55, 110, or 219 mg/kg/day 2,4-D DEA] in deionized distilled (10 mL/kg) daily, during days 6 through 15 of gestation. Concentration, homogeneity and stability of the test article/vehicle mixtures were determined prior to the initiation of the study.

Animals were observed for clinical signs of toxicity daily and were weighed on gestation days 0, 6, 9, 12, 16 and 20. Dams in each group were sacrificed on day 20 and postmortem examination included macroscopic examination of internal organs, with emphasis on the uterus, uterine contents, position of each fetus in the uterus, and corpora lutea counts. The uteri (and its contents) of all dams were weighed to determine corrected body weights. The fetuses were sexed, weighed, examined, and discarded.

3. RESULTS

i. Analysis of dosing solution

The mean concentrations found were 100.2%, 99.5%, 94.6% and 97.4% of the nominal concentration for the 37, 75, and 150 and 300 mg/kg dose groups, respectively. The homogeneity analysis showed that the sample solutions were within 5% of the nominal concentrations indicating that the dosing solutions were homogeneous. Stability analysis indicated that 2,4-D DEA was stable in distilled water at room temperature for up to eight days post-preparation.

ii. Maternal Toxicity

- While no mortality occurred at the 37 or 75 mg/kg/day dose groups, one female was found dead on gestation day 12 at 150 mg/kg/day, and one female was sacrificed moribund on gestation day 8 at 300 mg/kg/day.

- Clinical signs of toxicity observed in rats prior to death were decreased activity, tremors, slow breathing and prostration. Less severe clinical signs observed in the surviving dams at 150 and 300 mg/kg/day included reddish vaginal discharge and urine stains. No treatment-related clinical signs were seen at the lower doses.

- No treatment-related effects were noted in body weight gain and food consumptions of dams at 37 or 75 mg/kg/day. Decreases in body weight gain and food consumption were observed at 150 and 300 mg/kg/day. The decrease in maternal weight gain noted at 300 mg/kg/day during gestation days 12-16 and 16-20 was primarily attributable to the reduced gravid uterus weights in these dams.

- Necropsy revealed no treatment-related gross abnormalities and treatment had no adverse effects on the reproductive performances of the dams.

iii. Developmental Toxicity

- No developmental toxicity was observed at 37 or 75 mg/kg/day. Treatment-related effects observed at 150 and 300 mg/kg/day are tabulated below:

PARAMETER	0 mg/kg/day	150 mg/kg/day	300 mg/kg/day
Post implantation loss (%)	7	3	**41**
Viable fetuses (%)	93	87	**59**
Gravid uterine weight (g)	76.7±11.12	78.6± 9.98	**39.8±28.98**
Fetal weight (g)	3.6±0.2	3.2±0.2	**2.2±0.2**

- No developmental variations or malformations were observed in any fetuses at the tested doses.

4. CONCLUSION

Based on the results of this study [maternal and developmental toxicity at 300 mg/kg/day], dose levels selected for the main study [0, 15, 75 and 150 mg/kg/day] seem appropriate .

5. CORE CLASSIFICATION: Not applicable; study not required by guideline.

366

CASWELL FILE
009262

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II

DATA EVALUATION REPORT

6. TERATOLOGY STUDY IN RATS

STUDY TYPE: Teratogenicity/Rat **GUIDELINE:** 83-3(a)

Caswell No. 315 K MRID No. 419209-06 HED PROJECT No. 1-2159

TEST MATERIAL: Diethanolamine salt of 2,4-D

REGISTRANT: PBI Gordon Corporation, Kansas City, MO

TESTING LABORATORY: Springborn Laboratories, Inc. OH

STUDY IDENTIFICATION: SLS 3229.3

TITLE OF REPORT: Teratology Study in Rats with Diethanolamine Salt of 2,4-D.

AUTHORS: J.C. Siglin, M.D. Mercieca and D. E. Rodwell

REPORT DATE: December 12, 1990

SUMMARY: Oral administration of the diethanolamine salt of 2,4-D DEA [73.09%] at doses of 0, 15, 75, or 150 mg/kg/day [equivalent to 0, 11, 55, or 110 mg/kg/day] to mated rats during gestation days 6 through 15 resulted in maternal toxicity manifested by decreases in mean body weight gain and mean food consumption at 75 and 150 mg/kg/day. No maternal toxicity was observed at 15 mg/kg/day. No treatment-related differences were seen in cesarian section parameters between the treated and control groups. The singular fetotoxicity observed at 150 mg/kg/day was a statistically significant reduction in fetal body weight. No treatment-related fetal malformation or variation was observed at 15 mg/kg/day. Treatment-related fetal variations observed at the higher doses included increased incidence of reduced ossification of the skull, and increased incidence of 14th rudimentary ribs at 75 and 150 mg/kg/day groups. Based on the results of this study the following NOELs and LOELs are established for Maternal and Developmental Toxicity:

NOEL: 15 mg/kg/day or 11 mg 2,4-D DEA/kg/day
LOEL: 75 mg/kg/day or 55 mg 2,4-D DEA/kg/day

CORE CLASSIFICATION: Minimum; this study satisfies the requirements for a developmental toxicity study in rats (83-3 a) and is acceptable for regulatory purposes.

1. OBJECTIVE

The objective of this study was to assess the effects of the diethanolamine salt of 2,4-D [2,4-D DEA] on the embryonic and fetal development following oral administration to rats during the period of organogenesis.

2. MATERIALS AND METHODS

a. Test Material

Identity: Diethanolamine salt of 2,4-D
Batch No.: 0017
Purity: 73.09%
Description: Clear, light brown viscous liquid

b. Test Animals

Species/Sex: Female rats
Strain: Sprague-Dawley Crl:CD BR VAF/Plus
Age at Breeding: 84 days
Body Weight on gestation day 0: 228 to 291 g
Identification: Ear tags.
Acclimation: 11 days
Housing: Individually in cages with softwood bedding
Food: Purina Certified Laboratory Chow #5002 ad libitum.
Water: Tap water ad libitum
Environment: Temperature - 72 ± 6°C; Humidity - 30-70%;
Light/Dark- 12 hr.cycle

Group Assignment: 25 mated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

Following acclimation, females were housed with sexually mature males (1:1) until mating had been observed (designated day 0 post coitum).

d. Preparation of Dosing Solutions

The test material/vehicle mixtures (w/v) were prepared fresh daily prior to dosing. The vehicle, deionized distilled water, was added to a weighed amount of the test material and the mixture was kept homogenous by constant stirring on a magnetic stirrer for ten minutes prior to daily dispensation.

e. Analysis of the Dosing Solutions

Concentration, homogeneity, and stability analysis of the dosing solutions were determined once during the study.

f. Administration of Test Article -2 -

The test article was administered daily orally via gavage at nominal concentrations of 0, 15, 75, or 150 mg/kg/day [equivalent to 0, 11, 55, or 110 mg/kg/day] from day 6 through 15 of gestation. The control group received the vehicle only. All groups received a dosing volume of 10 mL/kg body weight, with a daily adjustment of the individual volume to the actual body weight.

g. Observations

All animals were observed once daily for clinical signs of toxicity including physical or behavioral abnormalities. In addition, during the treatment period, the rats were observed between one-half and two hours following dosing for detection of overt signs of toxicity. Body weights were obtained on days 0, 6, 9, 12, 16 and 20 of gestation. Food consumptions were measured for gestation days 0-6, 6-9, 9-12, 12-16, 6-16, 16-20.

h. Termination

Dams were sacrificed on gestation day 20, and post mortem examination included gross macroscopic examination of all internal organs with emphasis on uterus, uterine contents, position of fetuses in the uterus and the number of corpora lutea. The uteri (and its contents) of all females with live fetuses were weighed and the corrected body weights were calculated. Fetus was weighed, sexed and examined for gross external abnormalities and was prepared by Wilson's slicing technique for visceral examinations and stained with alizarin red S for skeletal examinations.

37

i. Statistical Analysis

Test Employed	Parameters Analyzed
ANOVA followed by Dunnett's Test	Maternal and fetal data including body weights, food consumption, number of viable fetuses, implantation sites, and corpora lutea
Mann-Whitney U test	Post-implantation loss, dead fetuses, and resorptions
Chi-square test	Fetal sex ratios
Fisher's Exact Test	Fetal malformations and variations.

j. Quality Assurance Measures:

A quality assurance statement was signed and dated 12/18/90. This date conforms to the review of the study phases and the draft and the final reports.

3. RESULTS

a. Analysis of the Dosing Solutions

The mean concentrations found were in the range of 98.5 to 100.3% of the nominal concentration. Homogeneity and stability analyses indicated that 2,4-D DEA was homogenous and stable in aqueous solution when stored for up to eight days at room temperature protected from light.

b. Maternal Mortality

No mortality occurred during the study.

c. Clinical Signs

With the possible exception of increased urine staining and vaginal discharge at 150 mg/kg/day, there were no clinical signs of toxicity that could be directly attributable to treatment.

d. Maternal Body Weight

- No differences in body weight or body weight gain were seen between the vehicle control and 15 mg/kg/day groups.
- At 75 mg/kg/day, a slight, but statistically significant [$p < 0.05$] decrease in mean body weight was seen on gestation day 12 [309 ± 17.9 g vs. 324 ± 23.3 g in vehicle controls]. Body weight gain was significantly [$p < 0.05$] reduced during days 6 - 9 of dosing [8 ± 9.5 g vs.14 ± 6.5 g in vehicle controls], and also during days 6-16 [47 ± 15.8 g vs. 58 ± 16.4 g]. Decreases in corrected mean body weight gain were seen during days 0 - 20 [73 ± 10.8] when compared to vehicle controls [86 ± 17.2].
- At 150 mg/kg/day, body weight gain was significantly [$p < 0.05$] reduced during days 6 - 9 [4 ± 10.2 vs. 14 ± 6.5 g in vehicle controls].

e. Maternal Food Consumption

Except for statistically significant [$p < 0.05$] decreases at 150 mg/kg/day during gestation days 6-9 and 6-16, no other differences in food consumption were noted.

f. Maternal Macroscopical Examination

No treatment-related macroscopical changes were observed in the dams sacrificed at termination.

g. Reproduction Data

There was an incidental statistically significant ($p < 0.05$) reduction in the mean post-implantation loss at 15 and 75 mg/kg/day, 4.4% and 4.9% respectively, when compared to vehicle controls 10.5%. This difference, however, was attributable to a higher than normal mean post-implantation loss in the vehicle control group due to one control female with 100% litter resorption. No other treatment-related differences in cesarean section parameters were seen between the treated and vehicle control groups.

h. Sex Ratios

The sex ratios of fetuses among the treated groups were comparable to that in the vehicle control group.

i. Fetal Weight and Viability

Mean fetal weight was significantly [$p < 0.01$] decreased at 150 mg/kg/day [3.4 ± 0.4] when compared to the vehicle control [3.7 ± 0.3].

j. External Examination

No statistically significant differences were seen in fetal malformations. Although total percentage of litters with malformations was slightly higher at 150 mg/kg/day [13%] compared to vehicle control [4.3%], the types of malformations were generally dissimilar and the incidence of individual malformations remained well within each respective historical control range~~s~~.

k. Visceral Examination (Wilson Technique)

No visceral abnormalities were noted in the 160, 185, 165 or 165 fetuses of the vehicle control, 15, 75, and 150 mg/kg/day groups, respectively.

1. Skeletal Examination

Significant fetal variations observed are tabulated below:

Fetal Variants	Dose Level [mg/kg/day]				
	0	15	75	150	Historical Controls Litter/Total (%)
Number of litters	23	24	21	23	511
Reduced ossification of the skull	5 22%	3 13%	**14*** **67%**	12 52%	17 3% Range: 0-20%
14th rudimentary rib(s)	4 17%	6 25%	9 43%	**15*** **65%**	93 18% Range:3.7- 58.3
7th cervical rib(s)	1 4%	1 4%	2 10%	**7*** **30%**	15 3% Range: 0 - 12%
Bent rib(s)	0 0%	0 0%	**6*** **29%**	4 17%	20 4% Range:0 - 16%

Reduced ossification of the skull: This anomaly is attributed to treatment since the increase is not only statistically significant [p <0.05] at 75 mg/kg/day but also greater than historical controls]. Additionally, the increase at 150 mg/kg/day [52%] while not statistically significant, is also higher than the concurrent control [22%], and outside the of the historical control range [0-20%].

14th rudimentary ribs: A clear treatment-related and nearly a dose-response effect is seen. The increases at 75 mg/kg/day [43%] and at 150 mg/kg/day [65%] are higher than both the concurrent control [17%] and historical controls [18%] with the increase reaching statistical significance [p <0.05] at 150 mg/kg/day.

7th cervical ribs: Although the increases at 75 and 150 mg/kg/day were minimal, it was statistically significant at the high dose.

Bent ribs: The increase was statistically significant [p <0.05] at 75 mg/kg/day [also outside the historical control range], and at 150 mg/kg/day while not statistically significant, the incidence [17%] was also higher than the of the historical controls [4%].

4. DISCUSSION

Oral administration of the diethanolamine salt of 2,4-D at 0, 15, 75, or 150 mg/kg/day to mated rats during days 6 through 15 of gestation resulted in maternal toxicity manifested by decreases in mean body weight, body weight gain, and mean food consumption at the 75 and 150 mg/kg/day groups. No maternal toxicity was observed at the 15 mg/kg dose. No treatment-related differences were seen in cesarian section parameters between the treated and control groups. The slight fetotoxicity observed at 150 mg/kg/day was a statistically significant reduction in fetal body weight.

No statistically significant or treatment-related fetal anomalies were seen at 15 mg/kg/day. Fetal anomalies were seen at the higher doses. The increased incidence of reduced ossification of the skull is attributable to treatment since the increase at 75 mg/kg/day is significantly higher than the concurrent control, and also is out side the historical control range. The incidence of this anomaly at 150 mg/kg/day while not statistically significant is also higher than the concurrent control, and is out side the historical control range. The increased incidence of 14th rudimentary ribs is also attributable to treatment as evidenced by a near dose-response relationship [17%, 25%, 43% and 65% at 0, 15, 75, and 150 mg/kg/day, respectively], with the increase reaching statistical significance at the high dose.. The anomalies of the 7th cervical ribs and the bent ribs is also attributable to treatment due to statistically significant increases at 150 and 75 mg/kg/day doses, respectively, [also outside the historical control range]; however, the biological significance of these variants are not known due to the lack of a dose-response.

The report concluded that "the biological significance of these changes [fetal variations] was not clear since the increases occurred only at maternally toxic levels". Based on this observation, the authors established a NOEL of 150 mg/kg/day for developmental toxicity.

This reviewer does not agree with the author's conclusion that the NOEL for developmental toxicity is 150 mg/kg/day. It is clear that two of the fetal anomalies, namely the increased incidence of reduced ossification of the skull and the 14th rudimentary ribs, seen at the 75 and 150 mg/kg/day are due to the adverse developmental effect of the test material. In addition, even if these effects can be associated with manifestations of maternal toxicity, such effects are still toxic manifestations and such are generally considered a reasonable basis for Agency regulation and/or risk assessment. Consequently, a NOEL of 15 mg/kg/day is established for developmental toxicity.

5. CONCLUSION

2,4-D DEA is a developmental toxin when administered orally to pregnant rats during the period of organogenesis. Based on the results of this study the following NOELs and LOELs are established:

Maternal Toxicity

NOEL: 15 mg/kg/day or 11 mg 2,4-D DEA/kg/day
LOEL: 75 mg/kg/day or 55 mg 2,4-D DEA/kg/day

Developmental Toxicity:

NOEL: 15 mg/kg/day or 11 mg 2,4-D DEA/kg/day

LOEL: 75 mg/kg/day or 55 mg 2,4-D DEA/kg/day

6. CORE CLASSIFICATION: Minimum; this study satisfies the requirements for a developmental toxicity study in rats (83-3 a) and is acceptable for regulatory purposes.

37

[DIETHANOLAMINE SALT OF 2,4-D] Developmental Toxicity Study - Rabbit [§83-3(b)/OPPTS 870.3700]

Amendment to DER for MRID 42055501/- Developmental Toxicity Study - Rabbit [2,4-D DEA]
42013501

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 009460].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/12/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 12/12/00
Reregistration Branch I, Health Effects Division (7509C)
TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rabbit — § 83-3(b)/OPPTS 870.3700
P.C. CODE: 030016 — REREGISTRATION CASE NO.: 818713
CASWELL NO.: 315K — CAS #: 5742-19-8
TEST MATERIAL (PURITY): Ethanolamine salt of 2,4-D [73.09%]
SYNONYMS:dichlorophenoxyacetic acid, ethanolamine salt; 2,4-D DEA
SPONSOR: PBI/Gordon Corporation, Kansas City, MO

CITATION: Rodwell, D. E. (1991). Teratology Study in Rabbits with Diethanolamine Salt of 2,4-D. Springborn Laboratories, Inc., OH. Study No. SLS 3229.13, September 4, 1991. MRID 42055501; Document No. 009460. Unpublished. Range-Finding Teratology Study in Rabbits with Diethanolamine Salt of 2,4-D. Study No. SLS 3229.12, June 27, 1991. MRID 42013501.

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 42055501], artificially-inseminated female New Zealand White rabbits [20/group] were administered 2,4-D diethanolamine salt [73.09%] at dose levels of 0 [distilled water], 15 mg/kg/day, 30 mg/kg/day, and 60 mg/kg/day from gestation day 6 through gestation day 18.

One high-dose doe was found dead on day 19. One high-dose doe [day 23] and three low-dose does [between days 19-22] aborted. Treatment-related clinical signs of toxicity were limited to decreased activity of one high-dose doe on 3 occasions, wobbly gait of one high-dose doe on one occasion, no feces for one high-dose doe on 2 occasions, and a low incidence of few feces and mucoid stool in all treatment groups compared to no incidence in the control. Decreased [slight, not statistically significant] body weight was observed at the mid- [96% of control, corrected for uterine weight] and high- [93% of control, corrected for uterine weight] dose levels. At the high-dose level, a negative body-weight gain was observed during days 6 through 9 of dosing, and statistically-significant decreases in body-weight gains were observed throughout the dosing period at the mid- and high-dose levels [days 6-19: mid 59%/high 49% of control] and overall [days 0-29:mid 74%/high 76% of control]. Corrected body-weight gain was decreased at all dose levels compared to the control [41%, 40%, and 24% of control] but statistical significance was not attained at any dose level due to the wide variation among the does in each group. There was a dose-related decrease in food consumption at the mid- and high-dose levels both during dosing and overall.

Pregnancy rates were comparable among the groups. Comparable numbers of corpora lutea, implantations, and live fetuses were observed among the groups, and there were no dead fetuses. There was an increase in the number of resorptions at the high-dose level [22] compared to the concurrent control [3], and the number of litters with resorptions was also increased [9 *vs* 3]. Consequently, post-implantation loss was significantly increased at the high-

dose level [1.3] compared to the control [0.2] and other dose levels [0.5 and 0.4] but within the historical control mean [0.2-1.9]. Gravid uterine weight was comparable among the groups.

There was no adverse effect on fetal body weight, and the sex ratio was comparable among the groups. There were no apparent, treatment-related, effects on external or visceral variation/anomalies/retardations/malformations, or on skeletal anomalies/retardations/variations. The incidence of the skeletal malformation 7th cervical rib(s) was increased at the high-dose level [7/128 fetuses in 4/17 litters (23.5%)] compared to the concurrent [0%] and historical [litter incidence: 0-6.7%] controls. NOTE: This particular skeletal malformation was also observed at an increased rate in rat pups in the rat developmental toxicity study on 2,4-D DEA at 150 mg/kg/day.

The maternal toxicity NOAEL is 15 mg/kg/day, based on decreased body-weight gain and food consumption at the maternal toxicity LOAEL of 30 mg/kg/day. At the highest dose tested, one doe died, and there was a slight increase in resorptions/post-implantation loss. The developmental toxicity NOAEL is 30 mg/kg/day, based on the number of litters with fetuses with 7th cervical rib(s) at the developmental toxicity LOAEL of 60 mg/kg/day.

This developmental toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement **[§83-3(b)/OPPTS 870.3700]** for a developmental toxicity study in rabbits.

[DIETHANOLAMINE SALT OF 2,4-D] Developmental Toxicity Study - Rabbit [§83-3(b)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Body-Weight Gain/Food Consumption

Dose/Time	0 mg/kg/day	15 mg/kg/day	30 mg/kg/day	60 mg/kg/day
Body Weight [g]				
gestation day 0	3573±308	3554±299	3533±258	3504±211
gestation day 6	3775±327	3792±341	3743±263	3702±259
gestation day 9	3819±321	3840±360	3749±278	3648±317 [96]
gestation day 12	3896±336	3919±368	3813±309 [98]♪	3672±327 [94]
gestation day 29	4240±376	4168±426	4029±298 [95]	3987±329 [94]
gestation day 29 (corrected)	3785±359	3674±400	3618±316 [96]	3533±283 [93]
Body-Weight Gain [g]				
days 0-6	203±75	238±106	210±71	198±83
days 6-9	43±64	48±78	7±57 [82]	-54**
days 9-12	78±59	79±48	64±71 [82]	24±76* [31]
days 12-15	87±45	77±51	77±49	85±140
days 15-19	97±32	62±42 [64]	34±88 [35]	66±107 [68]
days 19-24	104±40	25±80 [24]	93±124 [89]	74±81 [71]
days 24-29	54±86	12±109 [22]	11±128 [20]	66±102 [122]
days 6-19	306±83	302±92	182±117** [59]	149±121** [49]
days 19-29	158±100	64±97 [41]	105±197 [66]	141±136 [89]
days 0-29	667±151	580±201 [87]	496±167* [74]	505±164* [76]
days 0-29 (corrected)	212±178	86±200 [41]	85±275 [40]	51±107 [24]
Food Consumption [g/doe/day]				
days 0-6	176±29	198±30	183±26	181±24
days 6-9	183±25	191±33	161±43 [88]	120±55** [66]
days 9-12	188±32	200±31	162±45 [86]	130±65** [69]
days 12-15	187±32	197±40	165±36 [88]	141±54** [75]
days 15-19	201±35	189±39	160±45* [80]	154±53** [77]
days 19-24	179±34	164±46	145±43* [81]	142±42* [79]
days 24-29	123±39	94±42 [76]	96±57 [78]	97±48 [79]
days 6-19	191±28	194±30	162±30* [85]	138±42** [72]
days 19-29	151±33	127±40	121±45 [80]	119±33 [79]
days 0-29	174±28	173±27	152±24* [87]	145±20** [83]

* $p<0.05$; ** $p<0.01$; ♪ [% of control]; data from Tables 3, 4, 5 of the report

Table 2. Resorptions

# fetuses/# litters	0 mg/kg/day	15 mg/kg/day	30 mg/kg/day	60 mg/kg/day
resorptions				
early	2/2	6/4	7/6	13/7
late	1/1	2/1	0/0	9/3
# does w/ resorptions only	0	0	2	0

data from Tables 1, 8 [pages 22, 34] of the report

009460

PRIMARY REVIEWER: Jess Rowland M.S, Toxicologist
Section II, Toxicology Branch II 4/22/92

SECONDARY REVIEWER: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II 4/22/92

DATA EVALUATION REPORT

1. TERATOLOGY RANGE-FINDING STUDY

STUDY TYPE: Teratology Range-Finding **GUIDELINE: N/A**

Caswell No. 315 K MRID No. 420135-01 HED PROJECT No. 2-0751

TEST MATERIAL: Diethanolamine salt of 2,4-D Acid.

REGISTRANT: PBI Gordon Corporation, Kansas City, MO

TESTING LABORATORY: Springborn Laboratories, Inc. OH

STUDY IDENTIFICATION: SLS 3229.12

TITLE OF REPORT: Range-finding Teratology Study in Rabbits with Diethanolamine Salt of 2,4-D.

AUTHOR: Dean E. Rodwell, M.S **REPORT DATE:** June 27, 1991

SUMMARY: Impregnated New Zealand White rabbits were given oral administration of 2,4-D DEA [73.09%] at doses of 0, 15, 25, 50, 75, or 100 mg/kg/day during days 6 through 18 of gestation. Dams were sacrificed on gestation day 29. 2,4-D DEA did not induce maternal or developmental toxicity at 15 or 25 mg/kg/day. Treatment did cause maternal toxicity manifested by mortality [100 mg/kg/day], clinical signs of toxicity [100 mg/kg/day], and dose-dependent decreases in body weight gain and food consumption [50, 75 and 100 mg/kg/day]. Minimal developmental toxicity seen only at 100 mg/kg/day was a slight decrease in fetal weight. Cesarean sections did not show any treatment-related differences between the treated and control groups, and no external malformations or variations were seen in any of the fetuses of treated does. Based on the results of this study the NOELs and LOELs are:

MATERNAL TOXICITY NOEL = 25 mg/kg/day; LOEL = 50 mg/kg/day
DEVELOPMENTAL TOXICITY NOEL = 50 mg/kg/day; LOEL = 100 mg/kg/day

The dose levels selected for the main study were 0, 15, 30 and 60 mg/kg/day.

CORE CLASSIFICATION: Not applicable; range-finding study.

381

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1. OBJECTIVE

The objective of this range-finding study, was to establish appropriate dose levels of the Diethanolamine salt of 2,4-D for the main study.

2. PROTOCOL

Groups of five impregnated female New Zealand White rabbits [7 months old] were given oral administrations of 2,4-D DEA (73.09% pure; Batch no.0017) at doses of 0, 15, 25, 50, 75 or 100 mg/kg/day in distilled water [2 mL/kg] daily, during days 6 through 18 of gestation. Concentration, homogeneity and stability of the test article/vehicle mixtures were determined prior to the initiation of the study.

Animals were observed for clinical signs of toxicity daily, dams were weighed on gestation days 0, 6, 9, 12, 15, 19, 24 and 29, and individual food consumption was measured daily during gestation. Dams in each group were sacrificed on day 29 and postmortem examination included macroscopic examination of internal organs, with emphasis on the uterus, uterine contents, position of each fetus in the uterus, and corpora lutea counts. The uteri (and its contents) of all dams were weighed to determine corrected body weights. The fetuses were sexed, weighed, examined, and discarded.

3. RESULTS

i. Analysis of dosing solution

The mean concentrations found were 99.2%, 96.3%, 105.6%, 106.15, and 99.5% of the nominal concentration for the 15, 25, 50, 75 and 100 mg/kg dose groups, respectively. The 2,4-D DEA was homogeneous and stable in distilled water at room temperature for up to eight days post-preparation.

ii. Maternal Toxicity

- No mortality or abortion occurred at 15, 25, or 50 mg/kg/day groups. At 75 mg/kg/day, the two does that aborted, one each on gestation days 25 and 28, were sacrificed. At 100 mg/kg/day, one female was found dead on gestation day 18 and the two does that aborted, one each on gestation days 22 and 25 were sacrificed.

- Necropsy of the doe that died at 100 mg/kg/day showed reddish-green fluid contents in the urinary bladder while those that aborted/sacrificed had clear fluid in the thoracic cavity, reddish brown fluid contents in the urinary bladder and mottled lung. Other females that aborted showed no treatment-related changes.

390

- The pregnancy rate was 60% in the 15 mg/kg/day group and 100% in the control, 25, 50, 75 and 100 mg/kg/day groups.

- A clinical sign of toxicity observed primarily at 100 mg/kg/day was an increased incidence of few or no feces. The female that died at this dose, exhibited salivation, wobbly gait, limb rigidity and partial limb paralysis prior to death. A low incidence of few feces was seen at 25, 50, and 75 mg/kg/day. No other treatment-related clinical signs were seen.

- No treatment-related effects on body weight gain were seen in does at 15 or 25 mg/kg/day. Dose-dependent decreases in body weight gain were seen at 50 mg/kg/day during Days 6-9, at 75 mg/kg/day during days 6-9, 15-19, 19-24 and 6-19 and at 100 mg/kg/day during days 6-9, 12-15, 15-19, 19-24, 6-19, and 0-29.

- No treatment-related effects in food consumption [g/animal/day and g/kg/day] were noted in does at 15 or 25 mg/kg/day. Dose-dependent decreases in food consumption were seen at 50 mg/kg/day [Days 6-9 and 9-12], 75 mg/kg/day [Days 6-9, 9-12, 12-15, 15-19, 19-24 and 6-19] and 100 mg/kg/day [Days 6-9, 9-12, 12-15, 15- 19, 19-24, 6-19, 19-29, and 0-29].

- Necropsy revealed no treatment-related gross abnormalities in the does sacrificed on Day 29. No treatment-related effects were seen on the reproductive performances of the does.

iii. Developmental Toxicity

- No developmental toxicity was observed at any dose level; treatment had no effect on corpora lutea, implantation sites, viable fetuses, early and late resorptions and gravid uterus weight.

- Mean fetal body weight was slightly reduced at 100 mg/kg/day [41.4 ± 3.3 g] compared to controls [47.6 ± 5.7 g].

- No external malformations or variations were observed in any of the fetuses of treated females.

4. CONCLUSION

MATERNAL TOXICITY NOEL = 25 mg/kg/day; LOEL = 50 mg/kg/day
DEVELOPMENTAL TOXICITY NOEL = 50 mg/kg/day; LOEL = 100 mg/kg/day

The dose levels selected for the main study were 0, 15, 30 and 60 mg/kg/day.

5. CORE CLASSIFICATION: Not applicable; study not required.

383

009460

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist
Section II, Toxicology Branch II

Jess Rowland 4/22/92

SECONDARY REVIEWER: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II

DATA EVALUATION REPORT

TERATOLOGY STUDY

STUDY TYPE: Teratology Study / Rabbits **GUIDELINE:** 83-3(b)

Caswell No. 315 K **MRID No. 420555-01** **HED PROJECT No. 2-0751**

TEST MATERIAL: Diethanolamine salt of 2,4-D

REGISTRANT: PBI Gordon Corporation, Kansas City, MO

TESTING LABORATORY: Springborn Laboratories, Inc. OH

STUDY IDENTIFICATION: SLS 3229.13

TITLE OF REPORT: Teratology Study in Rabbits with Diethanolamine Salt of 2,4-D.

AUTHORS: Dean E. Rodwell **REPORT DATE:** September 4, 1991

SUMMARY: Inseminated New Zealand White rabbits were given oral administration of 2,4-D DEA **[73.09%]** at doses of 0, 15, 30, or 60 mg/kg/day during days 6 through 18 of gestation. No maternal toxicity was observed at 15 mg/kg/day. At 30 mg/kg/day, maternal toxicity was characterized by decreases in body weight gain and food consumption. At 60 mg/kg/day, maternal toxicity was manifested by mortality, clinical signs of toxicity, decreases in mean body weight gain and mean food consumption. No treatment-related differences were seen in reproductive parameters. No developmental toxicity was seen at 15 or 30 mg/kg/day. At 60 mg/kg/day, the number of litters with 7th cervical rib(s) was significantly increased when compared to concurrent controls and was outside the historical control range of the testing laboratory. **This particular variation was also seen in pups of pregnant rats** treated with oral doses of 150 mg/kg/day DEA; the incidence in treated rats was significantly higher than the concurrent controls and was outside the rat historical control range. Therefore, it is evident that 2,4-D DEA may be the cause for this skeletal variation due to the consistency of this occurrence in two species. Based on the results of this study the following NOELs and LOELs are established.

MATERNAL TOXICITY NOEL = 15 mg/kg/day
LOEL = 30 mg/kg/day
DEVELOPMENTAL TOXICITY NOEL = 30 mg/kg/day
LOEL = 60 mg/kg/day

384

I. OBJECTIVE

The objective of this study was to assess the effects of the **diethanolamine salt of 2,4-D [2,4-D DEA]** on the embryonic and fetal development following oral administration to rabbits during the period of organogenesis.

II. MATERIALS AND METHODS

a. Test Material

Identity: Diethanolamine salt of 2,4-D
Batch No.: 0017
Purity: 73.09%
Description: Clear, light brown viscous liquid

b. Test Animals

Species/Sex: Female rabbits
Strain: New Zealand White
Body Weight on Gestation Day 0: 3.0 to 4.3 kg
Identification: Ear tags.
Acclimation: 33 days
Housing: Individually in stainless steel cages
Food: Purina Certified Rabbit Chow #5322 ad libitum.
Water: Tap water ad libitum
Environment: Temperature - 61 -70°F; Humidity - 40-70%; Light/Dark- 12 hr.cycle
Group Assignment: 20 inseminated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

The females were artificially inseminated with semen from proven male breeders of the same strain, obtained from the same source. The semen was diluted with normal saline and approximately 0.5 mL of the diluted semen was introduced into each female's vagina. Semen from one male was used to inseminate an equal number of females in each study group. Immediately following insemination, the females were administered HCG via the marginal ear vein. The day of insemination was considered Day 0 of gestation.

d. Preparation of Dosing Solutions

The concentration of each dosing solution was adjusted based on a test article activity of 73.09%. The test material/vehicle mixtures (w/v) were prepared fresh weekly and dispensed into daily aliquots. The vehicle, distilled water, was added to a weighed amount of the test material and the mixture was kept homogenous by constant stirring on a magnetic stirrer for ten minutes prior to daily dispensation.

e. Analysis of the Dosing Solutions

Concentration, homogeneity, and stability analysis of the dosing solutions were determined once during the study.

f. Administration of Test Article

The test article was administered daily orally via gavage at doses of 0, 15, 30, or 60 mg/kg/day from day 6 through 18 of gestation. The control group received the vehicle only. All groups received a dosing volume of 2 mL/kg body weight, with a daily adjustment of the individual volume to the actual body weight.

g. Observations

All animals were observed once daily for clinical signs of toxicity including physical or behavioral abnormalities. In addition, during the treatment period, the rabbits were observed between one-half and two hours following dosing for detection of overt signs of toxicity. Individual body weights were obtained on days 0, 6, 9, 12, 15, 19, 24 and 29 of gestation. Individual food consumptions were measured daily during gestation.

h. Termination

Females found dead or showing sings of abortion during the study were necropsied. All surviving does were sacrificed on gestation day 29.

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i. Cesarean Section

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 rabbits were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantation, early and late resorptions, and live and dead fetuses were recorded.

j. Fetal Examinations

Each fetus was removed from the uterus and individually identified with a tag, weighed, and observed for gross external alterations. The crown-rump length of late resorptions was measured and the tissue discarded. Every fetus was examined to determine sex and soft tissue alterations. Fetuses were then eviscerated, stained with Alizarin red S, and examined for skeletal changes.

k. Statistical Analysis

Test Employed	Parameters Analyzed
ANOVA followed by Dunnett's Test	Maternal and fetal data including body weights, food consumption, number of viable fetuses, implantation sites, and corpora lutea
Mann-Whitney U test	Resorptions
Chi-square test	Fetal sex ratios
Fisher's Exact Test	Fetal malformations and variations.

j. Quality Assurance Measures:

A quality assurance statement was signed and dated 09/04/91. This date conforms to the review of the study phases and the draft and the final reports.

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III. RESULTS

Analysis of the Dosing Solutions

The mean concentrations of test article found were in the range of 98.4 to 102.5% of the nominal concentration. Homogeneity and stability analyses indicated that 2,4-D DEA was homogenous and stable in aqueous solution when stored for up to eight days at room temperature.

1. Maternal Toxicity

a. Mortality & Pregnancy Status

At 60 mg/kg/day, one female was found dead on Day 19 and one doe aborted on Day 23. Three does aborted at 15 mg/kg/day between gestation days 19 and 22; determined to be unrelated to treatment due to lack of dose-response. No mortality or abortion occurred at 30 mg/kg/day. The pregnancy rate was 90%, 100%, 90%, and 95% in the 0, 15, 30, and 60 mg/kg/day groups.

b. Clinical Signs

With the possible exception of low incidences of few feces and mucoid stool noted during the dosing period at 60 mg/kg/day, there were no clinical signs of toxicity that could be directly attributable to treatment.

c. Body Weight Changes

- No differences in mean body weight or body weight gain were seen between the vehicle control and 15 mg/kg/day groups.

- At 30 mg/kg/day, body weight gain was significantly [p <0.01] reduced during days 6 - 19 of dosing [182.0 ± 117.1 g vs. 306.0 ± 82.9 g in vehicle controls], and also during days 0 - 29 [496.0 ± 166.5 g vs. 667.0 ± 150.9 g].

- At 60 mg/kg/day, body weight gain was significantly [p <0.01] reduced during days 6 - 19 of dosing [149.0 ± 120.6 vs. 306.0 ± 82.9 g in vehicle controls], and also during days 0 - 29 [505.0 ± 163.7 g vs. 667.0 ± 150.9 g].

- No statistical differences in net maternal body weights or net body weight gains [corrected for gravid uterus weight] were observed at any dose levels.

d. Food Consumption

- No differences in mean food consumption [g/animal/day] were seen between the vehicle control and 15 mg/kg/day groups.
- At 30 mg/kg/day, food consumption [g/animal/day] was significantly [p <0.01] reduced during days 6 - 19 of dosing [162.0 ± 30.4 g vs. 191.0 ± 27.9 g in vehicle controls], and also during days 0 - 29 [152.0 ± 24.1 g vs. 174.0 ± 27.7 g].
- At 60 mg/kg/day, food consumption [g/animal/day] was significantly [p <0.05] reduced during days 6 - 19 of dosing [138.0 ± 42.4 vs. 191.0 ± 27.9 g in vehicle controls], and also during days 0 - 29 [145.0 ± 20.1 g vs. 174.0 ± 27.7 g].

e. Macroscopical Examination

No treatment-related macroscopical changes were observed in the dams sacrificed at termination. Necropsy of the doe that died at 60 mg/kg/day on gestation day 19 exhibited dark red foci and colored mucoid contents in the stomach, reddened mucosa an colored mucoid contents in the small intestine, mottled lungs.

f. Reproduction Data

Reproduction data are presented in Table 1. An incidental, but statistically (p< 0.05) significant increase [1.3 ± 1.9] in mean post-implantation loss was seen at 60 mg/kg/day when compared to vehicle controls [0.2 ± 0.4]. The author reported that this difference was attributable to a lower than normal control group value when compared to historical control range [mean, 0.2 - 1.9] of the testing laboratory, and due to one female at the high dose with seven late resorptions. Historical control data were provided to support this statement. No other treatment-related differences in cesarean section parameters were seen between the treated and vehicle control groups.

The statistically significant decrease [41.5 g] in mean body weight of fetuses at 15 mg/kg/day was not considered to be treatment induced since the control group mean fetal body weight [46.7 g] was higher than the historical control mean of 44.6 g and there was no dose response.

009460

Table 1. Cesarean Section Observations[a]

Observations	Dose Level [mg/kg/day]			
	0	15	30	60
No. Assigned	20	20	20	20
Females Gravid	18	17	18	17
Maternal Wastage				
# Died	0	0	0	1
# Aborted	0	3	0	1
# Non pregnant	2	0	2	1
Total Corpora Lutea	182	196	184	190
Corpora Lutea/dam	10.1±2.5	11.5±2.6	10.2±3.8	11.2±2.8
Total Implantation	130	155	134	150
Implantation/Dam	7.2±2.9	9.1±2.5	7.4±3.1	8.8±1.7
Total Live Fetuses	127	147	127	128
Live Fetuses/Dam	7.1±2.9	8.6±8.6	7.1±7.1	7.5±2.6
Total Dead Fetuses	0	0	0	0
Total Resorptions	3	8	7	22
Early	2	6	7	13
Late	1	2	0	9
Total Preimplantation Loss	52	41	50	40
Mean ± S.D	2.9±2.7	2.4±3.0	2.8±4.2	2.4±2.3
Total Postimplantation Loss	3	8	7	22
Mean ± S.D	0.2±0.4	0.5±0.9	0.4±0.6	1.3* ±1.9
Gravid Uterus Weight [g]	455±164	494±89	411±182	454±127
% Sex Ratio ♂ / ♀	52/48	48/52	56/44	51/49
Fetal Weight [g]	46.7±5.4	41.5* ± 6	42.2±6.4	42.4±5.1

* Significantly different from control at $p < 0.05$.

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2. Developmental Toxicity

Fetal malformations are summarized in Table 2 and fetal variations are summarized in Table 3.

a. External Examinations

No treatment-related or statistically significant external malformations or variations were seen between the control and treated groups.

b. Visceral Examinations

No treatment-related or statistically significant soft tissue malformations were noted in the 127, 147, 127 or 128 fetuses of the vehicle control, 15, 30, and 60 mg/kg/day groups, respectively.

The number of litters with absent or small gall bladder was significantly [p <0.05] increased at 30 mg/kg/day [4/16; 25%] when compared to controls [0%]. This fetal variation was considered to be unrelated to treatment because a similar increase was not observed at the high dose and the increase at the 15 mg/kg/day [11.8%] was within the historical control range [0 - 12.5%] of the testing laboratory.

Other soft tissue variations known to occur spontaneously in rabbits were seen with similar frequency in the control and treated groups.

c. Skeletal Examinations

No treatment-related or statistically significant skeletal malformations were seen between the control and the treated groups.

At the 60 mg/kg/day, a statistically significant [p <0.05] increase was observed in the number of litters with 7th cervical rib(s) [4/17; 23.5%] when compared to controls [0%]. This increase was outside the historical control range [0-6.7%] of the testing laboratory.

This particular skeletal variation was also seen in pups of pregnant rats given 2,4-D DEA at 150 mg/kg/day [MRID No. 419209-06]; the 30% incidence in treated rats was significantly higher than the vehicle control incidence [0%], and was outside the rat historical control range [0-12%] of the testing laboratory.

No other findings were significantly altered relative to the controls.

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Table 2. Summary of Fetal Malformations

Observations	Dose Level [mg/kg/day]			
No. of Fetuses / No. of Litters	0	15	30	60
No. Examined **Externally**	127/18	147/17	127/16	128/17
Abdominal Closure Defect.....	0/0	1/1	0/0	0/0
No. Examined **Viscerally**	127/18	147/17	127/16	128/17
Right-sided Retroesophageal Aortic Arch.....	0/0	0/0	0/0	1/1
Kidney(s) - Unascended.....	0/0	2/1	0/0	0/0
No. Examined **Skeletally**	127/18	147/17	127/16	128/17
Skull Anomaly.....	1/1	3/3	2/2	1/1
Vertebral Anomaly.....	0/0	1/1	0/0	1/1
Sternebra(e) Malaligned (severe)..	1/1	1/1	0/0	0/0
Costal Cartilage Anomaly.....	1/1	3/2	2/2	0/0
Rib Anomaly.....	1/1	0/0	0/0	0/0
Extra Site of Ossification Anterior to Sternebra #1.....	0/0	1/1	0/0	0/0
Sternum Anomaly.....	0/0	2/1	0/0	0/0
Total **Malformations**				
No. With **External** Malformations	0/0	1/1	0/0	0/0
No. with **Soft Tissue** Malformations	0/0	2/1	0/0	1/1
No. with **Skeletal** Malformations	4/4	9/6	4/4	2/2
Total Number Malformations	**4/4**	**9/6**	**4/4**	**3/3**

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Table 3. Summary of Fetal Variations

Observations	Dose Level [mg/kg/day]			
No. of Fetuses / No. of Litters	**0**	**15**	**30**	**60**
No. Examined **Externally**	127/18	147/17	127/16	128/17
No. With External Variations.....	0/0	0/0	0/0	0/0
No. Examined **Viscerally**	127/18	147/17	127/16	128/17
Major Blood Vessel Variation.....	3/3	13/8	2/1	2/2
Liver-Accentuated Lobular Markings	0/0	1/1	0/0	4/2
Gall Bladder Absent or Small.....	0/0	4/2	6/4*	1/1
Retrocaval Ureter.....	1/1	1/1	1/1	2/2
Periovarian Cyst(s).....	2/1	0/0	0/0	0/0
No. Examined **Skeletally**	127/18	147/17	124/16	128/17
Hyoid Arch(es) Bent.....	9/6	21/9	11/6	7/4
13th Full Rib(s).....	51/15	55/15	57/14	73/17
27 Presacral Vertebrae.....	22/11	20/8	20/10	27/10
13th Rudimentary Rib(s).....	23/11	35/15	17/11	11/6
7th Sternebrae.....	1/1	0/0	0/0	0/0
Sternebra #5 and/or #6 Ossified...	13/6	7/3	5/4	11/5
Sternebra Malaligned.....	6/6	4/3	5/4	5/5
Accessory Skull Bone(s).....	4/2	3/3	4/4	3/2
Hyoid Body and/or Arch(es) Unossified.....	0/0	1/1	0/0	0/0
Sternebrae with Thread-like Attachment.....	1/1	1/1	0/0	1/1
25 Presacral Vertebrae.....	0/0	1/1	0/0	1/1
Talus Unossified.....	0/0	0/0	0/0	1/1
7th Cervical Rib(s).....	**0/0**	**5/2**	**1/1**	**7/4***
Reduced Ossification of the Vertebral Arches.....	1/1	1/1	0/0	0/0

343

IV. DISCUSSION

Oral administration of the diethanolamine salt of 2,4-D at 0, 15, 30, or 60 mg/kg/day to inseminated during days 6 through 18 of gestation resulted in maternal toxicity manifested mortality, few feces and/or mucoid stool, and decreases in body weight gain and food consumption during the entire treatment and gestation periods at the 60 mg/kg/day. Mean maternal body weight and food consumption were also decreased during the entire treatment and gestation periods at 30 mg/kg/day. No maternal toxicity was observed at the 15 mg/kg dose. No treatment-related differences were seen in cesarian section parameters between the treated and control groups.

No treatment-related external, visceral, or skeletal malformation or variations were seen in the fetuses of does treated at 15 or 30 mg/kg/day. At 60 mg/kg/day, there was a statistically significant increase in the number of litters with 7th cervical rib(s). This increase was also outside the historical control range of the testing laboratory. An increase in this particular skeletal variation was also seen in the pups of rats given 150 mg/kg/day 2,4-D DEA. In both species, the increase at the highest dose tested was statistically significant when compared to concurrent controls, and was outside the historical control range of the testing laboratory.

Therefore, this reviewer does not agree with the author's statement that "the biological significance of this variation is not clear, however, an increase in a minor skeletal variant is not considered to be indicative of a teratogenic response". In contrast, it is clear that this skeletal variation may be treatment-related due to the consistency of its occurrence in two species where it was higher than the concurrent controls and outside the historical control ranges of the testing laboratory.

The author established a NOEL of 60 mg/kg/day for developmental toxicity. However, due to the occurance of the skeletal variation at this dose, a NOEL of 30 mg/kg/day is established for developmental toxicity.

5. CONCLUSION

2,4-D DEA did not induce maternal or developmental toxicyt at 15 mg/kg/day. At 30 mg/kg/day, only maternal toxicity was seen. At 60 mg/kg/day, 2,4-D DEA induced maternal toxicity and minor skeletal variations in rabbits. Based on the results of this study the following NOELs and LOELs are established:

Maternal Toxicity

NOEL: 15 mg/kg/day
LOEL: 30 mg/kg/day

Developmental Toxicity:

NOEL: 30 mg/kg/day
LOEL: 60 mg/kg/day

6. **CORE CLASSIFICATION: Minimum;** this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

395

Amendment to DER for MRID 42158704/42158702 Developmental Toxicity Study - Rabbit [2,4-D IPA]

This amendment provides an EXECUTIVE SUMMARY and a data table to upgrade the original DER [Document No. 009666].

EPA Reviewer: Linda L. Taylor, Ph.D. 2/13/03
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V. M. D., M. P. H. for VDobozy 2/14/03
Reregistration Branch I, Health Effects Division (7509C)

TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rabbit § 83-3(b)/OPPTS 870.3700
P.C. CODE: 030025
CASWELL NO.: 315U CAS #: 5742-17-6
TEST MATERIAL (PURITY): 2,4-D isopropylamine salt [50.1% (acid equivalent 39.6%)]
SYNONYMS: dichlorophenoxyacetic acid, isopropylamine salt; 2,4-D IPA
SPONSOR: DowElanco
CITATION: Breslin, W. J.; Liberacki, A. B.; and Yano, B. L. (1991). Isopropylamine Salt of 2,4-D: Oral Gavage Teratology Study in New Zealand White Rabbits. Toxicology Research Laboratory, Michigan. Study No. M-004725-013, November 18, 1991. MRID 42158704. Oral Gavage Teratology Probe Study in New Zealand White Rabbits. Study No. M-004725-012. November 7, 1991. MRID 42158702. Document No. 009666. Unpublished.

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 42158704], artificially-inseminated female New Zealand White rabbits [20/group] were administered 2,4-D isopropylamine salt [50.1% (acid equivalent 39.6%)] at dose levels of 0 [deionized distilled water control)], 13 mg/kg/day, 38 mg/kg/day, and 95 mg/kg/day [corresponding to approximately 10, 30, and 75 mg/kg/day of 2,4-D acid] from gestation day 7 through gestation day 19.

One mid-dose doe aborted on day 25 and two mid-dose does died [days 16 and 24]. Four high-dose does [days 12, 13, 14, and 20] were sacrificed moribund, three high-dose does died [days 12, 20, and 21], and one high-dose doe delivered early [day 28]. Treatment-related clinical signs of toxicity [myotonia, lateral recumbency, cold extremities, perineal soiling, blood in urine, decreased feces] were observed in the mid- and high-dose does.

In general, body weight was comparable among the groups throughout the study. There was a dose-related decrease in body-weight gain during the dosing period [days 7-10 (negative body-weight gain at all dose levels, but statistical significance attained only at the high dose); days 7-20 (negative body-weight gain at high dose; 56% of control at mid dose, 64% of control at low dose; days 7-16 (8% of control at high dose; 77% of control at mid dose; 75% of control at low dose)]. The overall body-weight gain at the high-dose level was 67% of the control, although statistical significance was not attained. Corrected [for uterine weight] body-weight gain was comparable among the groups. Food consumption was not monitored.

There were no treatment-related effects on pregnancy rate, numbers of corpora lutea, implantations, and total live fetuses, resorption rate, number and percent of litters with resorptions, fetal sex ratio, fetal body weight, or gravid uterine weights. Dead fetuses were not reported at any dose level, and there were no litters that were totally

resorbed. There were no apparent, treatment-related, effects on external, visceral, or skeletal variation/anomalies/ retardations/malformations at any dose level.

The maternal toxicity NOAEL could not be determined, based on decreased body-weight gain throughout the dosing period at all dose levels. At the mid- and high-dose levels, mortality/morbidity and clinical signs were observed. The developmental toxicity NOAEL is 95 mg/kg/day, the highest dose tested.

This developmental toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [**§83-3(b)/OPPTS 870.3700**] for a developmental toxicity study in rabbits.

NOTE: The maternal NOAEL/LOAEL differ from that of the original DER. It was determined in this subsequent review that the body-weight gain deficit at all dose levels was treatment-related. Additionally, in the original DER, decreased kidney weights [absolute and relative] were noted at all dose levels. **Increased** kidney weights were observed at all dose levels compared to the control [absolute: 9%, 13%, and 13%; relative: 8%, 13%, and 11% greater than control with increasing dose]. However, only the relative kidney weights at the mid- and high-dose levels were statistically significant. The increase in kidney weight is considered treatment-related, since the kidney has been shown to be a target organ in other studies on 2,4-D and/or its amines/esters.

PRIMARY REVIEWER: Jess Rowland, Toxicologist Jess Rowland 8/3/92
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 8/4/92
Section II, Toxicology Branch II

DATA EVALUATION REPORT

1. IPA---PROBE STUDY

STUDY TYPE: Developmental Toxicity **[Probe Study]** **GUIDELINE: N/A**

CASWELL NO. 315 U **MRID No. 421587-02** **HED PROJECT No. 2-1087**

TEST MATERIAL: ISOPROPYLAMINE Salt of 2,4-D ACID **[2,4-D IPA]**

REGISTRANT: DowElanco, Indianapolis, IN.

TESTING LABORATORY: The Toxicology Research Laboratory, Michigan

STUDY IDENTIFICATION: M-004725-012

TITLE OF REPORT: *ISOPROPYLAMINE SALT OF 2,4-D: ORAL GAVAGE TERATOLOGY PROBE STUDY IN NEW ZEALAND WHITE RABBITS.*

AUTHOR(S): A.B. Liberacki, W.J. Breslin and B.L. Yano

REPORT DATE: November 7, 1991

SUMMARY: Impregnated New Zealand White rabbits were given oral administration of **Technical 2,4-D IPA [50.2%]** at doses of 0, 64, 127, or 190 mg/kg/day during days 7 through 19 of gestation. These dose levels correspond to approximately 0, 50, 100 or 150 mg/kg/day of 2,4-D Acid. Does were sacrificed on gestation day 20. 2,4-D IPA induced dose-related maternal toxicity which included: mortality/morbidity [127 and 190 mg/kg/day]; clinical signs of toxicity [all dose levels]; decreases in body weight gain [all dose levels]; and decreases in absolute and relative kidney weights [all dose levels]. Treatment had no adverse effects at any dose level on pregnancy rate, pre- and post-implantation losses, the number of implantations, the number of resorptions or litter size. No fetal data were reported.

MATERNAL TOXICITY NOEL = Not Achieved
LOEL = 64 mg/kg/day [LDT]

DEVELOPMENTAL TOXICITY NOEL = 190 mg/kg/day [HDT]
LOEL = Not achieved

CORE CLASSIFICATION: Not applicable; range-finding study.

1. OBJECTIVE

The objective of this probe [range-finding] study, was to establish appropriate dose levels of the Isopropylamine Salt of 2,4-D for the main study.

2. PROTOCOL

Groups of seven inseminated female New Zealand White rabbits [approximately 5.5 months of age and 3.5 to 4.0 kg] were given oral administrations of **Technical 2,4-D IPA (50.2% pure;** Lot No. AGR276461) at doses of 0, 64, 127, or 190 mg/kg/day in deionized distilled water [1 mL/kg] daily, during days 7 through 19 of gestation. These dose levels correspond to approximately 0, 50, 100 or 150 mg/kg/day of 2,4-D Acid. Concentration, homogeneity and stability of the test article/vehicle mixtures were determined prior to the initiation of the study.

Animals were observed for clinical signs of toxicity daily, dams were weighed on gestation days 0, daily during the dosing period, and on day 20 of gestation. Dams in each group were sacrificed on day 20 and postmortem examination included macroscopic examination of internal organs, with emphasis on the uterus, uterine contents, position of each fetus in the uterus, and corpora lutea counts. In addition, on day 20, at necropsy, liver and kidney weights were recorded. Fetal examinations were not performed.

3. RESULTS

i. Analysis of dosing solution

The mean concentrations found were 105%, 105%, and 103% of the nominal concentration for the 64, 127 and 190 mg/kg/day dose groups, respectively. The 2,4-D IPA was homogeneous and stable in deionized distilled water for up to 45 days post-preparation.

ii. Maternal Toxicity

- 64 mg/kg/day: No mortality
- 127 mg/kg/day: 1 doe was sacrificed moribund on Day 14
- 190 mg/kg/day: Mortality occurred in 6 does [1 doe found dead on Day 13; 1 doe each sacrificed moribund on Days 9, 10, 13; and 1 doe each sacrificed moribund on Days 10 & 14]

- Prior to death or sacrifice, does exhibited myotonia, decreased feces, and/or transient lateral recumbency.

- Clinical sign observed in surviving animals at 64 and 127 mg/kg/day was limited to an increased incidence of decreased feces.

- Mean body weight was decreased in does at all dose levels on Days 10, 13, 16 and 20, with the decrease reaching statistical significance [p <0.05] at 127 and 190 mg/kg/day on Days 10 and 13.

- Mean body weight gains were decreased in a dose-related manner. The decrease was statistically significant [p <0.05] on Days 7-10 [all dose groups], 10-13 [190 mg/kg/day], 7-20 [127 mg/kg/day] and 0-20 [64 and 127 mg/kg/day].

- Organ weight data showed dose-related ~~de~~increases in both absolute and relative kidney weights at all dose levels, with the disparity reaching statistical significance [p <0.05] at 64 mg/kg/day [absolute] and 127 mg/kg/day [absolute and relative].

- At terminal sacrifice, necropsy revealed no treatment-related findings. Gross pathological alterations seen in rabbits found dead or sacrificed moribund included: dry cecal ingesta; watery intestinal contents; digestive tract gas; perineal soiling; pale multifocal areas of the liver; distended gall bladder; firm lung lobes; and distended urinary bladder with or without dark urine. These observations were considered secondary to anorexia or debilitation.

- The pregnancy rate was 85.7%, 100%, 85.7%, and 100% at 0, 64, 127 and 190 mg/kg/day dose groups, respectively.

- Treatment had no effect on corpora lutea, implantation sites, pre-and post-implantation losses, litter size or viable fetuses at any dose level.

iii. Developmental Toxicity

No data were reported for fetal examinations.

4. CONCLUSION:

MATERNAL TOXICITY:

NOEL = Not Achieved
LOEL = 64 mg/kg/day [LDT]

DEVELOPMENTAL TOXICITY:

NOEL = 190 mg/kg/day [HDT]
LOEL = Not Achieved

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PRIMARY REVIEWER: Jess Rowland, Toxicologist Jess Rowland 8/3/92
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 8/4/92
Section II, Toxicology Branch II

DATA EVALUATION REPORT

2. IPA---MAIN STUDY

STUDY TYPE: Developmental Toxicity **[Main Study]** **GUIDELINE:** 83-3(b)

CASWELL NO. 315 U **MRID No. 421587-04** **HED PROJECT No. 2-1087**

TEST MATERIAL: Isopropylamine Salt of 2,4-D **[2,4-D IPA]**

REGISTRANT: DowElanco **STUDY IDENTIFICATION:** M-004725-013

TESTING LABORATORY: Toxicology Research Laboratory, Michigan.

TITLE OF REPORT: *ISOPROPYLAMINE SALT OF 2,4-D: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS.*

AUTHORS: W.J. Breslin, A. B. Liberacki and B.L. Yano

REPORT DATE: November 18, 1991

SUMMARY: Groups of 20 inseminated New Zealand White rabbits were given oral doses of **2,4-D IPA [50.1% pure]** at 0, 13, 38, or 95 mg/kg/day during days 7 through 19 of gestation. These dose levels correspond to approximately 0, 10, 30 or 75 mg/kg/day of 2,4-D acid. No maternal toxicity was observed at 13 mg/kg/day. At 38 and 95 mg/kg/day, maternal toxicity was manifested by mortality/morbidity, clinical signs, decreases in body weight gain, and increases in relative kidney weights. Treatment had no effect on the pregnancy rate, implantations, total live fetuses, resorption rate, pre- and post-implantion losses, fetal sex ratio or fetal body weights. No treatment-related external, visceral, or skeletal malformation or variation was seen in any of the fetuses.

MATERNAL TOXICITY:

NOEL = 13 mg/kg/day
LOEL = 38 mg/kg/day

DEVELOPMENTAL TOXICITY:

NOEL = 95 mg/kg/day [HDT].
LOEL = Not Achieved

CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

I. OBJECTIVE

The objective of this study was to assess the effects of the **Isopropylamine Salt of 2,4-D [2,4-D IPA]** on the embryonic and fetal development following oral administration to rabbits during the period of organogenesis.

II. MATERIALS AND METHODS

a. Test Material

Identity: Isopropylamine Salt of 2,4-D [Technical]
Batch No.: AGR 276461
Purity: 50.2% [39.6% as Acid equivalent]
Description: Liquid

b. Test Animals

Species/Sex: Female rabbits
Strain: New Zealand White
Age on Gestation Day 0: 6.5 to 7.5 months
Weight on Gestation Day 0: 3.25 - 4.25 kg
Identification: Ear tags.
Acclimation Period: Two weeks.
Housing: Individually in stainless steel cages
Food: Purina Certified Rabbit Chow #5322 ad libitum.
Water: Tap water ad libitum
Environment: Temperature and humidity controlled rooms.

Group Assignment: 20 inseminated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

Adult females were artificially inseminated with the day of insemination considered Day 0 of gestation. Immediately following insemination, does received an intravenous injection of 100 IU of human chorionic gonadotropin .

d. Preparation of Dosing Solutions

2,4-D IPA was administered in deionized distilled water such that a dose volume of 1 mL/kg body weight yielded the appropriate dose. Dose solutions were prepared once prior to the start of the study.

e. Analysis of the Dosing Solutions

Concentration, homogeneity, and stability analysis of the dosing solutions were determined once during the study.

f. Administration of Test Article

The test article was administered daily orally via gavage at doses of 0, 13, 38, or 95 mg/kg/day during days 7 through 19 of gestation. These dose levels correspond to approximately 0, 10, 30, or 75 mg/kg/day of 2,4-D acid. The control group received the vehicle [deionized distilled water only. All groups received a dosing volume of 1 mL/kg body weight and the dose volumes were adjusted daily based on individual body weights.

g. Observations

All animals were observed once daily for clinical signs of toxicity including physical or behavioral abnormalities. Individual body weights were obtained on day 0, daily during the dosing period, and on days 20 and 28 of gestation. Individual food consumptions were **not** measured in this study.

h. Termination

Any animal which died, appeared moribund or showed indications of early termination of pregnancy was submitted for complete necropsy. All surviving does were sacrificed on gestation day 28, obvious gross pathologic alterations were recorded, and the liver, kidneys and gravid uterus were weighed.

i. Cesarean Section

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 rabbits were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantation, early and late resorptions, and live and dead fetuses were recorded.

j. Fetal Examinations

Each fetus was removed from the uterus and individually 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.

k. Statistical Analysis

Maternal body weights, body weight gains, fetal body weights, and organ weights were evaluated by Bartlett's test for equality of variances followed by a parametric or nonparametric analysis of variance [ANOVA]. If the ANOVA was significant, analysis by Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction was performed, respectively. Statistical evaluation of the frequency of pre-implantation loss and resorption among litters and fetal population was performed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea and implants, and litter size was evaluated using a nonparametric ANOVA followed by the Wilcoxon Rank-Sum test with Bonferroni's correction. Pregnancy rates were analyzed using the Fisher exact probability test.

l. Compliance Statements:

A signed Statement of No Confidentiality Claim was provided dated November 12, 1991.

A signed Statement of Compliance with EPA's GLP was provided that was dated November 18, 1991.

A signed Quality Assurance Statement was dated 11/11/91. This date conforms to the review of the study phases and the draft and the final reports.

A signed statement dated November 18, 1991 was provided, which indicated that the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to the study. This study neither reportedly met or exceeded any of these criteria.

III. RESULTS

Analysis of the Dosing Solutions

The mean concentrations of test article found were 95%, 96% and 91% of the nominal concentrations for the 13, 38 and 95 mg/kg/day doses, respectively. Homogeneity and stability analyses indicated that 2,4-D IPA was homogenous and stable in aqueous solution when stored for up to 29 days at room temperature.

1. Maternal Toxicity

a. Mortality & Pregnancy Status

Dose Level [mg/kg/day]	0	13	38	95
No. on Test	20	20	20	20
Aborted	0	0	1	0
Delivered Early	0	0	0	1
Moribund Sacrifice	0	0	0	4
Found Dead	0	0	2	3

No maternal mortality/morbidity or abortions occurred at 13 mg/kg/day.

At 38 mg/kg/day, the doe that aborted on Day 25 did not exhibit any adverse clinical signs of toxicity and necropsy did not identify the cause for abortion. Two does died at this dose [1 on Day 16 and the other on Day 24]; neither clinical signs nor gross pathology attributable to treatment was observed in the doe that died on Day 16 and the doe that died on Day 24 exhibited decreased feces, soft feces, myotonia, red urine and perineal soiling prior to death and watery intestinal contents, a gastric hair ball, perineal soiling and congested lungs at necropsy.

At 95 mg/kg/day, the doe that delivered early on Day 28 [prior to necropsy] had three partially cannibalized fetuses, one late resorption, and a fourth fetus in utero. The four does that were sacrificed moribund and the three does that died at this dose exhibited decreased feces, myotonia, lateral recumbency prior to death or euthanasia. Gross necropsy revealed gastric erosions/ulcers, gastric hemolyzed blood, gastric hairball, pale liver foci, and dark/bloody urine.

405

b. Clinical Signs

Treatment-related clinical signs of toxicity are tabulated below:

Dose Level [mg/kg/day]	**0**	**13**	**38**	**95**
No. on Test	**20**	**20**	**20**	**20**
Decreased feces in pan	1	2	5	14
Soft feces	0	0	1	0
Perineal soiling	0	0	1	1
Blood in urine	0	0	0	1
Lateral recumbency	0	0	0	7
Myotonia	0	0	1	8
Cold extremities	0	0	0	1

c. Body Weight Changes

No statistically significant differences in mean body weight were seen between the control and treated groups. The mean corrected bodyweight was 99%, 98%, and 99% of the control value for the low, mid, and high dose groups, respectively.

Dose mg/kg/day	Mean Body weight Gain [G]				
	Days 0-7	Days 7-10	Days 7-20	Days 20-28	Days 0-28
0	196.6	18.2	201.3	57.3	455.2
13	150.2	-18.4	128.9 64	127.9	406.9
38	200.9	-31.7	113.4 56	102.3	445.0
95	160.3 80	**-190.2***	**-90.3***	139.9	306.6

There was a dose-related decrease in maternal body weight gain during the dosing period. Although reductions in maternal body weight gain was noted at all dose levels during the initial phase of dosing [Days 7-10], the decrease reached statistical significance [p <0.05] only at 95 mg/kg/day at this interval. Similarly, decrease were noted in maternal body weight gain at all dose levels during Days 7-20; however, statistical significance was attained only at the high dose. Does gained weight during the post-dosing period [Days 20-28], and for the entire study period, the average maternal weight gains were 89%, 97% and 67% of the control value for the low, mid, and high dose groups.

d. Organ Weight

No treatment-related changes on final body weights or absolute or relative liver weights were observed at any dose level. Both the absolute and relative kidney weights were increased at all dose levels with the increase in relative weights reaching statistical significance [p <0.5] at 38 and 95 mg/kg/day.

	Kidney weights	
Dose [mg/kg/day]	Absolute [g]	Relative [g/100 g]
0	16.24 ± 2.36	0.38 ± 0.04
13	17.64 ± 2.80	0.41 ± 0.04
38	18.38 ± 2.67	0.43 ± 0.06*
95	18.37 ± 2.74	0.42 ± 0.04*

e. Macroscopical Examination

No treatment-related macroscopical changes were observed in the dams sacrificed at termination. Gross pathological alterations observed in the does that died or were sacrificed moribund are discussed under Mortality [see Page 9].

2. Developmental Toxicity

Reproduction data are presented in Table 1. No biologically or statistically significant effects were seen on pregnancy rate, number of corpora lutea, number of implantations, total live fetuses per litter, resorption rate, number and percent of litters with resorptions, fetal sex ratio, fetal body weights, or gravid uterine weights at any dose level. The incidences were reported to be within the historical control ranges of the testing laboratory. This was substantiated with historical control data from the testing laboratory.

Table 1. Cesarean Section Observations

Observations [Mean ± S.D]	Dose Level [mg/kg/day]			
	0	13	38	95
No. Assigned	20	20	20	20
Females Gravid	20	16	19	20
Maternal Wastage				
# Died	0	0	2	3
# Sacrificed	0	0	0	4
# Aborted	0	0	1	0
# Early delivery	0	0	0	1
# Non pregnant	0	4	1	0
Total Corpora Lutea	204	159	173	125
Corpora Lutea/Dam	10.2±3.0	9.9±1.9	10.8±3.1	9.6±1.7
Total Implantation	144	113	129	104
Implantation/Dam	7.2±2.0	7.1±2.4	8.1±3.5	8.0±2.4
Total Live Fetuses	132	106	122	101
Live Fetuses/Litter	6.6±2.2	6.6±2.2	7.6±3.3	7.1±1.4
Total Resorptions	12	7	7	3
Early	5	7	6	2
Late	7	0	1	1
Resorption/Dam	0.6±1.1	0.4±0.6	0.4±0.6	0.2±0.4
No. and % of Litters with Resorptions	6/20 30.0	6/16 37.5	6/16 37.5	3/13 23.1
Pre Implantation Loss [%]	27.0	29.2	26.9	16.7
Post Implantation Loss [%]	8.3	6.2	5.4	2.9
Gravid Uterus Weight [g]	427±106	425±120	430±144	477±110
Sex Ratio ♂ / ♀	52/48	55/45	50/50	58/42
Fetal Weight [g]	38.2±3.1	40.4±4.7	37.2± 7.2	38.0±4.8

Fetal malformations and variations summarized in Table 10 of the study report are appended to this DER. No treatment-related or statistically significant external, visceral, or skeletal malformations or variations were seen in any of the fetuses. A total of 26 fetuses were malformed; 9 of 132 [6.8%] fetuses at 0 mg/kg/day, 2 of 106 [1.9%] at 13 mg/kg/day, 9 of 122 [7.4%] at 38 mg/kg/day, and 6 of 98 [6.1%] at 95 mg/kg/day. The malformations occurred at low frequencies, occurred at incidences similar to the concurrent control or were within the incidence range for historical controls. This was substantiated with historical control data from the testing laboratory.

IV. DISCUSSION

Inseminated rabbits were given oral administration of Isopropylamine Salt of 2,4-D at 0, 13, 38, or 95 mg/kg/day during days 7 through 19 of gestation. These dose levels correspond to approximately 10, 30 or 75 mg/kg/day of 2,4-D Acid.

2,4-D IPA at 13 mg/kg/day did not induce maternal toxicity. At 38 and 95 mg/kg/day maternal toxicity was manifested by mortality/morbidity, clinical signs of toxicity characterized by decreased feces, myotonia, lateral recumbency, decreases in body weight gain during the dosing period, and increases in relative kidney weights. No treatment-related effects were observed in reproductive parameters at any dose level. No treatment-related external, visceral, or skeletal malformation or variations were seen in any of the fetuses of treated does.

In a developmental toxicity study [MRID No. 415271-03] with rats, pregnant Sprague-Dawley rats were given oral administration of 2,4-IPA [same lot number and purity [50.2%] as the rabbit study] at 0, 22, 65, or 190 mg/kg/day during days 6 - 15 of gestation. These dose levels corresponded to approximately 0, 17, 51 or 150 mg/kg/day of 2,4-D Acid. For maternal toxicity the NOEL was 65 mg/kg/day and the LOEL based on decreases in body weight gain and food consumption was 190 mg/kg/day. For developmental toxicity the NOEL was 65 mg/kg/day and the LOEL, based on mild skeletal variations, was 190 mg/kg/day.

V. CONCLUSION

2,4-D-IPA was maternally toxic to rabbits at doses of 38 and 95 mg/kg/day; no maternal toxicity was seen at 13 mg/kg/day. The compound did not induce developmental toxicity at 95 mg/kg/day, the highest dose tested. Based on the results of this study, the following NOELs and LOELs are established.

Maternal Toxicity

NOEL: 13 mg/kg/day
LOEL: 38 mg/kg/day

Developmental Toxicity:

NOEL: 95 mg/kg/day [HDT]
LOEL: Not Achieved

VI. CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

TABLE 10

ISOPROPYLAMINE SALT OF 2,4-D: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	13	38	95
		NUMBER OF FETUSES (NUMBER OF LITTERS) EXAMINED			
EXTERNAL EXAMINATION		132 (20)	106 (16)	122 (16)	98 (13)[a]
VISCERAL EXAMINATION		132 (20)	106 (16)	122 (16)	98 (13)
SKELETAL EXAMINATION		132 (20)	106 (16)	122 (16)	97 (12)
		PERCENT AFFECTED (NUMBER AFFECTED)			
EXTERNAL OBSERVATIONS					
EDEMA/HEMORRHAGE - ABDOMEN†	F[b]	0.8(1)[c]	0	0	0
	L	5.0(1)	0	0	0
FORELIMB FLEXURE	F	0.8(1)	0	0	0
	L	5.0(1)	0	0	0
ROTATED LIMB†	F	0.8(1)[d]	0	0	0
	L	5.0(1)	0	0	0
VISCERAL OBSERVATIONS					
DILATED CEREBRAL VENTICLES†	F	2.3(3)[c,e]	0	0	0
	1	5.0(1)	0	0	0
HYDROCEPHALY/ANECEPHALLY†	F	0.8(1)[d]	0	0	0
	1	5.0(1)	0	0	0

(16)

TABLE 10 (CONTINUED)

ISOPROPYLAMINE SALT OF 2,4-D: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	13	38	95
		PERCENT AFFECTED (NUMBER AFFECTED)			
VISCERAL OBSERVATIONS (CONTINUED)					
PERSISTENT TRUNCUS ARTERIOSIS[+]	F	0.8(1)[f]	0	0	0
	L	5.0(1)	0	0	0
VENTRICULAR SEPTAL DEFECT[+]	F	0.8(1)[f]	0	0	0
	L	5.0(1)	0	0	0
MISSING CAUDAL LUNG LOBE[+]	F	1.5(2)	1.9(2)	4.9(6)	4.1(4)
	L	10.0(2)	12.5(2)	31.3(5)	15.4(2)
RETROCAVAL URETER	F	0.8(1)	0	0	1.0(1)
	L	5.0(1)	0	0	7.7(1)
HEMORRHAGIC RENAL PELVIS	F	1.5(2)[c,e]	0	0	0
	L	5.0(1)	0	0	0
DIAPHRAGMATIC HERNIA[+]	F	0	0	0.8(1)	0
	L	0	0	6.3(1)	0
MULTIPLE[+,h]	F	0.8(1)[c]	0	0	0
	L	5.0(1)	0	0	0

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 420 of 959

TABLE 10 (CONTINUED)

ISOPROPYLAMINE SALT OF 2,4-D: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	13	38	95
		PERCENT AFFECTED (NUMBER AFFECTED)			
VISCERAL OBSERVATIONS (CONTINUED)					
FALLOPIAN CYSTS[1]	F	0	0	0	2.4 (1)
	L	0	0	0	10.0(1)
SKELETAL OBSERVATIONS					
INTERPARIETAL					
-DELAYED OSSIFICATION	F	0.8(1)	0	0	0
	L	5.0(1)	0	0	0
HYOID					
-DELAYED OSSIFICATION[1]	F	18.9(25)	13.2(14)	25.4(31)	12.5(12)
	L	65.0(13)	43.8(7)	56.3(9)	58.3(7)
-CROOKED	F	1.5(2)	0.9(1)	0	1.0(1)
	L	10.0(2)	6.3(1)	0	8.3(1)
VERTEBRAE					
DENTOID PROCESS					
- DELAYED OSSIFICATION	F	0.8(1)	0	0	0
	L	5.0(1)	0	0	0
-HEMIVERTEBRA[+]	F	1.5(2)	0	0	2.1(2)
	L	10.0(2)	0	0	16.7(2)

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 421 of 959

TABLE 10 (CONTINUED)

ISOPROPYLAMINE SALT OF 2,4-D: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	13	38	95
		PERCENT AFFECTED (NUMBER AFFECTED)			
SKELETAL OBSERVATIONS (CONTINUED)					
VERTEBRAE (CONTINUED)					
-MISSING[+]	F	0	0	0.8(1)[g]	0
	L	0	0	6.3(1)	0
CENTRA					
-DELAYED OSSIFICATION	F	0.8(1)	0	0.8(1)	0
	L	5.0(1)	0	6.3(1)	0
-FUSED	F	0.8(1)	0	0	0
	L	5.0(1)	0	0	0
RIBS					
-FUSED[+]	F	0	0	1.6(2)[g]	0
	L	0	0	12.5(2)	0
-FORKED[+]	F	0	0	0.8(1)[g]	0
	L	0	0	6.3(1)	0
-MISSING[+]	F	0.8(1)	0	0.8(1)[g]	0
	L	5.0(1)	0	6.3(1)	0
SPURS					
-CERVICAL	F	0	0.9(1)	0	0
	L	0	6.3(3)	0	0

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 422 of 959

TABLE 10 (CONTINUED)

ISOPROPYLAMINE SALT OF 2,4-D: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	13	38	95
		PERCENT AFFECTED (NUMBER AFFECTED)			
SKELETAL OBSERVATIONS (CONTINUED)					
SPURS (CONTINUED)					
-LUMBAR	F	31.8(42)	28.3(30)	15.6(19)	21.6(21)
	L	80.0(16)	68.8(11)	62.5(10)	83.3(10)
STERNEBRAE					
-DELAYED OSSIFICATION	F	29.5(39)	30.2(32)	36.1(44)	11.3(11)
	L	80.0(16)	68.8(11)	87.4(14)	58.3(7)
-EXTRA SITE OF OSSIFICATION	F	0.8(1)	0	0	0
	L	5.0(1)	0	0	0
-FUSED	F	0.8(1)	0	0	0
	L	5.0(1)	0	0	0
-IRREGULAR PATTERN OF OSSIFICATION	F	0.8(1)	0	0	0
	L	5.0(1)	0	0	0
TOTAL FETUSES MALFORMED		6.8(9)	1.9(2)	7.4(9)	6.1(6)
TOTAL LITTERS WITH MALFORMED FETUSES		30.0(6)	12.5(2)	43.8(7)	30.8(4)

† CONSIDERED TO BE A MALFORMATION

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 423 of 959

TABLE 10 (CONTINUED)

ISOPROPYLAMINE SALT OF 2,4-D: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

a ONE DAM STARTED TO DELIVER HER LITTER EARLY ON GESTATION DAY 28; THREE FETUSES WERE DELIVERED IN THE CAGE AND WERE FOUND PARTIALLY CANNIBALIZED. A FOURTH FETUS WAS RECOVERED IN UTERO. ALL FETUSES WERE EXAMINED EXTERNALLY AND VISCERALLY TO THE EXTENT POSSIBLE; HOWEVER, THEY WERE INADVERTENTLY NOT SAVED FOR SKELETAL EXAMINATION. ONLY THE ONE PUP RECOVERED IN UTERO WAS INCLUDED IN THE EXTERNAL AND VISCERAL STATISTICAL ANALYSES AS A COMPLETE EXTERNAL AND VISCERAL EXAMINATION COULD NOT BE CONDUCTED ON THE CANNIBALIZED FETUSES.

b F=FETUSES; L=LITTERS

c-g MALFORMATIONS IDENTIFIED BY THE SAME SUPERSCRIPT WERE OBSERVED IN THE SAME FETUS.

h ONE FETUS HAD MULTIPLE MALFORMATIONS THAT INCLUDED DILATED CEREBRAL VENTRICLES, EDEMA WITHIN THE ABDOMINAL CAVITY AND HEMORRHAGE WITHIN THE LUNGS, SPLEEN, RENAL PELVIS, RIBS AND TONGUE.

i ANALYSES BASED ON FEMALE FETUSES ONLY.

j THE HYOID FROM ONE FETUS IN THE 95 MG/KG/DAY DOSE GROUP WAS DAMAGED IN PROCESSING AND COULD NOT BE READ FOR THE DEGREE OF OSSIFICATION.

THERE WERE NO STATISTICALLY IDENTIFIED DIFFERENCES FROM THE CONTROL VALUE BY THE CENSORED WILCOXON TEST, α=0.05.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 424 of 959

[2,4-D TRIISOPROPANOLAMINE SALT] Developmental Toxicity Study - Rabbit [§83-3(b)/OPPTS 870.3700]

Amendment to DER for MRID 42158705/ Developmental Toxicity Study - Rabbit [2,4-D TIPA]
/42158701/

This amendment provides an EXECUTIVE SUMMARY and a data table to upgrade the original DER [Document No. 009666].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/20/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P. H. Virginia A Dobozy 12/21/00
Reregistration Branch I, Health Effects Division (7509C)
TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rabbit §83-3(b)/OPPTS 870.3700
P.C. CODE: 030035
CASWELL NO.: 315AE CAS #: 18584-79-7
TEST MATERIAL (PURITY): 2,4-D triisopropanolamine salt [73.1% (acid equivalent 39.2%)]
SYNONYMS: dichlorophenoxyacetic acid, triisopropanolamine salt; 2,4-D TIPA
SPONSOR: DowElanco

CITATION: Liberacki, A. B.; Yano, B. L.; and Breslin, W. J. (1991). Triisopropanolamine Salt of 2,4-D: Oral Gavage Teratology Study in New Zealand White Rabbits. Toxicology Research Laboratory, Michigan. Study No. K-008866-016, November 18, 1991. MRID 42158705; Document No. 009666. Unpublished. Oral Gavage Teratology Probe Study in New Zealand White Rabbits. Study No. K-008866-014, 11/7/91. MRID 42158701, Document No. 009666.

+42158701

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 42158705], artificially-inseminated female New Zealand White rabbits [18/group] were administered 2,4-D triisopropanolamine salt [73.1% (acid equivalent 39.2%)] at dose levels of 0 [deionized distilled water control)], 19 mg/kg/day, 56 mg/kg/day, and 140 mg/kg/day [corresponding to approximately 10, 30, and 75 mg/kg/day of 2,4-D acid] from gestation day 7 through gestation day 19.

There were no abortions. One control doe delivered early [day 28]. One mid-dose doe died on day 18, and three high-dose does [days 12, 14, and 15] were sacrificed moribund. Clinical signs of toxicity [myotonia, lateral recumbency, perineal soiling, blood in urine] were observed in the mid- and high-dose does.

In general, body weight was comparable among the control, low- , and mid-dose does. Decreased body weight was observed throughout the dosing period at the high-dose level [94%-96% of control]. During the initial days of dosing [days 7-10], negative body-weight gains were observed at the mid- [-8.5 grams] and high-[-125.3 grams] dose levels, and the low-dose group displayed gains that were 39% of the control value. The high-dose does displayed a significant decrease in body-weight gain throughout the dosing period [40% of control], and the mid- [54% of control] and low- [81% of control] dose groups displayed non-significant decreases during this period compared to the control. The overall [days 0-28] body-weight gain at the mid- [77% of control] and high-dose [81% of control] levels was reduced compared to the control, but there was no dose response, and statistical significance was not attained. Corrected [for uterine weight] body weight was comparable among the groups.

[2,4-D TRIISOPROPANOLAMINE SALT] Developmental Toxicity Study - Rabbit [§83-3(b)/OPPTS 870.3700]

There were no treatment-related effects on pregnancy rate, numbers of corpora lutea, implantations, and total live fetuses, resorption rate, number and percent of litters with resorptions, fetal sex ratio, fetal body weight, or gravid uterine weights. Dead fetuses were not reported at any dose level, and there were no litters that were totally resorbed. There were no apparent, treatment-related, effects on external, visceral, or skeletal variation/anomalies/ retardations/malformations at any dose level.

The maternal toxicity NOAEL is 19 mg/kg/day, based on mortality/morbidity, clinical signs, and decreased body-weight gain at the LOAEL of 56 mg/kg/day. The developmental toxicity NOAEL is 140 mg/kg/day, the highest dose tested.

This developmental toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [**§83-3(b)/OPPTS 870.3700**] for a developmental toxicity study in rabbits.

[2,4-D TRIISOPROPANOLAMINE SALT] Developmental Toxicity Study - Rabbit [§83-3(b)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Body-Weight Gain [grams]

Dose/Time	0 mg/kg/day	19 mg/kg/day	56 mg/kg/day	140 mg/kg/day
Body Weight [g]				
gestation day 0	3934.0±171.7	3862.4±157.9	3895.4±215.2	3880.2±199.1
gestation day 7	4163.5±193.9	4101.0±199.1	4109.1±206.0	4128.9±238.4
gestation day 10	4235.0±203.6	4128.9±216.8	4100.6±206.1	4003.6±268.5* [95]
gestation day 13	4280.4±197.0	4155.3±228.3	4126.3±219.8	4030.5±322.3* [94]
gestation day 20	4407.7±217.5	4297.9±288.8	4233.1±201.2	4218.4±316.7 [96]
gestation day 28	4459.4±221.6	4397.5±317.1	4290.2±313.4	4295.6±306.8 [96]
corrected	4067.5±281.4	3925.0±300.8	3875.3±281.1	3892.6±270.7 [96]
Body-Weight Gain [g]				
days 0-7	229.5±98.1	238.5±93.3	213.7±89.8	248.7±114.2
days 7-10	71.5±57.0	28.0±55.6 [39]√	-8.5±104.7*	-125.3±144.0*
days 10-13	45.4±44.9	26.4±37.6 [58]	25.7±46.1 [57]	19.8±135.6 [44]
days 13-16	46.9±88.3	79.6±67.0	40.8±95.5	97.5±79.6
days 16-20	80.4±81.8	63.0±67.8 [78]	19.2±94.0 [24]	36.8±137.0 [46]
days 20-28	51.7±108.9	99.6±193.4	57.1±238.3	77.1±186.0
days 7-20	244.2±98.3	196.9±158.7 [81]	132.0±90.0 [54]	98.9±189.2* [40]
days 0-28	525.4±181.9	535.1±220.6	403.9±252.3 [77]	425.1±261.5 [81]

√ [% of control]; * $p<0.05$; data from Tables 5-6, pages 27-28 of the report

418

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PRIMARY REVIEWER: Jess Rowland, Toxicologist Jess Rowland 8/3/92
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 8/4/92
Section II, Toxicology Branch II

DATA EVALUATION REPORT

3. TIPA---PROBE STUDY

STUDY TYPE: Developmental Toxicity **[Probe Study]** **GUIDELINE: N/A**

CASWELL NO. 315 AE **MRID No. 421587-01** **HED PROJECT No. 2-1087**

TEST MATERIAL: TRIISOPROPANOLAMINE Salt of 2,4-D ACID **[2,4-D TIPA]**

REGISTRANT: DowElanco, Indianapolis, IN.

TESTING LABORATORY: The Toxicology Research Laboratory, Michigan

STUDY IDENTIFICATION: K-008866-014

TITLE OF REPORT: *TRIISOPROPANOLAMINE SALT OF 2,4-D: ORAL GAVAGE TERATOLOGY PROBE STUDY IN NEW ZEALAND WHITE RABBITS.*

AUTHOR(S): A.B. Liberacki, W.J. Breslin and B.L. Yano

REPORT DATE: November 7, 1991

SUMMARY: Impregnated New Zealand White rabbits were given oral administration of **Technical 2,4-D TIPA [73.1%]** at doses of 0, 95, 185, or 280 mg/kg/day during days 7 through 19 of gestation. These dose levels correspond to approximately 0, 50, 100 or 150 mg/kg/day 2,4-D acid. Does were sacrificed on gestation day 20. 2,4-D TIPA induced dose-related maternal toxicity which included mortality/morbidity [185 and 280 mg/kg/day]; clinical signs of toxicity [all dose levels]; decreases in mean body weight and body weight gain [185 and 280 mg/kg/day]; and degenerative kidney tubular lesions [all dose levels]. Treatment had no adverse effects at any dose level on pregnancy rate, pre- and post-implantation losses, the number of implantations, the number of resorptions or litter size. No fetal data were reported.

MATERNAL TOXICITY NOEL = Not achieved
LOEL = 95 mg/kg/day [LDT]

DEVELOPMENTAL TOXICITY NOEL = 280 mg/kg/day [HDT]
LOEL = Not achieved

CORE CLASSIFICATION: Not applicable; range-finding study.

419

1. OBJECTIVE

The objective of this probe [range-finding] study, was to establish appropriate dose levels of the Triisopropanolamine Salt of 2,4-D for the main study.

2. PROTOCOL

Groups of seven inseminated female New Zealand White rabbits [approximately 7 months of age and 3.5 to 4.0 kg] were given oral administrations of **Technical 2,4-D TIPA (73.1% pure;** Lot No. AGR276428) at doses of 0, 95, 185, or 280 mg/kg/day in deionized distilled water [1 mL/kg] daily, during days 7 through 19 of gestation. These dose levels correspond to approximately 0, 50, 100 or 150 mg/kg/day of 2,4-D Acid. Concentration, homogeneity and stability of the test article/vehicle mixtures were determined prior to the initiation of the study.

Animals were observed for clinical signs of toxicity daily, dams were weighed on gestation days 0, daily during the dosing period, and on day 20 of gestation. Dams in each group were sacrificed on day 20 and postmortem examination included macroscopic examination of internal organs, with emphasis on the uterus, uterine contents, position of each fetus in the uterus, and corpora lutea counts. In addition, on day 20, at necropsy, liver and kidney weights were recorded. Fetal examinations were not performed.

3. RESULTS

i. Analysis of dosing solution

The mean concentrations found were 99%, 105%, and 100% of the nominal concentration for the 95, 185 and 280 mg/kg/day dose groups, respectively. The 2,4-D TIPA was homogeneous and stable in deionized distilled water for up to 45 days post-preparation.

ii. Maternal Toxicity

- 95 mg/kg/day: No mortality

  185 mg/kg/day: 1 doe sacrificed moribund on gestation Day 16

  280 mg/kg/day: Mortality occurred in 6 does [1 doe each was found dead on gestation Days 9, 19 and 20; 1 doe each was sacrificed moribund on gestation Days 10, 10 and 12].

470

- Prior to death or sacrifice, does exhibited myotonia, decreased feces, and/or transient lateral recumbency, and weight loss.

- Clinical sign observed in surviving animals at 185 mg/kg/day was limited to decreased amounts of feces. At 95 mg/kg/day, one doe exhibited myotonia, decreased amounts of feces, transient lateral recumbency, and significant body weight loss [-714 g] during the treatment period.

- Mean body weights were decreased in does at 185 and 280 mg/kg/day.

- Mean body weight gains were decreased in a dose-related manner on gestation Days 7-10, and at 185 mg/kg/day throughout the treatment period. Body weight gain of does at the high dose did not provide meaningful results due to high mortality/morbidity.

- Both the absolute and relative kidney weights were increased in one of two surviving does at 185 mg/kg/day.

- Gross pathological alterations seen in rabbits found dead or sacrificed moribund at 280 mg/kg/day included: dry cecal ingesta; dark urine, a gastric hair ball; distended gall bladder with or without erosions/ulcers and pale liver foci which were considered secondary to anorexia or debilitation. At termination, a few rabbits at 95 and 185 mg/kg/day exhibited gastric hyperemia and erosions and/or ulcers of the stomach.

- Histopathology revealed degenerative kidney tubular lesions in 1/6 rabbits at 95 mg/kg/day, 2/6 at 185 mg/kg/day, and 4/6 at 280 mg/kg/day.

- The pregnancy rate was 83.3%, 100%, 50%, and 100% at 0, 95, 185 and 280 mg/kg/day dose groups, respectively.

- Treatment had no effect on corpora lutea, implantation sites, pre-and post-implantation losses, litter size or viable fetuses at any dose level.

iii. Developmental Toxicity

No data were reported for fetal examinations.

4. CONCLUSION:

MATERNAL TOXICITY:
NOEL = Not achieved
LOEL = 95 mg/kg/day [LDT]

DEVELOPMENTAL TOXICITY:
NOEL = 280 mg/kg/day [HDT]
LOEL = Not achieved

PRIMARY REVIEWER: Jess Rowland, Toxicologist Jess Rowland 8/3/92
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 8/4/92
Section II, Toxicology Branch II

DATA EVALUATION REPORT

4. TIPA---MAIN STUDY

STUDY TYPE: Developmental Toxicity **[Main Study] GUIDELINE:** 83-3(b)

CASWELL NO. 315 AE MRID No. 421587-05 HED PROJECT No. 2-1087

TEST MATERIAL: Triisopropanolamine Salt of 2,4-D **[2,4-D TIPA]**

REGISTRANT: DowElanco **STUDY IDENTIFICATION:** K-008866-016

TESTING LABORATORY: Toxicology Research Laboratory, Michigan.

TITLE OF REPORT: *TRIISOPROPANOLAMINE SALT OF 2,4-D: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS.*

AUTHORS: A. B. Liberacki, B.L. Yano and W.J. Breslin

REPORT DATE: November 18, 1991

SUMMARY: Groups of 18 inseminated New Zealand White rabbits were given oral doses of **2,4-D TIPA [73.1% pure]** at 0, 19, 56, or 140 mg/kg/day during days 7 through 19 of gestation. These dose levels correspond to approximately 0, 10, 30 or 75 mg/kg/day of 2,4-D acid. No maternal toxicity was observed at 19 mg/kg/day. At 56 and 140 mg/kg/day, maternal toxicity was manifested by mortality/ morbidity, clinical signs, and decreases in body weight gain. Treatment had no effect on the pregnancy rate, number of implantations, total live fetuses, resorption rate, pre- and post-implantion losses, fetal sex ratio or fetal body weights. No treatment-related external, visceral, or skeletal malformation or variation was seen in any of the fetuses.

MATERNAL TOXICITY:

NOEL = 19 mg/kg/day
LOEL = 56 mg/kg/day

DEVELOPMENTAL TOXICITY

NOEL = 140 mg/kg/day [HDT].
LOEL = Not achieved.

<u>CORE CLASSIFICATION</u>: **Guideline;** this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

1287

I. OBJECTIVE

The objective of this study was to assess the effects of the **Triisopropanolamine Salt of 2,4-D [2,4-D TIPA]** on the embryonic and fetal development following oral administration to rabbits during the period of organogenesis.

II. MATERIALS AND METHODS

a. Test Material

Identity: Triisopropanolamine Salt of 2,4-D [Technical]
Batch No.: AGR 276428
Purity: 73.1% [39.2% as Acid equivalent]
Description: Liquid

b. Test Animals

Species/Sex: Female rabbits
Strain: New Zealand White
Age on Gestation Day 0: 6.5 to 7.5 months
Weight on Gestation Day 0: 3.25 - 4.25 kg
Identification: Ear tags.
Acclimation Period: Two weeks.
Housing: Individually in stainless steel cages
Food: Purina Certified Rabbit Chow #5322 ad libitum.
Water: Tap water ad libitum
Environment: Temperature and humidity controlled rooms.

Group Assignment: 18 inseminated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

Adult females were artificially inseminated with the day of insemination considered Day 0 of gestation. Immediately following insemination, does received an intravenous injection of 100 IU of human chorionic gonadotropin .

d. Preparation of Dosing Solutions

2,4-D TIPA was administered in deionized distilled water such that a dose volume of 1 mL/kg body weight yielded the

appropriate dose. Dose solutions were prepared once prior to the start of the study.

e. Analysis of the Dosing Solutions

Concentration [pre- and post-dosing], homogeneity [pre-dosing], and stability [Days 3, 13, 21 and 31] analysis of the dosing solutions were determined during the study.

f. Administration of Test Article

The test article was administered daily orally via gavage at doses of 0, 19, 56, or 140 mg/kg/day during days 7 through 19 of gestation. These dose levels correspond to approximately 0, 10, 30, or 75 mg/kg/day of 2,4-D acid. The control group received the vehicle [deionized distilled water only. All groups received a dosing volume of 1 mL/kg body weight and the dose volumes were adjusted daily based on individual body weights.

g. Observations

All animals were observed once daily for clinical signs of toxicity including physical or behavioral abnormalities. Individual body weights were obtained on day 0, daily during the dosing period, and on days 20 and 28 of gestation. Individual food consumptions were **not** measured in this study.

h. Termination

Any animal which died, appeared moribund or showed indications of early termination of pregnancy was submitted for complete necropsy. All surviving does were sacrificed on gestation day 28, obvious gross pathologic alterations were recorded, and the liver, kidneys and gravid uterus were weighed.

i. Cesarean Section

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 rabbits were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantation, early and late resorption, and live and dead fetuses were recorded.

j. Fetal Examinations

Each fetus was removed from the uterus and individually 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.

k. Statistical Analysis

Maternal body weights, body weight gains, fetal body weights, and organ weights were evaluated by Bartlett's test for equality of variances followed by a parametric or nonparametric analysis of variance [ANOVA]. If the ANOVA was significant, analysis by Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction was performed, respectively. Statistical evaluation of the frequency of pre-implantation loss and resorption among litters and fetal population was performed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea and implants, and litter size was evaluated using a nonparametric ANOVA followed by the Wilcoxon Rank-Sum test with Bonferroni's correction. Pregnancy rates were analyzed using the Fisher exact probability test.

l. Compliance Statements:

A signed Statement of No Confidentiality Claim was provided dated November 12, 1991.

A signed Statement of Compliance with EPA's GLP was provided that was dated November 18, 1991.

A signed Quality Assurance Statement was dated 11/11/91. This date conforms to the review of the study phases and the draft and the final reports.

A signed statement dated November 18, 1991 was provided, which indicated that the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to the study. This study neither reportedly met or exceeded any of these criteria.

III. RESULTS

Analysis of the Dosing Solutions

The mean concentrations of test article found were 97%, 96% and 93% pre-dosing and 94%, 98%, and 98% post-dosing of the nominal concentrations for the 19, 56 and 140 mg/kg/day doses, respectively. Homogeneity and stability analyses indicated that 2,4-D IPA was homogenous and stable in aqueous solution when stored for up to 31 days at room temperature.

1. Maternal Toxicity

a. Mortality & Pregnancy Status

Dose Level [mg/kg/day]	0	19	56	140
No. on Test	18	18	18	18
Aborted	0	0	0	0
Delivered Early	1	0	0	0
Moribund Sacrifice	0	0	0	3
Found Dead	0	0	1	0

The doe which delivered early on Day 28 of gestation showed no gross pathological alterations.

No maternal mortality/morbidity or abortions occurred at 19 mg/kg/day.

The doe that died at 56 mg/kg/day on Day 18 and the three does that were sacrificed moribund at 140 mg/kg/day on gestations Days 12, 14 and 15 exhibited myotonia and/or transient lateral recumbency, decreased amounts of feces, perineal soiling and significant weight loss prior to death or sacrifice. Necropsy revealed pale kidneys in the doe that died at 56 mg/kg/day, dosing-error related lesions in the lung in the doe sacrificed on Day 15, and decreased/ dry ingesta, watery stomach contents, dark urine, pale kidneys and pale liver foci in the doe sacrificed on Day 14. In addition, two rabbits at this level also exhibited erosions and/or ulcers of the stomach and dark stomach contents.

427

b. Clinical Signs

Treatment-related clinical signs of toxicity are tabulated below:

Dose Level [mg/kg/day]	**0**	**19**	**56**	**140**
No. on Test	**18**	**18**	**18**	**18**
Decreased feces in pan	5	4	8	8
Perineal soiling	0	0	0	3
Urine discoloration - red	0	0	0	2
Blood in thickened urine	0	0	1	0
Lateral recumbency	0	0	1	1
Myotonia	0	0	1	3

c. Body Weight Changes

No statistically significant differences in mean body weight were seen between does the control groups and the does in 19 or 56 mg/kg/day. The mean body weights of dams at 140 mg/kg/day were lower throughout the treatment period, with the decrease reaching statistical significance [p <0.05] on gestation Days 10 and 13. The mean corrected bodyweight was 96.5%, 95.2%, and 95.6% of the control value for the low, mid, and high dose groups, respectively.

Dose mg/kg/day	Mean Body Weight Gain [G]				
	Days 0-7	Days 7-10	Days 7-20	Days 20-28	Days 0-28
0	229.5	71.5	244.2	51.7	525.4
19	238.5	28.0	196.9	99.6	535.1
56	213.7	**-8.5***	132.0	57.1	403.9
140	248.7	**-125.3***	**98.9***	77.1	425.1

A dose-related decrease in maternal body weight gain was seen during the dosing period with the decrease reaching statistical significance at 56 mg/kg/day and 140 mg/kg/day during the initial phase of dosing [Days 7-10]. Similarly, decrease were noted in maternal body weight gain at all dose levels during Days 7-20; however, statistical significance was attained only at the high dose. Does gained weight during the post-dosing period [Days 20-28], and for the entire study period, the average maternal weight gains were 101.9%, 76.8% and 80.9% of the control value for the low, mid, and high dose groups.

d. Organ Weight

No treatment-related changes on final body weights, absolute/relative liver weights or absolute/relative kidney weights were observed at any dose level. The statistical significant decrease in both absolute and relative liver weights at 19 mg/kg/day was not considered to be treatment related due to the lack of effect at the higher doses.

e. Macroscopical Examination

No treatment-related macroscopical changes were observed in the dams sacrificed at termination. Gross pathological alterations observed in the does that died or were sacrificed moribund are discussed under Mortality [see Page 9].

2. Developmental Toxicity

Reproduction data are presented in Table 1. No biologically or statistically significant effects were seen on pregnancy rate, number of corpora lutea, number of implantations, total live fetuses per litter, resorption rate, number and percent of litters with resorptions, pre- and post implantation loses, fetal sex ratio, fetal body weights, or gravid uterine weights at any dose level.

Table 1. Cesarean Section Observations

Observations [Mean ± S.D]	Dose Level [mg/kg/day] 0	19	56	140
No. Assigned	18	18	18	18
Females Gravid	14	16	16	18
Maternal Wastage				
# Died	0	0	1	0
# Sacrificed	0	0	0	3
# Aborted	0	0	0	0
# Early delivery	1	0	0	0
# Non pregnant	4	2	2	0
Total Corpora Lutea	126	157	124	153
Corpora Lutea/Dam	9.7±2.1	9.8±2.4	9.5±1.9	10.2±3.7
Total Implantation	85	135	96	109
Implantation/Dam	6.5±2.8	8.4±2.4	6.4±2.8	7.1±1.7
Total Live Fetuses	83	121	91	101
Live Fetuses/Litter	6.4±2.8	7.6±2.4	6.1±3.0	6.7±1.5
Total Resorptions	2	14	5	6
Early	1	7	5	5
Late	1	7	0	1
Resorption/Dam	0.2±0.4	0.9±1.6	0.3±0.5	0.4±1.1
No. and % of Litters with Resorptions	2/13 15.4	5/16 31.3	5/15 33.3	3/15 20.0
Pre Implantation Loss [%]	30.8	14.0	23.6	25.0
Post Implantation Loss [%]	2.4	9.6	5.2	5.6
Gravid Uterus Weight [g]	392±121	472±125	415±104	403±71
Sex Ratio ♂ / ♀	46 38/45	49 59/62	39/52	55/46
Fetal Weight [g]	40.3±7.1	38.2±4.1	37.6± 5.3	37.9±6.6

Fetal malformations and variations summarized in Table 10 of the study report are appended to this DER. No treatment-related or statistically significant external, visceral, or skeletal malformations or variations were seen in any of the fetuses. A total of 29 fetuses were malformed; 9 of 83 [10.8%] fetuses at 0 mg/kg/day, 10 of 121 [8.3%] at 19 mg/kg/day, 8 of 91 [8.8%] at 56 mg/kg/day, and 2 of 101 [2%] at 140 mg/kg/day. The malformations occurred at low frequencies, occurred at incidences similar to the concurrent controls or were within the incidence range for historical controls. This was substantiated with historical control data from the testing laboratory.

IV. DISCUSSION

Inseminated rabbits were given oral administration of Triisopropanolamine Salt of 2,4-D at 0, 19, 56, or 140 mg/kg/day during days 7 through 19 of gestation. These dose levels correspond to 10, 30 or 75 mg/kg/day of 2,4-D Acid.

2,4-D TIPA at 19 mg/kg/day did not induce maternal toxicity. At 56 and 140 mg/kg/day maternal toxicity was manifested by mortality/morbidity, clinical signs of toxicity characterized by decreased feces, myotonia and/or lateral recumbency, and decreases in body weight gain during the dosing period. No treatment related effects were observed in reproductive parameters at any dose level. No treatment-related external, visceral, or skeletal malformation or variation was seen in any of the fetuses of treated does.

In a developmental toxicity study [MRID No. 415271-02] with rats, pregnant Sprague-Dawley rats were given oral doses of 2,4-TIPA [72.2%] at 0, 32.5, 100, or 325 mg/kg/day during days 6 - 15 of gestation. For maternal toxicity LOEL was 32.5 mg/kg/day based on decreases in body weight gain and food consumption; a NOEL was not achieved. For developmental toxicity the NOEL was 32.5 mg/k/day and the LOEL was 100 mg/kg/day based on skeletal malformations/variations.

5. CONCLUSION

2,4-D TPA was maternally toxic to rabbits at doses of 56 and 140 mg/kg/day but not at a dose of 19 mg/kg/day. The compound did not induce developmental toxicity at 140 mg/kg/day, the highest dose tested. Based on the results of this study, the following NOELs and LOELs are established.

Maternal Toxicity

NOEL: 19 mg/kg/day
LOEL: 56 mg/kg/day

Developmental Toxicity:

NOEL: 140 mg/kg/day [HDT]
LOEL: Not attained

6. CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

TABLE 10

2,4-D TIPA: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	19	56	140
		NUMBER OF FETUSES AFFECTED (NUMBER OF LITTERS) EXAMINED			
EXTERNAL EXAMINATION		83 (13)	121 (16)	91 (13)	101 (15)
VISCERAL EXAMINATION		83 (13)	121 (16)	91 (13)	101 (15)
SKELETAL EXAMINATION		83 (13)	121 (16)	91 (13)	101 (15)
		PERCENT AFFECTED (NUMBER AFFECTED)			
EXTERNAL OBSERVATIONS					
PETECHIAL HEMORRHAGE	F[a]	0	0.8(10)	0	0
	L	0	6.3(1)	0	0
VISCERAL OBSERVATIONS					
MULTIPLE CARDIAC[+,b]	F	1.2(1)	0	0	0
	L	7.7(1)	0	0	0
RIGHT SUBCLAVIAN ARISING FROM DESCENDING AORTA[+]	F	1.2(1)[c]	0	0	0
	L	7.7(1)	0	0	0
RETROESOPHAGEAL RIGHT SUBCLAVIAN ARTERY[+]	F	1.2(1)[c]	0	0.9(1)	0
	L	7.7(1)	0	7.7(1)	0
FUSED INNOMINATE AND CAROTID	F	1.2(1)	0	0.9(1)	0
	L	7.7(1)	0	7.7(1)	0

34

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 441 of 959

K1A TTERAT AM 688

TABLE 10 (CONTINUED)

2,4-D TIPA: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	19	56	140
		PERCENT AFFECTED (NUMBER AFFECTED)			
VISCERAL OBSERVATIONS CONTINUED					
MISSING CAUDAL LUNG LOBE†	F	2.4(2)	8.3(10)	4.4(4)	1.0(1)
	L	15.4(2)	31.3(5)	23.1(3)	6.7(1)
MISSING GALLBLADDER†	F	0	0	2.2(2)	0
	L	0	0	15.4(2)	0
HYPOPLASTIC GALLBLADDER	F	0	0	0	1.0(1)
	L	0	0	0	6.7(1)
RETROCAVAL URETER	F	3.6(3)	0.8(2)	0	2.0(2)
	L	23.1(3)	6.3(1)	0	6.7(1)
ECTOPIC KIDNEYS†	F	1.2(1)[d]	0	0	0
	L	7.7(1)	0	0	0
FUSED URETERS†	F	1.2(1)[d]	0	0	0
	L	7.7(1)	0	0	0
ACCESSORY ADRENAL GLAND	F	1.2(1)	0	0	0
	L	7.7(1)	0	0	0

35

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 442 of 959

KIR TIERRT AM 689

TABLE 10 (CONTINUED)

2,4-D TIPA: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	19	56	140
		PERCENT AFFECTED (NUMBER AFFECTED)			
SKELETAL OBSERVATIONS					
SKULL					
-DELAYED OSSIFICATION	F	4.8(4)	0	1.1(1)	5.0(5)
	L	30.8(4)	0	7.7(1)	20.0(3)
-FUSED BONES	F	0	0	1.1(1)	0
	L	0	0	7.7(1)	0
HYOID					
-DELAYED OSSIFICATION	F	43.4(36)	32.2(39)	40.7(37)	34.7(35)
	L	84.6(11)	81.3(13)	92.3(12)	93.3(14)
-CROOKED	F	8.4(7)	2.5(3)	6.6(6)	7.9(8)
	L	30.8(4)	12.5(2)	30.8(4)	26.7(4)
VERTEBRAE					
DENTOID PROCESS					
- DELAYED OSSIFICATION	F	0	0	1.1(1)	3.0(3)
	L	0	0	7.7(1)	13.3(2)
DENTOID PROCESS					
- IRREGULAR PATTERN OF	F	1.2(1)	0	0	0
OSSIFICATION	L	7.7(1)	0	0	0

36

KIA TTERAT AM 690

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 443 of 959

TABLE 10 (CONTINUED)

2,4-D TIPA: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	19	56	140
		PERCENT AFFECTED (NUMBER AFFECTED)			
SKELETAL OBSERVATIONS (CONTINUED)					
VERTEBRAE (CONTINUED)					
-HEMIVERTEBRA†	F	2.4(2)[e,f]	0	0	0
	L	15.4(2)	0	0	0
-FUSED†	F	0	0	1.1(1)	1.0(1)
	L	0	0	7.7(1)	6.7(1)
CENTRA					
-DELAYED OSSIFICATION	F	1.2(1)	0	0	0
	L	7.7(1)	0	0	0
-FUSED	F	1.2(1)	0	0	1.0(1)
	L	7.7(1)	0	0	6.7(1)
RIBS					
-FUSED†	F	2.4(2)[e,f]	0	0	0
	L	15.4(2)	0	0	0
-EXTRA (CERVICAL)†	F	1.2(1)[e]	0	0	0
	L	7.7(1)	0	0	0
-IRREGULAR PATTERN OF OSSIFICATION	F	0	0.8(1)	0	0
	L	0	6.3(1)	0	0

37

K1A TTERAT AM

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 444 of 959

TABLE 10 (CONTINUED)

2,4-D TIPA: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	19	56	140
		PERCENT AFFECTED (NUMBER AFFECTED)			
SKELETAL OBSERVATIONS (CONTINUED)					
SPURS					
-CERVICAL	F	2.4(2)	2.5(3)	1.1(1)	0
	L	15.4(2)	18.8(3)	7.7(1)	0
-LUMBAR	F	34.9(29)	47.1(57)	39.6(36)	43.6(44)
	L	92.3(12)	87.5(14)	92.3(12)	80.0(12)
-SACRAL	F	1.2(1)	0	1.1(1)	2.0(2)
	L	7.7(1)	0	7.7(1)	13.3(2)
STERNEBRAE					
-DELAYED OSSIFICATION	F	39.8(33)	48.8(59)	30.8(28)	28.7(29)
	L	84.6(11)	81.3(13)	84.6(11)	60.0(9)
-EXTRA SITE OF OSSIFICATION	F	0	0	1.1(1)	0
	L	0	0	7.1(1)	0
-FUSED	F	0	0.8(1)	1.1(1)	1.0(1)
	L	0	6.3(1)	7.1(1)	6.7(1)
-IRREGULAR PATTERN OF OSSIFICATION	F	1.2(1)	0.8(1)	2.2(2)	0
	L	7.7(1)	6.3(1)	15.4(2)	0
-EXTRA	F	0	0.8(1)	0	0
	L	0	6.3(1)	0	0

38

K1R TTERAT AM 692

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 445 of 959

TABLE 10 (CONTINUED)

2,4-D TIPA: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	19	56	140
		PERCENT AFFECTED (NUMBER AFFECTED)			
SKELETAL OBSERVATIONS (CONTINUED)					
PUBIS					
- DELAYED OSSIFICATION	F	0	0	0	4.0(4)
	L	0	0	0	6.7(1)

39

* CONSIDERED TO BE A MALFORMATION

a F=FETUSES; L=LITTERS

b MULTIPLE CARDIAC MALFORMATIONS ARE AS FOLLOWS: SINISTRAL ROTATION OF HEART, INTERRUPTION OF AORTIC ARCH, HYPERTROPHY OF RIGHT ATRIUM AND VENTRICLE AND MISSING DUCTUS ARTERIOSUS.

c,d,e,f MALFORMATIONS IDENTIFIED BY THE SAME SUPERSCRIPT WERE OBSERVED IN THE SAME FETUS.

THERE WERE NO STATISTICALLY IDENTIFIED DIFFERENCES FROM THE CONTROL VALUE BY THE CENSORED WILCOXON TEST, α=0.05.

[2,4-D-2-BUTOXYETHYL ESTER] Developmental Toxicity Study - Rabbit [§83-3(b)/OPPTS 870.3700]

Amendment to DER for MRID 42158706/42158703 - Developmental Toxicity Study - Rabbit [2,4-D BEE]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 009512].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/7/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P. H. Virginia A. Dobozy 12/11/00
Reregistration Branch I, Health Effects Division (7509C)
TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rabbit § 83-3(b)/OPPTS 870.3700
P.C. CODE: 030053 REREGISTRATION CASE NO.: 818618
CASWELL NO.: 315AI CAS #: 1929-73-3
TEST MATERIAL (PURITY): 2,4-D butoxyethyl ester [95.9% (acid equivalent 65.8%)]
SYNONYMS: dichlorophenoxyacetic acid, 2-butoxyethyl ester; 2,4-D BEE
SPONSOR: DowElanco
CITATION: Zablotny, C. L., Yano, B. L., and Breslin, W. J. (1991). 2,4-D 2-Butoxyethyl Ester: Oral Gavage Teratology Study in New Zealand White Rabbits. Toxicology Research Laboratory, Michigan. Study No. K-007722-021, November 22, 1991. MRID 42158706; Document No. 009512. Unpublished. Oral Gavage Teratology Probe Study in New Zealand White Rabbits. Study No. K-007722-020, November 7, 1991. MRID 42158703.

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 42158706], artificially-inseminated female New Zealand White rabbits [24/group] were administered 2,4-D butoxyethyl ester [95.9% (acid equivalent 65.8%)] at dose levels of 0 [corn oil control)], 15 mg/kg/day, 45 mg/kg/day, and 110 mg/kg/day from gestation day 7 through gestation day 19.

One mid-dose doe was sacrificed on day 21 and another mid-dose doe died on day 24. Four high-dose does [days 14, 15, 18, 21] were sacrificed moribund, and four high-dose does died [days 11, 12, 13, and 19]. One low-dose [day 25] and three mid-dose does [days 22, 23, and 24] aborted. It is to be noted that, although there were no abortions at the high-dose level, the abortions that occurred at the lower dose levels took place between days 22 and 25 and the 8 deaths/sacrifices that occurred at the high-dose level happened prior to day 22. Had these does survived, they may have aborted; therefore, it cannot be ruled out that abortions might have occurred at the high-dose level. One control female was sacrificed because of a dosing error. Treatment-related clinical signs of toxicity [decreased activity, prostration, myotonia, transient lateral recumbency, cold to touch, perineal soiling/blood, red urine] were observed in the mid- and high-dose does.

In general, body weight was comparable among the groups throughout the study, although the does that died or were sacrificed moribund exhibited body-weight loss during the exposure period, and these losses are considered treatment-related. During the dosing period, both the low- [71 grams] and mid-dose [6 grams] does displayed decreased body-weight gains compared to the controls [129 grams]. Corrected body-weight gain was comparable among the groups.

Pregnancy rates were comparable among the groups, although fewer females were pregnant at the mid- and high-

dose levels [83%] compared to the low dose [88%] and control [92%]. Dead fetuses were not reported at any dose level, and there were no litters that were totally resorbed. At the **mid-dose** level, there were decreases in the number of implantations per doe and in the number of live fetuses per doe. Additionally, increases in the percent of litters with resorption and increased pre- [42.8%] and post- [16.5%] implantation losses were observed at the mid-dose level compared to the control [21.8% and 3.7%, respectively] and other dose levels [low: 26.5% and 3.5%; high: 24.8% and 2.0%, respectively]. Uterine weight was decreased at the mid-dose level [69% of control] compared to the control. The sex ratio was comparable among the groups, and fetal body weight was significantly increased [a result of the decreased number of fetusus/litter] at the mid-dose level compared to the control [11% increase]. There were no apparent, treatment-related, effects on external, visceral, or skeletal variation/anomalies/retardations/ malformations at any dose level. Although the number of litters at the high-dose level was decreased compared to the control due to 8 deaths/moribund sacrifices, the number of litters at the high-dose level [14] was comparable to the number at the mid-dose level [15], and the effects on the numbers of implant/resorptions/live fetuses observed at the mid dose were not observed at the higher dose level. The observations at the mid-dose level are not considered treatment-related.

The maternal toxicity NOAEL is 15 mg/kg/day, based on mortality/morbidity, clinical signs, and decreased body-weight gain at the LOAEL of 45 mg/kg/day. At the high-dose level [110 mg/kg/day], there was an increased incidence of deaths. The developmental toxicity NOAEL is 110 mg/kg/day, the highest dose tested.

This guideline developmental toxicity study is classified ACCEPTABLE, and it satisfies the guideline requirement **[§83-3(b)/OPPTS 870.3700]** for a developmental toxicity study in rabbits.

[2,4-D-2-BUTOXYETHYL ESTER] Developmental Toxicity Study - Rabbit [§83-3(b)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Body-Weight Gain [grams]

Dose/Time	0 mg/kg/day	15 mg/kg/day	45 mg/kg/day	110 mg/kg/day
Body Weight [g]				
gestation day 0	3739.9±186.6	3811.4±204.7 [21]	3770.2±160.7 [20]	3761.7±138.2 [20]
gestation day 7	3958.5±212.7	4005.4±265.7 [21]	4042.3±194.4 [20]	4009.4±194.7 [20]
gestation day 20	4078.6±301.4	4076.6±290.7 [21]	4048.3±340.2 [20]	4197.2±227.7 [14]
gestation day 28√	3788.9±306.0 [21]	3823.1±287.4 [20]	4046.8±296.8 [15]	3929.5±275.0 [14]
Body-Weight Gain [g]				
days 0-7	218.6±105.1	194.0±190.4 [21]	272.1±75.1 [20]	247.8±104.3 [20]
days 7-10	17.8±57.4	-6.5±70.4 [21	-17.1±75.9 [20]	-42.0±109.3 [20]
days 10-13	42.5±60.8	51.1±74.6 [21]	61.9±61.8 [20]	14.5±122.3 [17]
days 7-20	127.9±201.8 [21]	71.3±232.7 [21]	5.9±302.1 [20]	111.7±121.4 [14]
days 20-28	155.6±166.6 [21]	147.1±145.3 [20]	168.8±112.9 [15]	184.6±113.4 [14]
days 0-28	507.7±241.7 [21]	445.9±205.1 [20]	568.9±165.7 [15]	562.0±223.4 [14]
Gravid Uterine Weight [g]	454.9±86.0	414.3±110.2	315.6±131.5*	459.5±72.3

* $p<0.05$; √ terminal body weight - gravid uterine weight; n=22 unless [ ]; data from Tables 6-7, pages 35-36 of the report

Table 2. Maternal Hematology Parameters

Dose/Parameter	0 mg/kg/day	15 mg/kg/day	45 mg/kg/day	110 mg/kg/day
RBC [x 10^6/mm^3]	5.73±0.52	5.63±0.47	5.34±0.70	5.27±0.53
HGB [g/dL]	12.6±1.0	12.4±0.9	11.9±1.3	11.8±1.0
HCT [%]	43.1±2.9	42.4±3.1	40.5±4.3	40.3±3.5
platelet [x 10^3/mm^3]	418±70	403±104	431±119	402±105
WBC [x 10^3/mm^3]	7.7±1.8	7.1±1.5	7.3±1.9	7.3±2.0

data from Table 8, page 37 of the report

Table 3. Maternal Organ Weights

Dose/Organ	0 mg/kg/day	15 mg/kg/day	45 mg/kg/day	110 mg/kg/day
liver				
grams	101.68±18.50	101.81±13.05	120.35±26.21*	109.01±17.21
g/100 grams	2.39±0.34	2.40±0.26	2.75±0.51*	2.48±0.31
kidneys				
grams	17.3±2.0	17.03±1.50	18.34±2.46	18.56±2.48
g/100 grams	0.41±0.03	0.40±0.03	0.42±0.04	0.42±0.04

data from Table 11, page 40 of the report; * $p<0.05$

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PRIMARY REVIEWER: Jess Rowland, Toxicologist
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5/15/92

SECONDARY REVIEWER: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II

DATA EVALUATION REPORT

1. PROBE STUDY

STUDY TYPE: Developmental Toxicity [Probe Study] **GUIDELINE: N/A**

Caswell No. 315 AI MRID No. 421587-03 HED PROJECT No. 2-1084

TEST MATERIAL: Butoxyethyl Ester of 2,4-D ACID [2,4-D BEE]

REGISTRANT: DowElanco, Indianapolis, IN.

TESTING LABORATORY: The Toxicology Research Laboratory, Michigan

STUDY IDENTIFICATION: K-007722-020

TITLE OF REPORT: 2,4-D 2-Butoxyethyl Ester: Oral Gavage Teratology Probe Study in New Zealand White Rabbits.

AUTHOR(S): W.J. Breslin, C.L. Zablotny, and B.L. Yano

REPORT DATE: November 7, 1991

SUMMARY: Impregnated New Zealand White rabbits were given oral administration of 2,4-D BEE [95.99%] at doses of 0, 73, 145, or 218 mg/kg/day during days 7 through 19 of gestation. Dams were sacrificed on gestation day 20. 2,4-D BEE induced maternal toxicity included mortality/morbidity and clinical signs [at all dose levels], decreases in body weight gain [145 and 218 mg/kg/day], increase in kidney weights [145 and 218 mg/kg/day], changes in hematology parameters [145 and 218 mg/kg/day], and increase in the degree of microscopic kidney tubular degeneration [all dose levels]. There was a dose-related increase in post-implantation loss [all dose levels] and an increase in the percentage of litters with resorption [145 and 218 mg/kg/day]. Based on the results of this study the NOELs and LOELs are:

MATERNAL TOXICITY NOEL = Not established; LOEL = 73 mg/kg/day

DEVELOPMENTAL TOXICITY NOEL = Not established; LOEL = 73 mg/kg/day

CORE CLASSIFICATION: Not applicable; range-finding study.

1. OBJECTIVE

The objective of this probe [range-finding] study, was to establish appropriate dose levels of the 2-Butoxyethyl Ester of 2,4-D for the main study.

2. PROTOCOL

Groups of seven inseminated female New Zealand White rabbits [7 months old] were given oral administrations of 2,4-D BEE (95.9% pure; Lot no.AGR276426) at doses of 0, 73, 145, or 218 mg/kg/day in corn oil [1 mL/kg] daily, during days 7 through 19 of gestation. These dose levels correspond to approximately 0, 50, 100 or 150 mg/kg/day of 2,4-D acid. Concentration, homogeneity and stability of the test article/vehicle mixtures were determined prior to the initiation of the study.

Animals were observed for clinical signs of toxicity daily, dams were weighed on gestation days 0, daily during the dosing period, and on day 20 of gestation. The eyes were examined in situ. Dams in each group were sacrificed on day 20 and postmortem examination included macroscopic examination of internal organs, with emphasis on the uterus, uterine contents, position of each fetus in the uterus, and corpora lutea counts. The uteri (and its contents) of all dams were weighed to determine corrected body weights. In addition, on day 20, blood samples were drawn for hematologic examination, at necropsy, liver and kidney weights were recorded, and these tissues underwent histopathological examination. No fetal examinations were performed.

3. RESULTS

i. Analysis of dosing solution

The mean concentrations found were 101%, 103%, and 103% of the nominal concentration for the 73, 145 and 218 mg/kg/day dose groups, respectively. The 2,4-D BEE was homogeneous and stable in corn oil for up to ninety days post-preparation.

ii. Maternal Toxicity

- 73 mg/kg/day: 1 doe sacrificed moribund on Day 17

  145 mg/kg/day: 1 doe sacrificed moribund on Day 13

  218 mg/kg/day: 1 doe found dead on Day 9
  1 doe found dead on Day 19
  1 doe sacrificed moribund on Day 16
  2 does sacrificed moribund on Day 17

- Prior to death or sacrifice, does exhibited decreased feces, decreased activity, transient lateral recumbency with myotonia, lack of righting reflex and prostration. Clinical signs observed in surviving animals were limited to decreased feces at 218 mg/kg/day.

- No treatment-related effects in body weight gain were seen in does at 73 mg/kg/day. Mean body weight was significantly [p <0.05] decreased in does at 145 mg/kg/day on Day 20 and in does at 218 mg/kg/day on Days 16 and 20. The body weight gains were statistically decreased on Days 7-10 and 0-20 in dams given 218 mg/kg/day.

- Hematology revealed decreases in RBC, HCT, HGB concentrations in does at 145 and 218 mg/kg/day.

- Organ weight data showed non statistically significant increases in absolute kidney weights and statistically significant increases in relative kidney weights at 145 and 218 mg/kg/day.

- In rabbits sacrificed at termination, necropsy showed increases in the degree of microscopic kidney tubular degeneration in **all dose groups.** Rabbits at 145 and 218 mg/kg/day also had decreased hepatocellular vacuolation that was considered secondary to anorexia or debilitation.

- The pregnancy rate was 100%, 86%, 57%, and 100% at 0, 73, 145 and 218 mg/kg/day dose groups, respectively.

- There was a **dose-related increase in post implantation loss; 7.5%, 20.0% and 29.4% at 73, 145 and 218 mg/kg/day, respectively, when compared to controls [5.4%].**

- There was a **increase in the percentage of litters with resorptions at 145 mg /kg/day [100%] and 218 mg/kg/day [50%] when compared to controls [29%]** with the increase reaching statistical significance only at 145 mg/kg/day.

- Treatment had no effect on corpora lutea, implantation sites, pre-implantation losses, or viable fetuses at any dose level.

iii. Developmental Toxicity

No data were reported for fetal examinations.

4. **CONCLUSION: MATERNAL TOXICITY NOEL = Not established**
LOEL = 73 mg/kg/day
DEVELOPMENTAL TOXICITY NOEL = Not established
LOEL = 73 mg/kg/day

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PRIMARY REVIEWER: Jess Rowland, Toxicologist
Section II, Toxicology Branch II

Jess Rowland 5/18/92

SECONDARY REVIEWER: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II

K. Clark Swentzel 5/19/9

DATA EVALUATION REPORT

MAIN STUDY

STUDY TYPE: Developmental Toxicity / Rabbits **GUIDELINE:** 83-3(b)

Caswell No. 315 AI **MRID No. 421587-06** **HED PROJECT No. 2-1084**

TEST MATERIAL: 2-Butoxyethyl Ester of 2,4-D [2,4-D BEE]

REGISTRANT: DowElanco **STUDY IDENTIFICATION:** K-007722-021

TESTING LABORATORY: Toxicology Research Laboratory, Michigan.

TITLE OF REPORT: 2,4-D 2-Butoxyethyl Ester: Oral Gavage Teratology Study in New Zealand White Rabbits.

AUTHORS: C.L. Zablotny, B.L. Yano and W.J. Breslin

REPORT DATE: November 22, 1991

SUMMARY: Inseminated New Zealand White rabbits were given oral doses of 2,4-D BEE [95.9%] at 0, 15, 45, or 110 mg/kg/day during days 7 through 19 of gestation. No maternal toxicity was observed at 15 mg/kg/day. At 45 and 110 mg/kg/day, maternal toxicity was manifested by mortality/morbidity, clinical signs, and decreases in body weight gain. Treatment had no effect on the pregnancy rate, number of corpora lutea, or fetal sex ratio. Cesarean section data at 45 mg/kg/day showed a non-statistically significant increase in percent preimplantation loss, statistically significant decreases in the number of implantations/dam, number of fetuses/litter, gravid uterine weight, as well as statistically significant increases in resorption rate and fetal weight. However, since these findings were not dose related and the incidences were within the historical control range of the testing laboratory they were considered to be unrelated to treatment. No treatment-related external, visceral, or skeletal malformation or variation was seen in any of the fetuses.

MATERNAL TOXICITY NOEL = 15 mg/kg/day; LOEL = 45 mg/kg/day

DEVELOPMENTAL TOXICITY NOEL = 110 mg/kg/day [HDT].
LOEL = Not established.

<u>CORE CLASSIFICATION</u>: **Minimum;** this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

I. OBJECTIVE

The objective of this study was to assess the effects of the **Butoxyethyl Ester of 2,4-D [2,4-D BEE]** on the embryonic and fetal development following oral administration to rabbits during the period of organogenesis.

II. MATERIALS AND METHODS

a. Test Material

Identity: Butoxyethyl Ester of 2,4-D
Batch No.: AGR 276426
Purity: 95.9% [Acid equivalent-65.8%]
Description: liquid

b. Test Animals

Species/Sex: Female rabbits
Strain: New Zealand White
Age on Gestation Day 0: 6.5 to 7.5 months
Weight on Gestation Day 0: 3.25 - 4.25 kg
Identification: Ear tags.
Acclimation Period: Not reported.
Housing: Individually in stainless steel cages
Food: Purina Certified Rabbit Chow #5322 ad libitum.
Water: Tap water ad libitum
Environment: Temperature and humidity controlled rooms.

Group Assignment: 24 inseminated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

Adult females were artificially inseminated with the day of insemination considered Day 0 of gestation. Immediately following insemination, the females were administered HCG.

d. Preparation of Dosing Solutions

2,4-D BEE was administered in corn oil such that a dose volume of 1 mL/kg body weight yielded the appropriate dose. Dose solutions were prepared once prior to the start of the study.

e. Analysis of the Dosing Solutions

Concentration, homogeneity, and stability analysis of the dosing solutions were determined once during the study.

f. Administration of Test Article

The test article was administered daily orally via gavage at doses of 0, 15, 45, or 110 mg/kg/day during days 7 through 19 of gestation. These dose levels correspond to approximately 0, 10, 30, or 75 mg/kg/day of 2,4-D acid. The control group received the vehicle [corn oil] only. All groups received a dosing volume of 1 mL/kg body weight and the dose volumes were adjusted daily based on individual body weights.

g. Observations

All animals were observed once daily for clinical signs of toxicity including physical or behavioral abnormalities. Individual body weights were obtained on day 0, daily during the dosing period, and on days 20 and 28 of gestation. Individual food consumptions were **not** measured in this study.

h. Hematology

Blood samples were drawn from the auricular artery on day 20 of gestation, approximately 24 hours following the final dose. Hematology parameters evaluated were: hemoglobin and hematocrit concentrations; red blood cell, white blood cell and platelet counts; mean corpuscular volume, mean corpuscular hemoglobin concentrations; and white blood cell differential counts.

i. Termination

Any animal which died, appeared moribund or showed indications of early termination of pregnancy was submitted for complete necropsy. All surviving does were sacrificed on gestation day 28, obvious gross pathologic alterations were recorded, and the liver, kidneys and gravid uterus were weighed.

j. Cesarean Section

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 rabbits were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantation, early and late resorption, and live and dead fetuses were recorded.

k. Fetal Examinations

Each fetus was removed from the uterus and individually 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.

l. Statistical Analysis

Maternal body weights, body weight gains, fetal body weights, hematologic parameters and organ weights were evaluated by Bartlett's test for equality of variances followed by a parametric or nonparametric analysis of variance [ANOVA]. If the ANOVA was significant, analysis by Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction was performed, respectively. Statistical evaluation of the frequency of pre-implantation loss and resorption among litters and fetal population was performed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea and implants, and litter size was evaluated using a nonparametric ANOVA followed by the Wilcoxon Rank-Sum test with Bonferroni's correction. Pregnancy rates were analyzed using the Fisher exact probability test.

j. Quality Assurance Measures:

A quality assurance statement was signed and dated 11/14/91. This date conforms to the review of the study phases and the draft and the final reports.

III. RESULTS

Analysis of the Dosing Solutions

The mean concentrations of test article found were 101%, 96% and 92% of the nominal concentrations for the 15, 45 and 110 mg/kg/day doses, respectively. Homogeneity and stability analyses indicated that 2,4-D BEE was homogenous and stable in aqueous solution when stored for up to 62 days at room temperature.

1. Maternal Toxicity

a. Mortality & Pregnancy Status

No. on Test	24	24	24	24
Dose level [mg/kg/day]	0	15	45	110
Found Dead	1	0	1	4
Moribund	0	0	1	4
Aborted	0	1	3	0

The death of the doe in the control group was due to dosing error. At 15 mg/kg/day, the rabbit that aborted on day 25 had decreased feces, soft feces and body weight loss; however, no treatment-related findings were seen at necropsy. At 45 mg/kg/day, all five rabbits [3 abortions on days 22, 23, and 24 + 1 death on day 24 + 1 moribund sacrifice on day 21] exhibited decreased feces and body weight loss prior to their death, euthanasia, or abortion. In addition, myotonia, transient lateral recumbency or decreased activity were seen in the doe that die spontaneously and in the doe that was sacrificed. Necropsy showed watery dark cecal contents, decreased ingesta, pale liver, gastric hairball, edema in the lungs and hemolyzed blood in the stomach. At 110 mg/kg/day, the four does that were sacrificed on days 14, 15, 18, and 21, and the four that died spontaneously on days 11, 12, 13, and 19 exhibited decreased feces, body weight loss, decreased activity, perineal soiling, myotonia, transient lateral recumbency or prostration. Necropsy revealed decreased or dry ingesta, intestinal gas, bloody/red urine, perineal soiling, gastric hairball, distended gall bladder, pale area or foci of the liver, congestion and/or edema of the lungs, and ulceration of the stomach. The pregnancy rate was 91.7%, 87.5%, 83.3%, and 87% in the 0, 15, 45, and 110 mg/kg/day groups. The one abortion at 15 mg/kg/day and the three abortions at 45 mg/kg/day were not considered to be treatment related due to lack of dose-response [no abortions at 110 mg/kg/day] and the incidences were within the historical control range [0-7.69] of the testing laboratory.

b. Clinical Signs

Treatment-related clinical signs of toxicity observed in does at 45 and 110 mg/kg/day are tabulated below:

No. on Test	24	24	24	24
Dose level [mg/kg/day]	0	15	45	110
Cold to touch	0	0	1	0
Decreased activity	0	0	2	5
Myotonia	0	0	2	6
Perineal soiling-Blood/red urine	0	0	0	4
Transient lateral recumbency	0	0	1	1
Prostration	0	0	0	4
Urine discoloration - red	0	0	1	5

c. Body Weight Changes

There were no statistically significant differences in mean body weight or body weight gain between does in the vehicle control and any treated groups. Body weight gains were comparable to that of the control group. However, the individual rabbits that died or were sacrificed moribund at 45 and 110 mg/kg/day groups exhibited body weight loss over their exposure period; these body weight loses were considered to be treatment related.

d. Macroscopical Examination

No treatment-related macroscopical changes were observed in the dams sacrificed at termination. Gross pathological alterations observed in the does that died or were sacrificed moribund are discussed under Mortality [see Page 9].

e. Hematology

No significant, treatment-related effects on any of the mean hematologic parameters or mean differential WBC counts were observed at any dose level.

f. Organ Weight

No treatment-related changes on final body weights or absolute or relative liver or kidney weights were observed at any dose level. The numerical and statistically significant increases noted in the absolute and relative liver weights at 45 mg/kg/day were not considered to be treatment related due to a lack of dose response and the absence of gross pathological alterations in the liver.

2. Developmental Toxicity

Reproduction data are presented below in Table 1. No treatment related effects were observed in pregnancy rate, number of corpora lutea, and fetal sex ratio at any dose level.

Does at 45 mg/kg/day, had a nonstatistically significant increase in the percent preimplantation loss, statistically significant decreases in the number of implantation/dam, number of fetuses/litter, gravid uterine weight, and statistically significant increases in the resorption rate and fetal body weight. Similar changes were not observed at the high-dose [110 mg/kg/day].

Table 1. Cesarean Section Observations

Observations [Mean ± S.D]	Dose Level [mg/kg/day]			
	0	15	45	110
No. Assigned	24	24	24	24
Females Gravid	22	21	20	20
Maternal Wastage				
# Died	1	0	1	4
# Sacrificed	0	0	1	4
# Aborted	0	1	3	0
# Non pregnant	2	3	4	4
Total Corpora Lutea	9.2 202	9.7 204	7.0 140	6.95 139
Corpora Lutea/Dam	10.1±2.2	10.2±2.0	9.3±1.0	9.9±2.0
Total Implantation	7.4 162	6.9 145	3.95 79	5.1 102
Implantation/Dam	7.7±1.7	7.3±2.2	**5.3±2.7***	7.3±1.5
Total Live Fetuses	156	140	66	100
Live Fetuses/Litter	7.4±1.7	7.0±2.3	**4.4±2.6***	7.1±1.4
Total Resorption	6	5	13	2
Early	4	4	12	2
Late	2	1	1	0
Resorption/Dam	0.3±0.6	0.3±0.4	0.9±1.2	0.1±0.4
No. and % of Litters with Resorption	5/21 23.8	5/20 25.0	7/15 **46.7***	2/14 14.3
Pre Implantation Loss [%]	21.8	26.5	**42.8***	24.8
Post Implantation Loss [%]	3.7	3.5	**16.5***	2.0
Gravid Uterus Weight [g]	455±86	414±110	**316±132***	459±73
Sex Ratio ♂ / ♀	48/52	50/50	55/46	51/49
Fetal Weight [g]	38.2±5.0	37.4±4.3	**42.3*± 5.7**	39.3±3.6

* Significantly different from control at $p < 0.05$.

Evaluation of individual reproduction data of does at 45 mg/kg/day [Table 8 in the report] showed that three does each had a reduced number implantations [2, 1, and 1 implantation for animal numbers 91A1788, 91A1793 and 91A1800, respectively], when compared to a mean of 5.3/doe.

Therefore, the cesarean section data were recalculated with 12 litters [i.e., excluding the three outliers], and the incidences were compared with the historical control data of the testing laboratory as shown below:

Observations	45 mg/kg/day		Historical Control Range [10 studies; 161 pregnant rabbits; 1987-1991
	With 15 litters	With 12 litters	
No.of Impantations/Dam	5.3 ± 2.7	6.3 ± 2.0	6.1 - 9.3
No. of Fetuses/Litter	4.4 ± 2.6	5.2 ± 2.3	6.0 - 8.6
% of Litters with Resorption	47	39	11 - 64
Pre-implantation Loss [%]	43	34	15 - 39
Post-implantation Loss [%]	17	17	2 - 19

As shown above, since the recalculated values were within the historical control range of the testing laboratory, it is apparent that the differences observed at 45 mg/kg/day are unrelated to treatment.

Fetal malformations and variations summarized in Table 14 of the study report are appended to this DER. No treatment-related or statistically significant external, visceral, or skeletal malformations or variations were seen in any of the fetuses; 156 at 0 mg/kg/day, 140 at 15 mg/kg/day, 66 at 45 mg/kg/day, and 100 at 110 mg/kg/day.

441

IV. DISCUSSION

Oral administration of the butoxyethyl ester of 2,4-D at 0, 15, 45, or 110 mg/kg/day to inseminated rabbits during days 7 through 19 of gestation resulted in no maternal toxicity at 15 mg/kg dose. Maternal toxicity at 45 and 110 mg/kg/day included mortality/morbidity, clinical signs characterized by decreased activity, myotonia, lateral recumbency, and decreases in body weight gain in does that died or were sacrificed moribund.

Caesarean section of dams at 45 mg/kg/day showed decreases in the number of implantations/dam, number of fetuses/litter and gravid uterine weight, and increases in the percentage of litters with resorptions, postimplantation losses, and fetal body weights. Similar changes were not observed in does at 110 mg/kg/day. However, analysis of this data, excluding three does with lower than average implantations, showed that the incidences observed at 45 mg/kg/day were within the historical control range of the testing laboratory. Consequently, because these findings were not dose related and the incidences were within the historical control range of the testing laboratory they were considered to be unrelated to treatment. No treatment-related external, visceral, or skeletal malformation or variations were seen in any of the fetuses of treated does.

In a rat developmental toxicity study [MRID 415271-01], pregnant Sprague-Dawley rats were given oral administration of 2,4-BEE [95.6%] at 0, 25, 75, or 185 mg/kg/day during days 6-15 of gestation. For maternal toxicity the NOEL was 75 mg/kg/day and the LOEL was 185 mg/kg/day based on decreases in body weight gain and food consumption. For developmental toxicity the NOEL was 25 mg/k/day and the LOEL was 75 mg/kg/day based on incompletely ossified interparietal, supraoccipital, squamosal(s), malar(s), and 4th sternebra, and the presence of 14th rib pairs and short rib(s).

5. CONCLUSION

2,4-D BEE was maternally toxic to rabbits at doses of 45 and 110 mg/kg/day; no maternal toxicity was seen at 15 mg/kg/day. The compound did not induce developmental toxicity at 110 mg/kg/day, the highest dose tested.

Maternal Toxicity	**Developmental Toxicity:**
NOEL: 15 mg/kg/day	**NOEL = 110 mg/kg/day [HDT**
LOEL: 45 mg/kg/day	**LOEL: Not established**

6. **CORE CLASSIFICATION: Minimum;** this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

TABLE 14

2,4-D 2-BUTOXYETHYL ESTER: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	15	45	110
		NUMBER OF FETUSES AFFECTED (NUMBER OF LITTERS) EXAMINED			
EXTERNAL EXAMINATION		156 (21)	140 (20)	66 (15)	100 (14)
VISCERAL EXAMINATION		156 (21)	140 (20)	66 (15)	100 (14)
SKELETAL EXAMINATION		156 (21)	140 (20)	66 (15)	100 (14)
		PERCENT AFFECTED (NUMBER AFFECTED)			
EXTERNAL OBSERVATIONS					
GENERALIZED EDEMA, PETICHIAL HEMORRHAGE	F[a]	0.6(1)	0	0	0
	L	4.8(1)	0	0	0
MULTIPLE[+,b]	F	0	0.7(1)[c]	0	0
	L	0	5.0(1)	0	0
VISCERAL OBSERVATIONS					
MERO-ANENCEPHALY[+]	F	0	0.7(1)[c]	0	0
	L	0	5.0(1)	0	0
APHAKIA[+], RUDIMENTARY IRIS[+]	F	0.6(1)[d]	0	0	0
	L	4.8(1)	0	0	0
MULTIPLE CARDIAC[+,e]	F	0.6(1)[d]	0	0	0
	L	4.8(1)	0	0	0

14

TABLE 14 (CONTINUED)

2,4-D 2-BUTOXYETHYL ESTER: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	15	45	110
		PERCENT AFFECTED (NUMBER AFFECTED)			
VISCERAL OBSERVATIONS (CONTINUED)					
MISSING CAUDAL LUNG LOBE[+]	F	1.3(2)	6.4(9)[f]	1.5(1)[g]	2.0(2)
	L	9.5(2)	15.0(3)	6.7(1)	7.1(1)
HEMORRHAGIC LIVER LOBE	F	0	0.7(1)	0	0
	L	0	5.0(1)	0	0
ECTOPIC KIDNEY[+]	F	0.6(1)	0	1.5(1)[g]	0
	L	4.8(1)	0	6.7(1)	0
DILATED RENAL PELVIS	F	0	0.7(1)	0	0
	L	0	5.0(1)	0	0
CONVOLUTED, DILATED URETERS	F	0	0.7(1)	0	0
	L	0	5.0(1)	0	0
RETROCAVAL URETER	F	0	1.4(2)	1.5(1)	0
	L	0	10.0(2)	6.7(1)	0
PARAOVARIAN CYST[h]	F	0	0	0	2.0(1)
	L	0	0	0	7.1(1)

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 463 of 959

TABLE 14 (CONTINUED)

2,4-D 2-BUTOXYETHYL ESTER: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	15	45	110
		PERCENT AFFECTED (NUMBER AFFECTED)			
SKELETAL OBSERVATIONS					
SKULL					
-DELAYED OSSIFICATION	F	0	0.7(1)	0	0
INTERPARIETAL	L	0	5.0(1)	0	0
-FUSED FRONTALS	F	0.6(1)	0	0	0
	L	4.8(1)	0	0	0
HYOID					
-DELAYED OSSIFICATION	F	26.3(41)	27.9(39)	12.1(8)	18.2(18)
	L	85.7(18)	75.0(15)	26.7(4)	64.3(9)
-CROOKED	F	1.3(2)	3.6(5)	4.5(3)	1.0(1)
	L	9.5(2)	25.0(5)	20.0(3)	7.1(1)
VERTEBRAE					
-FUSED (THORACIC)+	F	0.6(1)	0	0	0
	L	4.8(1)	0	0	0

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 464 of 959

TABLE 14 (CONTINUED)

2,4-D 2-BUTOXYETHYL ESTER: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	15	45	110
		PERCENT AFFECTED (NUMBER AFFECTED)			
SKELETAL OBSERVATIONS (CONTINUED)					
VERTEBRA					
-HEMIVERTEBRA[+]	F	0	2.1(3)[f,i,j]	3.0(2)[g]	1.0(1)[k]
	L	0	10.0(2)	13.3(2)	7.1(1)
-MISALIGNED (LUMBAR)[+]	F	0	0	1.5(1)[l]	0
	L	0	0	6.7(1)	0
ATLAS					
- DELAYED OSSIFICATION	F	0	0.7(1)	0	0
	L	0	5.0(1)	0	0
DENTOID PROCESS					
- DELAYED OSSIFICATION	F	0.6(1)	0.7(1)	0	0
	L	4.8(1)	5.0(1)	0	0
CENTRA					
-DELAYED OSSIFICATION CERVICAL	F	0	0.7(1)	0	0
	L	0	5.0(1)	0	0
-DELAYED OSSIFICATION THORACIC, LUMBAR	F	0.6(1)	0.7(1)	4.5(3)	0
	L	4.8(1)	5.0(1)	13.3(2)	0

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 465 of 959

TABLE 14 (CONTINUED)

2,4-D 2-BUTOXYETHYL ESTER: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	15	45	110
		PERCENT AFFECTED (NUMBER AFFECTED)			
SKELETAL OBSERVATIONS (CONTINUED)					
CENTRA					
-EXTRA SITE OF OSSIFICATION	F	0	0	1.5(1)	0
	L	0	0	6.7(1)	0
-FUSED	F	0.6(1)	0	3.0(2)	0
	L	4.8(1)	0	13.3(2)	0
-MISALIGNED	F	0	0	1.5(1)	0
	L	0	0	6.7(1)	0
RIBS					
-FORKED+	F	0	0.7(1) j	0	0
	L	0	5.0(1)	0	0
-FUSED+	F	0	0.7(1) f	1.5(1)	1.0(1) k
	L	0	5.0(1)	6.7(1)	7.1(1)
RIB					
-DISPLACED+	F	0.6(1)	0	1.5(1) l	0
	L	4.8(1)	0	6.7(1)	0

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 466 of 959

TABLE 14 (CONTINUED)

2,4-D 2-BUTOXYETHYL ESTER: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	15	45	110
		PERCENT AFFECTED (NUMBER AFFECTED)			
SKELETAL OBSERVATIONS (CONTINUED)					
RIB					
MISSING+	F	0	0.7(1)	0	0
	L	0	5.0(1)	0	0
-EXTRA	F	0	0	1.5(1)	0
	L	0	0	6.7(1)	0
-PARTIALLY OSSIFIED	F	0	0	1.5(1)	0
	L	0	0	6.7(1)	0
SPURS					
-LUMBAR	F	35.3(55)	26.4(37)	36.4(24)	37.0(37)
	L	81.0(17)	80.0(16)	86.7(13)	92.9(13)
STERNEBRAE					
-DELAYED OSSIFICATION	F	25.6(40)	27.9(39)	19.7(13)	19.0(19)
	L	71.4(15)	70.0(14)	46.7(7)	64.3(9)
-FUSED	F	0.6(1)	0	3.0(2)	0
	L	4.8(1)	0	13.3(2)	0

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 467 of 959

TABLE 14 (CONTINUED)

2,4-D 2-BUTOXYETHYL ESTER: ORAL GAVAGE TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS

DOSE LEVEL (MG/KG/DAY)		0	15	45	110
		PERCENT AFFECTED (NUMBER AFFECTED)			
SKELETAL OBSERVATIONS (CONTINUED)					
STERNEBRAE					
-EXTRA SITE OF OSSIF.	F	0.6(1)	0	0	0
	L	4.8(1)	0	0	0
SCAPULA					
-DELAYED OSSIFICATION	F	0	0.7(1)	0	0
	L	0	5.3(1)	0	0
TOTAL FETUSES WITH MALFORMATIONS[@]		3.2(5)	8.6(12)	6.1(4)	3.0(3)
TOTAL LITTERS WITH MALFORMED FETUSES[@]		23.8(5)	25.0(5)	20.0(3)	21.4(3)

+ CONSIDERED TO BE A MALFORMATION

a F=FETUSES; L=LITTERS

b 91A1775 HAD MULTIPLE EXTERNAL MALFORMATIONS WHICH INCLUDED ACAUDIA, GASTROSCHISIS, ABLEPHARIA, PEROMELIA - FORELIMBS AND APODIA OF THE HINDLIMBS

c,d,f,g,i,j,k,l MALFORMATIONS IDENTIFIED BY THE SAME SUPERSCRIPT WERE OBSERVED IN THE SAME FETUS.

e MULTIPLE CARDIAC MALFORMATIONS INCLUDED RETROESOPHAGEAL RIGHT SUBCLAVIAN ARTERY, VENTRICULAR SEPTAL DEFECT AND INTERRUPTION OF THE AORTIC ARCH.

h STATISTICAL ANALYSIS BASED ON FEMALE PUPS ONLY.

@ NOT STATISTICALLY COMPARED.

THERE WERE NO STATISTICALLY IDENTIFIED DIFFERENCES FROM THE CONTROL VALUE BY THE CENSORED WICOXON TEST, $\alpha = 0.05$.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 468 of 959

[DIMETHYLAMINE SALT OF 2,4-D] Developmental Toxicity Study - Rabbit [§83-3(b)/OPPTS 870.3700]

Amendment to DER for MRID 42224001 - Developmental Toxicity Study - Rabbit [2,4-D DMA]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 009635].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/6/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 12/8/00
Reregistration Branch I, Health Effects Division (7509C)

XR# 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rabbit § 83-3(b)/OPPTS 870.3700
P.C. CODE: 030019 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315O CAS #: 2008-39-1
TEST MATERIAL (PURITY): Dimethylamine salt of 2,4-D [66.18%; acid equivalent: 55.5%]
SYNONYMS: dichlorophenoxyacetic acid, dimethylamine salt; 2,4-D DMA
SPONSOR: Industry Task Force II

CITATION: Martin, T. (1991). Developmental Toxicity [Embryo-Fetal Toxicity and Teratogenic Potential] Study of 2,4-Dimethylamine Salt of 2,4-D [2,4-D DMA] Administered Orally *via* Stomach Tube to New Zealand White Rabbits. Argus Research Laboratories, Inc. Study No. Argus 320-004, December 12, 1991. MRID 42224001; Document No. 009635. Unpublished.

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 42224001], artificially-inseminated female New Zealand White rabbits [20/group] were administered 2,4-D dimethylamine salt [66.18% (same Lot # as MRID 41735201); 55.5% acid equivalent] at dose levels of 0 [deionized water], 10 mg/kg/day, 30 mg/kg/day, and 90 mg/kg/day [acid equivalents] from gestation day 6 through gestation day 18.

Two high-dose doe were found dead on day 10, and two high-dose does were found dead on day 18. One high-dose doe was sacrificed moribund on day 17. One low-dose doe delivered prematurely on day 29, and another aborted on day 21. One mid-dose doe aborted on day 23. Treatment-related clinical signs of toxicity were observed at the mid- and high-dose levels and first occurred on day 7 of gestation. At the mid-dose level, soft or liquid feces [2 does] and red substance in cage pan [1 doe] were observed, while at the high-dose level, decreased motor activity [9 does], myotonia [7 does], ataxia [5 does], and impaired or loss of the righting reflex [5 does] were observed in addition to soft or liquid feces [3 does] and red substance in cage pan [1 does]. Body weights were comparable among the groups, and when those does that died/sacrificed moribund are removed, body-weight gains were comparable also. All treatment groups displayed an initial [day 6-7] negative body-weight gain [+0.01, -0.02, -0.02, -0.04 grams with increasing dose]. Corrected body-weight gain could not be calculated since gravid uterine weights were not provided. Reduced food consumption was associated only with the high-dose does.

Pregnancy rates were comparable among the groups. On a per doe basis, comparable numbers of corpora lutea, implantations, and live fetuses were observed among the groups, and there were no dead fetuses. There was a slight increase in the number of resorptions at the high-dose level [13] compared to the concurrent control [7], and the number [%] of litters with resorptions was also increased [30% *vs* 57%]. Consequently, post-implantation loss was increased at the high-dose level [11.6%] compared to the control [4%] and other dose levels [5%], but statistical

450

significance was not attained, and the incidence was within the historical control. There was no adverse effect on fetal body weight, and the sex ratio was comparable among the groups. There were no apparent, treatment-related effects on external, visceral, or skeletal variation/anomalies/ retardations/malformations.

The maternal toxicity NOAEL is 30 mg/kg/day, based on mortality/morbidity, decreased body-weight gain and food consumption, and clinical signs of toxicity [decreased motor activity, myotonia, ataxia, impaired/loss of righting reflex] at the maternal toxicity LOAEL of 90 mg/kg/day. The developmental toxicity NOAEL is 90 mg/kg/day, the highest dose tested.

This developmental toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [**§83-3(b)/OPPTS 870.3700**] for a developmental toxicity study in rabbits.

[DIMETHYLAMINE SALT OF 2,4-D] Developmental Toxicity Study - Rabbit [§83-3(b)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Body-Weight Gain [grams]				
Dose/Time	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
Body Weight [g]				
gestation day 0	3.84±0.31	3.85±0.34	3.85±0.32	3.81±0.40
gestation day 7	3.98±0.30	3.96±0.34	3.95±0.34	3.93±0.39
gestation day 14	4.04±0.34	4.04±0.36	4.05±0.37	4.01±0.42 [17]
gestation day 20	4.09±0.36	4.06±0.42	4.10±0.42	4.14±0.48 [14]
gestation day 29	4.18±0.39	4.19±0.48 [16]	4.20±0.42 [18]	4.26±0.49 [14]
# pregnant	20	18	19	19
Body-Weight Gain [g]				
days 0-6	0.13±0.07	0.12±0.06	0.12±0.07	0.16±0.06
days 6-7	0.01±0.05	-0.02±0.02	-0.02±0.03	-0.04±0.05*
days 7-8	-0.01±0.05	0.02±0.06*	0.00±0.04	-0.03±0.04
days 9-12	0.04±0.06	0.01±0.07	0.02±0.08	0.05±0.04
days 15-19	0.02±0.07	0.04±0.10	0.02±0.06	0.07±0.05
days 6-19	0.10±0.13	0.10±0.19	0.12±0.10	0.14±0.12
days 0-29	0.34±0.18	0.36±0.28	0.35±0.18	0.45±0.21

* $p<0.05$; N=# pregnant, unless [ ]; data from Tables 4-5, pages 49-53 of the report

Table 2. Food Consumption				
interval [days]	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
grams/day				
0-6	168.7±13.7	165.3±19.7	169.0±18.1	171.4±14.6
6-7	160.7±27.4	159.9±21.3	157.2±30.4	120.4±54.9*
7-8	164.6±17.4	155.9±28.6	159.2±30.9	119.0±55.9
6-9	162.1±19.5	158.4±22.8	159.8±29.2	118.4±46.1**
15-19	144.2±38.1	136.0±63.8	147.4±41.4	150.2±30.0
6-19	149.4±29.0	145.7±41.8	153.6±28.1	143.3±32.0
0-29	139.2±20.8	144.2±28.5	141.1±24.5	145.5±22.8
grams/kg/day				
0-6	43.2±2.8	42.2±3.3	43.3±4.6	44.3±4.4
6-7	40.4±6.0	40.3±4.4	39.6±6.8	30.8±14.4
7-8	41.5±3.9	39.2±6.0	40.3±7.2	30.1±13.5*
6-9	40.9±4.2	39.8±4.4	40.3±6.4	30.0±11.1**
15-19	35.4±8.4	33.2±15.3	35.8±9.3	36.8±6.6
6-19	37.2±6.2	36.2±9.5	38.1±5.4	35.7±6.9
0-29	34.3±4.2	35.3±5.2	34.5±4.6	35.8±4.8

* $p<0.05$; ** $p<0.01$; data from Table 6-754-57, page of the report

Table 3. Litter Observations				
Parameter	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
fetal body weight [g]				
combined sexes	43.56±5.65	42.90±7.14	40.93±5.91	44.46±6.99
males	43.32±5.90	41.90±6.93	41.59±6.18	45.18±6.89
females	43.37±6.30	42.76±7.04	39.85±5.90	42.87±6.21
% males/litter	56.8±18.9	50.1±23.1	50.9±14.0	54.9±22.5
% resorbed conceptuses/litter	4.9±8.0	8.2±13.2	5.7±9.6	14.1±19.4

data from Table 9, page 59 of the report

152

[DIMETHYLAMINE SALT OF 2,4-D] Developmental Toxicity Study - Rabbit [§83-3(b)/OPPTS 870.3700]

Table 4. Fetal Alterations				
Parameter	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
# litters evaluated	20	16	18	14
# fetuses evaluated	170	126	155	99
FETAL ALTERATIONS				
litters w/ fetuses w/ any alteration (n [%])	18 [90.0]	13 [81.2]	16 [88.9]	12 [85.7]
fetuses w/ any alteration (n [%])	48 [28.2]	38 [30.2]	44 [28.4]	31 [31.3]
% fetuses w/ any alteration/litter	28.78±21.46	29.44±24.01	31.11±20.54	30.20±18.09

data from Table 10, page 60 of the report

009635

PRIMARY REVIEWER: Jess Rowland, Toxicologist
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II

DATA EVALUATION REPORT

1. RANGE-FINDING STUDY

STUDY TYPE: Developmental Toxicity **GUIDELINE: 83-3[b]**

CASWELL No. 315 O MRID No. 422240-01 HED PROJECT No. 2-1678

TEST MATERIAL: Dimethylamine salt of 2,4-D Acid.

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

TESTING LABORATORY: Argus Research Laboratories, Inc, PA.

STUDY IDENTIFICATION: 320-004P

TITLE OF REPORT: *DOSE RANGE-FINDING DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D-DIMETHYLAMINE SALT OF 2,4-D [2,4-D-DMA] ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS.*

AUTHOR: Terry Martin, D.V. M **REPORT DATE:** December 12, 1991

SUMMARY: Impregnated New Zealand White rabbits were given oral administration of technical 2,4-D DMA [55.5%] at doses of 0, 50, 100, 150, or 200 mg/kg/day during days 6 through 18 of gestation. Dams were sacrificed on gestation day 29. 2,4-D DMA induced maternal toxicity were decreases in body weight gain and food consumption at 50, 100 and 150 mg/kg/day. Does at 150 and 200 mg/kg/day died or were sacrificed. Developmental toxicity seen at 100 mg/kg/day included increases in the mean number of resorptions, number of does with any resorptions, and in the mean percent resorbed/litter; no developmental effects were seen at 50 mg/kg/day. In this study, the NOELs and LOELs are:

MATERNAL TOXICITY NOEL = Not achieved; LOEL = 50 mg/kg/day [LDT]
DEVELOPMENTAL TOXICITY NOEL = 50 mg/kg/day; LOEL = 100 mg/kg/day

Based on these results the dose levels selected for the main study were 0, 10, 30 or 90 mg/kg/day.

CORE CLASSIFICATION: Not applicable; range-finding study.

1. OBJECTIVE

The objective of this range-finding study, was to establish appropriate dose levels of the Dimethylamine salt of 2,4-D for the main study.

2. PROTOCOL

Groups of four impregnated female New Zealand White rabbits [7 months old] were given oral administrations of 2,4-D DMA (55.5%; Lot 04FD31349) at doses of 0, 50, 100, 150, or 200 mg [acid equivalent]/kg/day in deionized water [10 mL/kg] daily, during days 6 through 18 of gestation. Concentration and stability of the test article/vehicle mixtures were determined prior to the initiation of the study.

Animals were observed daily for clinical signs of toxicity and body weights and food consumption were obtained daily. Dams in each group were sacrificed on day 29 and postmortem examination included macroscopic examination of internal organs, with emphasis on the uterus, uterine contents, position of each fetus in the uterus, and corpora lutea counts. The uteri (and its contents) of all dams were weighed to determine corrected body weights. The fetuses were discarded.

3. RESULTS

i. Analysis of dosing solution

Concentrations ranged from 0% to 6%, -2% to 1%, -3% to -1% and -3% to 0% from the target concentration for the 50, 100, 150, and 200 mg/kg dose groups, respectively. The 2,4-D DMA was homogeneous and stable in distilled water at room temperature for up to seven days post-preparation.

ii. Maternal Toxicity

- No mortality or abortion occurred at 50 or 100 mg/kg/day. Two does died at 150 mg/kg/day on gestation days 8 and 12. Two does died at 200 mg/kg/day on gestation days 8 and 9. Necropsy showed reddish-fluid contents in the urinary bladder and partially gas-filled cecum.

- One doe at 50 mg/kg/day aborted on gestation day 23 and another prematurely delivered on gestation day 29; these were not considered to be treatment-related due to lack of dose response. The pregnancy rate was 75%, 100% and 100% in the control, 50, and 100 mg/kg/day groups. Since two does each died at 150 and 200 mg/kg/day, the surviving does at these groups were sacrificed and discarded; pregnancy status was not determined.

55

- Clinical signs of toxicity, observed only in the does that died, included decreased motor activity, impaired righting reflex, myotonia, loss of righting reflex and/or hyperpnea.

- Reduced body weight gain was seen in does at 50 and 100 mg/kg/day during the dosing period [days 6-19]; does gained weight during the post dosing period [days 19-29]. Termination of the 150 and 200 mg/kg/day dose groups precluded the evaluation of body weight data.

- Decreases in absolute [g/day] and relative [g/kg/day] food consumptions were seen in does at 50 and 100 mg/kg/day during the dosing period [days 6-19]; food consumption was increased during the post dosing period [days 19-29]. Termination of the 150 and 200 mg/kg/day dose groups precluded the evaluation of food consumption data.

- Necropsy revealed no treatment-related gross abnormalities in the does sacrificed on Day 29.

iii. Developmental Toxicity

- No adverse effects were seen at 50 mg/kg/day. At 100 mg/kg/day, the mean number of resorptions [1.0± 1.2], number of does with any resorptions [2] and the mean percent resorbed/litter [13.4±15.5] were increased when compared to controls [none].

4. CONCLUSION

MATERNAL TOXICITY NOEL = Not achieved; LOEL = 50 mg/kg/day
DEVELOPMENTAL TOXICITY NOEL = 50 mg/kg/day; LOEL = 100 mg/kg/day

Based on these finding, the dose levels selected for the main study were 0, 10, 30 and 90 mg/kg/day.

5. CORE CLASSIFICATION: Not applicable; not a guideline study.

009635

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II

DATA EVALUATION REPORT

TERATOLOGY STUDY

STUDY TYPE: Developmental Toxicity/Rabbits **GUIDELINE:** 83-3(b)

Caswell No. 315-O **MRID No. 422240-01** **HED PROJECT No. 2-1678**

TEST MATERIAL: Dimethylamine salt of 2,4-D

REGISTRANT: Industry Task Force II

TESTING LABORATORY: Argus Research Laboratories, Inc.

STUDY IDENTIFICATION: Argus 320-004

TITLE OF REPORT: *DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D-DIMETHYLAMINE SALT OF 2,4-D [2,4-D-DMA] ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS.*

AUTHORS: Terry Martin, DVM **REPORT DATE:** December 12, 1991

SUMMARY: Inseminated New Zealand White rabbits were given oral administration of technical 2,4-D DMA **[55.5%]** at doses of 0, 10, 30, or 90 mg/kg/day during days 6 through 18 of gestation. No maternal toxicity was observed at 10 or 30 mg/kg/day. At 90 mg/kg/day, maternal toxicity was characterized by mortality/morbidity, decreases in body weight gain and food consumption, and clinical signs of toxicity such as decreased motor activity, myotonia, ataxia and impaired/lost righting reflex. No treatment-related differences were seen in reproductive parameters. No developmental toxicity was seen at any dose level. Based on the results of this study the following NOELs and LOELs are established.

MATERNAL TOXICITY NOEL = 30 mg/kg/day
LOEL = 90 mg/kg/day

DEVELOPMENTAL TOXICITY NOEL = 90 mg/kg/day [HDT]
LOEL = Not achieved

CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

I. OBJECTIVE

The objective of this study was to assess the effects of the **dimethylamine salt of 2,4-D [2,4-D-DMA]** on the embryonic and fetal development following oral administration to rabbits during the period of organogenesis.

II. MATERIALS AND METHODS

a. Test Material

Identity: Dimethylamine salt of 2,4-D
Batch No: 04FD31349
Acid equivalent: 55.5%
Purity: Although not stated in the report it is believed to be 66.18% [based on the rat study] since the same lot.no was identified for both studies.
Description: Brown liquid

b. Test Animals

Species/Sex: Female rabbits
Strain: New Zealand White [Hra:(NZW)SPF]
Age: Approximately 5 months
Body Weight on Gestation Day 0: 3.0 to 4.7 kg
Identification: Ear tags.
Acclimation: Approximately 5 weeks
Housing: Individually in stainless steel cages
Food: Purina Certified Rabbit Chow #5322 ad libitum.
Water: Tap water ad libitum
Environment: Temperature - 61 -70°F; Humidity - 40-60%; Air Exchange- 10/hr.; Light/Dark- 12 hr.cycle
Group Assignment: 20 inseminated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

Adult females were artificially inseminated with spermtatozoa from untreated proven male rabbits used specifically for breeding. The day of insemination was considered Day 0 of gestation. Approximately three hours prior to insemination, does received an intravenous injection of 20 USP Units/kg of HCG. An estimated 0.25 mL of semen that had been diluted with normal saline to a concentration of 6.0×10^6 spermatozoa/0.25 mL saline was used to artificially inseminate each rabbit.

458

d. Preparation of Dosing Solutions

The concentration of each dosing solution was adjusted based on a test article activity of 55.5%. The test material/vehicle [reverse osmosis membrane processed deionized water] solutions were prepared once during the first three days of the dosage period. Beginning on the fourth day of the dosing period, the dosage solutions were prepared daily.

e. Analysis of the Dosing Solutions

Concentration and stability analysis of the dosing solutions were determined once during the study. Homogeneity was not performed since the test material is highly soluble in water.

f. Administration of Test Article

The test article was administered daily orally via gavage at doses of 0, 10, 30, or 90 mg/kg/day from day 6 through 18 of gestation. The control group received the vehicle only. All groups received a dosing volume of 10 mL/kg body weight, with a daily adjustment of the individual volume to the actual body weight.

g. Observations

All animals were observed for clinical signs of toxicity, abortions, premature deliveries and deaths immediately prior to dosing, approximately 1 hour, 2 hours and 3 hours post dosing and then once daily till termination. Individual body weights and food consumptions were obtained daily.

h. Termination

Females found dead or showing sings of abortion during the study were necropsied. All surviving does were sacrificed on gestation day 29.

i. Cesarean Section

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 rabbits were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantation, early and late resorptions, and live and dead fetuses were recorded.

459

j. Fetal Examinations

Each fetus was removed from the uterus and individually identified with a tag, 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 changes.

k. Statistical Analysis

Maternal and fetal incidence data were analyzed using the Variance Test for Homogeneity and Binomial Distribution. Maternal body weights, body weight changes and feed consumption data, and litter averages for percent male fetuses, percent dead or resorbed conceptuses, fetal body weights, anomaly average data and ossification site data were analyzed using Bartlett's Test of Homogeneity of Variances and ANOVA, when appropriate. If ANOVA was significant, Dunnett's Test was used to identify statistical significance of the individual groups. If ANOVA was not appropriate, the Kruskal-Wallis Test and the Fisher's Exact tests were used. In cases in which the Kruskal-Wallis Test was statistically significant, Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual group. All other cesarian-sectioning data were evaluated using the parameters described for the Kruskall-Wallis Test.

l. Compliance Statements

A signed Statement of No Confidentiality Claim was provided.

A signed Statement of Compliance with EPA's GLP was provided that was dated December 10, 1991.

A signed Quality Assurance Statement was dated September 4, 1991. This date conforms to the review of the study phases and the draft and the final reports.

A signed statement dated December 12, 1991 was provided, which indicated that the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to the study. This study reportedly neither met nor exceeded any of these criteria.

III. RESULTS

Analysis of the Dosing Solutions

Concentrations ranged from -1% to 3%, -3% to -1%, and -2% to -1% from the target concentration for the 10, 30, and 90 mg/kg dose groups, respectively. 2,4-D DMA was stable in distilled water at room temperature for up to seven days post-preparation.

1. Maternal Toxicity

a. Mortality & Pregnancy Status

No mortality occurred at 10 or 30 mg/kg/day. At 90 mg/kg/day, two does were found dead on Day 10 and two does on Day 18. At 10 mg/kg/day, one doe prematurely delivered on Day 29 and one doe aborted on Day 21. At 30 mg/kg/day, one doe aborted on day 23. These abortions were determined to be unrelated to treatment due to lack of dose-response. The pregnancy rate was 100%, 90%, 95%, and 95% in the 0, 10, 30, and 90 mg/kg/day groups.

b. Clinical Signs

Treatment-related clinical signs of toxicity observed in does at 30 and 90 mg/kg/day are tabulated below:

Dose level [mg/kg/day]	**0**	**10**	**30**	**90**
No. on Study	**20**	**20**	**20**	**20**
Soft or liquid feces	0	0	2	3
Decreased motor activity	0	0	0	9
Myotonia	0	0	0	7
Ataxia	0	0	0	5
Impaired/lost righting reflex	0	0	0	5
Red substance in cage pan	0	0	1	1

Does at the high dose exhibited the above noted clinical signs prior to death/or sacrifice.

c. Body Weight Changes

- No statistically significant differences in mean body weights were seen between does in the vehicle control and any treated groups.

- The three does that died and the one that was sacrificed moribund at 90 mg/kg/day exhibited body weight loss prior to death or sacrifice. Because of these animals, body weight gain was decreased at this dose during Days 6-9 with the reduction reaching statistical significance on Days 6-7. However, following the death of the affected rabbits [Day 10 and Day 18], body weight gains of the surviving does at this dose were comparable to that of the control group.

- Body weight gains were comparable in the four groups during the entire dosing period [Days 6-19] and the entire study [0-29].

Dose mg/kg/day	Mean Body weigh Gain [kg]					
	Days 0-6	Days 6-9	Days 9-12	Dosing	Post dosing	Entire study
				Days 6-19	Days 19-29	Days 0-29
0	0.13	-0.01	0.04	0.10	0.11	0.34
10	0.12	0.01	0.01	0.10	0.08	0.36
30	0.12	-0.01	0.02	0.12	0.10	0.35
90	0.16	-0.08	0.05	0.14	0.14	0.45

d. Food Consumption

- Absolute food consumption values were reduced at all dose levels during Days 6-9 with the difference reaching statistical significance at 90 mg/kg/day. Reduced food consumption was seen only at the high dose during days 9-12 and 12-15; values at 30 and 10 mg/kg/day were comparable to controls on these days.

- Relative food consumption values were significantly reduced relative only at 90 mg/kg/day on days 7-8, 8-9, and 6-9. Reduced food consumption values were also seen on days 9-12 and 12-15; the decreases were not statistically significant.

- Following the deaths of the does at the high dose, the absolute and relative food consumption values were comparable in the four groups.

- During days 19-29 [post-dosing period], does at 90 mg/kg/day, exhibited an increase in food consumption, a common compensatory observation that occurs following cessation of dosing.

e. Macroscopical Examination

No treatment-related macroscopical changes were observed in the dams sacrificed at termination. Treatment-related necropsy finding was limited to a red-brown substance filling the urinary bladder that was seen in one doe sacrificed moribund and in one doe that die at 90 mg/kg/day. Other necropsy finding which were considered unrelated to treatment included fluid-filled abdominal cavity, dark spleen, large or small gall bladder, pale liver or kidneys, hemorrhage areas in the gastric fundic, a gastric trichobezoar, small amounts of thickened dark green substance in the stomach and parovarian cysts.

2. Developmental Toxicity

As summarized in Table 1, cesarean data showed no treatment-related effects. No biologically or statistically significant findings were observed on pregnancy rate, number of corpora lutea, total live fetuses per litter, pre- and post implantation loses, fetal sex ratio or fetal body weights at any dose level. The incidences were within the historical control range of the testing laboratory.

The increase observed in the number of resorptions/litter at 90 mg/kg/day [mean, 0.9±1.1] was not considered to be treatment-related since the increase was not statistically significant when compared to controls [0.4±0.6], was not dose-dependent, and was within the historical control range [0-1.4] of the testing laboratory. Similarly, the increase in does with any resorptions at 90 mg/kg/day [57%] was not considered to be treatment-related due to lack of statistical significance [when compared to concurrent control, 30%] and dose-response.

Table 1. Cesarean Section Observations

Observations	Dose Level [mg/kg/day]			
	0	10	30	90
No. Assigned	20	20	20	20
Females Gravid	20	18	19	19
Maternal Wastage				
# Died	0	0	0	4
# Moribund sacrifice	0	0	0	1
# Aborted	0	1	1	0
# Premature delivery	0	1	0	0
# Non pregnant	0	2	1	1
Total Corpora Lutea	230	171	203	156
Corpora Lutea/dam	11.5±2.0	10.7±2.2	11.3±1.2	11.1±1.6
Total Implantations	177	133	163	112
Implantations/Dam	8.8±2.8	8.3±2.6	9.0±1.8	8.0±2.0
Total Live Fetuses	170	126	155	99
Live Fetuses/Dam	8.5±2.5	7.9±2.9	8.6±2.1	7.1±2.4
Total Dead Fetuses	0	0	0	0
Total Resorptions	7	7	8	13
Early	5	5	5	11
Late	2	2	3	2
Average No. of Resorption	0.4±0.6	0.4±0.6	0.4±0.6	0.9±1.1
No. and % of Litters with Resorptions	6/20 30.0	7/16 43.8	6/18 33.3	8/14 57.1
Pre-implantation Loss [%]	18.8	22.2	19.7	28.2
Post-implantation Loss [%]	4.0	5.3	4.9	11.6
Sex Ratio ♂ / ♀	55 94/76	65/61	57 80/75	52/47
Fetal Weight [g]	43.56	42.90	40.93	44.46

As summarized in Table 2, there were no treatment-related fetal alterations in the number of litters with fetuses with any alterations noted, the number of fetuses with any alterations noted or the average percent of fetuses with any alterations per litter.

Table 2. Summary of Fetal Alterations

Observations	Dose Level [mg/kg/day]			
	0	10	30	90
Litters Evaluated	20	16	18	14
Fetuses Evaluated	170	126	155	99
Litters with Fetuses with any Alterations Observed	18 90%	13 81.2%	16 88.9%	12 85.7%
Fetuses with any Alterations Observed	48 28.2%	38 30.2%	44 28.4%	31 31.3%
Percentage of Fetuses with any Alterations/Litter [Mean±S.D.]	28.78 ± 21.46	29.44 ± 24.01	31.11 ± 20.54	30.20 ± 18.09

The fetal malformations/alterations observed in the 170, 126, 155 and 99 fetuses of does at 0, 10, 30, and 90 mg/kg/day and summarized in Tables 10 thru 14 of the study report [pages 60-70] are appended to this DER. At 90 mg/kg/day, 1 of 99 fetuses [19173-1] had multiple gross external, soft tissue and skeletal malformations as discussed below.

a. External Examinations

The external malformations observed in one fetus at the high dose included: acephaly; thoracogastroschisis [defective closure of the thorax and abdomen with protrusion of the liver, intestines, heart and lungs]; forelimb malformations [the pollex and the nail of the third digit were absent in both forepaws]; and deformations [flexion or rotation] of the forepaws and left hind paw attributable to *in utero* compression.

No other treatment-related or statistically significant external malformations or variations were seen between the control and treated groups.

b. Visceral Examinations

The soft tissue malformations observed in the one fetus at the high dose were malformations of the vessels, kidneys and ureters [persistent truncus arteriosus, fused kidneys and marked dilation of the ureters].

No other treatment-related or statistically significant soft tissue malformations or variations were noted between the control and treated groups.

c. Skeletal Examinations

Skeletal anomalies observed in the one fetus at the high dose included: malformations of the skull, hyoid and sternum and forepaw digits [the skull, hyoid and sternum were absent (not ossified); only six of seven cervical vertebrae were present; the 3rd through 12th thoracic rib pairs were wavy; and the phalanges of the pollex and the third digit in both forepaws were absent]

No other treatment-related or statistically significant skeletal malformations were seen between the control and the treated groups.

Fetal malformations and/or alterations observed in the treated groups were not considered to be compound related since the litter and fetal incidences were: (i) not statistically significant when compared to concurrent controls; (ii) not dose-related; and (iii) within the historical control range for this species/strain/testing laboratory.

IV. DISCUSSION

Oral administration of the dimethylamine salt of 2,4-D to inseminated rabbits at 0, 10, 30, or 90 mg/kg/day during days 6 through 18 of gestation resulted in maternal toxicity manifested as mortality /morbidity, clinical signs [soft or liquid feces, decreased motor activity, myotonia, ataxia and impaired/loss of righting reflex], and decreases in body weight gain and food consumption at 90 mg/kg/day. Except for a slight but transient body weight loss on days 6-7, no adverse maternal toxicity was seen at 30 mg/kg/day. No maternal toxicity was observed at the 10 mg/kg dose.

464

No treatment-related differences were seen in cesarian section parameters between the treated and control groups. No treatment-related external, visceral, or skeletal malformation or variations were seen in any of the fetuses of treated does. The anomalies seen in treated groups were not considered to be treatment related due to lack of dose-response, statistical significance when compared to concurrent controls, and they did not exceed the ranges observed in historical controls of the testing laboratory. Historical control data were provided to support this statement.

In a developmental toxicity study in rats [MRID No.417352-01], 2,4-D DMA [same lot no.] was administered orally at 0, 12.5, 50 or 100 mg/kg/day during days 6-15. For maternal toxicity the NOEL was 12.5 mg/kg/day and the LOEL was 50 mg/kg/day, based on body weight loss, decrease in food consumption and clinical signs [decreased motor activity and ataxia]. For developmental toxicity, the NOEL was 50 mg/kg/day and the LOEL was 100 mg/kg/day, based on reduced litter weight, significant increase in the litter incidence of incompletely and unossified sternebrae, and significant increase in the incidence of wavy and/or incompletely ossified ribs. Developmental anomalies observed in rats at 100 mg/kg/day were not seen in rabbits at a comparable dose [90 mg/kg/day] demonstrating species difference in 2,4-D DMA induced developemental toxicity.

V. CONCLUSION

2,4-D DMA did not induce maternal toxicity at 15 or 30 mg/kg/day, but did at 90 mg/kg/day. 2,4-D DMA was not a developmental toxin in rabbits. Based on the results of this study the following NOELs and LOELs are established:

Maternal Toxicity

NOEL: 30 mg/kg/day
LOEL: 90 mg/kg/day

Developmental Toxicity:

NOEL: 90 mg/kg/day [HDT]
LOEL: Not established

6. CORE CLASSIFICATION

Guideline; this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

467

PROTOCOL 320-004: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D DIMETHYLAMINE SALT (2,4-D-DMA) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 10 (PAGE 1): FETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	20	16[b]	18	14
Fetuses Evaluated	N	170	126	155	99
Live Fetuses	N	170	126	155	99
Dead Fetuses	N	0	0	0	0
Litters with Fetuses with any Alteration Observed	N(%)	18(90.0)	13(81.2)	16(88.9)	12(85.7)
Fetuses with any Alteration Observed	N(%)	48(28.2)	38(30.2)	44(28.4)	31(31.3)
% Fetuses with any Alteration/Litter	$\bar{X}\pm$S.D.	28.78 ± 21.46	29.44 ± 24.01	31.11 ± 20.54	30.20 ± 18.09

a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported as the acid equivalent of 2,4-D contained in the 2,4-D Dimethylamine Salt.
b. Excludes rabbit 19140 which delivered on day 29 of gestation. Seven placentas were found in the cage pan; pups were presumed to be cannibalized.

PROTOCOL 320-004: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D DIMETHYLAMINE SALT (2,4-D-DMA) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 11 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)[a]		0(VEHICLE)	10	30	90
Litters Evaluated	N	20	16[b]	18	14
Fetuses Evaluated	N	170	126	155	99
Live Fetuses	N	170	126	155	99
Dead Fetuses	N	0	0	0	0
HEAD:					
Acephaly					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[c]
BODY:					
Meningomyelocele on Sacral Region of Back					
Litter Incidence	N(%)	0	0	1(5.6)	0
Fetal Incidence	N(%)	0	0	1(0.6)	0
Thoracogastroschisis					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[c]
FORELIMBS:					
Bilateral, Paws, Flexed and Pollex Absent					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[c]
Bilateral, Paws, Digit 3, Nail Absent					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[c]
HINDLIMB:					
Left, Paw, Rotated Medially					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[c]

a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported as the acid equivalent of 2,4-D contained in the 2,4-D Dimethylamine Salt.
b. Excludes rabbit 19140 which delivered on day 29 of gestation. Seven placentas were found in the cage pan; pups were presumed to be cannibalized.
c. Fetus 19173-1 also had other gross external alterations.

PROTOCOL 320-004: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D DIMETHYLAMINE SALT (2,4-D-DMA) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 12 (PAGE 1): FETAL SOFT TISSUE ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	20	16[b]	18	14
Fetuses Evaluated	N	170	126	155	99
Live Fetuses	N	170	126	155	99
Dead Fetuses	N	0	0	0	0
EYE:					
Right, Circumcorneal Hemorrhage					
Litter Incidence	N(%)	2(10.0)	0	0	0
Fetal Incidence	N(%)	2(1.2)	0	0	0
VESSELS:					
Persistent Truncus Arteriosus					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[c]
LUNGS:					
Intermediate Lobe, Agenesis					
Litter Incidence	N(%)	3(15.0)	1(6.2)	1(5.6)	0
Fetal Incidence	N(%)	3(1.8)	2(1.6)	1(0.6)	0
KIDNEYS:					
Fused					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[c]
URETERS:					
Marked Dilation					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[c]

a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported as the acid equivalent of 2,4-D contained in the 2,4-D Dimethylamine Salt.
b. Excludes rabbit 19140 which delivered on day 29 of gestation. Seven placentas were found in the cage pan; pups were presumed to be cannibalized.
c. Fetus 19173-1 also had other soft tissue alterations.

PROTOCOL 320-004: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D DIMETHYLAMINE SALT (2,4-D-DMA) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 1): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	20	16[b]	18	14
Fetuses Evaluated	N	170	126	155	99
Live Fetuses	N	170	126	155	99
Dead Fetuses	N	0	0	0	0
SKULL - IRREGULAR OSSIFICATION[c]:					
(SUMMARIZATION OF ALL IRREGULAR OSSIFICATION OF SKULL[d]; SUMMARIZED AND INDIVIDUAL SUBCATEGORIES CITED BELOW)					
Litter Incidence	N(%)	15(75.0)	12(75.0)	16(88.9)	12(85.7)
Fetal Incidence	N(%)	40(23.5)	34(27.0)	40(25.8)	26(26.3)
SKULL, NOT OSSIFIED					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[h]
NASAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF INTERNASALS; INTRANASALS; IRREGULAR SUTURE; FUSED; MIDLINE SUTURE DISPLACED; NASAL(S)-FRONTAL(S), IRREGULAR SUTURE)					
Litter Incidence	N(%)	13(65.0)	8(50.0)	13(72.2)	11(78.6)
Fetal Incidence	N(%)	23(13.5)	15(11.9)	23(14.8)	20(20.2)
Nasals, Internasal					
Litter Incidence	N(%)	0	1(6.2)	0	1(7.1)
Fetal Incidence	N(%)	0	1(0.8)	0	1(1.0)
Nasal(s), Intranasal					
Litter Incidence	N(%)	0	0	1(5.6)	0
Fetal Incidence	N(%)	0	0	1(0.6)	0
Nasals, Irregular Suture					
Litter Incidence	N(%)	2(10.0)	0	1(5.6)	2(14.3)
Fetal Incidence	N(%)	2(1.2)	0	1(0.6)	3(3.0)
Nasals, Partially Fused					
Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)	0	0

PROTOCOL 320-004: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D DIMETHYLAMINE SALT (2,4-D-DMA) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS.

TABLE 13 (PAGE 2): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	20	16[b]	18	14
Fetuses Evaluated	N	170	126	155	99
Live Fetuses	N	170	126	155	99
Dead Fetuses	N	0	0	0	0
SKULL - IRREGULAR OSSIFICATION (CONTINUED)[c]:					
Nasals, Midline Suture Displaced					
Litter Incidence	N(%)	9(45.0)	8(50.0)	10(55.6)	9(64.3)
Fetal Incidence	N(%)	16(9.4)	11(8.7)	15(9.7)[f]	14(14.1)[j]
Nasal(s)-Frontal(s), Irregular Suture					
Litter Incidence	N(%)	5(25.0)	3(18.8)	7(38.9)	3(21.4)
Fetal Incidence	N(%)	7(4.1)	3(2.4)	7(4.5)	3(3.0)
FRONTAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF INTERFRONTALS; INTRAFRONTALS; IRREGULAR SUTURE)					
Litter Incidence	N(%)	11(55.0)	11(68.8)	10(55.6)	5(35.7)
Fetal Incidence	N(%)	23(13.5)	22(17.5)	18(11.6)	7(7.1)
Frontals, Interfrontal					
Litter Incidence	N(%)	4(20.0)	5(31.2)	6(33.3)	2(14.3)
Fetal Incidence	N(%)	6(3.5)	7(5.6)	9(5.8)	2(2.0)
Frontal(s), Intrafrontal					
Litter Incidence	N(%)	1(5.0)	2(12.5)	2(11.1)	1(7.1)
Fetal Incidence	N(%)	2(1.2)	2(1.6)	2(1.3)	1(1.0)
Frontals, Irregular Suture					
Litter Incidence	N(%)	9(45.0)	9(56.2)	5(27.8)	4(28.6)
Fetal Incidence	N(%)	15(8.8)	13(10.3)[e]	7(4.5)	5(5.0)
PARIETAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF INTERPARIETAL; INTRAPARIETAL)					
Litter Incidence	N(%)	0	1(6.2)	0	1(7.1)
Fetal Incidence	N(%)	0	1(0.8)	0	1(1.0)

PROTOCOL 320-004: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D DIMETHYLAMINE SALT (2,4-D-DMA) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 3): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	20	16[b]	18	14
Fetuses Evaluated	N	170	126	155	99
Live Fetuses	N	170	126	155	99
Dead Fetuses	N	0	0	0	0
SKULL - IRREGULAR OSSIFICATION (CONTINUED)[c]:					
Parietals, Interparietal					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)
Parietal, Intraparietal					
Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)	0	0
HYOID[c]:					
Not Ossified					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[h]
Alae, Angulated					
Litter Incidence	N(%)	1(5.0)	0	1(5.6)	1(7.1)
Fetal Incidence	N(%)	1(0.6)	0	1(0.6)[g]	1(1.0)
Alae, Short					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)
VERTEBRAE:					
Cervical, 6 Present					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[h]
Thoracic, Hemivertebra					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[i]

PROTOCOL 320-004: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D DIMETHYLAMINE SALT (2,4-D-DMA) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 4): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	20	16[b]	18	14
Fetuses Evaluated	N	170	126	155	99
Live Fetuses	N	170	126	155	99
Dead Fetuses	N	0	0	0	0
VERTEBRAE (CONTINUED):					
Thoracic, Arch, Small					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[i]
Lumbar, Hemivertebra					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)
Caudal, Misaligned					
Litter Incidence	N(%)	1(5.0)	0	0	0
Fetal Incidence	N(%)	1(0.6)	0	0	0
Caudal, Fused					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[h]
VERTEBRAL/RIB MALFORMATIONS:					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[i]
RIBS:					
Fused					
Litter Incidence	N(%)	0	1(6.2)	1(5.6)	0
Fetal Incidence	N(%)	0	1(0.8)[e]	1(0.6)[f]	0
Split					
Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)[e]	0	0

PROTOCOL 320-004: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D DIMETHYLAMINE SALT (2,4-D-DMA) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 5): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	20	16[b]	18	14
Fetuses Evaluated	N	170	126	155	99
Live Fetuses	N	170	126	155	99
Dead Fetuses	N	0	0	0	0
RIBS (CONTINUED):					
Thickened					
Litter Incidence	N(%)	2(10.0)	2(12.5)	1(5.6)	0
Fetal Incidence	N(%)	2(1.2)	2(1.6)	1(0.6)	0
Wavy					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[h]
Proximate					
Litter Incidence	N(%)	1(5.0)	1(6.2)	0	1(7.1)
Fetal Incidence	N(%)	1(0.6)	1(0.8)[e]	0	1(1.0)[i]
Extra					
Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)[e]	0	0
MANUBRIUM:					
Fused to Sternebra					
Litter Incidence	N(%)	0	0	1(5.6)	0
Fetal Incidence	N(%)	0	0	1(0.6)[g]	0
Not Ossified					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[h]
STERNEBRAE:					
Incompletely Ossified					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)

PROTOCOL 320-004: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D DIMETHYLAMINE SALT (2,4-D-DMA) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 6): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	20	16[b]	18	14
Fetuses Evaluated	N	170	126	155	99
Live Fetuses	N	170	126	155	99
Dead Fetuses	N	0	0	0	0
STERNEBRAE (CONTINUED):					
Not Ossified					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[h]
Fused					
Litter Incidence	N(%)	1(5.0)	0	1(5.6)	1(7.1)
Fetal Incidence	N(%)	1(0.6)	0	1(0.6)[g]	1(1.0)[j]
Asymmetric					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)
XIPHOID:					
Not Ossified					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[h]
FOREPAWS:					
Pollex, Absent					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[h]
Digits, Short					
Litter Incidence	N(%)	0	0	0	1(7.1)
Fetal Incidence	N(%)	0	0	0	1(1.0)[h]

PROTOCOL 320-004: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D DIMETHYLAMINE SALT (2,4-D-DMA) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 7): FETAL SKELETAL ALTERATIONS - SUMMARY

FOOTNOTES

a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported as the acid equivalent of 2,4-D contained in the 2,4-D Dimethylamine Salt.
b. Excludes rabbit 19140 which delivered on day 29 of gestation. Seven placentas were found in the cage pan; pups were presumed to be cannibalized.
c. Fetuses with alterations of the skull and/or hyoid are not separately identified in this summary table, except when alterations of other ossification sites were also present.
d. Includes all findings noted for the skull except hyoid, ala(e), angulated; ala, short. These categories are excluded because they are not considered to result from irregular ossification.
e. Fetus 19149-4 also had other skeletal alterations.
f. Fetus 19160-8 also had other skeletal alterations.
g. Fetus 19164-1 also had other skeletal alterations.
h. Fetus 19173-1 also had other skeletal alterations.
i. Fetus 19185-2 also had other skeletal alterations.
j. Fetus 19186-8 also had other skeletal alterations.

PROTOCOL 320-004: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D DIMETHYLAMINE SALT (2,4-D-DMA) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 14 (PAGE 1): FETAL OSSIFICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
LITTERS EXAMINED	N	20	16	18	14
FETUSES EXAMINED	N	170	126	155	99
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	1.00 ± 0.02	0.99 ± 0.02	1.00 ± 0.00	1.00 ± 0.00
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	12.60 ± 0.27	12.64 ± 0.33	12.64 ± 0.32	12.71 ± 0.33
LUMBAR	MEAN±S.D.	6.40 ± 0.26	6.36 ± 0.33	6.36 ± 0.32	6.28 ± 0.33
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	16.92 ± 0.37	16.85 ± 0.41	16.93 ± 0.27	17.18 ± 0.32
RIBS (PAIRS)	MEAN±S.D.	12.50 ± 0.25	12.56 ± 0.32	12.59 ± 0.33	12.68 ± 0.34
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.96 ± 0.10	3.94 ± 0.09	3.92 ± 0.15	3.96 ± 0.08
XIPHOID	MEAN±S.D.	0.94 ± 0.09	0.92 ± 0.16	0.89 ± 0.17	0.93 ± 0.16
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	5.00 ± 0.00	4.93 ± 0.20	4.95 ± 0.13	4.98 ± 0.06
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	13.99 ± 0.04	13.92 ± 0.21	13.94 ± 0.10	13.99 ± 0.03
HINDLIMB b					
TARSALS	MEAN±S.D.	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
METATARSALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
PHALANGES	MEAN±S.D.	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00

a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported as the acid equivalent of 2,4-D contained in the 2,4-D Dimethylamine Salt.
b. Calculated as average per limb.

Skeletal Malformations

No. Fetuses Evaluated for Skeletal Malformations: 4,469
No. of Litters Evaluated: 526
No. of Study Groups: 23

Observations	No. Fetuses Affected	No. Litters with Malf. Fetus
7 lumbar vertebrae:	1	1
5 lumbar vertebrae:	5	5
Additional sternebrae:	1	1
Fused sternebrae:	1	1
Angulated rib(s):	2	2
Cervical rib:	1	1
Cervical vertebral transverse processes fused:	3	3
Exoccipital fused to 1st cervical vertebral transverse process:	1	1
Angulated/wavy ribs:	1	1
Wavy ribs:	27	21
Wavy ribs with distal portion unossified:	2	2
Thoracic vertebral transverse process missing (unilateral) and misaligned thoracic vertebral centra and fused ribs:	1	1
Thoracic vertebral centra - misaligned:	1	1
1st cervical vertebral transverse process absent:	1	1
Cranial bones reduced in size, some cranial bones are not present (supraoccipital, interparietal, exoccipital), clavicle angulated, cervical vertebral transverse processes absent (fetus noted externally with exencephaly):	1	1

Soft Tissue Malformations (cont.)

No. of Fetuses Evaluated for Soft Tissue Malformations: 3,757
No. of Litters Evaluated: 527
No. of Study Groups: 23

Observations	No. Fetuses Affected	No. Litters with Malf. Fetus
Liver - dark area (variation):	1	1
Liver - necrotic (variation):	3	3
Spleen - pale (variation):	1	1
Spleen - absent:	1	1
Uterus and vagina absent:	1	1
Lungs - red foci (variation):	3	3
Adrenal(s) absent:	1	1
- enlarged (variation):	1	1
- discolored (variation):	2	2
Testes		
- prominent vascularity (variation):	1	1
- absence of structure:	1	1
Stomach		
- contains no fluid (variation):	2	2
Kidney - distended pelvis without papillae present[1]	4	4
Absence of innominate artery off the aortic arch (variation):	2	2

[1] Not to be confused with distended renal pelvis with a papillae present (a soft tissue variation).

External Malformations

Total No. of Fetuses Evaluated Externally: 7,193
Total No. of Litters Evaluated: 527
Total No. of Study Groups: 23

Observations	No. Fetuses Affected	No. Litters with Malf. Fetus
Filamentous tail - alone:	1	1
Pale color (variation):	7	6
Tail short - alone:	1	1
Dark purple area (variation):		
- foreleg:	1	1
- tip of tail:	2	2
- lower thoracic:	1	1
- midlegs:	1	1
Umbilical hernia:	1	1
Omphalocele:	1	1
Glassy (shiny) appearance (variation):	18	5
- with dark purple area on dorsal surface	1	1
Indentation on side of head:	1	1
Swollen dark area - top of skull:	1	1
Exencephaly:	1	1
Cleft lip:	1	1

Q-3
Appendix Q (cont.)
Bio/dynamics, Inc.
Historical Control Data - CD Rat
1982-1987

Reproduction Data and Mean Fetal Weights

Study No.	No. Females P/NP (M)	Preg. Rate %	Mean/Pregnant Females C.L.	Impl.	Resorp.	L.F. (D.F.)	Fetal Weight (grams) Male	Female	Both
X	24/0 (0)	100.0	18.3	15.5	0.7	14.8 (0)	3.30	3.14	-
Y	24/1 (0)	96.0	16.4	15.4	0.8	14.6 (0)	3.70	3.53	-
Z	23/1 (0)	95.8	16.7	14.4	0.8	13.6 (0)	3.27	3.12	-
AA	24/0 (0)	100.0	16.4	14.1	0.5	13.6 (0)	3.23	3.07	-
BB	23/1 (1)	95.8	16.1	14.4	0.6	13.7 (0)	3.48	3.34	-
CC	22/2 (0)	91.7	15.6	14.3	0.8	13.5 (0)	3.61	3.40	-
DD	17/8 (0)	68.0	14.3	11.5	0.7	10.8 (0)	3.46	3.21	-
EE	24/2 (0)	92.3	15.9	14.8	1.0	13.8 (0)	3.77	3.56	3.67
FF	24/0 (0)	100.0	16.4	15.3	1.1	14.2 (0)	3.36	3.21	3.28
GG	23/1 (0)	95.8	14.8	13.5	0.7	12.8 (0)	3.75	3.57	3.67
HH	23/1 (0)	95.8	15.1	14.3	0.8	13.5 (0)	3.76	3.58	3.67
II	24/0 (0)	100.0	16.1	14.4	0.4	14.0 (0)	3.75	3.58	3.67
JJ	23/1 (0)	95.8	15.0	13.6	0.7	12.9 (0)	3.79	3.58	3.69
KK	21/3 (0)	87.5	15.4	14.4	0.9	13.5 (1)	3.66	3.47	3.58
LL	23/1 (0)	95.8	13.5	12.7	0.3	12.3 (0)	3.55	3.38	3.46
MM	24/0 (0)	100.0	14.5	13.3	0.8	12.5 (0)	3.87	3.66	3.76
NN	24/0 (0)	100.0	15.0	13.3	0.4	13.0 (0)	3.50	3.33	3.42
OO	23/1 (0)	95.8	15.2	13.9	0.3	13.6 (0)	3.67	3.47	3.56
PP	24/0 (0)	100.0	16.8	15.0	0.4	14.6 (0)	3.61	3.43	3.52
QQ	21/3 (0)	87.5	16.6	15.1	0.6	14.5 (0)	3.63	3.42	3.53
RR	24/0 (0)	100.0	16.3	14.7	0.5	14.1 (0)	3.50	3.31	3.41
SS	25/0 (0)	100.0	16.3	14.9	0.8	14.1 (0)	3.72	3.57	3.65
TT	22/2 (0)	91.7	16.3	15.1	0.7	14.4 (0)	3.86	3.63	3.74
Totals:	529/28	2185.3	363.0	327.9	15.3	312.4	82.80	78.56	57.28
Mean:	(95.0)	95.0	15.8	14.3	0.7	13.6	3.60	3.42	3.58
Range									
- low:		68.0	13.5	11.5	0.3	10.8	3.23	3.07	3.28
- high:		100.0	18.3	15.5	1.1	14.8	3.87	3.66	3.76
N:		23	23	23	23	23	23	23	16

Key: P = pregnant
C.L. = corpora lutea
L.F. = live fetuses
Resorp. = resorption sites
NP = not pregnant
Impl. = implantation sites
D.F. = dead fetuses
M = mortality

Appendix Q (cont.)
Bio/dynamics, Inc.
Historical Control Data - CD Rat
1982-1987

Dates of Sacrifice and Fetal Inventory

Study No.	Supplier	Dates of Sacrifice	Number of Fetuses Evaluated (Litters) External	Soft Tissue	Skeletal
X	Kin	6/7-24/82	355 (24)	182	355
Y	Por	10/18-11/4/82	350 (24)	180	350
Z	Kin	5/17-6/4/82	313 (23)	162	313
AA	Kin	7/6-21/82	326 (24)	170	326
BB	Kin	12/6-23/82	302 (22)	157	302
CC	Kin	7/5-18/83	298 (22)	155	143
DD	Kin	4/27-5/16/83	183 (16)	183	183
EE	Wil	12/6-20/83	331 (24)	173	159
FF	Kin	12/27/83-1/10/84	341 (24)	178	162
GG	Wil	6/5-18/84	295 (23)	151	144
HH	Wil	9/21-10/10/84	310 (23)	162	148
II	Wil	7/5-18/84	337 (24)	176	161
JJ	Wil	8/30-9/19/84	297 (23)	154	143
KK	Por	12/27/84-1/16/85	284 (21)	147	137
LL	Wil	3/14-28/85	284 (23)	147	137
MM	Wil	4/30-5/16/85	301 (24)	157	144
NN	Por	4/9-22/85	311 (24)	161	150
OO	Por	12/10-30/85	312 (23)	163	149
PP	Por	11/19-12/5/85	351 (24)	181	170
QQ	Por	9/3-18/85	304 (21)	94	210
RR	Por	5/29-6/26/86	339 (24)	176	163
SS	Por	9/2-17/86	353 (25)	185	168
TT	Por	12/30-1/15/87	316 (22)	163	153
		Totals:	7193 (527)	3757	4470

No. of Studies = 23
No. of Control Groups = 23

Key: Wil = Wilmington, Mass.
Por = Portage, Mich.
Kin = Kingston, N.Y.

Appendix Q (cont.)
Bio/dynamics Inc.
Historical Control Data - CD Rat
1983-1987

Pre- and Post-Implantation Loss Indices
and Incidence of Litters with Resorptions

Project No.[a]	Mean Pre-Implantation Loss Index	Mean Resorption to Implantation Ratio	Incidence of Litters with Resorptions No.	Incidence of Litters with Resorptions %
(EE)	0.071	0.061	10/24	41.7
(FF)	0.066	0.070	17/24	70.8
(GG)	0.084	0.049	11/23	47.8
(HH)	0.056	0.060	13/23	56.5
(II)	0.094	0.024	6/24	25.0
(JJ)	0.096	0.049	10/23	43.5
(KK)	0.080	0.062	11/21	52.4
(LL)	0.060	0.028	5/23	21.7
(MM)	0.077	0.052	13/24	54.2
(NN)	0.121	0.027	8/24	33.3
(OO)	0.081	0.025	6/23	26.1
(PP)	0.082	0.028	8/24	33.3
(QQ)	0.087	0.043	10/21	47.6
(RR)	0.090	0.037	9/24	37.5
(SS)	0.074	0.052	15/25	60.0
(TT)	0.071	0.051	11/22	50.0
Total:	1.290	0.718		701.4
Mean:	0.081	0.045		43.8
Range:	0.056 - 0.121	0.024 - 0.070		21.7 - 70.8

[a] Pre- and post-implantation loss indices were not calculated and presented in reports prior to project (EE).

Soft Tissue Malformations

No. of Fetuses Evaluated for Soft Tissue Malformations: 3,757
No. of Litters Evaluated: 527
No. of Study Groups: 23

Observations	No. Fetuses Affected	No. Litters with Malf. Fetus
Distended lateral ventricles of brain:	9	9
Distended cerebral ventricles and diencephalon portion of brain is small:	1	1
Red material surrounding cerebral hemispheres (variation):	1	1
Microphthalmia (unilateral) - - with distended lateral ventricles of the brain:	1	1
Anophthalmia - alone:	2	2
Folded retina:	1	1
Eye - conjunctival sac contains red material (variation):	2	2
Situs inversus: - of abdominal viscera, right lung exists as a single lobe and ectopic (R) adrenal:	1	1
- of abdominal viscera, persistent truncus arteriosus, heart misshapened, ventricular septal defect:	1	1
Cleft palate (not noted at external exam.):	1	1
Cleft lip:	1	1
Heart - indentation at apex:	1	1
Heart - cherry shaped:	1	1
Heart - persistent truncus arteriosus and interventricular septal defect:	1	1
Subclavian (R) artery passes dorsal to trachea/esophagus:	1	1

Appendix Q (cont.)
Bio/dynamics, Inc.
Historical Control Data - CD Rat
1982-1987

Soft Tissue Variation Data

	Number Fetuses (litters) with								
	Distended Renal Pelvis Only [Fetuses]			Distended Renal Pelvis & Distended Ureter [% Fetuses]			Distended or Tortuous Ureter [% Fetuses]		
X	4/182	(3/24)	[2.2]	0/182	(0/24)	[0.0]	1/182	(1/24)	[0.5]
Y	1/180	(1/24)	[0.6]	0/180	(0/24)	[0.0]	12/180	(8/24)	[6.7]
Z	2/162	(2/23)	[1.2]	1/162	(1/23)	[0.6]	4/162	(4/23)	[2.5]
AA	4/170	(4/24)	[2.4]	2/170	(2/24)	[1.2]	5/170	(5/24)	[2.9]
BB	5/157	(5/22)	[3.2]	8/157	(6/22)	[5.1]	13/157	(10/22)	[8.3]
CC	1/155	(1/22)	[0.6]	0/155	(0/22)	[0.0]	14/155	(7/22)	[9.0]
DD	1/183	(1/16)	[0.5]	3/183	(2/16)	[1.6]	23/183	(10/16)	[12.6]
EE	7/173	(4/24)	[4.0]	2/173	(2/24)	[1.2]	8/173	(5/24)	[4.6]
FF	1/178	(1/24)	[0.6]	3/178	(3/24)	[1.7]	4/178	(4/24)	[2.2]
GG	0/151	(0/23)	[0.0]	2/151	(2/23)	[1.3]	2/151	(2/23)	[1.3]
HH	1/162	(1/23)	[0.6]	2/162	(1/23)	[1.2]	9/162	(6/23)	[5.6]
II	1/176	(1/24)	[0.6]	2/176	(2/24)	[1.1]	4/176	(4/24)	[2.3]
JJ	0/154	(0/23)	[0.0]	0/154	(0/23)	[0.0]	1/154	(1/23)	[0.6]
KK	11/147	(7/21)	[7.5]	5/147	(5/21)	[3.4]	3/147	(2/21)	[2.0]
LL	3/147	(2/23)	[2.0]	1/147	(1/23)	[0.7]	5/147	(4/23)	[3.4]
MM	10/157	(7/24)	[6.4]	1/157	(1/24)	[0.6]	1/157	(1/24)	[0.6]
NN	4/161	(4/24)	[2.5]	3/161	(2/24)	[1.9]	5/161	(4/24)	[3.1]
OO	0/163	(0/23)	[0.0]	0/163	(0/23)	[0.0]	2/163	(2/23)	[1.2]
PP	1/181	(1/24)	[0.6]	0/181	(0/24)	[0.0]	2/181	(2/24)	[1.1]
QQ	3/94	(3/21)	[3.2]	0/94	(0/21)	[0.0]	2/94	(2/21)	[2.1]
RR	0/176	(0/24)	[0.0]	0/176	(0/24)	[0.0]	1/176	(1/24)	[0.6]
SS	1/185	(1/25)	[0.5]	0/185	(0/25)	[0.0]	5/185	(3/25)	[2.7]
TT	0/163	(0/22)	[0.0]	3/163	(2/22)	[1.8]	3/163	(1/22)	[2.5]
Fetuses	61/3757		[1.6]	38/3757		[1.0]	129/3757		[3.4]
Range:	0.0%-7.5%			Range: 0.0-5.1%			Range: [0.0-12.6]		

Litters: 49/1527 [3.2%]

No. of Studies = 23
No. of Control Groups = 23

[2-ETHYLHEXYL ESTER OF 2,4-D] Developmental Toxicity Study - Rat [OPPTS 870.3700]

Amendment to DER for MRID 42304601 - Developmental Toxicity Study - Rat [2-ETHYL HEXYL ESTER OF 2,4-D]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 009697]. MRID# 42383601

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 2/13/03
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Whylng for V. Dobozy 2/14/03
Reregistration Branch I, Health Effects Division (7509C)
TXR#005/360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rat § 83-3(a)/OPPTS 870.3700
P.C. CODE: 030063
CASWELL NO.:315AS CAS NO.: 1928-43-4
TEST MATERIAL (PURITY) : 2-ethylhexyl ester of 2,4-D [95%; 63.25% acid equivalent]
SYNONYMS: 2,4-D EHE; 2,4-Dichlorophenoxyacetic acid, isooctyl (2-ethylhexyl) ester
SPONSOR: Industry Task Force II on 2,4-D Research Data

CITATION: Martin, T. (1992). Developmental Toxicity [Embryo-Fetal Toxicity and Teratogenic Potential] Study of 2, 4-D 2-Ethylhexyl Ester [2,4-D Isooctyl Ester] Administered Orally *via* Gavage to Crl:CD BR VAF/Plus Presumed Pregnant Rats. Argus Research Laboratories, PA; Study No. Argus 320-005. MRID 42304601. Document No. 009697. Unpublished.

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 42304601], female Sprague-Dawley Crl:CD BR VAF/Plus rats [25/group] were administered the 2-ethylhexyl ester of 2,4-dichlorophenoxyacetic acid [95% a.i.; 63.25% acid equivalent] *via* gavage at dose levels of 0 [aqueous 1% methylcellulose], 10 mg/kg/day, 30 mg/kg/day, and 90 mg/kg/day [of the acid] from gestation day 6 through gestation day 15.

There were no deaths. Clinical signs attributed to treatment were ataxia in 4 high-dose dams [days 6, 9, 13-15; 6; 13; 13], decreased motor activity [1 high-dose dam on days 7, 9, 11, 14-16], bradypnea in one high-dose dam [days 14-15], and an increase in the presence of a red perivaginal substance during gestation days 13-15 in 8 mid- and 12 high-dose dams. Body weights were comparable among the groups throughout the study, but there was a decrease in body-weight gain initially after the start of dosing [days 6-9: 75%, 72%, and 72% of control with increasing dose] and for the entire dosing period at the high-dose level [88% of control]. Corrected body-weight gains were comparable among the groups. A lower food consumption was observed at the high-dose level during the dosing period.

There were no abortions or early deliveries, and the pregnancy rate was comparable among the groups. The numbers of corpora lutea, implantation, and live fetuses were comparable among the groups, and the numbers of resorptions, as well as pre- and post-implantation losses were not adversely affected. There were no litters with 100% resorptions. There were no dead fetuses. Fetal body weight was significantly reduced [94% of

control] at the high-dose level compared to the control, which may be attributed to the larger number of fetuses per litter. The sex ratio was comparable among the groups. There were no statistically-significant differences in the incidence of fetal external or visceral malformations or variations. There was an increased incidence of fetuses with any alteration observed at the mid- [23 fetuses; 6%] and high-[31 fetuses; 8%, statistically-significant] dose levels compared to the control [13 fetuses; 4%] and low dose [12 fetuses; 3%], and a dose-related increase in the percent of fetuses with any alteration/litter [3.4%, 4.5%, 6.1%, and 7.9% with increasing dose]. The litter incidence of fetuses with any alteration was increased at the mid- [62.5%] and high- [50%] dose levels compared to the control and low-dose groups [both 40.9%], although there was no dose-response. For the litter incidence, the historical control incidence was exceeded at both the mid- and high-dose levels. The fetal incidence of several skeletal malformations was increased at the high-dose level compared to the control, which displayed an incidence of 0% [wavy ribs: 3.2%, 1%, and 3.9%, at the low-, mid-, and high-dose levels, respectively; fused sternebrae: 1 fetus [0.5%] at high dose; bent scapulae: one fetus [0.5%] at high dose]. Delayed sternebrae ossification; i.e., incompletely or unossified sternebrae, occurred more frequently at the mid- [13 fetuses; 6.7%] and high- [18 fetuses; 8.7%; statistically significant] dose levels compared to the control [8 fetuses; 4.4%] and low- [3 fetuses; 1.6%] dose groups. On a litter basis, the incidence of incompletely or unossified sternebrae was increased at the mid- [12 litters; 50%, statistically significant] and high-dose [9 litters; 38%] levels compared to the control [6 litters; 27%] and low-dose [2 litters; 9%]. Although there was no dose-response for the litter incidence, the historical control incidence was exceeded at both dose levels. Additionally, the fetal incidence at the mid- and high-dose levels each exceeded the historical control.

The maternal toxicity NOAEL is 10 mg/kg/day, and the maternal toxicity LOAEL is 30 mg/kg/day, based on decreased body-weight gain [gestation days 6-9] and an increased incidence of clinical signs [perivaginal staining]. The NOAEL for developmental toxicity is 10 mg/kg/day, and the developmental toxicity LOAEL is 30 mg/kg/day, based on an increased incidence of delayed sternebrae ossification.

This developmental toxicity study is classified Acceptable/Guideline,and it satisfies the guideline requirement [OPPTS 870.3700; §83-3(a)] for a developmental toxicity study in the rodent.

COMMENT: Refer to MRID 42304602; TXR# 009697 for dose selection information.

[2-ETHYLHEXYL ESTER OF 2,4-D] Developmental Toxicity Study - Rat [OPPTS 870.3700]

Table 1. Maternal Body Weight/Gravid Uterine Weight/Corrected Body Weight/Gain [grams]

Dose✦/Day	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
body weight				
0	265±13	267±12	267±12	267±12
6	302±16	305±14	303±14	302±13
9	320±18	318±14	316±15	315±15
12	342±16	343±15	339±17	337±15
16	378±20	382±18	374±19	369±19
20	452±25	462±25	452±24	446±21
body-weight gain				
0-6	37±5.6	37±7.3	36±6.7	35±7.8
6-9	18±5.0	14±4.0* [77]√	13±4.2** [74]	13±7.0** [74]
9-12	22±5.8	25±6.0	23±5.5	22±4.5
12-16	36±8.9	30±9.8	35±6.5	32±11.7 [89]
16-20	74±11.0	80±12.2	78±9.3	77±11.2
6-16	76±9.4	77±12.0	71±10.8 [94]	67±13.1** [88]
0-20	188±10.0	194±10.9	186±19.8	179±14.2 [96]
gravid uterine weight	89.7±15.4	95.2±20.7	91.6±20.4	88.6±11.1
corrected body weight	363±20	366±19	361±18	358±16
corrected BWG [0-20]	98±14.3	99±13.1	94±11.4 [96]	91±11.6 [93]

✦ acid-equivalent dose; √[% of control]; * $p<0.05$; ** $p<0.01$; data from Tables 3-4, pages 44-46 of the report

Table 2. Maternal Food Consumption

Dose✦/Day	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
grams/rat/day				
0-6	27±2.2	27±2.2	26±3.1	26±1.0
6-9	27±3.0	27±2.2	26±2.5	261±3.2 [95]√
9-12	29±2.3	29±2.7	29±2.9	27±2.3* [93]
12-16	30±2.1	31±2.4	30±2.3	27±2.2** [90]
16-20	31±2.7	33±2.3	33±2.7	31±1.9
6-16	29±2.2	29±2.1	28±2.3	27±2.0** [92]
0-20	29±2.1	29±1.9	29±2.2	27±1.6* [95]
grams/kg/day				
0-6	94±4.7	93±5.7	92±10.2	91±5.3
6-9	90±3.6	87±4.5	85±6.4	83±8.7** [93]
6-16	86±3.5	86±4.1	85±4.6	80±4.3** [93]
0-20	82±2.8	82±3.5	82±4.0	79±2.8** [96]

✦ acid-equivalent dose; √[% of control]; * $p<0.05$; ** $p<0.01$; data from Tables 5-6, pages 47-48 of the report

[2-ETHYLHEXYL ESTER OF 2,4-D] Developmental Toxicity Study - Rat [OPPTS 870.3700]

Table 3. Fetal Skeletal Alterations

Dose✦/Day	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
VERTEBRAE				
thoracic, centra, unilateral ossification				
litter incidence N[%]	1[4.5]	0	0	0
fetal incidence N[%]	1[0.5]♪	0	0	0
thoracic centra, not ossified				
litter incidence N[%]	1[4.5]	0	0	0
fetal incidence N[%]	1[0.5]♪	0	0	0
thoracic, centra, bifid				
litter incidence N[%]	1[4.5]	1[4.5]	0	0
fetal incidence N[%]	1[0.5]♪	1[0.5]	0	0
lumbar, centrum, unilateral ossification				
litter incidence N[%]	1[4.5]	0	0	0
fetal incidence N[%]	1[0.5]♪	0	0	0
lumbar, arches, incompletely ossified				
litter incidence N[%]	1[4.5]	2[9.1]	0	2[8.3]
fetal incidence N[%]	1[0.5]♪	2[1.0]✪✯	0	2[1.0]
RIBS				
incompletely ossified (hypoplastic)				
litter incidence N[%]	0	2[9.1]	1[4.2]	2[8.3]
fetal incidence N[%]	0	3[1.6]✪✯✿	1[0.5]■	3[1.4]
wavy				
litter incidence N[%]	0	4[18.2]	1[4.2]	3[12.5]
fetal incidence N[%]	0	6[3.2]✪✯✿	2[1.0]■▼	8[3.9]
cervical rib at 7th cervical vertebrae				
litter incidence N[%]	0	0	1[4.2]	0
fetal incidence N[%]	0	0	1[0.5]	0
STERNEBRAE				
incompletely ossified				
litter incidence N[%]	5[22.7]	1[4.5]	9[37.5]	7[29.2]
fetal incidence N[%]	5[2.7]♬	2[1.0]	10[5.1]✢✣✤✥	9[4.3]
not ossified				
litter incidence N[%]	2[9.1]	1[4.5]	3[12.5]	5[20.8]
fetal incidence N[%]	3[1.6]✚	1[0.5]	3[1.5]◙	9[4.3]
fused				
litter incidence N[%]	0	0	0	1[4.2]
fetal incidence N[%]	0	0	0	1[0.5]
asymmetric				
litter incidence N[%]	0	0	1[4.2]	0
fetal incidence N[%]	0	0	1[0.5]◙	0
STERNEBRAE SUMMARIZATION				
litter incidence N[%]	6[27.3]	2[9.1]	12[50.0]*	9[37.5]
fetal incidence N[%]	8[4.4]	3[1.6]	13[6.7]	18[8.7]*
SCAPULAE				
bent				
litter incidence N[%]	0	0	0	1[4.2]
fetal incidence N[%]	0	0	0	1[0.5]

[2-ETHYLHEXYL ESTER OF 2,4-D] Developmental Toxicity Study - Rat [OPPTS 870.3700]

Table 3. Fetal Skeletal Alterations				
Dose✦/Day	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
PELVIS				
pubes, incompletely ossified				
litter incidence N[%]	3[13.6]	0	6[25.0]	4[16.7]
fetal incidence N[%]	4[2.2]✚♬	0	10[5.1]■▼✢✣✤✤	7[3.4]
ischia, incompletely ossified				
litter incidence N[%]	1[4.5]	1[4.5]	1[4.2]	4[16.7]
fetal incidence N[%]	1[0.5]♬	1[0.5]✯	1[0.5]✤	5[2.4]
pubes, not ossified				
litter incidence N[%]	1[4.5]	0	0	1[4.2]
fetal incidence N[%]	1[0.5]♪	0	0	1[0.5]
ischia, not ossified				
litter incidence N[%]	1[4.5]	0	0	0
fetal incidence N[%]	1[0.5]♪	0	0	0
PELVIS SUMMARIZATION				
litter incidence N[%]	4[18.2]	1[4.5]	6[25.0]	4[16.7]
fetal incidence N[%]	5[2.7]	1[0.5]	10[5.1]	9[4.3]
litters evaluated	22	22	24	24
fetuses evaluated	182	190	195	207

✦ acid-equivalent dose; * $p<0.05$; ♪♬✚ ■▼✢✣✤✤◙ fetus had other skeletal alterations; data from Table 12, pages 54-57 of the report

Table 4. Historical Control Data on Fetal Skeletal Alterations				
ALTERATION	number [%]		range/study # [%]	
	litter incidence	fetus incidence	litter incidence	fetus incidence
STERNEBRAE				
one or more incompletely ossified or not ossified	38 [6.85]	47 [1.10]	0-4 [0-18.2]	0-9 [0-5.5]
RIBS				
one of more incompletely ossified (hypoplastic) or not ossified	2 [0.36]	2 [0.05]	0-1 [0-4.3]	0-1 [0-0.6]
one or more wavy	4 [0.72]	4 [0.09]	0-1 [0-4.3]	0-1 [0-0.6]
PELVIS				
pubis(es) and/or ischium(a) incompletely or not ossified	50 [9.01]	71 [1.67]	0-5 [0-31.2]	0-9 [0-5.5]
pubis(es) incompletely ossified	43 [7.75]	58 [1.36]	0-4 [0-25.0]	0-8 [0-4.5]
pubis(es) not ossified	5 [0.90]	9 [0.21]	0-1 [0-6.2]	0-3 [0-1.8]
ischium(a) incompletely ossified or not ossified	13 [2.34]	14 [0.33]	0-2 [0-8.7]	0-2 [0-1.2]

26 studies from 1989-1990; 555 litters; 4258 fetuses; data from page 378 of the report

491

009697

PRIMARY REVIEWER: Jess Rowland, Toxicologist 8/14/92
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II 8/19/92

DATA EVALUATION REPORT

2. MAIN STUDY--RATS

STUDY TYPE: Developmental Toxicity **[Main Study] GUIDELINE:** 83-3(b)

CASWELL NO. 315 AS MRID No. 423036-01 DP Barcode: 179762

TEST MATERIAL: 2-Ethylhexyl Ester of 2,4-D **[2,4-D-EHE]**

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

STUDY IDENTIFICATION: Argus **320-005**

TESTING LABORATORY: Argus Research Laboratories, PA.

TITLE OF REPORT: *DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOOCTYL ESTER] ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD BR VAF/Plus PRESUMED PREGNANT RATS.*

AUTHOR: Terry Martin, D.V.M **REPORT DATE:** April 10, 1992

SUMMARY: Groups of 20 pregnant rats were given oral administration of technical 2,4-D-2-ethylhexyl ester [63.25% acid equivalent; 95% pure] in aqueous methylcellulose [1%] at acid equivalent doses of 0, 10, 30, or 90 mg/kg/day during days 6 through 15 of gestation. Does were sacrificed on Day 20. No maternal toxicity was seen at 10 mg/kg/day. At 30 mg/kg/day, maternal toxicity was limited to decreases in body weight gain and the presence of a red perivaginal substance which probably reflected reduced grooming. At 90 mg/kg/day maternal toxicity included clinical signs of toxicity, and decreases in body weight gain and food consumption. No treatment-related effects were observed in reproductive parameters at any dose level. No treatment-related external or visceral malformation or variations were seen in any of the fetuses of treated does. A skeletal variation attributable to treatment was the delayed sternal ossification observed at 30 and 90 mg/kg/day. Based on the results of this study, the following NOELs and LOELs are established:

MATERNAL TOXICITY NOEL = 10 mg/kg/day; LOEL = 30 mg/kg/day
DEVELOPMENTAL TOXICITY NOEL = 10 mg/kg/day; LOEL = 30 mg/kg/day.

CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rats (83-3) and is acceptable for regulatory purposes.

I. OBJECTIVE

The objective of this **study** was to assess the effects of the 2-Ethylhexyl ester of 2,4-D [2,4-D-EHE] on the embryonic and fetal development following oral administration to rats during the period of organogenesis.

II. MATERIALS AND METHODS

a. Test Material

Identity: 2-ethylhexyl ester of 2,4-D [Technical]
Batch No.: 04KF54479
Acid Equivalent: 63.25%
Purity: 95%
Description: Yellow liquid

b. Test Animals

Species/Sex: Female rats.
Strain: Crl:CD BR VAF/Plus
Age at receipt: Approximately 65 days
Weight on Day 0: 242 to 292 g
Identification: Ear tags.
Acclimation Period: Approximately 2 weeks.
Housing: Individually in stainless steel cages
Food: Purina Certified Rabbit Chow #5002 ad libitum.
Water: Tap water ad libitum
Environment: Temperature-70-78°F; humidity-40-70%; light cycle:12 hr. light/12 hr.dark.

Group Assignment: 25 inseminated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

Following an acclimation period, virgin females were placed in cohabitation with males in a 1:1 ratio. Female rats with spermatozoa observed in smears of vaginal contents and/or copulatory plugs observed in situ were considered to be at day 0 of presumed gestation and assigned to individual housing.

d. Preparation of Dosing Solutions

Suspensions of 2,4-D-EHE in aqueous 1% methylcellulose were prepared daily. Dosage calculations were corrected for the 63.25% acid equivalence of the test substance and expressed as mg of the acid.

e. Analysis of the Dosing Solutions

Concentration analysis of the dosing solutions were determined twice [on the first and the last days] during the study. Homogeneity analyses of the mid and high dosage concentrations were performed prior to the beginning of the dosage period, and the low dose was analyzed for homogeneity on the second day of dosing. Since dosing samples were prepared daily, no additional stability analyses were performed.

f. Administration of Test Article

The test article was administered daily orally via gavage at doses of 0, 10, 30, or 75 mg/kg/day during days 6 through 15 of gestation. All groups received a dosing volume of 10 mL/kg body weight and the dose volumes were adjusted daily based on individual body weights. Each daily dosage given to the rats was administered at approximately the same time each day.

g. Observations

All animals were observed daily during the dosing and post-dosing periods for clinical signs of toxicity, abortions, premature deliveries and deaths. Individual body weights were obtained on day 0 and daily during the dosing and post-dosing period. Individual food consumptions were measured daily during the study period.

h. Termination

Any animal which died, appeared moribund or showed indications of early termination of pregnancy was submitted for complete necropsy. All surviving dams were sacrificed on gestation day 20, obvious gross pathologic alterations were recorded, and the gravid uteri were weighed.

i. Cesarean Section

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 and late resorptions, and live and dead fetuses were recorded.

j. Fetal Examinations

Each fetus was removed from the uterus and individually 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.

k. Statistical Analysis

Maternal body weights, body weight gains, gravid uterine weights, feed consumption data, and litter averages for fetal body weights, percent male fetuses, percent resorbed conceptuses, fetal ossification sites and percent fetal alterations were analyzed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance [ANOVA]. If the ANOVA was significant, analysis by Dunnett's Test was used. IF the ANOVA was not significant, the Kruskal-Wallis Test or Fisher's Exact Test was used. All other cesarean-sectioning data were evaluated using the Kruskal-Wallis Test. Observations for aborted conceptuses and late resorptions were excluded from statistical analyses.

l. Compliance Statements:

A signed Statement of No Confidentiality Claim was provided and dated April 10, 1992.

A signed Statement of Compliance with EPA's GLP was provided that was dated April 10, 1992.

A signed Quality Assurance Statement was dated April 10, 1992. This date conforms to the review of the study phases and the draft and the final reports.

A signed statement dated April 10, 1992 was provided, which indicated that the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to the study. This study neither reportedly met or exceeded any of these criteria.

III. RESULTS

Analysis of the Dosing Solutions

Results of the concentration analyses of the dosing solutions are tabulated below:

Target Level		% Deviation from Target Dosage	
mg/mL	mg/kg/day	First Day Sample	Last Day Sample
1.0	10	4	-36
3.0	30	-1	-4
7.5	90	-4	-4

The -36% deviation from the target dose level for the low-dose on the last day of dosing was not considered to compromise the study because: (i) it represented a single preparation day; (ii) the concentration analyses of the 10 mg/kg/day dosing solution in the rabbit study, which used the same mixing procedures, yield a -7% deviation from the target level; and (iii) the analytical precision data collected on the third day of dosing at the 10 mg/kg/day level showed the concentration to be within ±10%.

Results of the homogeneity analyses showed a relative standard deviation of 8% for the 1.0 mg/mL level, 4% for the 5.0 mg/mL level, and 5% for 20.0 mg/mL.

1. Maternal Toxicity

a. Mortality & Pregnancy Status

No maternal mortality/morbidity, abortions or premature delivery occurred at any dose level.

b. Clinical Signs

No clinical signs of toxicity were seen at 10 mg/kg/day. Statistically significant, treatment-related clinical signs of toxicity observed were: the presence of a red perivaginal substance generally during Days 13, 14, or 15 of gestation in 8 dams at 30 mg/kg/day [$p < 0.05$] and in 12 dams at 90 mg/kg/day [$p < 0.1$]; ataxia in 4 dams at 90 mg/kg/day [$p < 0.1$]; and decreased motor activity and bradypnea in 1 dam at 90 mg/kg/day.

096

c. Mean Body Weight and Body Weight Gains

No treatment-related effects were seen in mean body weights at any dose level. Body weight gain was unaffected at 10 mg/kg/day. However, statistically significant [p <0.05 to p <0.01] differences in body weight gains were seen during the early dosing period [Days 6 to 9] at 30 and 90 mg/kg/day and for the entire dosing period [Days 6-16] at 90 mg/kg/day. When the body weights were corrected for the gravid uterine weights, body weight gains were comparable between the treated and the control group.

Dose mg/kg/day	Mean Body weight Gain [G]			
	Days 0 - 6	Days 6 - 9	Days 6 - 16	Days 6 -20
0	+37.2±5.6	+18±5.0	+76.2±9.4	+60.9±11.9
10	+37.2±7.3	+13.9±4.8	+77.3±12.0	+61.8±12.7
30	+36.1±6.7	**+13.3±4.2****	+71.3±10.8	+57.9±9.6
90	+35.2±7.8	**+13.3±7.0***	**+66.8±13.1****	+55.7±12.4

d. Food Consumption

No treatment-related or statistically significant differences were observed in absolute [g/day] or relative [g/kg/day] food consumption values at 10, or 30 mg/kg/day. At 90 mg/kg/day, both the absolute and relative food consumption values were significantly [p <0.01] reduced for the entire dosing period.

e. Macroscopical Examination

No treatment-related macroscopical changes were observed in the dams sacrificed at termination.

2. Developmental Toxicity

Reproduction data are presented in Table 1. No biologically or statistically significant effects were seen on pregnancy rate, number of implantations, total live fetuses per litter, resorption rate, number and percent of litters with resorptions, fetal sex ratio, or gravid uterine weights at any dose level. The incidences were reported to be within the historical control ranges of the testing laboratory. This was substantiated with historical control data from the testing laboratory. Fetal body weight was significantly [p<0.05] reduced at 90 mg/kg/day.

Table 1. Cesarean Section Observations

Observations [Mean ± S.D]	Dose Level [mg/kg/day] 0	10	30	90
No. Assigned	25	25	25	25
Females Gravid	22	22	24	24
Maternal Wastage				
# Died	0	0	0	0
# Sacrificed	0	0	0	0
# Aborted	0	0	0	0
# Early delivery	0	0	0	0
# Non pregnant	3	3	1	1
Total Corpora Lutea	432	431	504	456
Corpora Lutea/Dam	19.6±3.5	19.6±3.5	19.1±3.0	19.0±2.3
Total Implantations	385	381	411	421
Implantation/Dam	17.5±2.7	17.3±3.8	17.1±3.8	17.5±1.7
Total Live Fetuses	351	366	378	400
Live Fetuses/Litter	16.0±3.2	16.6±3.9	15.8±3.7	16.7±2.0
Total Resorptions	34	15	33	21
Early	33	14	33	21
Late	1	1	0	0
Resorption/Dam	1.5±1.3	0.7±0.9	1.4±1.2	0.9±0.9
No. and % of Litters with Resorptions	16/22 72.7	9/22 40.9	18/25 75.0	14/24 58.3
Pre Implantation Loss [%]	10.9	11.6	18.4	7.7
Post Implantation Loss [%]	8.8	3.9	8.0	5.0
Gravid Uterus Weight [g]	90 ± 15	95 ± 21	92 ± 20	89 ± 11
Sex Ratio ♂ / ♀	49 168/183	53 189/177	46 173/205	47 188/212
Fetal Weight [g]/Litter	3.49±0.2	3.6±0.2	3.6±0.4	**3.29±0.3***

94

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As summarized in Table 2, increases in fetuses with any alteration was observed at 30 and 90 mg/kg/day with the increase reaching statistical significance [p <0.05] at the high dose. These occurrences were due to the retarded sternal ossification [see discussion under skeletal examination]. No other statistically or biologically significant differences were seen in fetal alterations at any dose level.

Table 2. Summary of Fetal Alterations

Observations	Dose Level [mg/kg/day]			
	0	**10**	**30**	**90**
Litters Evaluated	22	22	24	24
Fetuses Evaluated	351	366	378	400
Litters with Fetuses with any Alterations Observed	9 40.9%	9 40.9%	15 62.5%	12 50.0%
Fetuses with any Alterations Observed	13 3.7%	12 3.3%	23 6.1%	**31** **7.8%** *
Percentage of Fetuses with any Alterations/Litter [Mean±S.D]	3.4± 5.2	4.5±7.8	6.1±6.5	7.9±11.6

The fetal malformations/alterations observed in the 351, 366, 378 and 400 fetuses of does at 0, 10, 30, and 90 mg/kg/day and summarized in Tables 10 thru 13 of the study report [pages 52-58] are appended to this DER.

a. External Examinations

No treatment-related or statistically significant gross external malformations or variations were seen at any dose level.

b. Visceral Examinations

No treatment-related or statistically significant soft tissue malformations or variations were observed.

c. Skeletal Examinations

No statistically significant or treatment-related skeletal alterations were seen at 10 mg/kg/day.

Delayed sternal ossification [i.e., incompletely or unossified sternebrae] occurred in 8, 3, 13 and 18 fetuses in 6, 2, 12 and 9 litters in the 0, 10, 30, and 90 mg/kg/day groups, respectively. At 30 mg/kg/day, the litter incidence [12/24, 50%] was significantly [p <0.5] increased when compared to controls [6/22, 27.3%], and also exceeded the historical control range [0-18.2%] of the testing laboratory. The fetal incidence [13/195, 6.7%] although, was not significantly increased when compared to controls [8/182, 4.4%] did slightly exceed the historical control range [0-5.5%] of the lab.

At 90 mg/kg/day, the fetal incidence [18/207, 8.7%] was significantly [p <0.5] increased when compared to controls [8/182, 4.4%], and also exceeded the historical control range [0-5.5%] of the testing laboratory. The litter incidence [9/24, 37.5%], although was not significantly increased when compared to controls [6/22, 27.3%] did exceed the historical control range [0-18.2%] of the lab. Additionally, the litter average for ossified sternal centers per fetus was significantly reduced at this level; 3.29 ± 0.34 in the treated compared to 3.67 ± 0.34 in the controls.

The **delayed sternal ossifications** observed **at 30 mg/kg/day** are **considered to be treatment-related since: (i) the litter incidence** [the parameter considered more appropriate for analyses] **was significant** when compared to concurrent controls; **(ii)** the **values were not within the ranges of the historical controls** [contrary to what the report author asserts on page 33, paragraph 3]; and (iii) **the fetal incidence also** slightly **exceeded the historical control range.** At 90 mg/kg/day, the delayed ossification was associated with reduced fetal body weights.

Other skeletal observations which showed statistical significance [fetal incidence of wavy ribs at 10 and 90 mg/kg/day and incompletely ossified pubes at 30 mg/kg/day] were not considered to be treatment-related since there was no dose-response and the values were within the historical control ranges of the testing laboratory.

IV. DISCUSSION

Pregnant rats were given oral administration of 2-ethylhexyl ester of 2,4-D at acid equivalent doses of 0, 10, 30, or 75 mg/kg/day during days 6 through 15 of gestation.

2,4-D-2-EHE at 10 mg/kg/day did not induce maternal toxicity. Treatment did not cause mortality, morbidity, abortions or premature delivery at any dose level. At 30 mg/kg/day, maternal toxicity was limited to decreases in body weight gain and the presence of a red perivaginal substance which probably reflected reduced grooming. At 90 mg/kg/day maternal toxicity was manifested by clinical signs of toxicity that included the presence of a red perivaginal substance, ataxia, decreased motor activity and bradypnea, and decreases in body weight gain and food consumption. No treatment-related effects were observed in reproductive parameters at any dose level. No treatment-related external or visceral malformation or variations were seen in any of the fetuses of treated does. No treatment-related skeletal malformations were seen. Skeletal variation attributable to treatment was the delayed ossification which was seen at 30 and 90 mg/kg/day dose levels. Although, these observations are generally considered reversible delays in ossification they were considered to be treatment related since the values were significant when compared to controls, exceeded the historical control ranges, and was associated with the significantly reduce fetal body weight at the high dose.

In a developmental toxicity study [MRID No. 423046-03] with rabbits, artificially inseminated rabbits were given oral administration of 2,4-2-EHE [same lot number and purity [95%] as the rat study] at acid equivalent doses of 0, 10, 30, or 75 mg/kg/day during days 6 - 18 of gestation. For maternal toxicity the NOEL was 30 mg/kg/day and the LOEL, based on mortality, clinical signs of toxicity and decreases in body weight gain and food consumption was 75 mg/kg/day. For developmental toxicity the NOEL was 75 mg/kg/day, the highest dose tested; a LOEL was not achieved. The lack of any developmental toxicity in rabbits compared to skeletal variations in rats observed in this study at comparable acid equivalent doses [75 mg/kg/day in rabbit vs 90 mg/kg/day in rats] indicate the species difference in 2,4-D-EHE induced developmental toxicity.

V. CONCLUSION

2,4-D-2-EHE was maternally toxic to rats at a dose of 30 and 90 mg/kg/day; no maternal toxicity was seen at 10 mg/kg/day. The compound did not induce developmental toxicity at 10 mg/kg/day, but did cause delays in sternal ossification at 30 and 90 mg/kg/day. Based on the results of this study, the following NOELs and LOELs are established.

Maternal Toxicity

NOEL: 10 mg/kg/day
LOEL: 30 mg/kg/day

Developmental Toxicity:

NOEL: 10 mg/kg/day
LOEL: 30 mg/kg/day

VI. CORE CLASSIFICATION: **Guideline;** this study satisfies the requirements for a developmental toxicity study in rats (83-3 a) and is acceptable for regulatory purposes.

PROTOCOL 320-005: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 10 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	22	22	24	24
Fetuses Evaluated	N	351	366	378	400
Live Fetuses	N	351	366	378	400
Dead Fetuses	N	0	0	0	0
BODY:					
Umbilical Hernia					
Litter Incidence	N(%)	0	0	1(4.2)	0
Fetal Incidence	N(%)	0	0	1(0.3)	0

a. Dosage occurred on days 6 through 15 of gestation.

PROTOCOL 320-005: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 11 (PAGE 1): FETAL SOFT TISSUE ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	22	22	24	24
Fetuses Evaluated	N	169	176	183	193
Live Fetuses	N	169	176	183	193
Dead Fetuses	N	0	0	0	0
NASAL PASSAGES:					
Dilated					
Litter Incidence	N(%)	0	1(4.5)	0	0
Fetal Incidence	N(%)	0	1(0.6)	0	0
KIDNEYS:					
Pelvis, Slight to Moderate Dilation					
Litter Incidence	N(%)	2(9.1)	1(4.5)	1(4.2)	1(4.2)
Fetal Incidence	N(%)	2(1.2)	1(0.6)	1(0.5)	1(0.5)
Small					
Litter Incidence	N(%)	0	0	1(4.2)	0
Fetal Incidence	N(%)	0	0	1(0.5)	0

a. Dosage occurred on days 6 through 15 of gestation.

PROTOCOL 320-005: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 12 (PAGE 1): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	22	22	24	24
Fetuses Evaluated	N	182	190	195	207
Live Fetuses	N	182	190	195	207
Dead Fetuses	N	0	0	0	0
VERTEBRAE:					
Thoracic, Centra, Unilateral Ossification					
Litter Incidence	N(%)	1(4.5)	0	0	0
Fetal Incidence	N(%)	1(0.5)[c]	0	0	0
Thoracic, Centra, Not Ossified					
Litter Incidence	N(%)	1(4.5)	0	0	0
Fetal Incidence	N(%)	1(0.5)[c]	0	0	0
Thoracic, Centra, Bifid					
Litter Incidence	N(%)	1(4.5)	1(4.5)	0	0
Fetal Incidence	N(%)	1(0.5)[c]	1(0.5)	0	0
Lumbar, Centrum, Unilateral Ossification					
Litter Incidence	N(%)	1(4.5)	0	0	0
Fetal Incidence	N(%)	1(0.5)[c]	0	0	0
Lumbar, Arches, Incompletely Ossified					
Litter Incidence	N(%)	1(4.5)	2(9.1)	0	2(8.3)
Fetal Incidence	N(%)	1(0.5)[c]	2(1.0)[e,f]	0	2(1.0)[t,u]
RIB(S):					
Incompletely Ossified (Hypoplastic)					
Litter Incidence	N(%)	0	2(9.1)	1(4.2)	2(8.3)
Fetal Incidence	N(%)	0	3(1.6)[e,f,g]	1(0.5)[n]	3(1.4)[r,s,u]
Wavy					
Litter Incidence	N(%)	0	4(18.2)	1(4.2)	3(12.5)
Fetal Incidence	N(%)	0	6(3.2)[e,f,g] **	2(1.0)[m,n]	8(3.9)[r,s,t,u,v] **

PROTOCOL 320-005: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 12 (PAGE 2): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	22	22	24	24
Fetuses Evaluated	N	182	190	195	207
Live Fetuses	N	182	190	195	207
Dead Fetuses	N	0	0	0	0
RIBS (CONTINUED):					
Cervical Rib at the 7th Cervical Vertebra					
Litter Incidence	N(%)	0	0	1(4.2)	0
Fetal Incidence	N(%)	0	0	1(0.5)	0
STERNEBRAE SUMMARIZATION (Includes incompletely or unossified sternebrae):					
Litter Incidence	N(%)	6(27.3)	2(9.1)	12(50.0)*	9(37.5)
Fetal Incidence	N(%)	8(4.4)	3(1.6)	13(6.7)	18(8.7)*
STERNEBRAE:					
Incompletely Ossified					
Litter Incidence	N(%)	5(22.7)	1(4.5)	9(37.5)	7(29.2)
Fetal Incidence	N(%)	5(2.7)[d]	2(1.0)	10(5.1)[i,j,k,l]	9(4.3)[t]
Not Ossified					
Litter Incidence	N(%)	2(9.1)	1(4.5)	3(12.5)	5(20.8)
Fetal Incidence	N(%)	3(1.6)[b]	1(0.5)	3(1.5)[h]	9(4.3)[o,p,v**]
Fused					
Litter Incidence	N(%)	0	0	0	1(4.2)
Fetal Incidence	N(%)	0	0	0	1(0.5)
Asymmetric					
Litter Incidence	N(%)	0	0	1(4.2)	0
Fetal Incidence	N(%)	0	0	1(0.5)[h]	0

PROTOCOL 320-005: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 12 (PAGE 3): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	90
Litters Evaluated	N	22	22	24	24
Fetuses Evaluated	N	182	190	195	207
Live Fetuses	N	182	190	195	207
Dead Fetuses	N	0	0	0	0
SCAPULAE:					
Bent					
Litter Incidence	N(%)	0	0	0	1(4.2)
Fetal Incidence	N(%)	0	0	0	1(0.5)[r]
PELVIS SUMMARIZATION (Includes incompletely or unossified pubes and ischia):					
Litter Incidence	N(%)	4(18.2)	1(4.5)	6(25.0)	4(16.7)
Fetal Incidence	N(%)	5(2.7)	1(0.5)	10(5.1)	9(4.3)
PELVIS:					
Pubes, Incompletely Ossified					
Litter Incidence	N(%)	3(13.6)	0	6(25.0)	4(16.7)
Fetal Incidence	N(%)	4(2.2)[b,d]	0	10(5.1)[i,j,k,l,m,n]	7(3.4)[o,q,u,v]
Ischia, Incompletely Ossified					
Litter Incidence	N(%)	1(4.5)	1(4.5)	1(4.2)	4(16.7)
Fetal Incidence	N(%)	1(0.5)[d]	1(0.5)[f]	1(0.5)[l]	5(2.4)[o,q,u,v]
Pubes, Not Ossified					
Litter Incidence	N(%)	1(4.5)	0	0	1(4.2)
Fetal Incidence	N(%)	1(0.5)[c]	0	0	1(0.5)[p]
Ischia, Not Ossified					
Litter Incidence	N(%)	1(4.5)	0	0	0
Fetal Incidence	N(%)	1(0.5)[c]440	0	0	0

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PROTOCOL 320-005: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2.4-D 2-ETHYLHEXYL ESTER (2.4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 12 (PAGE 4): FETAL SKELETAL ALTERATIONS - SUMMARY

FOOTNOTES

a. Dosage occurred on days 6 through 15 of gestation.
b. Fetus 18929-18 also had other skeletal alterations.
c. Fetus 18937-12 also had other skeletal alterations.
d. Fetus 18949-1 also had other skeletal alterations.
e. Fetus 18951-11 also had other skeletal alterations.
f. Fetus 18971-4 also had other skeletal alterations.
g. Fetus 18971-8 also had other skeletal alterations.
h. Fetus 18976-1 also had other skeletal alterations.
i. Fetus 18982-6 also had other skeletal alterations.
j. Fetus 18990-8 also had other skeletal alterations.
k. Fetus 18992-13 also had other skeletal alterations.
l. Fetus 18994-14 also had other skeletal alterations.
m. Fetus 19000-1 also had other skeletal alterations.
n. Fetus 19000-16 also had other skeletal alterations.
o. Fetus 19005-13 also had other skeletal alterations.
p. Fetus 19005-15 also had other skeletal alterations.
q. Fetus 19011-1 also had other skeletal alterations.
r. Fetus 19014-1 also had other skeletal alterations.
s. Fetus 19014-6 also had other skeletal alterations.
t. Fetus 19014-10 also had other skeletal alterations.
u. Fetus 19017-7 also had other skeletal alterations.
v. Fetus 19025-5 also had other skeletal alterations.
* Significantly different from the vehicle control group value ($P \leq 0.05$).
** Significantly different from the vehicle control group value ($P \leq 0.01$).

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PRIMARY REVIEWER: Jess Rowland, Toxicologist 8/14/92
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II 8/19/92

DATA EVALUATION REPORT

1. RANGE-FINDING STUDY--RATs

STUDY TYPE: Developmental Toxicity **[Range-Finding] GUIDELINE: N/A**

CASWELL NO. 315 AS MRID No. 423046-02 DP Barcode:D179762

TEST MATERIAL: 2-Ethylhexyl Ester of 2,4-D ACID **[2,4-D-EHE]**

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

TESTING LABORATORY: Argus Research Laboratories, PA

STUDY IDENTIFICATION: Argus **320-005P**

TITLE OF REPORT: *DOSAGE-RANGE DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOOCTYL ESTER] ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD BR VAF/Plus PRESUMED PREGNANT RATS.*

AUTHOR: Terry Martin, D.V.M **REPORT DATE:** April 10, 1992

SUMMARY: In a dose-range finding study, pregnant rats were given oral administration of technical 2,4-D-2-ethylhexyl ester [63.25% acid equivalent; 95% pure] in aqueous methylcellulose [1%] at acid equivalent doses of 0, 50, 100, 150 or 200 mg/kg/day during days 6 through 15 of gestation. Does were sacrificed on gestation day 20. No maternal toxicity was seen at 50 mg/kg/day. At 100, 150 and 200 mg/kg/day, 2,4-D-2-EHE induced maternal toxicity was evident from mortality/ morbidity, clinical signs of toxicity, decreases in body weight gain and food consumption, and gross pathological alterations. Treatment had no adverse effects on pregnancy rate, the number of implantations, the number of resorptions, litter size, or viability of fetuses at 50 or 100 mg/kg/day. Treatment-related effects seen at 100, 150 and/or 200 mg/kg/day included: a reduction in live litter sizes; increase in the mean number of resorptions per litter; increase in the percentage of resorbed fetuses per litter; and increases in the numbers of totally resorbed fetuses.

MATERNAL TOXICITY NOEL = 50 mg/kg/day [LDT]; LOEL = 100 mg/kg/day
DEVELOPMENTAL TOXICITY NOEL = 50 mg/kg/day; LOEL = 100 mg/kg/day

CORE CLASSIFICATION:: Not applicable; range-finding study.

510

009697

1. OBJECTIVE

The objective of this **range-finding study in rats** was to establish appropriate dose levels of the 2-ethylhexyl ester of 2,4-D [2,4-D-2-EHE] for the main study.

2. PROTOCOL

Groups of eight presumed pregnant Crl:CD BR VAF/Plus [Sprague-Dawley] rats [approximately 1 month of age and weighing 200 to 225 g were given oral administrations of **Technical 2,4-D-2-EHE [63.25% acid equivalent, 95% pure, Lot No.04KF54479]** at acid equivalent doses of 50, 100, 150, or 200 mg/kg/day in 1% methylcellulose [10 mL/kg] daily, during days 6 through 15 of gestation. A control group received the vehicle alone under the same schedule. Concentration and homogeneity of the test article/vehicle mixtures were determined prior to the initiation of the study.

Animals were observed daily for viability, clinical signs of toxicity, abortions, premature deliveries, body weights and food consumption during the dosing and post-dosing periods. Dams in each group were sacrificed on day 20 and postmortem examination included macroscopic examination of internal organs, with emphasis on the uterus, uterine contents, position of each fetus in the uterus, and corpora lutea counts. Fetal examinations were not performed.

511

3. RESULTS

i. Analysis of dosing solution

The mean concentrations found were 144%, 82%, 94% and 103% of the nominal concentration, respectively, on the first day of dosing, and 109%, 122%, 89%, and 74%, respectively, on the last day of dosing for the 50, 100 and 150 mg/kg/day groups. Dosing solution exhibited 100% homogeneity.

ii. Maternal Toxicity

- 50 mg/kg/day: No mortality or moribundity.
- 100 mg/kg/day: 1 dam found dead on Day 18.
- 150 mg/kg/day: No mortality or moribundity.
- 200 mg/kg/day: 1 doe each was found dead on Days 11 & 14 and 1 dam was sacrificed moribund on Day 14.
- No clinical signs of toxicity were seen at 50 mg/kg/day. Treatment-related clinical signs observed in surviving dams included ataxia, decreased motor activity, pinpoint pupils [100, 150 and 200 mg/kg/day]; impaired righting reflex [150 and 200 mg/kg/day]; and urine stains on the fur and red vaginal substance [200 mg/kg/day]. The severity and incidence of these signs generally remained constant throughout the course of the dosing period.
- When compared to controls, the mean body weight gain was unaffected at 50 mg/kg/day. Decreases in average weight gain were seen at 100, 150 and 200 mg/kg/day during the dosing-[Days 6 to 16] and post-dosing [Days 16 to 20] periods. Reduction in the corrected body weight gain was noted at 150 and 200 mg/kg/day. Average gravid uterine weights was reduced at 150 and 200 mg/kg/day.
- Food consumption was unaffected at 50 mg/kg/day. When compared to controls, decreases in absolute [g/day] and relative [g/kg/day] food consumption occurred during the entire dosing period at 100 mg/kg/day and higher dosage groups. During the post-dosing period, absolute food consumption was slightly reduced at 150 and 200 mg/kg/day.
- Treatment-related gross pathological alterations seen only in the rat that was found dead at 100 mg/kg/day were gastric erosions and a bright to dark red lungs. In rats that died or sacrificed at 200 mg/kg/day, necropsy revealed gastric erosions, bright to dark red lungs, consolidated lungs and a yellow to red mottled liver.

iii. Developmental Toxicity

- The pregnancy rate was 100% in all treatment groups.
- No marked treatment-related effects were observed in implantations, litter sizes, or viable fetuses at 50 mg/kg/day when compared to controls.
- The increase observed in the average number of resorptions [1.6±0.8] when compared to controls [1.1±1.1] was within the historical control range [0.3 to 2.0] of the testing laboratory.
- The increases in the number of dams with any resorptions at 100, 150 and 200 mg/kg/day was higher than the concurrent controls, and were outside the historical control range [25 to 82%]. Therefore, this finding was considered to be treatment related.
- Treatment-related effects observed in caesarean sectioning at 150 and 200 mg/kg/day are tabulated below:

C-Section Observations	mg/kg/day				
	0	50	100	150	200
Litter size [mean]	13	15	13	11	5
Total No. of live fetuses	102	116	90	85	27
Resorptions [mean]	1.1	0.6	1.6	4.4	9.4
Early Resorptions [mean]	1.1	0.6	1.6	4.4	9.2
Dams with any resorptions	63%	38%	100%	88%	100%
Mean percentage of resorbed conceptuses/Litter	10	4	11	17	41

- No data were reported for fetal examinations.

4. **CONCLUSION:**

MATERNAL TOXICITY: NOEL = 50 mg/kg/day [LDT]; LOEL = 100 mg/kg/day

DEVELOPMENTAL TOXICITY: NOEL = 50 mg/kg/day; LOEL = 100 mg/kg/day

5. **CORE CLASSIFICATION:**: Not applicable; range-finding study.

Amendment to DER for MRID 42304603 - Developmental Toxicity Study - Rabbit [2,4-D 2-ETHYL HEXYL ESTER]

This amendment provides an EXECUTIVE SUMMARY and a data table to upgrade the original DER [Document No. 009697].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/20/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 12/20/00
Reregistration Branch I, Health Effects Division (7509C)

TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rabbit § 83-3(b)/OPPTS 870.3700
P.C. CODE: 030063
CASWELL NO.: 315AS CAS #: 1928-43-4
TEST MATERIAL (PURITY): 2,4-D 2-ethylhexyl ester [95% (acid equivalent 63.25%)]
SYNONYMS: dichlorophenoxyacetic acid, isooctyl (2-ethylhexyl) ester ;2,4-D EHE
SPONSOR: Industry Task Force II on 2,4-D Research Data

CITATION: Martin, T. (1992). Developmental Toxicity [Embryo-Fetal Toxicity and Teratogenic Potential] Study of 2,4-D 2-Ethylhexyl Ester [2,4-D Isooctyl Ester] Administered Orally [stomach tube] to New Zealand White Rabbits. Argus Research Laboratories, PA; Study No. Argus 320-006. MRID 42304603. Document No. 009697. Unpublished.

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 42304603], artificially-inseminated female New Zealand White rabbits [20/group] were administered 2-ethylhexyl ester of 2,4-D [95% (acid equivalent 63.25%)] at acid equivalent doses of 0 [1% methyl cellulose], 10 mg/kg/day, 30 mg/kg/day, and 75 mg/kg/day from gestation day 7 through gestation day 18.

Two high-dose does were sacrificed moribund [gestation days 15 and 16] and one mid-dose doe was found dead on day 21. Clinical signs of toxicity {decreased motor activity [3 does (moribund sacrifices and aborted)], ataxia [doe that aborted], impaired righting reflex [1 moribund doe], loss of righting reflex [1 moribund doe], bradypnea [1 moribund doe], dried feces [doe that aborted]} were observed in the high-dose does only.

No treatment-related effects were observed on body weight or overall body-weight gain, although the high-dose does displayed a non-significant decrease [81% of control] in body-weight gain during the dosing period compared to the control. Food consumption was comparable among the groups. Corrected body-weight gain was comparable among the groups.

One high-dose doe aborted [day 23]. There were no early deliveries. There were no treatment-relatedor statistically-significant effects on pregnancy rate, numbers of corpora lutea, implantations, and total live fetuses, resorption rate, number and percent of litters with resorptions, pre- or post-implantation losses, fetal sex ratio, fetal body weight, or gravid uterine weights, although the high-dose group displayed the fewest implantations/litter [90% of control] and fetuses/litter [92% of control]. Dead fetuses were not observed at any dose level. There was one mid-dose litter that was totally resorbed.

There were no apparent treatment-related effects on external, visceral, or skeletal variations or malformations.

The maternal toxicity NOAEL is 30 mg/kg/day, based on mortality/morbidity, clinical signs, and decreased body-weight gain in does that died at the LOAEL of 75 mg/kg/day. The developmental toxicity NOAEL is 75 mg/kg/day, the highest dose tested.

This developmental toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [**§83-3(b)/OPPTS 870.3700**] for a developmental toxicity study in rabbits.

COMMENT: Refer to MRID 42304604;TXR# 9697 for dose selection information.

Table 1. Cesarean Section Observations				
Observation/Dose	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	75 mg/kg/day
# assigned	20	20	20	20
# pregnant	18	16	16	20
maternal wastage # died # sacrificed # aborted # early delivery # non pregnant	 0 0 0 0 2	 0 0 0 0 4	 1 0 **0** 0 4	 0 2 **1** 0 0
total corpora lutea corpora lutea/doe	183 10.2±2.8	159 9.9±1.5	151 10.1±2.4	181 10.6±2.1
total implantations implantations/doe	131 7.3±2.3	133 8.3±1.8	118 7.9±2.6	112 6.6±2.4
total live fetuses live fetuses/doe	128 **7.1±2.2**	131 **8.2±1.8**	114 **7.6±2.7**	110 **6.5±2.4**
total resorptions early late resorptions/doe	2 2 **1** 0.2±0.4	2 1 **]10** 0.1±0.3	4 4 0 0.3±0.4	2 2 0 0.1±0.5
# and % of litters w/ resorptions	3/18 [16.7]	2/16 [12.5]	4/**15]26.7]**	**1/17 [5.9]**
# does w/ all resorptions	**0**	**0**	**1**	**0**
preimplantation loss - %	28.4	16.4	21.9	38.1
postimplantation loss - %	2.3	1.5	3.4	1.8
gravid uterine weight [g]	456±101	460±127	483±159	421±119
sex ratio [% males]	**45.2±21.6**	**48.3±18.0**	**56.6±16.6**	**58.6±18.4**
fetal body weight [g] male female	**46.5±6.5** **45.9±6.0** **46.3±6.5**	**43.1±3.8** **43.9±4.2** **42.3±4.7**	**44.5±3.8** **45.8±4.2** **43.0±3.3**	**46.6±5.1** **46.7±5.8** **45.4±5.0**

bolded data are corrections/additions to original DER Table 1; data from Tables 4 [page 49], 8 and 9 [pages 54 and 55] of the report

514

PRIMARY REVIEWER: Jess Rowland, Toxicologist Jess Rowland 8/14/92
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 8/19/92
Section II, Toxicology Branch II

DATA EVALUATION REPORT

4. MAIN STUDY--RABBITS

STUDY TYPE: Developmental Toxicity **[Main Study] GUIDELINE:** 83-3(b)

CASWELL NO. 315 AS MRID No. 423036-03 DP Barcode: 179762

TEST MATERIAL: 2-Ethylhexyl Ester of 2,4-D **[2,4-D-EHE]**

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

STUDY IDENTIFICATION: Argus 320-006

TESTING LABORATORY: Argus Research Laboratories, PA.

TITLE OF REPORT: *DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOOCTYL ESTER] ADMINISTERED ORALLY [STOMACH TUBE] TO NEW ZEALAND WHITE RABBITS.*

AUTHOR: Terry Martin, D.V.M **REPORT DATE:** April 10, 1992

SUMMARY: Groups of 20 inseminated rabbits were given oral administration of 2-ethylhexyl ester of 2,4-D [2,4-D-2EHE] at acid equivalent doses of 0, 10, 30, or 75 mg/kg/day during days 6 through 18 of gestation. 2,4-D-2-EHE at 10 or 30 mg/kg/day did not induce maternal toxicity. At 75 mg/kg/day maternal toxicity was manifested by mortality/morbidity, decreases in mean body weight, body weight gain and food consumption in does that died or sacrificed moribund, and clinical signs of toxicity characterized by dried feces, decreased motor activity, ataxia, impaired righting reflex, lost righting reflex and bradypnea. No treatment-related effects were observed in reproductive parameters at any dose level. No treatment-related external, visceral, or skeletal malformation or variations were seen in any of the fetuses of treated does. Based on these results the following NOELs and LOELs are established.

MATERNAL TOXICITY: NOEL = 30 mg/kg/day; LOEL = 75 mg/kg/day [HDT]

DEVELOPMENTAL TOXICITY: NOEL = 75 mg/kg/day; LOEL= Not Achieved

CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

I. OBJECTIVE

The objective of this study was to assess the effects of the **2-Ethylhexyl ester of 2,4-D [2,4-D-EHE]** on the embryonic and fetal development following oral administration to rabbits during the period of organogenesis.

II. MATERIALS AND METHODS

a. Test Material

Identity: 2-ethylhexyl ester of 2,4-D [Technical]
Batch No.: 04KF54479
Acid Equivalent: 63.25%
Purity: 95%
Description: Yellow liquid

b. Test Animals

Species/Sex: Female rabbits
Strain: New Zealand White [Hra:(NZW)SPF]
Age at receipt: Approximately 6 months
Weight at randomization: 3.11 - 4.02 kg
Identification: Ear tags.
Acclimation Period: 28 days.
Housing: Individually in stainless steel cages
Food: Purina Certified Rabbit Chow #5322 ad libitum.
Water: Tap water ad libitum
Environment: Temperature-60-70°F; humidity-40-70%;light cycle: 12 hr.light/12 hr. dark

Group Assignment: 20 inseminated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

Adult females were artificially inseminated with the day of insemination considered Day 0 of gestation. Does were given intravenous injection of 20 USP Units/kg of human chorionic gonadotropin approximately three hours prior to insemination. An estimated 0.25 mL of semen that had been diluted with normal saline to a concentration of 6.0×10^{-6} spermatozoa/0.25 mL saline was used for insemination.

d. Preparation of Dosing Solutions

Suspensions of 2,4-D-EHE in aqueous 1% methylcellulose were prepared daily. Dosage calculations were corrected for the 63.25% acid equivalence of the test substance and expressed as mg of the acid.

e. Analysis of the Dosing Solutions

Concentration analysis of the dosing solutions were determined twice [on the first and the last days] during the study. Homogeneity was determined prior to the beginning of the dosage period. Since dosing samples were prepared daily, no additional stability analyses were performed.

f. Administration of Test Article

The test article was administered daily orally via gavage at doses of 0, 10, 30, or 75 mg/kg/day during days 6 through 18 of gestation. All groups received a dosing volume of 10 mL/kg body weight and the dose volumes were adjusted daily based on individual body weights. Each daily dosage given to the rabbits was administered at approximately the same time each day.

g. Observations

All animals were observed for clinical signs of toxicity, abortions, premature deliveries and deaths immediately prior to dosing, and approximately 60 minutes post-dosing. Individual body weights were obtained on day 0 and daily during the dosing and post-dosing period. Individual food consumptions were measured daily during the study period.

h. Termination

Any animal which died, appeared moribund or showed indications of early termination of pregnancy was submitted for complete necropsy. All surviving does were sacrificed on gestation day 29, obvious gross pathologic alterations were recorded, and the gravid uteri were weighed.

i. Cesarean Section

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 rabbits were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantations, early and late resorptions, and live and dead fetuses were recorded.

j. Fetal Examinations

Each fetus was removed from the uterus and individually 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.

k. Statistical Analysis

Maternal body weights, body weight gains, gravid uterine weights, feed consumption data, and litter averages for percent male fetuses, percent resorbed conceptuses, fetal body weights, anomaly average data and ossification site data were analyzed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance [ANOVA]. If the ANOVA was significant, analysis by Dunnett's Test was used. IF the ANOVA was not significant, the Kruskal-Wallis Test or Fisher's Exact Test was used. All other cesarean-sectioning data were evaluated using the Kruskal-Wallis Test. Observations for aborted conceptuses and late resorptions were excluded from statistical analyses.

l. Compliance Statements:

A signed Statement of No Confidentiality Claim was provided dated April 10, 1992.

A signed Statement of Compliance with EPA's GLP was provided that was dated April 10, 1992.

A signed Quality Assurance Statement was dated April 10, 1992. This date conforms to the review of the study phases and the draft and the final reports.

A signed statement dated April 10, 1992 was provided, which indicated that the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to the study. This study neither reportedly met or exceeded any of these criteria.

III. RESULTS

Analysis of the Dosing Solutions

Results of the concentration analyses of the dosing solutions are tabulated below:

Target Level		% Deviation from Target Dosage	
mg/mL	mg/kg/day	First Day Sample	Last Day Sample
1.0	10	-2	-7
3.0	30	-6	2
7.5	75	-11	4

Results of the homogeneity analyses showed a relative standard deviation of 8% for the 1.0 mg/mL level, 4% for the 5.0 mg/mL level, and 5% for the 20.0 mg/mL.

1. Maternal Toxicity

a. Mortality & Pregnancy Status

Dose Level [mg/kg/day]	0	10	30	75
No. on Test	20	20	20	20
Aborted	0	0	0	1
Delivered Early	0	0	0	0
Moribund Sacrifice	0	0	0	2
Found Dead	0	0	1	0

No maternal mortality/morbidity or abortions occurred at 10 mg/kg/day.

At 30 mg/kg/day, the doe that died on Day 21 did not exhibit any adverse clinical signs of toxicity however, did show body weight loss and reduced food consumption. Necropsy revealed a red substance in the perianal area that may have been interrelated with resorption of the litter [the litter of this doe had 9 late resorption].

At 75 mg/kg/day, the doe that aborted on Day 23 showed clinical signs, body weight loss, and decreased food consumption. No gross lesions were seen at necropsy. The litter of this doe had one early and six late resorptions. The one doe that was sacrificed moribund on Day 15 exhibited clinical signs, body weight loss, reduced food consumption, and red cardiac and fundic regions in the stomach and parovarian cysts. The litter of this doe had 10 early resorptions. The doe that was sacrificed on Day 16 exhibited body weight loss, reduced food consumption, clinical signs, distended bladder with red brown fluid, clear, red fluid in the thoracic cavity, brown spots in all lung lobes and parovarian cysts. The litter of this doe had eight early resorptions.

b. Clinical Signs

No clinical signs of toxicity were seen at 0, 10 or 30 mg/kg/day. Treatment-related clinical signs of toxicity observed at 75 mg/kg/day included dried feces, decreased motor activity, ataxia, impaired righting reflex, lost righting reflex and bradypnea.

c. Body Weight Changes

As shown below, no statistically or biologically significant differences were seen in mean body weight or body weight gains between the control and treated groups during the pre-dosing period [Days 0 to 6], the dosing period [Days 6 to 19], the post-dosing period [Days 6 to 19], and the entire study [Days 0 to 29].

Dose mg/kg/day	Mean Body weight Gain [G]			
	Days 0 - 6	Days 6 - 19	Days 19 - 29	Days 0 -29
0	+0.18±0.06	+0.21±0.10	+0.11±0.14	+0.03±0.17
10	+0.18±0.06	+0.18±0.06	+0.13±0.09	+0.03±0.15
30	+0.19±0.04	+0.20±0.03	+0.16±0.10	+0.09±0.19
75	+0.18±0.06	+0.17±0.16	+0.14±0.12	+0.09±0.14

d. Food Consumption

No treatment-related or statistically significant differences were observed in absolute [g/day] or relative [g/kg/day] food consumption values between treated and control groups during the pre-dosing, dosing, post-dosing or the entire study periods.

e. Macroscopical Examination

No treatment-related macroscopical changes were observed in the dams sacrificed at termination. Gross pathological alterations observed in the does that died or were sacrificed moribund are discussed under Mortality [see Page].

2. Developmental Toxicity

Reproduction data are presented in Table 1. No biologically or statistically significant effects were seen on pregnancy rate, number of implantations, total live fetuses per litter, resorption rate, number and percent of litters with resorptions, fetal sex ratio, fetal body weights, or gravid uterine weights at any dose level. The incidences were reported to be within the historical control ranges of the testing laboratory. This was substantiated with historical control data from the testing laboratory.

Table 1. Cesarean Section Observations

Observations [Mean ± S.D]	Dose Level [mg/kg/day] 0	10	30	75
No. Assigned	20	20	20	20
Females Gravid	18	16	16	20
Maternal Wastage				
# Died	0	0	~~0~~ 1	0
# Sacrificed	0	0	0	2
# Aborted	0	0	~~1~~	0 1
# Early delivery	0	0	0	0
# Non pregnant	2	4	4	0
Total Corpora Lutea	183	159	151	181
Corpora Lutea/Dam	10.2±2.8	9.9±1.5	10.1±2.4	10.6±2.1
Total Implantations	131	133	118	112
Implantations/Dam	7.3±2.3	8.3±1.8	7.9±2.6	6.6±2.4
Total Live Fetuses	128	131	114	110
Live Fetuses/Litter	~~6.6±2.2~~ 7.1±2.2	~~6.6±2.2~~ 8.2	7.6±~~3.3~~	~~7.1±1.4~~
Total Resorptions	2	2	4	2
Early	2	2	4	2
Late	0	0	0	0
Resorption/Dam	0.2±0.4	0.1±0.3	0.3±0.4	0.1±0.5
No. and % of Litters with Resorptions	3/18 16.7	2/16 12.5	4/16 25.0	1/20 5.0
Pre Implantation Loss [%]	28.4	16.4	21.9	38.1
Post Implantation Loss [%]	2.3	1.5	3.4	1.8
Gravid Uterus Weight [g]	456±101	460±127	483±159	421±119
Sex Ratio ♂ / ♀	49 63/65	47 62/69	64/50	53 61/49
Fetal Weight [g]	47 ± 7	48 ± 18	57 ± 17	59 ± 18

As summarized in Table 2, there were no treatment-related or statistically significant differences in fetal alterations in the number of litters with fetuses with any alterations noted, the number of fetuses with any alterations noted or the average percent of fetuses with any alterations per litter.

Table 2. Summary of Fetal Alterations

Observations	Dose Level [mg/kg/day]			
	0	10	30	75
Litters Evaluated	18	16	14	17
Fetuses Evaluated	128	131	114	110
Litters with Fetuses with any Alterations Observed	17 94.4%	15 93.8%	14 100%	16 94.1%
Fetuses with any Alterations Observed	59 46.1%	58 44.3%	53 46.5%	51 46.4%
Percentage of Fetuses with any Alterations/Litter [Mean±S.D]	47 ± 25	46 ± 24	45 ± 20	50 ± 30

The fetal malformations/alterations observed in the 128, 131, 114 and 110 fetuses of does at 0, 10, 30, and 75 mg/kg/day and summarized in Tables 11 thru 14 of the study report [pages 57-6] are appended to this DER.

a. External Examinations

No treatment-related or statistically significant gross external malformations or variations were seen.

b. Visceral Examinations

No treatment-related or statistically significant soft tissue malformations were observed. Statistically significant soft tissue variations included agenesis at 10 and 30 mg/kg/day and alterations in brain at 75 mg/kg/day.

Statistically significant increased incidences of agenesis of the intermediate lobe of the lungs were seen in 5 of 131 fetuses [3.8%] in two litters at 10 mg/kg/day [$p < 0.01$] and in 3 of 114 fetuses [2.6%] of in two litters at 30 mg/kg/day [$p<0.05$]. Although these incidences showed statistical significance at the fetal incidence, they were not considered to be treatment-related since: (i) the litter incidence [12.5% at 10 mg/kg/day and 14.3% at 30 mg/kg/day] was not significantly increased; (ii) this anomaly was not seen at the high dose; and (iii) both the fetal [0-5.15] and the litter [0-31%] incidences were within the historical control range of the testing laboratory.

Moderate dilation of the lateral ventricles of the brain was observed in 4 of 110 fetuses [3.6%] at 75 mg/kg/day compared to none in the vehicle controls [p <0.01]. Increase in this variation was not considered to be treatment-related since it occurred in only one high dose group litter, the litter incidence [1/17, 5.9%] was not statistically significant, and both the fetal [0-2.5%] and litter [0-17%] incidences were within the historical control range of the testing laboratory.

c. Skeletal Examinations

No other treatment-related or statistically significant skeletal malformations were seen between the control and the treated groups.

IV. DISCUSSION

Inseminated rabbits were given oral administration of 2-ethylhexyl ester of 2,4-D at acid equivalent doses of 0, 10, 30, or 75 mg/kg/day during days 6 through 18 of gestation.

2,4-D-2-EHE at 10 or 30 mg/kg/day did not induce maternal toxicity. At 75 mg/kg/day maternal toxicity was manifested by mortality/morbidity, decreases in mean body weight, body weight gain and food consumption in does that died or sacrificed moribund, and clinical signs of toxicity characterized by dried feces, decreased motor activity, ataxia, impaired righting reflex, lost righting reflex and bradypnea. No treatment-related effects were observed in reproductive parameters at any dose level. No treatment-related external, visceral, or skeletal malformation or variations were seen in any of the fetuses of treated does.

In a developmental toxicity study [MRID No. 423046-01] with rats, pregnant Sprague-Dawley rats were given oral administration of 2,4-2-EHE [same lot number and purity [98%] as the rabbit study] at acid equivalent doses of 0, 10, 30, or 90 mg/kg/day during days 6 - 15 of gestation. For maternal toxicity the NOEL was 10 mg/kg/day and the LOEL, based on decreases in body weight gain and food consumption, was 30 mg/kg/day. For developmental toxicity the NOEL was 10 mg/kg/day and the LOEL, based on delayed sternal ossification, was 30 mg/kg/day. The lack of any developmental toxicity in rabbits compared to skeletal variations in rats at comparable acid equivalent doses [75 mg/kg/day in rabbits vs 90 mg/kg/day in rats] indicate the species difference in 2,4-D-EHE induced developmental toxicity.

V. CONCLUSION

2,4-D-2-EHE was maternally toxic to rabbits at a dose of 75 mg/kg/day; no maternal toxicity was seen at 10 or 30 mg/kg/day. The compound did not induce developmental toxicity at 75 mg/kg/day., the highest dose tested. Based on the results of this study, the following NOELs and LOELs are established.

Maternal Toxicity

NOEL: 30 mg/kg/day
LOEL: 75 mg/kg/day [HDT]

Developmental Toxicity:

NOEL: 95 mg/kg/day [HDT]
LOEL: Not Achieved

VI. CORE CLASSIFICATION: **Guideline;** this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

PROTOCOL 320-006: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 11 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	75
Litters Evaluated	N	18[b]	16	14	17
Fetuses Evaluated	N	128[b]	131	114	110
Live Fetuses	N	128[b]	131	114	110
Dead Fetuses	N	0	0	0	0
HEAD:					
Encephalocele					
Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)	0	0
BODY:					
Meningomyelocele (Lumbar Region)					
Litter Incidence	N(%)	0	0	0	1(5.9)
Fetal Incidence	N(%)	0	0	0	1(0.9)
Subcutaneous Hemorrhage (Whole Body)					
Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9)[c]	0
FORELIMBS:					
Bilateral, Paws, Flexed					
Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9)[c]	0

a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported as the acid equivalent of 2,4-D contained in the 2,4-D 2-Ethylhexyl Ester.
b. Includes values for litter 19768, in which gross external examination was performed to the extent possible because the heads of these fetuses were skinned prior to examination.
c. Fetus 19799-4 also had other gross external alterations.

PROTOCOL 320-006: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 12 (PAGE 1): FETAL SOFT TISSUE ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0
BRAIN:					
Small					
Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)	0	0
Lateral Ventricles, Moderate Dilation					
Litter Incidence	N(%)	0	0	0	1(5.9)
Fetal Incidence	N(%)	0	0	0	4(3.6)**
LUNGS:					
Intermediate Lobe, Agenesis					
Litter Incidence	N(%)	0	2(12.5)	2(14.3)	0
Fetal Incidence	N(%)	0	5(3.8)**	3(2.6)b*	0
GALLBLADDER:					
Absent					
Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9)b	0
KIDNEY:					
Left, Pelvis, Marked Dilation					
Litter Incidence	N(%)	1(5.6)	0	0	0
Fetal Incidence	N(%)	1(0.8)	0	0	0

His

a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported as the acid equivalent of 2,4-D contained in the 2,4-D 2-Ethylhexyl Ester.
b. Fetus 19799-4 also had other soft tissue alterations.
* Significantly different from the vehicle control group value ($P \leq 0.05$).
** Significantly different from the vehicle control group value ($P \leq 0.01$).

PROTOCOL 320-006: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 1): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0
SKULL - IRREGULAR OSSIFICATION[b]:					
(SUMMARIZATION OF ALL IRREGULAR OSSIFICATION OF SKULL[c]; SUMMARIZED AND INDIVIDUAL SUBCATEGORIES CITED BELOW)					
Litter Incidence	N(%)	16(88.9)	15(93.8)	14(100.0)	15(88.2)
Fetal Incidence	N(%)	53(41.4)	52(39.7)	50(43.8)	48(43.6)
NASAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF INTERNASALS; INTRANASALS; IRREGULAR SUTURE; MIDLINE SUTURE DISPLACED; NASAL(S)-FRONTAL(S), IRREGULAR SUTURE)					
Litter Incidence	N(%)	15(83.3)	15(93.8)	12(85.7)	14(82.4)
Fetal Incidence	N(%)	35(27.3)	41(31.3)	33(28.9)	29(26.4)
Nasals, Internasal					
Litter Incidence	N(%)	1(5.6)	3(18.8)	0	2(11.8)
Fetal Incidence	N(%)	1(0.8)	3(2.3)	0	2(1.8)
Nasal(s), Intranasal					
Litter Incidence	N(%)	1(5.6)	1(6.2)	0	2(11.8)
Fetal Incidence	N(%)	1(0.8)	3(2.3)	0	2(1.8)
Nasals, Irregular Suture					
Litter Incidence	N(%)	1(5.6)	2(12.5)	2(14.3)	2(11.8)
Fetal Incidence	N(%)	1(0.8)	2(1.5)	2(1.8)	2(1.8)
Nasals, Midline Suture Displaced					
Litter Incidence	N(%)	15(83.3)	14(87.5)	12(85.7)	13(76.5)
Fetal Incidence	N(%)	27(21.1)[d]	28(21.4)	29(25.4)[g,h]	21(19.1)[j,l]
Nasal(s)-Frontal(s), Irregular Suture					
Litter Incidence	N(%)	5(27.8)	5(31.2)	3(21.4)	2(11.8)
Fetal Incidence	N(%)	7(5.5)	11(8.4)	4(3.5)	6(5.4)

PROTOCOL 320-006: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 2): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0
SKULL - IRREGULAR OSSIFICATION (CONTINUED)[b]:					
FRONTAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF INTERFRONTALS; INTRAFRONTALS; IRREGULAR SUTURE; SUTURE ENLARGED)					
Litter Incidence	N(%)	12(66.7)	12(75.0)	13(92.8)	12(70.6)
Fetal Incidence	N(%)	25(19.5)	19(14.5)	25(21.9)	27(24.5)
Frontals, Interfrontal					
Litter Incidence	N(%)	9(50.0)	6(37.5)	7(50.0)	6(35.3)
Fetal Incidence	N(%)	11(8.6)[e]	7(5.3)	9(7.9)	9(8.2)[k]
Frontal(s), Intrafrontal					
Litter Incidence	N(%)	4(22.2)	2(12.5)	1(7.1)	2(11.8)
Fetal Incidence	N(%)	4(3.1)	2(1.5)	1(0.9)	2(1.8)
Frontals, Irregular Suture					
Litter Incidence	N(%)	7(38.9)	8(50.0)	10(71.4)	9(52.9)
Fetal Incidence	N(%)	12(9.4)	9(6.9)	16(14.0)[g,i]	18(16.4)
Frontals, Suture Enlarged					
Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)	0	0
PARIETAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF INTRAPARIETAL; SUTURE ENLARGED)					
Litter Incidence	N(%)	1(5.6)	1(6.2)	0	0
Fetal Incidence	N(%)	1(0.8)	1(0.8)	0	0
Parietal, Intraparietal					
Litter Incidence	N(%)	1(5.6)	0	0	0
Fetal Incidence	N(%)	1(0.8)	0	0	0

PROTOCOL 320-006: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 3): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0
SKULL - IRREGULAR OSSIFICATION (CONTINUED)b:					
Parietals, Suture Enlarged					
Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)	0	0
SUPRAOCCIPITALS, IRREGULARLY SHAPED					
Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)	0	0
HYOIDb:					
Ala(e), Angulated					
Litter Incidence	N(%)	2(11.1)	1(6.2)	3(21.4)	3(17.6)
Fetal Incidence	N(%)	3(2.3)	1(0.8)	3(2.6)	3(2.7)k
Ala(e), Short					
Litter Incidence	N(%)	1(5.6)	1(6.2)	0	0
Fetal Incidence	N(%)	2(1.6)	1(0.8)	0	0
VERTEBRAE:					
Cervical, Hemivertebra					
Litter Incidence	N(%)	0	0	1(7.1)	1(5.9)
Fetal Incidence	N(%)	0	0	1(0.9)h	1(0.9)l
Cervical, Centrum, Unilateral Ossification					
Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9)h	0

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PROTOCOL 320-006: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 4): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0
VERTEBRAE (CONTINUED):					
Cervical, Centra, Fused					
Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9)[h]	0
Thoracic, Hemivertebra					
Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9)[f]	0
Thoracic, Centrum, Unilateral Ossification					
Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9)[h]	0
Thoracic, Centra, Fused					
Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9)[h]	0
Lumbar, Centra, Fused					
Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9)[f]	0
Lumbar, Arch, Small					
Litter Incidence	N(%)	0	0	0	1(5.9)
Fetal Incidence	N(%)	0	0	0	1(0.9)[j]
Caudal, Misaligned					
Litter Incidence	N(%)	1(5.6)	0	0	0
Fetal Incidence	N(%)	1(0.8)[e]	0	0	0

PROTOCOL 320-006: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 5): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0
RIBS:					
Split					
Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9)[h]	0
STERNEBRAE SUMMARIZATION (Includes incompletely ossified, fused and asymmetric sternebrae):					
Litter Incidence	N(%)	2(11.1)	2(12.5)	2(14.3)	3(17.6)
Fetal Incidence	N(%)	2(1.6)	4(3.0)	2(1.8)	3(2.7)
STERNEBRAE:					
Incompletely Ossified					
Litter Incidence	N(%)	0	0	0	2(11.8)
Fetal Incidence	N(%)	0	0	0	2(1.8)[k,l]
Fused					
Litter Incidence	N(%)	2(11.1)	2(12.5)	2(14.3)	1(5.9)
Fetal Incidence	N(%)	2(1.6)[d]	4(3.0)	2(1.8)[g,i]	1(0.9)
Asymmetric					
Litter Incidence	N(%)	1(5.6)	0	0	0
Fetal Incidence	N(%)	1(0.8)[d]	0	0	0

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TABLE 13 (PAGE 6): FETAL SKELETAL ALTERATIONS - SUMMARY

FOOTNOTES

a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported the acid equivalent of 2,4-D contained in the 2,4-D 2-Ethylhexyl Ester.
b. Fetuses with alterations of the skull and/or hyoid are not separately identified in this summary table, except when alterations of other ossification sites were also present.
c. Includes all findings noted for the skull except hyoid, ala(e), angulated; ala(e), short. These categories are excluded because they are not considered to result from irregular ossification.
d. Fetus 19757-2 also had other skeletal alterations.
e. Fetus 19770-5 also had other skeletal alterations.
f. Fetus 19796-1 also had other skeletal alterations.
g. Fetus 19801-8 also had other skeletal alterations.
h. Fetus 19803-4 also had other skeletal alterations.
i. Fetus 19803-9 also had other skeletal alterations.
j. Fetus 19818-3 also had other skeletal alterations.
k. Fetus 19818-4 also had other skeletal alterations.
l. Fetus 19825-7 also had other skeletal alterations.

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PROTOCOL 320-006: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2 ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 14 (PAGE 1): FETAL OSSIFICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	75
LITTERS EXAMINED	N	18	16	14	17
FETUSES EXAMINED	N	128	131	114	110
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	0.99 ± 0.02	1.00 ± 0.00	1.00 ± 0.00	0.99 ± 0.05
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	12.66 ± 0.28	12.54 ± 0.31	12.72 ± 0.32	12.68 ± 0.30
LUMBAR	MEAN±S.D.	6.34 ± 0.29	6.46 ± 0.31	6.26 ± 0.30	6.31 ± 0.31
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	17.14 ± 0.49	16.81 ± 0.46	17.07 ± 0.22	17.19 ± 0.48
RIBS (PAIRS)	MEAN±S.D.	12.57 ± 0.29	12.51 ± 0.30	12.61 ± 0.28	12.64 ± 0.30
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.99 ± 0.02	3.97 ± 0.10	3.98 ± 0.04	3.96 ± 0.12
XIPHOID	MEAN±S.D.	0.89 ± 0.19	0.92 ± 0.25	0.91 ± 0.18	0.86 ± 0.18
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.99 ± 0.02	4.99 ± 0.05	4.99 ± 0.03	5.00 ± 0.00
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	13.94 ± 0.09	13.96 ± 0.09	13.95 ± 0.09	13.87 ± 0.31
HINDLIMB b					
TARSALS	MEAN±S.D.	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
METATARSALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
PHALANGES	MEAN±S.D.	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	11.99 ± 0.05

a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported as the acid equivalent of 2,4-D contained in the 2,4-D 2-Ethylhexyl Ester.

b. Calculated as average per limb.

PRIMARY REVIEWER: Jess Rowland, Toxicologist
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II

DATA EVALUATION REPORT

3. RANGE-FINDING STUDY--RABBIT

STUDY TYPE: Developmental Toxicity **[Range-Finding] GUIDELINE: N/A**

CASWELL NO. 315 AS MRID No. 423046-04 DP Barcode:D179762

TEST MATERIAL: 2-Ethylhexyl Ester of 2,4-D ACID **[2,4-D-EHE]**

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

TESTING LABORATORY: Argus Research Laboratories, PA

STUDY IDENTIFICATION: Argus 320-006P

TITLE OF REPORT: *DOSAGE-RANGE DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOOCTYL ESTER] ADMINISTERED ORALLY [STOMACH TUBE] TO TO NEW ZEALAND WHITE RABBITS.*

AUTHOR: Terry Martin, D.V.M **REPORT DATE:** April 10, 1992

SUMMARY: In a dose range-finding study, impregnated New Zealand White rabbits were given oral administration of Technical 2,4-D-2-ethylhexyl ester [63.25% acid equivalent; 95% pure] in aqueous methylcellulose [1%] at acid equivalent doses of 0, 50, 100, 150 or 200 mg/kg/day during days 6 through 18 of gestation. Does were sacrificed on gestation day 29. No maternal toxicity was seen at 50 mg/kg/day. 2,4-D-2-EHE induced maternal toxicity which included mortality/morbidity, clinical signs of toxicity, decreases in body weight gain and food consumption, and gross pathological alterations at 100, 150 and 200 mg/kg/day. Treatment had no adverse effects on pregnancy rate, the number of implantations, the number of resorptions, litter size, or viability of fetuses at 50 mg/kg/day. However, maternal toxicity at higher dose levels precluded meaningful evaluation of these parameters at higher levels. No fetal data were reported.

MATERNAL TOXICITY NOEL = 50 mg/kg/day [LDT]; LOEL = 100 mg/kg/day

DEVELOPMENTAL TOXICITY NOEL = 50 mg/kg/day; LOEL = 100 mg/kg/day.

CORE CLASSIFICATION:: Not applicable; range-finding study.

1. OBJECTIVE

The objective of this **range-finding study in rabbits** was to establish appropriate dose levels of the 2-ethylhexyl ester of 2,4-D [2,4-D-2-EHE] for the main study.

2. PROTOCOL

Groups of five inseminated female New Zealand White rabbits [Hra:(NZW)SPF] approximately 5 months of age and weighing 2.5 to 5.5 kg were given oral administrations of **Technical 2,4-D-2-EHE [62,25% acid equivalent, 95% pure, Lot No.04KF54479]** at acid equivalent doses of 50, 100, 150, or 200 mg/kg/day in 1% methylcellulose [10 mL/kg] daily, during days 6 through 18 of gestation. A control group consisting of four rabbits received the vehicle alone under the same schedule. Concentration and homogeneity of the test article/vehicle mixtures were determined prior to the initiation of the study.

Animals were observed daily for viability, clinical signs of toxicity, abortions, premature deliveries, body weights and food consumptions during the dosing and post-dosing periods. Dams in each group were sacrificed on day 29 and postmortem examination included macroscopic examination of internal organs, with emphasis on the uterus, uterine contents, position of each fetus in the uterus, and corpora lutea counts. Fetal examinations were not performed.

3. RESULTS

i. Analysis of dosing solution

The mean concentrations found were 82%, 94%, 76% and 96% of the nominal concentration, respectively on the first day of dosing, and 96%, 87%, 75%, and 84% on the last day of dosing, respectively, for the 50, 100 and 150 mg/kg/day groups. Dosing solution exhibited 100% homogeneity.

ii. Maternal Toxicity

- 50 mg/kg/day: No mortality
- 100 mg/kg/day: 40% [2/5] mortality; 1 doe found dead on day 21 & 1 doe sacrificed moribund on Day 13.
- 150 mg/kg/day: 60% [3/5] mortality; 1 doe each found dead on Days 17 & 18 and 1 doe sacrificed moribund on Day 9.
- 200 mg/kg/day: 100% [5/5] mortality 1 doe each found dead on Days 9 & 11; 2 does found dead on Day 12 and 1 doe sacrificed on Day 10.
- Because of deaths/moribund sacrifices, the 100 and 150 mg/kg/day groups were not continued on study after Day 21 and 19, respectively.
- Treatment-related clinical signs of toxicity observed in does [at 100, 150 and 200 mg/kg/day] prior to death or sacrifice included hyperactivity, myotonia, decreased motor activity, lost righting reflex, impaired righting reflex, labored breathing and/or ataxia. No treatment-related clinical signs were seen at 50 mg/kg/day.
- Body weight gain was unaffected at 50 mg/kg/day. Body weight loss occurred at 100 mg/kg/day [Days 6 to 9 & Days 15 to 19], 150 mg/kg/day [Days 12 to 15 and Days 15 to 19], and 200 mg/kg/day [Days 6 to 9]. However, deaths and moribund sacrifices at these higher levels precluded further meaningful evaluation of body weights or body weight gains.

- Food consumption was unaffected at 50 mg/kg/day. Decreases in food consumption occurred in the 100 mg/kg/day [Days 6 to 9; 9 to 12; 15 to 19; 6-19], 150 mg/kg/day [Days 12 to 15 & Days 15 to 19], and 200 mg/kg/day [Days 6 to 9]. However, deaths and moribund sacrifices at these higher levels precluded further meaningful evaluation.

- Treatment-related gross pathological alterations seen in rabbits found dead or sacrificed moribund were a red substance in the anogenital area in one doe at 100 mg/kg/day; ulcerations in the gastric fundus in one doe at 150 and in another doe at 200 mg/kg/day; and distended urinary bladder in one doe at 100 mg/kg/day and two does at 200 mg/kg/day. All other necropsy observations were not considered to be treatment related.

- The pregnancy rate was 100%, 100%, 80%, 100% and 80% at 0, 50, 100, 150 and 200 mg/kg/day dose groups, respectively.

iii. Developmental Toxicity

- Since there were no surviving dams at 100, 150 or 200 mg/kg/day at termination, cesarean section data is based on the 50 mg/kg/day.

- No marked treatment-related effects were observed in implantations, litter sizes, or viable fetuses at 50 mg/kg/day when compared to controls. The average number of early and late resorptions and the number of does with any resorptions at 50 mg/kg/day were slightly higher than the concurrent control group values but were within the range of historical control values. No doe had a litter of only resorbed conceptuses, and there were no dead fetuses. Maternal deaths or sacrifices precluded examination of these parameters in does given higher dose levels.

- No data were reported for fetal examinations.

4. CONCLUSION:

MATERNAL TOXICITY: NOEL = 50 mg/kg/day [LDT]
LOEL = 100 mg/kg/day

DEVELOPMENTAL TOXICITY: NOEL = 50 mg/kg/day [LDT]
LOEL = 100 mg/kg/day

54

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150

Amendment to DER for MRID 42780001/42780004 - Subchronic Oral Toxicity Study [Dog] [2,4-DICHLOROPHENOXYACETIC ACID]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 010938].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 3/29/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 4/2/01
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: subchronic oral toxicity - dog — § 82-1(b)/OPPTS 870.3150
P.C. CODE: 030001 — REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315 — CAS NO.: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [96.7%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force on 2,4-D Research

CITATION: Dalgard, D. W. (1993). 13-Week Dietary Toxicity Study of 2,4-D in Dogs. Hazleton Washington, Inc.; Study No. HWA 2184-125, May 6, 1993. MRID 42780001. 4-Week Exploratory Rangefinding Study in Dogs with 2,4-D. Study No. 2184-121, September 17, 1992. MRID 42780004. Document No. 010938. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [MRID 42780001], 4 beagle dogs/sex/group were administered 2,4-dichlorophenoxyacetic acid [96.7%] *via* the diet for 13 weeks at concentrations of 0, 0.5 mg/kg/day, 1.0 mg/kg/day, 3.75 mg/kg/day, and 7.5 mg/kg/day. The dose levels were selected based on the results of a 4-week range-finding study [MRID 42780004]in which decreased body weight, food consumption, alterations in clinical chemistry parameters, and distended gall bladders were observed at ≥10 mg/kg/day.

There were no deaths, and no treatment-relatedclinical signs were observed. Decreased body weights were observed in males at the highest dose level [85% of control at week 14] and in females at the lowest and highest dose levels [91% of control] at week 14. Body-weight gains were decreased throughout the study in both sexes at the two highest dose levels but there was no dose-response [overall gain: males 52% and 55% of the control value/females 53% and 58% of the control value at 3.75 and 7.5 mg/kg/day, respectively]. Females at the two lowest dose levels also displayed decreased body-weight gains [overall gain: 74% and 79% of control at 0.5 and 1.0 mg/kg/day, respectively] compared to the control. Decreased food consumption was observed in males at the two highest dose levels and in all female groups compared to their respective controls, but there was no consistent dose response.

Ophthalmology findings were comparable among the groups for both sexes. There were no apparent treatment-related alterations in hematology among the groups for either sex, with the exception of decreased platelet counts. Decreased platelet counts [73% and 87% of control] were observed in males at the high-dose level at both [week 4 and week 13, respectively] time points. Elevations in blood urea nitrogen [two highest dose levels, both sexes], creatinine [three highest dose levels, both sexes], and alanine aminotransferase [two highest dose levels, both sexes] and decreased glucose levels [two highest dose levels, females] were observed at week 4 and/or week 13; however, a dose response was not always apparent. Although no corroborative histopathological changes were observed in

the liver or kidneys, similar changes in several of these clinical chemistry parameters have been observed in other studies on both 2,4-D and the 2,4-D salts and esters in rats and in an earlier subchronic toxicity study in dogs with 2,4-D [MRID 41737301]. In this latter study, microscopic lesions were observed in the kidneys of both sexes at 10 mg/kg/day.

Males at the two highest dose levels displayed a dose-related decrease in testes weight [absolute, relative to body, and relative to brain], although statistical significance was not attained [n=4]. Decreased adrenal weight [absolute and relative to brain] was observed in both sexes at the highest dose level, decreased liver weight [absolute and relative to brain] was observed in both sexes at the highest dose level, and decreased heart weight [absolute and relative to brain] was observed in both sexes at the two highest dose levels, but there was no dose response. Microscopically, hypospermatogenesis/ juvenile testis was observed in 1 control, 1 mid-high [3.75 mg/kg/day], and 2 high-dose [7.5 mg/kg/day] males, and inactive, juvenile prostate was observed in one male in the control, 0.5 mg/kg/day, 1 mg/kg/day, and 3.75 mg/kg/day dose groups and in all 4 high-dose males.

The NOAEL is 1.0 mg/kg/day. The LOAEL of 3.75 mg/kg/day is based on decreased body-weight gain (both sexes) and food consumption (males), as well as alterations in clinical chemistry parameters [increased BUN, creatinine, and alanine aminotransferase] in both sexes, and decreased testes weight.

This subchronic oral toxicity study is classified ACCEPTABLE/Guideline,and it satisfies the guideline requirement [OPPTS 870. 3150; §82-1(b)] for a subchronic non-rodent oral toxicity study.

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 1. Body-Weight and Food Consumption Data

Parameter/Week/Sex/Dose	0 mg/kg/day	0.5 mg/kg/day	1.0 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
BODY WEIGHT [kg]					
MALES					
1	8.0±1.1	8.3±1.3	8.0±1.0	9.2±1.1 [115]♪	7.5±0.8 [94]
2	8.0±1.0	8.8±1.4	8.2±0.5	9.1±1.2	7.6±0.7
3	8.3±1.2	8.8±1.1	8.6±0.4	9.4±1.1	7.9±0.8
4	8.9±1.3	9.2±1.0	9.0±0.9	9.8±1.3 [110]	8.1±0.7 [91]
6	9.3±1.0	9.6±1.1	9.8±0.8	10.2±1.2	8.5±0.6 [91]
9	10.3±1.1	10.1±1.0	10.2±1.0	10.4±1.7	8.7±0.6 [84]
11	10.5±1.1	10.3±1.0	10.6±1.1	10.6±1.7 [101]	9.2±0.5 [88]
14	10.8±1.2	10.8±1.1	11.1±1.3	10.6±1.5 [98]	9.2±0.8 [85]
FEMALES					
1	6.9±1.0	6.6±0.5 [96]	6.7±0.7	7.3±1.2	6.9±0.8
2	7.0±0.7	6.7±0.4	7.0±0.5	7.5±1.0	6.9±1.0
3	7.3±0.8	7.1±0.6	7.0±0.5	7.5±0.9	7.0±0.9
4	7.4±0.8	7.2±0.5	7.1±0.5	7.7±1.0	7.1±0.8
6	7.8±0.9	7.5±0.5	7.6±0.8	7.9±1.2	7.3±0.8 [94]
9	8.2±1.1	7.3±0.7	7.9±0.7	8.1±1.2	7.4±0.8 [90]
11	8.6±1.1	7.7±0.6	8.1±0.9	8.4±1.1	7.8±1.0 [91]
14	8.8±1.0	8.0±0.7 [91]	8.3±0.9	8.3±1.1	8.0±1.1 [91]
BODY-WEIGHT GAIN [kg]✖					
MALES	no data	no data	no data	no data	no data
0-1	0	0.5	0.2	-0.1	0.1
1-2	0.3	0	0.4	0.3	0.3
2-3	0.6	0.4	0.4	0.4	0.2
3-4	0.2	0.1	0.3	0.2	0.1
4-5	0.4	0.1	0.2	0.1	0.1
6-7	0.1	0.2	0.2	-0.1	0.2
9-10	0.2	0.4	0.3	-0.4	0
11-12	0	0.2	0.1	0.2	0
13-14	2.9±0.2	2.5±1.0 [86]	3.1±0.8	1.5±0.9 [52]	1.6±0.5 [55]
1-14✔					
FEMALES	no data	no data	no data	no data	no data
0-1	0.1	0.1	0.3	0.2	0
1-2	0.3	0.4	0	0	0.1
2-3	0.1	0.1	0.1	0.2	0.1
3-4	0.3	0.1	0.2	0	0.1
4-5	0.2	0.1	0.3	0.1	0
6-7	0.3	0.5	0.1	0.2	0.3
9-10	0	0.3	0	-0.1	0
11-12	0	0	0	0	0.2
13-14	1.9±0.3	1.4±0.4 [74]	1.5±0.7 [79]	1.0±1.0 [53]	1.1±0.4 [58]
1-14✔					

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150

Table 1. Body-Weight and Food Consumption Data

Parameter/Week/Sex/Dose	0 mg/kg/day	0.5 mg/kg/day	1.0 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
FOOD CONSUMPTION✖					
MALES					
1	1797±552	1827±306	1786±417	1560±435 [87]	1425±338 [79]
2	1895±308	1986±194	1933±476	1775±234 [94]	1670±268 [88]
3	2194±124	2096±308	2268±425	1894±231 [86]	1751±96 [80]
4	2063±298	1932±384	2211±302	1827±305 [89]	1622±226 [79]
8	2328±233	1959±316	2167±434	1807±398 [78]	1868±203 [80]
11	2199±191	2433±294	2185±426	1653±515 [75]	1980±452 [90]
13	2078±211	2346±288	2088±224	2062±362 [99]	1858±280 [89]
1-13✔	28085±2057	27512±2845	27820±3615	22755±3048 [81]	23716±2644 [84]
FEMALES					
1	1744±120	1575±400 [90]	1514±232 [87]	1388±302 [80]	1596±307 [92]
2	1898±351	1516±138 [80]	1604±178 [84]	1356±192 [71]	1650±142 [87]
3	1818±305	1708±217 [94]	1498±225 [82]	1532±391 [84]	1524±143 [84]
4	1925±297	1553±193 [81]	1577±128 [82]	1428±232 [74]	1564±109 [81]
8	1938±342	1506±247 [78]	1626±188 [84]	1610±311 [83]	1680±138 [87]
11	1877±214	1863±451	1650±196 [88]	1554±55 [83]	1757±287 [94]
13	2051±330	1729±510 [84]	1716±127 [84]	1527±130 [74]	1687±146 [82]
1-13✔	25078±3668	22427±3636 [89]	21377±1574 [85]	19933±3176 [79]	21855±2092 [87]

✖ calculated by this reviewer [not provided in report]; n=4; ♪ [% of control];1 ✔ interval provided in report;
data from Tables 3 and 4 [pages 48-49 and 51-52] of the report

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150

Table 2. Clinical Pathology Data - MALES					
Dose/Week/Parameter	0 mg/kg/day	0.5 mg/kg/day	1.0 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
platelets [TH/UL]					
-1	378±78	459±109	452±144	414±47	365±54 [97] √
4	350±50	408±80	348±56	325±68	254±104 [73]
13	300±86	360±97	296±57	343±127	261±84 [87]
WBC [TH/UL]					
-1	14.1±2.3	14.9±6.0	16.9±5.4	11.5±1.5	15.3±6.6
4	12.7±3.9	15.8±6.5	13.4±2.6	12.9±2.1	13.4±4.1
13	11.0±2.9	13.1±2.6	13.0±1.5	11.7±3.2	10.5±1.5 [95]
lymphocytes [TH/UL]					
-1	4.1±2.3	4.5±1.6	4.8±1.5	3.9±0.6	3.4±0.7 [83]
4	3.5±2.3	4.1±1.5	4.7±1.4	3.4±0.5	3.2±1.4 [91]
13	3.5±0.6	3.9±1.7	3.3±0.6	3.7±0.4	3.3±1.0 [94]
alanine aminotransferase [U/L]					
-1	24±3	28±4	29±5	32±3	36±16 [150]
4	28±3	266±470[a]	41±10	89±48* [318]	101±58* [361]
13	31±5	40±7	41±8* [132]	96±44* [310]	87±47* [281]
aspartate aminotransferase [U/L]					
-1	32±2	32±7	32±2	33±5	36±6
4	32±3	55±41	39±3	40±8	41±8
13	34±4	30±6	38±8	42±5	38±6
blood urea nitrogen [mg/dL]					
-1	10±2	11±1	11±3	11±4	9±1
4	11±2	11±2	14±1	18±3* [164]	19±3* [173]
13	13±3	16±1	17±3	20±5* [154]	22±2* [169]
creatinine [mg/dL]					
-1	0.7±0.0	0.7±0.10	0.7±0.08	0.8±0.06	0.7±0.08
4	0.7±0.05	0.8±0.16	0.9±0.05* [129]	1.0±0.13* [143]	1.0±0.06* [143]
13	0.8±9.06	1.0±0.08	1.1±0.05	1.3±0.26* [163]	1.3±0.06* [163]
alkaline phosphatase [U/L]					
-1	109±21	107±21	122±13	151±61	123±13
4	118±12	180±178	124±10	115±40	82±13 [69]
13	77±10	70±14	81±8	91±52	71±13 [92]
glucose [mg/dL]					
-1	102±4	104±8	98±11	106±8	108±10
4	102±7	103±5	106±11	103±3	101±11
13	96±7	100±10	99±13	96±7	97±9

√[% of control]; [a] values for 4 dogs were 30, 970, 34, 29; * $p<0.05$; data from Tables 6-7 [pages 57-79] of the report

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150

Table 3. Clinical Pathology Data - FEMALES					
Dose/Week/Parameter	0 mg/kg/day	0.5 mg/kg/day	1.0 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
platelets [TH/UL]					
-1	292±14	390±56 [134]	416±42* [142]	462±106* [158]	378±14 [129]
4	280±53	335±18 [120]	347±37 [124]	358±58 [128]	257±95 [92]
13	236±93	342±59 [145]	321±39 [136]	336±60 [142]	313±72 [133]
WBC [TH/UL]					
-1	9.9±3.3	8.8±1.8	9.9±0.8	9.6±1.4	9.9±1.7
4	12.1±3.2	13.1±5.5	13.3±3.0	12.6±3.1	11.7±0.8
13	10.9±2.8	11.4±4.0	10.6±1.8	12.0±2.9	9.2±1.7 [84]
lymphocytes [TH/UL]					
-1	3.3±1.3	3.0±0.3	3.5±0.3	2.9±1.0	3.3±1.0
4	2.8±1.0	3.5±0.8	3.3±0.5	3.5±1.9	3.9±0.5 [139]
13	3.4±1.1	3.0±0.7	3.5±1.2	2.9±1.4	3.1±1.0 [91]
alanine aminotransferase [U/L]					
-1	30±3	26±4	31±2	28±5	29±7
4	31±4	30±3	34±4	49±15* [158]	63±46* [203]
13	37±8	36±8	37±4	56±14 [151]	76±46* [205]
aspartate aminotransferase [U/L]					
-1	33±11	33±9	31±6	32±9	30±1
4	31±2	39±6	36±8	33±4	35±5
13	34±9	36±7	34±6	35±8	41±6 [121]
blood urea nitrogen [mg/dL]					
-1	9±1	11±1	10±2	12±1	11±1
4	11±2	12±2	13±2	19±2* [173]	19±5* [173]
13	13±3	15±1	17±2 [131]	23±5* [177]	22±2* [169]
creatinine [mg/dL]					
-1	0.7±0.1	0.7±0.1	0.7±0.1	0.8±0.1	0.8±0.1
4	0.7±0.1	0.8±0.1	0.9±0.1	1.1±0.2* [157]	1.0±0.1* [143]
13	0.8±0.1	0.8±0.1	1.1±0.1* [138]	1.4±0.2* [175]	1.2±0.1* [150]
alkaline phosphatase [U/L]					
-1	135±23	120±23 [89]	122±24 [90]	124±33	151±34
4	116±23	98±21 [84]	111±18	81±8 [70]	97±11 [84]
13	83±24	78±14 [94]	82±19	59±9 [71]	80±11
glucose [mg/dL]					
-1	104±9	106±9	104±7	105±6	103±8
4	104±8	105±2	102±3	98±2	97±6
13	97±5	96±9	94±7	83±6* [86]	85±1* [88]

√[% of control]; * $p<0.05$; data from Tables 6-7 [pages 57-79] of the report

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 4. Organ Weights					
Dose/Organ	0 mg/kg/day	0.5 mg/kg/day	1.0 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
ADRENAL					
males					
absolute	1.00±0.23	1.02±0.20	1.06±0.21	1.13±0.05	0.89±0.20 [89]♪
relative to body	0.010±0.002	0.010±0.001	0.010±0.001	0.011±0.002	0.010±0.002
relative to brain	0.013±0.004	0.013±0.002	0.014±0.003	0.015±0.001	0.012±0.003 [92]
females					
absolute	0.98±0.22	0.99±0.29	1.02±0.18	0.91±0.08	0.80±0.19 [82]
relative to body	0.012±0.002	0.013±0.005	0.013±0.003	0.011±0.001	0.011±0.002 [92]
relative to brain	0.014±0.003	0.014±0.003	0.015±0.003	0.013±0.001	0.011±0.003 [79]
LIVER					
males					
absolute	268±47	247±26	278±48	246±39	225±20 [84]
relative to body	2.6±0.2	2.4±0.2	2.6±0.4	2.4±0.1	2.5±0.2
relative to brain	3.6±0.7	3.2±0.3	3.5±0.5	3.2±0.6	3.1±0.1 [86]
females					
absolute	224±41	193±28 [86]	194±22 [87]	208±34 [93]	196±21 [88]
relative to body	2.7±0.3	2.6±0.4	2.4±0.2	2.5±0.3	2.7±0.2
relative to brain	3.3±0.6	2.8±0.4	2.8±0.5	3.0±0.3	2.7±0.3 [82]
HEART					
males					
absolute	91.6±8.6	87.0±7.0	87.2±7.4	73.4±7.0* [80]	71.4±4.4
relative to body	0.89±0.08	0.85±0.06	0.83±0.04	0.73±0.08* [82]	0.81±0.02
relative to brain	1.22±0.10	1.14±0.04	1.12±0.13	0.96±0.13* [79]	0.99±0.07
females					
absolute	76.9±14.1	74.7±10.2	72.1±4.9 [94]	62.3±7.6 [81]	67.8±10.6 [88]
relative to body	0.92±0.09	0.98±0.09	0.91±0.05	0.76±0.07* [83]	0.91±0.08
relative to brain	1.13±0.21	1.07±0.14	1.04±0.13	0.90±0.09 [80]	0.93±0.16 [82]
TESTES					
males					
absolute	17.0±3.3	16.0±3.7	16.1±1.4 [95]	13.2±3.4 [78]	10.6±0.7* [62]
relative to body	0.16±0.02	0.16±0.03	0.15±0.02	0.13±0.04 [81]	0.12±0.01 [75]
relative to brain	0.23±0.05	0.21±0.06	0.21±0.02	0.17±0.05 [74]	0.15±0.02 [65]
BRAIN					
males					
absolute	75.3±2.8	76.4±8.3	78.3±4.8	77.0±3.5	72.3±5.8 [96]
relative to body	0.73±0.08	0.75±0.05	0.75±0.08	0.77±0.13	0.82±0.07
females					
absolute	68.3±4.6	70.1±5.7	69.7±5.0	69.3±3.6	73.1±2.7 [107]
relative to body	0.83±0.10	0.93±0.14	0.89±0.14	0.86±0.11	1.00±0.15 [120]
TERMINAL BODY WEIGHT					
males	10350±1066	10250±915	10550±835	10175±1384	8875±619 [86]
females	8325±1040	7600±698	7950±772	8225±1389	7425±1011 [89]

N=4/sex; √[% of control]; * $p<0.05$; data from Table 9 [pages 89-97] of the report

[2,4-DICHLOROPHENOXYACETIC ACID] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150

Table 5. Histopathology

Dose/Organ	0 mg/kg/day	0.5 mg/kg/day	1.0 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
TESTES	♂	♂	♂	♂	♂
hypospermatogenesis/juvenile testis	1	0	0	1	2
giant cells	3	2	2	2	3
PROSTATE	♂	♂	♂	♂	♂
inflammation, chronic	0	0	1	0	1
inactive/juvenile prostate	1	1	1	1	4

n=4; data from Table 10 [pages 99-106 of the report]

508

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 4-25-94
Section IV, Tox. Branch II

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Section IV, Tox. Branch II

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral Toxicity Study - Dogs (§82-1B)

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid

SYNONYM: 2,4-D

PC Code: 030001 (2,4-D) **MRID Nos.:** 13-Week Study = 427800-01
4-Week Study = 427800-04

STUDY NUMBERS: 13-Week Study = HWA 2184-125
4-Week Rangefinding Study = HWA 2184-121

SPONSOR: Industry Task Force on 2,4-D Research

TESTING FACILITY: Hazleton Washington, Inc., Vienna, VA

TITLES OF REPORTS: 13-Week Dietary Toxicity Study of 2,4-D in Dogs
4-Week Exploratory Rangefinding Study in Dogs with 2,4-D

AUTHOR: Dan W. Dalgard

REPORTS ISSUED: 13-Week = May 6, 1993; 4-Week = September 17, 1992

EXECUTIVE SUMMARY:

In a subchronic toxicity study, 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 0.5, 1.0, 3.75 and 7.5 mg/kg/day for 13 weeks. The following parameters were examined: mortality, clinical signs, body weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, macroscopic pathology, organ weights and microscopic pathology.

Group mean body weight gains were 89, 111, 50 and 61% of the control value for males and 74, 84, 53 and 58% for females at 0.5, 1.0, 3.75 and 7.5 mg/kg/day, respectively. Both sexes of dogs at 3.75 and 7.5 mg/kg/day exhibited elevated levels of blood urea nitrogen, creatinine and alanine aminotransferase. However, no corroborative histopathological changes were seen in the liver or kidneys. **[MRID Nos. 427800-01 and 04]**

The NOEL is 1.0 mg/kg/day. The LOEL of 3.75 mg/kg/day is based on decreased body weight gain and food consumption as well as alterations in clinical chemistry parameters.

This study is core classified as **Minimum** and satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

THIS DER INCLUDES THE REVIEWS OF A 4-WEEK PALATABILITY AND TOXICITY STUDY IN FEMALE DOGS AND A 13-WEEK TOXICITY STUDY IN MALE AND FEMALE DOGS.

I. TEST ARTICLE

Name: 2,4-dichlorophenoxyacetic acid (2,4-D)

II. 4-WEEK EXPLORATORY RANGEFINDING STUDY (MRID No. 427800-04)

A. Test Article Description

Lot No.: 909
Storage: refrigerated
Physical property: off-white powder
Purity: 96.7%

B. Test Article Homogeneity, Stability and Concentration

Table 1

TEST ARTICLE HOMOGENEITY, STABILITY AND CONCENTRATION IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH 2,4-D

Time/Location	10 mg/kg/day		15 mg/kg/day		20 mg/kg/day	
	Target ppm	% of Target	Target ppm	% of Target	Target ppm	% of Target
DAY 0						
top	362	100	443	100	677	97
middle	362	99	443	98	677	99
bottom	362	100	443	99	677	100
DAY 7						
top	362	93	443	97	677	98
middle	362	96	443	96	677	95
bottom	362	99	443	97	677	98
CONCENTRATION						
week 1	349	99	523	98	697	99
week 4	349	97	523	99	697	108

All % of target are means of duplicate samples.
Data extracted from Report Table 1, pages 24-27.

Homogeneity, stability and concentration data were within acceptable limits.

C. Dietary Admixes

After the test article was ground to a fine powder (mortar and pestle), weighed material was combined with about 200 g of basal diet and mixed in a Waring blender. This premix was added to appropriate amounts of basal food and mixed in a Patterson-Kelly twin-shell blender. Fresh diets were prepared weekly and made available to the dogs ad libitum 7 days/week for at least 28 days.

D. Animals

Female beagles were received from Hazleton Research Products, Inc., Cumberland, VA. There was a 2-week period of acclimation. Dogs were individually housed in stainless-steel cages and exercised 3 times each week for at least 15 minutes. Room temperature and humidity were 62-80°F and 20-85%, respectively. There was a 12-hour light/dark cycle. Food and water were available ad libitum.

There were 2 control dogs and 3/treated group. The doses were 10, 15 and 20 mg/kg/day.

E. Observations

SURVIVAL AND CLINICAL SIGNS

Animals were observed A.M. and P.M. each day. A physical examination was performed weekly.

There was no mortality.

Primarily during study weeks 3 and 4, a relative dose-dependent increase was reported in the incidence of fecal abnormalities: few or no feces, diarrhea, soft feces, mucoid feces (with or without blood) and discolored (orange) feces. Hypoactivity was reported in one 20 mg/kg/day dog and emesis in one 10 and one 20 mg/kg/day dogs. Both controls were without clinical signs.

BODY WEIGHTS

These were recorded weekly. Table 2.

Table 2

INDIVIDUAL BODY WEIGHTS AND WEIGHT GAINS IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH 2,4-D

Dog No.	mg/kg/day	Body Weight (kg) - Week					Gain or Loss
		1	2	3	4	5	
390	0	7.2	7.4	7.6	7.7	7.5	+0.3
391	0	7.9	7.7	7.7	8.0	8.1	+0.2
392	10	7.7	7.7	7.8	7.6	7.7	0
393	10	8.4	8.4	8.1	7.8	7.7	-0.7
394	10	9.7	10.3	9.6	9.5	9.4	-0.3
395	15	7.4	7.6	7.3	7.0	6.8	-0.6
396	15	8.6	8.7	8.6	8.6	8.5	-0.1
397	15	9.0	8.8	8.8	8.6	8.5	-0.5
398	20	6.7	6.8	6.3	6.0	5.6	-1.1
399	20	8.0	7.8	7.0	6.2	6.0	-2.0
400	20	9.6	9.3	8.9	8.3	8.0	-1.6

Data extracted from Report Appendix 3, page 64.

The two controls gained weight and all but one 10 mg/kg/day treated dogs lost weight in a dose-response fashion.

FOOD CONSUMPTION

This was recorded weekly.

All treated groups ate less food than the controls, particularly during weeks 2,3 and 4. The 20 mg/kg/day dogs ate 1/3 to 1/2 as much as controls during this time period. There was a decrease in the amount of food eaten in each of the 4 groups from week 1 to week 4 (% decrease: 5, 20, 32 and 61 for 0, 10, 15 and 20 mg/kg/day).

TEST ARTICLE CONSUMPTION

This was calculated weekly and was based on body weight and food eaten. Table 3.

During the 1st week, when food consumption by treated groups was not much below the control value, test article intake was near the target doses. For the last 3 weeks, when food intake was reduced, there was a concomitant decrease in test article consumed so that during this 3-week period, the dogs actually received an average of 7.2, 10.5 and 9.0 mg/kg/day (target doses were 10, 15 and 20 mg/kg/day).

Table 3

GROUP MEAN TEST ARTICLE CONSUMPTION (mg/kg/day) IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH 2,4-D

mg/kg/day	No. of Dogs	Week			
		1	2	3	4
10	3	8.7	7.1	7.1	7.4
15	3	13.6	11.3	10.9	9.4
20	3	18.5	11.2	7.3	8.4

Data extracted from Report Table 5, page 35.

E. Clinical Pathology

Although none was originally scheduled for this study, the Report indicated that, because of results from other studies, limited serum chemistry parameters were to be examined. Blood was taken from the jugular vein of fasted (food and water withheld - no time stated) animals during the last treatment week. The following were measured: alkaline phosphatase, urea nitrogen, creatinine and total cholesterol. Table 4.

Table 4

GROUP MEAN CLINICAL CHEMISTRY VALUES IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH 2,4-D

mg/kg/day	Urea nitrogen (MG/DL)	Creatinine (MG/DL)	Alkaline Phos. (U/L)	Total Choles. (MG/DL)
0	12	0.6	65	156
10	28	1.3	50	195
15	37	1.2	38	180
20	33	1.2	23	202

No. of dogs: control = 2; treated = 3/group
Data extracted from Report Table 6, page 37.

There were increases in urea nitrogen, creatinine and total cholesterol group mean values of treated dogs compared with control. Alkaline phosphatase levels in animals administered the test article were lower than in the control group.

553

F. Sacrifice and Pathology

Following 4 treatment weeks, all dogs were fasted, weighed, anesthetized with sodium thiamylal (intravenous) and exsanguinated. Gross necropsies were performed and about 38 tissues were fixed in 10% formalin. No tissues were processed/ examined microscopically.

The only apparent test article related finding was a distended gallbladder in 0/2 controls, 2/3 at 10 mg/kg/day, 3/3 at 15 mg/kg/day and 2/3 at 20 mg/kg/day.

G. Discussion

No dogs died during the 4-week study. A dose-dependent increase in fecal abnormalities was noted during the 3rd and 4th weeks.

The 2 control dogs gained 0.3 and 0.2 kg of body weight during the 4 weeks; whereas, the 10 and 15 mg/kg/day animals lost 0-0.7 kg, with the 20 mg/kg/day dogs losing 1.1-2.0 kg.

Decreases in food consumption were noted for all 3 treated groups during weeks 2, 3 and 4, with the 20 mg/kg/day dogs eating 1/3 to 1/2 as much as the controls.

In treated animals, there were increases in urea nitrogen, creatine and total cholesterol levels and a decrease in alkaline phosphatase.

Distended gallbladders were noted only in treated dogs (2/3, 3/3 and 2/3 at 10, 15 and 20 mg/kg/day).

H. Conclusions

It was considered by the Report Author that doses greater than 10 mg/kg/day exceeded the maximum tolerated dose and levels below that would be appropriate for longer studies.

III. 13-WEEK STUDY (MRID No.: 427800-01)

A. Statistical Analyses

The following were subjected to statistical analysis: body weight changes, total feed consumption, clinical pathology data and organ weight data.

LEVENE'S test of homogeneity of variances

Homogeneous - ANOVA - if not significant "stop"; if significant, Dunnett's control versus treatment comparisons (for equal variances, for unequal variances, if heterogeneous)

Heterogeneous - Log 10 transformation, to square transformation, to square root transformation, to reciprocal transformation, to angular (arcsine) transformation, to rank transformation and to ANOVA. If any transformation is heterogeneous, directly to ANOVA.

B. Regulatory Compliance

A Good Laboratory Practice Compliance Statement, Quality Assurance Statement and a list of Quality Assurance inspections were included in the Report.

A signed statement of no confidentiality claim was provided.

The Report stated that, "The criteria for flagging studies, stipulated in 40 CFR 158.34, do not apply to this study." This was signed by the Testing Facility Study Director, but not by the Sponsor (person's name typed, but no signature or date).

C. Test Article Description

Lot Number: 909
Storage: refrigerated
Physical Property: off-white powder
Purity: 96.7%

D. Test Article Homogeneity, Stability and Concentration

Analyses were performed on all concentrations for male and female diets at weeks 1, 2, 3, 4, 8 and 12. Table 1 presents selected analytical data.

Data from all intervals indicated that concentrations were 93-105% of the targets.

Data for homogeneity, stability and concentration were within acceptable limits.

Table 1

TEST ARTICLE HOMOGENEITY, STABILITY AND CONCENTRATION IN A 13-WEEK STUDY IN DOGS WITH 2,4-D

Time/ Location	0.5 mg/kg/day		1.0 mg/kg/day		3.75 mg/kg/day		7.5 mg/kg/day	
	Targ ppm	% of Targ	Targ ppm	% of Targ	Targ ppm	% of Targ	Targ ppm	% of Targ
PRETEST								
top M	-	-	-	-	-	-	334.13	102
mid M	-	-	-	-	-	-	334.13	102
bot M	-	-	-	-	-	-	334.13	109
top F	17.44	98	-	-	-	-	-	-
mid F	17.44	96	-	-	-	-	-	-
bot F	17.44	99	-	-	-	-	-	-
PRETEST 7 day stabil. rm temp								
M	-	-	-	-	-	-	334.13	97
F	17.44	97	-	-	-	-	-	-
30 DAY STABIL freezer								
M	-	-	-	-	-	-	334.13	96
F	17.44	97	-	-	-	-	-	-
WEEK 1								
M	22.28	97	44.55	102	167.07	97	334.13	96
F	17.44	102	34.87	103	130.77	96	261.54	101
WEEK 12								
M	20	100	40	101	160	100	240	98
F	20	101	30	103	140	99	230	93

% of targets are mean of duplicate samples;
M = male; F = female
mid = middle; bot = bottom
rm temp = room temperature; stabil. = stability; Targ = Target
Data extracted from Report Table 1, pages 41-44.

Data from all intervals indicated that concentrations were 93-105% of the targets.

Data for homogeneity, stability and concentration were within acceptable limits.

E. Dose Selection

The doses for this 13-week study were selected based upon the results of the 4-week rangefinding study (reviewed earlier in this Data Evaluation Report). The doses in this 13-week study are 0, 0.5, 1.0, 3.75 and 7.5 mg/kg/day.

F. Dose Preparation

After the test article was ground to a fine powder (mortar and pestle), weighed material was combined with about 200 g of basal diet and mixed in a Waring blender. This premix was added to appropriate amounts of basal food and mixed in a Patterson-Kelly twin-shell blender. Fresh diets were prepared weekly and made available to the dogs ad libitum 7 days/week for 13 weeks.

G. Animals

Male and female beagle dogs were received from Hazleton Research Products, Inc., Cumberland, VA. Animals were acclimated for at least 2 weeks. Dogs were individually housed in stainless-steel cages in a room with temperature and humidity at 69-76°F and 35-70%, respectively. There was a 12-hour light/dark cycle. The animals were placed in an exercise pen, with members of the same sex and group, 3 days/week for about 15 minutes/day. Food and water were available ad libitum. After physical and ophthalmic examinations as well as clinical laboratory tests, the dogs were stratified by weight and assigned to groups by a computerized randomization program.

H. Survival and Clinical Observations

Dogs were observed twice each day for mortality, moribundity and clinical signs. Physical examinations were conducted once each week at the time of weighing.

All animals survived the 13-week study. No clinical signs were considered to have been the result of test article administration. Fecal changes (discoloration, mucoid, soft, diarrhea, few and none) were noted in dogs of all treated and control groups.

Male No. 29650 (3.75 mg/kg/day) lost 1.5 kg body weight (with a decrease in food consumption) during study week 11. The dog was examined by a veterinarian and was observed to be: hypoactive, hunched, abdominal discomfort, salivating and mucoid/bloody feces. There was an increase in the leukocyte count and a firm swelling (presumed a swollen lymph node) was noted on the neck. A repeat leukocyte count several days later was also elevated. An antibiotic was given for 5 days, after which appetite improved and the swelling subsided. Body weights increased at the week 13 and 14 weighings.

I. Body Weights

Body weights were recorded before treatment and weekly during the study. Table 2.

Table 2

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS IN A 13-WEEK STUDY IN DOGS WITH 2,4-D

Week	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0.5	1.0	3.75	7.5	0	0.5	1.0	3.75	7.5
B.W.										
1	8.0	8.3	8.0	9.2	7.5	6.9	6.6	6.7	7.3	6.9
2	8.0	8.8	8.2	9.1	7.6	7.0	6.7	7.0	7.5	6.9
3	8.3	8.8	8.6	9.4	7.9	7.3	7.1	7.0	7.5	7.0
4	8.9	9.2	9.0	9.8	8.1	7.4	7.2	7.1	7.7	7.1
6	9.3	9.6	9.8	10.2	8.5	7.8	7.5	7.6	7.9	7.3
8	9.9	10.0	10.1	10.4	8.7	8.2	7.8	7.9	7.9	7.5
10	10.4	10.3	10.4	10.3	8.9	8.5	7.8	8.0	8.3	7.7
12	10.7	10.7	10.9	10.2	9.2	8.6	8.0	8.1	8.3	7.8
14	10.8	10.8	11.1	10.6	9.2	8.8	8.0	8.3	8.3	8.0
B.W. GAIN										
1-4	0.9	0.9	1.0	0.6	0.6	0.5	0.6	0.4	0.4	0.2
4-8	1.0	0.8	1.1	0.6	0.6	0.8	0.6	0.8	0.2	0.4
8-14	0.9	0.8	1.0	0.2	0.5	0.6	0.2	0.4	0.4	0.5
1-14	2.8	2.5	3.1	1.4	1.7	1.9	1.4	1.6	1.0	1.1

4 dogs/sex/group
Body weight gains were calculated by the Reviewer for all intervals (the Report stated the gains for the weeks 1-14 interval).
Data extracted from Report Table 3, pages 48 and 49.

For both males and females at 3.75 and 7.5 mg/kg/day, group mean body weight gains were lower (not statistically significant) than control values.

J. Food Consumption

Data for this parameter were recorded each week.

In males, there appeared to be a decrease in group mean food consumption at 3.75 and 7.5 mg/kg/day compared with the control amounts. For females, there were lower group mean values at most measurement intervals in all treated groups versus the control group.

K. Test Article Intake (Report Table 5, pages 54 and 55)

This was calculated from body weight and food consumption data.

The group means for the entire 13-week period were as follows (mg/kg/day):

males = 0.5, 1.0, 3.8 and 7.8 for 0.5, 1.0, 3.75 and 7.5 mg/kg/day
females = 0.5, 1.0, 3.8 and 7.7 for 0.5, 1.0, 3.75 and 7.5 mg/kg/day

L. Ophthalmoscopic Examinations

Using indirect ophthalmoscopy, the eyes of all dogs were examined before the start of treatment and at study termination.

There were no abnormalities noted in any animal which were considered to be the result of test article administration. Dog No. 29628, male, control, had multifocal retinal fold of the left fundus only at termination. Dog No. 29634, male, 0.5 mg/kg/day, had multifocal retinal fold of the left fundus only at pretest. Dog No. 29642, male, 1.0 mg/kg/day, had multifocal retinal fold of the right fundus only at termination. Dog No. 29649, male, 3.75 mg/kg/day, had persistent hyaloid remnant of the left lens only at pretest. Dog No. 29658, male, 7.5 mg/kg/day, had pigment in the right cornea at pretest and right corneal opacity at termination.

M. Clinical Pathology

Blood was collected from the jugular vein of overnight-fasted dogs prior to treatment as well as during weeks 4 and 13 for hematology and clinical chemistry determinations. Urine samples were taken by catheterization prior to study initiation and during week 13 (feed and water available prior to the collection of urine).

HEMATOLOGY

The following parameters were examined:

Erythrocyte count*	Leukocyte count*
Hematocrit*	Corrected leukocyte count
Hemoglobin*	Leukocyte differential*
Platelet count*	Cell morphology
Reticulocyte count	Mean corpuscular volume
Absolute retic. count	Mean corpuscular hemoglobin
	Mean corpuscular hemoglobin concentration

* = EPA Guideline Requirement

There were no hematology parameters which appeared to be altered by test article administration.

CLINICAL CHEMISTRY

The following parameters were examined:

Alanine aminotransferase*
Aspartate aminotransferase*
Alkaline phosphatase
Albumin*
Globulin
Urea nitrogen*
Creatine kinase
Creatinine*
Total bilirubin*
Total cholesterol
Total protein*
Glucose*
Inorganic phosphorus*
Potassium*
Sodium*
Calcium*
Chloride*

* = EPA Guideline Requirement

The parameters which appeared to be altered by test article administration were urea nitrogen, creatinine and alanine aminotransferase. In addition, the Report stated that alkaline phosphatase levels, which usually decline with age, declined more rapidly in treated dogs than in controls. Table 3.

Table 3

SELECTED GROUP MEAN CLINICAL CHEMISTRY PARAMETERS IN A 13-WEEK STUDY IN DOGS WITH 2,4-D

mg/kg/day	Urea nitrogen MG/DL			Creatinine MG/DL			Alanine amino U/L			Alk. phos. U/L		
	-1a	4	13	-1	4	13	-1	4	13	-1	4	13
MALES												
0	10	11	13	0.7	0.7	0.8	24	28	31	109	118	77
0.5	11	11	16	0.7	0.8	1.0	28	266b	40	107	180c	70
1.0	11	14	17	0.7	0.9*	1.1	29	41	41*	122	124	81
3.75	11	18*	20*	0.8	1.0*	1.3*	32	89*	96*	151	115	91
7.5	9	19*	22*	0.7	1.0*	1.3*	36	101*	87*	123	82	71
FEMALES												
0	9	11	13	0.7	0.7	0.8	30	31	37	135	116	83
0.5	11	12	15	0.7	0.8	0.9	26	30	36	120	98	78
1.0	10	13	17	0.7	0.9	1.1*	31	34	37	122	111	82
3.75	12	19*	23*	0.8	1.1*	1.4*	28	49*	56	124	81	59
7.5	11	19*	22*	0.8	1.0*	1.2*	29	63*d	76*e	151	97	80

4 dogs/sex/group
a = week of determination
individual values - **b** = 30, 970, 34 and 29; **c** = 101, 447, 93 and 79
d = 131, 35, 47 and 38; **e** = 143, 41, 69 and 52
NOTE: Dog male 634 (0.5 mg/kg/day) at week 4, alkaline phosphatase = 447, aspartate aminotransferase = 116 (other 3, 26-39) and alanine aminotransferase, 970
Statistical Significance: * = ≤0.05
Data extracted from Report Table 7 and Appendix 7, pages 70-79 and 175-186.

URINALYSIS (not an EPA requirement)

The following parameters were examined:

Appearance	Occult blood	Bilirubin
pH	Glucose	Protein
Ketones	Specific gravity	Microscopic examination of sediment
Urobilinogen		

There were no parameters which appeared to be altered by test article administration.

N. Sacrifice and Pathology

At the end of the 13-week treatment period, the animals were weighed, anesthetized with sodium thiamylal and exsanguinated. Complete necropsies were performed. The following organs were weighed and organ-to-body weight as well as organ-to-brain weight ratios were calculated: adrenals, brain with stem, heart, kidneys, liver without gallbladder, ovaries, pituitary, testes without epididymides and thyroid with parathyroid.

The following tissues were removed, preserved in 10% neutral buffered formalin and the "x" organs weighed:

DIGESTIVE	RESPIRATORY	UROGENITAL
Salivary gland*	Trachea*	xKidneys*
Esophagus*	Lung*	Urinary bladder*
Stomach*		xTestes*
Duodenum*	CARDIOVASC/HEMAT	xOvaries
Jejunum*	Aorta*	Epididymides
Ileum*	xHeart*	Prostate
Cecum*	Bone marrow*	Uterus*
Colon*	Lymph nodes*	Lacrimal gland
xLiver*	Spleen*	Vagina
Pancreas*	Thymus*	
Gallbladder*		
Tongue		

NEUROLOGIC	GLANDULAR	OTHER
xBrain*	xAdrenals*	Bone*
Peripheral nerve*	Mammary gland*	Skeletal muscle*
Spinal cord (3 levels)*	xParathyroids*	Skin
xPituitary*	xThyroid*	Gross lesions and masses*
Eyes (with optic n.)*		

* = EPA Guideline Requirements [rectum required but not examined]

MACROSCOPIC

There were no gross pathology findings that were considered to have been related to test article administration.

ORGAN WEIGHTS

Statistically significant ($p \leq 0.05$) or apparent differences were noted in the weights of thyroids, heart and testes of treated versus control groups. Table 4.

Table 4

GROUP MEAN ABSOLUTE AND RELATIVE THYROID/PARATHYROID, HEART AND TESTES WEIGHTS IN A 13-WEEK STUDY IN DOGS WITH 2,4-D

mg/kg/day	Males				Females			
	Final B.W. (g)	Absolute (g)	to BW (%)	to brain (ratio)	Final B.W. (g)	Absolute (g)	to BW (%)	to brain (ratio)
THYROID								
0	10350	0.95	.009	.013	8325	0.70	.008	.010
0.5	10250	0.80	.008	.011	7600	0.73	.010	.010
1.0	10550	1.03	.010	.013	7950	0.75	.010	.011
3.75	10175	1.06	.010	.014	8225	0.75	.009	.011
7.5	8875	0.83	.009	.011	7425	1.03	.014*	.014
HEART								
0	10350	91.6	0.89	1.22	8325	76.9	0.92	1.13
0.5	10250	87.0	0.85	1.14	7600	74.7	0.98	1.07
1.0	10550	87.2	0.83	1.12	7950	72.1	0.91	1.04
3.75	10175	73.4*	0.73*	0.96*	8225	62.3	0.76*	0.90
7.5	8875	71.4*	0.81	0.99*	7425	67.8	0.91	0.93
TESTES								
0	10350	17.0	0.16	0.23				
0.5	10250	16.0	0.16	0.21				
1.0	10550	16.1	0.15	0.21				
3.75	10175	13.2	0.13	0.17				
7.5	8875	10.6*	0.12	0.15				

4 dogs/sex/group
Statistical Significance: * $=p \leq 0.05$
Data extracted from Report Table 9, pages 88-97.

Only female thyroid (absolute, to-body weight and to-brain weight) weights at 7.5 mg/kg/day appeared to be heavier than controls. This observation did not seem to be of toxicological significance.

Heart weights for males and females in the 3.75 and 7.5 mg/kg/day groups appeared to be below respective control values (except for female to-body weight at 7.5 mg/kg/day).

The Report Author indicated that, "The significance of the lower heart weights in Groups 4 and 5 [3.75 and 7.5 mg/kg/day] is not known."

Group mean testicular weights were observed to be lower than controls in the 3.75 and 7.5 mg/kg/day groups.

MICROSCOPIC

Report pages 38 and 39 (Pathology Report, Histopathology), indicated that there were no consistent lesions which were attributed to test article administration. One male and one female at 7.5 mg/kg/day were reported to have moderate perivascular, chronic active inflammation of the liver (male No. 658, female No. 661). Table 5.

Table 5

SEVERITY OF LIVER INFLAMMATION IN A 13-WEEK STUDY IN DOGS WITH 2,4-D

Parameter		Males (mg/kg/day)					Females (mg/kg/day)				
		0	0.5	1.0	3.75	7.5	0	0.5	1.0	3.75	7.5
No. Examined		4	4	4	4	4	4	4	4	4	4
No. not remarkable		0	0	0	0	0	0	1	0	0	1
Inflammation, chronic	->	0	0	1	0	1	0	2	0	0	1
	1>	4	4	3	4	2	3	2	3	4	3
	2>	0	0	0	0	1	1	0	1	0	0
	TL>	4	4	4	4	4	4	4	4	4	4
	MN>	1.0	1.0	0.8	1.0	1.0	1.3	0.5	1.3	1.0	0.8
Inflammation, chronic active, perivascular	->	0	2	2	0	1	1	2	1	1	1
	1>	3	1	1	4	1	3	2	3	2	2
	2>	1	1	1	0	1	0	0	0	1	0
	3>	0	0	0	0	1	0	0	0	0	1
	TL>	4	4	4	4	4	4	4	4	4	4
	MN>	1.3	0.8	0.8	1.0	1.5	0.8	0.5	0.8	1.0	1.3

-> = finding not present
1> = minimal
2> = slight
3> = moderate
TL> = total
MN> = mean of graded findings
Data extracted from Report Table 11, pages 106-107.

O. Discussion

Data for homogeneity, stability and concentration were within acceptable limits.

All dogs survived the 13-week study. There were no clinical signs which differentiated treated from control dogs. One 3.75 mg/kg/day male presumably had a swollen lymph node with accompanying leukocytosis (lost 1.5 kg body weight during week 11). The dog was given an antibiotic for 5 days and improved.

Body weight gains for both sexes at 3.75 and 7.5 mg/kg/day, were lower than controls, but were not statistically significant. Group body weight gains were 89, 111, 50 and 61% of the control values for males at 0.5, 1.0, 3.75 and 7.5 mg/kg/day, respectively. The corresponding values for females were 74, 84, 53 and 58%.

Food consumption was reduced in males at 3.75 and 7.5 mg/kg/day. In females, lower group mean values were noted at most intervals for all treated groups compared with the control group.

Test article intake (mg/kg/day) over the 13 weeks for the 0.5, 1.0, 3.75 and 7.5 mg/kg/day groups was as follows: males = 0.5, 1.0, 3.8 and 7.8; females = 0.5, 1.0, 3.8 and 7.7.

There were no ophthalmic, hematology or urinalysis changes which appeared related to the administration of the test article.

Blood urea nitrogen, creatinine and alanine aminotransferase values (with one exception) were significantly ($p \leq 0.05$) above control levels at the 4- and 13-week intervals for 3.75 and 7.5 mg/kg/day dogs of both sexes. Alkaline phosphatase levels declined with age more rapidly in treated groups than in the control groups. However, there were no corroborative histopathological changes in the kidneys or liver.

Group mean thyroid weights (absolute, to-body weight and to-brain weight) of 7.5 mg/kg/day females only, appeared to be heavier than the control value. The Report Author stated that the significance of the lower heart weights at 3.75 and 7.5 mg/kg/day was unknown. There were lower group mean testicular weights in the 3.75 and 7.5 mg/kg/day groups compared with the control group. No histopathological changes were seen in the thyroids or hearts. Therefore, these weight changes were not considered to be treatment related or of toxicological significance.

One male and one female at 7.5 mg/kg/day were reported to have <u>moderate</u> chronic active perivascular inflammation of the liver compared with a less severe finding (minimal or slight) in controls or lower dose groups.

IV. CONCLUSIONS

In a subchronic toxicity study, 2,4-dichlorophenoxyacetic acid (1,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 0.5, 1.0, 3.75 and 7.5 mg/kg/day for 13 weeks. The following parameters were examined: mortality, clinical signs, body weights, food consumption, ophthalmology, hematology, clinical chemistry. urinalysis, macroscopic pathology, organ weights and microscopic pathology.

Group mean body weight gains were 89, 111, 50 and 61% of the control value for males and 74, 84, 53 and 58% for females at 0.5, 1.0, 3.75 and 7.5 mg/kg/day, respectively. Both sexes of dogs at 3.75 and 7.5 mg/kg/day exhibited elevated levels of blood urea nitrogen, creatinine and alanine aminotransferase. However, no corroborative histopathological changes were seen in the liver or kidneys.

The NOEL is 1.0 mg/kg/day. The LOEL of 3.75 mg/kg/day is based on decreased body weight gain and food consumption as well as alterations in clinical chemistry parameters.

This study is core classified as **Minimum** and satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

[2,4-D DIMETHYLAMINE SALT] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Amendment to DER for MRID 42780002 / 42780006 Subchronic Oral Toxicity Study [Dog] [2,4-D-2-DIMETHYLAMINE SALT]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 008400].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 2/27/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 3/16/01
Reregistration Branch I, Health Effects Division (7509C)
TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: subchronic oral toxicity - dog § 82-1(b)/OPPTS 870.3150
P.C. CODE: 030019 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315O CAS NO.:2008-39-1
TEST MATERIAL (PURITY): 2,4-D Dimethylamine salt [66.18% (acid equivalent 55.45%)]
SYNONYMS: 2,4-D-dichlorophenoxyacetic acid, dimethylamine salt
SPONSOR: Industry Task Force II on 2,4-D Research

CITATION: Dalgard, D. W. (1993). 13-Week Dietary Toxicity Study with the Dimethylamine salt of 2,4-D in Dogs. Hazleton Washington, Inc., Vienna, VA; Study No. WHA 2184-126, May 7, 1993. MRID 42780002. 4-WeekExploratory Rangefinding Study in Dogs with the Dimethylamine Salt of 2,4-D. Study No. HWA 2184-123, December, 15, 1992. MRID 42780006. Document No. 008400. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [MRID 42780002], 4 beagle dogs/sex/group were administeredthe 2,4-dichlorophenoxyacetic acid, dimethylamine salt [66.18%; 55.45% acid equivalent] *via* the diet for 13 weeks at concentrations of 0, 1 mg/kg/day, 3.75 mg/kg/day, and 7.5 mg/kg/day [calculated for entire 13-week period: males 1.1, 4.0, and 8.3/females 1.0, 3.8, and 7.7 mg/kg/day, respectively]. The dose levels were selected based on the results of a 4-week range-finding study [MRID 42780006].

All dogs survived until study termination. Decreased body weights were observed in both sexes at the high-dose level [males 93%/females 83% of control] at study termination. Body-weight gains were decreased throughout the study in females [overall gain 78%, 61%, and 33% of the control value for females at 1.0, 3.75, and 7.5 mg/kg/day, respectively] and in the high-dose males [68% of control]. Decreased food consumption was observed in females at the high-dose level. There were no treatment-related alterations in hematology among the groups for either sex, with the possible exception of decreased platelet counts in the high-dose males at week 13. Similar decreases in platelet counts were observed in the subchronic dog study on 2,4-D, and this is a consistent finding in the subchronic studies in rats for 2,4-D, its salts and ester. Elevations in blood urea nitrogen [mid-and high-dose for both sexes and low-dose males], creatinine [all doses for males, mid- and high-dose females], aspartate aminotransferase[high-dose females] and alanine aminotransferase [high-dose level for females, all dose levels for males] were observed at week 4 and/or week 13. Although there were no apparent corroborative histopathological changes observed in the liver or kidneys, similar changes in several of these clinical chemistry parameters have been observed in other studies on both dimethylamine salt of 2,4-D and the other amines and ester of 2,4-D, and in some cases, histopathological changes were observed [2,4-D, ethylhexyl ester, diethanolamine, dimethylamine].

[2,4-D DIMETHYLAMINE SALT] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

The NOAEL is 1.0 mg/kg/day. The LOAEL of 3.75 mg/kg/day is based on decreased body-weight gain and alterations in clinical chemistry parameters [increased BUN, creatinine] in both sexes.

This subchronic oral toxicity study is classified ACCEPTABLE/Guideline,and it satisfies the guideline requirement [OPPTS 870. 3150; §82-1(b)] for a subchronic non-rodent oral toxicity study.

NOTE: Although creatinine levels were elevated in the low-dose males at 4 and 13 weeks, there were no other effects observed at this dose level, the low-dose female creatinine values were not elevated, and no histopathological effects were observed at a dose that was 7.5X higher; therefore, the low dose was considered the NOAEL.

[2,4-D DIMETHYLAMINE SALT] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 1. Hematology Parameters				
Dose/Week/Parameter	0 mg/kg/day	1 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
MALES **platelets [TH/UL]**				
-1	382±76	371±53	374±97	410±101
4	302±59	376±43	310±35	305±66
13	300±50	346±28	307±42	273±60 [91]√
FEMALES **platelets [TH/UL]**				
-1	406±92	373±107	407±56	368±102 [91]
4	356±62	330±83	322±46	303±91 [85]
13	305±59	320±87	335±37	294±48 [96]
MALES **RBC [MI/UL]**				
-1	6.29±0.62	6.29±0.45	6.26±0.54	6.69±0.44
4	6.11±0.80	6.91±0.57	6.43±0.48	6.69±0.51
13	6.60±0.86	7.01±0.35	7.02±0.43	7.08±0.47
FEMALES **RBC [MI/UL]**				
-1	6.46±0.51	5.82±0.34	6.43±0.21	6.18±0.68
4	6.50±0.70	6.36±0.48	6.49±0.38	6.34±0.20
13	7.43±0.98	6.59±0.57	6.89±0.29	6.45±0.69
MALES **HGB [G/DL]**				
-1	14.2±1.3	13.9±0.9	13.8±0.9	14.8±1.1
4	14.0±1.6	15.6±1.2	14.2±0.5	14.7±0.7
13	15.1±2.0	15.7±0.9	15.4±0.9	15.5±0.6
FEMALES **HGB [G/DL]**				
-1	14.5±1.3	13.0±0.7	14.7±0.6	13.9±2.1
4	14.7±2.0	14.3±0.7	14.9±0.9	14.3±0.7
13	16.9±2.4	14.8±1.2	15.8±0.8	14.4±1.0
MALES **HCT [%]**				
-1	41.2±3.8	40.7±2.6	40.6±2.6	42.8±2.8
4	40.1±4.9	45.1±3.6	41.2±1.4	42.5±2.0
13	43.0±5.6	45.2±2.6	44.7±2.3	44.6±1.6
FEMALES **HCT [%]**				
-1	41.7±3.3	37.7±2.1	42.4±1.8	40.3±5.7
4	42.0±5.1	41.3±2.2	42.8±2.9	41.2±1.0
13	48.3±6.8	42.5±3.4	45.2±2.0	41.7±3.5

√[% of control]; data from Table 6, page 58 of the report

[2,4-D DIMETHYLAMINE SALT] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 2. Clinical Chemistry Parameters - MALES				
Dose/Week/Parameter	0 mg/kg/day	1 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
alanine aminotransferase [U/L]				
-1	25±1.7	30±3.9 [120]√	28±8.1 [112]	34±5.5 [136]
4	28±3.6	39±7.8 [139]	61±46.9* [218]	223±171.3* [796]
13	35±2.6	51±10.7* [146]	88±74.1* [251]	388±464.2* [1109]
aspartate aminotransferase [U/L]				
-1	33±4.7	32±4.3	33±4.7	46±24.6
4	34±3.8	35±8.4	35±1.8	41±6.8
13	35±5.7	39±4.8	43±6.8	48±7.6
blood urea nitrogen [mg/dL]				
-1	10±1.7	12±1.7 [120]	11±1.0 [110]	12±3.1 [120]
4	11±2.9	19±2.1* [173]	18±2.4* [164]	22±4.9* [200]
13	12±0.5	17±5.4 [142]	22±6.0* [183]	25±2.9* [208]
creatinine [mg/dL]				
-1	0.6±0.05	0.6±0.06	0.6±0.06	0.7±0.00 [117]
4	0.7±0.05	1.0±0.05* [143]	1.0±0.13* [143]	1.1±0.08* [157]
13	0.9±0.10	1.2±0.17* [133]	1.2±0.22* [133]	1.3±0.06* [144]
alkaline phosphatase [U/L]				
-1	104±13.2	126±9.7	113±22.8	101±18.2
4	86±14.8	113±17.0	86±20.0	70±10.4
13	62±11.0	74±9.4	63±20.0	68±22.9
total protein [g/dL]				
-1	5.7±0.33	5.8±0.22	5.5±0.14	5.8±0.17
4	5.7±0.29	5.8±0.22	5.4±0.22	5.7±0.35
13	6.2±0.39	6.0±0.21	5.6±0.30	5.4±0.47* [87]√
glucose [mg/dL]				
-1	109±2.6	115±4.6	112±7.0	111±4.5
4	106±4.3	109±5.2	107±5.7	110±8.9
13	97±5.4	100±4.3	97±6.2	91±9.7

√[% of control]; * $p<0.05$; data from Table 7, pages 63-67 of the report

576

[2,4-D DIMETHYLAMINE SALT] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 3. Clinical Chemistry Parameters - FEMALES				
Dose/Week/Parameter	0 mg/kg/day	1 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
alanine aminotransferase [U/L]				
-1	29±5.4	32±2.9	30±8.3	30±7.0
4	36±3.6	36±5.7	50±18.4	74±28.3* [206]√
13	41±5.1	36±0.6	47±11.9	87±16.3*
aspartate aminotransferase [U/L]				
-1	28±7.3	37±5.9	36±6.6	35±8.9 [125]
4	31±9.5	47±10.4	40±11.1	42±4.9 [135]
13	34±4.2	40±3.2	42±7.8	49±9.2* [144]
blood urea nitrogen [mg/dL]				
-1	12±3.5	11±2.6	11±1.7	13±4.3 [108]
4	16±7.4	19±5.5 [119]	20±2.9 [125]	22±2.5 [138]
13	13±0.6	18±5.4 [138]	22±2.0* [169]	23±4.8* [177]
creatinine [mg/dL]				
-1	0.6±0.05	0.6±0.10	0.6±0.06	0.7±0.05 [117]
4	0.7±0.05	0.9±0.17 [129]	1.0±0.13* [143]	1.1±0.15* [157]
13	0.8±0.06	1.0±0.17 [125]	1.2±0.08* [150]	1.3±0.13 [163]
alkaline phosphatase [U/L]				
-1	134±23.0	100±4.8*	131±30.5	144±32.9
4	112±17.7	78±3.2	93±24.4	86±10.4
13	89±11.6	49±6.2*	63±18.0*	78±12.1
total protein [g/dL]				
-1	5.3±0.30	5.4±0.00	5.6±0.13	5.5±0.38
4	5.3±0.38	5.3±0.22	5.4±0.17	5.4±0.10
13	5.8±0.39	5.7±0.14	5.7±0.31	5.8±0.29
glucose [mg/dL]				
-1	105±2.1	107±2.4	108±11.3	103±4.1
4	114±19.6	107±9.7	101±4.8	86±8.7* [75]
13	101±9.5	93±4.8 [92]	92±5.3 [91]	84±4.8* [83]

√[% of control]; * $p<0.05$; data from Table 7, pages 63-67 of the report

571

[2,4-D DIMETHYLAMINE SALT] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 4. Organ Weights

Dose/Organ	0 mg/kg/day	1 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
ADRENAL				
males				
absolute	1.19±0.37	1.23±0.18	1.23±0.12	1.02±0.12 [86]√
relative to body	0.012±0.004	0.011±0.001	0.011±0.001	0.010±0.001
relative to brain	0.014±0.004	0.015±0.003	0.016±0.002	0.014±0.002
females				
absolute	1.13±0.34	1.17±0.20	0.98±0.18	0.90±0.09 [80]
relative to body	0.015±0.005	0.016±0.002	0.014±0.002	0.014±0.002 [93]
relative to brain	0.016±0.005	0.016±0.004	0.014±0.003	0.013±0.001 [81]
KIDNEY				
males				
absolute	59.0±8.4	55.1±8.2	52.4±4.8	46.6±5.3 [79]
relative to body	0.56±0.06	0.50±0.09	0.49±0.03	0.48±0.05 [86]
relative to brain	0.72±0.09	0.69±0.11	0.69±0.06	0.64±0.08 [89]
females				
absolute	37.0±2.7	41.6±6.0	38.7±5.7	34.3±4.2 [93]
relative to body	0.48±0.02	0.55±0.06	0.55±0.05	0.54±0.05
relative to brain	0.52±0.05	0.56±0.10	0.56±0.05	0.51±0.05
HEART				
males				
absolute	94.2±14.4	92.4±10.8	93.1±2.8	82.8±13.3 [88]
relative to body	0.89±0.07	0.84±0.05	0.87±0.07	0.85±0.08
relative to brain	1.16±0.24	1.15±0.16	1.22±0.04	1.14±0.17
females				
absolute	69.7±8.6	68.0±3.9	64.3±10.5	58.5±7.5 [84]
relative to body	0.91±0.10	0.91±0.03	0.92±0.08	0.92±0.09
relative to brain	0.99±0.17	0.92±0.12	0.93±0.11	0.87±0.09 [88]
LIVER				
males				
absolute	300±33	298±49	283±36	261±25 [87]
relative to body	2.9±0.0	2.7±0.6	2.6±0.1	2.7±0.4
relative to brain	3.7±0.6	3.7±0.5	3.7±0.5	3.6±0.4
females				
absolute	209±29	221±24	192±35	188±19 [90]
relative to body	2.7±0.3	2.9±0.3	2.7±0.3	3.0±0.3
relative to brain	2.9±0.3	3.0±0.3	2.8±0.4	2.8±0.3
TERMINAL BODY WEIGHT				
males	10500.1±1023.1	11000.0±983.2	10750.0±1047.2	9775.0±1537.0 [93]
females	7675.0±170.8	7500.0±476.1	7000.0±890.7	6400.0±820.6 [83]

√[% of control]; data from Table 9, pages 77-83 of the report

572

[2,4-D DIMETHYLAMINE SALT] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 5. Histopathology

Dose/Organ	0 mg/kg/day		1 mg/kg/day		3.75 mg/kg/day		7.5 mg/kg/day	
LIVER	**males**	**females**	**males**	**females**	**males**	**females**	**males**	**females**
inflammation, chronic								
->	1	2	1	1	1	2	0	2
1>	3	2	3	3	3	2	4	2
T	4	4	4	4	4	4	4	4
MN	0.8	0.5	0.8	0.8	0.8	0.5	1.0	0.5
inflammation, chronic active/perivascular								
->	0	1	3	4	1	3	1	2
1>	4	3	1	0	3	0	1	1
2>	0	0	0	0	0	1	2	0
3>	0	0	0	0	0	0	0	1
T	4	4	4	4	4	4	4	4
MN	1.0	0.8	0.3	0.0	0.8	0.5	1.3	1.0
inflammation, chronic active/multifocal								
->	4	4	4	4	4	4	4	3
2>	0	0	0	0	0	0	0	1
T	4	4	4	4	4	4	4	4
MN	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
necrosis, individual cell								
->	4	4	4	4	4	4	4	3
2>	0	0	0	0	0	0	0	1
T	4	4	4	4	4	4	4	4
MN	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
TESTES	**males**		**males**		**males**		**males**	
hypospermatogenesis/juvenile testis	1		0		1		3	
giant cells	1		3		1		3	
PROSTATE	**males**		**males**		**males**		**males**	
inactive/juvenile prostate	1		0		3		4	

Grade: -> finding not present; 1> minimal; 2> slight; 3> moderate; T total; MN mean of graded finding: √[% of control]; n=4
data from Tables 10-11, pages 89-95 of the report

593

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 4-25-94
Section IV, Tox. Branch II

Secondary reviewer: Jess Rowland, M.S. Jess Rowland 4/25/94
Section IV, Tox. Branch II

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral Toxicity Study - Dogs (§82-1B)

TEST MATERIAL: Dimethylamine salt of 2,4-dichlorophenoxyacetic acid

SYNONYM: DMA salt of 2,4-D

PC Code: 030001 (2,4-D) **MRID Nos.:** 13-Week Study = 427800-02
4-Week Study = 427800-06

STUDY NUMBERS: 13-Week Study = HWA 2184-126
4-Week Rangefinding Study = HWA 2184-123

SPONSOR: Industry Task Force on 2,4-D Research

TESTING FACILITY: Hazleton Washington, Inc., Vienna, VA

TITLES OF REPORTS: 13-Week Dietary Toxicity Study with the Dimethylamine Salt of 2,4-D in Dogs
4-Week Exploratory Rangefinding Study in Dogs with the Dimethylamine Salt of 2,4-D

AUTHOR: Dan W. Dalgard

REPORTS ISSUED: 13-Week = May 7, 1993; 4-Week = December 15, 1992

EXECUTIVE SUMMARY:

In a subchronic toxicity study, the dimethylamine salt of 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 1, 3.75 and 7.5 mg/kg/day for 13 weeks. The following parameters were examined: mortality, clinical signs, body weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, macroscopic pathology, organ weights and microscopic pathology.

Group mean body weight gains were 126, 126 and 68% of the control value for males and 78, 61 and 33% for females at 1, 3.75 and 7.5 mg/kg/day, respectively. There was decreased food consumption in 7.5 mg/kg/day females as well as elevated alanine aminotransferase, aspartate aminotransferase, urea nitrogen and creatinine at 3.75 and 7.5 mg/kg/day. However, no corroborative histopathological changes were seen in the liver or kidneys. **[MRID Nos. 427800-02 and 06]**

The NOEL is 1.0 mg/kg/day. The LOEL of 3.75 mg/kg/day is based on decreased body weight gain and food consumption as well as alterations in clinical chemistry parameters.

This study is core classified as **Minimum** and satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

THIS DER INCLUDES THE REVIEWS OF A 4-WEEK PALATABILITY AND TOXICITY STUDY IN FEMALE DOGS AND A 13-WEEK TOXICITY STUDY IN MALE AND FEMALE DOGS.

I. TEST ARTICLE

Name: Dimethylamine salt of 2,4-dichlorophenoxyacetic acid (DMA salt of 2,4-D)

II. 4-WEEK EXPLORATORY RANGEFINDING STUDY (MRID No. 427800-06)

A. Test Article Description

Lot No.: 04FD31349
Storage: refrigerated
Physical property: brown liquid
Purity: 55.45% 2,4-D acid equivalent ("dietary formulations adjusted accordingly")

B. Test Article Homogeneity, Stability and Concentration

Table 1

TEST ARTICLE HOMOGENEITY, STABILITY AND CONCENTRATION IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

Time/Location	10 mg/kg/day		15 mg/kg/day		20 mg/kg/day	
	Target ppm	% of Target	Target ppm	% of Target	Target ppm	% of Target
DAY 0						
top	362	96	443	94	677	98
middle	362	95	443	96	677	93
bottom	362	97	443	93	677	99
DAY 7						
top	362	91	443	94	677	92
middle	362	91	443	89	677	93
bottom	362	93	443	106	677	94
CONCENTRATION						
week 1	349	102	524	102	697	100
week 5	349	104	524	102	697	100

All % of target are means of duplicate samples.
Data extracted from Report Table 1, pages 24-26

Homogeneity, stability and concentration data are within acceptable limits.

C. Dietary Admixes

After shaking, the amount of test article needed was weighed, added to about 200 g of basal diet and mixed in a Waring blender. This premix was added to appropriate amounts of basal food in order to obtain expected diets (10, 15 or 20 mg/kg/day of the test article). Fresh diets were prepared weekly and made available to the dogs ad libitum 7 days/week for at least 28 days.

D. Animals

Female beagles were received from Hazleton Research Products, Inc., Cumberland, VA. There was a 2-week period of acclimation. The dogs were individually housed in stainless-steel cages and exercised 3 times each week for at least 15 minutes. Room temperature and humidity were 68-84°F and 12-82%, respectively. There was a 12-hour light/dark cycle. Food and water were available ad libitum.

There were 2 control dogs and 3/treated group. The doses were 0, 10, 15 and 20 mg/kg/day (2,4-D acid equivalent).

E. Observations

SURVIVAL AND CLINICAL SIGNS

Animals were observed A.M. and P.M. each day. A physical examination was performed weekly.

There was no mortality.

Primarily during the last 10 dosing days, a dose-dependent increase was reported in the incidence of fecal abnormalities (diarrhea, orange and/or bloody feces and mucoid feces). No controls had any of these signs.

One 20 mg/kg/day dog was hypoactive, thin (last week of the study), had decreased food consumption and lost weight.

BODY WEIGHTS

These were recorded weekly. Table 2.

Table 2

INDIVIDUAL BODY WEIGHTS AND WEIGHT GAINS IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

Dog No.	mg/kg/day	Body Weight (kg) - Week					Gain or Loss
		1	2	3	4	5	
412	0	6.6	6.7	6.7	6.7	6.8	+0.2
413	0	7.3	7.7	7.5	7.6	7.7	+0.4
414	10	6.8	7.0	6.9	6.9	6.8	0
415	10	7.3	7.3	7.2	7.2	7.2	-0.1
416	10	8.9	9.1	9.0	8.9	8.8	-0.1
417	15	6.7	6.7	6.6	6.2	6.1	-0.6
418	15	8.5	8.5	8.1	7.9	7.6	-0.9
419	15	8.7	8.7	8.2	8.0	8.1	-0.6
420	20	6.4	6.3	5.7	4.9	4.5	-1.9
421	20	7.4	7.4	7.0	6.6	5.9	-1.5
422	20	8.6	8.6	8.0	7.5	6.7	-1.9

Data extracted from Report Appendix 3, page 60.

The two controls gained a small amount of weight with one 10 mg/kg/day dog having the same terminal as initial weight and all other treated animals losing weight in a dose-response fashion.

FOOD CONSUMPTION

This was recorded weekly. Table 3.

With the exception of one 20 mg/kg/day dog (No. 420), animals in treated groups ate about the same amount of food as did controls during week one. During the 4-week study, the same amount of food was consumed each week by controls, 2/3 dogs at 10 mg/kg/day, 1/3 at 15 mg/kg/day and 0/3 at 20 mg/kg/day.

Table 3

INDIVIDUAL FOOD CONSUMPTION IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

Dog No.	mg/kg/day	Weekly Food Consumption (g)			
		1	2	3	4
412	0	1087	1229	1057	1109
413	0	1639	1599	1592	1650
414	10	1343	1191	1080	989
415	10	1341	1528	1316	1539
416	10	1703	1637	1386	1406
417	15	1278	1037	671	669
418	15	1683	1104	1085	1006
419	15	1550	1300	1318	1469
420	20	919	382	52	128
421	20	1339	985	801	234
422	20	1437	942	686	351

Data extracted from Report Appendix 4, page 62.

TEST ARTICLE CONSUMPTION

This was calculated weekly and was based on body weight and food eaten. Table 4.

Table 4

GROUP MEAN TEST ARTICLE CONSUMPTION (mg/kg/day) IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

mg/kg/day	No. of Dogs	Week			
		1	2	3	4
10	3	9.4	9.3	8.2	8.5
15	3	14.1	11.0	10.0	10.4
20	3	16.3	10.4	7.1	3.7

Data extracted from Report Table 5, page 34.

During the 1st week, when food consumption by treated groups was similar to the control group, test article intake was relatively near the target doses. For the last 3 weeks, especially in the 15 and 20 mg/kg/day groups, the amount of test article received was considerably less than the amount targeted (decreased food consumption and body weight decrease).

E. Clinical Pathology

During the last treatment week, blood was taken from the jugular vein of food-fasted dogs and the following clinical chemistry parameters were examined: alkaline phosphatase, urea nitrogen, creatinine and total cholesterol. Table 5.

Table 5

GROUP MEAN CLINICAL CHEMISTRY VALUES (± S.D.) IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

mg/kg/day	Urea nitrogen (mg/DL)	Creatinine (mg/DL)	Alkaline Phos. (U/L)	Total Choles. (mg/DL)
0	16±0.7	0.8±0.07	72±1.4	149±27.6
10	31±10.4	1.3±0.20	40±9.6	165±39.7
15	33±4.0	1.3±0.00	31±1.7	164±4.5
20	40±5.5	1.3±0.12	38±25.7a	161±46.4

a = Individual values were: 18, 29 and 67.
No. of dogs: control = 2; treated = 3/group
Data extracted from Report Table 6, page 36.

In treated groups, there were increases in group mean urea nitrogen and creatinine values. Alkaline phosphatase levels of 2/3 treated dogs in the 20 mg/kg/day group were below the control. The "sick" dog (No. 420) had a value of 18 U/L.

F. Sacrifice and Pathology

Following 4 treatment weeks, all dogs were fasted, weighed, anesthetized with sodium thiamylal (intravenous) and exsanguinated. Gross necropsies were performed and about 40 tissues were fixed in 10% formalin. No tissues were processed or examined microscopicaly.

The only apparent test article related finding was a distended gallbladder in 0/2 controls, 2/3 at 10 mg/kg/day, 3/3 at 15 mg/kg/day and 3/3 at 20 mg/kg/day.

G. Discussion

No dogs died during the 4 week study. A dose dependent increase in fecal abnormalities was noted primarily during the last 10 dosing days. Dog No. 420 (20 mg/kg/day) appeared to be "sick" throughout the study.

The 2 control dogs gained 0.2 and 0.4 kg of body weight during the 4 weeks; whereas, the 10 mg/kg/day animals lost 0-0.1 kg, the 15 mg/kg/day lost 0.6-0.9 kg and the 20 mg/kg/day lost 1.5-1.9 kg.

Most treated animals ate less food than did controls during the last 1 or 2 or 3 weeks of the study. The 20 mg/kg/day dogs, during week 4, ate only about 20% of the amount of food consumed by them during week one. The Report indicated that palatability was probably not the cause of decreased food intake as treated and control dogs ate about the same amount during the 1st study week.

In treated animals, there were increases in urea nitrogen and creatinine values as well as decreases in alkaline phosphatase levels.

Distended gallbladders were noted only in treated dogs (2/3, 3/3 and 3/3 at 10, 15 and 20 mg/kg/day).

G. Conclusions

All three dose levels were poorly tolerated and were considered too high for longer studies.

III. 13-WEEK STUDY (MRID No.: 427800-02

A. Statistical Analyses

The following were subjected to statistical analysis: body weight changes, total feed consumption, clinical pathology data and organ weight data.

LEVENE'S test of homogeneity of variances

<u>Homogeneous</u> - ANOVA - if not significant "stop"; if significant, Dunnett's control versus treatment comparisons (for equal variances, for unequal variances, if heterogeneous)

<u>Heterogeneous</u> - Log 10 transformation, to square transformation, to square root transformation, to reciprocal transformation, to angular (arcsine) transformation, to rank transformation and to ANOVA. If any transformation is heterogeneous, directly to ANOVA.

B. Regulatory Compliance

A Good Laboratory Practice compliance statement, Quality Assurance statement and a list of Quality Assurance inspections were included in the Report.

A signed statement of no confidentiality claim was provided.

The Report stated, "The criteria for flagging studies, stipulated in 40 CFR 158.34, do not apply to this study." This was signed by the Testing Facility Study Director, but not by the Sponsor (person's name typed, but no signature or date).

C. Test Article Description

Lot Number: 04FD31349

Storage: refrigerated

Physical Property: brown liquid

Purity: 55.45%, acid equivalent ("weight adjustment was calculated to 100% purity")

D. Test Article Homogeneity, Stability and Concentration

Analyses were performed on all concentrations for male and female diets at weeks 1, 2, 3, 4, 8 and 12. Table 1.

Data from all intervals indicated that concentrations were 89-115% of the targets.

Data for homogeneity, stability and concentration were within acceptable limits.

Table 1

TEST ARTICLE HOMOGENEITY, STABILITY AND CONCENTRATION IN A 13-WEEK STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

Time/Location	1.0 mg/kg/day		3.75 mg/kg/day		7.5 mg/kg/day	
	Target ppm	% of Target	Target ppm	% of Target	Target ppm	% of Target
PRETEST						
top M ..	-	-	-	-	334.13	98
middle M ..	-	-	-	-	334.13	99
bottom M ..	-	-	-	-	334.13	97
top F ..	34.87	95	-	-	-	-
middle F ..	34.87	96	-	-	-	-
bottom F ..	34.87	100	-	-	-	-
PRETEST (7 day stabil. room temper						
M ..	-	-	-	-	334.13	93
F ..	34.87	94	-	-	-	-
32 DAY STABIL freezer						
M ..	-	-	-	-	334.13	98
F ..	34.87	105	-	-	-	-
WEEK 1						
M ..	44.55	97	167.07	95	334.13	91
F ..	34.87	97	130.77	93	261.54	96
WEEK 12						
M ..	57.42	91	204.33	95	474.19	96
F ..	54.89	96	212.71	102	422.82	94

M = male; F = female
% of targets are means of duplicate samples
Data extracted from Report Table 1, pages 39-43.

E. Dose Selection

The doses for this 13-week study were selected based upon the results of the 4-week rangefinding study (reviewed earlier in this Data Evaluation Report). The doses in this 13-week study are 0, 1, 3.75 and 7.5 mg/kg/day.

F. Dose Preparation

After shaking, an amount of test article for each dose level was weighed, added to about 200 g of basal diet and mixed in a Waring blender. The premix was then added to appropriate amounts of basal diet and mixed in a Patterson-Kelly blender.

Fresh diets were prepared each week based on sex, body weight and food consumption.

G. Animals

Male and female beagle dogs were received from Hazleton Research Products, Inc., Cumberland, VA. Animals were acclimated for at least 2 weeks. Dogs were individually housed in stainless-steel cages in a room where the temperature was 68-77°F and the humidity was 42-77%. There was a 12-hour light/dark cycle. The animals were placed in an exercise pen, with members of the same sex and group, 3 days/week for about 15 minutes/day. Food and water were available ad libitum. After physical and ophthalmic examinations as well as clinical laboratory tests, the dogs were stratified by weight and assigned to groups by a computerized randomization program. Before the start of the study, males weighed 7.7-10.1 kg and females, 5.4-7.5 kg.

H. Survival and Clinical Observations

Dogs were observed twice each day for mortality, moribundity and clinical signs. Physical examinations were conducted once each week (at the time of weighing).

The Report (page 23) indicated that fecal abnormalities consisted of discoloration, mucoid, bloody, soft, diarrhea, few or none and that these were observed in both treated and control animals.

Because of the fecal "abnormalities" reported in the 4-week rangefinding study, these incidences in the 13-week study dogs are presented in Table 2.

Table 2

SUMMARY INCIDENCE OF FECAL "ABNORMALITIES" IN A 13-WEEK STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

Observation	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1	3.75	7.5	0	1	3.75	7.5
diarrhea	0	2	1	1	0	1	1	1
discoloration	2	2	1	2	0	2	0	3
few	1	1	0	1	1	1	2	0
mucoid	3	1	2	3	3	2	1	4
none	0	0	0	1	0	0	0	0
soft	4	4	4	4	4	4	3	4

4 dogs/sex/group
Data extracted from Report Table 2, page 45.

583

I. Body Weights

Body weights were recorded before treatment and weekly during the study. Table 3.

Table 3

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS IN A 13-WEEK STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

Week	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1	3.75	7.5	0	1	3.75	7.5
BODY WEIGHTS								
1	9.2	9.0	8.6	8.8	6.2	6.5	6.2	6.2
2	9.7	9.3	9.1	9.0	6.5	6.8	6.5	6.4
3	10.0	9.7	9.4	9.2	6.8	7.1	6.6	6.4
4	9.9	9.7	9.5	9.6	6.9	7.1	6.7	6.5
6	10.4	10.2	10.0	9.3	7.3	7.4	7.0	6.6
8	10.7	10.6	10.5	9.7	7.6	7.7	7.2	6.7
10	10.8	11.1	10.7	10.1	8.0	7.8	7.3	6.5
12	10.6	11.2	10.9	9.9	8.0	8.0	7.3	6.7
14	11.1	11.4	11.0	10.1	8.0	7.9	7.3	6.8
BODY WT GAIN								
1-4	0.7	0.7	0.9	0.8	0.7	0.6	0.5	0.3
4-8	0.8	0.9	1.0	0.1	0.7	0.6	0.5	0.2
8-14	0.4	0.8	0.5	0.4	0.4	0.2	0.1	0.1
1-14	1.9	2.4	2.4	1.3	1.8	1.4	1.1	0.6*

4 dogs/sex/group
Body weight gains were calculated by the Reviewer for all intervals except weeks 1-14 which were included in the Report.
Statistical Significance: * = ≤ 0.05; presented in the Report only for weeks 1-14 body weight gains.
Data extracted from Report Table 3, pages 47 and 48.

The only statistically significant ($p \leq 0.05$) difference between a treated group and the control, was a lesser body weight gain in the 7.5 mg/kg/day female group. Individual animal weight gains (kg) in the female groups were (mg/kg/day): 0 = 2.4, 1.9, 1.7 and 1.4; 1 = 1.8, 1.6, 1.3 and 0.9; 3.75 = 1.4, 1.1, 1.1 and 0.8; and 7.5 = 1.5, 0.4, 0.3 and 0.1.

J. Food Consumption

Data for this parameter were recorded each week.

For males, there was essentially no difference in the group mean g of food consumed by treated or control groups. An accurate evaluation regarding the 7.5 mg/kg/day males is not considered possible because of the number of weeks that 3 of 4 dogs had "exclude" in place of a value.

Though not statistically significant, the grams of food consumed/week by the 7.5 mg/kg/day females was less than the control at most weekly measurements. The 1 and 3.75 mg/kg/day female group mean values were more similar to the control.

K. Test Article Intake (Report Table 5, pages 53 and 54)

This was calculated from body weight and food consumption data.

The group means for the entire 13-week period were as follows (mg/kg/day):

males = 1.1, 4.0 and 8.3 for 1.0, 3.75 and 7.5
females = 1.0, 3.8 and 7.7 for 1.0, 3.75 and 7.5

L. Ophthalmoscopic Examinations

Using indirect ophthalmoscopy, the eyes of all dogs were examined before the start of treatment and at study termination.

There were no abnormalities noted in any animal at termination which were not reported prior to the beginning of dosing. Dog No. 29577, male, 3.75 mg/kg/day, had hemorrhage of the right fundus (pretest only). Dog No. 29578, male, 3.75 mg/kg/day, had pignment spots on the right fundus (pretest and at termination). Dog No. 29589, female, 7.5 mg/kg/day, had aberrant eyelashes, both conjunctiva (pretest and at termination). Dog No. 29590, female, 7.5 mg/kg/day, had had a non-tapetal hypopigmented area of the right fundus (pretest and at termination).

M. Clinical Pathology

Blood was collected from the jugular vein of overnight-fasted dogs prior to treatment as well as during weeks 4 and 13 for hematology and clinical chemistry determinations. Urine samples were taken by catheterization prior to study initiation and during week 13 (food and water available prior to the collection of urine).

HEMATOLOGY

The following parameters were examined:

Erythrocyte count*	Leukocyte count*
Hematocrit*	Corrected leukocyte count
Hemoglobin*	Leukocyte differential*
Platelet count*	Cell morphology
Reticulocyte count	Mean corpuscular volume
Absolute retic. count	Mean corpuscular hemoglobin
	Mean corpuscular hemoglobin concentration

* = EPA Guideline Requirement

There were no hematology parameters which appeared to be altered by test article administration.

CLINICAL CHEMISTRY

The following parameters were examined:

Alanine aminotransferase*	Total bilirubin*
Aspartate aminotransferase*	Total cholesterol
Alkaline phosphatase	Total protein*
Albumin*	Glucose*
Globulin	Inorganic phosphorus*
Urea nitrogen*	Potassium*
Creatine kinase	Sodium*
Creatinine*	Calcium*
	Chloride*

* = EPA Guideline Requirement

Those parameters which appeared to be affected by test article administration (some, $p \leq 0.05$) were alanine aminotransferase, aspartate aminotransferase, urea nitrogen and creatinine. [Table 4] The Report stated that alkaline phosphatase levels which decline normally with age, declined more rapidly in treated animals than in controls. [Table 5]

Table 4

SELECTED GROUP MEAN CLINICAL CHEMISTRY PARAMETERS IN A 13-WEEK STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

mg/kg/day	Alanine amino. U/L			Aspartate amino. U/L			Urea nitrogen MG/DL			Creatinine MG/DL		
	-1a	4	13	-1	4	13	-1	4	13	-1	4	13
MALE												
0	25	28	35	33	34	35	10	11	12	0.6	0.7	0.9
1	30	39	51*	32	35	39	12	19*	17	0.6	1.0*	1.2*
3.75	28	61*	88*	33	35	43	11	18*	22*	0.6	1.0*	1.2*
7.5	34	223*	388*	46	41	48	12	22*	25*	0.7	1.1*	1.3*
FEM												
0	29	36	41	28	31	34	12	16	13	0.6	0.7	0.8
1	32	36	36	37	47	40	11	19	18	0.6	0.9	1.0
3.75	30	50	47	36	40	42	11	20	22*	0.6	1.0*	1.2*
7.5	30	74*	87*	35.	42	49*	13	22	25*	0.7	1.1*	1.3*

4 dogs/sex/group
a = week of determination
Statistical Significance; * = $p \leq 0.05$
Data extracted from Report Table 7 and Appendix 7, pages 62-67 and 154-165.

The 7.5 mg/kg/day group mean alanine aminotransferase value of 388 U/L at week 13 (control = 35) consisted of the following 4 values: 1,081, 215, 136 and 118 (controls of 31-37). The 3.75 mg/kg/day group mean value for this enzyme at week 13 was 88 and consisted of the following 4 values: 199, 57, 54 and 43.

Table 5

GROUP MEAN ALKALINE PHOSPHATASE VALUES (U/L) IN A 13-WEEK STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

Week	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1	3.75	7.5	0	1	3.75	7.5
-1	104	126	113	101	134	100*	131	144
4	86/17	113/10	86/24	70/31	112/16	78/22	93/29	86/40
13	62/40	74/41	63/44	68/33	89/34	49*/51	63*/52	78/46

#/# = enzyme value Units per Liter/% reduction from week -1 value
4 dogs/sex/group
Statistical Significance: * = $p \leq 0.05$
Data extracted from Report Table 7, page 63.

URINALYSIS (Not an EPA requirement)

The following parameters were examined:

Appearance	Occult blood	Bilirubin
pH	Glucose	Protein
Ketones	Specific gravity	Microscopic examination of sediment
Urobilinogen		

There were no parameters which appeared to be altered by test article administration.

N. Sacrifice and Pathology

At the end of the 13-week treatment period, the animals were weighed, anesthetized with sodium thiamylal and exsanguinated. Complete necropsies were performed. The following organs were weighed and organ-to-body weight percents as well as organ-to-brain weight ratios were calculated: adrenals, brain with stem, heart, kidneys, liver with gallbladder, ovaries, pituitary, testes without epididymides and thyroid with parathyroids.

The following tissues were removed, preserved in 10% neutral-buffered formalin and the "x" organs were weighed.

DIGESTIVE
- Salivary gland*
- Esophagus*
- Stomach*
- Duodenum*
- Jejunum*
- Ileum*
- Cecum*
- Colon*
- xLiver*
- Pancreas*
- Gallbladder*
- Tongue

RESPIRATORY
- Trachea*
- Lung*

CARDIOVASC/HEMAT
- Aorta*
- xHeart*
- Bone marrow*
- Lymph nodes*
- Spleen*
- Thymus*

UROGENITAL
- xKidneys*
- Urinary bladder*
- xTestes*
- xOvaries
- Epididymides
- Prostate
- Uterus*
- Lacrimal gland
- Vagina

NEUROLOGIC
- xBrain*
- Peripheral nerve*
- Spinal cord (3 levels)*
- xPituitary*
- Eyes (with optic n.)*

GLANDULAR
- xAdrenals*
- Mammary gland*
- xParathyroids*
- xThyroids*

OTHER
- Bone*
- Skeletal muscle*
- Skin
- Gross lesions and masses*

* = EPA Guideline Requirements

MACROSCOPIC

There were no gross pathology findings that were considered to have been related to test article administration.

ORGAN WEIGHTS

Testicular weights (absolute, to-body weight and to-brain weight) of the 7.5 mg/kg/day dogs were lower (not statistically significant) than controls. Table 6.

Table 6

GROUP MEAN ± S.D. TESTICULAR WEIGHTS IN A 13-WEEK STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

	mg/kg/day			
	0	1	3.75	7.5
absolute g	17.0±3.9	15.7±3.0	15.1±4.4	9.8±2.3
to-body wt %	0.16±0.03	0.14±0.02	0.14±0.05	0.10±0.02
to-brain wt ratio	0.21±0.05	0.20±0.04	0.20±0.06	0.13±0.03

Data extracted from Report Table 9, page 85.

The group mean absolute brain weight of 7.5 mg/kg/day male dogs was lower ($p \leq 0.05$) than the control mean. The Report Author did not consider this to be of biological significance. Table 7.

Table 7

GROUP MEAN BRAIN WEIGHTS IN A 13-WEEK STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

	Male (mg/kg/day)				Female (mg/kg/day)			
	0	1	3.75	7.5	0	1	3.75	7.5
absolute g	82.1	80.3	76.2	72.7*	71.1	74.4	69.0	67.2
to-body wt %	0.79	0.74	0.71	0.76	0.93	1.00	0.99	1.06

Terminal body weights: males = 10.5, 11.0, 10.8 and 9.8 kg (0, 1, 3.75 and 7.5 mg/kg/day)
females = 7.7, 7.5, 7.0 and 6.4 kg (0, 1, 3.75 and 7.5 mg/kg/day)
Statistical Significance: * = $p \leq 0.05$
Data extracted from Report Table 9, page 82.

59

As the brain-to-body weight values for the control and 7.5 mg/kg/day are similar, the lower ($p \leq 0.05$) absolute weight is probably a reflection of a lower group mean terminal body weight.

MICROSCOPIC

There were no definitive histopathologic findings which were attributed to test article administration.

The Pathology Report (Report page 36) stated, "There was a minimal increase in the average severity of perivascular, chronic active inflammation in the liver of Group 4 (7.5 mg/kg/day) males and females when compared to Group 1 (control) males and females. This increase in severity was due to a slight severity in two males and a moderate severity in one female. Minimal perivascular, chronic active inflammation of the liver noted in controls, across all male treated groups, and Group 4 females, and the single slight occurrence in a Group 3 female, is considered unrelated to treatment."

O. Discussion

Data for homogeneity, stability and concentration were within acceptable limits.

There was no mortality.

An increased incidence of fecal "abnormalities" (as noted in the 4-week Rangefinding study) was not observed in this 13-week study.

The only statistically significant ($p \leq 0.05$) decrease in body weight gain occurred in the 7.5 mg/kg/day females, although in males, the controls gained a group mean of 1.9 kg and the 7.5 mg/kg/day dogs, 1.3 kg.

Food consumption in all treated male groups was similar to the control, although it was not possible to evaluate this parameter accurately because 3 of 4 dogs at 7.5 mg/kg/day did not have food consumption values for most of the weeks (no explanation was given for the lack of data). Females given 7.5 mg/kg/day ate less (not statistically significant) than the controls during most weeks.

Test article intake (mg/kg/day) over the 13 weeks for the 1, 3.75 and 7.5 mg/kg/day groups was as follows: males = 1.1, 4.0 and 8.3; females = 1.0, 3.8 and 7.7.

There were no ophthalmic, hematology or urinalysis changes which appeared related to the administration of the test article.

Statistically significant ($p \leq 0.05$) or apparent (not significant) increases in alanine aminotransferase, aspartate aminotransferase, urea nitrogen and creatinine were reported for males and females of the 7.5 mg/kg/day group at the 4 and 13 week intervals. The 3.75 mg/kg/day group also had values for some of these parameters increased over controls. There were no liver or kidney organ weights or histopathology findings which clearly corroborated these clinical chemistry results.

Testicular (absolute, to-body weight and to-brain weight) weights of the 7.5 mg/kg/day group were lower (not statistically significant) than the control. There were no histopathological findings.

The Pathology Report indicated that there was a minimal increase in the average severity of perivascular, chronic active inflammation in 7.5 mg/kg/day male and female livers (primarily due to 2 males and 1 female), but that this was not considered related to treatment.

IV. CONCLUSIONS

In a subchronic toxicity study, the dimethylamine salt of 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 1, 3.75 and 7.5 mg/kg/day for 13 weeks. The following parameters were examined: mortality, clinical signs, body weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, macroscopic pathology, organ weights and microscopic pathology.

Group mean body weight gains were 126, 126 and 68% of the control value for males and 78, 61 and 33% for females at 1, 3.75 and 7.5 mg/kg/day, respectively. There was decreased food consumption in 7.5 mg/kg/day females as well as elevated alanine aminotransferase, aspartate aminotransferase, urea nitrogen and creatinine at 3.75 and 7.5 mg/kg/day. However, no corroborative histopathological changes were seen in the liver or kidneys.

The NOEL is 1.0 mg/kg/day. The LOEL of 3.75 mg/kg/day is based on decreased body weight gain and food consumption as well as alterations in clinical chemistry parameters.

This study is core classified as **Minimum** and satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

[2,4-D-2-ETHYLHEXYL ESTER] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Amendment to DER for MRID 42780003/42780005 Subchronic Oral Toxicity Study [Dog] [2,4-D-2-ETHYLHEXYL ESTER]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 010938]. The NOAEL/LOAEL have been modified from those in the original DER.

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 1/25/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V. M.D., M. P.H. Virginia A. Dobozy 1/29/01
Reregistration Branch I, Health Effects Division (7509C)

TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: subchronic oral toxicity - dog § 82-1(b)/OPPTS 870.3150
P.C. CODE: 030063 REREGISTRATION CASE NO.: 818730
CASWELL NO.: 315AS CAS NO.: 1928-43-4
TEST MATERIAL (PURITY): 2,4-D-2-ethylhexyl ester [98%; (acid equivalent 62.7%)]
SYNONYMS: 2,4-D-dichlorophenoxyacetic acid, isooctyl (2-ethylhexyl) ester
SPONSOR: Industry Task Force II on 2,4-D Research

CITATION: Dalgard, D. W. (1993). 13-Week Dietary Toxicity Study with the 2-Ethylhexyl Ester of 2,4-D in Dogs. Hazleton Washington, Inc., Vienna, VA; Study No. WHA 2184-127, May 5, 1993. MRID 42780003. 4-Week Exploratory Rangefinding Study in Dogs with the 2-Ethylhexyl Ester of 2,4-D. Study No. WHA 2184-122, December 15, 1992. MRID 42780005. Document No. 010938. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [MRID 42780003], 4 beagle dogs/sex/group were administered the 2,4-dichlorophenoxyacetic acid-2-ethylhexyl ester of [98% (from 21-day dermal and subchronic study reports)]; 62.7% acid equivalent (63.25% acid equivalents listed in 21-day and subchronic rat studies)] *via* the diet for 13 weeks at nominal doses of 0, 1 mg/kg/day, 3.75 mg/kg/day, and 7.5 mg/kg/day [calculated for entire 13-week period: males 1.1, 3.9, and 7.8/females 1.0, 3.9, and 7.7 mg/kg/day, respectively]. Thyroid hormones [T3 and T4] were not monitored. The dose levels were selected based on the results of a 4-week range-finding study [MRID 42780005].

Overall group mean body-weight gains were 67%, 52%, and 15% of the control value for males and 75%, 38%, and 50% for females at 1.0, 3.75, and 7.5 mg/kg/day, respectively. Decreased food consumption was observed at the mid- [≈9% males; 13% females less than control] and high- [23% males; 10% females less than control] dose levels. Hematology parameters were comparable among the groups [both sexes]. There were increases in blood urea nitrogen [BUN] in both sexes at all dose levels at week 13 [statistical significance attained in all groups except the low-dose females], in creatinine in both sexes at all dose levels at week 13 [statistical significance attained in all groups except the low-dose males], and in alanine aminotransferase levels [females] at 3.75 and 7.5 mg/kg/day. In the liver, males at the high-dose level displayed a slight increase in the severity of perivascular chronic inflammation. Decreased testis [males] and ovary [females] weights were observed at the high-dose level and, although these were accompanied by decreased body weight, decreased gonadal weight is a consistent finding in studies on 2,4-D. The high-dose dogs displayed a slightly higher incidence of hypospermatogenesis/juvenile testis and inactive/juvenile prostate compared to the control dogs. There were no toxicologically-significant changes in

the kidney on microscopic examination.

No NOAEL was determined, based on increased BUN and creatinine values at all dose levels at study termination. The LOAEL is 1.0 mg/kg/day

This subchronic oral toxicity study is classified ACCEPTABLE/guideline, and it satisfies the guideline requirement [OPPTS 870. 3150; §82-1(b)] for a subchronic non-rodent oral toxicity study.

NOTE: The original DER set the NOAEL at 1.0 mg/kg/day and the LOAEL at 3.75 mg/kg/day, based on decreased body-weight gains and alterations in clinical chemistry parameters. In this subsequent review, it was determined that the increases observed in BUNand creatinine at the low-dose level were treatment-related. Although there were no histopathological lesions in the kidney to support an effect on this organ as suggested by the statistically-significant increases in the values for BUN and creatinine at all dose levels, the magnitude of the increase was greater with time on test in addition to occurring at a lower dose level with time. It cannot be determined whether further dosing with EHE beyond 13 weeks would result in lesions in the kidneys in dogs. Additionally, alterations in these clinical chemistry parameters were observed in other studies in rats following exposure to 2,4-D and/or the amines/ester of 2,4-D and in dogs following exposure to DMA. In the chronic dog study on 2,4-D, alterations in serum chemistry [BUN and creatinine] with corroborative histopathological lesions in the kidney were observed.

[2,4-D-2-ETHYLHEXYL ESTER] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 1. Clinical Chemistry Observations

Parameter/Week/Dose/Sex	0 mg/kg/day	1 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
MALES				
BUN [mg/dL]				
-1	9±1.9	10±1.4	11±1.2	10±2.4
4	13±1.7	16±1.7	21±2.9*	22±2.2*
13	13±2.4	18±2.4*	25±3.3*	27±5.3*
creatinine [mg/dL]				
-1	0.6±0.05	0.6±0.06	0.6±0.06	0.7±0.00
4	0.7±0.00	0.9±0.12	1.0±0.08*	1.2±0.12*
13	0.8±0.06	1.0±0.13	1.2±0.15*	1.3±0.10*
ALT [U/L]				
-1	41±14.3	32±9.4	22±5.4*	23±1.3*
4	42±12.5	46±18.0	35±11.8	70±67.4
13	47±14.7	45±14.8	39±7.8	71±46.4
glucose [mg/dL]				
-1	108±6.3	104±12.7	101±4.8	102±6.1
4	104±3.3	102±9.2	99±7.1	94±7.0
13	106±5.4	102±10.3	103±7.5	95±8.1
FEMALES				
BUN [mg/dL]				
-1	12±3.9	11±1.2	12±1.7	12±2.8
4	14±2.2	16±2.2	22±2.9*	25±4.8*
13	16±2.2	19±3.4	24±3.8*	25±3.3*
creatinine [mg/dL]				
-1	0.7±0.05	0.7±0.13	0.8±0.06	0.7±0.10
4	0.8±0.05	0.9±0.13	1.1±0.13*	1.1±0.17*
13	0.9±0.05	1.1±0.05*	1.3±0.10*	1.3±0.16*
ALT [U/L]				
-1	30±13.9	28±3.4	30±8.7	30±9.7
4	28±9.5	35±4.5	55±23.7	99±31.7*
13	31±11.0	36±3.9	66±24.2*	127±24.6*
glucose [mg/dL]				
-1	96±13.1	101±12.7	106±8.3	108±5.0
4	96±9.5	97±3.7	97±7.2	90±7.9
13	100±7.5	102±4.6	99±2.2	92±3.1

N=4; * p<-.05; data from Table 7 [pages 62-66] of the report

594

[2,4-D-2-ETHYLHEXYL ESTER] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 2. Organ Weights [grams]

Parameter/Dose/Sex/Week	0 mg/kg/day	1 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
LIVER				
MALES				
absolute	245±15	235±37	219±23	205±5
relative/body	2.5±0.1	2.7±0.4	2.6±0.4	2.7±0.3
relative/brain	3.1±0.2	3.2±0.5	3.1±0.5	2.7±0.1
FEMALES				
absolute	183±20	184±16	193±16	198±11
relative/body	2.4±0.2	2.6±0.1	2.7±0.3	2.8±0.2
relative/brain	2.4±0.1	2.7±0.3	2.9±0.1	2.8±0.2
KIDNEY				
MALES				
absolute	50.2±7.0	45.6±8.6	38.4±1.7	40.8±4.0
relative/body	0.51±0.04	0.52±0.07	0.46±0.02	0.54±0.02
relative/brain	0.64±0.10	0.62±0.09	0.54±0.03	0.54±0.03
FEMALES				
absolute	37.8±5.4	37.3±4.2	35.0±3.1	36.7±3.3
relative/body	0.49±0.05	0.53±0.02	0.49±0.03	0.52±0.02
relative/brain	0.50±0.06	0.55±0.08	0.53±0.05	0.51±0.04
BRAIN				
MALES				
absolute	79.0±0.9	72.9±2.9	71.8±5.2	75.5±3.9
relative/body	0.81±0.08	0.84±0.09	0.85±0.02	1.01±0.10*
FEMALES				
absolute	75.0±4.6	68.6±5.0	66.2±2.3*	71.3±3.3
relative/body	0.98±0.05	0.99±0.17	0.93±0.07	1.01±0.09
THYROID				
MALES				
absolute	0.77±0.08	1.05±0.26	0.97±0.30	0.90±0.12
relative/body	0.008±0.001	0.012±0.002	0.012±0.004	0.012±0.002
relative/brain	0.010±0.001	0.014±0.003	0.014±0.005	0.012±0.001
FEMALES				
absolute	0.64±0.14	0.95±0.32	0.87±0.11	0.92±0.31
relative/body	0.008±0.002	0.013±0.004	0.012±0.001	0.013±0.005
relative/brain	0.009±0.002	0.014±0.005	0.013±0.002	0.013±0.004
ADRENAL				
MALES				
absolute	1.02±0.13	1.15±0.31	0.84±0.14	0.91±0.06
relative/body	0.011±0.002	0.013±0.002	0.010±0.001	0.012±0.001
relative/brain	0.013±0.002	0.016±0.004	0.012±0.002	0.012±0.001
FEMALES				
absolute	1.00±0.12	1.03±0.31	0.98±0.12	0.95±0.21
relative/body	0.013±0.002	0.014±0.003	0.014±0.002	0.013±0.002
relative/brain	0.013±0.002	0.015±0.005	0.015±0.002	0.013±0.003
TESTES				
MALES				
absolute	13.3±1.3	12.0±2.5	11.5±0.8	9.4±3.7
relative/body	0.14±0.02	0.14±0.02	0.14±0.01	0.12±0.03
relative/brain	0.17±0.02	0.16±0.03	0.16±0.02	0.12±0.05

[2,4-D-2-ETHYLHEXYL ESTER] Subchronic Oral Toxicity Study - Dog [§82-1(b)/OPPTS 870.3150]

Table 2. Organ Weights [grams]				
Parameter/Dose/Sex/Week	0 mg/kg/day	1 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
OVARIES				
FEMALES				
absolute	0.89±0.48	0.86±0.20	0.77±0.08 [87]	0.73±0.16 [82]
relative/body	0.012±0.007	0.012±0.002	0.011±0.001	0.010±0.002
relative/brain	0.012±0.007	0.013±0.003	0.012±0.001	0.010±0.002
TERMINAL BODY WEIGHT				
MALES	9775±873	8750±988	8450±387 [86]	7575±1024* [77]♪
FEMALES	7650±700	7050±777	7150±332 [93]	7100±600 [94]

N=4; ♪ [% of control]; * p<0.05; data from Table 9 [pages 76-84] of the report

Table 3. Histopathology [number of dogs with finding]				
Organ/Sex/Dose	0 mg/kg/day	1 mg/kg/day	3.75 mg/kg/day	7.5 mg/kg/day
LIVER				
MALES				
inflammation, chronic active, perivascular				
->	1	2	1	1
1>	3	2	3	1
2>	0	0	0	2
mean grade	0.8	0.5	0.8	1.3
FEMALES				
inflammation, chronic active, perivascular				
->	3	3	2	2
1>	1	1	2	2
mean grade	0.3	0.3	0.5	0.5
TESTIS				
MALES				
hypospermatogenesis/juvenile testis	1	0	1	2
PROSTATE				
fibrosis	0	0	0	1
inactive/juvenile prostate	1	2	3	4
KIDNEY				
MALES				
tubule, microconcretion	4	4	4	2
inflammation, chronic	0	0	1	0
inflammation, subacute	0	0	0	0
FEMALES				
tubule, microconcretion	4	4	4	3
inflammation, chronic	0	0	0	0
inflammation, subacute	0	0	1	0

-> finding not present; 1> minimal; 2> slight; n=4; data from Table 10 [pages 86-92] and Table 11 [page 94] of the report

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 4-25-94
Section IV, Tox. Branch II

Secondary reviewer: Jess Rowland, M.S. Jess Rowland 4/25/94
Section IV, Tox. Branch II

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral Toxicity Study - Dogs (§82-1B)

TEST MATERIAL: 2-ethylhexyl ester of 2,4-dichlorophenoxyacetic acid

SYNONYMS: 2-EHE of 2,4-D

PC Code: 030001 (2,4-D) **MRID Nos:** 13-Week Study = 427800-03
4-Week Rangefinding Study = 427800-05

STUDY NUMBERS: 13-Week Study = WHA 2184-127
4-Week Rangefinding Study = WHA 2184-122

SPONSOR: Industry Task Force on 2,4-D Research

TESTING FACILITY: Hazleton Washington, Inc., Vienna, VA

TITLES OF REPORTS: 13-Week Dietary Toxicity Study with the 2-Ethylhexyl Ester of 2,4-D in Dogs
4-Week Exploratory Rangefinding Study in Dogs with the 2-Ethylhexyl Ester of 2,4-D

AUTHOR: Dan W. Dalgard

REPORTS ISSUED: 13-Week = May 5, 1993; 4-Week = December 15, 1992

EXECUTIVE SUMMARY:

In a subchronic toxicity study, the 2-ethylhexyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 1.0, 3.75 and 7.5 mg/kg/day for 13 weeks. The following parameters were examined: mortality, clinical signs, body weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, macroscopic pathology, organ weights and microscopic pathology.

Group mean body weight gains were 67, 52 and 15% of the control value for males and 75, 38 and 50% for females at 1, 3.75 and 7.5 mg/kg/day, respectively. There were elevations in blood urea nitrogen, creatinine and alanine aminotransferase levels at 3.75 and 7.5 mg/kg/day. However, no corroborative histopathological changes were seen in the liver or kidney. **[MRID Nos. 427800-03 and 05]**

The NOEL is 1.0 mg/kg/day. The LOEL of 3.75 mg/kg/day is based on decreased body weight gain as well as alterations in clinical chemistry parameters.

This study is core classified as **Minimum** and satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

THIS DER INCLUDES THE REVIEWS OF A 4-WEEK PALATABILITY AND TOXICITY STUDY IN FEMALE DOGS AND A 13-WEEK TOXICITY STUDY IN MALE AND FEMALE DOGS.

I. TEST ARTICLE

Name: 2-ethylhexyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D)

II. 4-WEEK EXPLORATORY RANGEFINDING STUDY (MRID No. 427800-05)

A. Test Article Description

Lot No.: 04KF54479
Storage: refrigerated
Physical Property: brown liquid
Purity: 62.7% 2,4-D acid equivalent

B. Test Article Homogeneity, Stability and Concentration

Table 1

TEST ARTICLE HOMOGENEITY, STABILITY AND CONCENTRATION IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE 2-ETHYLHEXYL ESTER OF 2,4-D

Time/Location	10 mg/kg/day		15 mg/kg/day		20 mg/kg/day	
	Target ppm	% of Target	Target ppm	% of Target	Target ppm	% of Target
DAY 0						
top	362	101	443	106	677	104
middle	362	101	443	104	677	106
bottom	362	101	443	105	677	105
DAY 7						
top	-	-	-	-	-	-
middle	362	97	443	98	677	99
bottom	-	-	-	-	-	-
WEEK 2						
top	349	100	523	98	697	99
middle	349	97	523	98	697	97
bottom	349	97	523	97	697	94
CONCENTRATION						
week 1	349	97	523	93	697	99
week 4	349	99	523	100	697	100

All % of target are means of duplicate samples.
Data extracted from Report Table 1, pages 24-28.

Homogeneity, stability and concentration data are within acceptable limits.

598

C. Dietary Admixes

After shaking, the amount of test article needed for one week's diets was weighed. The weighed material was mixed with a small amount of basal diet (Waring blender). This premix was added to appropriate amounts of basal food in order to obtain expected diets (10, 15 or 20 mg/kg/day of 2,4-D acid equivalent). Fresh diets were prepared weekly and made available to the dogs ad libitum 7 days/week for at least 28 days.

D. Animals

Female beagles were received from Hazleton Research Products, Inc., Cumberland, VA. There was a 2-week period of acclimation. The dogs were individually housed in stainless-steel cages and exercised 3 times each week for at least 15 minutes. Room temperature and humidity were 64-79°F and 25-81%, respectively. There was a 12-hour light/dark cycle. Food and water were available ad libitum.

There were 2 control dogs and 3/treated group. The doses were 0, 10, 15 and 20 mg/kg/day.

E. Observations

SURVIVAL AND CLINICAL SIGNS

Animals were observed A.M. and P.M. each day. A physical examination was performed weekly.

There was no mortality.

Primarily during study weeks 3 and 4, a dose-dependent increase was reported in the incidence of fecal abnormalities (orange/bile stained, mucoid, soft, at times with blood, diarrhea and few or no feces). One 15 mg/kg/day dog vomited food during week 1 and one 20 mg/kg/day dog had frothy emesis during week 3.

BODY WEIGHTS

These were recorded weekly. Table 2.

The two controls gained weight and all treated dogs lost weight in a dose-response fashion.

Table 2

INDIVIDUAL BODY WEIGHTS AND WEIGHT GAINS IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE 2-ETHYLHEXYL ESTER OF 2,4-D

Dog No.	mg/kg/day	Body Weight (kg) - Week					Gain or Loss
		1	2	3	4	5	
401	0	6.2	6.4	6.4	6.4	6.6	+0.4
402	0	7.8	7.9	7.8	7.7	7.9	+0.1
403	10	6.3	6.4	6.5	6.3	6.1	-0.2
404	10	6.8	6.9	6.6	6.6	6.7	-0.1
405	10	8.2	8.1	7.8	7.5	7.5	-0.7
406	15	6.6	6.3	6.3	6.0	6.0	-0.6
407	15	6.8	6.7	6.2	6.5	6.2	-0.6
408	15	8.2	8.1	7.5	7.3	7.2	-1.0
409	20	6.6	6.8	6.1	5.6	5.0	-1.6
410	20	7.4	7.1	6.0	5.8	5.5	-1.9
411	20	8.7	8.7	7.7	7.4	7.1	-1.6

Data extracted from Report Appendix 3, page 61.

The two controls gained weight and all treated dogs lost weight in a dose-response fashion.

FOOD CONSUMPTION

This was recorded weekly.

The amount of food consumed in the control and 3 treated groups during the 1st week was similar. During the last 3 weeks of the study, there was a dose-dependent decrease in food eaten so that, compared with control values, the decreases were about 20, 36 and 58% at 10, 15 and 20 mg/kg/day, respectively.

TEST ARTICLE CONSUMPTION

This was calculated weekly and was based on body weight and food eaten. Table 3.

Table 3

GROUP MEAN TEST ARTICLE CONSUMPTION (mg/kg/day) IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE 2-ETHYLHEXYL ESTER OF 2,4-D

mg/kg/day	No. of Dogs	Week			
		1	2	3	4
10	3	9.9	8.1	8.0	8.3
15	3	12.9	8.3	11.9	10.2
20	3	22.4	8.4	9.0	9.6

Data extracted from Report Table 5, page 36.

During the 1st week, when food consumption by treated groups was similar to the control group, test article intake was near the target doses. For the last 3 weeks, when food intake was reduced, there was a concomitant decrease in test article consumed so that during this 3-week period, the dogs actually received an average of 8.1, 10.1 and 9.0 mg/kg/day (target doses were 10, 15 and 20 mg/kg/day).

E. Clinical Pathology

Although none was originally scheduled for this study, the Report indicated that, because of results from other studies, limited serum chemistry parameters were to be examined. Blood was taken from the jugular vein of fasted (food and water withheld - no time stated) animals during the last treatment week. The following were measured: alkaline phosphatase, urea nitrogen, creatinine and total cholesterol. Table 4.

Table 4

GROUP MEAN CLINICAL CHEMISTRY VALUES IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE 2-ETHYLHEXYL ESTER OF 2,4-D

mg/kg/day	Urea Nitrogen (mg/DL)	Creatinine (mg/DL)	Alkaline Phos. (U/L)	Total Choles. (mg/DL)
0	14	0.8	85	125
10	32	1.3	43	222
15	40	1.3	37	158
20	35	1.2	50	180

No. of dogs: control = 2; treated = 3/group
Data extracted from Report Table 6, page 38.

There were increases in urea nitrogen, creatinine and total cholesterol group mean values of treated dogs compared with control. Alkaline phosphatase levels in animals administered the test article were lower than in the control group.

F. Sacrifice and Pathology

Following 4 treatment weeks, all dogs were fasted, weighed, anesthetized with sodium thiamylal (intravenous) and exsanguinated. Gross necropsies were performed and about 39 tissues were fixed in 10% formalin. No tissues were processed/ examined microscopically.

The only apparent test article related finding was a distended gallbladder in 0/2 controls, 2/3 at 10 mg/kg/day, 3/3 at 15 mg/kg/day and 3/3 at 20 mg/kg/day.

G. Discussion

No dogs died during the 4-week study. A dose-dependent increase in fecal abnormalities was noted during the 3rd and 4th weeks.

The 2 control dogs gained 0.4 and 0.1 kg of body weight during the 4 weeks; whereas, the 10 and 15 mg/kg/day animals lost 0.1-1.0 kg, with the 20 mg/kg/day dogs losing 1.6-1.9 kg.

Dose dependent decreases in food consumption were reported for weeks 2, 3 and 4. During this same time period, test article intake was severly reduced from the target values, especially during the 2nd week of the study.

In treated animals, there were increases in urea nitrogen, creatinine and total cholesterol levels and a decrease in alkaline phosphatase.

Distended gallbladders were noted only in treated dogs (2/3, 3/3 and 3/3 at 10, 15 and 20 mg/kg/day).

H. Conclusions

All three dose levels were poorly tolerated and were considered too high for longer studies.

III. 13-WEEK STUDY (MRID No.: 427800-03)

A. Statistical Analyses

The following were subjected to statistical analysis: body weight changes, total feed consumption, clinical pathology

data and organ weight data.

LEVENE'S TEST OF HOMOGENEITY OF VARIANCES:

Homogeneous - ANOVA - if not significant "stop"; if significant, Dunnett's control versus treatment comparisons (for equal variances, for unequal variances, if heterogeneous)

Heterogeneous - Log 10 transformation, to square transformation, to square root transformation, to reciprocal transformation, to angular (arcsine) transformation, to rank transformation and to ANOVA. If any transformation is homogeneous, directly to ANOVA.

B. Regulatory Compliance

A Good Laboratory Practice Compliance Statement, Quality Assurance Statement and a list of Quality Assurance inspections were included in the Report.

A signed statement of no confidentiality claim was provided.

The Report stated that, "the criteria for flagging studies, stipulated in 40 CFR 158.34, do not apply to this study." This was signed by the Testing Facility Study Director, but not by the Sponsor (person's name typed, but no signature or date).

C. Test Article Description

Lot Number: 04KF54479
Storage: refrigerated
Physical Property: brown liquid
Purity: 62.7% (acid equivalent) - weight adjustment calculated to 100% purity

D. Test Article Homogeneity, Stability and Concentration

Analyses were performed for male and female diets on all concentrations at weeks 1, 2, 3, 4, 8 and 12.

Report page 23 indicated that, during week 2, the initial results showed that the diets were about 60% of the desired amount, and a remix was done. Further investigation determined that a correction had been made for test article activity (62.7% of acid equivalent). It was concluded that the original mixes were correct and the remix diets were about 60% over target. Animals received the remix diet during the last 3 days of week 2.

Table 1 presents selected analytical data.

Table 1

TEST ARTICLE HOMOGENEITY, STABILITY AND CONCENTRATION IN A 13-WEEK STUDY IN DOGS WITH THE 2-ETHYLHEXYL ESTER OF 2,4-D

Time/Location	1 mg/kg/day		3.75 mg/kg/day		7.5 mg/kg/day	
	Target ppm	% of Target	Target ppm	% of Target	Target ppm	% of Target
PRETEST						
top M ..	-	-	-	-	334.13	98
middle M ..	-	-	-	-	334.13	96
bottom M ..	-	-	-	-	334.13	95
top F ..	34.87	96	-	-	-	-
middle F...	34.87	94	-	-	-	-
bottom F ..	34.87	91	-	-	-	-
PRETEST (7 day stabil. room temper						
M ..	-	-	-	-	334.13	95
F ..	34.87	93	-	-	-	-
30 DAY STABIL freezer						
M ..	-	-	-	-	334.13	93
F ..	34.87	95	-	-	-	-
WEEK 1						
M ..	44.55	97	167.07	97	334.13	95
F ..	34.87	95	130.77	98	261.54	95
WEEK 12						
M ..	43.48	96	194.95	96	410.69	99
F ..	45.80	95	208.30	98	400.39	97

Percent of targets are mean of duplicate samples.
M = male; F = female
Data extracted from Report Table 1, pages 39-42.

Data from all intervals indicated that concentrations were 91-100% of the targets (excludes week 2 data).

Data for homogeneity, stability and concentration were within acceptable limits.

E. Dose Selection

The doses for this 13-week study were selected based upon the results of the 4-week rangefinding study (reviewed earlier in this Data Evaluation Report). The doses in this 13-week study are 0, 1, 3.75 and 7.5 mg/kg/day.

F. Dose Preparation

After shaking, an amount of test article for each dose level for one week's feed was weighed, added to about 200 g of basal diet and mixed in a Waring blender. This premix was then added to appropriate amounts of basal diet and mixed in a Patterson-Kelly blender. After week 1, fresh diets were prepared each week based on sex, body weight and food consumption.

G. Animals

Male and female beagle dogs were received from Hazleton Research Products, Inc., Cumberland, VA. Animals were acclimated for at least 2 weeks. Dogs were individually housed in stainless-steel cages in a room with the environment set to maintain a temperature of 64-84°F, humidity of 41-70% and a 12-hour light/dark cycle. The animals were placed in an exercise pen, with members of the same sex and group 3 days/ week for about 15 minutes/day. Food and water were available ad libitum. After physical and ophthalmic examinations as well as clinical laboratory tests, the dogs were stratified by weight and assigned to groups by a computerized randomization program.

H. Survival and Clinical Observations

Dogs were observed twice each day for mortality, moribundity and clinical signs. Physical examinations were conducted once each week (at the time of weighing).

Female G29543, from the 1.0 mg/kg/day group, was found dead on the 3rd study day. There had been no previous signs of illness. The Sponsor requested that a replacement dog be put on study (day 7) using the deceased's animal number. No data from the original dog were included in the Report. There was no mention of a necropsy. Report page 128 stated, "Presumably from an epileptic seizure." The death was not considered to have been test article related. All other dogs survived the 13 weeks.

There were no clinical signs which were considered to have been related to test article administration.

I. Body Weights

Body weights were recorded before treatment and weekly during the study. Table 2.

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Table 2

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS IN A 13-WEEK STUDY IN DOGS WITH THE 2-ETHYLHEXYL ESTER OF 2,4-D

Week	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1.0	3.75	7.5	0	1.0	3.75	7.5
BODY WEIGHTS								
1	7.5	7.1	7.1	7.5	6.6	6.2	6.9	6.7
2	7.7	7.1	7.3	7.5	6.7	6.3	6.8	6.9
3	8.1	7.6	7.4	7.3	7.0	6.4	6.9	6.8
4	8.5	7.5	7.6	7.3	7.2	6.6	6.9	7.0
6	8.8	7.9	8.0	7.5	7.4	6.8	7.1	7.1
8	9.1	8.1	8.1	7.6	7.8	6.9	7.3	7.2
10	9.5	8.5	8.4	7.7	7.9	7.0	7.4	7.4
12	9.8	8.7	8.6	7.8	8.0	7.2	7.3	7.4
14	10.1	8.9	8.5	7.8	8.2	7.3	7.4	7.5
BODY WT GAIN								
1-4	1.0	0.4	0.5	-0.2	0.6	0.4	0	0.3
4-8	0.6	0.6	0.5	0.3	0.6	0.3	0.4	0.2
8-14	1.0	0.8	0.4	0.2	0.4	0.4	0.1	0.3
1-14	2.7	1.8	1.4*	0.4*	1.6	1.2	0.6*	0.8*

NOTE: 4 dogs/sex/group
Body weight gains were calculated by the Reviewer for all intervals except weeks 1-14 for which data were included in the Report.
Statistical Significance: * = $p \leq 0.05$; presented in the Report only for weeks 1-14 body weight gains.
Data extracted from Report Table 3, pages 46 and 47.

There were lower body weights in treated male and female groups during the study. Body weight gains for the 13 weeks of the study were significantly ($p \leq 0.05$) less than the controls for both sexes at 3.75 and 7.5 mg/kg/day. The 7.5 mg/kg/day dose males lost a group mean of 0.2 kg during the 1st four weeks; whereas, the controls gained a mean of 1.0 kg with the 1.0 and 3.75 mg/kg/day groups gaining a mean of 0.4 and 0.5 kg, respectively.

J. Food Consumption

Data for this parameter were recorded each week.

In males, there was relatively little difference in the amount of food consumed (g/dog/week) between the 1.0 mg/kg/day and the control group. The 3.75 mg/kg/day group means were about 9% less than controls and the 7.5 mg/kg/day group means, about 23% less.

For females, the 1.0 mg/kg/day dogs ate the same or slightly less (generally no more than 5-8%; 5/13 intervals about equal and 8/13 intervals, less). The 3.75 mg/kg/day group means were about 13% less than controls and the 7.5 mg/kg/day group means about 10% less.

K. Test Article Intake (Report Table 5, pages 52 and 53)

This was calculated by body weight and food consumption data.

The group means for the entire 13-week period were as follows (mg/kg/day):

males = 1.1, 3.9 and 7.8 for 1.0, 3.75 and 7.5 mg/kg/day
females = 1.0, 3.9 and 7.7 for 1.0, 3.75 and 7.5 mg/kg/day

L. Ophthalmoscopic Examinations

Using indirect ophthalmoscopy, the eyes of all dogs were examined before treatment and at study termination.

There were no abnormalities noted in any animal at termination which were not reported prior to the beginning of dosing. Dog No. G29538, male, 1.0 mg/kg/day, had focal retinal fold of the right fundus (pretest and at termination) and No. G29558, female 7.5 mg/kg/day, had persistent hyaloid remnant of both lenses (pretest and at termination).

M. Clinical Pathology

Blood was collected from the jugular vein of overnight-fasted dogs prior to treatment as well as during weeks 4 and 13 for hematology and clinical chemistry determinations. Urine samples were taken by catheterization prior to study initiation and during week 13 (food and water available prior to the collection of urine).

HEMATOLOGY

The following parameters were examined:

Erythrocyte count*	Leukocyte count*
Hematocrit*	Corrected leukocyte count
Hemoglobin*	Leukocyte differential*
Platelet count*	Cell morphology
Reticulocyte count	Mean corpuscular volume
Absolute retic. count	Mean corpuscular hemoglobin
	Mean corpuscular hemoglobin conc.

* = EPA Guideline Requirement

There were no hematology parameters which appeared to be altered by test article administration.

CLINICAL CHEMISTRY

The following parameters were examined:

Alanine aminotransferase*
Aspartate aminotransferase*
Alkaline phosphatase
Albumin*
Globulin
Urea nitrogen*
Creatine kinase
Creatinine*
Total bilirubin*
Total cholesterol
Total protein*
Glucose*
Inorganic phosphorus*
Potassium*
Sodium*
Calcium*
Chloride*

* = EPA Guideline Requirement

Those parameters which showed statistically significant ($p \leq 0.05$) differences between treated and control groups were urea nitrogen, creatinine and alanine aminotransferase. Table 3.

Table 3

SELECTED GROUP MEAN CLINICAL CHEMISTRY PARAMETERS IN A 13-WEEK STUDY IN DOGS WITH THE 2-ETHYLHEXYL ESTER OF 2,4-D

mg/kg/day	Urea nitrogen MG/DL			Creatinine MG/DL			Alanine amino. U/L		
	-1a	4	13	-1	4	13	-1	4	13
MALES									
0	9	13	13	0.6	0.7	0.8	41	42	47
1.0	10	16	18*	0.6	0.9	1.0	32	46	45
3.75	11	21*	25*	0.6	1.0*	1.2*	22*	35	39
7.5	10	22*	27*	0.7	1.2*	1.3*	23*	70	71
FEMALES									
0	12	14	16	0.7	0.8	0.9	30	28	31
1.0	11	16	19	0.7	0.9	1.1*	28	35	36
3.75	12	22*	24*	0.8	1.1*	1.3*	30	55	66*
7.5	12	25*	25*	0.7	1.1*	1.3*	38	99*	127*

4 dogs/sex/group
a = week of determination
Statistical Significance: * = $p \leq 0.05$
Data extracted from Report Table 7, pages 62-66.

Urea nitrogen and creatinine levels were elevated ($p \leq 0.05$) in males and females at 3.75 and 7.5 mg/kg/day at the weeks 4 and 13 intervals. Alanine aminotransferase was elevated (not significant) in 7.5 mg/kg/day males at weeks 4 and 13. In 3.75 and 7.5 mg/kg/day females at weeks 4 and 13, there were higher levels of this enzyme than in respective control dogs (all ≤ 0.05 except not significant for 3.75 mg/kg/day at week 4).

URINALYSIS (not an EPA requirement)

The following parameters were examined:

Appearance	Occult blood	Bilirubin
pH	Glucose	Protein
Ketones	Specific gravity	Microscopic examination of sediment
Urobilinogen		

There were no parameters which appeared to be altered by test article administration.

N. Sacrifice and Pathology

At the end of the 13-week treatment period, the animals were weighed, anesthetized with sodium thiamylal and exsanguinated. [The 1.0 mg/kg/day replacement dog that started on treatment day 7, was sacrificed with the other study dogs.] Complete necropsies were performed. The following organs were weighed and organ-to-body weight as well as organ-to-brain weight ratios were calculated: adrenals, brain with stem, heart, kidneys, liver without gallbladder, ovaries, pituitary, testes without epididymides and thyroid with parathyroid.

The following tissues were removed, preserved in 10% neutral-buffered formalin and the "x" organs weighed:

DIGESTIVE	RESPIRATORY	UROGENITAL
Salivary gland*	Trachea*	xKidneys*
Esophagus*	Lung*	Urinary bladder*
Stomach*		xTestes*
Duodenum*	CARDIOVASC/HEMAT	xOvaries
Jejunum*	Aorta*	Epididymides
Ileum*	xHeart*	Prostate
Cecum*	Bone marrow*	Uterus*
Colon*	Lymph nodes*	Lacrimal gland
xLiver*	Spleen*	Vagina
Pancreas*	Thymus*	
Gallbladder*		
Tongue		

NEUROLOGIC	GLANDULAR	OTHER
xBrain*	xAdrenals*	Bone*
Peripheral nerve*	Mammary gland*	Skeletal muscle*
Spinal cord (3 levels)*	xParathyroids*	Skin
xPituitary*	xThyroid*	Gross lesions and masses*
Eyes (with optic n.)*		

* = EPA Guideline Requirements [rectum required but not examined]

MACROSCOPIC

There were no gross pathology findings that were considered to have been related to test article administration.

ORGAN WEIGHTS

Questionable, non-statistically significant ($p \leq 0.05$), organ weight changes were noted for the thyroid/parathyroid and testes. Table 4.

Table 4

GROUP MEAN ABSOLUTE AND RELATIVE THYROID/PARATHYROID AND TESTES WEIGHTS IN A 13-WEEK STUDY IN DOGS WITH THE 2-ETHYLHEXYL ESTER OF 2,4-D

mg/kg/day	Males				Females			
	Final B.W. (g)	Absolute (g)	to BW (%)	to brain (ratio)	Final B.W. (g)	Absolute (g)	to BW (%)	to brain (ratio)
THYROID								
0	9775	0.77	.008	.010	7650	0.64	.008	.009
1.0	8750	1.05	.012	.014	7050	0.95	.013	.014
3.75	8450	0.97	.012	.014	7150	0.87	.012	.013
7.5	7575*	0.90	.012	.012	7100	0.92	.013	.013
TESTES								
0	9775	13.3	.14	.17				
1.0	8750	12.0	.14	.16				
3.75	8450	11.5	.14	.16				
7.5	7575*	9.4	.12	.12				

Statistical Significance: * = $p \leq 0.05$
Data extracted from Report Table 9, pages 76-84.

At most, the increase in group mean thyroid/parathyroid and decrease in group mean testicular weights, are only suggestive of a test article effect. There was an overlapping of individual organ weights between the control and treated groups. In addition, because the group mean terminal body weights, especially in the 7.5 mg/kg/day groups, were lower than the controls, individual variation of organ weights between animals and groups might be expected.

MICROSCOPIC

There were no histopathological findings which distinguished treated from control dogs.

O. Discussion

Data for homogeneity, stability and concentration were within acceptable limits.

Except for one female given 1.0 mg/kg/day which was found dead on study day 3 ("presumably from an epileptic seizure") and replaced on day 7, all dogs survived the 13-week study. There were no clinical signs which differentiated the treated from the control dogs.

During weeks 1-14, group mean body weight gains for males were 2.7, 1.8, 1.4 and 0.4 kg and for females, 1.6, 1.2, 0.6 and 0.8 kg for the 0, 1.0, 3.75 and 7.5 mg/kg/day groups. Food consumption for the 3.75 and 7.5 mg/kg/day males and females were at least 9% less than controls.

Test article intake (mg/kg/day) over the 13 weeks for the 1.0, 3.75 and 7.5 mg/kg/day groups was as follows: males = 1.1, 3.9 and 7.8; females = 1.0, 3.9 and 7.7.

There were no ophthalmic, hematology or urinalysis changes which appeared related to the administration of the test article.

Blood urea nitrogen and creatinine levels were elevated ($p \leq 0.05$) in males and females at both the 4 and 13 week intervals at doses of 3.75 and 7.5 mg/kg/day. Though not always statistically significant, alanine aminotransferase levels were elevated in males at 7.5 mg/kg/day and in females at 3.75 and 7.5 mg/kg/day at both 4 and 13 weeks. However, there were no corroborative histopathological changes in the kidneys or liver.

There were no macroscopic or microscopic pathology observations which were considered to have been related to test article administration. The suggestion of a decrease in testicular weights and an increase in thyroid/parathyroid

(both sexes) weights in one or more dose groups, appears to be mostly a reflection of body weights as there were no supporting microscopic findings.

IV. CONCLUSIONS

In a subchronic toxicity study, the 2-ethylhexyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 1.0, 3.75 and 7.5 mg/kg/day for 13 weeks. The following parameters were examined: mortality, clinical signs, body weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, macroscopic pathology, organ weights and microscopic pathology.

Group mean body weight gains were 67, 52 and 15% of the control value for males and 75, 38 and 50% for females at 1, 3.75 and 7.5 mg/kg/day, respectively. There were elevations in blood urea nitrogen, creatinine and alanine aminotransferase levels at 3.75 and 7.5 mg/kg/day. However, no corroborative histopathological changes were seen in the liver or kidneys.

The NOEL is 1.0 mg/kg/day. The LOEL of 3.75 mg/kg/day is based on decreased body weight gain as well as alterations in clinical chemistry parameters.

This study is core classified as **Minimum** and satisfies the data requairement (§82-1B) for a 13-week subchronic toxicity study in dogs.

Amendment to DER for MRID 43879801 and 43597201 - Carcinogenicity Study [Mouse] [2,4-DICHLORO-PHENOXYACETIC ACID]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 011934].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 11/19/02
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Whang Phang, Ph.D. 11/21/02
Reregistration Branch I, Health Effects Division (7509C)
TXR#0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: carcinogenicity - mouse § 83-2/OPPTS 870.4200
P.C. CODE: 030001 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315 CAS NO.: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [97.5%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force for 2,4-D Research Data

CITATION: Study in Male Mice: Sott, W. T.; Johnson, K. A.; Gilbert, K. S..; *et al.* (1995). 2,4-Dichlorophenoxyacetic acid: Dietary Oncogenicity Study in Male $B_6C_3F_1$ Mice. The Toxicology Research Laboratory, Dow Chemical Co.; Study No. K-002372-063**M** , 11/16/95. MRID 43879801. Document No. 011934. Unpublished.
Study in Female Mice: Sott, W. T.; Johnson, K. A.; Gilbert, K. S..; *et al.* (1995). 2,4-Dichlorophenoxyacetic acid: Dietary Oncogenicity Study in $B_6C_3F_1$ Mice. The Toxicology Research Laboratory, Dow Chemical Co.; Study No. K-002372-063**F** , 3/10/95. MRID 43597201. Document No. 011934. Unpublished.

EXECUTIVE SUMMARY: In a carcinogenicity study [MRID 43879801 and 43597201], 50 $B_6C_3F_1$ CRL BR mice/sex/group were administered 2,4-dichlorophenoxyacetic acid [96.4%] *via* the diet for 104 weeks at concentrations of 0, 5 mg/kg/day [both sexes], 62 [males]/150 [females] mg/kg/day, and 120 [males]/300 [females] mg/kg/day. Additionally, 10 mice/sex/group were sacrificed at 52 weeks [interim sacrifice].

There were no treatment-related deaths or clinical signs of toxicity in either sex. Body weight, body-weight gain, and food consumption were comparable among the male groups throughout the study. Females at the high-dose level displayed a slightly lower body weight at the 3- and 6-month intervals [96% of control]. Body-weight gains were decreased significantly at all dose levels in the females at the 3-month interval [93%, 94%, and 86% of control at the low-, mid-, and high-dose, respectively], and the high-dose females continued to display a reduced body-weight gain at the 6-month [91% of control] and 12-month [91% of control] intervals. There were no consistent changes in food consumption in the female groups.

Ophthalmology and hematology parameters [RBC, HGB, HCT, platelets] were comparable among the groups [both sexes]; no thyroid parameters were monitored.

There was a dose-related increase in kidney weights in both sexes. In males, increased kidney weights [absolute and relative] were observed at the mid- and high-dose levels at the terminal sacrifice only. In females, increased kidney

weights were observed at the mid- and high-dose levels at both the interim and terminal sacrifices.

Gross pathology findings were comparable among the groups [both sexes]. Microscopically, there was an increased incidence of lesions in the kidneys of both sexes at the mid- and high-dose levels. In males at the interim/terminal sacrifices, renal lesions were characterized as degeneration with regeneration of the descending limb of the proximal tubule in the mid- [20%/50%] and high-dose [100%/96%] males *vs* none in the low dose or control males; decreased vacuolization of the renal proximal tubule in mid- [80%/78%] and high-dose [100%/96%] males *vs* 0% in the low-dose and control males. Additionally at the terminal sacrifice, there was an increased incidence of mineralization of the tubule(s) in the mid- [58%] and high- [72%] dose males compared to the low and control groups [38% and 32%, respectively]. In females, the renal lesions were characterized by hypercellularity in the descending part of the proximal tubule at both the interim and terminal sacrifices at the mid- [80%/64%] and high-dose [100%/50%] levels *vs* 0% in the low- and control females. There was no treatment-related increase in any tumor type in either sex.

The NOAEL is 5 mg/kg/day. The LOAEL of 62 mg/kg/day [males]/150 mg/kg/day [females] is based on an increased absolute and/or relative kidney weights and an increased incidence of renal microscopic lesions. There was no treatment-related increase in the incidence of any tumor type.

This carcinogenicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [OPPTS 870. 4200; §83-2] for a carcinogenicity study in the mouse.

NOTE: In the mouse study, there was an increase in hemangiosarcomas of the spleen in male mice at the low and mid doses, which was not sustained at the highest dose; however, the slides from all male mice at the low and mid doses were not evaluated. The HED Carcinogenicity Peer Review Committee [CPRC; TXR No. 0050017] requested that the slides of the low- and mid-dose spleens of the males be evaluated. These additional data were submitted [MRID 44284502]. The spleen of 35 male mice in the low-dose [5 mg/kg/day] group and 33 in the mid-dose [62.5 mg/kg/day] group were examined by light microscopy. No neoplasms were observed. When all of the data on the 50 male mice/group were combined, there was no dose-related increase in hemangiosarcomas or in possible preneoplastic lesions, such as extramedullary hematopoiesis. It was concluded that the additional data do not alter the findings of the study. There was another mouse carcinogenicity study [MRID 40061801] conducted at dosages of 1, 15, and 45 mg/kg/day. No treatment-related increase in tumor incidence was found; however, this latter study was classified unacceptable because the high dose was considered inadequate to assess the carcinogenic potential of 2,4-D.

Table 1. Body Weight/Body-Weight Gain Data

Parameter/Day/Sex/Dose	0 mg/kg/day	5 mg/kg/day	62 or 150 mg/kg/day	120 or 300 mg/kg/day
BODY WEIGHT [g]				
MALES				
-3 days	22.2±1.3	22.3±1.2	21.9±1.2	21.8±1.3 [98] ✔
3 months	29.2±1.7	28.9±1.7	29.3±1.7	29.5±1.9
6 months	33.7±3.5	33.4±2.7	34.0±2.7	33.4±3.0
12 months	36.3±4.1	35.7±3.4	36.1±3.6	36.1±3.2
18 months	37.1±4.1	36.6±3.6	36.8±4.1	36.8±3.6
24 months	34.5±3.5	34.6±4.0	34.5±3.4	33.5±3.1 [97]
FEMALES				
-3 days	18.7±0.9	18.8±1.2	18.6±0.9	18.6±0.8
3 months	25.8±1.3√	25.2± *1.1	25.3±1.2	24.7± *1.3 [96]
6 months	28.1±2.2	27.9±1.9	27.4±1.6	27.1± *1.9 [96]
12 months	31.5±3.5√	32.1±3.1√	30.8±2.9	30.2±2.9
18 months	32.5±3.8	33.0±3.1	33.0±4.2	31.8±3.3
24 months	30.6±3.0	32.1±3.2	31.9±4.8	31.3±3.2
BODY-WEIGHT GAIN♪ [g]				
MALES				
3 months	7.0±1.2	6.7±1.1	7.3±1.3	7.6± *1.5
6 months	11.5±	11.2±	12.1±	11.5±
12 months	14.1±	13.5±	14.2±	14.3±
18 months	14.9±	14.5±	14.9±	14.9±
24 months	12.2±	12.4±	12.7±	11.6±
FEMALES				
3 months	8.4±	7.8± * (93)	7.9± * (94)	7.2± * (86)
6 months	10.6±	10.5±	10.0±	9.6± * (91)
12 months	14.1±	14.7±	13.4±	12.8± * (91)
18 months	15.1±	15.5±	15.5±	14.4± (95)
24 months	13.2±	14.7±	14.4±	13.9±
BODY-WEIGHT GAIN [g]✖				
MALES				
days -3-6	1.8	1.3 [72]	1.5 [83]	1.7 [94]
days 6-13	0.5	0.9	0.9	0.9
days 6-83	5.2	5.3	5.9	6.0
FEMALES				
days -3-4	1.4	1.3	1.4	1.2 [86]
days 4-11	0.8	1.0	0.6 [75]	0.7 [88]
days 4-88	5.7	5.1 [89]	5.3 [93]	4.9 [86]

♪ cumulative; ✖ calculated by reviewer using summary tables of report (s.d. not calculated); √ s.d. nor legible;
✔ [% of control]; * $p<0.05$; data from Tables 9-10 [males, pages 51-60; females pages 52-60]

Table 2. Hematology Data - MALES				
Dose/Month/Parameter	0 mg/kg/day	5 mg/kg/day	62 mg/kg/day	120 mg/kg/day
platelets [x10E3/cumm]				
12	1232±241 (8)	1238±125	1357±188	1237±169
18	1262±259	1384±128	1323±108	1357±104
24	1421±196	1356±170	1269±387	1471±301
RBC[x10E6/cumm]				
12	9.39±0.34	9.42±0.41	9.50±0.56	9.40±0.56
18	10.06±1.77	9.41±0.37	9.31±0.36	9.31±0.31
24	9.14±0.34	9.07±0.45	9.34±0.94	9.25±1.26
HGB [g/dL]				
12	14.8±0.3	14.7±0.6	14.7±1.0	14.7±0.8
18	15.4±1.9	14.8±0.5	14.9±0.5	14.8±0.4
24	14.4±0.5	14.1±0.7	14.4±1.2	14.2±1.3
HCT {%}				
12	47.0±1.9	46.2±1.9	46.5±2.6	46.7±2.8
18	48.5±6.4	46.8±1.7	46.6±2.0	46.7±1.4
24	44.9±1.6	44.4±2.6	45.2±4.3	44.5±4.1
WBC [x10^3/m^3]				
12	5.21±0.88	4.79±1.75	4.83±1.95	4.68±1.41
18	6.10±1.70	6.00±1.62	6.31±0.85	6.62±0.96
24	6.01±1.32	6.00±1.91	6.87±2.19	8.35±7.61

√[% of control]; n=10 unless (#); [12, 18 months]/n=20 [24 months]; nl not legible; * $p<0.05$;
data from Tables 14-16 [pages 67-69] of the report

Table 3. Hematology Data - FEMALES				
Dose/Month/Parameter	0 mg/kg/day	5 mg/kg/day	62 mg/kg/day	120 mg/kg/day
platelets [x10E3/cumm]				
12	800±48	813±85	783±94	784±76
18	1099±181	1063±128	1051±67	1058±147
24	899±263	936±226	843±277	754±269
RBC[x10E6/cumm]				
12	9.63±0.49	9.70±0.53	9.44±0.66	9.08±0.55
18	9.28±0.48	9.20±0.45	9.16±0.21	8.96±0.38
24	9.11±0.37	9.14±0.62	8.86±1.49	8.92±0.72
HGB [g/dL]				
12	15.1±0.7	15.1±0.7	15.0±1.0	14.4±0.9
18	14.9±0.5	14.7±0.4	14.7±0.3	14.7±0.3
24	14.0±0.8	14.2±0.8	13.7±2.1	14.0±1.2
HCT {%}				
12	53.5±3.2	54.2±2.7	53.4±4.0	51.2±3.3
18	46.4±1.6	46.1±1.5	46.0±0.9	45.8±1.3
24	41.6±2.7	41.4±2.3	40.3±5.5	41.0±3.3
WBC [x10^3/m^3]				
12	5.2±1.1	4.8±1.7	4.9±1.0	3.3±1.6* [63]√
18	3.78±1.75	4.00±1.52	3.27±0.61	4.35±3.41
24	3.70±1.63	3.92±2.03	4.25±4.76	3.66±2.10

√[% of control]; n=10 unless (#); [12, 18 months]/n=20 [24 months]; nl not legible; * $p<0.05$;
data from Tables 14-16 [pages 67-69] of the report

Table 4. Organ Weight Data - MALES [12 months]				
Dose/Organ	0 mg/kg/day (8)	5 mg/kg/day	62 mg/kg/day	120 mg/kg/day
brain				
absolute [g]	0.49±0.01	0.50±-0.01	0.49±0.01	0.48±0.015
relative-to-body [g/100]	1.32±0.19	1.36±0.15	1.38±0.13	1.37±0.167
liver				
absolute [g]	1.96±0.41	1.87±0.18	1.82±0.19	1.81±0.16 [92]
relative-to-body [g/100]	5.18±0.27	5.03±0.20	5.095±0.42	5.10±0.22
relative-to-brain [g/100]	403.88±86.48	373.55±36.37	372.664±41.7	374.42±36.42
kidney				
absolute [g]	0.71±0.11	0.73±0.06	0.69±0.07	0.71±0.05
relative-to-body [g/100]	1.92±0.35	1.97±0.16	1.92±0.16	2.01±0.19
relative-to-brain [g/100]	146.14±21.38	145.70±10.29	140.33±14.05	146.69±10.54
testes				
absolute [g]	♪0.39±0.47	0.24±0.01	0.23±0.01	0.22±0.03
relative-to-body [g/100]	♪0.93±0.8280.6	0.65±0.05	0.65±0.07	0.63±0.06
relative-to-brain [g/100]	♪80.69±96.25	48.16±2.87	47.30±2.75	46.03±5.64
terminal body weight [g]	37.6±6.5	37.2±3.9	35.8±3.7	35.7±3.9 [95]

√[% of control]; n=10 except (#): * p<0.05;
♪ with the exclusion of one diagnosed with large testicular tumor, the mean absolute, relative to body and brain weights are: 0.23±0.02, 0.64±0.07, and 46.69±4.24; data from Tables [pages] of the report

Table 5. Organ Weight Data - MALES [24 months]				
Dose/Organ	0 mg/kg/day	5 mg/kg/day	62.5 mg/kg/day	120 mg/kg/day
brain				
absolute [g]	0.49±0.02	0.49±0.02	0.49±0.02	0.49±0.02
relative-to-body [g/100]	1.41±0.12	1.44±0.15	1.40±0.12	1.45±0.12
liver				
absolute [g]	2.07±0.52	2.01±0.68	2.34±1.01	2.01±0.48
relative-to-body [g/100]	5.97±2.02	5.82±2.21	6.72±2.87	5.94±1.43
relative-to-brain [g/100]	423.51±115.58	407.63±147.38	484.35±218.04	410.88±102.61
kidney				
absolute [g]	0.74±0.07	0.72±0.06	0.78±0.07 [105]	0.80±0.08* [107]
relative-to-body [g/100]	2.12±0.22	2.09±0.18	2.24±0.22* [106]	2.34±0.18* [110]
relative-to-brain [g/100]	151.42±13.71	145.87±12.16	160.56±15.26* [106]	162.09±15.27* [107]
testes				
absolute [g]	0.20±0.02	0.26±0.35	0.21±0.03	0.21±0.02
relative-to-body [g/100]	0.57±0.05	0.79±1.26	0.60±0.10	0.60±0.05* [106]
relative-to-brain [g/100]	40.53±4.06	53.15±73.57	42.66±7.70	41.64±3.44 [1-3]
terminal body weight [g]	35.2±3.6	34.8±4.0	34.9±3.3	34.1±3.1

√[% of control];* p<0.05; n=28, 25, 33, 36 [control, low, mid, high, respectively];
data from Tables 25-26 [pages 83-84] of the report

Table 6. Organ Weight Data - FEMALES [12 months]				
Dose/Organ	0 mg/kg/day	5 mg/kg/day	150 mg/kg/day	300 mg/kg/day
brain				
absolute [g]	0.51±0.01	0.51±0.02	0.51±0.02	0.50±0.03
relative-to-body [g/100]	1.57±0.16	1.63±0.17	1.69±0.10	1.73±0.12* [110]√
liver				
absolute [g]	1.53±0.11	1.52±0.10	1.51±0.11	1.45±0.10 [95]
relative-to-body [g/100]	4.73±0.33	4.87±0.25	5.02±0.18* [106]	4.98±0.22
relative-to-brain [g/100]	303.47±21.92	299.65±20.73	298.96±23.07	289.67±21.23 [95]
kidney				
absolute [g]	0.43±0.03	0.42±0.03	0.49±0.03* [114]	0.50±0.03* [117]
relative-to-body [g/100]	1.32±0.12	1.35±0.10	1.62±0.10* [122]	1.72±0.12* [130]
relative-to-brain [g/100]	84.82±6.02	83.02±4.46	96.12±6.70* [113]	99.66±3.71* [117]]
terminal body weight [g]	32.5±3.3	31.3±3.0	30.1±1.8	29.2±1.8* [90]

√[% of control];* p<0.05; n=10; data from Tables 78-79 [pages 183-185] of the report

Table 7. Organ Weight Data - FEMALES [24 months]				
Dose/Organ	0 mg/kg/day	5 mg/kg/day	150 mg/kg/day	300 mg/kg/day
brain				
absolute [g]	0.52±0.02	0.52±0.02	0.52±0.02	0.52±0.02
relative-to-body [g/100]	1.68±0.13	1.62±0.13	1.64±0.21	1.65±0.14
liver				
absolute [g]	1.83±0.79	1.80±0.34	1.75±0.38	1.79±0.28
relative-to-body [g/100]	5.83±2.23	5.59±0.91	5.46±0.83	5.63±0.82
relative-to-brain [g/100]	351.40±145.16	346.75±65.50	337.81±71.38	
kidney				
absolute [g]	0.49±0.09	0.48±0.05	0.44±0.06* [114]√	0.60±0.08* [122]
relative-to-body [g/100]	1.56±0.19	1.49±0.13	1.74±0.18* [112]	1.87±0.22* [120]
relative-to-brain [g/100]	93.82±16.84	91.94±8.85	106.72±11.07* [114]	114.45±15.56* [122]
terminal body weight [g]	31.1±2.7	32.2±2.8	32.2±5.1	31.8±2.7

√[% of control]; n= 39, 42, 42, 35 for control, low, mid, and high doses, respectively; ; * p<0.05; data from Tables 25-26 [pages 83-84] of the report

011934

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).

011934

I. INTRODUCTION

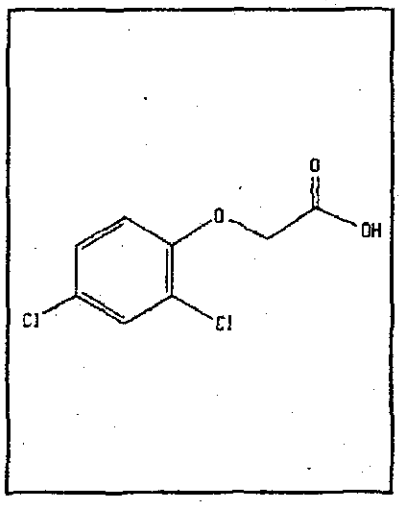
In 1988, the Agency required that rodent carcinogenicity testing with 2,4-dichlorophenoxyacetic acid (2,4-D) be repeated because a Maximum Tolerated Dose (MTD) had not been achieved in the Industry-sponsored studies. In the Data Call-In notice of 1989, the Agency formally requested that the carcinogenicity 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[a]
x Duodenum[a]	x Larynx[a]
x Jejunum[a]	Nose[a]
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]	
x Peripheral nerve[ab]	Urogenital System
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.

011934

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

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

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.

650

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

654

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.**

63

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

TABLE 34

2,4-DICHLOROPHENOXYACETIC ACID: DIETARY ONCOGENICITY STUDY IN B6C3F1 MALE MICE: TWO YEAR FINAL REPORT

TUMOR INCIDENCE-ALL MODES OF DEATH

SEX	MALES			
DOSE IN MG/KG/DAY	0	5	62.5	125
NUMBER OF MICE EXAMINED	50	50	50	50
ADRENALS (NO. OF TISSUES EXAMINED)	49	7	8	49
ADENOMA, CORTEX, BENIGN, PRIMARY:	1	0	0	1
EPIDIDYMIDES (NO. OF TISSUES EXAMINED)	50	8	8	50
LEYDIG CELL TUMOR, BENIGN, PRIMARY:	1	1	0	0
HEART (NO. OF TISSUES EXAMINED)	50	7	8	50
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, METASTASIS:	1	0	0	0
JEJUNUM (NO. OF TISSUES EXAMINED)	50	7	8	50
ADENOCARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	1	0	0	0
KIDNEYS (NO. OF TISSUES EXAMINED)	50	50	50	50
ADENOMA, TUBULE(S), BENIGN, PRIMARY:	0	0	1	0
CARCINOMA, MALIGNANT, PRIMARY, METASTASIS:	0	1	0	0
LACRIMAL/HARDERIAN GLAND(S) (NO. OF TISSUES EXAMINED)	50	18	17	50
ADENOMA, BENIGN, PRIMARY:	6	1	6	6
LIVER (NO. OF TISSUES EXAMINED)	50	50	50	50
ADENOMA, HEPATOCELLULAR, BENIGN, PRIMARY:	10	9	10	11

@ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
** THIS LINE REPRESENTS THE COMBINATION OF TWO OR MORE PRECEDING LINES WITH SIMILAR OBSERVATIONS AND LOCATORS.
- INDICATES NOT APPLICABLE.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 656 of 959

TABLE 34 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: DIETARY ONCOGENICITY STUDY IN B6C3F1 MALE MICE: TWO YEAR FINAL REPORT

TUMOR INCIDENCE-ALL MODES OF DEATH

SEX		MALES			
DOSE IN MG/KG/DAY		0	5	62.5	125
NUMBER OF MICE EXAMINED		50	50	50	50
LIVER (CONTINUED)					
ADENOMA, HEPATOCELLULAR, BENIGN, PRIMARY: (TWO)		1	0	3	4
ADENOMA, HEPATOCELLULAR, BENIGN, PRIMARY: (THREE)		1	0	0	1
ADENOMA, HEPATOCELLULAR, BENIGN, PRIMARY:	**	12	9	13	16
CARCINOMA, HEPATOCELLULAR, MALIGNANT, PRIMARY, NO METASTASIS:		4	3	5	4
CARCINOMA, HEPATOCELLULAR, MALIGNANT, PRIMARY, METASTASIS:		2	0	2	0
CARCINOMA, HEPATOCELLULAR, MALIGNANT, PRIMARY, METASTASIS OR NO METASTASIS:	**	6	3	7	4
NUMBER OF MICE WITH HEPATOCELLULAR ADENOMA: (ONE OR MORE) AND/OR CARCINOMA		17	11	18	20
HEMANGIOMA, BENIGN, PRIMARY:		0	1	1	1
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:		0	0	1	1
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, METASTASIS:		1	0	0	0
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, METASTASIS OR NO METASTASIS:	**	1	0	1	1
LUNGS (NO. OF TISSUES EXAMINED)		50	50	50	50
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY:		17	9	10	8
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY: (TWO)		1	1	3	2
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY: (THREE)		0	1	1	1
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY:	**	18	11	14	11
CARCINOMA, BRONCHIOLOALVEOLAR, MALIGNANT, PRIMARY, NO METASTASIS:		0	0	1	1
CARCINOMA, BRONCHIOLOALVEOLAR, MALIGNANT, PRIMARY, METASTASIS:		1	0	0	0

@ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
** THIS LINE REPRESENTS THE COMBINATION OF TWO OR MORE PRECEDING LINES WITH SIMILAR OBSERVATIONS AND LOCATORS.
- INDICATES NOT APPLICABLE.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 657 of 959

C11934

TABLE 34 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: DIETARY ONCOGENICITY STUDY IN B6C3F1 MALE MICE: TWO YEAR FINAL REPORT

TUMOR INCIDENCE-ALL MODES OF DEATH

SEX		MALES			
DOSE IN MG/KG/DAY		0	5	62.5	125
NUMBER OF MICE EXAMINED		50	50	50	50
LUNGS (CONTINUED)					
CARCINOMA, BRONCHIOLOALVEOLAR, MALIGNANT, PRIMARY, METASTASIS OR NO METASTASIS:	**	1	0	1	1
PAPILLARY ADENOMA, BRONCHIAL/BRONCHIOLAR EPITHELIUM, BENIGN, PRIMARY:		0	0	1	0
LYMPH NODE - MESENTERIC (NO. OF TISSUES EXAMINED)		49	14	20	50
HEMANGIOMA, BENIGN, PRIMARY:		0	0	1	0
MESENTERIC TISSUES (NO. OF TISSUES EXAMINED)		50	9	8	50
HEMANGIOMA, BENIGN, PRIMARY:		0	1	0	1
MULTIPLE ORGANS (NO. OF TISSUES EXAMINED)		8	4	6	5
LEUKEMIA - LYMPHOID CELL, MALIGNANT, PRIMARY:		5	1	1	3
LEUKEMIA - MYELOID CELL, MALIGNANT, PRIMARY, NO METASTASIS:		0	0	1	0
LEUKEMIA - UNCLASSIFIED, MALIGNANT, PRIMARY:		1	1	0	1
LYMPHOSARCOMA, MALIGNANT, PRIMARY:		1	1	3	1
SKIN AND SUBCUTIS (NO. OF TISSUES EXAMINED)		50	10	9	50
FIBROSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:		1	0	0	0
FIBROUS HISTIOCYTOMA, MALIGNANT, PRIMARY, NO METASTASIS:		0	1	0	1

@ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
** THIS LINE REPRESENTS THE COMBINATION OF TWO OR MORE PRECEDING LINES WITH SIMILAR OBSERVATIONS AND LOCATORS.
- INDICATES NOT APPLICABLE.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 658 of 959

TABLE 34 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: DIETARY ONCOGENICITY STUDY IN B6C3F1 MALE MICE: TWO YEAR FINAL REPORT

TUMOR INCIDENCE-ALL MODES OF DEATH

SEX		MALES			
DOSE IN MG/KG/DAY		0	5	62.5	125
NUMBER OF MICE EXAMINED		50	50	50	50
SKIN AND SUBCUTIS (CONTINUED)					
HEMANGIOMA, BENIGN, PRIMARY:		2	0	0	1
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:		0	1	0	0
UNDIFFERENTIATED SARCOMA, SUBCUTANEOUS, MALIGNANT, PRIMARY, NO METASTASIS:		1	0	1	0
SPLEEN (NO. OF TISSUES EXAMINED)		50	15	17	50
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:		0	6	3	1
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, METASTASIS:		0	0	1	1
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, METASTASIS OR NO METASTASIS:	**	0	6	4	2
LYMPHOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:		0	0	0	2
TESTES (NO. OF TISSUES EXAMINED)		50	8	9	50
LEYDIG CELL TUMOR, BENIGN, PRIMARY:		0	1	1	0
NUMBER OF MICE WITH HEMANGIOMA, BENIGN					
PRIMARY SITE IN LIVER		0	1	1	1
PRIMARY SITE IN LYMPH NODE - MESENTERIC		0	0	1	0
PRIMARY SITE IN MESENTERIC TISSUES		0	1	0	1
PRIMARY SITE IN SKIN AND SUBCUTIS		2	0	0	1
TOTAL NUMBER OF MICE WITH HEMANGIOMA, BENIGN - ANY SITE		2	2	2	3
NUMBER OF MICE WITH HEMANGIOSARCOMA					
PRIMARY SITE IN HEART		1	0	0	0
PRIMARY SITE IN LIVER		1	0	1	1
PRIMARY SITE IN SKIN		0	1	0	0
PRIMARY SITE IN SPLEEN		0	6	4	2
NUMBER OF MICE WITH HEMANGIOSARCOMA - ANY SITE		2	7	5	3
NUMBER OF MICE WITH HEMANGIOMA AND/OR HEMAGIOSARCOMA - ANY SITE		4	7	7	5

@ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
** THIS LINE REPRESENTS THE COMBINATION OF TWO OR MORE PRECEDING LINES WITH SIMILAR OBSERVATIONS AND LOCATORS.
- INDICATES NOT APPLICABLE.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 659 of 959

TABLE 35

2,4-DICHLOROPHENOXYACETIC ACID: DIETARY ONCOGENICITY STUDY IN B6C3F1 MICE: TWO YEAR FINAL REPORT

TUMOR INCIDENCE - ALL MODES OF DEATH

SEX	FEMALES			
DOSE IN MG/KG/DAY	0	5	150	300
NUMBER OF MICE EXAMINED	50	50	50	50
ADRENALS (NO. OF TISSUES EXAMINED)	50	8	8	50
PHEOCHROMOCYTOMA, BENIGN, PRIMARY:	1	0	0	1
BONE (NO. OF TISSUES EXAMINED)	50	9	8	50
HEMANGIOSARCOMA, VERTEBRAE, MALIGNANT, PRIMARY, NO METASTASIS:	1	0	0	0
HEMANGIOSARCOMA, VERTEBRAE, MALIGNANT, PRIMARY, METASTASIS:	1	0	0	0
OSTEOGENIC SARCOMA, (SKULL), MALIGNANT, PRIMARY, NO METASTASIS:	0	1	0	0
OSTEOGENIC SARCOMA, (VERTEBRA), MALIGNANT, PRIMARY, METASTASIS:	0	0	1	0
CERVIX (NO. OF TISSUES EXAMINED)	50	50	49	50
ADENOCARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	1
LEIOMYOMA, BENIGN, PRIMARY:	0	0	0	2
HISTIOCYTIC SARCOMA, MALIGNANT, PRIMARY, METASTASIS:	0	1	1	0
LACRIMAL/HARDERIAN GLAND(S) (NO. OF TISSUES EXAMINED)	50	10	8	50
ADENOCARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	1	0	0	0
ADENOMA, BENIGN, PRIMARY:	3	2	0	4
TOTAL MICE WITH ADENOMA OR ADENOCARCINOMA	4	2	0	4
LIVER (NO. OF TISSUES EXAMINED)	50	50	50	50
ADENOMA, HEPATOCELLULAR, BENIGN, PRIMARY:	4	11	7	7

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- INDICATES NOT APPLICABLE.

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 660 of 959

TABLE 35 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: DIETARY ONCOGENICITY STUDY IN B6C3F1 MICE: TWO YEAR FINAL REPORT

TUMOR INCIDENCE - ALL MODES OF DEATH

SEX	FEMALES			
DOSE IN MG/KG/DAY	0	5	150	300
NUMBER OF MICE EXAMINED	50	50	50	50
LIVER (CONTINUED)				
ADENOMA, HEPATOCELLULAR, BENIGN, PRIMARY: (TWO)	0	0	0	3
ADENOMA, HEPATOCELLULAR, BENIGN, PRIMARY: (THREE)	1	0	1	0
CARCINOMA, HEPATOCELLULAR, MALIGNANT, PRIMARY, NO METASTASIS:	1	2	0	1
TOTAL MICE WITH HEPATOCELLULAR ADENOMA (ONE OR MORE) OR CARCINOMA	5	13	8	11
HEMANGIOMA, BENIGN, PRIMARY:	0	0	0	1
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	1	0	0	0
LIPOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	1	0
HISTIOCYTIC SARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	1
LUNGS (NO. OF TISSUES EXAMINED)	50	50	50	50
ADENOCARCINOMA, BRONCHIOLOALVEOLAR, MALIGNANT, PRIMARY, NO METASTASIS:	2	1	1	0
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY:	4	5	1	4
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY: (TWO)	0	1	0	0
LYMPH NODE - CERVICAL (NO. OF TISSUES EXAMINED)	28	3	5	30
LYMPHOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	1	0	0	0
LYMPH NODE - MESENTERIC (NO. OF TISSUES EXAMINED)	46	18	19	47
HEMANGIOMA, BENIGN, PRIMARY:	2	0	1	0
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	1
LYMPHOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	1	0	0

0 DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION, - INDICATES NOT APPLICABLE.

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EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 661 of 959

TABLE 35 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: DIETARY ONCOGENICITY STUDY IN B6C3F1 MICE: TWO YEAR FINAL REPORT

TUMOR INCIDENCE - ALL MODES OF DEATH

SEX	FEMALES			
DOSE IN MG/KG/DAY	0	5	150	300
NUMBER OF MICE EXAMINED	50	50	50	50
LYMPH NODE - MISCELLANEOUS (NO. OF TISSUES EXAMINED)	23	8	10	23
HEMANGIOMA, LUMBAR, BENIGN, PRIMARY:	0	0	0	1
LYMPHOSARCOMA, RENAL NODE, MALIGNANT, PRIMARY, NO METASTASIS:	0	1	0	0
MAMMARY GLAND (NO. OF TISSUES EXAMINED)	49	8	7	49
ADENOCARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	1	0
MULTIPLE ORGANS (NO. OF TISSUES EXAMINED)				
LYMPHOSARCOMA, MALIGNANT, PRIMARY, METASTASIS:	8	7	13	8
OVARIES (NO. OF TISSUES EXAMINED)	50	50	50	50
ADENOCARCINOMA, GERMINAL EPITHELIUM, MALIGNANT, PRIMARY, METASTASIS:	0	1	0	1
ADENOMA, GERMINAL EPITHELIUM, BENIGN, PRIMARY:	0	0	0	1
CYSTADENOMA, GERMINAL EPITHELIUM, BENIGN, PRIMARY:	3	1	2	2
CYSTADENOMA, GERMINAL EPITHELIUM, BENIGN, PRIMARY: (TWO)	1	0	0	0
GRANULOSA - THECAL CELL TUMOR, BENIGN, PRIMARY:	0	1	3	0
GRANULOSA - THECAL CELL TUMOR, MALIGNANT, PRIMARY, NO METASTASIS:	0	1	0	0
HEMANGIOMA, BENIGN, PRIMARY:	0	2	1	0
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	1	0
TERATOMA, BENIGN, PRIMARY:	1	0	0	1

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EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 662 of 959

TABLE 35 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: DIETARY ONCOGENICITY STUDY IN B6C3F1 MICE: TWO YEAR FINAL REPORT

TUMOR INCIDENCE - ALL MODES OF DEATH

SEX	FEMALES			
DOSE IN MG/KG/DAY	0	5	150	300
NUMBER OF MICE EXAMINED	50	50	50	50
PARATHYROID GLANDS (NO. OF TISSUES EXAMINED)	49	7	8	43
ADENOMA, BENIGN, PRIMARY:	0	0	1	0
PITUITARY (NO. OF TISSUES EXAMINED)	50	9	13	46
ADENOMA, ANTERIOR (PARS DISTALIS), BENIGN, PRIMARY:	13	2	6	7
ADENOMA, ANTERIOR (PARS DISTALIS), BENIGN, PRIMARY: (TWO)	1	0	0	0
SKELETAL MUSCLE (NO. OF TISSUES EXAMINED)	50	9	8	50
UNDIFFERENTIATED SARCOMA, MALIGNANT, PRIMARY, METASTASIS:	0	1	0	0
SKIN AND SUBCUTIS (NO. OF TISSUES EXAMINED)	50	11	10	50
ADENOMA, SEBACEOUS GLANDS, BENIGN, PRIMARY:	0	1	0	0
BASAL CELL CARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	1	0	0
SQUAMOUS PAPILLOMA, LIP, BENIGN, PRIMARY:	0	0	0	1

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EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 663 of 959

TABLE 35 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: DIETARY ONCOGENICITY STUDY IN B6C3F1 MICE: TWO YEAR FINAL REPORT

TUMOR INCIDENCE - ALL MODES OF DEATH

SEX	FEMALES			
DOSE IN MG/KG/DAY	0	5	150	300
NUMBER OF MICE EXAMINED	50	50	50	50
SKIN AND SUBCUTIS (CONTINUED)				
HEMANGIOSARCOMA, SUBCUTANEOUS, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	1
HEMANGIOSARCOMA, SUBCUTANEOUS, MALIGNANT, PRIMARY, METASTASIS:	2	0	0	1
LEIOMYOSARCOMA, SUBCUTANEOUS, MALIGNANT, PRIMARY, NO METASTASIS:	0	1	0	0
UNDIFFERENTIATED SARCOMA, SUBCUTANEOUS, MALIGNANT, PRIMARY, NO METASTASIS:	1	0	1	0
SPLEEN (NO. OF TISSUES EXAMINED)	50	20	21	49
HISTIOCYTIC SARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	1	0	0
LYMPHOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	1	0	1	2
STOMACH (NO. OF TISSUES EXAMINED)	50	10	8	50
SQUAMOUS CELL CARCINOMA, NONGLANDULAR MUCOSA, MALIGNANT, PRIMARY, NO METASTASIS:	0	2	0	0
URINARY BLADDER (NO. OF TISSUES EXAMINED)	50	49	49	48
TRANSITIONAL CELL CARCINOMA, MUCOSA, MALIGNANT, PRIMARY, NO METASTASIS:	0	1	0	0
UTERUS (NO. OF TISSUES EXAMINED)	50	50	50	50
ADENOCARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	2	1	1
CARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	1

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EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 664 of 959

TABLE 35 (CONTINUED)

2,4-DICHLOROPHENOXYACETIC ACID: DIETARY ONCOGENICITY STUDY IN B6C3F1 MICE: TWO YEAR FINAL REPORT

TUMOR INCIDENCE - ALL MODES OF DEATH

SEX	FEMALES			
DOSE IN MG/KG/DAY	0	5	150	300
NUMBER OF MICE EXAMINED	50	50	50	50
UTERUS (CONTINUED)				
ENDOMETRIAL STROMAL POLYP, BENIGN, PRIMARY:	1	3	4	3
HEMANGIOMA, BENIGN, PRIMARY:	0	0	0	2
HEMANGIOPERICYTOMA, BENIGN, PRIMARY:	0	0	1	0
HEMANGIOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	0	2
LEIOMYOMA, BENIGN, PRIMARY:	1	0	1	0
LEIOMYOSARCOMA, MALIGNANT, PRIMARY, NO METASTASIS:	0	0	1	0
HISTIOCYTIC SARCOMA, MALIGNANT, PRIMARY, METASTASIS:	1	1	1	0
POLYPOID ADENOMA, BENIGN, PRIMARY:	1	0	1	0
VAGINA (NO. OF TISSUES EXAMINED)	50	50	50	50
ADENOCARCINOMA, MALIGNANT, PRIMARY, METASTASIS:	0	1	0	0

@ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
- INDICATES NOT APPLICABLE.

(1193)

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 665 of 959

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

MEMORANDUM

SUBJECT: 2,4 Dichlorophenoxyacetic acid (2,4-D) - Review of Additional Histopathology Data from Mouse Carcinogenicity Study and Rat Combined Chronic Toxicity/Carcinogenicity Study

DP Barcode: D236685; D239909
PC Code: 030001
Case: 838294; 818706
Submission: S525309; S531709

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer
Reregistration Branch I, Health Effects Division (7509C) Virginia A. Dobozy 2/9/99

TO: Richard Dumas/Jill Bloom
Special Review and Reregistration Division

THRU: Whang Phang, Ph.D., Branch Senior Scientist
Reregistration Branch I, Health Effects Division (7509C) Whang Phang 2/9/99

Action Requested: Review additional data from Rat Combined Chronic Toxicity/Carcinogenicity Study (MRID 44284501) and Mouse Carcinogenicity Study (MRID 44284502)

Recommendation: The additional data do not alter the conclusions of either study concerning the carcinogenic potential of 2,4-D. The chemical should continue to be classified as a Group D carcinogen pending receipt of the additional epidemiological data referred to in the July 17, 1996 Cancer Peer Review Committee report.

BACKGROUND

Additional data from the mouse carcinogenicity study and rat combined chronic toxicity/carcinogenicity study were requested in the HED Carcinogenicity Peer Review Committee (CPRC) report of the July 17, 1998 meeting. In the mouse carcinogenicity study, there was an increase in hemangiosarcomas of the spleen in male mice at the low and mid doses, which was not sustained at the highest dose, however the slides from all animals at the low and mid doses were not evaluated. There were two rat combined chronic toxicity/carcinogenicity studies reviewed by the CPRC. In a 1986 study, there was a statistically significant trend for astrocytomas in male rats, but there was no pair-wise significance. The number of tumors in the control, 1, 5, 15 and 45 mg/kg/day groups was 1/60, 0/60, 0/60, 2/60 and 6/60, respectively. The incidence in both the treated and control animals exceeded the historical control incidences for this tumor in studies conducted by the National Toxicology Program. In addition, the CPRC concluded that the high dose was not adequate for testing the chemical's carcinogenicity potential. Therefore, a 1995 study was conducted at higher doses. In this study, there was one astrocytoma in the brain of males at the high dose vs. none in the control males. There was one tumor each in the control group and the high dose group for females. However, all the brains of animals in the low- and mid-dose groups were not examined. The CPRC concluded that these tissues from male animals should be examined. In addition to requesting the additional tissue examinations, the report also stated that a better characterization of the impurities in the test materials used in the original and new study might be helpful in explaining the conflicting results (i.e., astrocytomas in male rats).

DATA REVIEW

1) Rat Brains - MRID 44284501

The brains of 24 male rats dosed at 5 mg/kg/day and 32 males dosed at 75 mg/kg/day were examined by standard light microscopy. No additional neoplasms were observed. The incidence of astrocytomas can be found on page 7 of the July 17, 1996 CPRC report. The findings were consistent with rats of this strain (Fischer 344) and age (> 2 years). When the data from all the rats at the 24-month sacrifice were compared, the only observations which were statistically significantly increased were swollen axon, very slight, in the mid-dose group and swollen axon, gracile nucleus, very slight, in the low- and mid-dose groups.

2) Mice Spleens - MRID 44284502

The spleens of 35 male mice in the 5 mg/kg/day group and 33 in the 62.5 mg/kg/day group were examined by light microscopy. No neoplasms were observed. When all the data on the 50 animals/group were combined, there was no dose-related increase in hemangiosarcomas or in possible preneoplastic lesions, such as splenic extramedullary hematopoiesis, as presented in the following Table 1.

Table 1: Incidence of Splenic Lesions in Male Mice[a,b]

	Dosage Levels (ppm)			
	0	5	62.5	125
Increased extramedullary hematopoiesis, slight	10	4	5	4
Increased extramedullary hematopoiesis, moderate	3	4	11	3
Increased extramedullary hematopoiesis, any severity**	13	8	16	7
Hemangiosarcoma, malignant, primary, no metastasis	0	5#	3	1
Hemangiosarcoma, malignant, primary, metastasis	0	1#	1	1
Hemangiosarcoma, malignant, primary, metastasis or no metastasis**	0	6	4	2
Hemangiosarcoma (liver), malignant, secondary	1	0	0	0
Lymphosarcoma, malignant, primary, no metastasis	0	0	0	2

a Extracted from Table 2 (page 10) of MRID 44284502
b Fifty mice were examined in all dose groups.
** This line represents the combination of two or more preceding lines with similar observations and locators.
# Change from previously reported data. One of the hemangiosarcomas from the 5 mg/kg/day dose group had a metastasis to the liver; the total remains six for this dose group, five primary splenic hemangiosarcomas without metastasis and one with metastasis.

The registrant argues that the splenic hemangiosarcomas should not be considered treatment-related for several reasons:

1) There was no dose response.

2) A similar pattern of vascular tumors was not present in any other organ. Since neoplasms of blood vessels may arise in any organ, it is misleading to tabulate and analyze hemangiosarcomas by an individual organ. It is preferable to examine the incidence from all sites. In this study, when the incidence of hemangiosarcomas from all sites, or of total vascular tumors (hemangiomas and hemangiosarcomas) from all sites, is examined, there is no pattern of dose responsiveness. These data are presented in the Table 2 below.

Table 2: Incidence of Vascular Tumors[a,b]

	Dose Levels (ppm)			
	0	5	62.5	125
Total number of mice with hemangioma, benign, any site	2	2	2	3
Number of mice with hemangiosarcoma				
Primary site in heart	1	0	0	0
Primary site in liver	1	0	1	1
Primary site in skin	0	1	0	0
Primary site in spleen	0	6	4	2
Total number of mice with hemangiosarcoma, malignant, any site	2	7	5	3
Total number of mice with hemangioma and/or hemangiosarcoma, any site	4	7	7	5

a Extracted from Table 3 (page 11) of MRID 44284502
b Fifty animals were examined in all dose groups.

3) Data from all dose groups fall within the historical control range. Historical control data were submitted from 5 dietary studies conducted from 7/90 to 8/95. Using only those studies which are within two to three years of the study in question in accordance with the proposed *Guidelines for Carcinogen Risk Assessment*, the following data are applicable.

Study & Date Reported	B (8/95)	C (5/95)	D(2/95)
Total Number of Mice with Hemangioma, Benign, Any Site	N.R.	0/50	N.R.
Total Number of Mice with Hemangiosarcoma, Malignant, Any Site	N.R.	8/50	N.R.
Total Number of Mice with Hemangioma and/or Hemangiosarcoma, Any Site	6/50	8/50	5/50

N.R. Data not reported for this category in final report (data reported for benign or malignant vascular neoplasms for each organ separately).

4) The incidence of splenic hemangiosarcomas was not statistically significantly increased.

CONCLUSIONS

The additional data do not alter the findings of the study. The data were discussed with the present Chairman of the Cancer Assessment Review Committee and it was concluded that another meeting of the Committee is not required. The chemical should remain a Group D carcinogen, as classified at July 17, 1996 CPRC meeting.

4) The incidence of splenic hemangiosarcomas was not statistically significantly increased.

CONCLUSIONS

The additional data do not alter the findings of the study. The data were discussed with the present Chairman of the Cancer Assessment Review Committee and it was concluded that another meeting of the Committee is not required. The chemical should continue to be classified as a Group D carcinogen pending receipt of the additional epidemiological data referred to in the July 17, 1996 Cancer Peer Review Committee report.

cc: William Burnam, SAB
Cancer File, SAB

653

[2,4-D ISOPROPYLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Amendment to DER for MRID 42021401 - Subchronic Oral Toxicity Study [Rat] [2,4-D ISOPROPYLAMINE SALT]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 009405].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/20/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 12/20/00
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: subchronic oral toxicity - rat § 82-1/OPPTS 870.3100
P.C. CODE: 030025
CASWELL NO.: 315U CAS NO.: 5742-17-6
TEST MATERIAL (PURITY): 2,4-D isopropylamine salt [10.8% (acid equivalent 35.6%)]
SYNONYMS: 2,4-D IPA; dichlorophenoxyacetic acid, isopropylamine salt
SPONSOR: DowElanco

CITATION: Yano, B. L.; Cosse, P. F.; Atkin, L.; *et al.* (1991). 2,4-D Isopropylamine Salt [2,4-D IPA]: A 13-Week Dietary Toxicity Study in Fischer 344 Rats. Toxicology Research Lab. Dow Chem. Co.; Study No. HET M-004725-006, September 4, 1991. MRID 42021401. Document No. 009405. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [MRID 42021401], 10 Fischer 344 rats/sex/group were administered the isopropylamine salt of 2,4-dichlorophenoxyacetic acid [10.8%] *via* the diet for 13 weeks at concentrations of 0, 1 mg/kg/day, 19 mg/kg/day, 127 mg/kg/day, and 380 mg/kg/day [acid equivalents: 1, 15, 100, and 300 mg/kg/day, respectively, according to study report].

There were no deaths or treatment-related clinical signs of toxicity during the study or during the limited Functional Observational Battery conducted one week prior to necropsy. Decreased body weight [males 76%-94%/females 67%-83% of control] was observed in the high-dose [380 mg/kg/day] rats throughout the study, with the magnitude of the deficit increasing with time. Females at the next highest dose level [127 mg/kg/day] also displayed a slight [91%-93% of control] decrease in body weight throughout the study. Overall body-weight gain was decreased in both sexes at the highest dose level [males 47%/females 8% of control] and in females at the next to the highest dose level [77% of control]. Food consumption for both sexes at the two highest dose levels was decreased throughout the study, which may have resulted from a palatability problem and/or due to slight anorexia secondary to toxicity.

Statistically-significant decreases in red blood cell, hemoglobin, hematocrit, platelet values, and white blood cell values were observed at study termination in the mid-high dose males and in the high-dose females, and the mid-high dose females also displayed a significant decrease in HCT and platelet counts. Several of the clinical chemistry parameters [increased ALP activity, total cholesterol, triglycerides, AST/ALT activity, and BUN; decreased glucose, total protein, globulin, and calcium concentrations] were altered at the high-dose level in one or both, but many of these changes can be attributed to the severe debilitation of both sexes as evidenced by the lack of food consumption and decreased body weight. Males at the high-dose level displayed an elevated T3 concentration [142% of control] and a decrease in T4 concentrations [21% of control]. Females at the two highest dose levels displayed decreases

in T4 concentrations [22%/19% of control at 127 and 380 mg/kg/day, respectively]. The iris of one high-dose male and 10 high-dose females appeared pale at the end of the study, and this was considered to be the result of slight anemia.

Increased thyroid and liver weights were observed at the high-dose level in both sexes. Decreased absolute and increased relative adrenal, brain, kidney, and heart weights were observed in both sexes at the high-dose level also. Additionally decreased absolute testes weight and decreased absolute and relative ovarian weights were observed at the high-dose level in the respective sexes, and increased absolute and relative kidney weights were noted in the mid-high males. Eye lesions were observed at the high-dose level [males and females] and consisted of bilateral retinal degeneration [focal or diffuse unilateral or diffuse bilateral], which varied from slight to severe, characterized by a complete or partial loss of the rod and cone layer and the outer nuclear layer, according to the study report. Liver lesions [increased size of hepatocytes in the centrilobular and midzonal regions] were observed at the high dose level in both sexes, and kidney lesions [degeneration, proximal tubule, descending part (both sexes), decreased protein droplets, proximal tubule (males)] were observed at the two high dose level in both sexes. Thyroid lesions consisted of slightly increased size of epithelial cell lining the thyroid follicles [hypertrophy-columnversus cuboidal shape] with a vacuolated cytoplasm in females at the mid-high and in both sexes at the high-dose level. The cellularity of erythroid, myeloid, and megakaryocyte cell lines in the bone marrow was slightly decreased, and fat spaces were more readily apparent in both sexes at the high-dose level. In the adrenal glands, the cells of the zona glomerulosa were increased slightly in size [hypertrophy] with the cytoplasm being more vacuolated in the mid-high dose females and in both sexes at the high-dose level than in the controls. Other lesions in both sexes at the high-dose level were reported in the lungs [slight increase in multifocal alveolar histiocytosis], spleen [generalized lymphoid atrophy], thymus [generalized lymphoid atrophy], and the testes [slight testicular degeneration] in males.

The NOAEL is 19 mg/kg/day, and the LOAEL is 127 mg/kg/day, based on decreased body-weight gain, food consumption, and histopathological lesions in the kidneys [both sexes], adrenal gland [females], and thyroid [females]. At the highest dose level [380 mg/kg/day], females displayed alterations in hematology parameters, both sexes displayed alterations in clinical chemistry parameters, increased liver and thyroid weights, decreased testes and ovarian weights [in the respective sex], and lesions in the eyes, liver, thyroids, kidneys, adrenal, spleen, thymus, and lungs, and the high-dose males displayed testicular lesions.

This subchronic oral toxicity study is classified **Acceptable/Guideline**, and the study satisfies the guideline requirement [OPPTS 870.3100; §82-1] for a subchronic oral toxicity study in the rodent.

[2,4-D ISOPROPYLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Table 1. Hematology Parameters

Dose/Sex/Parameter	0 mg/kg/day	1.0 mg/kg/day	19 mg/kg/day	127 mg/kg/day	380 mg/kg/day
MALES					
RBC [x10^6]	7.80±0.34	7.87±0.38	7.97±0.37	6.86±0.85*[88]√	7.11±1.01
HGB [mg/dL]	15.5±2.9	15.9±0.7	15.6±0.6	14.3±1.8	14.3±1.5
HCT [%]	46.0±1.8	46.3±2.3	46.7±2.5	39.7±5.1*[86]	41.7±6.1
platelets [x10^3/mm^3]	786±64	689±190	756±48	672±73*[85]	724±100
WBC [x10^3/mm^3]	8.3±1.1	7.8±1.3	7.8±1.5	6.2±1.6*[75]	7.0±2.3 [84]
FEMALES					
RBC [x10^6]	7.07±0.43	7.08±0.23	6.87±0.42	6.80±0.39	6.09±0.34*[86]
HGB [mg/dL]	14.8±1.3	14.3±2.0	14.4±0.6	14.7±0.9	13.6±1.0*[92]
HCT [%]	42.7±2.6	42.7±1.3	41.6±2.8	39.9±2.5*[93]	34.6±2.1*[81]
platelets [x10^3/mm^3]	825±110	764±131	814±140	604±111*[73]	549±67*[67]
WBC [x10^3/mm^3]	5.2±0.9	4.8±1.0	5.0±1.1	5.1±1.2	3.4±0.9*[65]

Data from Tables 14 and 17, pages 67 and 71 of the report; n=10; * p<0.05; √ [% of control]

Table 2. Clinical Chemistry Parameters

Dose/Sex/Parameter	0 mg/kg/day	1 mg/kg/day	19 mg/kg/day	127 mg/kg/day	380 mg/kg/day
MALES					
alkaline phosphatase [mU/mL]	123±10	123±17	123±10	119±10	132±22
ALT [mU/mL]	49±10	46±9	51±8	59±15	63±33 [129]√
AST [mU/mL]	76±15	78±27	77±16	88±20	103±37 [136]
glucose [mg/dL]	144±19	145±13	144±19	140±32	101±10*[70]
BUN [mg/dL]	16±1	16±1	15±1	16±2	22±3*[138]
total protein [g/dL]	6.5±0.3	6.2±0.3	6.4±0.1	6.2±0.2	5.8±0.4*[89]
albumin [g/dL]	3.4±0.2	3.3±0.1	3.4±0.1	3.3±0.1	3.4±0.2
globulin [g/dL]	3.1±0.2	2.9±0.2	3.0±0.1	2.9±0.2	2.4±0.2*[77]
cholesterol [mg/dL]	50±5	49±5	48±5	45±4	68±13*[136]
triglycerides [mg/dL]	75±20	79±30	80±18	83±17	92±18 [123]
calcium [mg/dL]	9.4±0.5	9.6±0.5	9.7±0.6	9.5±0.4	9.0±0.4 [96]
FEMALES					
alkaline phosphatase [U/L]	99±15	93±13	100±14	94±13	130±26*[131]
ALT [U/L]	40±12	37±6	42±22	58±23	51±19 [128]
AST [U/L]	73±28	71±14	73±26	136±110	127±35*[174]
glucose [mg/dL]	103±8	108±10	108±8	95±8	82±11*[80]
BUN [mg/dL]	16±2	15±2	16±2	17±1	21±3*[131]
total protein [g/dL]	6.1±0.2	6.1±0.3	6.2±0.2	6.1±0.3	5.4±0.4*[89]
albumin [g/dL]	3.3±0.2	3.3±0.1	3.3±0.2	3.5±0.2*[106]	3.3±0.2
globulin [g/dL]	2.9±0.1	2.8±0.2	2.9±0.1	2.7±0.2	2.1±0.3*[72]
cholesterol [mg/dL]	86±6	86±9	82±7	59±7*[69]	95±9 [112]
triglycerides [mg/dL]	48±8	47±10	49±16	57±14	95±14*[198]
calcium [μg/L]	9.5±0.4	9.7±0.3	9.6±0.4	9.4±0.4	9.0±0.2*[96]

Data from Tables 24-27, pages 84, 86, 87, 89 of the report; n=10; * p<0.05; √ [% of control]

Table 3. Thyroid Parameters

Dose/Sex/Parameter	0 mg/kg/day	1 mg/kg/day	19 mg/kg/day	127 mg/kg/day	380 mg/kg/day
MALES					
thyroid weight					
grams	0.017±0.001	0.017±0.002	0.017±0.001	0.017±0.002	0.023±0.002*[135]√
g/100 g	0.0058±0.0003	0.0056±0.0005	0.0057±0.0004	0.0062±0.0004	0.0104±0.0013*[179]
T3 [ng/mL]	69±22	67±17	58±18	62±21	98±32*[142]
T4 [μg/dL]	4.3±0.8	3.9±0.6	4.2±0.8	3.5±0.7	0.9±0.5*[21]
FEMALES					
thyroid weight					
grams	0.013±0.002	0.014±0.001	0.014±0.002	0.014±0.002	0.016±0.002*[123]
g/100 g	0.0078±0.0009	0.0083±0.0008	0.0083±0.0015	0.0094±0.0011*[121]	0.0142±0.0020*[182]
T3 [ng/mL]	101±15	103±21	84±16	64±17*[63]	104±23
T4 [μg/dL]	3.2±0.7	3.1±0.8	2.9±0.7	0.7±0.5*[22]	0.6±0.4*[19]

Data from Tables 24, 26, 28, 29 [pages 85, 88, 91, 93] of the report; n=10; * $p<0.05$; √ [% of control]

Table 4. Organ-Weight Data [grams]

Dose/Sex/Parameter	0 mg/kg/day	1 mg/kg/day	19 mg/kg/day	127 mg/kg/day	380 mg/kg/day
MALES					
ADRENAL					
grams	0.047±0.004	0.043±0.005	0.044±0.003	0.042±0.003*[89]	0.040±0.003*[85]
g/100 g	0.0160±0.0014	0.0147±0.0018	0.0149±0.0011	0.0151±0.0016	0.0180±0.0016*[113]
BRAIN					
grams	1.877±0.043	1.844±0.113	1.890±0.031	1.884±0.053	1.781±0.041*[95]
g/100 g	0.640±0.024	0.621±0.030	0.641±0.020	0.673±0.041	0.807±0.083*[126]
HEART					
grams	0.788±0.043	0.810±0.043	0.802±0.023	0.755±0.072	0.663±0.058*[84]
g/100 g	0.268±0.015	0.273±0.014	0.272±0.007	0.269±0.016	0.298±0.012*[111]
KIDNEYS					
grams	1.950±0.091	1.962±0.125	2.066±0.163	2.263±0.289*[116]	1.618±0.102*[83]
g/100 g	0.665±0.032	0.661±0.028	0.699±0.034	0.806±0.098*[121]	0.730±0.051*[110]
LIVER					
grams	8.008±0.309	8.095±0.440	8.304±0.548	7.554±0.506	8.701±0.549*[109]
g/100 g	2.729±0.113	2.727±0.101	2.812±0.101	2.690±0.104	3.929±0.287*[144]
TESTES					
grams	3.090±0.065	3.132±0.139	3.109±0.122	3.115±0.100	2.386±0.326*[77]
g/100 g	1.053±0.030	1.056±0.039	1.054±0.047	1.112±0.053*[106]	1.070±0.076
FEMALES					
ADRENAL					
grams	0.054±0.004	0.053±0.005	0.050±0.005	0.049±0.006	0.031±0.003*[57]
g/100 g	0.0315±0.0032	0.0313±0.0030	0.0299±0.0029	0.0316±0.0040	0.0274±0.0013*[87]
BRAIN					
grams	1.784±0.048	1.771±0.040	1.792±0.061	1.759±0.063	1.594±0.031*[89]
g/100 g	1.047±0.052	1.051±0.038	1.067±0.065	1.147±0.056*[110]	1.424±0.096*[136]
HEART					
grams	0.549±0.032	0.546±0.041	0.537±0.018	0.514±0.068	0.439±0.022*[80]
g/100 g	0.322±0.019	0.323±0.020	0.320±0.014	0.334±0.033	0.391±0.023*[121]
KIDNEYS					
grams	1.219±0.059	1.224±0.060	1.243±0.064	1.215±0.091	0.986±0.058*[81]
g/100 g	0.715±0.036	0.725±0.026	0.740±0.052	0.790±0.029*[110]	0.878±0.029*[123]
LIVER					
grams	4.685±0.253	4.540±0.266	4.659±0.274	4.511±0.159	5.489±0.271*[117]
g/100 g	2.745±0.085	2.691±0.120	2.774±0.211	2.942±0.177	4.893±0.253*[178]
OVARIES					
grams	0.064±0.007	0.063±0.007	0.067±0.004	0.058±0.008	0.025±0.007*[39]
g/100 g	0.037±0.004	0.037±0.004	0.040±0.003	0.038±0.005	0.023±0.006*[62]
Terminal fasted body weight					
males	293.5±7.0	296.9±14.2	295.1±11.0	281.0±19.4	222.8±23.5*[76]
females	170.7±8.8	168.7±7.0	168.2±6.6	153.8±9.1*	112.4±7.8*[66]

Data from Tables 28-29, pages 63-64 of the report; n=10 for groups 145 mg/kg/day and lower; 9 for high dose; √ [% of control]; * $p<0.05$

Table 5. Microscopic Lesions

Dose/Sex/Organ/Lesion	0 mg/kg/day	1 mg/kg/day	19 mg/kg/day	127 mg/kg/day	380 mg/kg/day
MALES					
ADRENAL					
hypertrophy, zona glomerulosa					
very slight	0	0	0	0	0
slight	0	0	0	0	10
BONE MARROW					
decreased hematopoiesis, slight	0	0	0	0	7
EYES					
degeneration, retina					
unilateral, focal					
slight	0	0	0	0	1
moderate	0	0	0	0	1
unilateral diffuse					
moderate	0	0	0	0	1
bilateral diffuse					
moderate	0	0	0	0	1
severe	0	0	0	0	0
mineralization, cornea, unilateral, very slight	5	3	3	6	4
mineralization, cornea, bilateral, very slight	0	5	6	4	3
KIDNEYS✔					
degeneration, proximal tubule, descending part					
very slight	0	0	0	0	0
slight	0	0	0	1	10
moderate	0	0	0	0	0
vacuolation, tubule, slight	0	0	0	0	1
decreased protein droplets, proximal tubule	0	0	0	0	10
LIVER					
increased size of hepatocytes	0	0	0	0	10
LUNGS					
alveolar histiocytosis, focal, very slight	1	0	0	0	1
alveolar histiocytosis, multifocal					
very slight	0	0	0	0	6
slight	0	0	0	0	1
SPLEEN					
atrophy	0	0	0	0	10
TESTES					
degeneration, tubule, bilateral✱	0	0	0	0	10
THYMUS					
atrophy	0	0	0	0	10
THYROID					
hypertrophy, epithelial cells					
very slight	0	0	0	0	3
slight	0	0	0	0	7

Table 5. Microscopic Lesions

Dose/Sex/Organ/Lesion	0 mg/kg/day	1 mg/kg/day	19 mg/kg/day	127 mg/kg/day	380 mg/kg/day
FEMALES					
ADRENAL					
hypertrophy, zona glomerulosa					
very slight	1	0	0	6	0
slight	0	0	0	2	10
BONE MARROW					
decreased hematopoiesis, slight	0	0	0	0	10
EYES					
degeneration, retina					
unilateral, focal					
slight	1	0	0	0	0
moderate	0	0	0	0	0
unilateral diffuse					
moderate	0	0	0	0	1
bilateral diffuse					
moderate	0	0	0	0	8
severe	0	0	0	0	1
mineralization, cornea, unilateral, very slight	4	5	5	3	8
mineralization, cornea, bilateral, very slight	3	2	3	4	1
KIDNEYS✔					
degeneration, proximal tubule, descending part					
very slight	0	0	0	8	0
slight	0	0	0	0	1
moderate	0	0	0	0	9
vacuolation, tubule, slight	0	0	0	0	0
decreased protein droplets, proximal tubule	0	0	0	0	0
LIVER					
increased size of hepatocytes	0	0	0	0	10
LUNGS					
alveolar histiocytosis, focal, very slight	1	0	0	1	0
alveolar histiocytosis, multifocal					
very slight	0	0	0	0	8
slight	0	0	0	0	2
SPLEEN					
atrophy	0	0	0	0	10
THYMUS					
atrophy	0	0	0	0	10
THYROID					
hypertrophy, epithelial cells					
very slight	0	0	0	0	1
slight	0	0	0	1	9

Data from Table 31 [pages 97-106] of the report; n=10; ✔degenerative effects in the kidneys were described as: very slight to moderate and characterized by the loss of the brush border of the straight descending portion of the proximal tubules, increased vacuolation of epithelial cell cytoplasm, occasionally accompanied by focal tubular basement membrane thickening, fibrosis, and individual cell atrophy [page 25 of the report]; ✱ characterized by an absence of spermatozoa, variable decreases in the number of spermatids, spermatocytes, and spermatogonia, and the presence of occasional multinucleated spermatids [page 26 of the report]; .

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DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding-Rodent **GUIDELINE:** 82-1(a)

TOX.CHEM. No.: 315-U MRID No.: 420214-01

HED Project No. 2-0319 **Registrant:** DowElanco.

TEST MATERIAL: Isopropylamine Salt of 2,4-D **(2,4-D IPA)**

TESTING LABORATORY: Toxicology Research Lab, Dow Chem. Co.

STUDY IDENTIFICATION: HET M-004725-006

TITLE OF REPORT: 2,4-D Isopropylamine Salt [2,4-D IPA]: A 13-Week Dietary Toxicity Study in Fischer 344 rats.

REPORT AUTHORS: B. L. Yano, P.F. Cosse, L. Atkin and R.A. Corley

REPORT DATE: September 4, 1991.

SUMMARY: In groups of 10 male and 10 female Fischer-344 rats fed diets containing the Isopropylamine salt of 2,4-Dichlorophenoxy-acetic acid at 0, 1, 19, 127, or 380 mg/kg/day for 13 weeks, no treatment-related effects were seen at 1 or 19 mg/kg/day. Treatment-related effects observed at 127 mg/kg/day were: decreases in mean body weight and body weight gain; reduction in the mean absolute food consumption; minor alterations in hematology and clinical chemistry parameters; decrease in urine specific gravity; increase in relative kidney weights; and histopathologic changes in the liver, kidneys and thyroid and adrenal glands. At 380 mg/kg/day, primary treatment-related effects included: decreases in mean body weight and body weight gain and food consumption; alterations in hematology and clinical chemistry, and urinalysis; changes in organ weights; gross pathology; and histopathological changes in the, eyes, kidneys, liver, and thyroid glands of male and female rats. Effects secondary to decreased weight gain, the debilitated condition of the rats, and/or toxicity in other organs occurred in the adrenals, bone marrow, mesenteric fat, lungs, spleen, thymus, and testes.

Under the conditions of this study, a NOEL of 19 mg/kg/day and a LOEL of 127 mg/kg/day is established for the 90-day oral toxicity of the Isopropylamine Salt of 2,4-Dichlorophenoxyacetic acid to male and female rats. female rats.

I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the subchronic toxicity of the **Isopropylamine Salt of 2,4-Dichlorophenoxyacetic acid [2,4-D IPA]** following dietary administration to rats.

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: Isopropylamine salt of 2,4-D.
Purity: 35.6 Wt% 2,4-D acid and 10.8 Wt% IPA
Lot No.: AGR 276461
Description: Aqueous amber liquid.
Vehicle: Acetone

2. Test Animals

Species: Rats
Strain: Fischer-344
Sex: Males and females
Weight at Initiation: 155 - 185 g (M); 107 - 130 g (F)
Identification: Ear tags.
Acclimation: Minimum of seven days
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Chow #5002.
Water: Tap water ad libitum
Environment: Temperature and humidity controlled rooms with a photocycle regulated for rats.

3. Study Design

Group No.	Treatment	No. of Animals		Dose level [mg/kg/day]	Acid Equivalent [mg/kg/day]
		Males	Females		
1	Control	10	10	0	0
2	Low	10	10	1	1
3	Mid-1	10	10	19	15
4	Mid-2	10	10	127	100
5	High	10	10	380	300

4. Test Article Formulation and Analyses

a. Formulation: Test material was dissolved in a relatively small amount of acetone to facilitate its uniform dispersion in the diet. The test diets were prepared weekly by serial dilution of the high-dose to attain the lower doses. Initial concentrations of test material in the diet were calculated from the pretest body weights and feed consumption data. Thereafter, the most recent body weight and food consumption data were used to adjust the concentration of the test material in the diet to maintain the dose levels on the targeted mg/kg/day basis. Reference samples [one/dose/sex/mix plus premix] were retained and stored at ambient temperature.

b. Analysis: Difficulties were encountered in analyzing the diets for 2,4-D IPA due to the complete disassociation of the test material to the conjugate base [2,4-D] and ammonium ion [IPA] moieties in aqueous solution. Therefore, verification of the stability of 2,4-D IPA in test diets were conducted in two separate analyses based on the stability of the 2,4-D acid and IPA moieties for at least 21 days. Homogeneity and concentration analysis based on analysis of 2,4-D acid were performed at the start of the study and bi-weekly thereafter.

5. Treatment

Rats were fed the control and test diets 7 days per week for at least 13 weeks. The oral route of administration was chosen because it is the route of potential human exposure.

6. Experimental Procedures

Mortality and moribundity checks were performed once daily. Body weights and food consumption were measured prior to initiation and once weekly. A complete physical examination was performed weekly. Ophthalmologic examinations were conducted prior to initiation [day -21] and at termination. A functional observation battery [FOB] was conducted on all surviving rats during the week prior to necropsy. Blood and urine were collected from all animals at termination for hematology, clinical chemistry and urinalysis. 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]**
x Mean corpuscular HGB (MCH)	**x Mean corpuscular HGB Concentration (MCHC)**
x Mean corpuscular volume (MCV)	Blood clotting measurements
Corrected leukocyte count COR WBC)	**x 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 Cholesterol[a]**
x Potassium[a]	**x Globulins**
x Sodium	**x Glucose[a]**
	x Total bilirubin[a]
Enzymes:	**x Total serum protein[a]**
	x Triglycerides
x Alkaline phosphatase	Serum protein electrophoresis
x Alanine aminotransferase (SGPT)[a]	**x Triiodothyronine [T_3]**
x Aspartate aminotransferase (SGOT)[a]	**x Thyroxine [T_4]**
Cholinesterase[b]	x A/G Ratio
x Creatinine phosphatase[a]	
Lactic acid dehydrogenase	
Gama glutamyl transferase	

Urinalysis

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

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

7. Termination

After 13 weeks of treatment, all surviving animals were weighed, anesthetized with sodium pentobarbital and exsanguinated. Necropsies were performed on each animal, all gross pathological changes were recorded, and the following organs were weighed.

Adrenals	Brain	Heart	Kidneys
Liver	Ovaries	Thyroid	Testes

8. Histopathology

The checked (X) tissues from all animals in the control and high-dose groups, and the liver, kidneys, lungs, thyroid, all gross lesions and target organs identified in the high-dose group were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Salivary glands[a]	x Trachea[a]
x Esophagus[a]	x Lung[a]
x Duodenum[a]	Pharynx[e]
x Jejunum[a]	Larynx[e]
x Cecum[a]	Nose[e]
x Colon[a]	
x Rectum[a]	Cardiovascular/Hemo.System
x Liver[ac]	
x Pancreas[a]	x Aorta (thoracic)[a]
Gall bladder[ab]	x Heart[a]
	x Bone marrow[a]
Neurological System	x Lymph nodes[a]
	x Spleen[a]
x Brain[ac]	x Thymus[a]
x Pituitary[a]	
x Peripheral nerve[ac]	Urinogenital System
x Spinal cord (3 levels)[ac]	x Kidneys[ac]
x Eyes (optical nerve)[ac]	x Urinary bladder[a]
	x Testes[ac]
Glandular System	x Epididymides
	x Uterus[a]
x Adrenals[a]	x Ovaries[ac]
Lacrimal glands[c]	Others
x Parathyroids[ad]	
x Thyroids[ad]	x All gross lesions and masses
	x Skeletal muscle[a]

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.

665

9. Statistical Analyses

Only means and standard deviations were reported for food consumption, WBC differential counts, RBC indices and reticulocytes. Bartlett's test for equality of variances was employed for body weights, organ weights, clinical chemistry data, appropriate hematology data and urinary specific gravity. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric analysis of variance [ANOVA], followed respectively by Dunnnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of The Toxicology Research Laboratory, and the Study Protocol. A quality assurance statement was signed and dated August, 29, 1991.

III. RESULTS

1. Analysis of Diet Mix

Concentrations of test diets, based on analysis of 2,4-D indicated that the diets were accurately prepared throughout the study. The mean assay values were 87%, 116%, 103% and 104 respectively, of the nominal concentrations for males and 89%, 107 %, 113% and 109% respectively, of the nominal concentrations for females at 1, 19, 127 and 380 mg/kg/day diets. The time-weighted average dose levels for targeted dose levels of 1, 19, 127 395 mg/kg/day were 0.9, 22, 131 and 395 mg/kg/day, respectively, for males and 0.9, 20, 144 and 414 mg/kg/day, respectively, for females.

Homogeneity of the test diets, based on analysis of 2,4-D indicated that diets formulated to deliver 127 or 380 mg/kg/day were homogenous. The mean values [% of target] were 71%, 90% and 92%, respectively, and the coefficient of variations were 30.1%, 24% and 6.64%, respectively for the 1, 19, and 127 mg/kg/day diets.

Stability analyses for the acid moiety indicated that the test material was stable for at least 14 days with 93% recovery of Day 0. The stability analysis of the acid and amine moieties resulted in the recovery of 90.38 ± 1.30% and 99.15 ± 3.99% after 21 days in separate experiments using ^{14}C-labelled 2,4-D acid and ^{14}C-labeled IPA.

2. Survival

All rats survived the study.

3. Clinical Observations

No treatment-related clinical signs of toxicity were seen.

4. Functional Observational Battery:

No treatment-related effects were seen in either sex of rats at any dose levels.

5. Ophthalmology Examination

The iris of one male and 10 females at 380 mg/kg/day appeared pale towards the end of the study which was considered to be the result of slight anemia.

V64

6. Body Weights and Body Weight Changes

Mean body weight data are summarized in Tables 1 and 2 for males and females, respectively. Decrease in mean body weights were seen in:

- o Males at 380 mg/kg/day from Weeks 3 - 13,
- o Females at 380 mg/kg/day from Weeks 1 - 13;
- o Males at 127 mg/kg/day from Weeks 10 - 13, and
- o Females at 127 mg/kg/day from Weeks 1 - 13.
- o Mean body weights of males and females at 1 and 19 mg/kg/day were comparable to that of the control rats.

Table 1. Mean Body Weights and S.D (G) for Male Rats.

Dose Level [mg/kg/day]	Week: 0	Week: 6	Week: 10	Week: 13[a]
0	166.7 ± 7.4	277.6 ± 8.0	308.1 ± 5.3	318.7 ± 6.7
1.0	172.1 ± 12.1	282.6 ± 16.6	308.1± 21.4	320.3±14.2
19.0	171.3 ± 6.4	281.9 ± 7.5	310.5 ± 9.6	317.7±11.8
127.0	167.3 ± 8.0	272.1 ± 14.7	298.0± 19.1	305.0±19.7
380.0	171.7 ± 10.3	**261.8* ±15.2**	**259.3* ±22.4**	**243.4* ±24.3**

* Significantly different from control value at $p \leq 0.05$.

Table 2. Mean Body Weights and S.D (G) for Female Rats.

Dose Level [mg/kg/day]	Week: 0	Week: 6	Week: 10	Week: 13
0	121.7 ± 6.1	169.8 ± 8.7	184.4 ± 9.4	187.1 ± 9.3
1.0	119.7 ± 4.2	171.7 ± 5.4	181.3 ± 6.3	184.7 ± 5.9
19.0	119.6 ± 3.5	168.3 ± 4.4	183.1 ± 5.9	184.8 ± 6.4
127.0	119.5 ± 5.2	**158.4* ± 7.7**	**168.8* ± 7.8**	**169.6* ± 8.3**
380.0	120.5 ± 4.4	**140.4* ± 5.8**	**138.2* ± 7.8**	**125.6* ± 8.7**

* Significantly different from control value at $p \leq 0.01$

Mean body weight change data are presented in Tables 3 and 4 for males and females, respectively. Decreases in body weight gain were noted in:

- o Males and females at 380 mg/kg/day during the entire study.
- o Males and females at 127 mg/kg/day during the entire study.
- o Body weight gain was comparable for both sexes of rats at the lower [1 or 19 mg/kg/day] doses.

Table 3. Mean Body Weight Change (G) in Male Rats.

Dose Level [mg/kg/day]	Weeks: 0 - 4	Weeks: 0 - 8	Weeks: 0 - 13
0	81.8 ± 2.6	130.0 ± 7.0	152.0 ± 7.3
1.0	85.6 ± 4.3	127.5 ± 7.3	148.1 ± 6.6
19.0	85.8 ± 5.6	127.7 ± 8.1	146.4 ± 11.5
127.0	**84.5 ± 9.0**	**117.5 ± 16.2** 90	**137.7 ± 18.0**
380.0	**71.4 ± 7.5** 87	**95.1 ± 17.2** 73	**71.7 ± 23.0** 47

Table 4. Mean Body Weight Change (G) in Female Rats.

Dose Level [mg/kg/day]	Weeks: 0 - 4	Weeks: 0 - 8	Weeks: 0 - 13
0	38.0 ± 4.2	58.2 ± 6.2	65.3 ± 7.1
1.0	39.6 ± 1.7	56.8 ± 4.0	65.0 ± 4.8
19.0	39.0 ± 2.3	57.6 ± 3.9	65.2 ± 5.4
127.0	**28.1 ± 2.4** 74	**43.4 ± 3.1** 75	**50.1 ± 5.6** 77
380.0	**14.7 ± 2.9** 39	**23.2 ± 4.2** 40	**5.1 ± 7.3** 8

7. Food Consumption

The mean absolute [g//rat/day] food consumption values were reduced for males and females at 127 and 380 mg/kg/day. The decrease in food consumption was related to the reduced body weights observed in these rats. The decrease in feed consumption may have resulted from unpalatability of the feed and/or due to slight anorexia secondary to toxicity. No differences were seen in feed consumption of males and females at 1 or 19 mg/kg/day.

648

8. Hematology and Clinical Chemistry

Treatment-related, statistically significant [$p < 0.05$] changes observed in hematology and clinical chemistry parameters are tabulated in Tables 5 and 6, respectively.

Table 5. Changes in Hematology Parameters.

Dose Level [mg/kg/day] Sex	Parameter Affected	Effect
127 / ♂	RBC WBC HCT Platelet	Decreased Decreased Decreased Decreased
127 / ♀	HCT Platelet	Decreased Decreased
380 / ♀	RBC WBC HCT Platelet	Decreased Decreased Decreased Decreased

Table 6. Changes in Clinical Chemistry Parameters

Dose Level [mg/kg/day] Sex	Parameter Affected	Effect
127 / ♀	Albumin Cholesterol T_4 T_3	Increased Decreased Decreased Decreased
380 / ♂	BUN Tot.Prot. Globulin Glucose Cholesterol T_4 T_3	Increased Decreased Decreased Decreased Increased Decreased Increased
380 / ♀	BUN AP AST Tot.Prot. Globulin Glucose Triglycerides Calcium T_4	Increased Increased Increased Decreased Decreased Decreased Increased Decreased Decreased

9. Urinalysis

Except for the statistically significant [p <0.05] decreases in urine specific gravity in males at 380 mg/kg/day and in females at 19, 127, and 380 mg/kg/day [sex, dose-dependent], urinalysis revealed no other treatment-related changes.

10. Organ Weights

Statistically significant [p < 0.05] differences observed in the absolute and relative organ weight are summarized below:

Absolute Organ Weights			
Organ	**Dose Level [mg/kg/day]**	**Sex**	**Effect**
Adrenal	127 380	M M, F	Decreased Decreased
Brain	380	M, F	Decreased
Heart	380	M, F	Decreased
Kidneys	127 380	M M, F	Increased Decreased
Liver	380	M, F	Increased
Thyroid	380	M, F	Increased
Testes	380	M	Decreased
Ovaries	380	F	Decreased

Relative Organ Weights			
Organ	**Dose Level [mg/kg/day]**	**Sex**	**Effect**
Adrenal	380	M F	Increased Decreased
Brain	127 380	F M, F	Increased Increased
Heart	380	M, F	Increased
Kidneys	127 & 380	M,F	Increased
Liver	380	M, F	Increased
Thyroid	127 380	F M, f	Increased Increased
Testes	127	M	Increased
Ovaries	380	F	Decreased

676

11. Gross Pathology

No treatment-related gross pathological changes were seen either in either sexes at 1, 19, or 127 mg/kg/day. At 380 mg/kg/day, gross pathological changes included: decrease in testicular size [4 ♂]; accentuated lobular pattern of the liver [10 ♂ and 10 ♀]; increased cecal size [6 ♂ and 5 ♀]; decreased amount of general fat [3 ♂ and 9 ♀]; pale foci in the lungs [1 ♂ and 5 ♀]; and hemolyzed blood in the lumen of the stomach [6 ♂ and 3 ♀]. Other gross necropsy findings were considered incidental in nature.

12. Histopathology

No treatment-related histopathological changes were seen in male or female rats at 1 or 19 mg/kg/day. Treatment-related histopathological changes observed at 127 and 380 mg/kg/day are tabulated in Tables 7 and 8 and are summarized below:

Dose Level [mg/kg/day]	Sex	Organs with Lesions
127	M	Kidneys and Liver
	F	Adrenal, Fat [mesenteric], Kidneys, and Thyroid
380	M & F	Adrenals, Bone marrow, Eyes, Fat [mesenteric], Kidneys, Liver, Lungs, Spleen, Thymus, Thyroid, and Testes

Adrenal glands: Cells of the adrenal zona gomerulosa were slightly increased in size [hypertrophy] with the cytoplasm being more vacuolated than the controls.

Bone marrow: The cellularity of erythroid, myeloid and megakaryocyte cell lines in the bone marrow was slightly decreased and fat spaces were more readily observed.

Eyes: Bilateral retinal degeneration which varied from slight to severe was characterized by a complete or partial loss of the rod an cone layer and the outer nuclear layer.

Kidneys: Degenerative kidney effects were slight to moderate and were characterized by a loss of the brush border of the straight descending portion of the proximal tubules, increased vacuolation of epithelial cell cytoplasm and were occasionally accompanied by focal tubular basement membrane thickening, fibrosis and individual cell atrophy.

Liver: Lesions were characterized by an increase in the size and altered tinctorial properties [increased cytoplasmic eosinophilia] of hepatocytes in the centrilobular and midzonal regions of the hepatic lobule. This effect was not accompanied by hepatocellular degeneration or necrosis.

Lungs: Very slight to slight increases in numbers of subpleural alveolar macrophages were seen.

Spleen: Generalized lymphoid atrophy was seen along with decreases in the populations of T- and B-lymphocytes. Splenic red pulp was also reduced in amount.

Thymus: Generalized lymphoid atrophy.

Thyroid: Epithelial cells lining thyroid follicles were very slightly to slightly increased in size [hypertrophy-column versus cuboidal shape] with a vacuolated cytoplasm.

Testes: Testicular degeneration was slight and characterized by an absence of spermatozoa, variable decreases in the number of spermatids, spermatocytes and spermatogonia and presence of occasional multinucleated spermatids.

Other microscopical changes were considered incidental and unrelated to treatment.

Table 7. Histopathological Findings in Male Rats.

Organ / Lesion	Dose Level [mg/kg/day]				
No.of animals= 10 / dose levels	0	1	19	127	380
Adrenals -Hypertrophy, Zona Glomerulosa	0	0	0	0	10
Bone Marrow -Hematopoiesis, decreased	0	0	0	0	7
Eye - Retinal degeneration, Bilateral	0	0	0	0	4
Kidney -Degeneration, Proximal Tubules	0	0	0	1	10
Liver, Centrilobular -Altered Tinctorial Properties, very slight	0	0	0	2	0
-Increased size of hepatocytes with Altered Tinctorial Properties	0	0	0	0	10
Lung -Alveolar Histosis	1	0	0	0	8
Mesenteric Tissue -Adipose tissue, Atrophy	0	0	0	0	10
Spleen -Lymphoid Atrophy	0	0	0	0	10
Testes -Degeneration, Tubules, Bilateral	0	0	0	0	10
Thymus -Atrophy	0	0	0	0	10
Thyroid -Hypertrophy, Epithelial cells	0	0	0	0	10

Table 8. Histopathological Findings in Female Rats.

Organ / Lesion	Dose Level [mg/kg/day]				
No.of animals= 10 / dose levels	0	1	19	127	380
Adrenals -Hypertrophy, Zona Glomerulosa	0	0	0	2	10
Bone Marrow -Hematopoiesis, decreased	0	0	0	0	10
Eye - Retinal degeneration, Bilateral	0	0	0	0	10
Kidney -Degeneration, Proximal Tubules	0	0	0	8	10
Liver, Centrilobular -Increased size of hepatocytes with Altered Tinctorial Properties	0	0	0	0	10
Lung -Alveolar Histosis	1	0	0	1	10
Mesenteric Tissue -Adipose tissue, Atrophy	0	4	2	10	10
Spleen -Lymphoid Atrophy	0	0	0	0	10
Thymus -Atrophy	0	0	0	0	10
Thyroid -Hypertrophy, Epithelial cells	0	0	0	1	10

IV. DISCUSSION

Male and female Fischer-344 rats were fed diets containing the Isopropylamine salt of 2,4-D at concentrations of 0, 1, 19, 127, or 380 mg/kg/day for 13 weeks.

Analytical data showed that the diet mixes were homogeneous, stable at room temperature for up to 21 days and the mean concentrations were within 10% of the target level.

No mortality occurred during the study and no treatment-related clinical signs of toxicity or functional abnormalities were seen at any dose level. **No treatment-related effects were seen at 1 or 19 mg/kg/day** on mean body weight, mean body weight gain, mean food consumption, ophthalmology, hematology, clinical chemistry, organ weights, gross pathology or histopathology.

Treatment-related effects observed at 127 were: decreases in mean body weight, body weight gain [both sexes]; reduction in the mean absolute food consumption [both sexes]; minor alterations in hematology [decreases in RBC, WBC, HCT and platelet counts in males and decreases in HCT and platelet counts in females] clinical chemistry parameters [decreases in cholesterol, T_4 and T_3 and increase in albumin in females]; decrease in urine specific gravity [females]; changes in organ weights [increase in relative kidney weights in both sexes] and histopathologic changes in the liver and kidneys [males] and kidneys, adrenal and thyroid glands [females]. Minor alterations of a secondary nature also occurred in the mesenteric fat of females at this dose.

Primary treatment-related effects observed at 380 mg/kg/day included: decreases in mean body weight and body weight gain [both sexes] ; reduction in food consumption [both sexes]; alterations in hematology and clinical chemistry [both sexes], and urinalysis [decrease in urine specific gravity in both sexes]; changes in organ weights, gross pathology; and histopathological changes in several organs of males and females.

Alterations in hematology parameters were limited to decreases in RBC, WBC, HCT and platelet counts in females only. Abnormalities in urine were limited decreased specific gravity in both sexes.

Treatment-related alterations in clinical chemistry parameters were decreases in Total protein, Glucose, Globulin and T_4, and increases in BUN, Cholesterol and T_3 in males and decreases in Total protein, Globulin, Glucose, Calcium and T_4 in males and increases in BUN, AP, AST, Triglycerides in females. These changes correlated with histopathological lesions of the kidneys, liver, and thyroids. Remaining differences in clinical chemistry values were considered to be related to the effect on body weight and the condition of the rats.

Differences in absolute and relative weights were seen in a number of organs at 127 and 380 mg/kg/day. However, only the increases in relative kidney, thyroid, and liver weights were considered to be treatment related due to collaborative clinical chemistry alterations and histopathological changes in these tissues. Differences seen in other organ weights were attributed to effects secondary to decreased body weights and the debilitated condition of the rat at the high dose.

Although, histopathology revealed alterations in several organs such as the adrenals, bone marrow, eyes, kidneys, liver, lung, mesenteric fat, spleen, thyroid, thymus, and testes, only the changes seen in the eyes, liver, kidneys, and thyroid glands were considered to be clearly treatment-related. Alterations seen in the other organs were considered to be secondary changes possibly associated with decreased body weight, poor nutrition due to unpalatability of the food, the debilitated condition of the rats, and general toxicity involving multiple organs.

The bilateral retinal degeneration observed in both sexes of rats at the high dose in this study was also seen in rats fed diets containing the 2,4-D acid, salts [dimethylamine and diethanolamine], and esters [butoxyethyl and 2-ethylhexyl ester]. The increase in size and altered tinctorial properties of hepatocytes seen in the centrilobular and midzonal regions of the hepatic nodule were not accompanied by hepatocellular degeneration and necrosis and therefore can probably be attributed to the induction of hepatic peroxisomes by 2,4-D IPA. The elevated BUN values and the increase in relative kidney weights may be related to the renal alterations in the descending proximal tubules. Hypertrophy of the follicular epithelial cells of the thyroid glands may be associated with a decrease in serum T_4 and increase in thyroid weights since there was no evidence of any degenerative changes involving the thyroid glands and the colloid appeared normal.

The hypertrophy and increased vacuolation of the adrenal gland zona glomerulosa was interpreted to be a secondary change possibly associated with the renal alterations since there were no specific microscopic alterations which corresponded with the decrease in absolute and relative organ weights. The decreased bone marrow cellularity associated with decreases in RBC, WBC and platelet counts and can be attributed to the interaction of the animals debilitated condition and possible secondary nutritional anemia. Similarly, the generalized lymphoid atrophy of the spleen and thymus, and the testicular degeneration can be attributed to poor nutrition, debilitated conditions, and general toxicity involving multiple organ systems.

The effects seen in this study were parallel to those seen in rats fed diets of 2,4-D acid at similar dose levels [1, 15, 100, or 300 mg/kg/day]. Therefore, it is suggested that the toxicity observed with 2,4-D IPA is primarily the result of the acid moiety and not the isopropylamine salt.

V. CONCLUSION

Under the conditions of this study, a NOEL of 19 mg/kg/day and a LOEL of 127 mg/kg/day is established for the 90-day oral toxicity of the Isopropylamine Salt of 2,4-Dichlorophenoxyacetic acid to male and female rats. The LOEL is based on decreases in mean body weight gain and mean absolute food consumption, increase in relative kidney weights, minor alterations in hematology and clinical chemistry, and histopathological alterations in the kidneys and liver of males, and kidneys, liver, and thyroid glands of female rats.

VI. **CORE CLASSIFICATION:** Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

[2,4-D TRIISOPROPANOLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Amendment to DER for MRID 42021402 - Subchronic Oral Toxicity Study [Rat] [2,4-D TRIISOPROPANOLAMINE SALT]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 009446].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/29/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P.H. Virginia A Dobozy 12/20/00
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: subchronic oral toxicity - rat § 82-1/OPPTS 870.3100
P.C. CODE: 030025 35
CASWELL NO.: 315AE CAS NO.: 18584-79-7
TEST MATERIAL (PURITY): 2,4-D Triisopropanolamine salt [72.2% (acid equivalent 38.7%)]
SYNONYMS: 2,4-D TIPA; dichlorophenoxyacetic acid, triisopropanolamine salt
SPONSOR: DowElanco

CITATION: Yano, B. L.; Cosse, P. F.; Atkin, L.; *et al.* (1991). 2,4-D Triisopropanolamine Salt [2,4-D TIPA]: A 13-Week Dietary Toxicity Study in Fischer 344 Rats. Toxicology Research Lab. Dow Chem. Co.; Study No. K-008866-006, September 4, 1991. MRID 42021402. Document No. 009446. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [MRID 42021401], 10 Fischer 344 rats/sex/group were administered the triisopropanolamine salt [72.2%, a.i.; 38.7% acid equivalent] of 2,4-dichlorophenoxyacetic acid *via* the diet for 13 weeks at concentrations of 0, 2 mg/kg/day, 28 mg/kg/day, 187 mg/kg/day, and 560 mg/kg/day [acid equivalents: 1, 15, 100, 300 mg/kg/day, respectively (calculated by this reviewer)].

There were no deaths. The only treatment-related clinical sign of toxicity observed during the study and during the limited Functional Observational Battery was unilateral or bilateral opaque eyes, which were diagnosed as cataracts and occurred in 8 high-dose males and all 10 high-dose females. Decreased body weight [males 66%-75%/females 69%-79% of control] was observed in the high-dose [560 mg/kg/day] rats throughout the study, with the magnitude of the deficit increasing with time in both sexes. Females at the next highest dose level [187 mg/kg/day] also displayed a slight [94% of control] decrease in body weight at week 6. Overall body-weight gain was decreased in both sexes at the highest dose level [males 49%/females 47% of control]. Food consumption for both sexes at the high-dose level was decreased throughout the study [males 67%-76%/females 65%-74% of control], which may have resulted from a palatability problem and/or due to slight anorexia secondary to toxicity.

Both sexes at the high-dose level displayed decreased red blood cell, hemoglobin, hematocrit, white blood cell, and platelet values at study termination. Females at the next highest dose level also displayed decreased RBC and platelet values at study termination. Several of the clinical chemistry parameters [increased AP activity, total cholesterol, triglycerides, albumin, AST/ALT activity, and BUN; decreased glucose, total protein, globulin, and calcium concentrations] were altered mainly at the high-dose level in one or both sexes, but many of these changes can be attributed to the severe debilitation of both sexes as evidenced by the lack of food consumption and decreased body weight. At the next highest dose level, increased ALT/AST activity, albumin, and triglycerides values were

observed mainly in the females. Changes in urinalysis were limited to decreased specific gravity at the high-dose level. Males at the high-dose level displayed a slightly elevated T3 concentration [128% of control] and a decrease in T4 concentrations [13% of control]. Females at the two highest dose levels displayed comparable decreases in T4 concentrations [17% of control], and the high-dose females displayed elevated T3 values [159% of control] compared to the control.

Increased thyroid and liver weights were observed at the high-dose level in both sexes, and decreased absolute and relative testes and ovarian weights were observed at the high-dose level in the respective sexes. Decreased absolute and increased relative adrenal, brain, kidney, and heart weights were observed in both sexes at the high-dose level also. Histopathological lesions were observed in the eye [cataracts and retinal degeneration], liver [increased size of centrilobular and midzonal hepatocytes with altered tinctorial properties], kidney [degeneration of proximal tubules], adrenals [hypertrophy of zona glomerulosa], bone marrow [decreased hematopoiesis], lung [alveolar histiocytosis], and spleen and thymus [atrophy] at the high-dose level in both sexes. Thyroid lesions [hypertrophy of the follicular epithelial cells] were observed in all high-dose males [very slight] and all high-dose [slight] females. Testicular degeneration was moderate to severe and was characterized by an absence of spermatozoa, variable decreases in the number of spermatids, spermatocytes, and spermatogonia and the presence of occasional multinucleated spermatids. Several additional organ weight and microscopic alterations were observed at the high-dose level that are considered secondary and associated with decreased body weight and poor nutrition possibly due to the unpalatability of the feed, the debilitated condition of the rats, and general toxicity involving multiple organs. At the next highest dose level, histopathological lesions were observed in the kidneys [degeneration of proximal tubules in both male (1/10) and female (8/10) rats], adrenals [hypertrophy of zona glomerulosa in females (7/10)], and eye [retinal degeneration in 1/10 females].

The NOAEL is 28 mg/kg/day, and the LOAEL is 187 mg/kg/day, based on alterations in some hematological and clinical chemistry parameters and histopathological lesions in the adrenal [females], kidney [both sexes], and eye [females]. At the highest dose level [560 mg/kg/day], decreased body-weight gain, alterations in some hematological, clinical chemistry, and urinalysis parameters, increased liver weights, decreased testes and ovarian weights, and histopathological lesions in the kidneys, adrenal, thyroid, adrenal, testes [males], lung, spleen, thymus, bone marrow, and eyes were observed in both sexes.

This subchronic oral toxicity study is classified **Acceptable/Guideline**, and the study satisfies the guideline requirement [OPPTS 870.3100; §82-1] for a subchronic oral toxicity study in the rodent.

[2,4-D TRIISOPROPANOLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Table 1. Hematology Parameters

Dose/Sex/Parameter	0 mg/kg/day	2 mg/kg/day	28 mg/kg/day	187 mg/kg/day	560 mg/kg/day
MALES					
RBC [x10^6/mm^3]	8.52±0.35	8.56±0.29	8.81±0.52	8.54±0.20	6.96±0.34* [82]
HGB [g/dL]	16.2±0.9	16.8±0.6	17.0±1.4	16.6±1.2	15.6±0.9
HCT [%]	47.4±2.2	47.8±1.8	49.2±2.8	47.5±1.3	39.6±2.5* [84]
platelets [x10^3/mm^3]	904±59	888±58	912±67	863±30	579±91* [64]
WBC [x10^3/mm^3]	10.3±1.3	11.5±0.8	11.3±1.4	12.6±1.8* [122]√	5.0±1.2* [49]
FEMALES				(9)	
RBC [x10^6/mm^3]	7.92±0.30	7.93±0.19	7.96±0.23	7.62±0.22* [96]	7.01±0.20* [89]
HGB [mg/dL]	16.8±0.6	16.8±0.7	16.8±0.5	16.4±0.6	15.7±0.6* [93]
HCT [%]	48.0±1.8	48.2±1.4	48.2±1.4	46.3±1.4	40.7±2.2* [85]
platelets [x10^3/mm^3]	945±86	985±68	974±61	763±30* [81]	588±28* [62]
WBC [x10^3/mm^3]	6.9±1.5	7.0±1.9	7.6±1.2	7.4±2.0	3.8±0.9* [55]

Data from Tables 15&17, pages 70-72 of the report; n=10 unless (); * p<0.05

Table 2. Clinical Chemistry Parameters

Dose/Sex/Parameter	0 mg/kg/day	2 mg/kg/day	28 mg/kg/day	187 mg/kg/day	560 mg/kg/day
MALES					
BUN [mg/dL]	17±1	17±2	17±2	18±1	18±1
ALT [U/L]	39±3	39±3	44±7	51±5* [131]√	41±8
alkaline phosphatase [U/L]	150±9	152±10	152±17	148±11	157±16
AST [U/L]	60±5	60±5	67±8	71±9* [118]	69±7* [115]
glucose [mg/dL]	125±17	122±11	123±16	121±29	104±10* [83]
total protein [g/dL]	6.9±0.3	6.8±0.3	6.9±0.3	6.7±0.3	6.2±0.3* [90]
albumin [g/dL]	3.5±0.1	3.5±0.1	3.5±0.1	3.4±0.1	3.6±0.2
globulin [g/dL]	3.4±0.2	3.4±0.2	3.3±0.3	3.3±0.3	2.6±0.3* [76]
cholesterol [mg/dL]	55±6	51±4	54±5	48±5* [87]	77±9* [140]
triglycerides [mg/dL]	105±30	119±23	120±28	115±29	87±8 [83]
creatinine [mg/dL]	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.1	0.6±0.1
calcium [μg/L]	9.6±0.3	9.9±0.3	10.0±0.5	9.5±0.5	9.0±0.4* [94]
FEMALES				(9)	
BUN [mg/dL]	16±1	17±1	16±1	18±2	22±4* [138]
ALT [U/L]	36±6	39±8	39±5	58±9* [161]	47±14* [131]
alkaline phosphatase [U/L]	105±7	107±13	103±10	106±14	173±23* [165]
AST [U/L]	66±13	64±9	67±12	95±15	93±23* 141]
glucose [mg/dL]	96±7	99±7	97±9	88±6	82±11* [85]
total protein [g/dL]	6.6±0.3	6.6±0.2	6.6±0.2	6.6±0.3	6.1±0.3* [92]
albumin [g/dL]	3.4±0.1	3.3±0.1	3.4±0.1	3.6±0.1* [106]	3.6±0.1* [106]
globulin [g/dL]	3.2±0.2	3.3±0.1	3.2±0.2	3.0±0.2	2.5±0.2* [78]
cholesterol [mg/dL]	84±6	89±7	84±8	61±7* [73]	92±11
triglycerides [mg/dL]	45±8	45±6	47±9	54±5* [120]	89±9* [198]
creatinine [mg/dL]	0.6±0.0	0.6±0.0	0.6±0.0	0.7±0.0* [117]	0.6±0.1
calcium [μg/L]	9.8±0.2	10.1±0.1* [103]	9.9±0.2	9.9±0.2	9.5±0.3* [97]

Data from Tables 23-26, pages 80-85 of the report; n=10 unless (); * p<0.05

[2,4-D TRIISOPROPANOLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Table 3. Thyroid Parameters

Dose/Sex/Parameter	0 mg/kg/day	2 mg/kg/day	28 mg/kg/day	187 mg/kg/day	560 mg/kg/day
MALES					
thyroid weight					
grams	0.020±0.006	0.020±0.003	0.022±0.004	0.022±0.004	0.027±0.004* [135]
g/100 g	0.0073±0.0020	0.0072±0.0009	0.0080±0.0014	0.0082±0.0013	0.0157±0.0024* [215]
T3 [ng/mL]	110±26	113±23	118±25	111±27	141±34 [128]
T4 [μg/dL]	4.8±1.5	4.7±1.0	4.8±0.9	3.7±0.9 [77]√	0.6±0.5* [13]
FEMALES				(9)	
thyroid weight					
grams	0.015±0.002	0.015±0.002	0.016±0.004	0.018±0.003	0.022±0.0041* [147]
g/100 g	0.0097±0.0014	0.0093±0.0013	0.097±0.0027	0.0116±0.0025	0.0201*±0.0042 [207]
T3 [ng/mL]	107±20	115±16	105±9	83±25 [78]	170±42* [159]
T4 [μg/dL]	2.9±0.8	2.7±0.7	2.7±0.3	0.5±0.3* [17]	0.5±0.2 [17]

√ [% of control]; n=10, unless (); * $p<0.05$; data from Tables 23, 25, 27, 28, pages 81, 84, 87, 89 of the report

[2,4-D TRIISOPROPANOLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Table 4. Organ-Weight Data

Dose/Sex/Parameter	0 mg/kg/day	2 mg/kg/day	28 mg/kg/day	187 mg/kg/day	560 mg/kg/day
MALES					
ADRENAL					
grams	0.048±0.004	0.047±0.005	0.046±0.004	0.046±0.004	0.034±0.005* [71]
g/100 g	0.0179±0.0012	0.0172±0.0018	0.0173±0.0017	0.0172±0.0012	0.0193±0.0034
BRAIN					
grams	1.855±0.021	1.867±0.036	1.842±0.049	1.858±0.050	1.656±0.057* [89]
g/100 g	0.692±0.027	0.689±0.036	0.692±0.036	0.703±0.038	0.955±0.111* [138]
HEART					
grams	0.784±0.048	0.789±0.053	0.794±0.050	0.753±0.069	0.634±0.054* [81]
g/100 g	0.292±0.016	0.291±0.015	0.299±0.030	0.284±0.022	0.364±0.029* [125]
KIDNEYS					
grams	1.799±0.073	1.822±0.074	1.858±0.077	1.975±0.081* [110]	1.449±0.165* [81]
g/100 g	0.670±0.018	0.672±0.030	0.698±0.028	0.746±0.026* [111]	0.827±0.039* [123]
LIVER					
grams	7.625±0.414	7.766±0.484	7.648±0.465	7.468±0.472	8.676±0.730* [114]
g/100 g	2.839±0.092	2.860±0.061	2.868±0.059	2.818±0.053	4.970±0.338* [175]
TESTES					
grams	2.987±0.075	3.090±0.201	3.030±0.159	2.959±0.084	0.944±0.056* [32]
g/100 g	1.114±0.047	1.141±0.094	1.138±0.057	1.119±0.054	0.544±0.068* [49]
FEMALES					
ADRENAL					
grams	0.048±0.003	0.049±0.003	0.050±0.003	0.045±0.003	0.025±0.004* [52]
g/100 g	0.0306±0.0022	0.0298±0.0017	0.0308±0.0028	0.0294±0.0020	0.0234±0.0012* [76]
BRAIN					
grams	1.726±0.025	1.738±0.035	1.765±0.027* [102]	1.730±0.017	1.534±0.042* [89]
g/100 g	1.094±0.057	1.060±0.038	1.083±0.055	1.135±0.052	1.436±0.171* [131]
HEART					
grams	0.533±0.034	0.529±0.030	0.540±0.020	0.500±0.021	0.469±0.046* [88]
g/100 g	0.337±0.016	0.323±0.017	0.331±0.018	0.328±0.014	0.435±0.033* [129]
KIDNEYS					
grams	1.157±0.054	1.162±0.057	1.176±0.055	1.189±0.055	1.024±0.118* [89]
g/100 g	0.732±0.026	0.708±0.032	0.721±0.032	0.779±0.026* [106]	0.948±0.042* [130]
LIVER					
grams	4.222±0.253	4.384±0.231	4.396±0.143	4.310±0.181	5.868±0.434* [139]
g/100 g	2.671±0.089	2.672±0.083	2.692±0.062	2.824±0.059* [106]	5.467±0.504* [205]
OVARIES					
grams	0.053±0.005	0.056±0.005	0.055±0.004	0.050±0.004	0.020±0.004* [38]
g/100 g	0.033±0.003	0.034±0.004	0.034±0.002	0.033±0.003	0.019±0.003* [58]
Terminal fasted body weight [g]				(9)	
males	268.5±9.5	271.5±14.7	266.8±16.6	264.9±13.2	175.9±23.9* [66]
females	158.1±7.6	164.1±6.4	163.4±7.1	152.6±6.2	108.4±14.9* [69]

Data from Tables 27-28, pages 86&88 of the report; n=10 unless (); √ [% of control]; * p<0.05

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DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding-Rodent **GUIDELINE:** 82-1(a)

TOX.CHEM. No.: 315-AE MRID No.: 420214-02

HED Project No. 2-0320 Registrant: DowElanco.

TEST MATERIAL: Triisopropanolamine Salt of 2,4-D **(2,4-D TIPA)**

TESTING LABORATORY: Toxicology Research Lab, Dow Chem. Co.

STUDY IDENTIFICATION: K-008866-006

TITLE OF REPORT: 2,4-D Triisopropanolamine Salt [2,4-D TIPA]: A 13-Week Dietary Toxicity Study in Fischer 344 rats.

REPORT AUTHORS: B. L. Yano, P.F. Cosse, L. Atkin and R.A. Corley

STUDY COMPLETION DATE: September 4, 1991.

SUMMARY: In groups of 10 male and 10 female Fischer-344 rats fed diets containing the Triisopropanolamine salt of 2,4-Dichlorophenoxy-acetic acid at 0, 2, 28, 187, or 560 mg/kg/day for 13 weeks, no treatment-related effects were seen at 2 or 28 mg/kg/day. Treatment-related effects observed at 187 mg/kg/day were: decreases in mean body weight gain; alterations in hematology, clinical chemistry and urinalysis; changes in organ weights; and mild histopathologic changes in the liver, kidneys and adrenal glands.

Primary treatment-related effects observed at 560 mg/kg/day included: decreases in mean body weight and body weight gain and food consumption; alterations in hematology, clinical chemistry and urinalysis; changes in organ weights, gross pathology; and histopathological changes in the eyes, kidneys, liver and thyroids of male and female rats. Effects secondary to decreased weight gain, the debilitated condition of the rats, and/or toxicity in other organs occurred in the adrenals, bone marrow, fat [mesenteric], lungs, spleen, thymus, and testes.

Under the conditions of this study, a NOEL of 28 mg/kg/day and a LOEL of 187 mg/kg/day is established for the 90-day oral toxicity of the Triisopropanolamine Salt of 2,4-Dichlorophenoxyacetic acid to male and female rats.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the subchronic toxicity of the **Triisopropanolamine Salt of 2,4-Dichlorophenoxyacetic acid [2,4-D TIPA]** following dietary administration to rats.

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: Triisopropanolamine salt of 2,4-D.
Purity: 72.2% TIPA and 38.7% 2,4-D acid.
Lot No.: AGR 276428
Description: Aqueous amber liquid.
Vehicle: Acetone

2. Test Animals

Species: Rats
Strain: Fischer-344
Sex: Males and females
Weight at Initiation: 70 - 83 g (M); 60 - 75 g (F)
Identification: Ear tags.
Acclimation: Minimum of seven days
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Chow #5002.
Water: Tap water ad libitum
Environment: Temperature and humidity controlled rooms with a photocycle regulated for rats.

3. Study Design

Group No.	Treatment	No. of Animals		Dose level [mg/kg/day]	Acid Equivalent [mg/kg/day]
		Males	Females		
1	Control	10	10	0	0
2	Low	10	10	2	1
3	Mid-1	10	10	28	15
4	Mid-2	10	10	187	100
5	High	10	10	560	300

4. Test Article Formulation and Analyses

a. Formulation: Test material was dissolved in a relatively small amount of acetone to facilitate its uniform dispersion in the diet. The test diets were prepared weekly by serial dilution of the high-dose to attain the lower doses. Initial concentrations of test material in the diet were calculated from the pretest body weights and feed consumption data. Thereafter, the most recent body weight and food consumption data were used to adjust the concentration of the test material in the diet to maintain the dose levels on the targeted mg/kg/day basis. Reference samples [one/dose/sex/mix plus premix] were retained and stored at ambient temperature.

b. Analysis: Difficulties were encountered in analyzing the diets for 2,4-D TIPA due to the complete disassociation of the test material to the conjugate base [2,4-D] and ammonium ion [TIPA] moieties in aqueous solution. Therefore, verification of the stability of 2,4-D TIPA in test diets were conducted in two separate analyses based on the stability of the 2,4-D acid and TIPA moieties for at least 21 days. Homogeneity and concentration analysis based on analysis of 2,4-D acid were performed at the start of the study and monthly thereafter.

5. Treatment

Rats were fed the control and test diets 7 days per week for at least 13 weeks. The oral route of administration was chosen because it is the route of potential human exposure.

6. Experimental Procedures

Mortality and moribundity checks were performed once daily. Body weights and food consumption were measured prior to initiation and once weekly. A complete physical examination was performed weekly. Ophthalmologic examinations were conducted prior to initiation [Day - 5] and at termination [Day-90]. A functional observation battery [FOB] was conducted on all surviving rats on Day 90. Blood and urine were collected from all animals at termination for hematology, clinical chemistry and urinalysis. 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]**
x Mean corpuscular HGB (MCH)	**x Mean corpuscular HGB Concentration (MCHC)**
x Mean corpuscular volume (MCV)	Blood clotting measurements
Corrected leukocyte count (COR WBC)	**x Cell morphology**

Clinical Chemistry

Electrolytes:	Other
x Calcium[a] **x Chloride[a]** Magnesium[a] **x Phosphorus[a]** **x Potassium[a]** **x Sodium**	**x Albumin[a]** **x Blood creatinine[a]** **x Blood urea nitrogen[a]** **x Cholesterol[a]** **x Globulins** **x Glucose[a]** **x Total bilirubin[a]** **x Total serum protein[a]** **x Triglycerides** Serum protein electrophoresis **x Triiodothyronine [T_3]** **x Thyroxine [T_4]** x A/G Ratio
Enzymes:	
x Alkaline phosphatase **x Alanine aminotransferase (SGPT)[a]** **x Aspartate aminotransferase (SGOT)[a]** Cholinesterase[b] **x Creatinine phosphatase[a]** Lactic acid dehydrogenase Gama glutamyl transferase	

Urinalysis

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

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

7. Termination

After 13 weeks of treatment, all surviving animals were weighed, anesthetized with sodium pentobarbital and exsanguinated. Necropsies were performed on each animal, all gross pathological changes were recorded, and the following organs were weighed.

Adrenals	Brain	Heart	Kidneys
Liver	Ovaries	Thyroid	Testes

8. Histopathology

The checked (X) tissues from all animals in the control and high-dose groups were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Salivary glands[a]	x Trachea[a]
x Esophagus[a]	x Lung[a]
x Duodenum[a]	Pharynx[e]
x Jejunum[a]	Larynx[e]
x Cecum[a]	Nose[e]
x Colon[a]	
x Rectum[a]	Cardiovascular/Hemo.System
x Liver[ac]	
x Pancreas[a]	x Aorta (thoracic)[a]
Gall bladder[ab]	x Heart[a]
	x Bone marrow[a]
Neurological System	x Lymph nodes[a]
	x Spleen[a]
x Brain[ac]	x Thymus[a]
x Pituitary[a]	
x Peripheral nerve[ac]	Urinogenital System
x Spinal cord (3 levels)[ac]	
x Eyes (optical nerve)[ac]	x Kidneys[ac]
	x Urinary bladder[a]
Glandular System	x Testes[ac]
	x Epididymides
x Adrenals[a]	x Uterus[a]
Lacrimal glands[c]	x Ovaries[ac]
x Parathyroids[ad]	Others
x Thyroids[ad]	
	x All gross lesions and masses
	x Skeletal muscle[a]

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.

686

In addition, the liver, kidneys, lung, thyroid, adrenal, thymus, mesenteric lymph node, eyes, bone marrow with bone, cecum, colon, mesenteric tissue, spleen, testes, ovaries and all gross lesions from the lower dose groups also underwent histopathological examination.

9. Statistical Analyses

Only means and standard deviations were reported for food consumption, WBC differential counts, RBC indices and reticulocyte counts. Bartlett's test for equality of variances was employed for body weights, organ weights, clinical chemistry data, appropriate hematology data and urinary specific gravity. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric analysis of variance [ANOVA], followed respectively by Dunnnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of The Toxicology Research Laboratory, and the Study Protocol. A quality assurance statement was signed and dated August, 28, 1991.

III. RESULTS

1. Analysis of Diet Mix

Concentrations of test diets, based on analysis of 2,4-D indicated that the diets were accurately prepared throughout the study. The mean assay values were 116%, 115%, 98% and 104% respectively, of the nominal concentrations for males and 116%, 119 %, 104% and 102% respectively, of the nominal concentrations for females at 2, 28, 187 and 560 mg/kg/day diets.

Homogeneity of the test diets, based on analysis of 2,4-D indicated that diets formulated to deliver 112 or 187 mg/kg/day were homogenous. The mean values [% of target] were 89% and 47% respectively for the 112 and 187 mg/kg/day diets.

Stability analyses for the acid moiety indicated that the test material was stable with the recovery of 90.38 ± 1.30% and 110.88 ± 2.28% acid and amine moieties, respectively after 21 days in separate experiments using ^{14}C-labelled 2,4-D acid and ^{14}C-labeled TIPA.

2. Survival

All rats survived the study.

3. Clinical Observations

Treatment-related clinical signs of toxicity were limited to unilateral or bilateral opaque eyes noted in 8 males and 10 females at 560 mg/kg/day.

4. Functional Observational Battery:

Except for opaque eyes [lens] identical to those noted on in males and females at 560 mg/kg/day during ophthalmic examination [see below], no other treatment-related effects were seen in either sex of rats at any dose levels.

5. Ophthalmology Examination

Bilateral lens opacities that were considered to be cataracts were seen in 8 males and 10 females at 560 mg/kg/day. The differences in the incidences of corneal opacities between the treated and control group showed no dose response.

6. Body Weights and Body Weight Changes

Mean body weight data are summarized in Tables 1 and 2 for males and females, respectively. Statistical [p<0.05] differences were seen in:

- o Males at 560 mg/kg/day from Weeks 2 - 13,
- o Females at 560 mg/kg/day from Weeks 1 - 13;
- o Females at 187 mg/kg/day from Weeks 3 - 6.

Mean body weights of males and females at 2 and 28, and in males at 187 mg/kg/day were comparable to that of the control rats.

Table 1. Mean Body Weights and S.D (G) for Male Rats.

Dose Level [mg/kg/day]	Week: 0	Week: 6	Week: 10	Week: 13[a]
0	103.0 ± 4.8	230.2 ± 15.6	280.0± 11.9	291.7± 10.6
2.0	102.8 ± 5.5	231.6 ± 13.4	279.9± 14.5	295.6±15.8
28.0	103.0± 5.5	227.2 ± 15.9	276.1± 16.7	290.9±17.9
187.0	103.2 ± 6.0	227.5 ± 9.6	274.7± 11.8	288.5±15.3
560.0	98.7 ± 5.0	**172.2* ±13.5**	**191.4* ±21.1**	**191.4* ±25.2**

* Significantly different from control value at p ≤ 0.05.

Table 2. Mean Body Weights and S.D (G) for Female Rats.

Dose Level [mg/kg/day]	Week: 0	Week: 6	Week: 10	Week: 13
0	87.9 ± 2.2	145.7 ± 6.5	166.2 ± 8.4	173.9 ± 9.2
2.0	89.6 ± 4.6	148.4 ± 6.5	172.0 ± 7.3	180.8 ± 6.6
28.0	87.3 ± 4.0	149.2 ± 7.3	171.1 ± 6.4	179.4 ±6.6
187.0	85.7 ± 3.1	**137.0* ± 5.6**	162.1 ± 7.8	169.6 ± 8.3
560.0	80.3 ± 3.7	**115.6* ± 9.9**	**123.9* ±14.4**	**120.7* ± 16.0**

* Significantly different from control value at p ≤ 0.01

Mean body weight gain of male and female rats at 2, 28, and 187 mg/kg/day were comparable to those of the respective controls. Mean body weight gain was lower for both sexes of rats at 560 mg/kg/day as shown in Tables 3 and 4.

Table 3. Mean Body Weight Change (G) in Male Rats.

Dose Level [mg/kg/day]	Weeks: 0 - 4	Weeks: 0 - 8	Weeks: 0 - 13
0	92 ± 11	149 ± 9	189 ± 8
2.0	93 ± 13	150 ± 11	193 ± 13
28.0	87 ± 14	148 ± 11	188 ± 14
187	89 ± 8	146 ± 12	185 ± 15
560	**55 ± 6**	**85 ± 15**	**93 ± 23**

Table 4. Mean Body Weight Change (G) in Female Rats.

Dose Level [mg/kg/day]	Weeks: 0 - 4	Weeks: 0 - 8	Weeks: 0 - 13
0	44 ± 4	66 ± 7	86 ± 9
2	45 ± 3	69 ± 3	91 ± 4
28	47 ± 4	72 ± 4	92 ± 5
187	38 ± 3	64 ± 9	83 ± 6
560	**28 ± 5**	**38 ± 11**	**40 ± 16**

7. Food Consumption

No differences were seen in feed consumption of males and females at 2,28, or 187 mg/kg/day. Male and female rats at 560 mg/kg/day exhbited a 30% reduction in mean absolute [g//rat/day] food consumption values when compared to controls. The decrease in food consumption was related to the reduced body weights observed in these rats. The decrease in feed consumption may have resulted from unpalatability of the feed and/or due to slight anorexia secondary to toxicity.

8. Hematology and Clinical Chemistry

Treatment-related, statistically significant [$p < 0.05$] changes observed in hematology included decreases in red blood cell and platelet counts in females at 187 mg/kg/day, and decreases in red blood cell, white blood cell and platelet counts and decreases hemoglobin and hematocrit concentrations in male and females at 560 mg/kg/day. Alterations noted in the other hematological parameters were not considered to be treatment related since they were within normal limits and/or showed no dose response relationship.

Changes in observed in clinical chemistry parameters are summarized below:

Dose Level [mg/kg/day] Sex	Parameter Affected	Effect
187 / ♂	AST	Increased
187 / ♀	ALT	Increased
	AST	Increased
	Albumin	Increased
	Triglycerides	Increased
	T_4	Decreased
560 / ♂	AST	Increased
	Tot.Prot.	Decreased
	Globulin	Decreased
	Glucose	Decreased
	Cholesterol	Increased
	Calcium	Decreased
	T_4	Decreased
560 / ♀	BUN	Increased
	ALT	Increased
	AST	Increased
	AP	Increased
	Tot.Prot.	Decreased
	Albumin	Increased
	Globulin	Decreased
	Glucose	Decreased
	Triglycerides	Increased
	Calcium	Decreased
	T_4	Decreased
	T_3	Increased

9. Urinalysis

Except for the statistically significant [p <0.05], dose-related decreases in urine specific gravity in males and females at 187 and 560 mg/kg/day, urinalysis revealed no other treatment related changes.

10. Organ Weights

Statistically significant [p < 0.05] differences observed in the absolute and relative organ weight are summarized below:

Absolute Organ Weights			
Organ	**Dose Level [mg/kg/day]**	**Sex**	**Effect**
Adrenal	560	M & F	Decreased
Brain	560	M & F	Decreased
Heart	560	M & F	Decreased
Kidneys	187 560	M M & F	Increased Decreased
Liver	560	M & F	Increased
Thyroid	560	M & F	Increased
Testes	560	M	Decreased
Ovaries	560	F	Decreased

Relative Organ Weights			
Organ	**Dose Level [mg/kg/day]**	**Sex**	**Effect**
Adrenal	560	F	Decreased
Brain	560	M & F	Increased
Heart	560	M & F	Increased
Kidneys	**187 & 560**	**M & F**	**Increased**
Liver	560	M & F	Increased
Thyroid	560	M & F	Increased
Testes	560	M	Decreased
Ovaries	560	F	Decreased

11. Gross Pathology

No treatment-related gross pathological changes were seen in either sexes at 2, 28, or 187 mg/kg/day. At 560 mg/kg/day, gross pathological changes included: lenticular opacity of the eyes [9 ♂ and 10 ♀]; accentuated lobular pattern of the liver [10 ♂ and 10 ♀]; increased liver size [2 ♂ and 7 ♀]; pale foci in the lungs [10 ♂ and 10 ♀]; dark foci in the glandular mucosa of the stomach [2 ♂ and 3 ♀]; hemolyzed blood in the lumen of the stomach [2 ♂ and 2 ♀]; increased cecal size [4 ♂ and 6 ♀]; decreased amount of general fat [10 ♂ 10 ♀]; increased thyroid gland size [3 ♂]; and decreased testicular size [10 ♂]. Other gross necropsy findings were considered incidental in nature.

12. Histopathology

No treatment-related histopathological changes were seen in male or female rats at 2 or 28 mg/kg/day. Treatment-related histopathological changes observed at 187 and 560 mg/kg/day are tabulated in Tables 5 and 6 and are summarized below:

Dose Level [mg/kg/day]	Sex	Organs with Lesions
187	M	Kidneys and Liver
	F	Adrenal, Fat [mesenteric], and Kidneys
560	M & F	Adrenals, Bone marrow, Eyes, Fat [mesenteric], Kidneys, Liver, Lungs, Lymph node [♀], Spleen, Thymus, Thyroid, and Testes [♂]

Adrenal glands: Cells of the adrenal zona gomerulosa were very slightly to slightly increased in size [hypertrophy] with the cytoplasm being more vacuolated than the controls. No microscopic lesions which corresponded with the decreased absolute and relative weights were seen.

Bone marrow: The cellularity of erythroid, myeloid and megakaryocyte cell lines in the bone marrow was slightly decreased and fat spaces were more readily observed.

Eyes: Cataracts were mostly bilateral and diffused. Retinal degeneration was almost exclusively bilateral, diffuse, and varied from slight to severe in degree and was characterized by complete or partial loss of the rod and cone layer and the outer nuclear layer.

Kidneys: Degenerative kidney effects were very slight to slight and were characterized by a loss of the brush border of the straight descending portion of the proximal tubules, increased vacuolation of epithelial cell cytoplasm and were occasionally accompanied by focal tubular basement membrane thickening, fibrosis and individual cell atrophy.

Liver: Lesions were characterized by an increase in the size and altered tinctorial properties [increased cytoplasmic eosinophilia] of hepatocytes in the centrilobular and midzonal regions of the hepatic lobule. This effect was not accompanied by hepatocellular degeneration or necrosis.

Lungs: Very slight to slight increases in numbers of subpleural alveolar macrophages were seen.

Spleen: Generalized lymphoid atrophy was seen along with decreases in the populations of T- and B-lymphocytes. Splenic red pulp was also reduced in amount.

Thymus: Generalized lymphoid atrophy.

Thyroid: Epithelial cells lining thyroid follicles were very slightly to slightly increased in size [hypertrophy-column versus cuboidal shape] with a vacuolated cytoplasm. These alterations were most likely the result of a compensatory response due to decreased serum T_4 levels.

Testes: Testicular degeneration was moderate to severe and characterized by an absence of spermatozoa, variable decreases in the number of spermatids, spermatocytes and spermatogonia and presence of occasional multinucleated spermatids.

Other microscopical changes were considered incidental and unrelated to treatment.

Table 5. Histopathological Findings in Male Rats.

Organ / Lesion	Dose Level [mg/kg/day]				
No.of animals= 10 / dose levels	0	2	28	187	560
Adrenals					
-Hypertrophy, Zona Glomerulosa	0	0	0	0	9
Bone Marrow					
-Hematopoiesis, decreased	0	0	0	0	9
Eye					
- Retinal degeneration	0	0	0	0	2
- Cataract,lens, bilateral	0	0	0	0	7
- Cataract,lens, unilateral	0	0	0	0	1
Kidney					
-Degeneration, Proximal Tubules	0	0	0	1	10
Liver, Centrilobular and mid zonal					
-Increased size of hepatocytes with Altered Tinctorial Properties	0	0	0	0	10
-Altered Tinctorial Properties with increased eosinophilla.	0	0	0	2	0
-Necrosis, very slight	0	0	0	1	0
Lung					
-Alveolar Histosis,	0	0	0	0	10
Mesenteric Tissue					
-Adipose tissue, Atrophy	0	0	0	0	10
Spleen					
-Atrophy	0	0	0	0	3
Testes					
-Degeneration, Tubules, Bilateral	0	0	0	0	10
Thymus					
-Atrophy	0	0	0	0	4
Thyroid					
-Hypertrophy, Epithelial cells	0	0	0	0	10

Table 6. Histopathological Findings in Female Rats.

Organ / Lesion	Dose Level [mg/kg/day]				
No.of animals= 10 / dose levels	0	2	28	187	560
Adrenals					
-Hypertrophy, Zona Glomerulosa	0	0	0	7	10
Bone Marrow					
-Hematopoiesis, decreased	0	0	0	0	9
Eye					
-Retinal degeneration	0	0	0	1	7
-Cataract, bilateral	0	0	0	0	9
-Cataract, unilateral	0	0	0	0	1
Kidney					
-Degeneration, Proximal Tubules	0	0	0	8	10
Liver, Centrilobular and midzonal					
-Increased size of hepatocytes with Altered Tinctorial Properties	0	0	0	0	10
Lung					
-Alveolar Histosis	0	0	0	0	10
Lymph Node Mesenteric					
-Atrophy	0	0	0	0	10
Mesenteric Tissue					
-Adipose tissue, Atrophy	1	1	0	9	10
Spleen					
-Atrophy	0	0	0	0	8
Thymus					
-Atrophy	0	0	0	0	9
Thyroid					
-Hypertrophy, Epithelial cells	0	0	0	0	10

IV. DISCUSSION

Male and female Fischer-344 rats were fed diets containing the Triisopropanolamine salt of 2,4-D at concentrations of 0, 2, 28, 187, or 560 mg/kg/day for 13 weeks.

Analytical data showed that the diet mixes were homogeneous, stable at room temperature for up to 21 days. The concentrations of test material in the diet approximated the targeted concentrations with the mean concentrations of target levels being between 98 to 116%.

No mortality occurred during the study. **No treatment-related effects were seen at 2 or 28 mg/kg/day** on mean body weight, mean body weight gain, mean food consumption, ophthalmology, hematology, clinical chemistry, organ weights, gross pathology or histopathology.

Treatment-related effects observed at 187 were: slight but statistically significant decreases in mean body weight [females]; minor alterations in hematology [decreases in RBC and platelet counts in females] and clinical chemistry parameters [increases in ALT activity, AST activity, albumin and triglycerides, and decreased T_4 levels in females]; decrease in urine specific gravity [both sexes]; changes in organ weights [increase in relative kidney weights in both sexes] and mild histopathologic changes in the liver and kidneys of males and in the kidneys and adrenal glands of females.

Primary treatment-related effects observed at 560 mg/kg/day included: decreases in mean body weight and body weight gain; a 30% reduction in food consumption; alterations in hematology, clinical chemistry, and urinalysis; changes in organ weights, gross pathology; and histopathological changes in several organs of both sexes of rats.

Treatment-related alterations in hematology parameters were limited to decreases in RBC, WBC, HCT and platelet counts and decreases in hemoglobin and hematocrit concentrations in both sexes which correlated with the decreased bone marrow cellularity observed in animals at this dose.

Treatment-related alterations in clinical chemistry parameters were decreases in Total protein, Glucose, Globulin, calcium, and T_4, and increases in AST activity and Cholesterol in males, and decreases in Total protein, Globulin, Glucose, Calcium and T_4, and increases in BUN, AP, ALT, AST, Triglycerides, Albumin, and T_3 in females. These changes correlated with histopathological lesions in the kidneys, liver, and thyroids. The remaining differences in clinical chemistry values were considered to be non treatment related and were attributed to the influence of body weight loss and the debilitated condition of the rats.

Abnormalities in urine were limited decreased specific gravity in both sexes.

Differences seen in the relative weights of kidneys, liver and thyroid were considered to be treatment related due to collaborative clinical chemistry alterations and/or histopathological changes in these tissues. Differences seen in other organ weights were attributed to effects secondary to decreased body weights and the debilitated condition of the rat at the high dose.

Although, histopathology revealed alterations in several organs such as the adrenals, bone marrow, eyes, kidneys, liver, lung, mesenteric fat, spleen, thyroid, thymus, and testes, only the changes seen in the eyes, liver, kidneys, and thyroid glands were considered to be clearly treatment-related. Alterations seen in the other organs were considered to be secondary changes possibly associated with decreased body weight, poor nutrition due to unpalatability of the food, the debilitated condition of the rats, and general toxicity involving multiple organs.

The bilateral cataracts and retinal degeneration observed in both sexes of rats at the high dose in this study were also seen in rats fed diets containing the 2,4-D acid, salts [dimethylamine, diethanolamine, and isopropylamine], and esters [butoxyethyl and 2-ethylhexyl].

The increase in size and altered tinctorial properties of hepatocytes seen in the centrilobular and midzonal regions of the hepatic nodule were not accompanied by hepatocellular degeneration and necrosis and therefore may be attributed to the induction of hepatic peroxisomes by 2,4-D TIPA. Similar liver lesions were also seen in rats fed 2,4-D IPA.

The elevated BUN values and the increase in relative kidney weights may be related to the renal alterations in the descending proximal tubules. The hypertrophy and increased vacuolation of the adrenal gland zona glomerulosa was interpreted to be a secondary change possibly associated with the renal alterations since there were no specific microscopic alterations which corresponded with the decrease in absolute and relative organ weights.

Hypertrophy of the follicular epithelial cells of the thyroid glands may be associated with a decrease in serum T_4 and increase in thyroid weights since there was no evidence of any degenerative changes involving the thyroid glands and the colloid appeared normal.

The decreased bone marrow cellularity associated with decreases in RBC, WBC and platelet counts and can be attributed to the interaction of the animals debilitated condition and possible secondary nutritional anemia. Similarly, the generalized lymphoid atrophy of the spleen and thymus, and the testicular degeneration may be attributed to poor nutrition, debilitated conditions, and general toxicity involving multiple organ systems.

The effects seen in this study were parallel to those seen in rats fed diets of 2,4-D acid at similar dose levels [1, 15, 100, or 300 mg/kg/day]. Therefore, it is suggested that the toxicity observed with 2,4-D TIPA is probably the result of the acid moiety and not the triisopropanolamine salt. Similarly, a subchronic toxicity study evaluating the effects of 2,4-D isopropylamine salt at comparable dose levels also demonstrated that the toxicity observed with 2,4-IPA was probably due to the acid moiety.

V. *CONCLUSION*

Under the conditions of this study, a NOEL of 28 mg/kg/day and a LOEL of 187 mg/kg/day is established for the 90-day oral toxicity of the Triisopropylamine Salt of 2,4-Dichlorophenoxyacetic acid to male and female rats. The LOEL is based on decreases in mean body weight gain, alterations in hematology, clinical chemistry and urinalysis, increase in relative kidney weights and histopathological alterations in the kidneys and liver of males, and in the kidneys and adrenal glands of female rats.

VI. **CORE CLASSIFICATION:** Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

Amendment to DER for MRID 43049001 - Chronic Oral Toxicity Study [Dog] [2,4-DICHLOROPHENOXYACETIC ACID]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 011271].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 2/13/03
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. for V. Dobozy 2/14/03
Reregistration Branch I, Health Effects Division (7509C)
TXR# 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: chronic oral toxicity - dog § 83-1(b)/OPPTS 870.4100
P.C. CODE: 030001 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315 CAS NO.: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [96.7%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force II on 2,4-D Research Data

CITATION: Dalgard, D. W. (1993). 52-Week Dietary Toxicity Study of 2,4-D in Dogs. Hazleton Washington, Inc.; Study No. HWA 2184-124, December 2, 1993. MRID 43049001. Document No. 011271. Unpublished.

EXECUTIVE SUMMARY: In a chronic oral toxicity study [MRID 43049001], 5 beagle dogs/sex/group were administered 2,4-dichlorophenoxyacetic acid [96.7%] *via* the diet for 52 weeks at concentrations of 0, 1 mg/kg/day, 5 mg/kg/day, and 7.5 mg/kg/day. The target high-dose level of 10 mg/kg/day was reduced to 7.5 mg/kg/day during week 8, due to loss of body weight/failure to gain weight early in the study.

There were no treatment-related deaths or clinical signs. One female at 5 mg/kg/day was sacrificed on Day 130 in a moribund condition; however, death was attributed to an abdominal inflammatory condition. Decreased body weight was observed throughout the study at the high-dose level in both sexes, with females displaying the greater effect. By week 3, high-dose females displayed a 13% deficit in body weight compared to the control females. At the high-dose level at 8 weeks when the dose level was lowered, both sexes at the high-dose level displayed decreased body weight compared to the controls [males 90%/females 82% of control]. At week 13, the high-dose males were 94% of control and the high-dose females were 82% of control. At study termination, both sexes displayed decreased body weight at the mid- [males 89%/females 87% of control] and high- [males 87%/females 75% of control] dose levels compared to the controls. The low-dose females also displayed a decrease [87% of control] in body weight at study termination. Body-weight gain was decreased throughout the study in females at all dose levels [weeks 1-13 (75%, 70%, and 35% of control); 1-52 (73%, 64%, and 36% of control) at the low-, mid-, and high-dose levels, respectively]. Similarly, males displayed decreased body-weight gains throughout the study (except

the low-dose group during weeks 1-13) compared to the control, although there was no dose-response [weeks 1-13 (100%, 70%, 74% of control); 1-52 (78%, 63%, 67% of control]. Food consumption was decreased throughout most of the study, mainly at the high-dose level [both sexes], but statistical significance was not attained.

Ophthalmology findings were comparable among the groups for both sexes. There were no apparent treatment-related alterations in hematology or urinalysis among the groups for either sex. Elevations in blood urea nitrogen, creatinine, and alanine aminotransferase, and decreased glucose levels [mid-dose males, high-dose both sexes] were observed throughout the study [mid- and high-dose levels, both sexes], although a dose response was not always apparent, especially in the males. Alkaline phosphatase was decreased in both sexes at the high-dose level at week 4. Similar alterations in these clinical chemistry parameters have been observed in subchronic studies in rats and dogs on 2,4-D, and its amine salts and esters.

There was a dose-related decrease in absolute brain weight in females, with statistical significance being attained at the mid- and high-dose levels. Males at the high dose also displayed decreased brain weight, but statistical significance was not attained. In general, the organ-weight effects observed can be attributed to the lower body weight, although the decreased testes weights at the high-dose level and decreased ovarian weights at the mid- and high-dose levels are consistent findings in other studies on 2,4-D and its salts/esters. Microscopically, liver [perivascular, chronic active inflammation (males 1/5, 1/5, 3/5, 4/5; females 0/5, 0/4, 4/5, 3/5 with increasing dose) and sinusoidal lining cell pigment (females; 1/5, 2/4, 5/5, 4/5 with increasing dose)] and kidney [minimal increase in the frequency and average severity of pigment in the tubular epithelium (males 2/5, 4/5, 5/5, 5/5; females 1/5, 1/4, 5/5, 5/5 with increasing dose)] lesions were observed at the mid- and high-dose levels in both sexes. Aspermatogenesis and degeneration were observed in one male at the high-dose level compared to none in the other groups.

The NOAEL is 1 mg/kg/day. The LOAEL of 5 mg/kg/day is based on decreased body-weight gain (both sexes) and food consumption (females), as well as alterations in clinical chemistry parameters [increased BUN, creatinine, and alanine aminotransferase, decreased glucose] in both sexes, decreased brain weight in females, and histopathological lesions in the liver and kidneys.

This subchronic oral toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [OPPTS 870. 3150; §82-1(b)] for a subchronic, non-rodent, oral toxicity study.

Table 1. Body-Weight and Food Consumption Data

Parameter/Week/Sex/Dose	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10/7.5 mg/kg/day
BODY WEIGHT [kg]				
MALES				
1	8.2±0.4	9.0±0.4	8.5±1.3	8.2±0.4
2	8.5±0.5	9.1±0.3	8.6±1.3	8.6±0.3
3	8.9±0.4	9.4±0.4	8.8±1.4	8.6±0.3
4	9.3±0.4	9.6±0.4	9.1±1.6	8.9±0.5 [96]♪
6	9.7±0.3	10.3±0.5	9.7±1.7	9.1±0.4 [94]
8✖	10.3±0.2	10.7±0.4	10.2±1.8	9.3±0.6 [90]
13	10.9±0.3	11.7±0.7	10.4±1.9	10.2±0.9 [94]
26	12.2±0.8	12.5±0.9	11.4±2.4	11.1±1.2 [91]
52	13.1±1.0	12.7±1.2	11.7±2.4	11.5±1.5 [88]
FEMALES				
1	7.8±0.6	7.6±0.5	7.9±0.6	7.3±0.6 [94]
2	8.0±0.5	7.7±0.5	8.0±0.6	7.5±0.6 [94]
3	8.4±0.7	7.7±0.4 [92]	8.2±0.6	7.3±0.9 [87]
4	8.4±0.6	8.0±0.4 [95]	8.4±0.7	7.4±0.9 [88]
6	8.8±0.6	8.2±0.5 [93]	8.7±0.8	7.0±1.4 [80]
8✖	9.1±0.8	8.5±0.4 [93]	9.0±0.8	7.5±0.8 [82]
13	9.8±1.0	9.1±0.5 [93]	9.3±0.9	8.0±0.9 [82]
26	10.6±1.3	9.6±1.0 [91]	9.7±1.0	8.6±0.5]81]
52	11.1±1.9	10.0±0.7 [90]	10.1±0.9 [91]	8.5±0.4 [77]
BODY-WEIGHT GAIN [kg]				
MALES				
1-8✔	2.1	1.7 [81]	1.7 [81]	1.1 [52]
1-13	2.7±0.4	2.7±1.0	1.9±0.8 [70]	2.0±1.0 [74]
1-26	4.1±1.0	3.5±1.2 [85]	2.9±1.4 [71]	2.8±1.4 [68]
1-39	4.4±1.0	3.7±1.2 [84]	3.0±1.2 [68]	3.0±1.3 [68]
1-52	4.9±1.1	3.8±1.4 [78]	3.1±1.2 [63]	3.3±1.5 [67]
FEMALES				
1-8✔	1.3	0.9 [69]	1.1 [85]	0.2 [15]
1-13	2.0±0.4	1.5±0.5 [75]	1.4±0.5 [70]	0.7±0.5* [35]
1-26	2.8±0.8	2.0±0.5 [71]	1.8±0.7 [64]	1.3±0.4* [46]
1-39	3.1±0.8	1.9±0.2* [61]	2.0±0.6* [65]	1.4±0.4 [45]
1-52	3.3±1.3	2.4±0.3 [73]	2.1±0.6 [64]	1.2±0.7* [36]
FOOD CONSUMPTION [g]				
MALES				
1	1969±331	2182±325	1975±316	1880±187 [95]♪
2	1804±97	2056±402	1945±401	1677±250 [93]
3	2133±69	2218±453	2068±320	1843±306 [86]
6	2158±100	2175±501	2117±389	1784±191 [83]
8✖	2176±133	2726±1138	1992±425	2008±356 [92]
13	2299±117	2408±611	1912±626 [83]	2259±444 [98]
51	2524±136	2193±343	2401±438	2095±227 [83]
52	2255±82	2276±334	2203±356	2014±381 [89]
FEMALES				
1	1932±209	1619±162 [84]	1646±117 [85]	1788±228 [93]
2	1702±401	1637±183	1556±207 [91]	1314±349 [77]
3	1770±309	1746±236	1757±99	1405±347 [79]
6	1742±330	1821±234	1727±246	1702±275 [98]
8✖	1855±291	1790±455	1725±117 [93]	1621±211 [87]
13	2174±277	1722±559	1897±296 [87]	1694±275 [78]
51	1899±387	1728±474	1746±169 [92]	1516±337 [80]
52	1983±277	1561±463	1538±114 [78]	1469±198 [74]

✖Week 8, target dose was lowered from 10 to 7.5 mg/kg/day; n=5 [except low-dose females (4); one sacrificed day 130)]; ♪ [% of control];1 ✔calculated by reviewer using mean body-weight data; data from Tables 4A and 4 B [pages 58-66] and Table 5 [pages 68-74] of the report

Table 2. Clinical Pathology Data - MALES

Dose/Week/Parameter	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10/7.5 mg/kg/day
platelets [TH/UL]				
-1	413±37	369±35	446±37	433±58
4	303±74	292±32	292±26	261±81 [86]√
13	333±53	264±15*	295±32	309±76 [90]
26	347±78	266±17	307±55	314±57
52	342±57	273±39	335±48	339±42
alanine aminotransferase [U/L]				
-1	34±11	28±5	33±10	30±4
4	32±8	35±7	97±54* [303]	176±225** [550]
13	38±5	41±3	117±65* [308]	116±83* [270]
26	43±9	46±15	119±60* [277]	152±83* [353]
39	41±9	42±7	111±49* [271]	107±64* [261]
52	43±9	46±7	114±62* [265]	138±98* [321]
aspartate aminotransferase [U/L]				
-1	27±6	26±5	27±6	28±3
4	32±2	31±4	38±7	34±8
13	36±5	31±4	47±11 [131]	37±8
26	36±3	31±3	49±14 [136]	40±11
39	32±2	30±5	50±8* [156]	36±8
52	37±3	33±5	51±10 [138]	42±12
blood urea nitrogen [mg/dL]				
-1	9±3	9±3	10±1	8±2 [88]
4	11±2	14±2	22±6* [200]	24±5* [218]
13	12±2	16±3	25±5* [208]	25±6* [208]
26	12±3	15±2	26±11* [217]	23±4* [192]
39	13±3	16±1	28±10* [215]	22±4* [169]
52	12±2	15±2	26±10* [217]	24±8* [200]
creatinine [mg/dL]				
-1	0.7±0.04	0.6±0.08	0.7±0.04	0.7±0.04
4	0.8±0.04	0.9±0.12	1.2±0.09* [150]	1.2±0.18* [150]
13	0.8±0.04	0.9±0.05	1.2±0.11* [150]	1.2±0.13* [150]
26	0.9±0.05	1.0±0.00	1.4±0.09* [156]	1.3±0.10* [144]
39	0.9±0.04	1.0±0.10	1.5±0.16* [167]	1.3±0.18* [144]
52	1.0±0.04	1.0±0.11	1.5±0.19* [150]	1.4±0.12* [140]
alkaline phosphatase [U/L]				
-1	129±14	127±30	129±28	134±28
4	107±10	99±20	76±16* [71]	60±15* [56]
13	82±11	66±11	61±19 [74]	63±11
26	54±9	42±10	43±12	46±8
39	42±8	34±7	37±8	44±11
52	38±9	30±9	34±2	42±13
glucose [mg/dL]				
-1	114±8	118±7	112±8	116±8
4	101±4	106±4	102±5	94±5
13	100±6	97±9	89±5	91±8
26	104±5	101±7	94±8	93±4* [89]
39	96±1	96±5	85±5* [89]	81±6* [84]
52	94±3	92±9	82±3* [87]	80±3* [85]

Table 2. Clinical Pathology Data - MALES				
Dose/Week/Parameter	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10/7.5 mg/kg/day
cholesterol [mg/dL]				
-1	186±31	168±43	190±23	191±37
4	158±21	169±30	201±18* [127]	193±18
13	153±15	157±22	205±25* [134]	190±26
26	130±19	143±16	194±27* [149]	171±22* [132]
39	126±7	133±17	175±29* [139]	164±15* [130]
52	127±11	129±17	174±37* [137]	167±24* [131]

√[% of control]; n=5; [a] values for dogs were 88, 143, 36, 41, 570; * p<0.05; data from Tables 7 [page 89] and 8 [pages 98-114] of the report

Table 3. Clinical Pathology Data - FEMALES				
Dose/Week/Parameter	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10/7.5 mg/kg/day
platelets [TH/UL]				
-1	374±54	369±98	378±87	460±54
4	289±38	303±53	288±40	292±19
13	276±26	288±66	307±37	357±33*
26	296±57	307±90	293±88	355±42
52	354±49	314±73	326±41	372±60
alanine aminotransferase [U/L]				
-1	30±7	28±9	28±4	26±4
4	35±10	33±8	59±12	79±62* [226]√
13	36±6	34±13	72±20* [200]	115±48* [319]
26	33±7	34±11	104±88 [137]	109±68 [330]
39	36±4	28±5	72±43 [200]	70±28 [194]
52	34±10	28±6	59±4 [174]	119±87* [350]
aspartate aminotransferase [U/L]				
-1	26±5	27±5	24±4	27±3
4	33±5	33±10	33±4	31±6
13	31±2	33±9	37±4	44±10 [142]
26	29±4	39±9	39±7	46±8* [159]
39	32±6	34±12	35±7	38±5
52	32±5	30±6	38±6	48±9* [150]
blood urea nitrogen [mg/dL]				
-1	10±2	11±3	9±2	11±3
4	12±2	16±3	18±3* [150]	30±5* [250]
13	14±2	16±6	20±1* [143]	27±4* [193]
26	15±3	17±3	20±2 [133]	28±7* [187]
39	15±2	16±3	22±1* [147]	27±5* [180]
52	13±2	16±4	20±3* [154]	30±4* [231]
creatinine [mg/dL]				
-1	0.7±0.04	0.7±0.09	0.7±0.05	0.7±0.04
4	0.9±0.05	1.0±0.08* [111]	1.1±0.08* [122]	1.4±0.13* [156]
13	0.9±0.08	0.9±0.17	1.2±0.11* [133]	1.4±0.22* [156]
26	1.0±0.08	1.0±0.05	1.3±0.15* [130]	1.5±0.28* [150]
39	0.9±0.08	0.9±0.10	1.3±0.07* [144]	1.5±0.30* [167]
52	0.9±0.12	0.9±0.06	1.5±0.05* [167]	1.7±0.27* [189]
alkaline phosphatase [U/L]				
-1	106±12	113±28	111±19	113±12
4	92±12	89±13	79±17	58±19* [63]
13	60±5	90±71	54±17	66±10
26	33±8	37±7	35±11	62±11* [188]
39	29±3	32±5	28±7	50±18* [172]
52	31±9	20±9	25±5	47±18

Table 3. Clinical Pathology Data - FEMALES				
Dose/Week/Parameter	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10/7.5 mg/kg/day
glucose [mg/dL]				
-1	114±2	110±5	109±3	111±5
4	107±4	108±5	104±6	90±12* [84]
13	98±6	99±3	91±8	90±5 [92]
26	107±3	105±6	97±9	94±10* [88]
39	97±4	94±7	88±7	81±6* [84]
52	94±6	97±2	81±6	79±3* [84]
cholesterol [mg/dL]				
-1	154±27	184±59 [119]	181±27 [118]	167±8
4	137±10	172±11* [126]	189±15* [138]	194±41 [142]
13	139±19	187±60 [135]	192±20 [138]	177±37
26	159±33	146±21	198±24 [125]	102±35 [64]
39	132±22	133±12	193±44* [146]	160±39
52	178±100	200±87	187±16	160±42

√[% of control]; * p<0.05; data from Tables 7 [page 89] and 8 [pages 98-114] of the report

Table 4. Organ Weight Data - MALES				
Dose/Organ	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10/7.5 mg/kg/day
brain w/ stem				
absolute [g]	84.9±6.5	83.6±6.1	83.9±7.7	80.7±1.2 [95]
relative-to-body [%]	0.67±0.09	0.67±0.08	0.76±0.14	0.74±0.08 [110]
liver				
absolute [g]	286±21	296±46	261±45 [91]	267±38 [93]
relative-to-body [%]	2.3±0.3	2.4±0.3	2.3±0.1	2.4±0.3
relative-to-brain [ratio]	3.4±0.4	3.5±0.6	3.1±0.4	3.3±0.5
kidney				
absolute [g]	64.6±9.2	69.7±9.5	68.3±14.5	58.9±7.4
relative-to-body [%]	0.51±0.11	0.56±0.06	0.62±0.14	0.54±0.04
relative-to-brain [ratio]	0.76±0.07	0.83±0.09	0.82±0.21	0.73±0.09
testes				
absolute [g]	16.8±3.8	18.2±2.9	16.1±5.2 [96]	14.5±1.5 [86]
relative-to-body [%]	0.13±0.03	0.15±0.02	0.14±0.03	0.13±0.03
relative-to-brain [ratio]	0.20±0.06	0.22±0.03	0.19±0.06 [95]	0.18±0.02 [90]
thyroid				
absolute [g]	1.15±0.30	1.24±0.20 [108]	1.25±0.39 [109]	1.04±0.10 [90]
relative-to-body [%]	0.009±0.002	0.010±0.001	0.011±0.002	0.009±0.000
relative-to-brain [ratio]	0.014±0.004	0.015±0.002	0.015±0.003	0.013±0.001
terminal body weight [g]	12740±945	12540±1240	11280±2326 [89]	11000±1287 [86]

N=5, except low-dose female group (4); √[% of control];* p<0.05; data from Table 10 [pages 116-124] of the report

Table 5. Organ Weight Data - FEMALES				
Dose/Organ	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10/7.5 mg/kg/day
brain w/ stem				
absolute [g]	81.1±4.1	74.0±6.8	72.8±4.9* [90]	71.5±1.4* [88]
relative-to-body [%]	0.75±0.10	0.78±0.05	0.75±0.08	0.87±0.03* [116]
liver				
absolute [g]	273±80	232±56	237±32	203±22 [74]
relative-to-body [%]	2.5±0.4	2.4±0.4	2.4±0.2	2.5±0.2
relative-to-brain [ratio]	3.4±0.9	3.1±0.6	3.3±0.5	2.8±0.3 [82]

Table 5. Organ Weight Data - FEMALES

Dose/Organ	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10/7.5 mg/kg/day
kidney				
absolute [g]	49.7±7.8	45.6±7.9	45.7±2.8	41.3±4.1
relative-to-body [%]	0.46±0.04	0.48±0.05	0.47±0.06	0.50±0.05
relative-to-brain [ratio]	0.61±0.08	0.61±0.07	0.63±0.05	0.58±0.06
ovaries				
absolute [g]	1.41±0.66	1.36±0.68	0.72±0.17 [51]	0.80±0.15 [57]
relative-to-body [%]	0.012±0.004	0.014±0.007	0.007±0.001 [58]	0.010±0.002
relative-to-brain [ratio]	0.017±0.007	0.018±0.008	0.010±0.002 [59]	0.011±0.002 [65]
thyroid				
absolute [g]	1.07±0.13	0.96±0.18	1.09±0.15	0.92±0.17* [86]
relative-to-body [%]	0.010±0.002	0.010±0.002	0.011±0.002	0.011±0.002
relative-to-brain [ratio]	0.013±0.001	0.013±0.003	0.015±0.002	0.013±0.002
terminal body weight [g]	10940±1799	9525±670	9700±748	8260±422* [76]

n=5, except low-dose female group (4); √[% of control];* $p<0.05$; data from Table 10 [pages 116-124] of the report

Table 6. Histopathology Data

Dose/Organ	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10/7.5 mg/kg/day
thyroid				
hyperplasia, "C" cell				
males	2	2	1	1
females	1	2	0	2
liver				
inflammation, chronic, active, perivascular				
males	1	1	3	4
females	0	0	4	3
pigment, sinusoidal lining cell				
males	1	0	1	1
females	1	2	5	4
kidney				
pigment, tubular epithelium				
males	2	4	5	5
females	1	1	5	5
testes				
aspermatogenesis	0	0	0	1[a]
degeneration	0	0	0	1[a]
prostate				
inflammation, chronic active	1	1	3	2[a]

N=5, except low-dose female group (4); [a] high-dose male with both testicular lesions also displayed inflammation of prostate; data from Table 11B [pages 133-140] of the report

011271

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

MEMORANDUM 1994

#315

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT **2,4-DICHLOROPHENOXYACETIC ACID:** Chronic Toxicity Study in **DOGS**.

FROM: Jess Rowland, M.S, Toxicologist 9/29/94
Section II, Toxicology Branch II, Health Effects Division (7509C)

TO: Walter Waldrop / Judy Coombs
Product Manger 71, Reregistration Division

THRU: K. Clark Swentzel, Head 9/30/94
Section II, Toxicology Branch II, Health Effects Division (7509C)
and
Marcia van Gemert, Ph.D., Chief 9/30/94
Toxicology Branch II, Health Effects Division (H7509C)

TASK IDENTIFICATIONS: Submission: S455698 DP Barcode: D197867
PC Code: 030001 Caswell No: 315 MRID No: 430490-01

ACTION REQUESTED: Review a 1-year chronic toxicity study in dogs.

RESPONSE: A Data Evaluation Record is attached and an Executive Summary follows:

EXECUTIVE SUMMARY: In a chronic toxicity study, beagle dogs [5/Sex/Dose] were fed diets containing 2,4-dichlorophenoxyacetic acid at 0, 1, 5, or 7.5 m/kg/day for 52 weeks. Parameters investigated included: mortality; clinical observations; body weights; food consumption; ophthalmology; hematology; clinical chemistry; urinalysis; organ weights; and gross and histopathology. No treatment-related effects were see on survival, clinical signs, ophthalmology, hematology, urinalysis, organ weight or gross pathology at any dose level. Body weight gains of dogs at 1 mg/kg/day were comparable to those of the controls. Body weight gains were impaired in both sexes of dogs at 5 and 7.5 mg/kg/day with the effect being more pronounced in females at the high dose. Alterations seen in serum chemistry parameters in dogs at 5 and 7.5 mg/kg/day were increases in BUN, creatinine and total cholesterol levels and elevations in alanine aminotransferase activity [ALT]. These alterations were corroborated with histopathological changes in the liver and kidneys of these dogs. The increases in BUN and creatinine are compatible with either dehydration or mild renal tubular epithelial compromise while the elevations in ALT activity are indicative of hepatocellular injury. The increases in total cholesterol are nonspecific, but are typically seen with alterations in lipid metabolism by the liver. Histopathology revealed a minimal increase in the frequency and average severity of sinusoidal lining cells of the liver of females only at 5 and 7.5 mg/kg/day and minimal increases in the frequency and average severity of perivascular, chronic active inflammation of the liver and of pigment in the tubular epithelium of the kidneys in both sexes of dogs at 5 and 7.5 mg/kg/day. **Under the conditions of this study, the NOEL is 1 mg/kg/day. The LOEL is 5.0 mg/kg/day, based on alterations in serum chemistry with corroborative histopathological lesions in the liver and kidneys.**

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (83-1b) for a chronic feeding study in nonrodents.

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PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist 9/29/94
Section II, Toxicology Branch II (7509C)

SECONDARY REVIEWER: K. Clark Swentzel, Head 9/30/94
Section II, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity - Non Rodent. **GUIDELINE:** 83-1(b)

Chemical No. 030001 **MRID No. 430490-01**

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

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

TESTING LABORATORY: Hazleton Washington, Inc, Vienna, VA

TITLE: *"52-WEEK DIETARY TOXICITY STUDY WITH 2,4-D IN DOGS"*

STUDY IDENTIFICATION: HWA 2184-124

AUTHOR: Dan W. Dalgard, D.V.M. **REPORT DATE:** December 2, 1993

EXECUTIVE SUMMARY: In a chronic toxicity study, beagle dogs [5/Sex/Dose] were fed diets containing 2,4-dichlorophenoxyacetic acid at 0, 1, 5, or 7.5 mg/kg/day for 52 weeks. Parameters investigated included: mortality; clinical observations; body weights; food consumption; ophthalmoscopic examinations; clinical pathology including hematology, clinical chemistries, and urinalysis; gross pathology; organ weights; and histopathological evaluation. No treatment-related effects were see on survival, clinical signs of toxicity, ophthalmology, hematology, urinalysis, organ weight or gross pathology at any dose level. While body weight gains of dogs at 1 mg/kg/day were comparable to those of the controls, body weight gains were impaired in both sexes of dogs at 5 and 7.5 mg/kg/day with the effect being more pronounced at 7.5 mg/kg/day, especially in females. Alterations seen in serum chemistry parameters in dogs at 5 and 7.5 mg/kg/day were increases in BUN, creatinine and total cholesterol levels and elevations in ALT. These alterations were corroborated with histopathological changes in the liver and kidneys of these dogs. The increases in BUN and creatinine are compatible with either dehydration or mild renal tubular epithelial compromise. The elevations in ALT activity are indicative of hepatocellular injury. The increases in total cholesterol are nonspecific, but are typically seen with alterations in lipid metabolism by the liver. Histopathology revealed a minimal increase in the frequency and average severity of sinusoidal lining cells of the liver of females only at 5 and 7.5 mg/kg/day and minimal increases in the frequency and average severity of perivascular, chronic active inflammation of the liver and of pigment in the tubular epithelium of the kidneys in both sexes of dogs at 5 and 7.5 mg/kg/day. **Under the conditions of this study, the NOEL is 1 mg/kg/day; the LOEL of 5.0 mg/kg/day is based on alterations in serum chemistry with histopathological lesions in the liver and kidneys.**

CORE CLASSIFICATION: **Guideline;** this study satisfies the Guideline requirement [§83-1(b)] for a chronic feeding study in nonrodents,

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a chronic toxicity study in dogs with **2,4-Dichlorophenoxyacetic acid.**

II. MATERIALS AND METHODS

1. Test Material

Chemical Name: 2,4-Dichlorophenoxyacetic acid.
Purity: 96.7%
Lot No.: 909
Description: Off white powder.

2. Test Animals

Species: Dogs
Strain: Beagle
Sex: Males and females
Age At Initiation: 4-6 months
Weight At Initiation: 6.5 - 10.1 kg (M) and 6.5 - 8.9 kg,(F)
Identification: A permanent number in the form of a BioMedic, implantable microchip device inserted subcutaneously into the nape of neck.
Acclimation: At least 2 weeks
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Canine Diet Meal #5007.
Water: Tap water ad libitum
Environment: Temperature, 66-82°F; Humidity, 20-93%; Light cycle, 12 hr.on/off; Air flow, 10 air changes/hour.

3. Test Material Formulation and Concentration Analysis

The test material was ground into a fine powder using a mortar and pestle. For the purpose of dosage calculations, the test material was assumed to be 100%. The amount of test material necessary for each test group was weighted on an appropriate balance and premixed in a Waring blender with approximately 200 g of feed for approximately 2-3 minutes. The premix was then added to the required amount of feed for each level and mixed at a rate of approximately 1 minute/kg in a Patterson-Kelly twin-shell blender. Test diets were prepared weekly and dietary levels were adjusted weekly in order to achieve target dose levels, based on the most recently recorded body weight and feed consumption values.

Concentration analyses of each dose level was determined weekly for the first four weeks of the study and every fourth week thereafter. Homogeneity analyses was performed once prior to the start of the study and at Week 12. Stability analysis initiated with the start of the study analyzed stability at room temperature and under refrigeration on Day 7 and at freezer-temperature on Day 43.

4. Treatment

Male and female dogs were fed diets containing 2,4-D acid at 1, 5, or 10 mg/kg/day days per week for a period of up to 52 Weeks. Control animals received standard laboratory diet on the same schedule. During study Week 8, the high-dose was decreased from 10 to 7.5 mg/kg/day because of loss of body weight in dogs at this dose.

5. Study Design

Group No.	Treatment	No. of Animals Males	Females	Dose Level mg/kg/day
1	Control	5	5	0
2	Low	5	5	1
3	Mid	5	5	5
4	High	5	5	7.5/10*

* = During Week 8, this dose was reduced to 7.5 mg/kg/day due to toxicity.

6. Experimental Procedures

Mortality and moribundity checks were performed twice daily. A through weekly physical examination was conducted at each weighing interval. Body weights and food consumption were measured once prior to treatment and weekly thereafter. Ophthalmologic examinations were conducted prior to initiation and at termination. The following hematology and clinical chemistry parameters were measured once prior to treatment and during Weeks 4, 13, 26, 39, and 52; urinalysis tests were performed once prior to initiation and at weeks 26 and 52. The **checked (x)** parameters **were measured.**

Hematology

x Hematocrit (HCT)*	x Leukocyte count (WBC)*
x Hemoglobin (HGB)*	x Platelet count*
x Erythrocyte count (RBC)*	x Leukocyte differential*
x Mean corpuscular HGB (MCH)	x Mean corpuscular HGB Concentration (MCHC)
x Mean corpuscular volume (MCV)	x Corrected leukocyte count
x Cell morphology	x Reticulocyte count

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]**
	Triglycerides
x Alkaline phosphatase	Serum Protein Electrophoresis
x Alanine aminotransferase (SGPT)[a]	Triiodothyronine (T_3)
x Aspartate aminotransferase (SGOT)[a]	Thyroxine (T_4)
Cholinesterase[b]	A/G Ratio
x Creatinine phosphatase[a]	
Lactic acid dehydrogenase	
γ-Glutamyl transpeptidase [GGPT]	

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]	Microscopic examination of sediment[c]

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

7. Termination

After 523 weeks of treatment, all surviving animals were weighed, anesthetized with sodium thiamylal, and exsanguinated. Necropsies were performed on each animal, all gross pathological changes were recorded, and the following organs were weighed.

Adrenals	Brain	Heart	Kidneys	Liver	Pituitary	Thyroid/ parathyroid	Testes	Ovaries

8. Histopathology

The **checked (X)** tissues from all animals were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Tongue	x Trachea*
x Salivary gland*	x Lung*
x Esophagus*	Pharynx +
x Stomach	Larynx +
x Duodenum*	Nose +
x Jejunum*	
x Cecum*	Cardiovascular/Hemo.System
x Colon*	x Aorta (thoracic)*
x Rectum*	x Heart*
x Liver* †	x Bone marrow*
x Gall bladder*	x Lymph nodes*
x Pancreas	x Spleen*
	x Thymus*
Neurological System	
x Brain* †	Urinogenital System
x Pituitary*	x Kidneys* ‡
Peripheral nerve*#	x Urinary bladder*
x Spinal cord	x Tests* †
(3 levels)*#	x Prostate
x Eyes (optical nerve)*#	x Seminal vesicle
	x Uterus*
Glandular System	x Ovaries* †
x Adrenals*	
x Lacrimal glands#	Others
x Parathyroids* ‡	x All gross lesions and masses
x Thyroids* ‡	x Skeletal muscle*
x Mammary glands	x Femur [with articulate surface]
	x Sciatic nerve

* Required for subchronic and chronic studies.
\+ Required for chronic inhalation study.
\# In subchronic studies examined only if indicated by toxicity or target organ involvement.
† Organ weights required in subchronic and chronic studies.
‡ Organ weights required for nonrodent studies.

9. Statistical Analyses

Mean body weights and body weight changes, mean weekly food consumption, total food consumption, clinical pathology data (with the exception of cell morphology and routine urinalysis data), and organ weight data of treated groups were compared statistically against data of the control group of same sex by ANOVA followed by Dunnett's test. All analyses were conducted using two-tailed tests with a minimum significance level of 5%.

10. Regulatory Compliance

A signed statement of No Data Confidentiality Claim was dated 12-2-93. A signed statement dated 12-2-93 indicated that this study was conducted in accordance with the promulgation of the principles of EPA's Good Laboratory Practices [40CR.160]. A signed statement for Potential Adverse Effects, signed and dated 12-2-93 indicated that this study neither meets nor exceeds any of the applicable criteria stipulated in 40 CFR 158.34. A Quality Assurance Statement was signed and dated 11-24-93.

III. RESULTS

Analysis of Diet Mix

Stability analyses of test diet samples indicated 2,4-D to be stable for at least 7 days under refrigeration and at room temperature and for at least 43 days at -20°; percent of target values ranged from 96.2% to 104%,. Homogeneity of analysis within each diet group showed little variation from top, middle and bottom sampling; relative standard deviation values were within 3%. Results of the concentration analyses indicated that all formulations were within ±10% of target. Selected data are tabulated below:

	% of Target					
Week	**Males**			**Females**		
mg/kg/day	1	5	7.5	1	5	7.5
1	107	99	100	102	101	101
4	97	98	102	98	103	101
16	101	104	100	95	101	99
28	103	100	110	97	104	95
40	98	100	100	99	101	98
52	104	104	100	104	103	101

1. Survival

Except for one female dog at 5 mg/kg/day that was sacrificed on Day 130 in a moribund condition following a 6-week period of decreased appetite and progressive weight loss, all dogs survived the duration of the study. "Beagle pain syndrome" and/or aseptic meningitis were among conditions considered in the differential diagnosis. Histopathology revealed a non-specific inflammatory condition involving various abdominal organs.

2. Clinical Observations

No treatment-related clinical signs of toxicity were observed. Both sexes of treated as well as control dogs had mucoid, soft or sanguineous feces and other signs frequently seen in this strain/age of dog.

3. Body Weights and Body Weight Changes

Mean body weight data are summarized in Table 1 show that the mean body weights of both sexes of dogs at the high dose [7.5 mg/kg/day] were lower than the respective controls throughout the study with the decrease being more pronounced in females. Females at the high dose showed no appreciable weight gain during the first several weeks of the study. Thereafter, they showed a slow gradual increase but body weights remained well below the controls for the remainder of the study. Because of earlier loss of body weight in dogs at the high dose, the target dose of 10 mg/kg/day was reduced to 7.5 mg/kg/day during Week 8. This change was made following discussion with the toxicologists at the Agency and their concurrence. Mean body weights of both sexes of dogs at 1 or 5 mg/kg/day were comparable to their respective controls.

Table 1. Mean Body Weights (kg) in Dogs Fed 2,4-D for 52 Weeks.

Week	Males				Females			
mg/kg/day	0	1	5	7.5	0	1	5	7.5
1	8.2	9.0	8.5	8.2	7.8	7.6	7.9	7.394
2	8.5	9.1	8.6	8.6	8.0	7.7	8.0	7.590
3	8.9	9.4	8.8	8.6	8.4	7.7	8.2	7.387
4	9.3	9.6	9.1	8.9	8.4	8.0	8.4	7.4
5	9.3	9.9	9.4	8.9	8.6	8.0	8.5	7.9
6	9.7	10.3	9.7	9.1	8.8	8.2	8.7	7.080
7	9.9	10.4	9.8	9.1	8.9	8.3	8.7	7.382
8[a]	10.3	10.7	10.2	9.3	9.1	8.5	9.0	7.582
13	10.9	11.7	10.4	10.2	9.8	9.1	9.3	8.082
19	11.9	12.3	11.1	10.8	10.4	9.1	9.4	8.5
25	12.0	12.2	11.4	10.9	10.6	9.3	9.4	8.580
31	12.5	12.4	11.3	11.1	10.6	9.5	9.8	8.6
37	12.5	12.6	11.6	11.2	10.7	9.6	10.0	8.8
43	12.8	12.7	11.5	11.4	10.9	10.0	9.9	8.7
49	13.0	12.9	11.8	11.5	11.2	10.2	10.0	8.778
53	13.2	12.8	11.8	11.5	11.4	9.9	9.9	8.675

a = Dose level changed from 10 to 7.5 mg/kg/day.

Body weight change data are presented in Tables 2. Although both sexes of dogs at the high dose gained less weight when compared to controls, the decreases reached statistical significance ($p < 0.05$) only in females. Additionally, females at the low- and mid-doses also exhibited statistically significant lower body weight gains during Weeks 1-39.

Table 2. Mean Body Weight Gain (kg) in Dogs Fed 2,4-D for 52 Weeks.

Week	Males				Females			
mg/kg/day	0	1	5	7.5	0	1	5	7.5
1-13	2.7	2.7	1.9	2.0	2.0	1.5	1.4	0.7*
1-26	4.1	3.5	2.9	2.8	2.8	2.0	1.8	1.3*
1-39	4.4	3.7	3.0	3.0	3.1	1.9*	2.0*	1.4*
1-52	4.9	3.8	3.1	3.3	3.3	2.4	2.1	1.2*

* Significantly different from control at $p < 0.05$.

4. Food and Compound Consumption

No statistically significant differences were observed in mean food consumption for either sex at the three dose levels when compared to respective controls. The dose levels achieved for both sexes of dogs at 1 and 5 mg/kg/day were generally quite close to target levels. For the 1 and 5 mg/kg/day dose levels, the mean dose level achieved for Weeks 1-52 were 1.0 and 5.2 for males and 1.0 and 5.0 for females, respectively. More fluctuations were seen at the high-dose, especially during the first several weeks. When the targeted dose was 10 mg/kg/day during Weeks 1-8, the achieved doses were 11.4 and 10.4 for males and females, respectively. Once the target dose was lowered to 7.5 mg/kg/day, the actual dose level was close to the target levels for the duration of the study. Achieved dose during Weeks 9-52 were 8.2 and 7.9 mg/kg/day for males and females, respectively. The overall mean doses achieved at the high dose for the entire study [Weeks 1-52] were 8.2 and 7.9 mg/kg/day for males and females, respectively.

5. Ophthalmology Examination

No treatment-related ocular changes were seen at any dose level.

6. Clinical Pathology

a. Hematology: No treatment-related alterations were seen in any of the hematological parameters. Although various statistically significant findings were noted, they generally were not dose-and/or time-dependent and/or were within laboratory historical control data and were considered to be normal biologic variations.

b. Clinical Chemistry: Treatment-related, statistically significant [p <0.05] increases observed in ALT activity [Table 3] and BUN [Table 4], creatinine [Table 5] and cholesterol [Table 6] concentrations in both sexes of dogs at 5 and 7.5 mg/kg/day. Mean glucose values were significantly decreased at 5 mg/kg/day in males during Weeks 39 and 52 and in females at Week 52, and in both sexes at 7.5 mg/kg/day during Weeks 26, 39 and 52 [Table 7. Differences observed in other clinical chemistry parameters were not dose-and/or time-dependent and/or were within laboratory historical control data and were considered to be normal biologic variations.

Table 3. ALT Levels in Dogs Fed 2,4-D for 52 Weeks.

		ALT [U/L]					
mg/kg/day	Sex	-1	4	13	26	39	52
0.0	M	34	32	38	43	41	43
1.0	M	28	35	41	46	42	46
5.0	M	33	97*	117*	119*	111*	114*
7.5	M	30	176*	116*	152*	107*	138*
0.0	F	30	35	36	33	36	34
1.0	F	28	33	34	34	28	28
5.0	F	28	59	72*	104	72	59
10.0	F	26	79*	115*	109	70	119*

Table 4. BUN Levels in Dogs Fed 2,4-D for 52 Weeks.

		BUN [mg/dL]					
mg/kg/day	Sex	-1	4	13	26	39	52
0.0	M	9	11	12	12	13	12
1.0	M	9	14	16	15	16	15
5.0	M	10	22*	25	26*	28*	26*
7.5	M	8	24*	25*	23*	22*	24*
0.0	F	10	12	14	15	15	13
1.0	F	11	16	16	17	16	16
5.0	F	9	18*	20*	20*	22*	20*
7.5	F	11	30*	27*	28*	27*	30*

Table 5. Creatinine Levels in Dogs Fed 2,4-D for 52 Weeks.

		CREATININE [mg/dL]					
mg/kg/day	Sex	-1	4	13	26	39	52
0.0	M	0.7	0.8	0.8	0.9	0.9	1.0
1.0	M	0.6	0.9	0.9	1.0	1.0	1.0
5.0	M	0.7	1.2*	1.2*	1.4*	1.5*	1.5*
7.5	M	0.7	1.2*	1.2*	1.3*	1.3*	1.4*
0.0	F	0.7	0.9	0.9	1.0	0.9	0.9
1.0	F	0.7	1.0*	0.9	1.0	0.9	0.9
5.0	F	0.7	1.1*	1.2*	1.3*	1.3*	1.5*
7.5	F	0.7	1.4*	1.4*	1.5*	1.5*	1.7*

Table 6. Total Cholesterol Levels in Dogs Fed 2,4-D for 52 Weeks.

		TOTAL CHOLESTEROL [mg/dL]					
mg/kg/day	Sex	-1	4	13	26	39	52
0.0	M	186	158	153	130	126	127
1.0	M	168	169	157	143	133	129
5.0	M	190	201*	205*	194*	175*	174*
7.5	M	191	193	190	171*	164*	167*
0.0	F	154	137	139	159	132	178
1.0	F	184	172*	187	146	133	200
5.0	F	181	189*	192	198	193*	187
7.5	F	167	194	177	182	168	160

Table 7. Glucose Levels in Dogs Fed 2,4-D for 52 Weeks.

		GLUCOSE [mg/dL]					
mg/kg/day	Sex	-1	4	13	26	39	52
0.0	M	114	101	100	104	96	94
1.0	M	118	106	97	101	96	92
5.0	M	112	102	89	94	85*	82*
7.5	M	116	94	91	93*	81*	80*
0.0	F	114	107	98	107	97	94
1.0	F	110	108	99	105	94	97
5.0	F	109	104	91	97	88	81*
7.5	F	111	90*	90	94*	81*	79*

c.Urinalysis: The urinalyses were generally comparable between control and treated groups at all intervals [-1, 26 and 52 Weeks].

8. Organ Weights

Terminal body weights [fasted] were 14% lower than controls for males and 24% [$p < 0.05$] for females at 7.5 mg/kg/day. No treatment-related differences were seen in group mean absolute and relative organ weight values between the treated and control groups.

9. Gross Pathology

No treatment-related gross pathological changes observed at terminal sacrifice.

10. Histopathology

No treatment-related histopathological alterations were seen in either sex at 1.0 mg/kg/day. Histopathological lesions seen in the liver and kidneys of dogs at 5 and 7.5 mg/kg/day are summarized in Table 8. Liver lesions were characterized as a minimal increases in the frequency and average severity of perivascular, chronic active inflammation and sinusoidal lining cell pigment. Kidney lesions consisted of a minimal increase in the frequency and average severity of pigment in the tubular epithelium of the kidneys. Other alterations observed were considered typical findings in clinically normal dogs of this strain and age.

Table 8. Non-neoplastic Lesions in the Liver and Kidneys of Dogs Fed 2,4-D for 52 Weeks.

No. Examined: 5/Sex/Dose	Males				Females			
mg/kg/day	0	1	5	7.5	0	1	5	7.5
Liver: Inflammation, Chronic active, Perivascular	1	1	3	4	0	0	4	3
Pigment, Sinusoidal lining cells	1	0	1	1	1	2	5	4
Kidneys: Pigment, Tubular epithelium	2	4	5	5	1	1	5	5

IV. DISCUSSION

Dietary administration of 2,4-D acid at 1, 5, or 7.5 m/kg/day for 52 weeks did not cause in any adverse effects on survival, clinical signs of toxicity, ophthalmology, hematology, urinalysis, organ weight or gross pathology. Body weight gains were impaired in both sexes at 5 and 7.5 mg/kg/day with the effect being more pronounced at 7.5 mg/kg/day, especially in females where the differences showed statistical significance. The alterations seen in serum chemistry parameters [increases in BUN, creatinine and total cholesterol levels and ALT activity] in dogs at 5 and 7.5 mg/kg/day were corroborated with histopathological changes in the liver and kidneys of these dogs. The increases in BUN and creatinine are compatible with either dehydration or mild renal tubular epithelial compromise. The elevations in ALT activity are indicative of hepatocellular injury. The increases in total cholesterol are nonspecific, but are typically seen with alterations in lipid metabolism by the liver. The decreases in glucose levels in dogs at these doses are attributed to treatment; however, the mechanism is not apparent from the remainder of the clinical pathology data. Histopathology revealed a minimal increase in the frequency and average severity of sinusoidal lining cells of the liver of females only at 5 and 7.5 mg/kg/day and minimal increases in the frequency and average severity of perivascular, chronic active inflammation of the liver and of pigment in the tubular epithelium of the kidneys in both sexes of dogs at 5 and 7.5 mg/kg/day. It is interesting to note that the effects seen in clinical chemistry [i.,e increases in BUN, creatinine, cholesterol and ALT activity] at 5 mg/kg/day in this study were also seen in a 90-day feeding study [MRID No. 427800-01] at a lower dose [3.75 mg/kg/day]. Similarly, the liver lesions [perivascular chronic active inflammation] seen in this study at 5 mg/kg/day were also seen in the 90-day study at a higher dose [7.5 mg/kg/day]. Based on these findings it is concluded that the dose levels used in this study were adequate to assess the chronic toxicity of 2,4-D in this species.

V. CONCLUSION

Under the conditions of this study, a NOEL of 1 mg/kg/day and a LOEL of 5.0 mg/kg/day is established. The LOEL is based on alterations in serum chemistry with corroborative histopathological lesions in the liver and kidneys.

VI. CORE CLASSIFICATION: Guideline; this study satisfies the Guideline requirement [§ 82-1(b) for a 1-year feeding study in dogs.

Amendment to DER for MRID 43115201 - Acute Neurotoxicity Screening Battery [Rat] [2,4-DICHLOROPHENOXYACETIC ACID]

This amendment provides an EXECUTIVE SUMMARY to upgrade the original DER [Document No. 011273].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 11/19/02
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Whang Phang, Ph.D. 11/20/02
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: acute neurotoxicity - rat § 82-7/OPPTS 870.6200
P.C. CODE: 030001 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315 CAS NO.: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [96.7%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force II on 2,4-D Research Data

CITATION: Mattsson, J. L., McGuirk, R. J., Yano, B. L. (1994). 2,4-Dichlorophenoxyacetic acid: Acute Neurotoxicity Study in Fischer 344 Rats. The Toxicology Research Laboratory, Dow Chemical Co.; Study No. K-002372-066, January 5, 1994. MRID 43115201. Document No. 011273. Unpublished.

EXECUTIVE SUMMARY: In an acute neurotoxicity study [MRID 43115201], Fischer 344 rats (10/sex/dose) were orally gavaged once with 2,4-D [96.6% a.i.] at doses of 0 (corn oil), 15, 75, or 250 mg/kg (actual: 0, 13, 67 or 227 mg/kg). Neurobehavioral evaluations, consisting of Functional Observational Battery (FOB) and motor activity, were conducted at Day -1 (prestudy), Day 1 (approximately 5-6 hrs postdosing, peak time of effect) and Days 8 and 15. At terminal sacrifice (Day 15), animals were euthanized and neuropathological examination performed on control and treated animals (5/sex/dose).

There were no treatment-related mortalities, and no significant differences were noted in the mean body weights or mean body-weight gains. Treatment-related, clinical signs [uncoordinated movement/behavior] were observed in the high-dose rats only. During the Day 2 and 3 clinical examinations, incoordination was noted in high-dose animals of both sexes [Day 2, 2/10 males and 5/10 females; Day 3, 2/10 males, 1/10 females; Day 4, 0/10 males, 1/10 females]. The incidence of incoordination decreased to control levels by Day 4 in males and Day 5 in females.

Neurobehavioral evaluation revealed treatment-related changes. During the Day 1 FOB evaluations, increased incidences of incoordination (6/10, males; 4/10, females) and slight gait abnormalities, described as forepaw flexing or knuckling, were observed in high-dose animals (8/10, males; 8/10 females). Slight gait abnormalities, observed in a single mid-dose female, were not judged to be treatment-related since no other signs of toxicity were evident. Minimal [equivocal] gait abnormalities, not judged to be treatment-related, were observed in one low-dose female and one each mid- and high-dose male. In high-dose animals, total motor activity was significantly lower at Day 1 only. No treatment-related gross or neuropathological findings were present at any dose level in either sex.

The NOAEL for neurotoxicity is 67 mg/kg, based on an increased incidence of incoordination and slight gait abnormalities [described as forepaw flexing or knuckling] and decreased total motor activity at the LOAEL of 227 mg/kg. The NOAEL for systemic toxicity was 227 mg/kg [the highest dose tested] in males and females.

This acute neurotoxicity study in the rat is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [OPPTS 870. 6200] for an acute neurotoxicity study in the rat.

011273

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 4 Oct 94
Section IV, Tox. Branch II (7509C)

Secondary Reviewer: Susan L. Makris, M.S. Susan L Makris 10/4/94
Section IV, Tox. Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity Screening Battery - Rat (81-8)

EPA ID NOs.: MRID No.: 431152-01
Pesticide Chemical Code: 030001
Toxicology Chemical Code: 315
DP Barcode: D200086
Submission No.: S459751

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid

SYNONYMS: 2,4-D

STUDY NUMBERS: K-002372-066

SPONSOR: Industry Task Force II on 2,4-D Research Data

TESTING LAB: The Toxicology Research Laboratory
Health and Environmental Sciences
The Dow Chemical Company, Midland, MI

REPORT TITLE: 2,4-Dichlorophenoxyacetic Acid (2,4-D): Acute Neurotoxicity Study in Fischer 344 Rats

AUTHORS: J.L. Mattsson, R.J. McGuirk and B.L. Yano

REPORT ISSUED: 5 January 1994

EXECUTIVE SUMMARY: In this acute neurotoxicity study, Fischer 344 rats (10/sex/dose) were orally gavaged once with 2,4-D at doses of 0 (corn oil), 15, 75, or 250 mg/kg (actual: 0, 13, 67 or 227 mg/kg). Neurobehavioral evaluations, consisting of Functional Observational Battery (FOB) and motor activity, were conducted at Day -1 (prestudy), Day 1 (approximately 5-6 hrs postdosing, peak time of effect) and Days 8 and 15. At terminal sacrifice (Day 15), animals were euthanized and neuropathological examination performed on control and treated animals (5/sex/dose).

No treatment-related mortalities occurred during the study.

No significant differences were noted in the mean body weights or mean body weight gains.

Clinical signs and neurobehavioral evaluation revealed treatment-related changes. During the Day 1 FOB evaluations, increased incidences of incoordination (6/10, males; 4/10, females) and slight gait abnormalities, described as forepaw flexing or knuckling, were observed in high-dose animals (8/10, males; 8/10 females). Slight gait abnormalities, observed in a single mid-

dose female, were not judged to be treatment-related since no other signs of toxicity were evident. Minimal gait abnormalities, not judged to be treatment-related, were observed in one low-dose female and one each mid- and high-dose male. On the Day 2 and 3 clinical examinations, incoordination was noted in high-dose animals. The incidence of incoordination decreased to control levels by Day 4 in males and Day 5 in females. In high-dose animals total motor activity was significantly lower at Day 1 only.

No treatment-related gross or neuropathological findings were present.

Based on the results of this study, the LOEL for systemic toxicity was not determined in males and females; the NOEL for systemic toxicity was 227 mg/kg in males and females. The LOEL for neurobehavioral effects was 227 mg/kg in males and females; the NOEL for neurobehavioral effects was 67 mg/kg in males and females.

This study is classified as Core - Guideline and satisfies guideline requirements (§81-8) for an acute neurotoxicity screening battery in the rat.

I. MATERIALS

A. Test Compound: 2,4-D, technical Description: white solid Lot No.: 909 (air-milled) Purity: 96.6% Contaminants: Not given

B. Test Animals: Species: Rat Strain: Fischer 344, Age: 8 weeks Weight (g): 132.2 - 155.5 (males), 95.3 - 118.9 (females) Source: Charles River Laboratories, Kingston, NY Housing: Individually in mesh-bottom cages Feed: Certified Purina Rodent Chow #5002 (Purina Mills, Inc., St. Louis, MO), *ad libitum* Water: tap water, *ad libitum* Environment: Temperature, 22.2 ± 0.3°C; Humidity, 50.3 ± 0.6%; Light/dark cycle, 12 hr/12 hr

II. METHODS

A. Preliminary Study: A preliminary study was carried out to determine the time of peak effect and the highest non-lethal toxic dose. Animals (3/sex/dose) were orally gavaged with corn oil suspensions of 2,4-D at doses of 250, 500, 750 or 1000 mg/kg. Doses of 500 mg/kg or greater produced lethality. Toxicity, present in all treated animals, consisted of decreased muscle tone, increased lacrimation, fecal soiling (probably due to vehicle), incoordination, and knuckling of forepaws. To further refine the doses, a second preliminary study using 3 males/group was conducted at 0, 50, 100, 150 and 200 mg/kg dose levels. The benchmark dose was selected as 250 mg/kg based on increased salivation, incoordination and knuckling of forepaws. The time of peak effect occurred approximately 5 to 6 hours postdosing.

B. Study Design: Ten animals/sex/dose were randomly assigned to control and treatment groups (Table 1). Because of the complexity of study, the animals were further assigned to subgroups of 20 animals each; the subgroups were counter-balanced over the different doses and sexes. Each subgroup was stagger-started over a four day period. Following an overnight fast, animals were orally gavaged once with either corn oil (vehicle) or corn oil solution of 2,4-D followed by a 15-day observation period.

Table 1: Animal Assignment to Study Groups

Test Group	Dose Level[a] (mg/kg)	Number Assigned	
		Male	Female
Control	0	10	10
Low	15 (13)	10	10
Mid	75 (67)	10	10
High	250 (227)	10	10

[a] Values in parentheses are the actual doses based on chemical analysis.

C. Dosing Preparations: A sufficient amount of 2,4-D was dissolved in corn oil to yield doses of 15, 75 and 250 mg/kg when administered in a dose volume of 10 ml/kg. Control animals received corn oil only. Dosing preparations were analyzed for stability and concentration.

D. Observations: Starting the day before exposure (Day -1) through terminal sacrifice, cageside observations were performed twice a day on all animals. On the days that the detailed clinical examinations (Days 2, 3, and 4) or behavioral testing (Days -1, 1, 8 and 15) were performed, the cageside observations were performed only once a day.

E. Body Weights: Body weights were determined on preexposure Day -1 and study Days 1, 2, 8, and 15.

F. Ophthalmological Examinations: Ophthalmological examinations were performed approximately two weeks prestudy and on Day 14.

G. Behavioral Tests: Behavioral tests consisted of the Functional Observational Battery (FOB) and evaluation of motor activity. Behavioral tests were performed Day -1, the day of dosing (Day 1), at the peak time of effect (approximately 5-6 hrs postdosing), and on Days 8 and 15.

1. Functional Observational Battery: The following parameters were evaluated:

Hand-held observations
- General
- Palpebral closure
- Pupil size
- Lacrimation
- Salivation
- Skin/haircoat abnormalities
- Perineal staining
- Abnormal movements
  - Convulsions, tremors
  - muscle tone, etc.
- Abnormal respiration
- Reactivity to handling

Open-field observations
- Level of activity
- Startle response
- Touch response
- Tail pinch response
- Gait abnormalities
- Abnormal/stereotypic behavior
- Quantity of urine/fecal pellets

Measurements/counts
- Hindlimb grip strength
- Forelimb grip strength
- Landing foot splay

2. Motor activity: Motor activity was measured using automated photobeam activity recording devices; devices were calibrated before use each day. Motor activity was evaluated approximately five hours after dosing and consisted of six 8-minute epochs, totalling 48 minutes (asymptote at 30 to 40 minutes).

H. Pathological Examinations

1. Neuropathology: At Day 15, five animals/sex/dose were randomly selected for neuropathological examination. Heparinized animals were anesthetized and perfused transcardially, first with phosphate buffer containing 0.7% sodium nitrite, then with 1.5% glutaraldehyde/4% formaldehyde. The tissues collected for neuropathological examination are listed below.

Tissues Selected for Neuropathological Examination

Brain	Spinal Cord
Olfactory lobe	Cervical & lumbar swelling
Cerebral cortex (frontal, parietal, temporal & occipital lobes)	Peripheral Nerves
Thalamus/hypothalamus	Sciatic, tibial, sural, caudal
Midbrain	Skeletal Muscle
Pons	Gastrocnemius, anterior tibial
Cerebellum	Other
Medulla oblongata	Pituitary gland
Nucleus gracilis/cuneatus	Trigeminal ganglia & nerve
Dorsal Root Ganglia	Eyes with optic nerves
Cervical & lumbar swelling	Nasal tissues with olfactory epithelium
Dorsal & Ventral Roots	
Cervical & lumbar swelling	

2. Routine histopathology: Routine histopathological examination was performed on the remaining animals, following an overnight fast. The remaining animals were necropsied and the tissues listed below immersion fixed in neutral, phosphate-buffered 10% formalin.

Tissues Selected for Routine Histopathology

Digestive System	Cardiovascular/Hematology	Neurologic
Tongue	Aorta	Brain (cerebrum, brainstem, cerebellum)
Salivary glands	Heart	Peripheral nerve
Esophagus	Bone marrow	Spinal cord (cervical, thoracic, lumbar)
Stomach	Mediastinal & mesenteric lymph nodes and tissue	Pituitary
Duodenum	Spleen	Eyes
Jejunum	Thymus	Glandular
Ileum	Urogenital	Adrenals
Cecum	Kidneys	Mammary gland
Colon	Urinary bladder	Parathyroids
Rectum	Testes	Thyroids
Liver	Epididymides	Auditory sebaceous glands
Pancreas	Prostate	Harderian/lacrimal glands
Oral tissues	Seminal vesicles	Coagulating glands
Respiratory	Ovaries	Other
Trachea	Uterus	Bone with joint
Lungs	Vagina	Skeletal muscle
Nasal tissues	Cervix	Skin and subcutis
Larynx	Oviducts	Gross lesions

H. Positive Controls: Positive control data were included in the study. For neurobehavioral evaluation, the following positive controls were used: Amphetamine (0.1, 0.32 or 1 mg/kg, ip) and chlorpromazine (0.5, 2.24 or 5 mg/kg, ip) for motor activity; amphetamine (8 mg/kg, ip), chlorpromazine (4 mg/kg, ip), or atropine (2 mg/kg, ip) plus physostigmine (0.75 mg/kg, sc) for FOB. Positive control neuropathology animals were treated with trimethyltin (7 mg/kg, po) on Day 1 followed by treatment with acrylamide (35 mg/kg, po) 5 times/week for 3 weeks. The positive control materials

accurately validated the FOB, motor activity and neuropathology findings.

I. Statistical Evaluations: The means and standard deviations of parametric data (body weight, hindlimb and forelimb grip strength, landing foot splay, and motor activity) were determined and variances (F-max test) evaluated for homogeneity. Grip strength was normalized for body weight; motor activity was expressed as the square roots of the counts. Repeated measures analyses (ANOVA or MANOVA) were used to evaluate different interactions (treatment × time, treatment × time × sex, and/or treatment × time × epoch). The type I error rate (α) per comparison was set at 0.02 to decrease the rate of false positive responses.

III. REGULATORY COMPLIANCES

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

IV. RESULTS

A. Analytical Chemistry: After 17 days, the low- mid- and high-dosing solutions were 93, 95 and 91% of the initial concentrations, respectively. The observed concentrations of the low-, mid- and high-dosing solutions were 13, 67 and 227 mg/kg, respectively.

B. Clinical Observations: On Day 1, one control and one high-dose female died as a result of gavage error; the high-dose female was replaced, while the control female was not. Treatment-related clinical signs were limited to high-dose animals, which showed uncoordinated movement/behavior (Table 2). On Day 2, 2/10 males and 5/10 females were affected. On Day 3, the incidence of affected males was unchanged at 2/10, while the number of affected females decreased to 1/10. A single female was affected on Day 4. Perineal soiling, noted in all dose groups, was attributed to the vehicle (corn oil).

Table 2: Clinical Signs (number affected/total number of animals)*

Observation	Sex	Day	Dose Level (mg/kg)			
			0	15	75	250
Incoordination	Male	2	0/10	0/10	0/10	2/10
		3	0/10	0/10	0/10	2/10
		4	0/10	0/10	0/10	0/10
	Female	2	0/9	0/10	0/10	5/10
		3	0/9	0/10	0/10	1/10
		4	0/9	0/10	0/10	1/10

* Data summarized from Table V-1 of the study.

C. Body Weights: No treatment-related differences in body weights were noted.

D. Ophthalmology: Ophthalmological examinations at Day 14 did not reveal any treatment-related effects.

E. Neurobehavioral Evaluations

1. FOB results: At the peak time of effect on Day 1, treatment-related FOB findings consisted of increased incidence of incoordination and gait abnormalities in high-dose animals; gait abnormalities were also observed in a single mid-dose female (Table 3). Gait abnormalities, graded as minimal, were not considered treatment-related because they were judged to be equivocal findings. Neither fore- and hindlimb grip strength nor landing foot splay were affected by treatment.

Table 3: FOB Findings (number affected/total number of animals)[a]

Observation	Sex	Day	Dose Level (mg/kg)			
			0	15	75	250
Incoordination	Male	-1	0/10	0/10	0/10	0/10
		1	0/10	0/10	0/10	6/10
		8	0/10	0/10	0/10	0/10
	Female	-1	0/9	0/10	0/10	0/9[b]
		1	0/9	0/10	0/10	4/10
		8	0/9	0/10	0/10	0/10
Gait Abnormalities, (Grade = slight)	Male	-1	0/10	0/10	0/10	0/10
		1	0/10	0/10	0/10	8/10
		8	0/10	0/10	0/10	0/10
	Female	-1	0/9	0/10	0/10	0/9
		1	0/9	0/10	1/10	8/10
		8	0/9	0/10	0/10	0/10

[a] Data summarized from Table V-2 of the study.
[b] One high-dose females died of gavage error and was immediately replaced. The replacement animal was not evaluated during the prestudy.

2. Motor activity results: On Day 1 at the peak time of effect, total motor activity was significantly decreased in high-dose animals.

Table 4: Total Motor Activity (square root of total beam breaks) Results[a]

Sex	Day	Dose Level (mg/kg)			
		0	15	75	250
Male	-1	11.29	11.01	11.19	11.45
	1	7.50	8.61	8.46	4.89*
	8	12.14	11.94	11.95	10.73
Female	-1	12.94	13.43	12.36	13.84
	1	9.12	8.98	9.64	6.98*
	8	12.75	12.66	11.68	12.56

[a] Data summarized from Table V-12 of the study.
* $p = 0.015$

F. Sacrifice and Pathology

1. Gross pathology: The incidence of gross pathological observations did not show any treatment-related effects.

2. Neuropathology: Microscopic evaluation of central and peripheral nervous system tissues did not reveal any treatment-related effects.

V. DISCUSSION and CONCLUSIONS: In this acute neurotoxicity study, Fischer 344 rats (10/sex/dose) were orally gavaged once with 2,4-D at doses of 0 (corn oil), 13, 67, or 227 mg/kg. Neurobehavioral evaluations, consisting of Functional Observational Battery (FOB) and motor activity, were conducted at Day -1 (prestudy), Day 1 (approximately 5-6 hrs postdosing, peak time of effect) and Days 8 and 15. At terminal sacrifice (Day 15), animals were euthanized; neuropathological examinations were performed on 5 animals/sex/dose.

No treatment-related mortalities occurred during the study.

No significant differences were noted in the mean body weights or mean body weight gains.

Clinical signs and neurobehavioral evaluation revealed treatment-related changes. During the Day 1 FOB evaluations, increased incidences of incoordination (6/10, males; 4/10, females) and slight gait abnormalities, described as forepaw flexing or knuckling, was observed in high-dose animals (8/10, males; 8/10 females). Slight gait abnormalities, observed in a single mid-dose female, were not judged to be treatment-related since no other signs of toxicity were evident. Minimal gait abnormalities, not judged to be treatment-related, were observed in one low-dose female and one each mid- and high-dose male. On the Day 2 and 3 clinical examinations, incoordination was noted in high-dose animals. The incidence of incoordination decreased to control levels by Day 4 in males and Day 5 in females. In high-dose animals total motor activity was significantly lower at Day 1 only.

No treatment-related gross or neuropathological findings were present.

Based on the results of this study, the LOEL for systemic toxicity was not determined in males and females; the NOEL for systemic toxicity was 227 mg/kg in males and females. The LOEL for neurobehavioral effects was 227 mg/kg in males and females; the NOEL for neurobehavioral effects was 67 mg/kg in males and females.

This study is classified as Core - Guideline and satisfies guideline requirements (§81-8) for an acute neurotoxicity screening battery in the rat.

Amendment to DER for MRID 43293901 - Chronic Neurotoxicity Screening Battery [Rat] [2,4-DICHLOROPHENOXYACETIC ACID]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 011614].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 11/19/02
Reregistration Branch I, Health Effects Division (7509C)
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Reregistration Branch I, Health Effects Division (7509C)
TXR #0051340

AMENDED DATA EVALUATION RECORD

STUDY TYPE: chronic neurotoxicity - rat § 82-7/OPPTS 870.6200
P.C. CODE: 030001 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315 CAS NO.: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [96.7%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force II on 2,4-D Research Data

CITATION: Mattsson, J. L., Jeffries, T. K., Yano, B. L. (1994). 2,4-Dichlorophenoxyacetic acid: Subchronic Neurotoxicity Study in Fischer 344 Rats. The Toxicology Research Laboratory, Dow Chemical Co.; Study No. K-002372-064N, June 28, 1994. MRID 43293901. Document No. 011614. Unpublished.

Jeffries, T. K., Yano, B. L., and Ormand, J. R. (1995). 2,4-Dichlorophenoxyacetic acid: Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats. 1-Year Interim Report. The Toxicology Research Laboratory, Dow Chemical Co.; Study No. K-002372-064, June 23, 1993. MRID 43293901. Document No. 011614. Unpublished.

EXECUTIVE SUMMARY: In this chronic toxicity/carcinogenicity study with **a chronic neurotoxicity screening battery substudy** [MRID 43293901], 50 Fischer 344 rats/sex/group [main study]; 15/sex/group [substudy] were administered 2,4-dichlorophenoxyacetic acid [96.4%] *via* the diet for up to 24 months at concentrations of 0 [basal diet], 5 mg/kg/day, 75 mg/kg/day, and 150 mg/kg/day [achieved doses were 4.6, 71.2, and 141.1 mg/kg/day [males] and 4.6, 68.0, and 138.9 mg/kg/day [females], respectively. Additionally, 10 rats/sex/group were sacrificed at 12 months [interim sacrifice]. *NOTE: This DER presents the results of the interim [one-year] sacrifice and neurotoxicity substudy.*

There were no treatment-relateddeaths or clinical signs of toxicity. Decreased body weight was observed throughout the first year of the study at the high-dose level in both sexes [males 91%/females 88% of control at the interim sacrifice] and at the mid-dose level in females [94% of control at the interim sacrifice]. At 90 days, the decrease in body weight was very slight in the high-dose males [96% of control] and somewhat greater in the mid- [95% of control] and high-dose [90% of control] females. Body-weight gains were decreased throughout the first year at the mid- and high-dose levels in both sexes, although statistical significance was not always attained in males at the mid-dose level [2-week interval (males: mid-dose 90%/high-dose 84%; females: mid-dose 81%/high-dose 63% of control); 3-month interval (males: mid-dose 98%/high-dose 88%; females: mid-dose 87%/high-dose 75% of control); and 1-year interval (males: mid-dose 95%/high-dose 82%; females: mid-dose 89%/high-dose 73% of

control). Consistent with the decreased body-weight gains was a slight decrease in food consumption, which was observed in both sexes at the high-dose level.

Ophthalmology findings were comparable among the groups at the interim sacrifice. Decreased RBC [mid- and high-dose females (6 & 12 months) and high-dose males (12 months)], HCT [mid- and high-dose females and high-dose males (6 & 12 months)], HGB [high-dose females (6 months)], WBC 9high-dose females (6 months)], and platelet counts [mid- and high-dose females and high-dose males (6 & 12 months) were observed. A dose-related increase in aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase was observed in the mid- and high-dose males at 6 months but not at 12 months. The mid- and high-dose females displayed a dose-related increase in alkaline phosphatase values at both the 6 and 12-month intervals. Cholesterol levels were decreased in the mid- (males at 12 months only) and high-dose rats of both sexes at both time intervals. T4 values were decreased at both time points in both sexes at the mid- and high-dose levels, although the mid-dose males at 6 months did not attain statistical significance. The only urinalysis finding was a decrease in specific gravity , which was observed in both sexes and time points at the mid- and high-dose levels.

Thyroid weights [absolute and relative] were statistically-significantly increased in the mid-dose females [≈20% greater than control] and in both sexes at the high-dose level [≈20% greater than control] at the 12-month interim sacrifice. Decreased testes weights [absolute and relative to brain] were observed at the high-dose level at 12 months [≈15% lower than control]. Kidney weights [absolute and relative] were increased at the 12-months sacrifice in males at the mid- [7%-12% greater than control] and high-dose [8%-16% greater than control] levels [dose-related].

Gross pathology findings included decreased fat in high-dose females [4/10] and multifocal pale foci in the lungs [1/10 mid-dose, 10/10 high-dose females]. Microscopically, there were increased incidences of lesions in the bone marrow [decreased hematopoiesis in high-dose females], eyes [bilateral retina degeneration in 1 male and 10 females at high dose], kidney [proximal tubule degeneration in mid- and high-dose males and females], liver [altered tinctorial properties in mid-dose females and both sexes at high dose], lungs [multifocal , subacute to chronic inflammation (mid-dose females, both sexes at high dose), alveolar histiocytosis in females at high dose], adipose tissue [atrophy in mid- and high-dose females], testes [atrophy at high dose], and thyroid [decreased secretory material, epithelial cells-high-dose females].

The NOAEL is 5 mg/kg/day. The LOAEL of 75 mg/kg/day is based on decreased body weight (females)/body-weight gain (both sexes), alterations in hematology [decreased RBC, HCT, and platelets (females)], clinical chemistry parameters [increased alanine and aspartate aminotransferases (males), alkaline phosphatase (both sexes), decreased cholesterol (both sexes), and decreased T4 (both sexes)], and urinalysis [decreased specific gravity (both sexes)], increased kidney weights (males), increased incidence of degeneration of the descending proximal tubules (both sexes), hepatocellular hypertrophy with altered tinctorial properties (females), lung inflammation (females), adipose tissue atrophy (females). At the high-dose level, there also were microscopic lesions in the eyes (both sexes), liver (males), testes (males), thyroid (females), and lungs (males).

NEUROTOXICITY STUDY

No treatment-related, hand-held, FOB observations were noted at any of the evaluation periods. Relative forelimb grip strength was significantly increased in both sexes at the high-dose level, but there was no treatment-related change in absolute grip strength. There was no treatment-related effect on motor activity. In agreement with the chronic toxicity portion of the study, an increased incidence of bilateral retinal degeneration was observed in the high-dose females.

The NOAEL for neurotoxicity is 75 mg/kg/day, based on increased relative forelimb grip strength at the LOAEL of 150 mg/kg/day.

This chronic toxicity/neurotoxicity study in the rat is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [OPPTS 870. 6200; §82-7] for a subchronic neurotoxicity study in the rat.

Table 1. Body-Weight/Body-Weight Gain				
Parameter/Day/Sex/Dose	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
BODY WEIGHT [g]				
MALES				
-2	197.6±11.5	197.6±9.9	197.1±9.2	197.8±10.6
6	217.5±13.1	216.8±12.0	214.4±11.1	213.5±11.9
27	246.0±15.9	247.7±13.9	243.3±14.6	239.5±14.6*[97]
90	308.3±22.1	311.8±20.0	305.3±20.4	295.2±19.4*[96]
365	409.8±28.5	415.5±23.5	400.0±30.2	372.5±26.6*[91]
FEMALES				
-2	129.2±4.9	127.9±5.6	126.6±5.0*[98]✓	126.2±5.2*[98]
6	139.2±5.6	138.4±5.6	134.1±5.7*[96]	130.7±5.1*[94]
27	160.5±7.4	160.3±7.6	151.1±6.3*[94]	145.8±6.1*[91]
90	187.0±9.1	186.3±8.6	176.8±8.0*[95]	169.3±7.3*[91]
365	217.5±10.9	217.6±12.4	205.6±11.8*[95]	190.7±9.1*[88]
BODY-WEIGHT GAIN [g]				
MALES				
-2-6	19.9±4.1	19.2±5.8	17.3±3.5*[87]	15.7±3.6*[79]
-2-13	33.0±5.7	33.3±5.0	29.8±6.4*[90]	28.0±5.6*[85]
-2-90	110.7±15.0	114.3±14.7	108.3±14.6	97.4±12.9*[88]
-2-365	212.2±20.4	217.9±19.2	202.4±25.8*[95]	175.0±20.5*[82]
FEMALES				
-2-6	10.0±2.7	10.5±2.8	7.5±3.5*[75]	4.5±3.7*[45]
-2-13	17.5±3.4	19.1±3.1*	14.2±3.3*[81]	11.0±3.5*[63]
-2-90	57.8±7.6	58.4±6.5	50.2±6.6*[87]	43.1±5.9*[75]
-2-365	88.3±10.4	89.7±10.7	79.0±10.6*[89]	64.5±8.0*[73]

✓ [% of control]; data from Tables 9-12 [pages 96-107] of the report

Table 2. Clinical Pathology Data - MALES				
Dose/Month/Parameter	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
platelets [x10E3/cumm]				
6	615±41	613±32	604±36	529±25*[86]
12	586±65	598±25	572±33	474±45*[81]
RBC[x10E6/cumm]				
6	9.82±0.35	9.85±0.30	10.11±0.26	9.60±0.34
12	9.31±0.28	9.20±0.22	9.36±0.19	8.55±0.35*[92]
HGB [g/dL]				
6	17.2±0.5	17.2±0.6	17.5±0.5	17.1±0.4
12	15.0±0.5	15.1±0.3	15.1±0.3	14.9±0.7
HCT {%}				
6	61.9±2.2	61.9±2.1	62.6±1.7	59.3±1.7*[86]
12	58.5±1.8	58.1±1.5	58.2±1.2	55.3±2.7*[95]
alanine aminotransferase [U/L]				
6	65±8	63±14	120±61*[185]	122±26*[188]
12	66±18	61±15	91±21*[138]	68±15
aspartate aminotransferase [U/L]				
6	116±16	111±21	162±64*[140]	172±27*[148]
12	106±21	95±20	132±39 [125]	103±15
blood urea nitrogen [mg/dL]				
6	17±1	17±1	18±1	18±3
12	15±2	13±1 [87]	14±2	13±1 [87]
creatinine [mg/dL]				
6	0.7±0.0	0.8±0.1	0.8±0.1	0.9±0.1*[129]
12	0.6±0.0	0.6±0.0	0.7±0.1*[117]	0.7±0.1*[117]
alkaline phosphatase [U/L]				
6	73±7	75±7	85±11*[116]	92±9*[126]
12	57±4	57±5	58±6	55±7
glucose [mg/dL]				
6	112±16	116±17	108±15	102±15 [91]
12	117±9	124±4	111±7	100±10*[85]
cholesterol [mg/dL]				
6	86±10	80±3	84±10	71±8*[83]
12	87±17	83±11	69±11*[79]	53±6*[61]
triglycerides [mg/dL]				
6	140±27	139±36	171±38	129±12
12	101±29	105±29	89±21 [88]	70±9*[69]

√[% of control]; n=10; * p<0.05;
data from Tables 15-16 [pages 112-115] and Tables23-26 [pages 128-144] of the report

Table 3. Clinical Pathology Data - FEMALES				
Dose/Month/Parameter	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
platelets {x10E3/cumm}]				
6	599±39	609±44	461±30*[77]	403±30*[67]
12	583±23	548±120 [94]	471±48*[81]	486±44*[84]
18	591±40	613±47	496±33*[84]	491±33*[83]
24	562±92	549±139	551±130	523±135 [93]
RBC[x10E6/cumm]				
6	9.11±0.21	9.05±0.22	8.71±0.16*[96]	8.22±0.28
12	8.37±0.29	8.46±0.50	7.78±0.48*[93]	7.59±0.25
18	8.61±0.18	8.41±0.21	7.83±0.93*[91]	7.61±0.25*[88]
24	8.28±1.60	7.91±1.53 [96]	7.44±1.49*[90]	7.01±1.77*[85]
HGB [g/dL]				
6	17.2±0.3	17.1±0.3	16.9±0.4	16.6±0.4*[97]
12	14.8±0.4	15.0±0.9	14.4±0.9	14.5±0.6
18	16.1±0.3	15.8±0.3*[98]	14.8±2.1*[92]	14.9±0.3*[93]
24	14.7±2.6	14.6±1.9	13.9±2.3[95]	13.3±3.0 [90]
HCT [&]				
6	60.6±1.2	60.3±2.0	58.4±1.2*[96]	55.9±2.3*[92]
12	57.3±1.9	57.7±3.4	52.6±3.1*[92]	51.8±2.2*[90]
18	46.8±1.2	xx.5±0.9✖	44.0±4.4	43.5±1.4*[93]
24	43.6±7.6	43.6±4.3	41.6±6.4 [95]	40.9±6.5 [94]
alanine aminotransferase [U/L]				
6	51±8	50±19	56±16	40±6*
12	37±10	42±20	37±8	33±10
18	42±8	40±5	34±12	36±4
24	48±28	50±28	61±78	55±62
aspartate aminotransferase [U/L]				
6	96±11	101±35	103±14	98±13
12	71±15	80±34	63±10	68±16
18	80±15	70±12	72±8	81±9
24	92±60	105±110	136±291 [148]	134±216 [146]
blood urea nitrogen [mg/dL]				
6	20±3	20±3	21±3	21±2
12	14±1	14±1	15±2	13±2
18	20±1	18±2	21±3	19±2
24	20±10	16±2	20±6	25±28
creatinine [mg/dL]				
6	0.8±0.1	0.8±0.1	0.9±0.1*[113]	0.9±0.1*[113]
12	0.7±0.1	0.7±0.1	0.8±0.1*[114]	0.7±0.0*
18	0.7±0.0	0.7±0.0*	0.9±0.0*[129]	0.9±0.1*[129]
24	0.7±0.1	0.7±0.0	0.8±0.1*[114]	0.8±0.1*[114]
alkaline phosphatase [U/L]				
6	47±9	46±4	59±7*[126]	73±16*[155]
12	32±3	32±6	47±8*[147]	54±10*[169]
18	37±5	40±10	69±48*[186]	76±18*[205]
24	47±28	63±50	84±77*[179]	99±52*[211]
glucose [mg/dL]				
6	96±12	96±10	88±9 [92]	72±8[75]
12	109±8	114±10	112±9	113±11
18	97±6	103±10	86±11*[89]	79±6*[81]
24	94±18	94±11	82±18*[87]	77±11*[82]

Table 3. Clinical Pathology Data - FEMALES				
Dose/Month/Parameter	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
cholesterol [mg/dL]				
6	116±11	123±5	81±6*[70]	76±8*[66]
12	105±13	110±16	54±7*[51]	57±7*[54]
18	155±9	148±14	76±11*[49]	81±4*[52]
24	169±38	166±29	104±57*[62]	109±36*[64]
triglycerides [mg/dL]				
6	77±14	88±22	88±16	83±6
12	52±8	56±18	54±6	56±4
18	136±46	129±34	93±14*[68]	88±14*[65]
24	183±85	180±98	126±52*[69]	105±21*[57]

√[% of control]; n=10 [6, 12, 18 months]/n=20 [24 months]; * p<0.05;
data from Tables 15-16 [pages 112-115] and Tables23-26 [pages 128-144] of the report

Table 4. T4 Results [µg/dL]				
Dose/Sex/Month	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
MALES				
6	3.2±0.5	3.4±0.6	2.7±0.2 [84]	2.1±0.9*[66]
12	3.0±0.4	3.1±0.3	2.6±0.3*[87]	0.9±0.2*[30]
FE MALES				
6	2.6±0.5	2.7±0.4	1.1±0.3*[42]	0.9±0.2*[35]
12	2.3±0.4	2.2±0.4	0.8±0.2*[35]	0.7±0.2*[30]

√[% of control]; n=10 [6, 12, 18 months]/n=20 [24 months]; * p<0.05; data from Tables 27-28 [pages 136-139] of the report

756

Table 5. Organ Weight Data - MALES [12 months]				
Dose/Organ	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	150 mg/kg/day
brain				
absolute [g]	2.039±0.105	2.000±0.066	2.025±0.062	1.988±0.095 [97]√
relative-to-body [g/100]	0.536±0.016	0.515±0.017	0.557±0.040	0.056±0.031
liver				
absolute [g]	9.508±0.662	9.630±0.661	8.543±0.677*[90]	8.942±0.370
relative-to-body [g/100]	2.496±0.062	2.476±0.111	2.338±0.080*[94]	2.520±0.125
relative-to-brain [g/100]	466.26±19.50	481.45±30.38	421.69±28.47*[90]	450.74±30.15
kidney				
absolute [g]	2.231±0.105	2.255±0.111	2.391±0.150*[107]	2.409±0.125*[108]
relative-to-body [g/100]	0.586±0.019	0.580±0.017	0.655±0.026*[112]	0.678±0.013*[116]
relative-to-brain [g/100]	109.47±3.49	112.75±4.24	118.09±6.45*[108]	121.32±6.73*[111]
testes				
absolute [g]	3.158±0.293	3.247±0.128	3.198±0.193	2.669±0.540*[85]
relative-to-body [g/100]	0.832±0.092	0.836±0.032	0.877±0.052	0.748±0.139 [90]
relative-to-brain [g/100]	155.10±14.49	162.35±5.67	157.96±8.63	134.09±26.32*[86]
thyroid				
absolute [g]	0.027±0.002	0.025±0.002	0.026±0.003	0.032±0.003*[119]
relative-to-body [g/100]	0.0071±0.0006	0.0064±0.0006	0.0070±0.0006	0.0091±0.0009*[128]
relative-to-brain [g/100]	1.3178±0.1091	1.2517±0.1068	1.2582±0.1198	1.6308±0.1805*[124]
terminal body weight [g]	380.8±21.9	388.6±14.8	365.5±28.3	355.4±18.8*[93]

√[% of control]; n=10; * p<0.05; data from Tables 27-28 [pages 136-139] of the report

Table 6. Organ Weight Data - FEMALES [12 months]				
Dose/Organ	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	7.5 mg/kg/day
brain				
absolute [g]	1.833±0.058	1.852±0.051	1.837±0.049	1.796±0.056
relative-to-body [g/100]	0.926±0.037	0.887±0.045	0.942±0.060	1.013±0.056*[109] √
liver				
absolute [g]	5.294±0.350	5.560±0.418	5.354±0.445	5.499±0.422
relative-to-body [g/100]	2.673±0.154	2.655±0.125	2.735±0.118	3.094±0.171*[116]
relative-to-brain [g/100]	288.80±15.35	300.34±23.66	291.59±24.39	305.97±19.45 [106]
kidney				
absolute [g]	1.440±0.069	1.481±0.112	1.558±0.092	1.544±0.148 [107]
relative-to-body [g/100]	0.727±0.029	0.708±0.041	0.797±0.036*[110]	0.868±0.061*[119]
relative-to-brain [g/100]	78.55±2.53	80.05±6.75	84.89±5.60 [108]	85.94±7.63*[109]
ovaries				
absolute [g]	0.057±0.013	0.056±0.012	0.058±0.005	0.052±0.009 [91]
relative-to-body [g/100]	0.029±0.006	0.027±0.005	0.029±0.003	0.029±0.005
relative-to-brain [g/100]	3.11±0.70	3.03±0.64	3.13±0.24	2.87±0.44 [92]
thyroid				
absolute [g]	0.018±0.001	0.019±0.002	0.022±0.001*[122]	0.022±0.003*[122]
relative-to-body [g/100]	0.0093±0.0008	0.0091±0.0010	0.0111±0.0006*[119]	0.0126±0.0012*[135]
relative-to-brain [g/100]	1.0052±0.0872	1.0276±0.1280	1.1816±0.0659*[118]	1.2463±0.1265*[124]
terminal body weight [g]	198.2±10.5	209.3±10.5	195.7±13.1	177.7±9.8*[90]

√[% of control];* p<0.05; n=10; data from Tables 27-28 [pages 136-139] of the report

757

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 10 July 95
Section II, Tox. Branch II (7509C)

Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 7/10/95
Section II, Tox. Branch II (7509C)

011614

DATA EVALUATION RECORD

STUDY TYPES: Chronic (1-year) Neurotoxicity Screening Battery - Rat
Chronic Feeding/Oncogenicity Study - Interim Report (§83-5)

EPA ID NOs.: MRID No.: 432939-01
Pesticide Chemical Code: 030001
Toxicology Chemical Code: 315
DP Barcode: D205344
Submission No.: S469648

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid

SYNONYMS: 2,4-D

STUDY NOs: K-002372-064N (§82-7)
K-002372-064I (§83-5)

SPONSOR: Industry Task Force II on 2,4-D Research Data

TESTING LAB: The Toxicology Research Laboratory
Health and Environmental Sciences
The Dow Chemical Company, Midland, MI

REPORT TITLE: 2,4-Dichlorophenoxyacetic Acid: Subchronic Neurotoxicity Study in Fischer 344 Rats

2,4-Dichlorophenoxyacetic Acid: Chronic Toxicity/ Oncogenicity Study in Fischer 344 Rats - 1-Year Interim Report

AUTHORS: T.K. Jeffries, B.L. Yano and J.R. Ormand (§83-5)
J.L. Mattsson, T.K. Jeffries, and B.L. Yano (§82-7)

REPORTS ISSUED: 23 June 1993 (§83-5), 28 June 1994 (§82-7)

EXECUTIVE SUMMARY: In this chronic feeding/oncogenicity study with a chronic neurotoxicity screening battery substudy, Fischer 344 rats (50/sex/dose, main study; 15/dose/sex, substudy) were fed diets containing 0 (basal diet), 5, 75, or 150 mg/kg/day 2,4-D (achieved dosages: 4.6, 71.2, or 141.1 mg/kg, males; 4.6, 68.0 or 138.9 mg/kg/day, females). Body weights, feed consumption and clinical signs were evaluated in all animals. Clinical pathology was performed at 6 and 12

months. Neurobehavioral evaluations, consisting of Functional Observational Battery (FOB) and motor activity, were conducted at prestudy and after 3, 6, 9 and 12 months of treatment. After one-year of treatment, chronic study animals (10/sex/dose) were euthanized and routine histopathological examination, neurohistopathology was performed on neurotoxicity study animals (5/sex/dose).

Chronic Toxicity/Oncogenicity Study

Statistically significant, treatment-related decreases in mean body weights and body weight gains were observed in high-dose males and females. Compared to the control values, the mean body weights of high-dose animals were 9.1% lower in males and 12.4% in females; in mid-dose animals the decreases were 2.4% in males and 5.5% in females. Feed consumption was slightly, but not significantly, lower in high-dose males (2 to 7%) and females (5 to 14%).

During the study, one high- and two mid-dose males died or were sacrificed in moribund condition; the deaths were not considered treatment-related. All females survived through one-year of treatment.

Clinical pathological effects included alterations in hematology, clinical chemistry and urinalysis parameters. Hematological effects included decreased RBC counts, hematocrit and platelet counts in mid-dose females and high-dose males and females and decreased hemoglobin concentration and WBC counts in high dose females. Elevated alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities were observed in mid- and high-dose males at the 6-month evaluation; values were within control range at 12 months. Thyroxine and cholesterol levels were decreased at 6 months in mid-dose females and high-dose males and females, at 12 months both of these parameters were decreased in mid- and high-dose males and females. Alkaline phosphatase activities were increased at 6 months in mid- and high-dose males and females and at 12 months in mid- and high-dose females. Urine specific activity was significantly decreased at the 6 and 12 month evaluations in both mid- and high-dose animals.

At the interim sacrifice, treatment-related changes were observed in organ weights and gross pathological and histopathological findings. Mid- and high-dose animals had significant changes in absolute and/or relative kidney, thyroid, liver (females) and testes (males) weights. Gross pathological findings in high dose males (bilateral, flaccid testes) was confirmed at histopathological examination, which revealed diffuse, bilateral tubule atrophy in the testes. Gross findings in high-dose females (atrophy of mesenteric fat tissue and pale multifocal lung lesions) were also confirmed at histopathological examination, which revealed adipose tissue atrophy in the mesenteric tissues and inflammation of the lungs of mid- and high-dose females. Mid- and high-dose males and females had degeneration of the descending renal proximal tubules. Histopathological lesions in the thyroid consisted of decreased amount secretory material in high-dose females. Although

the ophthalmological examinations did not reveal any treatment-related effects, histopathological examinations in both the chronic toxicity and neurotoxicity studies showed an increased incidence of unilateral retinal degeneration in high-dose males and mid-dose females and bilateral retinal degeneration in high-dose males and females. Mid-dose females and high-dose males and females had increased incidence of liver lesions. In males the lesions were described as altered tinctorial properties resulting from increased eosinophilia and females hepatoceullar hypertrophy with altered tinctorial properties.

Based on the results of the chronic toxicity study (deceased body weight/body weight gains, clinical pathology, histopathology), the LOEL for systemic toxicity was 75 mg/kg/day in males and females; the NOEL for systemic toxicity was 5 mg/kg /day in males and females.

The chronic toxicity/oncogenicity study is classified as **Core - Supplementary** and does not satisfy guideline requirements (§83-5) since it is an interim, one-year report.

Chronic Neurotoxicity Study

No treatment-related FOB handheld observations were noted at either the 3-, 6-, 9- or 12-month evaluations. Relative forelimb grip strength (g of grip/g BW) was significantly increased in high-dose males and females; no treatment-related changes were noted in the absolute grip strength. Mean body weights (measured in conjunction with the chronic toxicity study) were significantly and biologically lower in high-dose males and females. A higher incidence of minimal to pronounced urination was noted in high-dose females at 9 and 12 months

No treatment-related changes were noted in motor activity and any of the evaluation times.

In agreement with the histopathology findings of the chronic toxicity study, neuropathological examination revealed an increased incidence of bilateral retinal degeneration in high-dose females; males were not affected.

Based on the results of the neurotoxicity study (decreased body weight and increased relative forelimb grip strength), the LOEL was 150 mg/kg/day in males and females; the NOEL was 75 mg/kg/day in males and females.

The chronic neurotoxicity study is classified as **Core - Acceptable** and satisfies the guideline requirements (§82-7) for a subchronic neurotoxicity study.

I. MATERIALS

A. **Test Compound:** 2,4-D, technical; Description: white solid; Lot No.: 909; Purity: 96.45%; Contaminants: Not given

B. **Test Animals:** Species: Rat; Strain: Fischer 344; Age: Approximately 7 weeks; Weight (g): 175.7 - 218.4 (males), 117.7- 137.4 (females); Source: Charles River Research Laboratories, Kingston, NY; Housing: Two/cage in mesh-bottom cages; Feed: Certified Purina Rodent Chow #5002, *ad libitum*; Water: tap water, *ad libitum*; Environment Conditions: Temperature: 19.9 - 24.8°C; Humidity: 47 - 68%; Light/dark cycle: 12hr/12hr; Air changes: 10 - 12/hr

II. STUDY DESIGN: Fischer 344 rats were randomly assigned to the chronic toxicity/oncogenicity (50/sex/dose) study and neurotoxicity screening battery (15/sex/dose) and fed test diets, formulated to yield 0 (basal diet), 5, 75 or 150 mg/kg/day (Table 1). Animals in the chronic toxicity/oncogenicity study were scheduled to receive test diets for two years, with an interim, one year, sacrifice; neurotoxicity study animals were fed test diets for one year.

Table 1: Animal Assignment to Chronic Toxicity/Oncogenicity Study and Neurotoxicity Screening Battery Groups

Test Group	Dosage (mg/kg/day)	Chronic Toxicity Study		Neurotoxicity Study	
		Male	Female	Male	Female
Control	0	50	50	15	15
Low	5	50	50	15	15
Mid	75	50	50	15	15
High	150	50	50	15	15

III. REGULATORY COMPLIANCES

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

C. A statement of "no confidentiality claims" was provided.

IV. STATISTICAL EVALUATIONS: *The means and standard deviations of* parametric data (body weight, hindlimb and forelimb grip strength, landing foot splay, and motor activity) *were determined and variances (F-max test)* evaluated for homogeneity. Grip strength was normalized for body weight; motor activity was expressed as *the square roots of the counts.* Repeated measures analyses (ANOVA or MANOVA) were used to evaluate different interactions (treatment × time, treatment × time × sex, and/or treatment × time × epoch). The type I error rate (α) per comparison was set at 0.02 to decrease the rate of false positive responses.

V. METHODS and RESULTS

A. Diet Preparation and Analysis: Test diets were formulated by serial dilution of a concentrated premix and were prepared weekly through week 13 and every two weeks, thereafter. The initial concentration of 2,4-D in the diets was determined from the pretest body weight and feed consumption data. During the study, the most recent body weight and feed consumption data were used to calculate the concentration of 2,4-D in the diets. Test diet stability and homogeneity were determined prior to initiation of the study; achieved concentrations were determined at weekly intervals through week four and at least quarterly, thereafter.

Results: Excluding one, non-typical aliquot, samples taken from the top and bottom of the low-dose female diet indicated that 2,4-D was homogeneously distributed (relative standard deviations of 2.91, 3.67, and 6.05% at prestudy, week 7 and week 38, respectively). A test diet containing 0.00215% 2,4-D was stable for at least 48 days (91% of day 0 value). 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%, 83-102% and 70-104%, respectively, for females.

B. Daily Observations and Clinical Evaluations: Cageside observations were performed on all animals twice daily during the study. Detailed clinical examinations were performed at prestudy and weekly during the study.

Results: At various times during the study, perineal soiling was noted in 6.2% (5/65), 4.6% (3/65), 23.1% (15/65) and 26.2% (17/65) of the control, low-, mid- and high-dose males, respectively. Unilateral ocular opacities were noted in 3.1% (2/65), 0% (0/65), 12.3% (8/65) and 9.2% (6/65) of the control, low-, mid- and high-dose females, respectively.

Two mid-dose males died on days 104 and 361, one high-dose male was sacrificed *in extremis* on day 333. During the first year of the study, no female deaths or moribund sacrifices occurred.

C. Ophthalmological Examinations (Chronic Toxicity Study): All animals were examined for ophthalmological abnormalities three weeks prior to initiation of the study and those with eye lesions were rejected from the study. At the interim (one-year) sacrifice, 10 animals/sex/dose underwent ophthalmological examinations.

Results: At the interim sacrifice, the incidence of ophthalmological abnormalities was not suggestive of any treatment-related effect.

D. Body Weights and Body Weight Gains (Chronic Toxicity and Neurotoxicity Studies): Body weights were determined at prestudy, at weekly intervals through week 13 and at monthly intervals, thereafter.

Results: Statistically significant decreases in mean body weights were observed in high-dose males from study days 27 through 365 and in mid- and high-dose females from study days -2 to 365 (Table 2). Compared to the control values, the mean body weights of high-dose animals were 9.1% lower in males and 12.4% in females.

Cumulative body weight gains were significantly lower in high-dose males and females and mid-dose females at all of the time intervals measured; representative data are presented in Table 3.

Table 2: Representative Mean Body Weights (g)[a]

Sex	Day	Dosage (mg/kg/day)			
		0	5	75	150
Male	-2	198	198	197 (0.5)[b]	198 (0.0)
	27	246	248	243 (1.2)	240*(2.4)
	90	308	312	305 (1.0)	295*(4.2)
	168	350	356	348 (0.6)	331*(5.4)
	365	410	416	400 (2.4)	373*(9.1)
Female	-2	129	128	127*(1.6)	126*(2.3)
	27	161	160	151*(6.2)	146*(9.3)
	90	187	186	177*(5.4)	169*(9.6)
	168	197	198	186*(5.6)	176*(10.7)
	365	218	218	206*(5.5)	191*(12.4)

[a] Data summarized from study Tables 9 and 10 (pp. 96 through 99, respectively).
[b] *Values in parentheses are the % decrease from control value.*
$ $p \leq 0.05$ compared to control (Wilcoxon's)
* $p \leq 0.05$ compared to control (Dunnett's)

Table 3: Representative Cumulative Mean Body Weight Gains (g)[a]

Sex	Day -2 to	Dosage (mg/kg/day)			
		0	5	75	150
Male	6	19.9	19.2	17.3$(13.1)[b]	15.7$(21.1)
	13	33.0	33.3	29.8*(9.7)	28.0*(15.2)
	27	48.3	50.1	46.2 (4.4)	41.7*(13.7)
	90	110.7	114.3	108.3 (2.2)	97.4*(12.0)
	168	152.3	157.9	150.6 (1.1)	132.7*(12.9)
	365	212.2	217.9	202.4*(4.6)	175.0*(17.5)
Female	6	10.0	10.5	7.5*(25.0)	4.5*(55.0)
	13	17.5	19.1*	14.2*(18.9)	11.0*(37.1)
	27	31.3	32.4	24.5*(21.7)	19.6*(37.4)
	90	57.8	58.4	50.2*(13.2)	43.1*(25.4)
	168	67.9	69.6	59.7*(12.1)	49.6*(27.0)
	365	88.3	89.7	79.0*(10.5)	64.5*(27.0)

[a] Data summarized from study Tables 11 and 12 (pp. 100 through 107, respectively).
[b] Values in parentheses are the % decrease from control value.
* $p \leq 0.05$ compared to control (Dunnett's)
$ $p \leq 0.05$ compared to control (Wilcoxon's)

E. Feed Consumption and Achieved Compound Intake: Feed consumption was determined at prestudy, at weekly intervals through week 13, and for a one-week period each month, thereafter.

1. Feed consumption: Mean feed consumption was slightly, but not significantly, lower in high-dose males (2 to 7%) and females (5 to 14%); the differences were not great enough to be treatment-related. Mean food consumption of low- and mid-dose animals were comparable to control values.

2. Achieved compound intake: The achieved compound intakes are summarized in Table 4.

Table 4: Achieved Compound Intake (% of Target)[a]

Sex	Dosage (mg/kg/day)		
	5	75	150
Male	4.6[b] (92%)	71.2 (95%)	141.1 (94%)
Female	4.6 (92%)	68.0 (91%)	138.9 (93%)

[a] Summarized from study Table 32 (p. 159)
[b] Time weighted average compound intake

F. Clinical Pathology (Chronic Toxicity Study)

1. Hematology: Orbital sinus blood was collected from 10 animals/sex/dose after approximately 6 and 12 months of treatment. The following hematological parameters were evaluated:

Hematocrit (HCT)
Hemoglobin (HGB)
Erythrocyte count (RBC)
Platelet count (PLAT)
Total leukocyte count (WBC)
Leukocyte differential count & cell morphology

Results: Hematological findings are summarized in Table 5. High-dose males had statistically significant decreases in hematocrit and platelet counts at the 6- and 12-month evaluations; RBC counts were decreased at 12 months only. Mid- and high-dose females had statistically significant decreases in RBC counts, hematocrit, and platelet counts at 6 and 12 months. High-dose females had statistically significant decreases in hemoglobin concentrations and WBC counts at 6 months; values at 12 months were comparable to controls. No treatment-related effects were noted in WBC differential counts.

Table 5: Hematology[a]

Sex	Parameter	Month	Dosage (mg/kg/day)			
			0	5	75	150
Male	RBC ($10^6/mm^3$)	6	9.82	9.85	10.11	9.69
		12	9.31	9.20	9.36	8.55* 97
	HCT (%)	6	61.9	61.9	62.6	59.3* 96
		12	58.5	58.1	58.2	55.3* 95
	PLAT ($10^3/mm^3$)	6	615	613	604 98	529* 86
		12	586	598	572	474* 81
Female	RBC ($10^6/mm^3$)	6	9.11	9.05	8.71* 96	8.22* 90
		12	8.37	8.46	7.78* 93	7.59* 91
	HGB (g/dl)	6	17.2	17.1	16.9	16.6*
		12	14.8	15.0	14.4	14.5
	HCT (%)	6	60.6	60.3	58.4*	55.9*
		12	57.3	57.7	52.6*	51.8*
	PLAT ($10^3/mm^3$)	6	599	609	461*	403*
		12	583	548	471$	486$
	WBC ($10^3/mm^3$)	6	3.3	3.3	3.3	2.4*
		12	2.7	3.0	2.9	2.5

[a] Data summarized from Tables 15 and 16 (pp. 112 through 115, respectively) of the study.
$ $p \leq 0.05$ (Wilcoxon's Test)
* $p \leq 0.05$ (Dunnett's Test)

2. **Clinical chemistry:** Clinical chemistry evaluations were performed after approximately 6 and 12 months of treatment on 10 animals/sex/dose. The following parameters were evaluated:

ELECTROLYTES
- Calcium
- Chloride
- Phosphorous
- Potassium
- Sodium

ENZYME ACTIVITIES
- Alkaline phosphatase (AP)
- Creatinine phosphokinase
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)

OTHER
- Albumin
- Creatinine
- Blood urea nitrogen
- Cholesterol (CHOL)
- Globulins
- Glucose
- Total bilirubin
- Triglycerides
- Total protein
- Thyroxine (T4)

Results: Clinical chemistry findings are summarized in Table 6. At the 6-month evaluation, mid- and high-dose males had increased ALT and AST activities compared to both the concurrent and historical control values. At 12 months, ALT and AST activities were comparable to control values. AP activities were significantly

Table 6: Clinical Chemistry Results[a]

Sex	Parameter	Month	Dosage (mg/kg/day)			
			0	5	75	150
Male	ALT (mU/l)	6	65	63	120$[b]	122$[b]
		12	66	61	91	68
	AST (mU/l)	6	116	111	162$[c]	172$[c]
		12	106	95	132	103
	AP (mU/l)	6	73	75	85*	92*
		12	57	57	58	55
	T4 (μg/ml)	6	3.2	3.4	2.7	2.1$
		12	3.0	3.1	2.6*	0.9*
	CHOL (mg/dl)	6	86	80	84	71*
		12	87	83	69*	53*
Female	AP (mU/l)	6	47	46	58$	73$
		12	32	32	47*	54*
	T4 (μg/ml)	6	2.6	2.7	1.1*	0.9*
		12	2.3	2.2	0.8*	0.7*
	CHOL (mg/dl)	6	116	123	81*	76*
		12	105	110	54*	57*

[a] Data summarized from study Tables 23, 24, 25 and 26 (pp. 128, 130, 133 and 144, respectively)
[b] ALT historical control values: 27.45 to 84.33 U/l
[c] AST historical control values: 53.30 to 145.82 U/l
$ $p \leq 0.05$ (Wilcoxon's Test)
* $p \leq 0.05$ (Dunnett's Test)

elevated in mid- and high-dose males and females at the 6-months and mid- and high-dose females, but not males, at 12 months. High-dose males and mid- and high-dose females had significantly decreased T4 levels at 6 months and mid- and high-dose males and females had decreased levels at 12 months. At the 6-month evaluation, high-dose males and mid- and high-dose females had significantly decreased cholesterol level; at 12 months, decreased levels were noted in mid- and high-dose males and females. Other statistically significant findings were observed in both mid- and high-dose males and females but were not considered toxicologically or biologically significant.

3. Urinalysis: Urine was collected, using manual compression of the bladder, approximately 1 to 2 weeks before the 6- and 12-month blood collections. Microsediment was characterized from pooled urine samples from each group. The following parameters were examined:

Specific gravity	Appearance	Occult blood	Glucose
	Sediment	Urobilinogen	Ketone Bodies
Protein	pH	Bilirubin	

Results: Urinalysis results were limited to statistically significant decreases in specific gravity in mid- and high-dose males at 6 and 12 months and mid- and high-dose females at 6 months (Table 7). A statistically significant decrease was noted in mid-dose females at 12 months. No other treatment-related urinalysis findings were observed.

Table 7: Urine Specific Gravity[a]

Sex	Month	Dosage (mg/kg/day)			
		0	5	75	150
Male	6	1.058	1.059	1.052*	1.049*
	12	1.053	1.053	1.040*	1.038*
Female	6	1.062	1.053	1.044$	1.046$
	12	1.048	1.046	1.038*	1.045

[a] Data summarized from study Tables 19 and 20 (pp. 120 through 123).
$ $p \leq 0.05$ (Wilcoxon's Test)
* $p \leq 0.05$ (Dunnett's Test)

G. Behavioral Tests (Neurotoxicity Study): Behavioral tests, performed at preexposure and after 3, 6, 9 and 12 months of treatment, consisted of evaluation of Functional Observational Battery (FOB) and motor activity.

1. **Functional Observational Battery:** FOB evaluations included handheld and open-field observations and measurement of grip strength (absolute and normalized for body weight) and landing foot splay. Body weights were determined as part of the chronic toxicity study; body weights taken nearest in time to the FOB evaluations were evaluated statistically and presented with the FOB results. The following parameters were evaluated:

HANDHELD OBSERVATIONS
- General
- Palpebral closure
- Pupil size
- Lacrimation
- Salivation
- Skin/haircoat abnormalities
- Perineal staining
- Abnormal movements
  - Convulsions, tremors
  - muscle tone, etc.
- Abnormal respiration
- Reactivity to handling

OPEN-FIELD OBSERVATIONS
- Level of activity
- Startle response
- Touch response
- Tail pinch response
- Gait abnormalities
- Abnormal/stereotypic behavior
- Quantity of urine/fecal pellets

MEASUREMENTS
- Body weight
- Hindlimb grip strength
- Forelimb grip strength
- Landing foot splay

Results: No treatment-related FOB handheld observations were noted at either the 3-, 6-, 9- or 12-month evaluations. Relative forelimb grip strength (g of grip/g BW) of high-dose males and females was significantly increased (Appendix 1); no treatment-related changes were noted in the absolute grip strength. A higher incidence of minimal to pronounced urination was noted in high-dose females at 9 and 12 months (Table 8).

Table 8: FOB Results - Female Rats[a]

Observation		Month	Dosage (mg/kg/day)			
			0	5	75	150
Urination	None	9	8/10	8/10	7/10	2/10
		12	7/10	7/10	5/10	4/10
	Present	9	2/10	2/10	3/10	8/10
		12	3/10	3/10	5/10	6/10

[a] Summarized from study Table V-3b (pp. 62 through 64)

2. **Motor activity:** Motor activity was measured in circular chambers fitted with a calibrated photobeam activity recording device; data were automatically recorded over a 48-min session, consisting of six

8-min epochs. Data were square root transformed prior to statistical evaluation. Evaluations were counterbalanced by dose and sex.

Results: *No treatment-related differences were noted in either the* total motor activity or activity within each epoch. Motor activity at the 3-, 6-, 9-, and 12-month evaluations were comparable to the prestudy values.

3. **Neurobehavioral positive controls:** Positive control data were included in the study. For validation of neurobehavioral effects, the following positive controls were used: Amphetamine (0.1, 0.32 or 1 mg/kg, ip) and chlorpromazine (0.5, 2.24 or 5 mg/kg, ip) for motor activity; amphetamine (8 mg/kg, ip), chlorpromazine (4 mg/kg, ip), or atropine (2 mg/kg, ip) plus physostigmine (0.75 mg/kg, sc) for FOB. Positive control neuropathology animals were treated with trimethyltin (7 mg/kg, po) on Day 1 followed by treatment with acrylamide (35 mg/kg, po) 5 times/week for 3 weeks. The positive control materials accurately validated the FOB and motor activity findings.

H. Pathological Examinations (Chronic Toxicity Study): After approximately 12 months of treatment, fasted animals (10/sex/dose) were weighed and sacrificed for gross and histopathological examination. Selected tissues (listed below) from the control and high-dose animals were examined histologically. Brains (excluding olfactory lobes) were examined in intermediate dose males.

TISSUES SELECTED FOR HISTOPATHOLOGY[a]

DIGESTIVE SYSTEM	CARDIOVASCULAR/HEMATOLOGY	NEUROLOGIC
Tongue	Aorta	**BRAIN** (cerebrum, brainstem, & cerebellum)
Salivary glands	HEART	Peripheral nerve
Esophagus	**Bone marrow**	Spinal cord (cervical, thoracic, & lumbar)
Stomach	Mediastinal & mesenteric lymph nodes & tissues	Eyes
Duodenum	Spleen	GLANDULAR
Jejunum	Thymus	Adrenals
Ileum	UROGENITAL	Auditory sebaceous glands
Cecum	**KIDNEYS**	Coagulating glands
Colon	Urinary bladder	Harderian/lacrimal glands
Rectum	**TESTES**	Mammary gland
LIVER	**Epididymides**	Pituitary
Pancreas	Prostate	***THYROIDS*** with parathyroids
Oral tissues	Seminal vesicles	OTHER
RESPIRATORY	OVARIES	Bone with joint
Trachea	Uterus	Skeletal muscle
Lungs	Vagina	Skin and subcutis
Nasal tissues	Cervix	**Gross lesions**
Larynx	Oviducts	

[a] Tissues in **bold** type were examined in all dose groups; the absolute and relative organ weights were measured in tissues in CAPITALIZED type.

1. **Terminal body and organ weights:** Terminal (fasted) body weights and selected absolute and relative organ weights are summarized in Table 9. Terminal body weights of high-dose animals were significantly lower (7%, males; 10%, females) than control values. Mid- and high-dose animals had significantly increased relative and absolute (males only) kidney weights. Relative and absolute thyroid weights were significantly increased in high-dose males and mid- and high-dose females. Absolute, but not relative testes weights were significantly lower than control values. Two high-dose males (92A1243 and 92A1246) had markedly lower absolute (1.96 and 1.49 g, respectively) and relative (0.525 and 0.483 g/100 g BW, respectively) testes weights. Excluding these values, the mean absolute and relative testes weights for the remaining eight animals were 2.904 g and 0.810 g/100 g BW, respectively. In high-dose females, absolute adrenal gland weights were significantly decreased; relative liver and brain weights slightly increased.

Table 9: Terminal Body Weights (g) and Absolute (g) and Relative (g/100 g BW) Organ Weights[a]

Sex	Parameter		Dosage (mg/kg/day)			
			0	5	75	150
Male	Body Weight (fasted)		381	389	366	355*
	Kidneys	Absolute	2.231	2.255	2.391*	2.409*
		Relative	0.586	0.580	0.655*	0.678*
	Thyroid	Absolute	0.027	0.025	0.026	0.032*
		Relative	0.0071	0.0064	0.0070	0.0091*
	Testes	Absolute	3.158	3.247	3.198	2.669$
		Relative	0.832	0.836	0.877	0.748
Female	Body Weight (fasted)		198	209	196	178*
	Kidneys	Absolute	1.440	1.481	1.558	1.544
		Relative	0.727	0.708	0.797*	0.868*
	Liver	Absolute	5.294	5.560	5.354	5.499
		Relative	2.673	2.655	2.735	3.094*
	Adrenals	Absolute	0.053	0.055	0.054	0.045*
		Relative	0.0266	0.0264	0.0275	0.0255
	Thyroid	Absolute	0.018	0.019	0.022*	0.022*
		Relative	0.0093	0.0091	0.0111*	0.0126*
	Brain	Absolute	1.833	1.852	1.837	1.796
		Relative	0.926	0.887	0.942	1.013*

[a] Data summarized from Tables 27 and 28 (pp. 136 through 139) of the study.
$ $p \leq 0.05$ (Wilcoxon's Test)
* $p \leq 0.05$ (Dunnett's Test)

2. **Gross pathology:** High-dose females had increased incidences of decreased body fat (4/10) and pale multifocal lesions in the lung (10/10). Two high-dose males (92A1243 and 92A1246) had bilateral flaccid testes.

3. **Histopathology:** Histopathological data are summarized in Table 10; a complete histopathological report is enclosed (Appendix 2).

Increased incidences of retinal degeneration were observed in high-dose males and mid- and high-dose females. High-dose males showed 2/10 showed severe unilateral retinal degeneration and 1/10, moderate bilateral degeneration. Females were more severely affected with 6/10 mid-dose animals showing very slight to severe unilateral degeneration and 10/10 high-dose animals showing very slight to severe bilateral degeneration. The degeneration was described in the study (p. 30) as "... partial to complete loss of the rod and cone layer and the outer nuclear layer."

Liver evaluations revealed an increased incidence of hepatocellular hypertrophy and/or altered tinctorial properties in high-dose males and mid- and high-dose females. In males the centrilobular and midzonal regions were affected, while in females the effects were panlobular, affecting all hepatocytes within the hepatic lobule. No hepatocellular degeneration or necrosis was noted.

Kidney lesions, noted in mid- and high-dose males and females, were described in the study (p. 29) as "...very slight to slight loss of the brush border and increased vacuolation of the epithelial cell cytoplasm of the straight of descending portion of the proximal convoluted tubules."

Thyroid lesions, consisting of decreases in secretory material (thyroglobulin) within the thyroid follicles, were observed in 8/10 high-dose females; males were not affected. The study (p. 30) describes the thyroid alterations as consisting of "... a population of smaller follicles, which frequently contained clear, resorption vacuoles adjacent to the epithelial cells."

Two high-dose males (92A1243 and 92A1246) had bilateral, diffuse testicular atrophy of the seminiferous tubules, which was graded as moderate to severe. The study (p. 31) states: "The seminiferous tubules of these rats did not contain normal developing sperm in adequate numbers and the tubules were frequently lined only by Sertoli cells."

Other lesions included increased incidence of alveolar histiocytosis and subacute to chronic pulmonary inflammation in high-dose males and mid- and high-dose females, adipose tissue atrophy in the mesenteric tissues of mid- and high-dose females, and increased incidence of decreased hematopoiesis in the bone marrow of high-dose females.

Table 10: Histopathology Findings - Chronic Toxicity Study[a]

Histopathological Observation			Dosage (mg/kg/day)			
			0	5	75	150
MALE						
EYES	Retinal Degeneration	Unilateral	1/10	1/10	0/9	2/10
		Bilateral	0/10	0/10	0/9	1/10
KIDNEYS	Degeneration, Proximal Tubules, Descending		0/10	0/10	8/10	10/10
LIVER	Reticuloendothelial Cell Aggregates		1/10	1/10	1/10	3/10
	Altered Tinctorial Properties, Increased Eosinophilia, Centrilobular, Midzonal		0/10	0/10	0/10	10/10
	Focus (I) of Altered Cells, Hepatocellular		0/10	0/10	3/10	3/10
LUNGS	Inflammation, Subacute to Chronic, Multifocal		0/10	0/10	0/10	2/10
TESTES	Tubule Atrophy, Bilateral, Diffuse		0/10	0/10	0/10	2/10
FEMALE						
BONE MARROW	Decreased Hematopoiesis		0/10	0/10	0/9	4/10
EYES	Retinal Degeneration	Unilateral	0/10	1/10	6/10	0/10[b]
		Bilateral	0/10	0/10	0/10	10/10
KIDNEYS	Degeneration, Proximal Tubules, Descending		1/10	0/10	7/10	9/10
LIVER	Hepatocellular Hypertrophy with Altered Tinctorial Properties, Panlobular		0/10	0/10	8/10	10/10
LUNGS	Alveolar Histiocytosis		5/10	5/10	7/10	10/10
	Inflammation, Subacute to Chronic, Multifocal		0/10	1/10	4/10	8/10
THYROID	Decreased Secretory Material, Epithelial Cells		0/10	0/10	0/10	8/10
MESENTERIC TISSUES, Adipose Tissue Atrophy			0/10	0/10	5/10	8/10

[a] Data summarized from Table 30 (pp. 143 through 157) of the study.
[b] One high-dose female had very slight degeneration in one eye and severe degeneration in the other. The pathology report (Appendix 2) summarized the data as two separate incidences of unilateral degeneration, rather than a single incidence of bilateral degeneration as summarized in this table.

I. **Neuropathology (Neurotoxicity Study):** After 12 months of treatment, five animals/sex/dose, randomly selected at prestudy, were heparinized and anesthetized. Animals were then perfused transcardially with 0.05 M phosphate buffer containing 0.7% sodium nitrite, followed by 1.5% glutaraldehyde/4% formaldehyde. Tissues (see list from chronic toxicity study) were collected for possible histopathological examination. The tissues listed below were collected; those from control and high-dose animals were examined microscopically.

TISSUES SELECTED FOR NEUROPATHOLOGICAL EXAMINATION

BRAIN	SPINAL CORD
Olfactory lobe	Cervical & lumbar swelling
Cerebral cortex	PERIPHERAL NERVES
Thalamus/hypothalamus	Proximal, tibial, peroneal, sural,
Midbrain	caudal, & optic
Cerebellum	SKELETAL MUSCLE
Medulla oblongata	Gastrocnemius, anterior tibial
Nucleus gracilis/cuneatus	OTHER
DORSAL ROOT GANGLIA	Pituitary gland
Cervical & lumbar swelling	Trigeminal ganglia & nerve
DORSAL & VENTRAL ROOTS	Eyes with optic nerves
Cervical & lumbar swelling	Nasal tissues with olfactory epithelium

Positive control neuropathology animals were treated with trimethyltin (7 mg/kg, po) on Day 1 followed by treatment with acrylamide (35 mg/kg, po) 5 times/week for 3 weeks. The positive control materials accurately validated the neuropathology findings.

Results: In agreement with the histopathology findings of the chronic toxicity study, neuropathological examination revealed an increased incidence of bilateral retinal degeneration in high-dose females; males were not affected. The ocular lesions were graded as moderate in 1/5 animals and severe in 4/5; 1/5 control females had severe unilateral retinal degeneration. The lesions were described on page 40 of the study as "... partial to complete loss of the rod and cone layer and the outer and inner nucleus layers."

V. DISCUSSION: In this chronic feeding/oncogenicity study with a chronic neurotoxicity screening battery substudy, Fischer 344 rats (50/sex/dose, main study; 15/dose/sex, substudy) were fed diets containing 0 (basal diet), 5, 75, or 150 mg/kg/day 2,4-D (achieved dosages: 4.6, 71.2, or 141.1 mg/kg, males; 4.6, 68.0 or 138.9 mg/kg/day, females). Body weights, feed consumption and clinical signs were evaluated in all animals. Clinical pathology was performed at 6 and 12 months. Neurobehavioral evaluations, consisting of Functional Observational Battery (FOB) and motor activity, were conducted at prestudy and after 3, 6, 9 and 12 months of treatment. After one-year of treatment, chronic study animals

(10/sex/dose) were euthanized and routine histopathological examination, neurohistopathology was performed on neurotoxicity study animals (5/sex/dose).

Chronic Toxicity/Oncogenicity Study

Statistically significant, treatment-related decreases in mean body weights and body weight gains were observed in high-dose males and females. Compared to the control values, the mean body weights of high-dose animals were 9.1% lower in males and 12.4% in females; in mid-dose animals the decreases were 2.4% in males and 5.5% in females. Feed consumption was slightly, but not significantly, lower in high-dose males (2 to 7%) and females (5 to 14%).

During the study, one high- and two mid-dose males died or were sacrificed in moribund condition; the deaths were not considered treatment-related. All females survived through one-year of treatment.

Clinical pathological effects included alterations in hematology, clinical chemistry and urinalysis parameters. Hematological effects included decreased RBC counts, hematocrit and platelet counts in mid-dose females and high-dose males and females and decreased hemoglobin concentration and WBC counts in high dose females. Elevated alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities were observed in mid- and high-dose males at the 6-month evaluation; values were within control range at 12 months. Thyroxine and cholesterol levels were decreased at 6 months in mid-dose females and high-dose males and females, at 12 months both of these parameters were decreased in mid- and high-dose males and females. Alkaline phosphatase activities were increased at 6 months in mid- and high-dose males and females and at 12 months in mid- and high-dose females. Urine specific activity was significantly decreased at the 6 and 12 month evaluations in both mid- and high-dose animals.

At the interim sacrifice, treatment-related changes were observed in organ weights and gross pathological and histopathological findings. Mid- and high-dose animals had significant changes in absolute and/or relative kidney, thyroid, liver (females) and testes (males) weights. Gross pathological findings in high dose males (bilateral, flaccid testes) was confirmed at histopathological examination, which revealed diffuse, bilateral tubule atrophy in the testes. Gross findings in high-dose females (atrophy of mesenteric fat tissue and pale multifocal lung lesions) were also confirmed at histopathological examination, which revealed adipose tissue atrophy in the mesenteric tissues and inflammation of the lungs of mid- and high-dose females. Mid- and high-dose males and females had degeneration of the descending renal proximal tubules. Histopathological lesions in the thyroid consisted of decreased amount secretory material in high-dose females. Although the ophthalmological examinations did not reveal any treatment-related effects, histopathological examinations in both the chronic toxicity and neurotoxicity studies showed an increased incidence of unilateral retinal degeneration in high-

dose males and mid-dose females and bilateral retinal degeneration in high-dose males and females. Mid-dose females and high-dose males and females had increased incidence of liver lesions. In males the lesions were described as altered tinctorial properties resulting from increased eosinophilia and females hepatoceullar hypertrophy with altered tinctorial properties.

Based on the results of the chronic toxicity study (deceased body weigh/body weight gains, clinical pathology, histopathology), the LOEL for systemic toxicity was 75 mg/kg/day in males and females; the NOEL for systemic toxicity was 5 mg/kg /day in males and females.

The chronic toxicity/oncogenicity study is classified as **Core - Supplementary** and does not satisfy guideline requirements (§83-5) since it is an interim, one-year report.

CHRONIC NEUROTOXICITY STUDY

No treatment-related FOB handheld observations were noted at either the 3-, 6-, 9- or 12-month evaluations. Relative forelimb grip strength (g of grip/g BW) was significantly increased in high-dose males and females; no treatment-related changes were noted in the absolute grip strength. Mean body weights (measured in conjunction with the chronic toxicity study) were significantly and biologically lower in high-dose males and females. A higher incidence of minimal to pronounced urination was noted in high-dose females at 9 and 12 months

No treatment-related changes were noted in motor activity and any of the evaluation times.

In agreement with the histopathology findings of the chronic toxicity study, neuropathological examination revealed an increased incidence of bilateral retinal degeneration in high-dose females; males were not affected.

Based on the results of the neurotoxicity study (decreased body weight and increased relative forelimb grip strength), the LOEL was 150 mg/kg/day in males and females; the NOEL was 75 mg/kg/day in males and females.

The chronic neurotoxicity study is classified as **Core - Acceptable** and satisfies the guideline requirements (§82-7) for a subchronic neurotoxicity study.

APPENDIX 1: FOB: FORELIMB GRIP STRENGTH

Table V-8. Forelimb Grip Performance (FLGP/BW) - Repeated-Measure Analysis

Sources of Variation	P values Forelimb (n = 80)
Treatment x Month	0.0048
Treatment x Month x Sex	0.4918
Contrasts	
5 mg/kg/day vs Control	0.3585
75 mg/kg/day vs Control	0.5370
150 mg/kg/day vs Control	0.0017

The overall treatment-by-month interaction was significant ($p = 0.0048$; $\alpha = 0.02$); i.e., forelimb grip ratios were affected by treatment during the study.

The treatment-by-month-by-sex interaction was not significant ($p = 0.4918$; $\alpha = 0.02$); i.e., treatment did not cause a significantly different grip response in males versus females at any test period.

Treatment versus control contrasts were evaluated because of the significant overall treatment-by-month interaction. The treatment-by-month contrast of 150 mg/kg/day vs control was statistically significant ($p = 0.0017$; $\alpha = 0.02$).

Table V-9. Forelimb Grip Performance (FLGP/BW) - Descriptive Statistics

		Dose (mg/kg/day)			
Males		0	5	75	150
Preexposure	Mean	2.06	1.94	2.20	1.65
	S.D.	0.56	0.19	0.36	0.30
	N	10	10	10	10
Month 3	Mean	1.87	2.16	2.20	1.90
	S.D.	0.51	0.39	0.27	0.48
	N	10	10	10	10
Month 6	Mean	1.51	1.67	1.59	1.70
	S.D.	0.41	0.44	0.44	0.46
	N	10	10	10	10
Month 9	Mean	1.46	1.38	1.47	1.70
	S.D.	0.39	0.50	0.38	0.39
	N	10	10	10	10
Month 12	Mean	1.27	1.31	1.36	1.56
	S.D.	0.29	0.34	0.37	0.32
	N	10	10	10	10
Females					
Preexposure	Mean	2.43	2.32	2.76	2.23
	S.D.	0.46	0.47	0.63	0.39
	N	10	10	10	10
Month 3	Mean	2.50	2.48	2.86	2.88
	S.D.	0.48	0.49	0.47	0.45
	N	10	10	10	10
Month 6	Mean	2.60	2.44	2.61	2.96
	S.D.	0.40	0.38	0.44	0.64
	N	10	10	10	10
Month 9	Mean	2.51	2.17	2.66	2.33
	S.D.	0.66	0.49	0.52	0.33
	N	10	10	10	10
Month 12	Mean	1.89	1.84	2.18	2.40
	S.D.	0.26	0.32	0.31	0.57
	N	10	10	10	10

Amendment to DER for MRID 41991501 - Subchronic Oral Toxicity Study [Rat] [2,4-DICHLOROPHENOXYACETIC ACID]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document Nos. 008754].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 2/27/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P.H. Virginia A Dobozy 3/16/01
Reregistration Branch I, Health Effects Division (7509C)
TXR# 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: subchronic oral toxicity - rat § 82-1/OPPTS 870.3100
P.C. CODE: 030001 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315 CAS NO.: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [96.1%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force II

CITATION: Schulze, G. E. (1991). Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid. Hazleton Laboratories America, Inc. Study No. HLA 2184-116, August 7, 1991. MRID 41991501. Document No. 008754. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [MRID 41991501], 10 Fischer 344 rats/sex/group were administered 2,4-dichlorophenoxyacetic acid [96.1% a.i.] *via* the diet for 13 weeks at concentrations of 0, 1 mg/kg/day, 15 mg/kg/day, 100 mg/kg/day, and 300 mg/kg/day.

There were no treatment-related deaths. Clinical signs of toxicity occurred mainly in the high-dose females and included hunched posture [1 high-dose female during weeks 13-14], depressed activity [all high-dose females during first week], and few/no feces [4 high-dose males and all high-dose females during week 1; 5 high-dose females during week 12; 1 high-dose female during week 13]. There was a dose-related increase in the incidence of pale/opaque eyes in both sexes [1/10 controls, 2/10 at 1, 15, and 100 mg/kg/day and 4/10 at 300 mg/kg/day in males; 1/10 controls, 2/10 at 1 mg/kg/day, 1/10 at 15 mg/kg/day, 4/10 at 100 mg/kg/day, and 8/10 at 300 mg/kg/day in females].

Decreased body weights were observed throughout the study at the high-dose level [both sexes], with the magnitude of the deficit increasing with time [males 85%/77% and females 79%/72% of control at weeks 6/13, respectively], and decreased body-weight gains [weeks 0-6: males 72%/females 50% of control; overall: males 63%/females 43% of control] were observed in both sexes at the highest dose level throughout the study. At the next highest dose level, decreased body weight [93% of control at 13 weeks; both sexes] and body-weight gain [overall males 91%/females 89% of control] were observed. A corresponding decrease in food consumption was observed in both sexes at the two highest dose levels.

Complete cataract formation was observed in 7 high-dose [300 mg/kg/day] females and in one female at the next highest [100 mg/kg/day] dose, and posterior subcapsular cataract was observed in 5 high-dose females. Treatment-

related alterations in hematology [statistically significant decreases in RBC, HGB, HCT, platelet count, absolute and corrected leukocyte counts, and lymphocyte counts] were observed at the 6- and/or 13-week intervals at the high-dose level [both sexes], and decreased platelet counts were observed in both sexes at the next highest dose level at week 13. Alterations in clinical chemistry [decreased thyroxine and triiodothyronine levels] were observed at 100 and 300 mg/kg/day at both intervals in one or both sexes.

Changes in absolute and/or relative organ weights [adrenals, brain, thymus, heart, kidneys, testes with epididymides (males), ovaries (females), pituitary, liver (increased), thyroids/parathyroids (increased) were observed primarily at the high-dose level [both sexes], and many of these changes may be attributable to decreased body weight. Gross findings, mainly in the high-dose group, included small testes and epididymis and opaque eyes [females]. Treatment-related histopathological changes were observed primarily in the high-dose group and included centrilobular hepatocellular hypertrophy [liver], bilateral retinal degeneration and cataract formation [females], atrophy of the testes [males], hypertrophy of the zona glomerulose [adrenal cortex, both sexes] and follicular cells [thyroid, females], atrophy of the thymus [both sexes] and spleen [both sexes], congestion and edema of the bone marrow [both sexes], brush border loss in proximal tubular cells [kidney, both sexes]. Many of the lesions correlated well with the alterations observed in hematology and clinical chemistry parameters and/or organ-weight data of the high-dose groups.

The NOAEL is 15 mg/kg/day, based on decreased body weight/body-weight gain, alterations in some hematology [decreased platelets (both sexes)] and clinical chemistry [decreased T_3 (females) and T_4 (both sexes)] parameters, and cataract formation in females at the LOAEL of 100 mg/kg/day.

This subchronic oral toxicity study is classified **Acceptable/Guideline**, and the study satisfies the guideline requirement [OPPTS 870.3100; §82-1] for a subchronic oral toxicity study in the rodent.

Table 1. Clinical signs

Signs/Sex/Dose	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
MALES					
few or no feces	0	0	0	0	2
pale/opaque eyes	1	2	2	2	4
FEMALES					
depressed activity	0	0	0	0	10
hunched	0	0	0	0	1
few or no feces	0	0	0	0	10
pale/opaque eyes	1	2	1	4	8

N=10; data from Tables 3A-3B, pages 164-177 of the report

Table 2. Body Weight (g)/Food Consumption (g/wk) Data

Dose/Sex/Week	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
MALES (body wt.)					
0	116±4	111±6	113±6	110±5	115±4
1	146±5	142±9	146±10	137±8 [94]	136±6 [93]
2	181±8	178±11	181±12	170±8 [94]	161±6 [89]
3	207±10	201±11	203±13	195±9 [94]	182±8 [88]
6	252±15	245±10	243±19	234±10* [93]♪	213±12* [85]
11	306±18	302±11	291±25	285±13	241±20 [79]
13	310±20	306±11	294±24	287±12 [93]	238±21* [77]
FEMALES (body wt.)					
0	97±3	96±4	95±4	96±2	94±4
1	114±3	111±4	113±4	107±3	93±4 [82]
2	128±4	128±5	129±5	119±4	108±6
3	138±4	136±5	139±6	121±5	111±7 [81]
6	156±7	154±7	158±10	145±8* [93]	123±9* [79]
11	181±8	179±10	179±8	167±8 [93]	130±15 [72]
13	181±8	180±9	178±9	170±9 [94]	129±16* [72]
MALES (body wt. gain)					
0-1 ✔	30.3	30.5	32.4	27.4 [90]	20.6 [68]♪
1-2 ✔	34.7	34.1	34.7	33.0	25.4 [73]
2-3 ✔	26.4	24.9	22.0	24.7 [94]	21.2 [80]
0-6	138.5±12.6	133.7±8.2	129.2±15.8	124.7±7.8 [90]	97.9±11.8* [72]
0-13	194.1±18.5	194.1±10.7	179.5±21.9	176.8±10.3* [91]	122.3±19.5* [63]
FEMALES (body wt. gain)					
0-1 ✔	16.5	15.7	17.6	11.4 [69]	-0.3
1-2 ✔	14.5	15.2	16.1	11.9 [82]	12.9 [89]
2-3 ✔	9.5	9.8	9.9	1.2 [13]	4.8 [51]
0-6	58.5±6.5	57.7±6.4	63.0±7.6	48.7±7.9* [83]	29.5±5.7* [50]
0-13	83.5±8.1	83.7±8.2	82.3±7.2	73.9±9.0 [89]	35.6±13.2* [43]
MALES (food consumption)					
1	108±6	103±7	104±8	98±4	96±0 {n=1}
2	122±5	117±8	119±8	109±5	99±5
3	126±6	118±7	118±8	114±5	103±9
6	109±7	100±6*	97±11*	95±6*[88]	81±8* [74]
13	108±12	101±6	95±6	106±33	81±11* [75]
1-6	711±38	670±34	670±50	646±26* [91]	591 {1 rat} [83]
1-13	1550±99	1478±60	1456±127	1438±74 [93]	1299 {1 rat} [84]

Table 2. Body Weight (g)/Food Consumption (g/wk) Data

Dose/Sex/Week	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
FEMALES (food consumption)					
1	90±10	87±4	90±4	77±5	-
2	94±4	94±3	94±3	84±6	74±5
3	95±5	97±4	97±4	86±5	74±8
6	75±6	73±5	72±6	65±6* [88]	56±5* [74]
13	71±6	73±2	70±4	69±4	67±18
1-6	537±30	535±18	541±25	462±47* [86]	-
1-13	1132±57	1155±43	1137±44	1017±115* [90]	-

♪[% of control]; * p<0.05; ✔ calculated by this reviewer using data from Table 4A, page 81(statistics not performed); - no data due to feed spillage; data from Tables 4B [pages 83-84] and Tables 5A [page 86] and 5B [pages 88-89] of the report

Table 3. Hematology Data

Parameter/Week/Dose/Sex	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
MALES					
RBC [MI/UL]					
6	8.85±0.55	9.30±0.31* [105]♪	9.30±0.29* [105]	9.28±0.27* [105]	8.61±0.17 [97]
13	9.69±0.10	9.62±0.14	9.69±0.23	9.80±0.19	8.58±0.11* [97]
HGB [g/dL]					
6	16.8±0.86	17.5±0.63	17.4±0.57	17.6±0.40* [105]	17.1±0.37
13	17.2±0.26	17.1±0.21	17.2±0.49	17.3±0.49	16.5±0.30* [96]
HCT [%]					
6	47.3±2.08	49.3±1.67*	49.1±1.58	49.6±1.69* [105]	48.7±0.87
13	49.1±0.77	48.9±0.87	49.3±1.16	50.1±0.20	48.8±0.79
platelets [TH/UL]					
6	953±156	821±136	820±208	789±205	659±130* [69]
13	839±22	831±26	753±219	792±38* [94]	676±28* [81]
WBC [TH/UL]					
6	10.7±0.7	11.5±2.2	9.4±1.5	10.3±1.4	8.1±1.7* [76]
13	8.3±1.2	8.3±1.0	9.1±1.2	8.5±0.6	7.1±1.2* [86]
cor WBC [TH/UL]					
6	10.6±0.7	11.5±2.2	9.4±1.5	10.3±1.4	8.1±1.8* [76]
13	8.3±1.2	8.3±1.0	9.1±1.3	8.5±0.5	7.1±1.2* [86]
lymphocytes [TH/UL]					
6	9.5±0.9	9.9±2.0	8.2±1.3	8.9±1.5	6.9±1.4* [73]
13	6.7±1.2	6.6±0.6	7.5±1.1	6.8±0.8	5.7±1.0 [85]
FEMALES					
RBC [MI/UL]					
6	8.61±0.25	8.62±0.29	8.76±0.19	8.58±0.29	7.81±0.21* [91]
13	9.01±0.20	9.01±0.18	9.09±0.18	8.79±0.17	7.85±0.33* [87]
HGB [g/dL]					
6	16.8±0.44	17.0±0.57	17.1±0.35	16.6±0.66	15.3±0.51* [91]
13	17.0±0.52	17.0±0.36	16.9±0.37	16.7±0.34	15.4±0.77* [91]

Table 3. Hematology Data

Parameter/Week/Dose/Sex	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
HCT [%]					
6	46.1±1.5	46.4±1.7	47.1±1.5	46.3±1.4	44.0±1.2* [95]
13	48.8±1.2	49.1±1.0	49.1±1.5	48.8±0.7	45.8±2.1* [94]
platelets [TH/UL]					
6	852±111	922±40	820±194	827±29.2	576±186* [68]
13	852±46	862±48	804±152	708±122* [83]	667±49* [78]
WBC [TH/UL]					
6	8.4±1.6	7.8±1.6	8.6±1.4	7.1±2.1	5.0±1.1* [60]
13	6.4±1.0	7.0±1.2	6.9±1.3	6.8±1.6	7.6±1.1* [119]
cor WBC [TH/UL]					
6	8.4±1.7	7.8±1.6	8.6±1.4	7.1±2.1	5.0±1.1* [60]
13	6.4±1.0	7.0±1.2	6.9±1.3	6.8±1.6	8.6±1.1* [134]
lymphocytes [TH/UL]					
6	7.2±1.7	6.8±1.3	8.0±1.4	6.4±2.2	4.5±1.0* [63]
13	5.3±0.7	5.8±1.1	5.8±1.1	5.7±1.4	2.8±1.0* [53]
seg [TH/UL]					
6	1.1±0.6	1.0±0.7	0.5±0.3 [45]	0.5±0.3 [45]	0.4±0.1* [36]
13	1.1±0.3	1.2±0.3	1.0±0.5	1.1±0.4	0.8±0.2 [73]

N=10 (except 15 mg/kg/day males; 9); * $p<0.05$; data from Table 8 [pages 98-103] of the report

Table 4. Clinical Chemistry Data

Parameter/Week/Dose/Sex	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
MALES					
BUN [mg/dL]					
6	14±1.1	15±1.6	14±2.0	15±1.5	14±1.4
13	15±1.1	15±1.1	13±1.2* [87]	14±1.3	15±1.9
creatinine [mg/dL]					
6	0.6±0.04	0.6±0.06	0.5±0.05 (9)	0.6±0.06	0.6±0.05
13	0.6±0.05	0.6±0.05	0.6±0.06 (9)	0.7±0.05	0.7±0.03
ALT [U/L]					
6	41±3	52±3	42±4	46±7	41±5
13	55±5	55±5	51±3	70±14*	39±5*
AST [U/L]					
6	105±14	102±7	113±30	125±40	113±23
13	100±11	113±19	97±12	125±65	90±14
glucose [mg/dL]					
6	99±15	113±16	110±19 (9)	112±10	85±7 [86]
13	110±6	110±7	107±6 (9)	100±7* [91]	92±7* [84]
total protein					
6	6.8±0.2	6.7±0.2	6.6±0.2 (9)	6.5±0.1* [96]	6.5±0.2* [96]
13	6.9±0.2	7.0±0.3	6.8±0.2 (9)	6.6±0.1* [96]	6.9±0.3*
albumin [g/dL]					
6	4.9±0.3	5.0±0.2	5.0±0.3	4.8±0.2	5.0±0.2
13	4.8±0.2	4.8±0.2	4.8±0.2	4.7±0.2	5.1±0.4

Table 4. Clinical Chemistry Data

Parameter/Week/Dose/Sex	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
globulin [g/dL]					
6	1.8±0.2	1.6±0.2	1.6±0.2	1.6±0.2	1.5±0.3
13	2.1±0.2	2.2±0.2	2.1±0.2	1.9±0.2	1.8±0.2*
calcium [mg/dL]					
6	10.6±0.4	10.5±0.4	10.5±0.3	10.1±0.5*	10.1±0.5
13	11.0±0.3	10.9±0.3	10.7±0.7	10.6±0.3	10.8±0.3
T3 [mg/dL]					
6	89.0±26.6	79.0±18.5	84.4±16.3	82.8±21.1	55.8±14.1* [63]
13	76.9±10.7	69.3±9.8	80.7±12.1	77.4±25.1	56.3±8.3* [73]
T4 [mg/dL]					
6	4.6±0.3	4.5±0.5	4.7±0.3	3.8±0.3* [83]	1.1±0.2* [24]
13	3.9±0.5	3.6±0.3	3.7±0.3	2.9±0.6* [74]	1.0±0.0* [26]
FEMALES					
BUN [mg/dL]					
6	14±2.1	14±1.5	14±1.1	16±3.7	?✖±2.3
13	15±1.4	14±1.6	16±2.4	16±1.7	18±7.1
creatinine [mg/dL]					
6	0.5±0.05	0.6±0.07	0.6±0.04	0.6±0.05*	0.6±0.03
13	0.6±0.05	0.6±0.05	0.6±0.05	0.7±0.06*	0.6±0.05
ALT [U/L]					
6	35±2.5	36±2.5	37±3.1	43±6.1*	42±3.1*
13	44±11.4	38±3.5	43±5.7	50±8.0	41±9.2
AST [U/L]					
6	95±14.4	93±6.5	102±23.5	101±16.6	109±14.6
13	96±9.9	85±8.2	87±9.7	95±12.0	93±20.1
glucose [mg/dL]					
6	97±16	96±11	97±7	97±12	87±8
13	102±8	104±8	99±12	88±7*	94±14
total protein					
6	6.3±0.2	6.3±0.5	6.3±0.3	6.4±0.2	6.5±0.3
13	6.8±0.2	6.7±0.2	6.8±0.4	6.7±0.3	6.6✖±0.3
albumin [g/dL]					
6	4.8±0.2	4.8±0.4	4.8±0.3	4.8±0.3	5.1±0.3
13	4.8±0.2	4.8±0.2	4.9±0.4	4.8±0.3	5.1±0.2
globulin [g/dL]					
6	1.5±0.2	1.5±0.3	1.5±0.2	1.6±0.3	1.4±0.4
13	2.0±0.1	2.0±0.2	1.9±0.1	1.8±0.2	1.5±0.3* [75]✔
calcium [mg/dL]					
6	10.0±0.6	9.9±0.3	9.8±0.5	10.1±0.3	9.7±0.3
13	10.7±0.4	10.6±0.3	10.8±0.5	10.9±0.4	10.6±0.6
T3 [mg/dL]					
6	89.3±24.7	92.2±24.5	85.6±20.3	65.0±13.5* [73]	77.3±13.3 [87]
13	87.6±9.7	96.0±19.4	99.3±17.4	65.7±12.7* [75]	57.6±9.7* [66]

Table 4. Clinical Chemistry Data					
Parameter/Week/Dose/Sex	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
T4 [mg/dL]					
6	3.3±0.7	3.1±0.7	2.9±0.6	1.2±0.2* [36]	1.0±0.03* [30]
13	2.4±0.5	2.6±0.8	2.1±0.5	1.0±0.0* [42]	1.0±0.00* [42]

N=10 (except 15 mg/kg/day males; 9); ✔ [% of control]; ✖ illegible in report; data from Table 9 [pages 105-110] of the report

Table 5. Organ Weights [grams]

Parameter/Dose/Sex/Week	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
LIVER					
MALES					
absolute	7.39±0.48	7.36±0.57	7.23±1.00	7.49±0.72	7.92±0.74 [107]♪
relative/body	2.57±0.09	2.60±0.13	2.64±0.25	2.83±0.30* [110]	3.67±✖* [143]
relative/brain	3.91±0.36	3.95±0.24	3.86±0.53	3.94±0.35	4.30±0.40 [110]
FEMALES					
absolute	5.20±0.71	4.89±0.65	4.97±0.67	5.02±0.82	5.60±0.45 [108]
relative/body	3.13±0.49	2.96±0.34	3.05±0.40	3.23±0.49	4.82±0.89* [154]
relative/brain	2.94±0.41	2.77±0.33	2.81±0.34	2.83±0.39	3.37±0.26* [115]
KIDNEY					
MALES					
absolute	1.99±0.06	1.95±0.09	2.02±0.22	2.22±0.18* [112]	1.81±0.18* [91]
relative/body	0.69±0.03	0.69±0.02	0.79±0.04* [114]	0.84±0.06* [122]	0.83±0.06*[120]
relative/brain	1.05±0.05	1.04±0.04	1.08±0.11	1.17±0.09*[111]	0.98±0.07* [93]
FEMALES					
absolute	1.27 ✖±0.04	1.26 ✖±0.09	1.28±0.08	1.31✖±0.08	1.16±0.10*
relative/body	0.76±0.03	0.77±0.05	0.79±0.05	0.85±0.05* [111]	1/01±0.09* [132]
relative/brain	0.72±0.04	0.72±0.04	0.73±0.04	0.74±0.03	0.71±0.05
BRAIN w/ STEM					
MALES					
absolute	1.89±0.07	1.87±0.07	1.87±0.04	1.90±0.07	1.84±0.08
relative/body	0.66±0.06	0.66±0.02	0.69±0.05	0.72±0.04* [108]	0.85±0.05* [129]
FEMALES					
absolute	1.77±0.07	1.77±0.06	1.77±0.06	1.77±0.07	1.63±0.07* [94]
relative/body	1.06±0.07	1.07±0.05	1.08±0.05	1.14±0.04* [107]	1.42±0.19* [134]
THYROID					
MALES					
absolute	0.020±0.003	0.022±0.003	0.022±0.033	0.024±0.004	0.028±0.003* [140]
relative/body	0.007±0.001	0.008±0.001	0.008±0.001	0.009±0.001* [127]	0.013±0.002* [186]
relative/brain	0.0108±0.001	0.0118±0.002	0.0116±0.002	0.0126±0.002	0.0155±0.002* [144]
FEMALES					
absolute	0.021±0.006	0.021±0.005	0.020±0.003	0.022±0.004	0.024±0.003
relative/body	0.0126±0.004	0.0130±0.003	0.0122±0.002	0.0140±0.002	0.0212±0.004* [168]
relative/brain	0.0118±0.003	0.0121±0.003	0.0113±0.002	0.0124±0.002	0.0149±0.002* [126]
TESTES					
MALES					
absolute	4.10±0.19	4.08±0.13	4.01±0.21	3.93±0.17	2.09±0.67* [51]
relative/body	1.43±0.07	1.44±0.05	1.47±0.07	1.49±0.07	0.95±0.24* [67]
relative/brain	2.17±0.17	2.19±0.10	2.14±0.12	2.07±0.11	1.13±0.21* [52]
OVARIES					
FEMALES					
absolute	0.113±0.01	0.122±0.02	0.117±0.01	0.104±0.02 [92]	0.066±0.02* [58]
relative/body	0.068±0.006	0.074±0.011	0.072±0.008	0.067±0.009	0.056✖±0.011*
relative/brain	0.064±0.008	0.068±0.010	0.066±0.007	0.059±0.008 [92]	0.040±0.010* [63]

Table 5. Organ Weights [grams]

Parameter/Dose/Sex/Week	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
PITUITARY					
MALES					
absolute	0.009±0.001	0.010±0.001	0.009±0.002	0.010±0.002	0.009±0.001
relative/body	0.0033±0.0004	0.0034±0.0004	0.0034±0.0005	0.0037±0.0005	0.0042±0.0005* [127]
relative/brain	0.0050±0.0006	0.0051±0.0007	0.0050±0.0009	0.0052±0.0008	0.0049±0.0005
FEMALES					
absolute	0.013±0.002	0.014±0.001	0.013±0.002	0.012±0.002	0.008±0.002 [62]
relative/body	0.0080±0.0013	0.0083±0.0007	0.0080±0.0010	0.0076±0.0010	0.0065±0.0010* [81]
relative/brain	0.0075±0.0010	0.0078±0.0008	0.0074±0.0011	0.0067±0.0009	0.0047±0.0010 [63]
ADRENAL					
MALES					
absolute	0.047±0.005	0.047±0.005	0.046±0.004	0.050±0.003	0.046±0.007
relative/body	0.016±0.002	0.017±0.002	0.017±0.001	0.019±0.001* [115]	0.021±0.003* [130]
relative/brain	0.0249±0.003	0.0253±0.003	0.0247±0.002	0.0263±0.002	0.0252±0.003
FEMALES					
absolute	0.059±0.006	0.058±0.006	0.058±0.009	0.054±0.004	0.038±0.006* [64]
relative/body	0.036±0.005	0.035±0.005	0.036±0.004	0.035±0.002	0.033±0.005 [93]
relative/brain	0.0334±0.003	0.0329±0.004	0.0329±0.004	0.0308±0.002	0.0234±0.004* [70]
HEART					
MALES					
absolute	0.90±0.06	0.87±0.05	0.87±0.05	0.83±0.03* [92]	0.76±0.06* [84]
relative/body	0.315±0.02	0.308±0.02	0.321±0.02	0.315±0.02	0.349±0.02
relative/brain	0.478±0.04	0.468±0.04	0.467±0.03	0.439±0.02* [92]	0.410±0.03 [86]
FEMALES					
absolute	0.61±0.03	0.64±0.05	0.63±0.04	0.59±0.04	0.52±0.05* [85]
relative/body	0.366±0.02	0.390±0.03	0.385±0.02	0.383±0.03	0.453±0.05* [124]
relative/brain	0.345±0.02	0.365±0.03	0.355±0.02	0.336±0.02	0.320±0.03* [93]
THYMUS					
MALES					
absolute	0.24±0.06	0.24±0.05	0.24±0.06	0.21±0.04	0.14±0.04* [58]
relative/body	0.082±0.02	0.084±0.02	0.090±0.03	0.079±0.01	0.066±0.02
relative/brain	0.125±0.03	0.127±0.03	0.130±0.03	0.110±0.02	0.078±0.02* [62]
FEMALES					
absolute	0.21±0.02	0.23±0.03	0.21±0.02	0.19±0.02	0.08±0.03* [38]
relative/body	0.126±0.01	0.140±0.02	0.128±0.01	0.128±0.01	0.125±0.01
relative/brain	0.119±0.02	0.131±0.02	0.118±0.01	0.110±0.01	0.052±0.02* [44]
TERMINAL BODY WEIGHT					
MALES	287.6±18.5	282.8±9.8	273.3±21.9	264.9±13.3* [92]	217.5±19.1* [76]
FEMALES	166.6±7.6	165.2±8.2	163.2±8.2	155.5±7.5 [93]	116.7±16.6* [70]

✖ illegible in report; N=10 unless (); ♪ [% of control]; * p<0.05; data from Tables 11, 12, 13 [pages 127-134] of the report

Table 6. Gross/Histopathology Observations

Signs/Sex/Dose	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
MALES n=10			(9)		
gross findings					
adrenal cortex - pale	0	0	0	0	5
lung - pale area	0	0	0	0	0
liver - pale	0	0	0	0	1
liver - prominent reticular area	0	0	0	0	2
eye - internal, opaque	0	0	1	1	1
testis - small	0	0	0	0	8
testis - soft	0	0	0	0	7
epididymis - small	0	0	0	0	8
epididymis- soft	0	0	0	0	6
FEMALES n=10		(9)			
gross findings					
adrenal cortex - pale	0	0	0	1	2
lung - pale area	0	0	0	0	4
liver - pale	0	0	0	0	1
liver - prominent reticular area	0	0	0	0	0
eye - internal, opaque	0	0	1	1	7
MALES					
histopathology findings					
adrenal cortex - hypertrophy✔	0	0	0	1	8
thyroid - hypertrophy	0	0	0	0	0
lung - alveolar macrophage accumulation	0	0	0	0	5
liver - hypertrophy, centrolobular	0	0	0	3	7
kidney - brush border loss, tubular	0	0	0	0	6
thymus - atrophy	0	0	0	0	4
thymus - necrosis	0	0	0	0	0
marrow, femur - congestion	0	0	0	0	0
marrow - edema	0	0	0	0	0
eye - cataractous change, bilateral	0	0	0	0	0
eye - cataractous change, unilateral	0	0	0	1	0
eye - degeneration, retinal, bilateral	0	0	0	0	0
abdominal cavity - atrophy, adipose tissue	0	0	0	0	10
testis - atrophy	0	0	0	0	8
spleen - atrophy	0	0	0	0	10
FEMALES					
histopathology findings					
adrenal cortex - hypertrophy✔	0	0	0	10	10
thyroid - hypertrophy	3	0	0	0	8
lung - alveolar macrophage accumulation	0	0	0	0	10
liver - hypertrophy, centrolobular	0	0	0	0	9
kidney - brush border loss, tubular	0	0	0	1	10
thymus - atrophy	0	0	0	0	8
thymus - necrosis	0	0	0	0	1
marrow, femur - congestion	0	0	0	0	2
marrow - edema	0	0	0	0	2
eye - cataractous change, bilateral	0	0	0	0	5
eye - cataractous change, unilateral	0	0	0	0	2
eye - degeneration, retinal, bilateral	0	0	0	2	2
abdominal cavity - atrophy, adipose tissue	0	0	0	0	10
spleen - atrophy	0	0	0	0	10

N=10 unless (); ✔ zona glomerulosa; data from Table 10B [pages 119-125] and Table 14B [pages 142-147] of the report;

765

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

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NOV 4 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT 2,4-Dichlorophenoxyacetic acid: Adverse Data Section 6(a) (2) Submission.

FROM: Jess Rowland, M.S, Toxicologist Jess Rowland 10/29/91
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

TO: Walter Waldrop / Judy Coombs
Product Manager (71)
Reregistration Division

THRU: K.Clark Swentzel, Section Head K. Clark Swentzel 10/29/91
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)
and
Marcia van Gemert, Ph.D., Chief Marcia Gemert 10/30/9
Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

STUDY IDENTIFICATIONS: Case No: 818706 Submission No: 5402220

HED Project No.1-2254 Caswell No. 315

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

ACTION REQUESTED: Review of subchronic feeding studies (MRID # 419915-01, mice and 419915-02, rats) for 2,4-Dichlorophenoxyacetic acid (2,4-D Acid). 6 (a) (2) data was submitted and reviewed by Tox.Branch for these guidelines on 6/30/90.

RESPONSE: A separate Data Evaluation Report (DER) for each of the above referenced studies is attached. A summary of each study is as follows:

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109

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1. Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic acid. [MRID No. 419915].

SUMMARY: Male and female Fischer-344 rats were fed diets containing 2,4-Dichlorophenoxyacetic acid at 0, 1, 15, 100 or 300 mg/kg/day for 13 weeks.

Treatment did not cause any adverse effects at 1 or 15 mg/kg/day, but did cause decreases in mean body weight, body weight gain, alterations in some of the hematology and clinical chemistry parameters, changes in various organ weights, and histopathological changes in the liver, adrenals and kidneys at 100 mg/kg/day.

The highest dietary dose level of 300 mg/kg/day was associated with weight loss, alterations in hematology and clinical chemistry parameters, changes in various organ weights, and histopathological lesions in the eye, liver, testes, adrenals, kidneys, thymus, bone marrow, spleen, thyroid, and lungs.

Under the conditions of this study, a No-Observable-Effect Level (NOEL) of 15 mg/kg/day and a Lowest-Observable-Effect Level (LOEL) of 100 mg/kg/day are established. The LOEL is based on decreases in mean body weight and body weight gain, alterations in hematology and clinical chemistry parameters, and histopathological lesions observed at 100 mg/kg/day.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

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2. Subchronic Toxicity Study in Mice with 2,4-Dichlorophenoxyacetic acid. [MRID No. 419915-02]

SUMMARY: Male and female B6C3F1 mice were fed diets containing 2,4-D at concentrations of 0, 1, 15, 100, or 300 mg/kg/day for 13 weeks. Treatment had no adverse effect on survival, body weight, body weight gain, food consumption, ophthalmoscopic examination, hematology and gross pathology at 1, 15, or 100 mg/kg/day dose levels.

Treatment-related changes at 100 mg/kg/day included decreased glucose (females) and decreased thyroxine (males) levels, increases in mean absolute and relative kidney weights (females), and a liver lesion in 1 female.

Treatment-related changes at 300 mg/kg/day included: transient decreases in food consumption (only up to Week 7); decreases in glucose (females) and thyroxine levels (males); significant decrease in kidney-to-brain weight ratios in males); correlating histopathological changes in the kidneys (karyomegaly, loss of brush order and decreased size of tubular lining cells) in males; and histopathological changes in the liver characterized by nuclear hyperchromatism, and decreased glycogen in periportal hepatocytes.

Under the conditions of this study, a NOEL of 15 mg/kg/day and a LOEL of 100.0 mg/kg/day is established for the 90-day oral toxicity of 2,4-Dichlorophenoxyacetic acid to male and female mice. The LOEL is based on decreases in glucose and thyroxine levels, and increases in mean and relative kidney weight observed at 100 mg/kg/day.

CORE CLASSIFICATION: Not applicable since a subchronic study in mouse is not a Guideline requirement.

171

008754

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist Jess Rowland 10/22/91
Section II, Toxicology Branch II (H7509C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 10/2
Section II, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding-Rodent **GUIDELINE:** 82-1(a)

TOX.CHEM. No.: 315 **MRID No.:** 419915-01

HED Project No. 1-2254 **Registrant:** Industry Task Force II

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

STUDY IDENTIFICATION: HLA Study No. 2184-116

TESTING LABORATORY: Hazleton Laboratories America, Rockville, Md.

TITLE OF REPORT: Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid.

REPORT AUTHOR: Gene E. Schulze, Ph.D., D.A.B.T

REPORT DATE: August 7, 1991

SUMMARY: Male and female Fischer-344 rats were fed diets containing 2,4-Dichlorophenoxyacetic acid at 0, 1, 15, 100 or 300 mg/kg/day for 13 weeks.

Treatment did not cause any adverse effects at 1 or 15 mg/kg/day, but did cause decreases in mean body weight, body weight gain, alterations in some of the hematology and clinical chemistry parameters, and changes in various organ weights, and histopathological changes in the liver, adrenals and kidneys at 100 mg/kg/day.

The highest dietary dose level of 300 mg/kg/day was associated with weight loss, alterations in hematology and clinical chemistry parameters, changes in various organ weights, and histopathological lesions in the eye, liver, testes, adrenals, kidneys, thymus, bone marrow, spleen, thyroid, and lungs.

Under the conditions of this study, a No-Observable-Effect Level (NOEL) of 15 mg/kg/day and a Lowest-Observable-Effect Level (LOEL) of 100 mg/kg/day are established. The LOEL is based on decreases in mean body weight, body weight gain and food consumption, alterations in hematology and clinical chemistry parameters, and histopathological lesions observed at 100 mg/kg/day.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

792

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I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the subchronic toxicity of 2,4-Dichlorophenoxyacetic acid (2,4-D) following dietary administration to rats.

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: 2,4-dichlorophenoxyacetic acid.
Purity: 96.1%
Lot No.: 909
Description: Off-white powder.

2. Test Animals

Species: Rats
Strain: Fischer-344
Sex: Males and females
Age: Approximately 42 days at initiation.
Weight at initiation: 103.6-122.3 g (M); 84.4-104.2 g (F)
Identification: Tail tatoo and cage cards.
Acclimation: Approximately 2 weeks
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Chow #5002.
Water: Tap water ad libitum
Environment: Temperature, 72 ± 6°F and humidity 50 ± 20 %
Light/dark cycles: 12 hour photocycle.

3. Study Design

Group No.	Treatment	No. of Animals Males	No. of Animals Females	Dose Level (mg/kg/day)
1	Control	10	10	0
2	Low	10	10	1
3	Mid-1	10	10	15
4	Mid-2	10	10	100
5	High	10	10	300

173

4. Test Article Formulation and Analyses

The test material was ground into fine powder with a mortar and pestle and a calculated amount for each dose level (adjusted to a purity of 100%) was weighed. A premix was prepared for each dose level with approximately 200 g of feed for approximately two minutes. The premixes of the respective dose levels were then added to the required amount of feed and mixed in a Hobart mixer for approximately 10 minutes.

Homogeneity was determined by a pretest mix analysis and stability tests were conducted prior to initiation at Days 0, 7 and 14. Control and test diets were analyzed for 2,4-D levels at Weeks 1, 2, 3, 4, 8 and 12.

5. Treatment

Rats were fed the control and test diets 7 days per week for at least 91 days. The oral route of administration was chosen because it is the route of potential human exposure.

6. Experimental Procedures

Mortality and moribundity checks were performed twice daily. Cageside observations for clinical signs of toxicity were performed once daily. Body weights were obtained prior to initiation and once weekly thereafter. Food consumption was measured weekly. Physical examinations were performed and detailed clinical observations were recorded once per week at each weighing interval. Ophthalmologic examinations were performed on all rats once prior to initiation of dosing and prior to necropsy.

Blood and urine were collected from all animals at termination for hematology, clinical chemistry and urinalysis. 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)	Erythrocyte fragility
x Corrected leukocyte count (COR WBC)	x Cell morphology

Clinical Chemistry

Electrolytes:	Other
x Calcium[a] x Chloride[a] Magnesium[a] x Phosphorus[a] x Potassium[a] x Sodium	x Albumin[a] x Blood creatinine[a] x Blood urea nitrogen[a] Cholesterol[a] x Globulins x Glucose[a] x Total bilirubin[a] x Total serum protein[a] Triglycerides Serum protein electrophoresi x Triiodothyronine (T_3 x Thyroxine (T_4)
Enzymes:	
Alkaline phosphatase x Alanine aminotransferase (SGPT)[a] x Aspartate aminotransferase (SGOT)[a] Cholinesterase[b] Creatinine phosphatase[a] Lactic acid dehydrogenase Gama glutamyl transferase	

Urinalysis

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

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

7. Termination

All surviving animals were fasted, weighed, anes sodium pentabarbital and exsanguinated. A gross performed on Study Day 92 (males) and Study Day 93 (gross pathological changes were recorded, and organs were weighed.

Adrenals	Brain	Heart	Kidneys
Ovaries	Pituitary	Testes	Thyroids/parathyro

8. Histopathology

The checked (X) tissues from all rats were processed for histopathological evaluation.

Digestive System	Respiratory System
x Salivary glands[a]	Trachea[a]
x Esophagus[a]	x Lung[a]
x Duodenum[a]	Pharynx[a]
x Jejunum[a]	Larynx[a]
x Cecum[a]	Nose[a]
x Colon[a]	
x Rectum[a]	Cardiovascular/Hemo.Sy
x Liver[ac]	
x Pancreas[a]	x Aorta (thoracic)[a]
Gall bladder[ab]	x Heart[a]
	x Bone marrow[a]
Neurological System	x Lymph nodes[a]
	x Spleen[a]
x Brain[ac]	x Thymus[a]
x Pituitary[a]	
Peripheral nerve[ab]	Urinogenital System
Spinal cord (3 levels)[ab]	x Kidneys[ac]
x Eyes (optical nerve)[ab]	x Urinary bladder[a]
	x Testes[ac]
Glandular System	x Epididymides
	x Uterus[a]
x Adrenals[a]	x Ovaries[ac]
Lacrimal glands[b]	Others
x Parathyroids[ad]	x All gross lesions ar
x Thyroids[ad]	x Skeletal muscle[a]

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

008754

9. Statistical Analyses

In-life body weights, food consumption, hematologic and clinical chemistry parameters, and organ weight data were compared for differences of statistical significance from the same sex of treated groups. If variances of untransformed data were heterogeneous, analyses were performed on transformed data to achieve variance homogeneity. When the series of transformations were not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were performed routinely at the 5% two-tailed probability level.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Laboratories Inc, and the Study Protocol. A quality assurance statement was signed and dated August 6,1991.

008754

II. RESULTS

1. Analysis of Diet Mix

Analytical results are presented in Table 1 (Appended). Routine concentration analyses showed that the mean concentration was within 10% of target level confirming accuracy of formulation. Homogeneity analyses indicated that the highest and lowest concentrations for Week 0 were homogeneous, and the stability analyses showed that the compound was stable in treated diet for at least a 14 day period.

2. Survival

Except for the accidental deaths of one male at 15 mg/kg/day and one female at 1 mg/kg/day during orbital sinus bleeding at Week 6, all animal survived to the end of the study.

3. Clinical Signs

Treatment-related clinical signs of toxicity observed in males and/or females at 300 mg/kg/day included few and/or no feces, pale and/or opaque eyes (also in females at 100 mg/kg/day), and depressed activity and hunched body posture (females only) . Other clinical signs occurred with low frequency sporadically throughout the groups.

4. Body Weights and Body Weight Changes

Mean body weights of both sexes of treated rats were lower than their respective controls during the study with the difference reaching statistical significance ($p < 0.05$) at Weeks 6 and 13. At Week 6, the decrease was 93 and 85% of control for males at 100 and 300 mg/kg/day, respectively, and 93 and 79% of controls for females at 100 and 300 mg/kg/day, respectively. This reduction can be atttributed to the overnight fast prior to scheduled bleeding. At week 13, the decrease was 92 and 77% of control for males at 100 and 300 mg/kg/day, respectively, and 94 and 72% of controls for males and females at 300 mg/kg/day, respectively.

008754

Mean body weight change values are summarized in Tables 2 and 3 for males and females, respectively. Statistically significant ($p < 0.05$) reductions in body weight gain were observed in males at 300 mg/kg/day at Weeks 0 through 6 (72% of control) and in males at 100 and 300 mg/kg/day groups at Weeks 0 through 13 (91 and 63% of control, respectively). Significant ($p < 0.05$) decreases were seen in females at 100 and 300 mg/kg/day at Weeks 0 through 6 (83 and 50% of control, respectively) and in females at 300 mg/kg/day at Weeks 0 through 13 (43% of control). No reductions in body weight gain were seen in rats at 1 or 15 mg/kg/day groups.

Table 2. Mean Body Weight Change (G) in Male Rats

Dose (mg/kg/day)	0-6 Week	0-13 Week
0	136.5 ± 12.62	194.1 ± 18.51
1	133.7 ± 8.21	194.1 ± 10.69
15	129.2 ± 15.81	179.5 ± 21.89
100	124.7 ± 7.79	176.8 ± 10.25*
300	97.9 ± 11.76*	122.3 ± 19.47*

Table 3. Mean Body Weight Change (G) in Female Rats.

Dose (mg/kg/day)	0-6 Week	0-13 Week
0	58.5 ± 6.46	83.5 ± 8.11
1	57.7 ± 6.39	83.7 ± 8.24
15	63.0 ± 7.55	82.3 ± 7.23
100	48.7 ± 7.85*	73.9 ± 8.96
300	29.5 ± 5.73*	35.6 ± 13.16*

5. Food Consumption

No conclusions can be drawn from the food consumption data due to a number of confounding factors such as: an overnight fast prior to scheduled blood collections at Weeks 6 which led to statistically significant differences in mean food consumption values between treated and control groups at Weeks 1 through 6; the decreased food consumption at 100 and 300 mg/kg/day groups due to non palatability of the diet; and the numerous instances of spilled feed at the highest dose level. Overall mean compound consumption (Weeks 1-13) was within 10% of target (mg/kg) values. The average daily compound intake during the study was 0.93, 13.98, 93.93, and 278.39 mg/kg/day for males and 0.96, 14.39, 96.16, and 293.42 mg/kg/day for females.

008754

5. Ophthalmology Examination

Ophthalmoscopic findings are summarized in Tables 4 and 5 for males and females respectively. 2,4-D induced complete cataract formation in 7 females and posterior subcapsular cataract in 5 females at 300 mg/kg/day.

Table 4 Ophthalmoscopic Observations In Male Rats.

Observations	Dose Level (mg/kg/day)				
No.of animals = 10/dose level	0	1	15	100	300
Corneal Dystrophy	10	10	9	10	10
Retinal Linear Atrophy	1	1	1	0	0
Retinal Degeneration	0	1	1	1	0
Chromodacryorhhea	0	0	0	0	0
Complete Cataract	0	0	0	1	0
Posterior Subcapsular Cataract	0	0	0	0	0
Phthisis Bulbi	0	0	0	1	0
Corneal Ulcer	0	0	0	0	1

Table 5 Ophthalmoscopic Observations In Female Rats.

Observations	Dose Level (mg/kg/day)				
No.of animals = 10/dose level	0	1	15	100	300
Corneal Dystrophy	10	9	10	10	10
Retinal Linear Atrophy	9	9	10	10	9
Retinal Degeneration	2	2	0	1	1
Chromodacryorhhea	0	0	1	1	0
Complete Cataract	0	0	0	1	7
Posterior Subcapsular Cataract	0	0	0	0	5
Phthisis Bulbi	0	0	0	0	0
Corneal Ulcer	0	0	0	0	0

18

008754

6. Hematology and Clinical Chemistry

Treatment-related, statistically significant ($p \leq 0.05$) increases (↑) or decreases (↓) observed in hematology parameters are summarized below:

Hematology

Parameter	Males				Females			
	100 mg/kg/day		300 mg/kg/day		100 mg/kg/day		300 mg/kg/day	
Week	6	13	6	13	6	13	6	13
RBC	↑	-	-	↓	-	-	↓	↓
HGB	↑	-	-	↓	-	-	↓	↓
HCT	↑	-	-	-	-	-	↓	↓
Platelet	-	↓	↓	↓	-	↓	↓	↓
WBC	-	-	↓	↓	-	-	↓	↓
COR WBC	-	-	↓	↓	-	-	↓	↓
LYMPH	-	-	↓	-	-	-	↓	-
SEG	-	-	-	-	-	-	↓	-

Clinical Chemistry

Parameter	Males				Females			
	100 mg/kg/day		300 mg/kg/day		100 mg/kg/day		300 mg/kg/day	
Week	6	13	6	13	6	13	6	13
Glucose	-	↓	-	↓	-	↓	-	-
CREAT	-	-	-	-	↑	↑	↑	-
ALT	-	↑	-	↓	↑	-	↑	-
T PROT	↓	↓	↓	-	-	-	-	-
Globulin	-	-	-	↓	-	-	-	↓
Calcium	↓	-	-	-	-	-	-	-
T3	-	-	↓	↓	↓	↓	-	↓
T4	↓	↓	↓	↓	↓	↓	↓	↓

008754

7. Gross Pathology

Treatment-related gross necropsy findings included: various eye lesions (primarily in females at 300 mg/kg/day); pale adrenal (males and females at 300 mg/kg/day); pale lung (females at 300 mg/kg/day); pale and/or prominent reticular pattern in liver (males and females at 300 mg/kg/day); and small testes and epididymides (males at 300 mg/kg/day).

8. Organ Weights

Treatment-related, statistically significant ($p \leq 0.05$) increases (↑) or decreases (↓) in absolute and relative organ weight primarily seen in male and/or female rats at 100 and 300 mg/kg/day are tabulated below:

Changes in Absolute and Relative Organ Weights in Male Rats

	Males			
	100 mg/kg/day		300 mg/kg/day	
Oragan	Absolute	Relative	Absolute	Relative
Adrenal	-	↑	-	↑
Brain	-	↑	-	↑
Heart	↓	-	↓	↑
Kidney	↑	↑	↓	↑
Liver	-	↑	-	↑
Pituitary	-	-	-	↑
Testes	-	-	↓	↓
Thymus	-	-	↓	-
Thyroid	-	↑	↑	↑

008754

Changes in Absolute and Relative Organ Weights in Female Rats

	Females			
	100 mg/kg/day		300 mg/kg/day	
Oragan	Absolute	Relative	Absolute	Relative
Adrenal	-	↑	↓	-
Brain	-	↑	↓	↑
Heart	-	-	↓	↑
Kidney	-	↑	↓	↑
Liver	-	↑	-	↑
Pituitary	-	-	↓	↓
Ovary	-	-	↓	↓
Thymus	-	-	↓	↓
Thyroid	-	↑	-	↑

9. Histopathology

Treatment-related histopathological changes (Tables 6 and 7) observed at 100 and 300 mg/kg/day included:

1. Bilateral cataract formation (cataractous change) and retinal degeneration in females at 300 mg/kg/day.

2. Centrilobular hepatocellular hypertrophy of the liver in males at 100 and 300 mg/kg/day and in females at 300 mg/kg/day.

3. Atrophy of the testes in males at 300 mg/kg/day.

4. Hypertrophy of the zona glomerulose of the adrenal cortex in males and females at 100 and 300 mg/kg/day.

5. Brush border loss in proximal tubular cells of kidneys in males at 100 and 300 mg/kg/day and in females at 300 mg/kg/day.

6. Atrophy of the thymus in females at 300 mg/kg/day.,

7. Alveolar macrophage accumulation of the lung in males and females at 300 mg/kg/day.

8. Hypocellularity of bone marrow in males and females at 300 mg/kg/day.

9. Atrophy of the spleen in males and females at 300 mg/kg/day.

10. Follicular cell hyperplasia of thyroid in females at 300 mg/kg/day.

Histopathological evaluation of the eye revealed cataractous changes (cataracts) in the lens which were moderately severe in 4 rats and severe in 3 rats at 300 mg/kg/day. Among females at 100 mg/kg/day, this finding was moderate in 1, minimal in another, but unilateral in both. Bilateral retinal degeneration observed only in 2 females at 300 mg/kg/day.

In some cases, the histopathological changes correlated well with alterations observed in hematology and clinical chemistry parameters and organ weight data. Increase in liver weight, alanine aminotransferase, and aspartate aminotransferase were associated with centrilobular hepatocellular hypertrophy of the liver. Decreased thyroxine levels can be correlated with follicular cell hypertrophy of the thyroid gland. Increase in adrenal weight can be correlated with hypertrophy of cells of the zona glomerulose. Decreased mean thymic weight can be histologically correlated with atrophy observed in both sexes at the high dose. Atrophy of mesenteric adipose tissue in the peritoneal cavity can be correlated with mean body weight decrease in males and females at the high dose. Other microscopical changes were considered incidental and unrelated to treatment.

008754

Table 7. Histopathological Findings in Male Rats At Sacrifice.

Organ / Lesion	Dose Level (mg/kg/day)				
No.of animals= 10/dose levels	0	1	15	100	300
Adrenal, cortex -Hypertrophy, zona glomerulose	0	0	0	1	8
Thyroid -Hypertrophy	0	0	0	0	0
Liver -Hypertrophy, centrilobular	0	0	0	3	7
Lung -Alveolar macrophage accumulation	0	0	0	0	5
Spleen -Atrophy	0	0	0	0	10
Kidney -Brush border loss, tubular	0	0	0	0	6
Testes -Atrophy, bilateral	0	0	0	0	8
Thymus -Atrophy	0	0	0	0	4
Eye -Cataractous change, unilateral	0	0	0	1	0

185

008754

Table 8. Histopathological Findings in Female Rats At Sacrifice.

Organ / Lesion	Dose Level (mg/kg/day)				
No.of animals= 10/dose levels	0	1	15	100	300
Adrenal, cortex -Hypertrophy, zona glomerulose	0	0	0	10	10
Thyroid -Hypertrophy	3	0	0	0	8
Liver -Hypertrophy, centrilobular	0	0	0	0	9
Lung -Alveolar macrophage accumulation	0	0	0	0	10
Spleen -Atrophy	0	0	0	0	10
Kidney -Brush border loss, tubular	0	0	0	1	10
Thymus -Atrophy	0	0	0	0	8
Eye -Cataractous change, bilateral -Cataractous change, unilateral -Degeneration, retinal, bilateral	 0 0 0	 0 0 0	 0 0 0	 0 2 0	 5 2 2

008754

IV. DISCUSSION

Male and female Fischer-344 rats were fed diets containing 2,4-D at concentrations of 0, 1, 15, 100, or 300 mg/kg/day for 13 weeks.

Analytical data showed that the diet mixes were homogeneous, stable at room temperature for up to 14 days and the mean concentrations were within 10% of the target level.

Treatment had no adverse effect on survival. No treatment-related effects were seen at 1 mg/kg/day or 15 mg/kg/day dose levels.

Treatment-related clinical signs were observed at the high-dose included few or no feces, pale and/or opaque eyes (also in males at 100 mg/kg/day) depressed activity and hunched body posture (female at the high-dose only).

Decreases in mean body weight and body weight gain were observed in males at 100 mg/kg/day and in both sexes at 300 mg/kg/day.

Ophthalmology examinations revealed bilateral cataract formation only in females at 300 mg/kg/day; no ocular changes were seen in male at the high dose or in either sex at the lower doses.

Treatment-related alterations in hematology parameters were confined primarily to the high dose included: decreased red cell mass (erythrocyte, counts, and hemoglobin), decreased white cell and lymphocyte count values, decreased platelet counts (also in males and females at 100 mg/kg/day) and decreased incidence/grade for echinocytes.

Also, treatment-related alterations in clinical chemistry parameters were primarily seen at 100 and 300 mg/kg/day groups which included: decreased glucose, globulin, and thyroxine levels, and decreased triiodothyronine levels. No biologically important changes were seen from urinalysis.

Treatment-related gross pathological changes were limited to the high dose group and included small and soft testes, opaque eyes, pale adrenals, pale areas of lung, and pale and/or prominent reticular pattern in the liver.

Treatment-related changes were observed in organ weight data at 100 and 300 mg/kg/day and included decreased mean relative and absolute ovary weights, increased relative thyroid weights, decreased relative and absolute testes with epididymides weights, increased absolute kidney weight, increased relative adrenal weights, and increased relative liver weights.

In most cases, alterations noted in hematology, clinical chemistry, gross pathology, and organ weights correlated well with histopathological changes. Treatment-related histopathological changes which were limited to 100 and 300 mg/kg/day group and included bilateral cataract formation and retinal degeneration (females at high dose only), centrilobular hepatocellular hypertrophy, atrophy of the testes, hypertrophy in the zona glomerulose of the adrenal cortex, brush order loss in proximal tubular cells in the kidneys, atrophy of the thymus, hypocellularity of bone marrow, splenic atrophy, follicular cell hypertrophy of the thyroid, and alveolar macrophage accumulation of the lung.

V. CONCLUSION

Under the conditions of this study, a NOEL of 15 mg/kg/day and a LOEL of 100.0 mg/kg/day is established for the 90-day oral toxicity of 2,4-Dichlorophenoxyacetic acid to male and female rats. The LOEL is based on decreases in mean body weight, body weight gain, alterations in hematology and clinical chemistry parameter, changes in organ weights, and histopathological changes observed at 100 mg/kg/day.

VI. **CORE CLASSIFICATION:** Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

008754

APPENDIX

008754

Table 1
Analytical Chemistry Results
Subchronic Toxicity Study in Rats with
2,4-Dichlorophenoxyacetic Acid

190

008754

Table 1
Results of Test Diet Analyses
Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid

Homogeneity (Pretest mix)

	Target Concentration (ppm)				Assayed Level (ppm)			
	0	8	6195		0	8	6195	0
	0	-	-		ND	-	-	-
Top	-	8	6195	A	-	7.182	6514	-
	-	8	6195	B	-	6.897	6550	-
Middle	-	8	6195	A	-	7.461	6352	-
	-	8	6195	B	-	7.461	6045	-
Bottom	-	8	6195	A	-	7.461	6438	-
	-	8	6195	B	-	7.182	6201	-
								Mean
								S.D.
								% RSD

NOTES: A and B are duplicate samples.
ND = Not detected.

23

791

008754

Table 1 - Continued
Results of Test Diet Analyses
Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid

Pretest Stability

	Target Concentration (ppm)				Assayed Level (ppm)			
	0	8	6193		0	8	6193	
Day 0[a]	0	-	-		-	-	-	-
	0	8	6193	A	ND	7.368	-	-
	-	8	6193	B	ND	7.188	-	-
Day 7	0	-	-		-	-	-	-
	0	8.00	6193	A	ND	8.500	6272	-
	-	8.00	6193	B	ND	8.750	6272	-
Day 14	0	-	-		-	-	-	-
	0	8.00	6193	A	ND	8.883	5944	-
	-	8.00	6193	B	ND	7.786	6156	-

NOTES: A and B are duplicate samples.
ND = Not detected.

[a] Mean of homogeneity mix.

24

-98

008754

Table 1 - Continued
Results of Test Diet Analyses
Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid

Males

		Target Concentration (ppm)					Assayed Level (ppm)				
	Group:	2	3	4	5		2	3	4	5	2
	Dose Level (mg/kg/day):	1	15	100	300		1	15	100	300	1
Week											
1		8.2099	125.3589	888.0947	2545.8632	A	7.747	124.2	812.0	2481	94.
						B	8.289	124.2	819.1	2481	101
2		9.6099	146.9174	978.4731	2545.8632	A	9.396[a]	147.5	1025	2595	97.
						B	10.18[a]	147.5	991.3	2595	105
3		10.5645	159.4834	1096.4216	3415.7568	A	11.19	169.3	1077	3695	106
						B	10.67	158.8	1118	3533	101
4		11.9388	188.7356	1197.3313	3784.8643	A	13.09	198.9	1279[a]	3692	110
						B	12.81	186.9	1317[a]	3376	107
8		15.2344	235.8272	1540.1973	4549.9297	A	14.24[a]	244.7	1595	4571	95.
						B	13.86[a]	226.1	1536	4807	90.
12		18.3899	278.0094	1726.9937	5259.3750	A	19.34[a]	262.2	1703	5281	105
						B	17.74[a]	262.2	1589	5427	96.

NOTES: Group 1 basal diet was analyzed for all weeks with no compound detected.
A and B are duplicate samples.

[a] Results from re-extraction.

25

008754

Table 1 - Continued
Results of Test Diet Analyses
Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid

Females

		Target Concentration (ppm)					Assayed Level (ppm)				
	Group:	2	3	4	5		2	3	4	5	
	Dose Level (mg/kg/day):	1	15	100	300		1	15	100	300	
Week											
1		9.5500	142.9950	960.5000	2888.6000	A	9.571	144.2	941.9	2717	1
						B	9.317	137.6	970.7	2717	1
2		8.9249	131.5401	981.8285	2888.6000	A	9.337	128.5	1025	2940	1
						B	9.337	132.3	1025	2768	1
3		9.4388	144.6328	994.8966	3836.9565	A	9.861[a]	151.9	974.0	2964	1
						B	10.36[a]	151.9	1036	3046	1
4		9.8154	150.9036	1052.6824	3151.8462	A	10.25	162.8[a]	1025	3165	1
						B	9.962	166.7[a]	1052	3165	1
8		11.8967	182.1635	1292.8747	3789.0547	A	10.87[a]	177.2	1355	3750	1
						B	10.87[a]	194.5	1338	3892	1
12		14.3254	223.8384	1586.8513	4321.6943	A	14.06[a]	201.5[a]	1292	4317	1
						B	13.79[a]	223.2[a]	1399	4048	1

NOTES: Group 1 basal diet was analyzed for all weeks with no compound detected.
A and B are duplicate samples.

[a] Results from re-extraction.

26

794

[2,4-D-2-BUTOXYETHYL ESTER] 21-Day Dermal Toxicity Study - Rabbit [§82-2/OPPTS 870.3200]

Amendment to DER for MRID 41407901 - 21-Day Dermal Toxicity Study [Rabbit] [2,4-D-2-BUTOXYETHYL ESTER]

This amendment provides an EXECUTIVE SUMMARY to upgrade the original DER [Document No. 008185].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/4/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P. H. Virginia A. Dobozy 12/6/00
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 21-day dermal toxicity - rabbit § 82-2/OPPTS 870.3200
P.C. CODE: 030053 REREGISTRATION CASE NO.: 818726
CASWELL NO.: 315AI CAS #: 1929-73-3
TEST MATERIAL (PURITY): 2,4-D butoxyethyl ester [94.6%]
SYNONYMS: 2,4-D BEE; dichlorophenoxyacetic acid, 2-butoxyethyl ester
SPONSOR: DowElanco

CITATION: Mizell, M. J.; Atkin, L.; and Crissman, J. W. (1990). 2,4-Dichlorophenoxyacetic acid butoxyethyl ester: 21-Day Dermal Toxicity Study in New Zealand White Rabbits. The Toxicology Research Laboratory, The Dow Chemical Company; Study No. K-007722-008, February 21, 1990. MRID 41407901. Document No. 008185. Unpublished.

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study [MRID 41407901], 5 New Zealand White rabbits/sex/group were administered 2,4-dichlorophenoxyacetic acid, butoxyethyl ester [94.6%] *via* dermal application in corn oil [once a day for 6 hours, 5 days/week] for 15 days at concentrations of 0, 50, 150, or 500 mg/kg/day.

There was no evidence of systemic toxicity; no treatment-related effects were observed on survival, clinical signs of toxicity, body-weight gain, hematology, clinical chemistry, organ weights, gross pathology, or histopathology. Dermal irritation, which was observed at the site of application in all dose groups, including the control, was characterized by erythema, edema, and scaling. Histopathology revealed slight fibrosis, epithelial hyperplasia and acute inflammation of the treated skin of some rabbits of both sexes from all groups.

The NOAEL for systemic effects and dermal irritation is 500 mg/kg/day, the highest dose tested.

This 21-day dermal toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [§82-2; 870.3200] for a 21-day dermal toxicity study.

CASWELL 315

PRIMARY REVIEWER: Jess Rowland, Toxicologist Jess Rowland 11/28/90
Toxicology Branch II, Section II (H7059C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 11/29/90
Toxicology Branch II, Section II (H7059C)

DATA EVALUATION REPORT

STUDY: 21-Day Dermal, Rabbit

MRID NO. 414079-01 **CASWELL NO.:** 315AI

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

SYNONYMS: 2,4-D BEE, 2,4-D butoxyethylester

STUDY NUMBER: K-007722-008

SPONSOR: DowElanco

TESTING FACILITY: The Toxicology Research Laboratory
The Dow Chemical Company
Midland, MI

TITLE OF REPORT: 2,4-Dichlorophenoxyacetic acid butoxyethyl ester: 21-Day Dermal Toxicity Study in New Zealand White Rabbits

AUTHORS: M.J. Mizell, L. Atkin and J. W. Crissman

REPORT ISSUED: February 21, 1990

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSION: Groups of five male and five female New Zealand White rabbits received 15 six hour dermal applications of 2,4-Dichlorophenoxyacetic acid, butoxyethyl ester (2,4-D BEE) in corn oil at 0, 50, 150 or 500 mg/kg/day. Dermal irritation observed at the site of application of various animals in all dose groups, including the controls, was characterized by erythema, edema and scaling. Histopathology revealed slight fibrosis, epithelial hyperplasia and acute inflammation at the treated skin of some males and females from all dose groups, including the corn oil control. There was no evidence of systemic toxicity; no treatment-related effects were observed on survival, clinical signs of toxicity, body weight gain, hematology and clinical chemistry, organ weights, gross pathology or histopathology. Under the conditions of this study, a no-observed-effect-level (NOEL) of 500 mg/kg/day was established for the dermal irritation and systemic toxicity of 2,4-D BEE in rabbits.

CORE CLASSIFICATION: Minimum; this study satisfies the guideline requirements (82-2) for a 21-day dermal toxicity study.

I. INTRODUCTION

This Data Evaluation Report (DER) summarizes the results of a 21-day dermal toxicity study in rabbits with 2,4-Dichlorophenoxyacetic acid, butoxyethyl ester (2,4-D BEE).

II. MATERIALS AND METHODS

1. Test Material: 2,4-Dichlorophenoxyacetic Acid,butoxyethyl ester (2,4-D BEE)

Lot No.	AGR 276426
ID No.:	K-007722-008
Description:	Dark Amber
Purity:	94.6%
Homogeneity:	stability and homogeneity of 2,4-D BEE dose solutions was determined concurrently with this study

2. Test Animals:

Species:	Rabbit
Strain:	New Zealand White
Age:	Five Months
Weight:	not provided
Source:	Hazleton Research Products Inc., Denver Pa.
Housing:	In individual cages
Food:	Purina Certified Chow #5322
Water:	ad libitum

3. Experimental Design

(i). A probe study was conducted to evaluate the dermal irritation potential of undiluted 2,4-D BEE following repeated application to aid in determining dose levels for the 21-day study. One male rabbit received four dermal applications of the undiluted test material at a rate of 1.7 ml/kg. This corresponded to a dose level of 2024 mg 2,4-D BEE/kg/day or 1300 mg/kg/day 2,4-D acid equivalent. Owing to irritation at this level, the material was diluted 1:3 with corn oil and applied to another rabbit at a rate of 1.7 ml/kg which corresponded to a dose of 562 mg 2,4 D BEE or 361 mg/kg 2,4 D acid equivalent.

(ii). Based on the results of the probe study, the dose levels selected for the 21-day study were 50, 150, and 500 mg/kg/day. The test material was diluted in corn oil, and the dosing volume was 1.7 ml/kg. Groups of five male and five female rabbits received a total of 15 dermal applications during a 21-day interval (weekends and holidays excluded). A control group received corn oil (1.7 ml/kg) only.

4. Treatment Procedure

Animals were acclimated for at least 14 days prior to the initiation of the study. All rabbits were acclimated to an elastic jacket (used to hold the test material and dressing in dermal contact) for at least four days prior to the first application. An area, approximately 10 x 15 cm on the back of each rabbit was clipped free of fur prior to the initiation of the study and as necessary thereafter. A dressing consisting of absorbent gauze and non-absorbent cotton was used to hold the test material in dermal contact. The jacket and dressing were removed approximately six hours after application and the test site was wiped with a water-dampened disposable towel to remove any residual test material.

5. Experimental Procedures

Parameter	Time measured
General appearance	Daily
Dermal observations	Daily
Body weight	Weekly
Hematology	At termination
Hematocrit (HCT)	
Hemoglobin (HGB)	
Leukocyte count (WBC)	
Erythrocyte Count (RBC)	
Platelets (PLAT)	
Leukocyte Differential count	
Cellular morphology	
Clinical chemistry:	At termination
Calcium	
Chloride	
Phosphorous	
Potassium	
Sodium	
Alkaline Phosphatase (AP)	
Serum Alanine aminotransferase (SGPT)	
Serum Aspartate Aminotransferase (SGOT)	
Albumin (ALB)	
Blood creatinine	
Blood Urea Nitrogen	
Globulin (GLOB)	
Glucose (GLCU)	
Total bilirubin (TBILI)	
Total protein	
Albumin/globulin ratio	

79

6. Termination

At termination, body weights were obtained, and all animals were subjected to a complete gross necropsy. Liver, kidney and testes were weighed, and organ weight to final weight ratios were calculated on all animals. Representative samples of all tissues/organs were preserved in 10% formalin.

7. Histopathology

Histologic evaluation of normal and treated skin, liver, kidney and any masses or lesions were made on all control and high-dose animals. Histopathologic examination was extended to lower dose animals and consisted of skin from the application site and skin from an adjacent site, as well as any grossly visible lesions.

8. Statistical Analyses

Body weights, absolute and relative organ weights, hematology and clinical chemistry data were analyzed by Bartlett's test for equality of variances. If the Barlett's test rejects the equality of variances, the parameter was flagged for careful evaluation of results. Body weights were then analyzed by a three-way repeated measures ANOVA, hematology and clinical chemistry parameters, terminal body weights, organ weights (absolute and relative) were evaluated by a two-way analysis of variance. If significant dose effects were determined in the one-way ANOVA, then separate doses were compared to controls using one-way ANOVA's with Bonferroni's Correction.

III. RESULTS

(i). Probe study

In the probe study, when tested at 2024 mg/kg/day, irritation and moderate to severe erythema, and well defined edema and moderate to severe fissuring was observed at the site of application. When diluted to 1:3 with corn oil, and applied at 562 mg/kg/day, well defined erythema was seen at the test site. No systemic toxicity was observed. Based on these results, 50, 100, or 500 mg/kg was selected as the dose levels for the 21-day study.

(ii). 21-Day Study

1. Survival: No mortality occurred during the study

2. Clinical Signs: No treatment-related clinical signs of toxicity were observed.

3. Dermal Observations: Signs of localized reaction to treatment consisted of dermal irritation at the site of application in various animals in all dose groups including the corn oil control. Two male and two female controls had slight, transient edema and/or scaling. One female control had very slight erythema at the test site. At the 50 mg/kg/day level, 4 males and 3 females showed very slight erythema and edema, and one female had slight scaling at the test site. All animals at the 150 mg/kg /day level developed slight erythema and edema, and 4 females had slight scaling at the test site. Well defined erythema and edema were present in 5 males and 4 females at the high-dose (500 mg/kg/day). In addition, slight scaling at the test site was observed in both sexes of rabbits at the high dose.

4. Body Weight: Treatment had no adverse effect on body weight; all animals gained weight or were near their pretreatment body weights at termination.

5. Clinical Pathology: No biologically or statistically significant changes were seen in mean hematology or clinical chemistry parameters in treated groups when compared to appropriate corresponding control group values.

6. Absolute and Relative Organ Weights: No treatment-related effects were noted on absolute or relative organ weights. The statistically significant increase observed in absolute liver weights of both sexes of rabbits at the 100 mg/kg/day was not considered to be toxicologically significant since the increase was not dose-dependent, there was no effect on relative liver weights, and histopathology showed no evidence of liver damage.

7. Gross Pathology: Treatment-related gross pathological changes were limited to the very slight to slight dermal thickening seen in male rabbits at the mid-and high-dose groups. Slight erythema, scabs and scales were observed in an incidence unrelated to exposure. The authors stated that the apparent discrepancy between gross pathological observations and dermal observations (dermal scoring) was most likely due to exsanguination of the animals and the subsequent blood loss which occurs.

8. Histopathology: There was no evidence of pathological changes attributable to 2,4-D BEE. Lesions observed at the application site were acute inflammation, epithelial hyperplasia and fibrosis; these lesions were seen in both the control and treated groups and showed neither a dose response nor trend (Table 1). Histopathology of the liver and kidneys from high dose animals revealed no evidence of systemic toxicity.

IV. DISCUSSION

In a 21-day dermal toxicity study, 15 repeated dermal applications of 2,4-D BEE at 0, 50, 150, or 500 mg/kg/day produced dermal irritation (at the application site) characterized by erythema, edema, and scaling. Histologically, the dermal lesions were diagnosed as slight fibrosis, epithelial hyperplasia and acute inflammation. These lesions were seen in some males and some females in all test groups including the corn oil control. Since the dermal lesions were seen both in the control and treated groups and showed no dose-response the dermal lesions were not considered to be compound-induced. 2,4-D BEE did not induce adverse effects on survival, body weight, hematology and clinical chemistry parameters, absolute and relative organ weights, or gross or histopathology.

V. CONCLUSION

Under the conditions of this study, a NOEL of 500 mg/kg/day is established for both dermal irritation and systemic toxicity of 2,4-D BEE.

VI. CORE CLASSIFICATION

Minimum; this study satisfies the guideline requirements (82-2) for a dermal toxicity study.

Table 1. DERMAL LESIONS AT APPLICATION SITE IN RABBITS RECEIVING 15-DERMAL APPLICATIONS OF 2,4-D BEE.

Sex	Males				Females			
Dose (mg/kg/day)	0	50	150	500	0	50	150	500
No.Examined	5	5	5	5	5	5	5	5
Dermal lesions								
Fibrosis, very slight	1	0	0	0	2	2	1	0
Fibrosis, slight	0	0	0	0	0	0	1	0
Hyperplasia, epithelial								
very slight	3	5	5	5	4	2	3	4
slight	2	0	0	0	1	2	2	0
Inflammation acute								
very slight	1	5	2	4	3	1	1	5
slight	0	0	1	1	0	2	2	0
moderate	0	0	1	0	0	1	1	0
severe	1	0	1	0	2	1	1	0

Data are the number of animals with specified observations.

808

[TRIISOPROPANOLAMINE SALT OF 2,4-D] 21-Day Dermal Toxicity Study - Rabbit [§82-2/OPPTS 870.3200

Amendment to DER for MRID 41407902 - 21-Day Dermal Toxicity Study [Rabbit] [TRIISOPROPANOLAMINE SALT OF 2,4-D]

This amendment provides an EXECUTIVE SUMMARY *to upgrade the original* DER [Document No. 008185].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/6/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P. H. Virginia A Dobozy 12/11/00
Reregistration Branch I, Health Effects Division (7509C)
TXR# 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 21-day dermal toxicity - rabbit § 82-2/OPPTS 870.3200
P.C. CODE: 030025
CASWELL NO.: 315AE CAS #: 18585-79-7
TEST MATERIAL (PURITY): Triisopropanolamine salt of 2,4-D [72.2%; 38.7% acid equivalent]
SYNONYMS: 2,4-D TIPA; dichlorophenoxyacetic acid, triisopropanolamine salt
SPONSOR: DowElanco

CITATION: Mizell, M. J. ; Atkin, L.; Haut, K. T.; *et al.* (1989). 2,4-D Triisopropanolamine Salt: 21-Day Dermal Toxicity Study in New Zealand White Rabbits. The Toxicology Research Laboratory, The Dow Chemical Company; Study No. K-008866-004, December 8, 1989. MRID 41407902. Document No. 008185. Unpublished.

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study [MRID 41407902], 5 New Zealand White rabbits/sex/group were administered the triisopropanolamine salt of 2,4-dichlorophenoxyacetic acid [72.2%] *via* dermal application in distilled water [6 hours/day for 15 dermal applications during a 21-day period] at concentrations of 0, 100, 350, and 1000 mg/kg/day.

Treatment had no adverse effect on survival, clinical signs, mean body weight, hematology, clinical chemistry, organ weight, gross pathology, or histopathology of the liver or kidney. Very slight, transient erythema at the application site was observed at all dose levels during the first week of dosing. Histologically, the dermal lesions were diagnosed as very slight to slight multifocal inflammatory reactions.

The NOAEL for systemic toxicity is 1000 mg/kg/day, the highest dose tested. The dermal irritation NOAEL is 1000 mg/kg/day also.

This 21-day dermal toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [§82-2; 870.3200] for a 21-day dermal toxicity study.

CASWELL FILE

PRIMARY REVIEWER: Jess Rowland, Toxicologist Jess Rowland 11/28/90
Toxicology Branch II, Section II (H7059C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 11/29/90
Toxicology Branch II, Section II (H7059C)

DATA EVALUATION REPORT

STUDY: 21-Day Dermal, Rabbit

MRID NO. 414079-02 **CASWELL NO.:** 315Ø AE

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid, triisopropanolamine salt

SYNONYMS: 2,4-D triisopropanolamine salt; (2,4-D TIPA)

STUDY NUMBER: K-008866-004

SPONSOR: DowElanco

TESTING FACILITY: The Toxicology Research Laboratory
The Dow Chemical Company
Midland, MI

TITLE OF REPORT: 2,4-D Triisopropanolamine salt: 21-Day Dermal Toxicity Study in New Zealand White Rabbits.

AUTHORS: M.J. Mizell, L. Atkin, K.T. Haut and K.E. Stebbins

REPORT ISSUED: December 8, 1989

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSION: Groups of five male and five female New Zealand White rabbits received 15 six hour dermal applications of 2,4-D Triisopropanolamine salt (2,4-D TIPA) in distilled water at 0, 100, 350 or 1000 mg/kg/day. Dermal irritation was limited to very slight, transient erythema at the application site. Histologically they were characterized as very slight or slight inflammatory reaction of the dermis; inflammation was present in two low-dose, four mid-dose, and five high-dose animals. The inflammatory reaction was confined to the superficial dermis; no epidermal lesions were seen. There was no evidence of systemic toxicity; no treatment-related effects were seen on survival, clinical signs, body weight gain, hematology or clinical chemistry parameters, organ weights, gross pathology or histopathology. Under the conditions of this study, a no-observed-effect-level (NOEL) of 1000 mg/kg/day was established for the dermal irritation and systemic toxicity of 2,4-D TIPA in rabbits.

CORE CLASSIFICATION: Minimum; this study satisfies the guideline requirements (82-2) for a 21-day dermal toxicity study.

I. INTRODUCTION

This Data Evaluation Report (DER) summarizes the results of a 21-day dermal toxicity study in rabbits with 2,4-D Triisopropanolamine salt (2,4-D TIPA).

II. MATERIALS AND METHODS

1. Test Material: 2,4-D Triisopropanolamine salt (2,4-D TIPA)

Lot No.	AGR 276428
ID No.:	K-008866-004
Description:	Amber
Purity:	72.2%
Homogeneity:	stability and homogeneity of 2,4-D TIPA dose solutions was determined concurrently with the study.

2. Test Animals:

Species:	Rabbit
Strain:	New Zealand White
Age:	Five Months
Weight:	not provided
Source:	Hazleton Research Products Inc., Denver Pa.
Housing:	In individual cages
Food:	Purina Certified Chow #5322
Water:	ad libitum

3. Experimental Design

(i). A probe study was conducted to evaluate the dermal irritation potential of undiluted 2,4-D TIPA following repeated application to aid in determining dose levels for the 21-day study. One male rabbit received eight dermal applications of the undiluted test material at a rate of 1.7 ml/kg. This corresponded to a dose level of 1474 mg 2,4-D TIPA/kg/day or 815 mg/kg/day 2,4-D acid equivalent.

(ii). Based on the results of the probe study, the dose levels selected for the 21-day study were 100, 350, or 1000 mg/kg/day. The test material was diluted in distilled water, and the dosing volume was 1.7 ml/kg. Groups of five male and five female rabbits received a total of 15 dermal applications during a 21-day interval (weekends and holidays excluded). A control group was dosed with distilled water at a rate of 1.7 ml/kg; this control group was also used for the 21-day dermal toxicity study with 2,4-D isopropylamine salt (MRID No. 414079-03).

4. Treatment Procedure

Animals were acclimated for at least 14 days prior to the initiation of the study. All rabbits were acclimated to an elastic jacket (used to hold the test material and dressing in dermal contact) for at least four days prior to the first application. An area, approximately 10 x 15 cm on the back of each rabbit was clipped free of fur prior to the initiation of the study and as necessary thereafter. A dressing consisting of absorbent gauze and non-absorbent cotton was used to hold the test material in dermal contact. The jacket and dressing were removed approximately six hours after application and the test site was wiped with a water-dampened disposable towel to remove any residual test material.

5. Experimental Procedures

Parameter	Time measured
General appearance	Daily
Dermal observations	Daily
Body weight	Weekly
Hematology	At termination
Hematocrit (HCT)	
Hemoglobin (HGB)	
Leukocyte count (WBC)	
Erythrocyte Count (RBC)	
Platelets (PLAT)	
Leukocyte Differential count	
Cellular morphology	
Clinical chemistry:	At termination
Calcium	
Chloride	
Phosphorous	
Potassium	
Sodium	
Alkaline Phosphatase (AP)	
Serum Alanine aminotransferase (SGPT)	
Serum Aspartate Aminotransferase (SGOT)	
Albumin (ALB)	
Blood creatinine	
Blood Urea Nitrogen (BUN)	
Globulin (GLOB)	
Glucose (GLCU)	
Total bilirubin (TBILI)	
Total protein	
Albumin/globulin ratio	

804

6. Termination

At termination, body weights were obtained, and all animals were subjected to a complete gross necropsy. Liver, kidney and testes were weighed, and organ weight to final weight ratios were calculated on all animals. Representative samples of all tissues/organs were preserved in 10% formalin.

7. Histopathology

Histologic evaluation of normal and treated skin, liver, kidney and any masses or lesions were made on all control and high-dose animals. Histopathologic examination was extended to lower dose animals and consisted of skin from the application site and skin from an adjacent site, as well as any grossly visible lesions.

8. Statistical Analyses

Body weights, absolute and relative organ weights, hematology and clinical chemistry data were analyzed by Bartlett's test for equality of variances. If the Barlett's test rejects the equality of variances, the parameter was flagged for careful evaluation of results. Body weights were then analyzed by a three-way repeated measures ANOVA, hematology and clinical chemistry parameters, terminal body weights, organ weights (absolute and relative) were evaluated by a two-way analysis of variance. If significant dose effects were determined in the one-way ANOVA, then separate doses were compared to controls using one-way ANOVA's with Bonferroni's Correction.

III. RESULTS

(i). Probe study

Dermal application of 1474 mg 2,4-D TIPA/kg/day caused very slight erythema at the dermal site after the first application; there were no other signs of dermal irritation at any time, and there were no signs of systemic toxicity. Based on these results, 100, 350 and 1000 mg/kg was selected as the dose levels for the 21-day study.

(ii). 21-Day Study

1. Survival: No mortality occurred during the study

2. Clinical Signs: No treatment-related clinical signs of toxicity were observed.

3. Dermal Observations: Localized dermal irritation at the application site was limited to very slight, transient erythema observed in five animals at the 100 mg/kg/day group, two animals at the 350 mg/kg/day groups, and three animals at the 1000 mg/kg/day group during days 1 through 6. After day six, there were no signs of dermal irritation at any dose level.

4. Body Weight: No adverse effects were seen on body weight in either sex at any dose level; all animals gained weight or were near their pretreatment body weights at termination.

5. Clinical Pathology: No biologically or statistically significant changes were seen on hematologic parameters. Sporadic, statistically significant differences were seen in a few clinical chemistry parameters (TBILI, ALT, BUN, TP) in all treated groups when compared to appropriate corresponding control group values. However, since the values were within the range of normal healthy rabbits, and there was no evidence of dose-response, these differences were not considered to be toxicologically significant.

6. Absolute and Relative Organ Weights: No treatment-related effects were noted on absolute or relative organ weights for male or female rabbits.

7. Gross Pathology: No treatment-related gross pathological changes were seen.

8. Histopathology: Histopathology revealed very slight, multifocal inflammation of the dermis at the test site in 1/5 males and 1/5 females at 100 mg/kg/day; 1/5 males and 3/5 females at 350 mg/kg/day; and 3/5 males and 1/5 females at 1000 mg/kg/day; in addition, 1 female at the high-dose exhibited slight, multifocal inflammation at the treatment area. The inflammatory lesions were confined to the superficial dermis, and no epidermal lesions were seen. Histopathology of the liver and kidneys from high dose animals revealed no evidence of systemic toxicity.

IV. DISCUSSION

In a 21-day dermal toxicity study, 15 repeated dermal applications of 2,4-D TIPA at 0, 100, 350 or 1000 mg/kg/day produced no severe dermal irritation. Localized signs at the application site were limited to very slight, transient erythema in a few animals up to treatment day 6. The application site of all treated rabbits was within normal limits during the remainder of the study. Histologically, the dermal lesions were diagnosed as very slight or slight, multifocal inflammatory reactions. These lesions were seen in 2 low dose, 4 intermediate dose, and 5 high dose rabbits. Some animals in all three treatment groups had no lesions at the application site. 2,4-D TIPA did not induce adverse effects on survival, body weight, hematology and clinical chemistry parameters, absolute and relative organ weights, or gross or histopathology.

V. CONCLUSION

Under the conditions of this study, a NOEL of 1000 mg/kg/day is established for both dermal irritation and systemic toxicity of 2,4-D BEE.

VI. CORE CLASSIFICATION

Minimum; this study satisfies the guideline requirements (82-2) for a dermal toxicity study.

Amendment to DER for MRID 41407903 - 21-Day Dermal Toxicity Study [Rabbit][ISOPROPYL AMINE SALT OF 2,4-D]

This amendment provides an EXECUTIVE SUMMARY to upgrade the original DER [Document No. 008185].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/7/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A. Dobozy 12/11/00
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 21-day dermal toxicity - rabbit § 82-2/OPPTS 870.3200
P.C. CODE: 030025
CASWELL NO.: 315U CAS #: 5742-17-6
TEST MATERIAL (PURITY): Isopropylamine salt of 2,4-D [50.2%]
SYNONYMS: 2,4-D IPA; dichlorophenoxyacetic acid, isopropylamine salt
SPONSOR: DowElanco

CITATION: Mizell, M. J., Atkin, L., Haut, K. T., *et al.* (1990). 2,4-D Isopropylamine Salt: 21-Day Dermal Toxicity Study in New Zealand White Rabbits. The Toxicology Research Laboratory, The Dow Chemical Company; Study No. M-004725-004, February 20, 1990. MRID 41407903. Document No. 008185. Unpublished.

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study [MRID 41407903], 5 New Zealand White rabbits/sex/group were administered the isopropylamine salt of 2,4-dichlorophenoxyacetic acid [50.2%] *via* dermal application in distilled water [6 hours/day for 15 dermal applications during a 21-day period] at concentrations of 0, 50, 125, and 350 mg/kg/day.

Treatment had no adverse effect on survival, clinical signs, mean body weight, hematology, clinical chemistry, organ weight, gross pathology, or histopathology of the liver or kidney. On gross pathological examination, thickened skin, scales, scabs, and hyperemia were observed at the two highest dose levels.

There was a dose-related increase in the incidence and severity of the signs of dermal irritation. Dose-dependent dermal irritation ranged from slight, transient erythema at the low dose, slight erythema and scaling at the mid dose, and slight to moderate erythema and scaling at the high dose. Histologically, dermal lesions characterized as focal or multifocal irritative effects [inflammation and epidermal hyperplasia] were observed only at 125 and 350 mg/kg/day.

The NOAEL for systemic toxicity is 350 mg/kg/day, the highest dose tested. The dermal irritation NOAEL is 50 mg/kg/day, based on microscopic dermal lesions at the dermal irritation LOAEL of 125 mg/kg/day.

This 21-day dermal toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [§82-2; 870.3200] for a 21-day dermal toxicity study.

CASWELL FILE

PRIMARY REVIEWER: Jess Rowland, Toxicologist 11/28/90
Toxicology Branch II, Section II (H7059C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head 11/29/90
Toxicology Branch II, Section II (H7059C)

DATA EVALUATION REPORT

STUDY: 21-Day Dermal, Rabbit

MRID NO. 414079-03 **CASWELL NO.:** 315[redacted]

TEST MATERIAL: 2,4-Dichlorophenoxyacetic Acid, Isopropylamine salt

SYNONYMS: 2,4-D Isopropylamine salt; (2,4-D IPA)

STUDY NUMBER: M-004725-004

SPONSOR: DowElanco

TESTING FACILITY: The Toxicology Research Laboratory
The Dow Chemical Company
Midland, MI

TITLE OF REPORT: 2,4-D Isopropylamine salt: 21-Day Dermal Toxicity Study in New Zealand White Rabbits.

AUTHORS: M.J. Mizell, L. Atkin, K.T. Haut and K.E. Stebbins

REPORT ISSUED: February 20,1990

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSION: Groups of five male and five female New Zealand White rabbits received 15 six hour dermal applications of 2,4-D Isopropylamine salt (2,4-D IPA) in distilled water at 0, 50, 125 or 350 mg/kg body weight/day. Dose-dependent dermal irritation observed were slight, transient erythema at the low-dose, slight erythema and scaling at the mid-dose, and slight to moderate erythema and scaling at the high-dose. Histologically, dermal lesions characterized as focal or multifocal irritative effects (inflammation and epidermal hyperplasia) were seen only at the 125 and 350 mg/kg/day groups; no histopathological dermal lesions were seen at 50 mg/kg/day group. There was no evidence of systemic toxicity; no treatment-related effects were seen on survival, clinical signs, body weight gain, hematology or clinical chemistry parameters, organ weights, gross pathology or histopathology of kidneys and liver. Under the conditions of this study, the no-observed-effect-levels (NOELs) established were 50 mg/kg/day for dermal irritation and 350 mg/kg /day for the systemic toxicity of 2,4-D TIPA in rabbits. The lowest-observed-effect-level (LOEL) for dermal irritation was 125 mg/kg/day.

CORE CLASSIFICATION: Minimum; this study satisfies the guideline requirements (82-2) for a 21-day dermal toxicity study.

I. INTRODUCTION

This Data Evaluation Report (DER) summarizes the results of a 21-day dermal toxicity study in rabbits with 2,4-D Isopropylamine salt (2,4-D IPA).

II. MATERIALS AND METHODS

1. Test Material: 2,4-D Isopropylamine salt (2,4-D IPA)

Lot No.	AGR 276461
ID No.:	M-00475-004
Description:	Amber
Purity:	50.2%
Homogeneity:	Stability and homogeneity of 2,4-D IPA dose solutions was determined concurrently with the study.

2. Test Animals:

Species:	Rabbit
Strain:	New Zealand White
Age:	Five Months
Weight:	not provided
Source:	Hazleton Research Products Inc., Denver Pa.
Housing:	In individual cages
Food:	Purina Certified Chow #5322
Water:	ad libitum

3. Experimental Design

(i). A probe study was conducted to evaluate the dermal irritation potential of undiluted 2,4-D IPA following repeated application to aid in determining dose levels for the 21-day study. One male rabbit received three dermal applications of the undiluted test material at a rate of 1.7 ml/kg. This corresponded to a dose of approximately 1008 mg 2,4-D IPA/kg/day or 788 mg/kg/day 2,4-D acid equivalent. Owing to irritation observed at this level, the material was diluted 1:3 with distilled water and applied to another rabbit at a rate of 1.7 ml/kg which corresponded to a dose of 301 mg 2,4-D IPA/kg/day or 235 mg 2,4-D acid equivalent.

(ii). Based on the results of the probe study, the dose levels selected for the 21-day study were 50, 125 or 350 mg/kg/day. The test material was diluted in distilled water, and the dosing volume was 1.7 ml/kg. Groups of five male and five female rabbits received a total of 15 dermal applications during a 21-day interval (weekends and holidays excluded). Since this study was conducted concurrent with the 21-day study with 2,4-D Triisopropanolamine salt (MRID No. 414079-02), one control group dosed with distilled water at a rate of 1.7 ml/kg was employed for both studies.

818

4. Treatment Procedure

Animals were acclimated for at least 14 days prior to the initiation of the study. All rabbits were acclimated to an elastic jacket (used to hold the test material and dressing in dermal contact) for at least four days prior to the first application. An area, approximately 10 x 15 cm on the back of each rabbit was clipped free of fur prior to the initiation of the study and as necessary thereafter. A dressing consisting of absorbent gauze and non-absorbent cotton was used to hold the test material in dermal contact. The jacket and dressing were removed approximately six hours after application and the test site was wiped with a water-dampened disposable towel to remove any residual test material.

5. Experimental Procedures

Parameter	Time measured
General appearance	Daily
Dermal observations	Daily
Body weight	Weekly
Hematology	At termination
Hematocrit (HCT)	
Hemoglobin (HGB)	
Leukocyte count (WBC)	
Erythrocyte Count (RBC)	
Platelets (PLAT)	
Leukocyte Differential count	
Cellular morphology	
Clinical chemistry:	At termination
Calcium	
Chloride	
Phosphorous	
Potassium	
Sodium	
Alkaline Phosphatase (AP)	
Serum Alanine aminotransferase (SGPT)	
Serum Aspartate Aminotransferase (SGOT)	
Albumin (ALB)	
Blood creatinine	
Blood Urea Nitrogen	
Globulin (GLOB)	
Glucose (GLCU)	
Total bilirubin (TBILI)	
Total protein	
Albumin/globulin ratio	

6. Termination

At termination, body weights were obtained, and all animals were subjected to a complete gross necropsy. Liver, kidney and testes were weighed, and organ weight to final weight ratios were calculated on all animals. Representative samples of all tissues/organs were preserved in 10% formalin.

7. Histopathology

Histologic evaluation of normal and treated skin, liver, kidney and any masses or lesions were made on all control and high-dose animals. Histopathologic examination was extended to lower dose animals and consisted of skin from the application site and skin from an adjacent site, as well as any grossly visible lesions.

8. Statistical Analyses

Body weights, absolute and relative organ weights, hematology and clinical chemistry data were analyzed by Bartlett's test for equality of variances. If the Barlett's test rejects the equality of variances, the parameter was flagged for careful evaluation of results. Body weights were then analyzed by a three-way repeated measures ANOVA, hematology and clinical chemistry parameters, terminal body weights, organ weights (absolute and relative) were evaluated by a two-way analysis of variance. If significant dose effects were determined in the one-way ANOVA, then separate doses were compared to controls using one-way Bonferroni's Correction.

III. RESULTS

(i). Probe study

In the probe study, when tested at 1008 mg/kg/day, slight erythema and slight edema, as well as necrosis was seen at the treatment site. When diluted to 1:3 with distilled water, and applied at 301 mg/kg/day, slight erythema was present in the treated area. No systemic toxicity was observed at either level. Based on these results, 50, 125, and 350 mg/kg was selected as the dose levels for the 21-day study.

(ii). 21-Day Study

1. Survival: No mortality occurred during the study

2. Clinical Signs: No treatment-related clinical signs of toxicity were observed.

3. Dermal Observations: Dose-dependent dermal irritation observed at the application site was characterized as slight,transient erythema at the low-dose (50 mg/kg/day), slight erythema and scaling at the mid-dose (125 mg/kg/day), and slight to moderate erythema at the high-dose (350 mg/kg/day). In addition, three males and one female at the high dose had scabs/crusts and scattered necrosis at the application site. No dermal irritation was seen in rabbits receiving distilled water only. One male rabbit at the high dose was not treated after the second application due to clipper abrasions which became necrotic and edematous.

4. Body Weight: No adverse effect on mean body weight was seen in either sex at any dose level; all animals gained weight or were near their pretreatment body weights at termination.

5. Clinical Pathology: No biologically or statistically significant changes were seen in hematology parameters. A statistically significant difference between high dose and control phosphorous levels was attributed to the normal variability of this parameter in rabbits and was considered to be of no toxicological significance.

6. Absolute and Relative Organ Weights: No treatment-related effects were noted on absolute or relative organ weights for male or female rabbits.

7. Gross Pathology: No treatment-related gross pathological alterations of the skin were seen in rabbits receiving distilled water or the low-dose (50 mg/kg/day). In rabbits at the 125 and 350 mg/kg/day dose levels, dermal lesions confined to the treatment area consisted of one, or a combination of more than one, of the following: thickened skin, scales, scabs, and hyperemia. The cutaneous lesions were dose-dependent; 4/5 males and 2/5 females at the 125 mg/kg/day group and 5/5 and 4/5 females at the 350 mg/kg/day group. No other treatment-related gross pathological changes were seen at any level.

8. Histopathology: No histopathological lesions were seen in the skin of rabbits receiving the vehicle or the 50 mg 2,4-D IPA/kg/day. Dermal lesions observed at the 125 mg/kg/day group included slight multifocal dermal inflammation, multifocal hyperkeratosis, focal keratosis, multifocal parakeratosis, and focal parakeratosis. Cutaneous lesions at the 350 mg/kg/day included very slight multifocal dermal inflammation, very slight to moderate epidermal hyperplasia, multifocal parakeratosis, multifocal hyperkeratosis, moderate focal suppurative epidermal inflammation, very slight to moderate dermal fibrosis (Table 21). Histopathology of the liver and kidneys from high dose animals revealed no evidence of systemic toxicity.

IV. DISCUSSION

In a 21-day dermal toxicity study, rabbits received 15 repeated dermal applications of 2,4-D IPA at 0, 50, 125 or 350 mg/kg/day. Dose-dependent dermal irritation ranged from slight, transient erythema at the 50 mg/kg/day group to moderate erythema, edema, and scaling at the 350 mg/kg/day group. No gross or histological dermal lesions were seen in rabbits at the low-dose. However, at the mid- (125 mg/kg/day) and high-dose (350 mg/kg/day), histopathology revealed focal or multifocal irritative effects at the dermal site. The dermal lesions consisted of inflammation and epidermal hyperplasia; the majority of these lesions were graded as very slight or slight. One female rabbit at the high-dose exhibited moderate suppurative inflammation of the epidermis, moderate epidermal hyperplasia, and moderate dermal fibrosis. 2,4-D IPA did not induce adverse effects on survival, body weight, hematology and clinical chemistry parameters, absolute and relative organ weights, or gross or histopathological changes in the liver and kidneys.

V. CONCLUSION

Under the conditions of this study, the NOELs established were 50 mg/kg/day for dermal irritation and 350 mg/kg/day for systemic toxicity. The LOEL was 125 mg/kg/day for dermal irritation.

VI. CORE CLASSIFICATION

Minimum; this study satisfies the guideline requirements (82-2) for a dermal toxicity study.

Table 1. Dermal Lesions At Application Site in Rabbits Receiving 15-Dermal Applications of 2,4-D Isopropylamine salt (2,4-D IPA).

Sex	Males				Females			
Dose (mg/kg/day)	0	50	125	350	0	50	125	350
No.Examined	5	5	5	5	5	5	5	5
Dermal lesions								
Normal	5	5	1	0	5	5	2	1
Fibrosis, dermis, focal	0	0	0	0	0	0	0	1
Fibrosis, dermis, multifocal	0	0	0	0	0	0	0	1
Hyperkertosis, epidermis focal	0	0	1	0	0	0	0	0
multifocal	0	0	2	4	0	0	1	2
Hyperplasia, epidermis, multifocal: very slight	0	0	0	2	0	0	1	1
slight	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Hyperplasia,epidermis,diffuse	0	0	0	0	0	0	0	1
Inflammation multifocal very slight	0	0	2	3	0	0	2	2
slight	0	0	0	0	0	0	1	2
Inflammation suppurative,								
Epidermis, focal, moderate	0	0	0	0	0	0	0	1
Parakeratosis, epidermis focal	0	0	1	0	0	0	0	0
multifocal	0	0	2	3	0	0	1	3

Data are number of animals with the specified observations.

Amendment to DER for MRID 41896701 - Subchronic Oral Toxicity Study [Rat] [2,4-D-2-ETHYLHEXYL ESTER]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document Nos. 008580 and 009628].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 1/25/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P.H. Virginia A. Dobozy 1/29/01
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: subchronic oral toxicity - rat § 82-1/OPPTS 870.3100
P.C. CODE: 030063 REREGISTRATION CASE NO.: 818730
CASWELL NO.: 315AS CAS NO.: 1928-43-4
TEST MATERIAL (PURITY): 2,4-D-2-ethylhexyl ester [98% (acid equivalent 63.25%)]
SYNONYMS: 2,4-D-dichlorophenoxyacetic acid, isooctyl (2-ethylhexyl) ester
SPONSOR: Industry Task Force II

CITATION: Schulze, G. E. (1991). Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid-2-Ethylhexyl Ester. Hazleton Laboratories America, Inc. Study No. HLA 2184-112, April 20, 1991. MRID 41896701. Document No. 008580. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [MRID 41896701], 10 Fischer 344 rats/sex/group were administered the 2,4-dichlorophenoxyacetic acid-2-ethylhexyl ester of [98%; 63.25% acid equivalent] *via* the diet for 13 weeks at concentrations of 0, 1.5 mg/kg/day, 22.6 mg/kg/day, 150 mg/kg/day, and 452 mg/kg/day.

At the high-dose level, five males [one during week 7, two during week 8, and one each during weeks 10 and 11] and three females [one during week 10 and two during week 11] died. Clinical signs of toxicity occurred at the high-dose level only and included thin appearance [9 males/10 females], hunched posture [10 males/10 females], languid behavior [2 females], ataxia [1 female], anorexia [1 male], squinted eyes [4 males/3 females], rough haircoat [4 males/4 females], and few/no feces [3 males]. Opaque eyes, observed only in the females, were increased at the high-dose level [1/10 controls, 1/10 at 150 mg/kg/day, and 9/10 at 452 mg/kg/day] compared to the control.

Decreased body weights [males 55%females 64% of control at week 13] and body-weight gains [overall males 21%/females 12% of control] were observed in both sexes only at the highest dose level throughout the study. Additionally, the magnitude of the deficit increased with time on test, especially for the males. A negative body-weight gain was observed during the 6-13 week interval in both sexes. A corresponding decrease in food consumption was observed in both sexes at the highest dose level.

Bilateral cataract formation was observed in 7 high-dose females, and retinal degeneration was observed in 3 high-dose males. Treatment-related alterations in hematology [decreased RBC, HGB, HCT, platelet count, absolute and corrected leukocyte counts, and lymphocyte counts] were observed at the 6- and 14-week intervals at the high-dose level [both sexes], and nucleated RBC were increased in both sexes at 6 months [only males attained statistical significance] and in females at 14 weeks [statistical significance not attained]. Alterations in clinical chemistry

Amendment to DER for MRID 41896701 - Subchronic Oral Toxicity Study [Rat] [2,4-D-2-ETHYLHEXYL ESTER]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document Nos. 008580 and 009628].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 1/25/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M. P.H. Virginia A. Dobozy 1/29/01
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

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Bilateral cataract formation was observed in 7 high-dose females, and retinal degeneration was observed in 3 high-dose males. Treatment-related alterations in hematology [decreased RBC, HGB, HCT, platelet count, absolute and corrected leukocyte counts, and lymphocyte counts] were observed at the 6- and 14-week intervals at the high-dose level [both sexes], and nucleated RBC were increased in both sexes at 6 months [only males attained statistical significance] and in females at 14 weeks [statistical significance not attained]. Alterations in clinical chemistry

[decreased glucose, globulin levels, thyroxine levels, total protein, and serum calcium, increased ALT] were observed at 150 and 452 mg/kg/day at both intervals in one or both sexes, and increased BUN (both sexes) and AST (females) were observed at 452 mg/kg/day at both intervals. Changes in absolute and/or relative organ weights [adrenals, brain, thymus, heart, kidneys, testes with epididymides (males), ovaries (females), pituitary, liver (increased in both sexes), thyroids/parathyroids (increased in males) were observed primarily at the high-dose level [both sexes] and many of these changes may be attributable to decreased body weight. Treatment-related histopathological changes were observed primarily in the high-dose group and included centrilobular hepatocellular hypertrophy [liver], bilateral retinal degeneration and cataract formation [females], atrophy of the testes, prostate, and seminal vesicles [males], hypertrophy of the zona glomerulose [adrenal cortex, both sexes] and follicular cells [thyroid, both sexes], lymphoid hypoplasia of thymus [both sexes] and spleen [both sexes], hypoplasia of bone marrow [both sexes], vacuolation of tubular cells and brush border loss in proximal tubular cells [kidney, females]. Many of the lesions correlated well with the alterations observed in hematology and clinical chemistry parameters and/or organ-weight data of the high-dose groups.

The NOAEL is 22.6 mg/kg/day, based on alterations in clinical chemistry [decreased total protein (both sexes), calcium and albumin (males), globulin and T_4 (females), increased ALT (females)] parameters at the LOAEL of 150 mg/kg/day.

This subchronic oral toxicity study is classified **Acceptable/Guideline**, and the study satisfies the guideline requirement [OPPTS 870.3100; §82-1] for a subchronic oral toxicity study in the rodent.

Table 1. Clinical signs

Signs/Sex/Dose	0 mg/kg/day	1.5 mg/kg/day	22.6 mg/kg/day	150 mg/kg/day	452 mg/kg/day
MALES✔					
hunched	0	0	0	0	10
thin	0	0	0	0	9
anorexia	0	0	0	0	1
few or no feces	0	0	0	0	3
urine stains	0	0	0	2	9
rough haircoat	0	0	0	0	4
squinted eyes	0	0	0	0	4
pale body	0	0	0	0	1
swollen♪	3	0	1	2	6
FEMALES✔					
hunched	0	0	0	0	10
thin	0	0	0	0	10
languid	0	0	0	0	2
ataxia	0	0	0	0	1
urine stains	5	3	5	3	6
rough haircoat	0	0	0	0	4
squinted eyes	0	0	0	0	3
opaque eyes	1	0	1	0	9
pale body	0	0	0	0	1
MALES✖					
hunched	0	0	0	0	8
languid	0	0	0	0	2
soft feces	0	0	0	0	1
few/no feces	0	0	0	0	4
FEMALES✖					
hunched	0	0	0	0	7
languid	0	0	0	0	3

N=10; ♪observations included swollen paw, genital, abdomen, or penis; ✔ data from Table 2A, pages 78-79 of the report; ✖ cageside observations [Table 2B of report (page 81)];

Table 2. Hematology Data

Parameter/Week/Dose/Sex	0 mg/kg/day	1.5 mg/kg/day	22.6 mg/kg/day	150 mg/kg/day	452 mg/kg/day
MALES					
RBC [MI/UL]					
6	9.50±0.25	9.41±0.29	9.34±0.37	9.47±0.21	8.50±0.30*
14	9.65±0.25	9.71±0.31	9.67±0.28	9.66±0.20 (9)	7.45±0.36* (5)
HGB [g/dL]					
6	16.9±0.29	16.7±0.40	16.8±0.47	17.0±0.39	15.8±0.83*
14	16.7±0.48	16.9±0.52	16.8±0.46	16.9±0.27 (9)	13.8±0.82* (5)
HCT [%]					
6	48.5±1.22	48.3±1.38	48.1±1.62	48.8±1.20	46.8±2.18*
14	48.7±1.39	49.2±1.34	49.0±1.48	49.3±1.14 (9)	42.2±2.06* (5)
platelets [TH/UL]					
6	954±65	968±57	1002±95	862±125	642±81*
14	897±50	865±66	916±20	824±38* (9)	694±57* (5)
WBC [TH/UL]					
6	9.4±1.9	8.9±1.2	8.9±1.1	9.0±1.6	6.0±1.3*
14	6.3±1.2	6.2±1.1	5.6±0.9	6.3±0.7 (9)	3.2±0.4* (5)
NRBC [/100 WBC]					
6	0±0	0±0.3	1±1	0±0.7	1±1.5*
14	0±0	0±0	0±0	0±0 (9)	0±0 (5)
cor WBC [TH/UL]					
6	9.4±1.9	8.9±1.2	8.8±1.1	9.0±1.6	5.9±1.3*
14	6.3±1.2	6.2±1.1	5.6±0.9	6.3±0.7 (9)	3.2±1.3* (5)
lymphocytes [TH/UL]					
6	7.9±1.9	7.6±1.1	7.5±0.8	7.3±1.6	4.7±1.2*
14	5.1±1.1	4.8±1.0	4.3±0.7	4.8±0.8 (9)	1.7±0.6* (5)
FEMALES					
RBC [MI/UL]					
6	8.87±0.33	8.93±0.26	8.82±0.28	8.73±0.20	7.92±0.65*
14	8.99±0.17	9.05±0.17	9.06±0.22	8.67±0.21*	7.66±0.30* (7)
HGB [g/dL]					
6	16.5±0.6	16.8±0.5	16.8±0.5	16.1±0.3	15.0±1.2*
14	16.7±0.2	16.7±0.4	16.8±0.5	16.4±0.6	14.6±0.8* (7)
HCT [%]					
6	46.9±1.9	47.2±1.7	46.6±1.3	46.3±1.3	44.1±2.9*
14	48.5±0.9	48.8±1.0	49.0±1.3	47.9±1.6	44.5±2.3* (7)
platelets [TH/UL]					
6	880±254	976±63	809±241	821±124	628±108*
14	867±79	910±44	889±53	725±68*	643±68* (7)
WBC [TH/UL]					
6	6.9±1.5	7.2±1.8	7.2±1.5	7.4±1.3	4.5±0.8*
14	4.1±0.6	4.7±1.0	4.8±1.0	4.9±0.8	2.8±1.0* (7)
NRBC [/100 WBC]					
6	1.0±0.7	1.0±0.7	0.0±0.4	0.0±0.3	2.0±5.3
14	0±0	0±0	0±0	0±0	1.0±1.5 (7)

Table 2. Hematology Data

Parameter/Week/Dose/Sex	0 mg/kg/day	1.5 mg/kg/day	22.6 mg/kg/day	150 mg/kg/day	452 mg/kg/day
cor WBC [TH/UL]					
6	6.9±1.6	7.2±1.8	7.2±1.5	7.3±1.3	4.5±0.8*
14	4.1±0.6	4.7±1.0	4.9±1.0	4.9±0.8	2.8±1.0* (7)
lymphocytes [TH/UL]					
6	6.1±1.4	6.2±1.7	5.9±1.5	6.4±1.4	3.7±0.9*
14	3.3±0.6	3.9±0.9	3.9±0.9	4.0±0.7	1.7±0.6* (7)

N=10 unless (); * $p<0.05$; data from Table 7 [pages 98-103] of the report; many numbers were illegible in available hardcopy of report

Table 3. Clinical Chemistry Data

Parameter/Week/Dose/Sex	0 mg/kg/day	1.5 mg/kg/day	22.6 mg/kg/day	150 mg/kg/day	452 mg/kg/day
MALES					
BUN [mg/dL]					
6	15±1	14±2	14±1	13±2	18±4*
14	16±2	15±2	16±1	14±2	22±4* (5)
creatinine [mg/dL]					
6	0.5±0.04	0.5±0.05	0.5±0.05	0.5±0.08	0.6±0.10
14	0.6±0.05	0.6±0.03	0.6±0.04	0.6±0.08	0.5±0.07*
ALT [U/L]					
6	40±3	43±5	42±4	44±5	59±35
14	58±10	53±9	56±10	65±7	69±34 (5)
AST [U/L]					
6	85±8	86±6	84±9	89±10	139±126
14	113±22	101±23	105±24	126±33	240±236 (5)
glucose [mg/dL]					
6	121±13	111±11	113±9	108±7*	104±9*
14	115±15	103±8	109±6	100±11*	88±10* (5)
total protein					
6	6.7±0.2	6.5±0.2	6.6±0.2	6.4±0.1*	6.0±0.6*
14	6.9±0.3	6.7±0.2	6.8±0.2	6.5±0.2*	5.4±0.5* (5)
albumin [g/dL]					
6	4.8±0.1	4.6±0.2*	4.7±0.2	4.6±0.1*	4.6±0.5
14	4.9±0.2	4.8±0.1	4.8±0.2	4.6±0.2*	4.3±0.5* (5)
globulin [g/dL]					
6	1.9±0.1	1.9±0.1	1.9±0.1	1.8±0.1	1.4±0.2*
14	2.0±0.2	1.9±0.1	1.9±0.2	1.8±0.1	1.1±0.1* (5)
calcium [mg/dL]					
6	10.8±0.8	10.5±0.7	10.7±0.6	10.6±0.8	9.7±0.6*
14	10.9±0.4	10.3±0.7	10.5±0.4	10.3±0.6*	9.7±0.6* (5)
T3 [mg/dL]					
6	101.1±16.5	97.8±24.7	105.5±19.7	92.2±16.3	88.0±24.5
14	94.2±19.7	106.0±17.3	93.5±19.0	94.9±18.4	113.2±32.5 (5)
T4 [mg/dL]					
6	4.0±0.8	4.1±0.9	4.6±0.8	3.8±0.7	0.9±0.1*
14	3.8±0.7	3.9±0.6	3.7±0.5	3.5±0.5	0.9±0.0* (5)
FEMALES					
BUN [mg/dL]					
6	16±2	17±2	18±2*	18±2	20±2*
14	17±2	17±2	18±2	16±1	26±3* (7)
creatinine [mg/dL]					
6	0.5±0.07	0.5±0.05	0.5±0.07	0.6±0.06*	0.6±0.05
14	0.6±0.06	0.6±0.03	0.6±0.0	0.7±0.05*	0.5±0.08 (7)
ALT [U/L]					
6	36±2	36±4	36±6	45±4*	47±5*
14	42±6	41±7	46±7	57±10*	67±34* (7)

Table 3. Clinical Chemistry Data

Parameter/Week/Dose/Sex	0 mg/kg/day	1.5 mg/kg/day	22.6 mg/kg/day	150 mg/kg/day	452 mg/kg/day
AST [U/L]					
6	81±9	85±17	84±14	88±11	102±12*
14	111±33	100±23	101±25	120±30	154±50* (7)
glucose [mg/dL]					
6	108±12	103±6	108±10	112±11	96±6*
14	99±7	99±10	103±8	92±10	93±14 (7)
total protein					
6	6.2±0.2	6.1±0.2	6.0±0.2	5.9±0.3*	6.3±0.2
14	6.6±0.2	6.3±0.2*	6.5±0.1	6.4±0.1	6.0±0.4* (7)
albumin [g/dL]					
6	4.5±0.2	4.5±0.2	4.3±0.1	4.4±0.3	5.0±0.1*
14	4.8±0.1	4.6±0.2	4.7±0.1	4.8±0.1	4.9±0.3 (7)
globulin [g/dL]					
6	1.8±0.2	1.7±0.1	1.7±0.1	1.5±0.1*	1.3±0.1*
14	1.8±0.1	1.6±0.1*	1.8±0.1	1.6±0.1*	1.1±0.1* (7)
calcium [mg/dL]					
6	10.4±0.4	10.3±0.6	10.1±0.6	10.2±0.4	9.9±0.5
14	10.3±0.3	10.2±0.4	10.3±0.3	10.2±0.4	9.7±0.5* (7)
T3 [mg/dL]					
6	92.1±13.6	95.9±20.8	99.0±19.2	71.9±12.1	104.1±21.8
14	92.3±11.0 (8)	78.2±7.9 (8)	90.1±8.3 (8)	69.0±16.3* (9)	96.0±28.9 (6)
T4 [mg/dL]					
6	2.4±0.7	2.4±1.0	2.6±0.8	0.9±0.1*	0.9±0.0*
14	2.3±0.7 (9)	2.0±0.6	2.1±0.8 (9)	0.9±0.0*	0.9±0.0* (7)

N=10 unless (); data from Table 8 [pages 105-112] of the report

Table 4. Organ Weights [grams]

Parameter/Dose/Sex/Week	0 mg/kg/day	1.5 mg/kg/day	22.6 mg/kg/day	150 mg/kg/day	452 mg/kg/day
LIVER					
MALES					
absolute	6.94±0.60	6.57±0.71	7.10±0.38	6.76±0.55	8.42±1.07* (5)
relative/body	2.57±0.08	2.61±0.29	2.90±0.49	2.61±0.16	5.78±0.57* (5)
relative/brain	3.61±0.25	3.44±0.44	3.74±0.29	3.50±0.43	4.77±0.50* (5)
FEMALES					
absolute	4.56±0.41	4.53±0.22	4.53±0.37	4.61±0.42	5.73±0.38* (7)
relative/body	3.07±0.46	2.86±0.20	3.07±0.52	3.13±0.35	6.01±0.59* (7)
relative/brain	2.52±0.14	2.48±0.13	2.46±0.12	2.59±0.24	3.64±0.33* (7)
KIDNEY					
MALES					
absolute	1.95±0.21	1.86±0.14	2.04±0.14	2.19±0.12*	1.43±0.13* (5)
relative/body	0.721±0.057	0.741±0.046	0.822±0.138	0.848±0.051*	0.985±0.068* (5)
relative/brain	1.013±0.081	0.976±0.101	1.061±0.107	1.130±0.078*	0.812±0.050* (5)
FEMALES					
absolute	1.22±0.06	1.24±0.09	1.30±0.12	1.31±0.11	1.09±0.05* (7)
relative/body	0.818±0.102	0.780±0.045	0.883±0.161	0.891±0.106	1.147±0.105* (7)
relative/brain	0.673±0.030	0.677±0.038	0.705±0.040	0.735±0.053*	0.695±0.054 (7)
BRAIN w/ STEM					
MALES					
absolute	1.92±0.11	1.92±0.15	1.93±0.10	1.94±0.13	1.76±0.06 (5)
relative/body	0.713±0.041	0.764±0.069	0.786±0.188	0.753±0.064	1.219±0.132* (5)
FEMALES					
absolute	1.81±0.08	1.83±0.06	1.84±0.10	1.79±0.09	1.58±0.08* (7)
relative/body	1.217±0.151	1.153±0.050	1.256±0.246	1.214±0.133	1.650±0.090* (7)
THYROID					
MALES					
absolute	0.017±0.003	0.018±0.003	0.017±0.004	0.017±0.005	0.028±0.003* (5)
relative/body	0.0064±0.0010	0.0072±0.0013	0.0068±0.0016	0.0067±0.0019	0.0196±0.0019* (5)
relative/brain	0.0089±0.0014	0.0095±0.0021	0.0090±0.0023	0.0089±0.0025	0.0162±0.0018* (5)
FEMALES					
absolute	0.23±0.011	0.026±0.009	0.022±0.006	0.024±0.005	0.023±0.004
relative/body	0.0151±0.0068	0.0166±0.0056	0.0146±0.0030	0.0163±0.0036	0.0244±0.0035* (7)
relative/brain	0.0127±0.0063	0.0144±0.0050	0.0121±0.0035	0.0134±0.0027	0.0149±0.0024 (7)
TESTES					
MALES					
absolute	4.11±0.23	3.94±0.19	4.03±0.17	3.99±0.28	1.37±0.28* (5)
relative/body	1.524±0.070	1.570±0.107	1.645±0.374	1.543±0.121	0.933±0.152* (5)
relative/brain	2.139±0.062	2.062±0.143	2.097±0.110	2.053±0.106	0.772±0.139* (5)
OVARIES					
FEMALES					
absolute	0.081±0.010	0.081±0.014	0.085±0.015	0.075±0.018	0.037±0.011* (6)
relative/body	0.054±0.005	0.051±0.010	0.058±0.013	0.050±0.009	0.038±0.008* (7)
relative/brain	0.045±0.007	0.045±0.009	0.046±0.010	0.042±0.011	0.023±0.006* (7)

Table 4. Organ Weights [grams]

Parameter/Dose/Sex/Week	0 mg/kg/day	1.5 mg/kg/day	22.6 mg/kg/day	150 mg/kg/day	452 mg/kg/day
ADRENAL					
MALES					
absolute	0.042±0.004	0.042±0.005	0.042±0.004	0.044±0.003	0.033±0.003* (5)
relative/body	0.0156±0.0014	0.0166±0.0019	0.0171±0.0048	0.0169±0.0011	0.0228±0.0018* (5)
relative/brain	0.0219±0.0025	0.0218±0.0029	0.0217±0.0018	0.0226±0.0026	0.0188±0.0013 (5)
FEMALES					
absolute	0.048±0.004	0.048±0.002	0.052±0.005	0.049±0.005	0.029±0.005* (7)
relative/body	0.0324±0.0034	0.0302±0.0014	0.0348±0.0051	0.0330±0.0030	0.0298±0.0041 (7)
relative/brain	0.0267±0.0020	0.0263±0.0019	0.0280±0.0032	0.0274±0.0030	0.0181±0.0029* (7)
PITUITARY					
MALES					
absolute	0.009±0.003	0.009±0.001	0.009±0.001	0.009±0.001	0.007±0.001 (5)
relative/body	0.0033±0.0009	0.0035±0.0003	0.0035±0.0007	0.0036±0.0004	0.0045±0.0004* (5)
relative/brain	0.0046±0.0013	0.0047±0.0005	0.0045±0.0006	0.0048±0.0007	0.0037±0.0006 (5)
FEMALES					
absolute	0.012±0.003	0.012±0.002	0.012±0.002	0.012±0.002	0.006±0/001* (7)
relative/body	0.0081±0.0026	0.0074±0.0012	0.0084±0.0021	0.0081±0.0008	0.0061±0.0009 (7)
relative/brain	0.0066±0.0016	0.0065±0.0012	0.0067±0.0010	0.0067±0.0010	0.0037±0.0006* (7)
HEART					
MALES					
absolute	0.93±0.08	0.92±0.08	0.94±0.10	0.89±0.04	0.69±0.05* (5)
relative/body	0.347±0.030	0.367±0.029	0.379±0.070	0.344±0.021	0.474±0.038* (5)
relative/brain	0.487±0.036	0.483±0.051	0.499±0.060	0.459±0.033	0.391±0.024* (5)
FEMALES					
absolute	0.65±0.06	0.67±0.03	0.71±0.07	0.68±0.06	0.60±0.05 (7)
relative/body	0.438±0.072	0.424±0.022	0.476±0.071	0.458±0.050	0.626±0.068* (7)
relative/brain	0.361±0.024	0.369±0.026	0.383±0.040	0.379±0.036	0.379±0.035 (7)
THYMUS					
MALES					
absolute	0.25±0.04	0.23±0.04	0.25±0.05	0.23±0.04	0.08±0.03* (5)
relative/body	0.091±0.013	0.091±0.014	0.101±0.031	0.088±0.018	0.053±0.025* (5)
relative/brain	0.129±0.022	0.119±0.019	0.129±0.023	0.117±0.022	0.043±0.019* (5)
FEMALES					
absolute	0.22±0.03	0.22±0.03	0.23±0.04	0.19±0.03	0.08±0.04* (7)
relative/body	0.149±0.029	0.137±0.016	0.158±0.046	0.126±0.024	0.086±0.032* (7)
relative/brain	0.122±0.015	0.119±0.015	0.125±0.010	0.104±0.015	0.053±0.022* (7)
TERMINAL BODY WEIGHT					
MALES	270.2±17.8	251.7±13.8	253.2±39.4	258.6±9.2	146.4±21.4* (5) [54]
FEMALES	150.2±13.6	158.7±6.0	150.5±21.9	140.2±12.3	96.1±8.9* (7) [64]

N=10 unless (); ♪ [% of control]; * p<0.05; data from Tables 10A, 10B, 10C [pages 130-137] of the report

824

[2,4-D-2-ETHYLHEXYL ESTER] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Table 5. Histopathological Findings [addition/correction to original DER Table 10]					
Organ/Dose	0 mg/kg/day	1.5 mg/kg/day	22.6 mg/kg/day	150 mg/kg/day	452 mg/kg/day
Testes atrophy, bilateral	D/T ♪ 0/0	D/T 0/1	D/T 0/0	D/T 0/0	D/T 5✖/5
Prostate atrophy	D/T 0/0	D/T 0/0	D/T 0/0	D/T 0/0	D/T 2/2
Seminal Vesicle atrophy	D/T 0/0	D/T 0/0	D/T 0/0	D/T 0/0	D/T 1/1

♪ D/T [death/terminal sacrifice]; ✖ listed as 0 in original DER Table 10; data from Tables 11A and 11 B, pages 139-151 of the report

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DATA EVALUATION REPORT

008580

STUDY TYPE: 90-day Feeding-Rodent **GUIDELINE:** 82-1(a)

TOX.CHEM. No.: 315-AS MRID No.: 418967-01

HED Project No. 1-1442 **Registrant:** Industry Task Force II

TEST MATERIAL: 2,4-Dichlorophenoxyacetic Acid-2-Ethylhexyl Ester

STUDY IDENTIFICATION: HLA Study No. 2184-112

TESTING LABORATORY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid-2-Ethylhexyl Ester.

REPORT AUTHOR: Gene E. Schulze, Ph.D

REPORT DATE: April 20, 1991

SUMMARY: Male and female Fischer-344 rats were fed diets containing 2,4-Dichlorophenoxyacetic acid-2-ethylhexyl ester (2,4-D IOE) at 0, 1.5, 22.61 150.0 or 452.0 mg/kg/day for 13 weeks. Treatment did not cause any adverse effects at 1.5 or 22.6 mg/kg/day, but did cause decreases in mean body weight, body weight gain, and food consumption, and alterations in some of the hematology and clinical chemistry parameters at 150.0 mg/kg/day. The highest dietary dose level of 452.0 mg/kg/day was associated with weight loss, reduced food consumption, alterations in hematology and clinical chemistry parameters, changes in various organ weights, and histopathological changes of the eye, liver, testes, adrenal cortex, thyroid, thymus, spleen, and kidneys. Under the conditions of this study, a NOEL of 22.6 mg/kg/day and a LOEL of 150.0 mg/kg/day were established.

Due to compound losses at the low dose, the concentration was increased by 15% to compensate for the loss (11 to 18%) starting with the Week 2 mix. The report, however, does not indicate at what point during the mixing procedure this increase was initiated. This information must be provided to the Agency for review. It is apparent that stability problems were encountered at the low dose; however, the toxic effects observed at at the mid- and high-dose levels in this study were similar to the toxicity seen in a subchronic dietary study in Fischer-344 rats employing comparable doses (120 and 361 mg/kg/day) of the Dimethylamine Salt of 2,4-D [MRID #418967-02]. Therefore, this study can be upgraded upon submission and review of the additional data.

CORE CLASSIFICATION: Supplementary; this study does not satisfy the requirement (82-1a) for a 90-day feeding study in rodents, but can be upgraded following submission and review of additional data.

I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the subchronic toxicity of **2,4- Dichlorophenoxyacetic Acid-2-Ethylhexyl Ester** (2,4-D IOE) following dietary administration to rats.

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: 2,4-Dichlorophenoxyacetic Acid-2-Ethylhexyl Ester.
Purity: 98%
Lot No.: 04KF54479
Description: Yellow liquid.
Acid equivalent: 63.25%

2. Test Animals

Species: Rats
Strain: CDF (F-344)/CrljBR Fischer-344 VAF/Plus
Sex: Males and females
Age: Approximately 6 weeks at initiation
Weight at initiation: 113.8-135.1 g (M); 93-105 g (F)
Identification: Tail tattoo .
Acclimation: Approximately 2 weeks
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Chow #5002.
Water: Tap water ad libitum
Environment: Temperature- 69 - 75° F; Humidity- 41 - 79 %;
Light/dark cycles: 12 hr light/12 dark cycle

3. Study Design

Group No.	Treatment	No. of Animals		Dose Level mg/kg/day
		Males	Females	
1	Control	10	10	0
2	Low	10	10	1.5
3	Mid-1	10	10	22.6
4	Mid-2	10	10	150.0
5	High	10	10	452.0

4. Test Article Formulation and Analyses

The purity (98%) of 2,4 D IOE was adjusted to 100% active compound. A premix for each concentration was prepared by mixing the total required amount of 2,4-D IOE with a portion of the required amount of powdered feed (200 g)) in a Warning blender for approximately 2 minutes or until a homogeneous mixture was achieved. Final diets were prepared by mixing the premixes with additional powdered feed in a Hobart mixer for 15 minutes to achieve the appropriate concentration. Fresh diets were prepared weekly and reserve samples of each mixed batch were taken on the day of preparation and stored in a freezer.

Concentration analyses were performed for each dose level at the beginning of Weeks 1-4, 8, and 12. Prior to initiation of dosing, samples from the top, middle, and bottom were analyzed for homogeneity and Day 0 stability at the lowest and highest dose levels.

The pretest (#1) stability analyses indicated a possible lack of stability or binding to the feed following storage at room temperature for 7 days. Therefore, the mixing procedure was changed by dissolving the test article in acetone and then mixing it with dry feed (pretest #2). However, analytical data indicated that there was no improvement in stability when this procedure was used. Samples were formulated using the old mixing procedures (Pretest #3) and stability analyses were performed daily for 7 days on samples that were stored at room temperature, refrigerated, and frozen. In addition, stability analyses were performed on the lowest and highest dose mixes after 4 days at room temperature. Due to the potential stability problem, additional stability analyses were performed during Week 1 on the lowest and highest dose levels after 4 days at room temperature, after 4 days in the freezer, and after 3 days at room temperature following 4 days in the freezer (for a total of 7-day stability).

5. Treatment

Rats were fed the control and test diets 7 days per week for at least 13 weeks. Because of stability problems at room temperature, freshly prepared feed was presented to the animals on Wednesday of each week and the remaining feed was placed into freezer until the following Sunday when the feed was removed from the freezer and given to the animals. The control diet was also stored in the freezer and given to the control group twice weekly. The oral route of administration was chosen because it is the route of potential human exposure.

6. Experimental Procedures

Mortality and moribundity checks were performed twice daily. Cageside observations for clinical signs of toxicity were performed once daily. Body weights were measured prior to initiation and once weekly thereafter. Food consumption was recorded twice weekly and physical examinations were performed weekly at the body weight interval. Ophthalmologic examinations were conducted on all animals once prior to initiation and at termination.

Blood and urine were collected from all animals during Weeks 6 and 14 for hematology, clinical chemistry and urinalysis. 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
x Corrected leukocyte count (COR WBC)	x Cell morphology

Clinical Chemistry

Electrolytes:	Other
x Calcium[a] x Chloride[a] Magnesium[a] x Phosphorus[a] x Potassium[a] x Sodium	x Albumin[a] x Blood creatinine[a] x Blood urea nitrogen[a] Cholesterol[a] x Globulins x Glucose[a] x Total bilirubin[a] x Total serum protein[a] Triglycerides Serum protein electrophoresis x Triiodothyronine (T_3 x Thyroxine (T_4)
Enzymes:	
x Alkaline phosphatase x Alanine aminotransferase (SGPT)[a] x Aspartate aminotransferase (SGOT)[a] Cholinesterase[b] x Creatinine phosphatase[a] Lactic acid dehydrogenase Gama glutamyl transferase	

Urinalysis

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

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

7. Termination

After 13 weeks of treatment, all surviving animals were weighed, anesthetized with sodium pentobarbital and exsanguinated. Necropsies were performed on each animal, all gross pathological changes were recorded, and the following organs were weighed.

Adrenals	Brain	Heart	Kidneys	Liver
Ovaries	Thymus	Thyroid	Pituitary	Testes

8. Histopathology

The checked (X) tissues from all animals were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Salivary glands[a]	Trachea[a]
x Esophagus[a]	x Lung[a]
x Duodenum[a]	Pharynx[e]
x Jejunum[a]	Larynx[e]
x Cecum[a]	Nose[e]
x Colon[a]	
x Rectum[a]	Cardiovascular/Hemo.System
x Liver[ac]	
x Pancreas[a]	x Aorta (thoracic)[a]
Gall bladder[ab]	x Heart[a]
	x Bone marrow[a]
Neurological System	x Lymph nodes[a]
	x Spleen[a]
x Brain[ac]	x Thymus[a]
x Pituitary[a]	
Peripheral nerve[ab]	Urinogenital System
Spinal cord (3 levels)[ab]	
x Eyes (optical nerve)[ab]	x Kidneys[ac]
	x Urinary bladder[a]
	x Testes[ac]
Glandular System	x Epididymides
	x Uterus[a]
x Adrenals[a]	x Ovaries[ac]
Lacrimal glands[c]	
x Parathyroids[ad]	Others
x Thyroids[ad]	
	x All gross lesions and masses
	x Skeletal muscle[a]

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

Mean absolute body weight, body weight change, mean weekly food consumption, total food consumption, clinical pathology data (with the exception of urinalyses and cell morphology), and organ weight data of the control group were compared statistically to the data from the compound-treated groups.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Labs, and the Study Protocol. A quality assurance statement was signed and dated April 20, 1991.

III. RESULTS

1. Analysis of Diet Mix

a. Concentraion

Concentration analyses summarized in Table 1 showed that the mean concentration was within 10% of target level confirming accuracy of formulation.

Table 1. Concentration Analysis

Group/Sex	Target Level (ppm)	Target Level (Mean%)					
		Week 1	Week 2	Week 3	Week 4	Week 8	Week 12
2 / M	21	109	105	92.3	91.5	110	93
2 / F	18	98.4	107	98.6	105	102	96.1
3 / M	270	101	104	96.7	98.7	94.6	95.9
3 / F	230	102	101	96.7	98.4	106	97.6
4 / M	1800	102	102	101	101	105	101
4 / F	1597	102	103	97.1	101	104	108
5 / M	5646	99.3	104	106	99.1	103	99
5 / F	5041	102	101	98.1	101	105	98.7

b. Stability

The pretest (#1) stability analyses indicated a possible lack of stability or binding to the feed following storage at room temperature for 7 days. Therefore, stability analyses were performed daily for 7 days for the pretest # 3 samples (low and high dose mixes). The analytical results are summarized in Table 2.

Table 2. Daily Stability Analyses (Mean %) of Pretest Feed Mixes.

Time Period	Room Temperature		Refrigerator		Freezer	
Dose	Low	High	Low	High	Low	High
Day 1	-5	-7	-3	-7.8	+6	-1
Day 2	-3	-8.2	-1	-2	+7	-4
Day 3	-11	-5	+2	-1	+3	-3
Day 4	-10	-8	-1	-8	+24	0
Day 5	-9	-9	-1	-7	+21	-7
Day 6	-11	-12	-2	-8	-9	-6
Day 7	-2	-15	-3	-4	-11	-8

Daily stability data indicate that the test article is not stable at room temperature for more than 2 days, storing the feed mixes in the freezer seem to improve stability. Consequently, feed was presented to the animals twice weekly; freshly prepared feed was presented on the Wednesday of each week and the remaining feed was placed into a freezer until the following Sunday when the feed was removed from the freezer and given to the animals.

Due to stability problems, the diet mixes were stored in the freezer during the study. Table 3 summarizes the stability analyses performed during Week 1 on the lowest and highest dose levels after 4 days at room temperature, after 4 days in the freezer, and after 3 days at room temperature following 4 days in the freezer (for a total of 7-day stability).

Table 3. Stability Analysis of Week 1 Diet Mixes.

Interval	Dose Level (mg/kg/day)	Target Level (ppm)	Target Level (Mean%)	Change from Day 0 Mean (%)
Week 14-Day Stability (room temperature)	1.5	11.5	97.4	- 11.6
	452	3935	98.1	- 3.9
4-Day Stability (freezer)	1.5	11.55	110	+ 1.0
	452	3935	103	+ 1.0
7-Day Stability (4 days frozen / 3 days room temperature)	1.5	11.5	90.4	- 18.6
	452	3935	98.9	- 3.1

Due to compound losses of 11.6% and 18.6% in the low dose, the concentration was increased by 15% to compensate for the loss. This increase began with the Week 2 mix. However, the report does not indicate at what point during the mixing procedure this increase was initiated. Therefore, it is unclear as to what was the actual concentration ingested by the low dose group rats due to this increase (i.e., immediately after the increase and prior to the degradation process). This information must be submitted for Agency review.

2. Survival

No mortality occurred at 1.5, 2.6, or 150 mg/kg/day groups. At the high dose (452 mg/kg/day), five males and three females died during the study. One male died during Week 7, two during Week 8, and one each during Weeks 10 and 11. The female deaths occurred during Weeks 10 (one rat) and 11 (two rats).

2. Clinical Observations

Treatment-related clinical signs of toxicity limited to the high dose animals were thin appearance (9 males and 10 females), hunched posture (10 males and 10 females), languid behavior (2 females), ataxia (1 males), squinted eyes (4 males and 3 females), and few/no feces (3 males). Opaque eyes were noted in the females only and were seen in 1/10 controls, 1/10 at 150 mg/kg/day and in 9/10 at 452 mg/kg/day. Other frequently seen signs (urine stains, alopecia, rough hair coat, lacrimation) did not appear to be dose- or compound-related.

3. Body Weights and Body Weight Changes

Mean body weight data are summarized in Tables 4 and 5 for males and females, respectively. Statistically significant ($p < 0.05$) reduction in mean body weight was observed for males females at 452 mg/kg/day at Weeks 6 and 13, and for males at 1.5 mg/kg/day at Week 13. Although not statistically significant, biologically significant decreases in mean body weights were also observed at Week 10 for both males (-41.5%) and females (-38%).

Table 4. Mean Body Weights and S.D (G) for Male Rats.

Dose Level (mg/kg/day)	Week: 0	Week: 6	Week: 10	Week: 13
0	127.0 ± 4.4	240.1 ± 12.6	275.8 ± 18.1	285.8 ± 18.2
1.5	123.5 ± 5.4	230.3 ± 9.6	256.4±13.6	**266.0 ± 15.2***
22.6	123.6 ± 4.8	238.7 ± 11.6	278.8 ± 13.9	287.1± 15.9
150.0	123.9 ± 4.8	231.0 ± 6.7	264.2 ± 11.3	273.5 ±11.0
452.0	127.5 ± 4.6	**181.0* ± 22.6**	161.8 ± 29.8	**157.8 ± 21.4***

* Significantly different from control value at $p \leq 0.05$.

Table 5. Mean Body Weights and S.D (G) for Female Rats.

Dose Level (mg/kg/day)	Week: 0	Week: 6	Week: 10	Week: 13
0	99.2 ± 2.9	142.2 ± 8.3	161.1 ± 6.7	167.6 ± 8.0
1.5	99.1 ± 3.3	145.5 ± 8.3	163.9 ± 6.8	172.1 ± 6.9
22.6	98.8 ± 3.1	144.4 ± 7.0	164.4 ± 9.2	171.0 ± 10.0
150.0	99.1 ± 3.8	139.2 ± 4.1	157.9 ± 6.9	163.0 ± 9.0
452.0	99.5 ± 2.6	**112.7* ±6.5**	100.3 ± 12.5	**107.2 ± 9.6***

* Significantly different from control value at $p \leq 0.05$

Mean body weight change data are presented in Tables 6 and 7 for males and females, respectively. Statistically significant ($p < 0.05$) decreases in mean body weight gains were seen in both males and females only at 452 mg/kg/day during Weeks 0-6, 6-13, and 0-13 intervals.

Table 6. Mean Body Weight Change (G) in Male Rats.

Dose Level (mg/kg/day)	Weeks: 0 - 6	Weeks: 6 - 13	Weeks: 0 - 13
0	113.2 ± 11.4	45.6 ± 7.9	158.6 ± 7.94
1.5	107.2 ± 8.8	35.7 ± 11.5	142.9 ± 17.1
22.6	115.1 ± 11.4	48.4 ± 6.9	163.5 ± 16.3
150.0	107.1 ± 6.1	42.5 ± 6.3	149.6 ± 10.6
452.0	**56.4* ± 22.52**	**-34.0* ± 19.6**	**33.1* ± 23.6**

* Significantly different from control at $p \leq 0.05$.

Table 7. Mean Body Weight Change (G) in Female Rats.

Dose Level (mg/kg/day)	Weeks: 0 - 6	Weeks: 6 - 13	Weeks: 0 - 13
0	43.0 ± 8.1	25.4 ± 5.3	68.4 ± 8.3
1.5	46.4 ± 4.6	26.6 ± 4.4	73.0 ± 6.1
22.6	45.7 ± 5.3	26.6 ± 5.8	72.3 ± 8.7
150.0	40.1 ± 5.5	23.8 ± 6.3	64.0 ± 8.3
452.0	**13.2* ± 6.4**	**-7.7* ± 7.1**	**8.0* ± 8.5**

* Significantly different from control at $p \leq 0.05$.

4. Food and Compound Consumption

The mean total food consumption was significantly ($p < 0.05$) decreased in males and females at 452 mg/kg/day during Weeks 1-6, 6-13, and 1-13. The mean total food consumption was significantly increased in females at 150 mg/kg/day during Weeks 1-6 and 1-13. A significant negative trend was noted for males during Weeks 1-6 and 1-13. No changes were seen in males or females at 1.5 and 22.6 mg/kg/day groups or in males at 150 mg/kg/day. The average daily compound consumption for Weeks 1-13 was 1.41 (18.27 ppm), 21.31 (277 ppm), 141.68 (1842 ppm), and 428.80 mg/kg/day (5656 ppm) for males and 1.46 (9 ppm), 22.01 (286 ppm), 144.87 (1883 ppm), and 444.80 mg/kg/day (5782 ppm) for females.

5. Ophthalmology Examination

Ophthalmoscopic findings are summarized in Tables 8 and 9 for males and females respectively. 2,4-D IOE induced bilateral cataract formation in 7 females and retinal degeneration in 3 males at 452 mg/kg/day. In females, retinal degeneration appears to precede complete cataract formation. No other treatment-related ophthalmoscopic changes were seen.

Table 8. Ophthalmoscopic Observations In Male Rats.

Observations	Dose Level (mg/kg/day)				
No.of animals = 10/dose level	0	1.5	22.6	150	452
Conjunctiva - chromodacryorrhea	1	0	0	0	0
Cornea - corneal dystrophy	0	2	0	0	2
Fundus -retinal linear dystrophy	0	2	0	0	0
Fundus - retinal degeneration	0	1	0	0	3
Lens - posterior subcapsular cataract	0	0	0	0	3

Table 9. Ophthalmoscopic Observations In Female Rats.

Observations	Dose Level (mg/kg/day)				
No. of animals = 10/dose level	0	1.5	22.6	150	452
Cornea - corneal dystrophy	0	1	1	3	0
Fundus -retinal linear dystrophy	0	0	1	0	-[a]
Fundus - retinal degeneration	0	1	0	2	-[a]
Lens - complete cataract	1	0	0	0	7
Phthisis bulbi	0	0	0	2	0

[a] The retinas could not be examined because of complete cataract.

6. Hematology and Clinical Chemistry

Treatment-related, statistically significant ($p \leq 0.05$) decreases observed in hematology parameters are summarized below:

Dose Level / Sex	Parameters Affected	Study Weeks
150.0 mg/kg/day (M)	↓ in Platelet	14
150.0 mg/kg/day (F)	↓ in RBC	14
	↓ in Platelet	14
		14
452.0 mg/kg/day (M)	↓ in RBC	14
	↓ in HGB	6,14
	↓ in HCT	6,14
	↓ in PLATELET	6,14
	↓ in WBC	6,14
	↓ in NRBC	6
	↓ in COR WBC	6,14
	↓ in LYMPH	6,14
452.0 mg/kg/day (F)	↓ in RBC	6,14
	↓ in HGB	6,14
	↓ in HCT	6,14
	↓ in PLATELET	6,14
	↓ in WBC	6,14
	↓ in COR WBC	6,14
	↓ in LYMPH	6,14

Alterations observed in hematology parameters correlated with histopathological changes observed in the bone marrow and spleen (see Histopathology).

Treatment-related, statistically significant ($p \leq 0.05$) differences observed in clinical chemistry parameters are summarized below:

Dose Level / Sex	Parameters Affected	Study Weeks
1.5 mg/kg/day (F)	↓ in T.Protein	14
22.61 mg/kg/day (F)	↓ in Globulin	14
150.0 mg/kg/day (M)	↓ in Glucose-	6,14
	↓ in T.Protein	6,14
	↓in Albumin	6,14
	↓ in Calcium	14
150.0 mg/kg/day (F)	↑ in Creatinine	6,14
	↑ in ALT	6,14
	↓ T.Protein	6
	↓ in Globulin	6,14
	↓ in Chloride	14
	↓ in T_3	14
	↓ in T_4	6,14
452.0 mg/kg/day (M)	↓ in Glucose	6,14
	↑ in BUN	6,14
	↓ in Creatinine	14
	↓ in Total protein	6,14
	↓ in Albumin	14
	↓ in Globulin	6,14
	↓ in Calcium	6,14
	↓ in Phos.	14
	↓ in T_4	6,14
452.0 mg/kg/day (F)	↓ in Glucose	6
	↑ in BUN	6,14
	↑ in AST	6,14
	↑ in ALT	6,14
	↓ in T.Protein	14
	↑ in Albumin	6
	↓ in Globulin	6,14
	↓ in Calcium	14
	↓ in Potassium	14
	↓ in T_4	6,14

Alterations seen in clinical chemistry parameters correlated well with histopathological changes observed in the liver, thyroids and adrenal glands (see Histopathology).

Urinalysis showed a decreased incidence/severity of ketones and protein at Week 6 and of ketones only at Week 14 in males at 452 mg/kg/day. An increased incidence of urine pH values of 7.5 or greater occurred in the high-dose females only at Week 6. The biological significance of these findings are not known

7. Gross Pathology

Gross necropsy findings were limited to the high-dose groups animals which included dark areas on the glandular stomach , small testes, small thymus, opaque eyes, and small prostrates. Observations noted in animals that died during the study appeared to be incidental in nature with the exception of dark areas on the stomach seen in 1 male and 2 females. Other gross necropsy findings were considered incidental in nature.

8. Organ Weights

Statistically significant differences in absolute and relative organ weight were primarily seen in male and/or female rats at the high dose. The absolute weights of adrenals (both sexes) thymus (both sexes), brain (females), heart (males), kidneys (both sexes), testes with epididymides, ovaries and pituitary (females) were decreased while absolute liver weights (both sexes) and thyroid/parathyroids (males) were increased. The relative weights of adrenals (males), brain (both sexes), heart (both sexes), kidneys (both sexes),liver (both sexes), thyroid/parathyroid (both sexes), and pituitary (males) were increased while the relative weights of thymus (both sexes) testes with epididymides, ovaries, and pituitary (females) were decreased.

9. Histopathology

Treatment-related histopathological changes (Tables 10 and 11) were primarily observed in the high dose group which included:

1. Centrilobular hepatocellular hypertrophy of the liver,
2. Bilateral retinal degeneration and cataract formation of the eye
3. Atrophy of the testes,
4. Hypertrophy of the zona glomerulose of the adrenal cortex,
5. Hypertrophy of thyroid follicular cells,
6. Lymphoid hypoplasia of the thymus,
7. Lymphoid hypoplasia of the spleen,
8. Hypoplasia of bone marrow,
9. Vacuolization of tubular cells of the kidney, and
10. Brush border loss in proximal tubular cells of kidneys,

Histopathology of the eye revealed degenerative changes in the retina and lens of females at the high dose. Retinal changes were moderate in two, slight in three and minimal in one animal. Degeneration of the lens fibers (cataract changes) was severe in four, moderately severe in four and moderate in two animals.

The dark areas on the glandular stomach observed in the high dose animals showed no collaborative pathological lesions.

In most cases, the histopathological changes correlated well with alterations observed in hematology and clinical chemistry parameters and organ weight data of the high dose group. Treatment-related decreases in red cell mass correlated with hypoplasia of the bone marrow, and the deceases in absolute lymphocyte count correlated with lymphoid hypoplasia of the thymus, spleen, and bone marrow. Decreases in circulating thyroxine levels correlated with the hypertrophy of follicular cells in the thyroid. Increases in liver weight, ALT and AST correlated with centrilobular hypertrophy of the liver. Decreases in testes weights correlated with testicular atrophy, while decreased ovarian weights and size could not be correlated with any microscopic changes.

Table 10. Histopathological Findings in Male Rats.

Organ / Lesion	Dose Level (mg/kg/day)									
No.of animals= 10/dose levels D =Death T = Terminal Sacrifice	0		1.5		22.6		150		452	
	D	T	D	T	D	T	D	T	D	T
Adrenal, cortex -Hypertrophy, zona glomerulose	0	0	0	0	0	0	0	0	1	5
Thyroid -Follicular cell hypertrophy	0	0	0	0	0	0	0	0	5	5
Kidney -Casts, proteinaceous	0	0	0	0	0	0	0	0	2	5
-Vacuolization, tubular	0	0	0	0	0	0	0	0	0	5
-Tubule, microconcretion	0	0	0	0	0	0	0	0	0	1
Testes -Atrophy, bilateral	0	0	0	1	0	0	0	0	0 5	5
Marrow, femur -Hypoplasia	0	0	0	0	0	0	0	0	5	1
Spleen-Hypoplasia, lymphoid	0	0	0	0	0	0	0	0	4	0
Liver- Hypertrophy, hepatocellular, centrilobular	0	0	0	0	0	0	0	0	1	5
Thymus- Hypoplasia, lymphoid	0	0	0	0	0	0	0	0	1	2
Eye-Corneal dystrophy	0	6	0	6	0	5	0	7	2	1
-Degeneration, retinal, bilateral	0	0	0	1	0	0	0	0	0	0
-Cataractous change	0	0	0	0	0	0	0	0	0	0

Table 11. Histopathological Findings in Female Rats.

Organ / Lesion	Dose Level (mg/kg/day)									
No.of animals= 10/dose levels D = Death T = Terminal Sacrifice	0		1.5		22.6		150		452	
	D	T	D	T	D	T	D	T	D	T
Adrenal, cortex -Hypertrophy, zona glomerulose	0	0	0	0	0	0	0	0	3	7
Thyroid -Follicular cell hypertrophy	0	0	0	0	0	0	0	0	1	7
Kidney -Brush border loss, tubular	0	0	0	0	0	0	0	0	0	6
-Vacuolization, tubular	0	0	0	0	0	0	0	0	0	6
Marrow, femur -Hypoplasia	0	0	0	0	0	0	0	0	1	5
Spleen-Hypoplasia, lymphoid	0	0	0	0	0	0	0	0	3	5
Liver- Hypertrophy, hepatocellular, centrilobular	0	0	0	0	0	0	0	0	1	7
Thymus- Hypoplasia, lymphoid	0	0	0	0	0	0	0	0	2	4
Eye-Corneal dystrophy	0	5	0	6	0	7	0	6	1	3
-Degeneration, retinal, bilateral	0	0	0	1	0	0	0	0	1	5
-Cataractous change	0	0	0	0	0	0	0	0	3	7

Male and female Fischer-344 rats were fed diets containing 2,4-D IOE at 0, 1.5, 22.6, 150.0, or 452.0 mg/kg/day for 13 weeks.

Analytical data indicated lack of stability or binding of the test article to the feed at room temperature at the low dose. Therefore, the low dose concentration was increased to compensate for the compound loss creating some uncertainty as to when the increase was initiated and what concentrations these animals were exposed to immediately after the increase and prior to introduction of the degradation process. This information must be made available for Agency review.

No treatment-related effects were seen at the low- (1.5 mg/kg/day) or the mid-1- dose (22.6 mg/kg/day). Decreases in mean body weight, body weight gain, and reduction in food consumption were observed in rats fed the mid-2- (150 mg/kg/day) and the high- (452 mg/kg/day) dose.

Ophthalmology examinations revealed bilateral cataract formation in females and retinal degeneration in males at 452 mg/kg/day; no ocular changes were seen at the lower doses.

Treatment-related alterations in hematology parameters were essentially confined to the 452 mg/kg/day group which included: decreased red cell mass, decreased platelet count, decreased absolute and corrected leukocyte counts, decreased lymphocyte counts, and decreased segmented neutrophil counts at both Week 6 and 14 intervals.

Treatment-related alterations clinical chemistry parameters were seen at 150 and 452 mg/kg/day groups which included: decreased glucose and globulin levels, increased BUN, decreased thyroxin levels, decreased triiodothyronine, decreased total protein, increased ALT, and decreased serum calcium, potassium and chloride levels. The significant decreases in total protein (6.3 g/DL vs 6.6 g/DL in controls) and globulin (1.6 g/Dl vs 1.8 g/DL) levels seen at 1.5 mg/kg/day at Week 14 and the significant increase in BUN (18 mg/DL vs 16 mg/DL) level noted at 22.6 mg/kg/day were all within the reference range (4.9 to 6.6 g/DL, 1.4 to 2.6 g/DL, and 13 to 33 mg/DL, respectively, for total protein, globulin, and BUN) and therefore were not considered to be treatment related.

Treatment-related gross pathological changes were limited to the high-dose group rats which included: dark areas on the glandular stomach, small testes, small thymus, opaque eyes, and small prostate. Compound-induced increases or decreases in absolute and relative organ weights included (but were not limited to) the thymus, liver, kidneys, testes with epididymides, ovaries, and thyroid with parathyroid in males and females at 452 mg/kg/day.

In most cases, alterations noted in hematology, clinical chemistry, gross pathology, and organ weights correlated well with histopathological changes. Treatment-related histopathological changes which were limited to the high-dose group included bilateral retinal degeneration and cataract formation (females), lymphoid hypoplasia of the thymus (females) and spleen (males and females), centrilobular hepatocellular hypertrophy of the liver (males and females); hypoplasia of the bone marrow (males and females), hypertrophy of thyroid follicular cells (males and females), atrophy of the testes (males), vacuolization of the tubular cells of the kidney (males and females), and brush order loss in proximal tubular cells in the kidney (females).

V. CONCLUSION

Under the conditions of this study, a NOEL of 22.6 mg/kg/day and a LOEL of 150.0 mg/kg/day is established for the 90-day oral toxicity of 2,4-Dichlorophenoxyacetic acid-2-ethylhexyl ester to male and female rats. The LOEL is based on decreases in mean body weight, body weight gain and food consumption and alterations in hematology and clinical chemistry parameters.

VI. **CORE CLASSIFICATION:** Supplementary; this study does not satisfy the requirement (82-1a) for a 90-day feeding study in rodents, but can be upgraded following submission and review of additional data.

Tox Chem No. 315 AS File Last Updated _____ Current Date 1/9/91 (NEW INPUT)

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA MRID NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
B2-1(a) Feeding - 90 Days Species: Rats Hazleton 2184-112; 4/11/91	2,4-D IOE Purity:98% Acid Equivalent: 63.25%	418967-01	Dose levels:0,1.5,22.6, 150, or 452 mg/kg/day for 13 weeks. No adverse effects at 1.5 or 22.6 mg/kg/day. At 150 mg/kg/day: Decreases in mean body weight, body weight gain and food consumptions and alterations in hematology and clinical chemistry parameters. At 452 mg/kg/day: Mortality, body weight loss, retinal degeneration and cataract formation, altered hematology and clinical chemistry parameters; changes in various organ weights, and pathologic changes in the eye, liver, testes, adrenal cortex, thyroid, thymus, spleen, and kidney. NOEL = 22.6 mg/kg/day LOEL = 150 mg/kg/day. Minor stability problems; additional data requested.	NA	Supplementary TR
			Study can be upgraded		

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 871 of 959

[2,4-D DIMETHYLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Amendment to DER for MRID 41896702 - Subchronic Oral Toxicity Study [Rat] [2,4-D DIMETHYL AMINE SALT]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 008581].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/12/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A. Dobozy 12/13/00
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: subchronic oral toxicity - rat § 82-1/OPPTS 870.3100
P.C. CODE: 030019 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315O CAS NO.:2008-39-1
TEST MATERIAL (PURITY): 2,4-D dimethylamine salt [66.18%; 55.5% acid equivalents]
SYNONYMS: 2,4-D DMA; dichlorophenoxyacetic acid, dimethylamine salt
SPONSOR: Industry Task Force II

CITATION: Shulze, G. E. (1991). Subchronic Toxicity Study in Rats with the Dimethylamine Salt of 2,4-Dichlorophenoxyacetic Acid. Hazleton Laboratories America, Inc. Study No. HLA 2184-113; April 20, 1991. MRID 41896702. Document No. 008581. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [MRID 41896702], 10 Fischer 344 rats/sex/group were administered the dimethylamine salt of 2,4-dichlorophenoxyaceticacid [66.18%; 55.5% acid equivalents] *via* the diet for 13 weeks at concentrations of 0, 1.2 mg/kg/day, 18.1 mg/kg/day, 120 mg/kg/day, and 361 mg/kg/day. NOTE: DMA was 66.18% and was adjusted to 100% ai. [acid equivalents: 1, 15, 101, 303 mg/kg/day, calculated by this reviewer].

One high-dose female who died during week 6 following blood sample collection displayed lesions in the liver [hepatocellular hypertrophy], thyroid [follicular cell hypertrophy], and spleen [hypoplasia]. Treatment-related clinical signs of toxicity were limited to the high-dose level and consisted of opaque eyes in 6/10 females. There was a treatment-related decrease in body weight throughout the study in both sexes at the high-dose level [males: 74%-96%/females 68%-87% of control]. Overall body-weight gain was decreased in both sexes at the high-dose level [males 58%/females 35% of control], and initially during weeks 0-6, the high-dose displayed a marked deficit [males 54%/females 13% of control] in body-weight gain compared to the control. Females at the next highest dose level [120 mg/kg/day] also displayed a body-weight gain deficit [76% of control] during weeks 0-6. Food consumption was decreased throughout the study from the first week on for both sexes at the highest dose level, suggesting a palatability problem. There was also a slight decrease in food consumption in females at the next highest dose level.

At the highest dose level [361 mg/kg/day], females displayed bilateral cataract formation and retinal degeneration. Alterations in hematology [decrease in red blood cell, hemoglobin, and hematocrit, white blood cell and platelet values] and clinical chemistry parameters [decreased T3/T4 concentrations, increased ALT] were observed at the highest dose level in one or both sexes, and these were consistent with changes observed in organ weights and/or

histopathology. Decreased RBC, platelets, T3, and T4 and increased ALT were observed in the females at the next highest dose level [120 mg/kg/day]. Females at the highest dose level displayed increased liver weight, decreased adrenal [absolute, relative to body and brain], pituitary [absolute, relative to body and brain], ovary [absolute, relative to body and brain], and thymus [absolute, relative to body and brain] weights. High-dose males displayed decreased testes [absolute, relative to body and brain] weight, and both sexes displayed increased thyroid weights. At the next highest dose level, males displayed increased kidney [absolute, relative to body and brain] weights, and females displayed decreased ovarian [absolute and relative to brain] weights.

Eye lesions were observed at the highest dose level in females and consisted of bilateral retinal degeneration and bilateral cataractous change. Lesions in the adrenal [hypertrophy (zona glomerulose)], thyroids [follicular cell hypertrophy], kidney [brush border loss in proximal tubular cells], and bone marrow [hypoplasia] were observed in both sexes at the highest dose level only, and testes [atrophy; males] and liver [centrilobular hepatocellular hypertrophy; females] lesions were observed only at the highest dose.

The NOAEL is 18.1 mg/kg/day, and the LOAEL is 120 mg/kg/day, based on decreased body-weight gain and food consumption in females, alterations in hematology parameters [females], decreased T3/T4 [females], increased kidney weights in males and decreased ovarian weights in females. At the highest dose tested [361 mg/kg/day], changes in several organ weights, and histopathological changes that included bilateral retinal degeneration and cataracts [females], centrilobular hepatocellular hypertrophy [females], atrophy of the testes [males], hypertrophy of thyroid follicular cells [both sexes], brush border loss in proximal tubular cells in the kidney [both sexes], and hypoplasia of the spleen [females] and bone marrow [both sexes] were observed.

This subchronic oral toxicity study is classified **Acceptable/Guideline**, and the study satisfies the guideline requirement [OPPTS 870.3100; §82-1] for a subchronic oral toxicity study in the rodent.

Table 1. Body Weight/Body-Weight Gain and Food Consumption [grams]

Dose/Time	0 mg/kg/day	1.2 mg/kg/day	18 mg/kg/day	120 mg/kg/day	361 mg/kg/day
Body Weight					
MALES					
0	133.3±4.1	132.5±5.0	128.9±3.0	133.9±3.8	133.5±5.1
1	168.2±6.6	167.0±7.2	163.5±4.8	168.9±4.0	160.9±7.4 [96]√
2	195.8±6.8	191.5±10.4	191.0±6.9	196.3±6.2	117.8±9.8 [91]
3	220.3±6.4	213.3±13.6	215.3±6.6	219.5±7.1	193.0±12.1 [88]
4	237.8±5.6	229.8±14.6	230.6±7.7	235.4±8.1	202.3±12.8 [85]
9	287.1±10.5	277.8±21.3	277.0±11.3	283.4±13.9	212.9±18.4 [74]
13	305.9±12.2	301.0±21.0	301.0±12.1	307.0±14.5	234.0±18.0* [76]
FEMALES					
0	99.2±2.5	99.1±3.9	99.5±2.0	101.1±2.7	98.9±2.2
1	116.1±2.1	115.4±5.7	116.7±2.7	112.2±4.1	101.4±1.8 [87]
2	124.3±1.8	125.9±8.5	126.3±2.5	121.5±3.0	101.1±3.1 [81]
3	134.0±3.0	136.2±9.4	135.1±2.8	129.1±5.0	110.1±5.1 [82]
4	141.2±2.8	143.6±10.1	141.9±2.7	134.5±5.1	110.8±7.0 [78]
9	159.7±7.4	164.1±12.6	161.1±3.5	152.4±6.1	108.9±10.9 [68]{9}
13	169.4±9.1	174.0±12.5	169.5±6.9	162.5±6.6	123.3±15.2* [73]{9}
Body-Weight Gain (weeks)					
MALES					
0-1✖	34.9	34.5	34.6	35.0	27.4 [79]
1-2✖	27.6	24.5	27.5	27.4	16.9 [61]
0-6	116.8±5.6	109.8±13.4	113.4±8.7	114.4±11.3	63.1±8.9* [54]
6-13	55.7±7.4	58.7±7.4	58.7±6.3	58.7±9.0	37.4±10.0* [67]
0-13	172.6±11.7	168.5±18.3	172.1±11.3	173.1±15.3	100.5±15.2* [58]
FEMALES					
0-1✖	16.9	16.3	17.2	13.1[78]	2.5 [15]
1-2✖	8.2	10.5	9.6	7.3 [89]	-0.3
0-6	41.4±5.9	43.9±7.0	43.1±2.3	31.5±4.7* [76]	5.4±8.0* [13]
6-13	28.8±4.3	31.0±5.3	27.0±6.9	30.0±3.4	18.8±8.5* [65]{9}
0-13	70.2±9.0	74.9±10.7	70.1±6.1	61.4±5.9 [87]	24.2±14.1* [35]
Food Consumption [g/wk]					
MALES					
1	105.8±6.1	104.9±5.1	103.4±4.1	106.6±4.2	97.7±4.1 [92]
2	113.6±7.6	110.6±5.8	112.2±4.5	111.9±5.4	96.6±7.7 [85]
3	115.4±7.8	112.3±7.5	114.3±3.5	111.0±5.3	94.4±7.2 [82]
4	112.5±7.4	107.3±6.6	108.0±4.4	108.7±5.0	89.2±6.3 [79]
7	117.3±11.0	108.4±7.1	111.0±5.7	110.1±8.8	75.0±8.4 [64]
13	103.0±5.4	107.1±6.1	105.4±3.9	110.7±6.8	88.3±8.1 [86]
FEMALES					
1	76.8±3.7	78.6±3.5	80.0±1.9	71.6±2.7 [93]	55.5±3.5 [72]
2	78.8±3.4	83.1±5.2	83.4±2.9	74.7±2.5	55.4±7.3 [70]
3	77.4±3.6	80.7±5.8	81.8±3.4	72.1±3.6	57.6±5.2 [74]
4	78.6±5.3	79.4±8.1	76.8±4.6	69.3±3.1 [88]	51.1±4.6 [65]
7	74.1±8.2	80.0±13.6	77.1±1.7	67.3±4.8 [91]	45.4±4.6 [61]
13	72.5±6.8	80.9±10.4	76.1±5.2	78.5±5.0	59.2±7.5 [82]

853

[2,4-D DIMETHYLAMINE SALT] Subchronic Oral Toxicity Study - Rat [§82-1/OPPTS 870.3100]

Table 1. Body Weight/Body-Weight Gain and Food Consumption [grams]					
Dose/Time	0 mg/kg/day	1.2 mg/kg/day	18 mg/kg/day	120 mg/kg/day	361 mg/kg/day
Total Food Consumption [g]					
MALES					
week 1-6	652.6±29.7	641.0±34.9	642.8±19.8	649.4±26.4	541.9±27.6* [83]
week 6-13	873.3±63.8	859.0±59.1	852.9±33.1	868.1±42.3	655.7±52.8* [75]
week 1-13	1417.2±76.1	1405.5±86.8	1402.4±44.9	1420.1±61.5	1131.1±53.6* [80]
FEMALES					
week 1-6	448.2±19.7	460.3±26.3	465.1±12.1	413.6±14.0* [92]	311.8±15.7* [70]
week 6-13	601.5±51.7	637.1±72.6	609.1±21.3	578.5±34.0 [96]	414.7±35.8* [69]
week 1-13	981.3±61.6	1014.8±69.6	1008.8±24.4	933.0±42.8 [95]	691.8±20.2* [70]

[% of control]; ✖ calculated by reviewer; * p<0.05; N=10, unless { }; data from Tables 3A, 3B, 4A, 4B [pages 74, 76, 78, 80 of the report]

Table 2. Hematology Parameters					
Parameter/Dose/Sex/Week	0 mg/kg/day	1.2 mg/kg/day	18.1 mg/kg/day	120 mg/kg/day	361 mg/kg/day
red blood cells [MI/UL]					
week 6					
MALES	9.12±1.04	9.51±0.35	9.48±0.21	9.32±0.19	8.71±0.24
FEMALES	8.71±0.34	8.88±0.22	8.84±0.18	8.71±0.32	7.80±0.20* [88]
week 14					
MALES	9.62±0.18	9.64±0.14	9.51±0.28	9.51±0.13	8.47±0.13* [88]
FEMALES	8.98±0.24	8.83±0.23	8.98±0.17	8.57±0.15* [95]√	7.78±0.43* [88]
hemoglobin [g/dL]					
week 6					
MALES	15.8±3.0	16.8±0.5	16.8±0.3	16.7±0.3	16.4±0.3
FEMALES	16.3±0.6	16.6±0.4	16.5±0.3	16.0±0.6	14.6±0.4* [90]
week 14					
MALES	16.9±0.4	16.9±0.2	16.8±0.4	16.8±0.3	16.4±0.2* [97]
FEMALES	16.7±0.4	16.4±0.4	16.7±0.3	16.3±0.3	15.4±1.1* [92]
hematocrit [%]					
week 6					
MALES	46.9±5.1	48.7±1.6	48.8±1.0	48.3±1.1	48.1±0.9
FEMALES	46.2±1.2	46.8±1.1	46.5±1.0	46.3±1.5	43.3±1.0* [94]
week 14					
MALES	48.4±1.1	48.7±0.8	48.1±1.3	48.5±0.7	48.0±0.7
FEMALES	48.0±1.3	47.2±1.4	47.7±1.1	47.3±0.8	45.6±2.6* [95]
platelets [TH/UL]					
week 6					
MALES	928±118	985±50	980±50	920±98	726±60* [78]
FEMALES	942±125	1016±49	988±58	848±43* [90]	614±86* [65]
week 14					
MALES	854±37	813±138	833±39	845±90	708±28* [83]
FEMALES	838±62	882±33	882±36	705±52* [84]	669±31* [80]
white blood cells [TH/UL]					
week 6					
MALES	8.9±2.1	9.7±1.5	9.2±1.0	9.9±1.7	6.7±0.7* [75]
FEMALES	7.7±2.0	7.9±1.6	7.6±1.0	7.5±0.9	4.3±1.0* [56]
week 14					
MALES	6.1±0.8	5.6±1.1	6.1±1.1	6.2±0.3	4.6±1.0* [75]
FEMALES	5.4±1.1	5.0±0.7	5.1±1.5	4.7±0.8 [87]	3.9±0.8* [75]

[% of control]; ✖ calculated by reviewer; * p<0.05; N=8-10; data from Table 7, pages 89 & 92 of the report

Table 3. Clinical Chemistry Parameters

Parameter/Dose/Sex/Week	0 mg/kg/day	1.2 mg/kg/day	18.1 mg/kg/day	120 mg/kg/day	361 mg/kg/day
glucose [mg/dL]					
week 6					
MALES	119±15	117±8	112±10	110±13	99±9* [83]
FEMALES	102±8	101±12	100±7	91±13* [89]√	86±6* [84]
week 14					
MALES	104±14	100±9	103±10	101±6	83±9* [80]
FEMALES	96±10	90±6	95±16	87±8 [89]	77±15* [80]
blood urea nitrogen [mg/dL]					
week 6					
MALES	17±1.2	16±1.2	15±1.3* [88]	15±1.2* [88]	17±1.6
FEMALES	17±3.4	17±1.2	17±1.8	17±1.1	19±1.6
week 14					
MALES	17±1.2	16±1.2	15±1.6	16±1.6	15±2.2 [88]
FEMALES	17±1.6	17±1.1	17±1.1	16±1.7	21±3.1* [124]
AST [U/L]					
week 6					
MALES	98±11	96±11	102±22	109±13	83±11*
FEMALES	96±37	96±13	93±14	106±17	108±20
week 14					
MALES	125±25	116±19	124±29	138±26	104±21
FEMALES	99±17	104±17	101±21	113±23	108±35
ALT [U/L]					
week 6					
MALES	39±3	40±3	40±5	43±4	35±4
FEMALES	34±6	34±3	34±3	42±4* [124]	39±5
week 14					
MALES	68±16	63±9	66±13	80±12 [118]	43±4*
FEMALES	39±4	38±3	42±5	51±5* [131]	48±11* [123]
total protein [G/dL]					
week 6					
MALES	7.0±0.3	6.7±0.2	6.8±0.2	6.6±0.2* [94]	6.5±0.3* [93]
FEMALES	6.3±0.2	6.3±0.3	6.3±0.3	6.3±0.2	6.3±0.2
week 14					
MALES	6.9±0.3	6.8±0.2	6.8±0.3	6.7±0.1	6.6±0.2
FEMALES	6.7±0.2	6.7±0.3	6.7±0.2	6.7±0.3	6.3±0.2* [94]
T3 [ng/dL]					
week 6					
MALES	95.2±21.7	94.5±19.3	94.0±21.4	90.6±22.4	75.2±17.9
FEMALES	92.3±21.3	76.4±16.7	81.5±17.1	57.3±15.2* [62]	95.7±17.7
week 14					
MALES	85.9±24.4	99.9±28.0	97.4±16.1	89.4±18.3	75.3±17.8 [88]
FEMALES	96.8±14.0	92.9±17.4	94.9±11.7	75.7±12.7* [78]	71.1±19.8* [73]
T4 [μg/dL]					
week 6					
MALES	4.6±0.5	4.8±0.7	4.8±0.5	3.9±0.5* [85]	0.9±0.2* [20]
FEMALES	3.0±0.5	2.4±0.6	2.3±0.3* [77]	0.9±0.2* [30]	0.9±0.0* [30]
week 14					
MALES	3.9±0.6	4.5±0.5	4.2±0.7	3.4±0.6	1.0±-.1* [26]
FEMALES	2.7±0.6	2.4±0.5	2.5±0.6	0.9±0.1* [33]	0.9±0.0* [33]

[% of control]; * $p<0.05$; N=10; data from Table 8, pages 96-103 of the report

Table 4. Organ Weights [grams]

Parameter/Dose/Sex/Week	0 mg/kg/day	1.2 mg/kg/day	18.1 mg/kg/day	120 mg/kg/day	361 mg/kg/day
LIVER					
MALES					
absolute	7.23±0.53	7.19±0.60	7.35±0.50	7.53±0.60	6.76±0.49
relative/body	2.41±0.16	2.61±0.14	2.58±0.16	2.58±0.13	3.06±0.25
relative/brain	3.66±0.29	3.79±0.25	3.91±0.31	3.80±0.34	3.70±0.26
FEMALES					
absolute	4.28±0.27	4.50±0.37	4.33±0.26	4.23±0.26	4.61±0.24* [108]
relative/body	2.72±0.11	2.77±0.10	2.73±0.13	2.00±0.11	4.10±0.69* [151]
relative/brain	2.43±0.18	2.49±0.21	2.50±0.15	2.38±0.14	2.99±0.35* [123]
KIDNEY					
MALES					
absolute	1.95±0.11	1.91±0.16	2.01±0.09	2.19±0.11* [112]	1.70±0.12* [87]
relative/body	0.673±0.036	0.667±0.030	0.706±0.026	0.750±0.029* [111]	0.768±0.032* [114]
relative/brain	0.990±0.065	1.004±0.058	1.068±0.052* [108]	1.106±0.069* [112]	0.932±0.067 [94]
FEMALES					
absolute	1.18±0.07	1.19±0.09	1.18±0.08	1.20±0.05	1.06±0.15 [90]
relative/body	0.753±0.046	0.731±0.029	0.744±0.046	0.796±0.031	0.933±0.101 [124]
relative/brain	0.671±0.045	0.657±0.057	0.682±0.050	0.676±0.035	0.688±0.107
BRAIN					
MALES					
absolute	1.98±0.05	1.90±0.10* [96]	1.89±0.06* [95]	1.98±0.07	1.83±0.05* [92]
relative	0.682±0.033	0.665±0.032	0.662±0.030	0.681±0.044	0.827±0.063* [121]
FEMALES					
absolute	1.77±0.06	1.81±0.05	1.73±0.07	1.78±0.03	1.56±0.17* [88]
relative	1.126±0.069	1.119±0.081	1.094±0.064	1.179±0.048	1.375±0.197* [122]
HEART					
MALES					
absolute	0.93±0.09	0.92±0.04	0.89±0.06	0.96±0.12	0.78±0.08* [84]
relative/body	0.319±0.025	0.322±0.024	0.314±0.023	0.327±0.036	0.350±0.028* [110]
relative/brain	0.469±0.047	0.485±0.030	0.474±0.027	0.482±0.055	0.425±0.046 [91]
FEMALES					
absolute	0.63±0.07	0.67±0.06	0.54±0.05	0.60±0.04	0.54±0.06* [86]
relative/body	0.400±0.037	0.410±0.030	0.401±0.031	0.396±0.025	0.473±0.055* [118]
relative/brain	0.357±0.042	0.368±0.035	0.368±0.029	0.336±0.024	0.347±0.041
THYMUS					
MALES					
absolute	0.26±0.05	0.27±0.05	0.26±0.05	0.27±0.07	0.19±0.03* [73]
relative/body	0.089±0.019	0.095±0.011	0.090±0.017	0.093±0.023	0.084±0.011
relative/brain	0.130±0.028	0.143±0.020	0.136±0.028	0.138±0.037	0.103±0.017
FEMALES					
absolute	0.22±0.02	0.23±0.03	0.22±0.04	0.21±0.04	0.14±0.04* [64]
relative/body	0.138±0.014	0.136±0.014	0.136±0.024	0.136±0.021	0.123±0.031 [89]
relative/brain	0.122±0.008	0.122±0.016	0.126±0.028	0.116±0.031	0.092±0.031* [75]
TESTES					
MALES					
absolute	4.08±0.14	3.97±0.30	4.04±0.21	4.13±0.23	1.77±0.20* [43]
relative/body	1.407±0.964	1.387±0.961	1.416±0.966	1.419±0.108	0.799±0.076* [57]
relative/brain	2.067±0.088	2.087±0.088	2.142±0.108	2.079±0.087	0.970±0.115* [47]

Table 4. Organ Weights [grams]

Parameter/Dose/Sex/Week	0 mg/kg/day	1.2 mg/kg/day	18.1 mg/kg/day	120 mg/kg/day	361 mg/kg/day
OVARIES					
FEMALES					
absolute	0.088±0.008	0.095±0.017	0.083±0.008	0.074±0.010* [84]	0.049±0.008* [56]
relative/body	0.056±0.0043	0.0581±0.0078	0.0522±0.0055	0.0490±0.0057 [88]	0.0430±0.0070* [77]
relative/brain	0.0496±0.0048	0.0524±0.0093	0.0478±0.0045	0.0416±0.0056* [84]	0.0317±0.0065* [64]
ADRENAL					
MALES					
absolute	0.041±0.007	0.043±0.004	0.044±0.004	0.038±0.006	0.038±0.003
relative/body	0.0140±0.0021	0.0150±0.0019	0.0154±0.0015	0.0131±0.0022	0.0172±0.0015* [123]
relative/brain	0.0206±0.0033	0.0225±0.0024	0.0232±0.0024	0.0194±0.0032	0.0209±0.0019
FEMALES					
absolute	0.049±0.009	0.050±0.007	0.047±0.002	0.042±0.004	0.029±0.005* [59]
relative/body	0.0313±0.0058	0.0306±0.0043	0.0294±0.0019	0.0281±0.0018	0.0252±0.0034* [81]
relative/brain	0.0279±0.0046	0.0275±0.0043	0.0269±0.0017	0.0239±0.0021	0.0186±0.0038* [67]
THYROID/PARATHYROID					
MALES					
absolute	0.016±0.002	0.016±0.002	0.016±0.002	0.016±0.003	0.017±0.004
relative/body	0.0054±0.0008	0.0057±0.0006	0.0056±0.0007	0.0056±0.0010	0.0079±0.0020* [146]
relative/brain	0.0079±0.0013	0.0086±0.0009	0.0085±0.0013	0.0083±0.0017	0.0095±0.0022 [120]
FEMALES					
absolute	0.010±0.002	0.012±0.003	0.011±0.004	0.013±0.004	0.014±0.003
relative/body	0.0062±0.0013	0.0076±0.0013	0.0071±0.0027	0.0085±0.0023	0.0121±0.0030* [195]
relative/brain	0.0054±0.0010	0.0068±0.0014	0.0066±0.0027	0.0072±0.0022	0.0088±0.0015* [163]
PITUITARY					
MALES					
absolute	0.009±0.001	0.009±0.002	0.009±0.001	0.009±0.000	0.009±0.001
relative/body	0.0030±0.0005	0.0030±0.0004	0.0033±0.0003	0.0032±0.0001	0.0038±0.0005* [127]
relative/brain	0.0045±0.0007	0.0045±0.0008	0.0050±0.0004	0.0048±0.0003	0.0047±0.0008
FEMALES					
absolute	0.011±0.001	0.011±0.002	0.012±0.002	0.010±0.001	0.006±0.001* [55]
relative/body	0.0069±0.0006	0.0068±0.0007	0.0073±0.0013	0.0064±0.0008	0.0054±0.0011* [78]
relative/brain	0.0062±0.0003	0.0062±0.0009	0.0067±0.0010	0.0055±0.0007	0.0040±0.0011* [65]
TERMINAL BODY WEIGHT					
MALES n=10	290.3±10.7	286.0±20.7	285.2±10.7	292.4±16.0	221.7±15.2* [76]
FEMALES n=9	157.3±7.6	162.5±11.0	158.4±6.5	150.9±5.5	114.4±13.9* [73]

[% of control]; * $p<0.05$; data from Tables 10A, 10B, & 10C, pages 120-127 of the report

857

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008581

DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding-Rodent **GUIDELINE:** 82-1(a)

TOX.CHEM. No.: 315-O MRID No.: 418967-02

HED Project No. 1-1443 **Registrant:** Industry Task Force II

TEST MATERIAL: Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid (2,4-D **DMA)**

STUDY IDENTIFICATION: HLA Study No. 2184-113

TESTING LABORATORY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: Subchronic Toxicity Study in Rats with the Dimethylamine Salt of 2,4-Dichlorophenoxyacetic Acid.

REPORT AUTHOR: Gene E. Schulze, Ph.D

REPORT DATE: April 20, 1991

SUMMARY: Male and female Fischer-344 rats were fed diets containing the Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid at 0, 1.2, 18.1, 120.0, and 361.0 mg/kg/day for 13 weeks. Treatment did not cause any adverse effects at 1.2 or 18.1 mg/kg/day, but did induce decreases in mean body weight, body weight gain, and food consumption, and alterations in some of the hematology and clinical chemistry parameters at 120.0 mg/kg/day. The highest dietary dose level of 361.0 mg/kg/day was associated with weight loss, reduced food consumption, alterations in hematology and clinical chemistry parameters, changes in various organ weights, and histopathological changes that included bilateral retinal degeneration and cataract formation (females); centrilobular hepatocellular hypertrophy (females); atrophy of the testes (males); hypertrophy of thyroid follicular cells, and brush order loss in proximal tubular cells in the kidney (males and females); and hypoplasia of the spleen (females) and bone marrow (males and females).

Under the conditions of this study, a NOEL of 18.1 mg/kg/day and a LOEL of 120.0 mg/kg/day are established. The LOEL is based on decreases in mean body weight, body weight gain and food consumption and alterations in hematology and clinical chemistry parameters observed at 120 mg/kg/day.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the subchronic toxicity of **Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid** following dietary administration to rats.

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: Dimethylamine Salt of 2,4-Dichlorophenoxyacetic acid.
Purity: 66.18%
Lot No.: 04FD31349
Description: Brown liquid.
Acid equivalent: 55.5%
Vehicle: Acetone, reaction grade, Mallenckrodt
Lot. No: 2440 KDMS

2. Test Animals

Species: Rats
Strain: CDF (F-344)/CrljBR Fischer-344 VAF/Plus
Sex: Males and females
Age: Approximately 6 weeks at initiation
Weight at initiation: 123.5-142.4 g (M); 92.0-104.2 g (F)
Identification: Tail tattoo.
Acclimation: Approximately 2 weeks
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Chow #5002.
Water: Tap water ad libitum
Environment: Temperature- 70 - 78° F; Humidity- 45 - 70 %;
Light/dark cycles: 12 hr light/12 dark cycle

3. Study Design

Group No.	Treatment	No. of Animals Males	No. of Animals Females	Dose Level mg/kg/day
1	Control	10	10	0
2	Low	10	10	1.2
3	Mid-1	10	10	18.1
4	Mid-2	10	10	120.0
5	High	10	10	361.0

85

4. Test Article Formulation and Analyses

The purity (66.18%) of 2,4 D DMA was adjusted to 100% active compound. The test article was stirred with a spatula and put on a shaker for approximately 1 hour to ensure an apparently homogeneous liquid state.

For each dose level, an appropriate amount of the test article was weighed to which an appropriate volume of acetone was added, and the test article/acetone mixture was then stirred on a magnetic stirrer for approximately 2 minutes or until an apparently homogeneous mixture was achieved. Final diets were prepared by mixing the test article/acetone mixture with additional powdered feed in a Hobart mixer for 20 minutes. The control diet was prepared in the same manner as the test diets using acetone only at the same level utilized in the test diets. Fresh diets were prepared weekly and reserve samples of each mixed batch were taken on the day of preparation and stored in a freezer.

Concentration analyses of the low and high dose levels were performed prior to initiation of treatment based on historic body weight and food consumption data. Homogeneity was determined of samples from the top, middle, and bottom of each diet mix. Stability analyses were performed on days 0, 7, and 14. Freshly prepared samples were collected from each dose level of the mixed for Weeks 1-4, 8, and 12 and were analyzed for verification of concentration.

5. Treatment

Rats were fed the control and test diets 7 days per week for at least 13 weeks. The oral route of administration was chosen because it is the route of potential human exposure.

6. Experimental Procedures

Mortality and moribundity checks were performed twice daily. Cageside observations for clinical signs of toxicity were performed once daily. Body weights were measured prior to initiation and once weekly. Food consumption was recorded weekly and physical examinations were performed weekly at the body weight intervals. Ophthalmologic examinations were conducted prior to initiation and during Weeks 11 and 13.

Blood and urine were collected from all animals during Weeks 6 and 14 for hematology, clinical chemistry and urinalysis. 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
x Corrected leukocyte count (COR WBC)	x Cell morphology

Clinical Chemistry

Electrolytes:	Other
x Calcium[a] x Chloride[a] Magnesium[a] x Phosphorus[a] x Potassium[a] x Sodium	x Albumin[a] x Blood creatinine[a] x Blood urea nitrogen[a] Cholesterol[a] x Globulins x Glucose[a] x Total bilirubin[a] x Total serum protein[a] Triglycerides Serum protein electrophoresis x Triiodothyronine (T_3 x Thyroxine (T_4)
Enzymes:	
x Alkaline phosphatase x Alanine aminotransferase (SGPT)[a] x Aspartate aminotransferase (SGOT)[a] Cholinesterase[b] x Creatinine phosphatase[a] Lactic acid dehydrogenase Gama glutamyl transferase	

Urinalysis

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

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

7. Termination

After 13 weeks of treatment, all surviving animals were weighed, anesthetized with sodium pentobarbital and exsanguinated. Necropsies were performed on each animal, all gross pathological changes were recorded, and the following organs were weighed.

Adrenals	Brain	Heart	Kidneys	Liver
Ovaries	Thymus	Thyroid	Pituitary	Testes

8. Histopathology

The checked (X) tissues from all animals were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Salivary glands[a]	Trachea[a]
x Esophagus[a]	x Lung[a]
x Duodenum[a]	Pharynx[e]
x Jejunum[a]	Larynx[e]
x Cecum[a]	Nose[e]
x Colon[a]	
x Rectum[a]	Cardiovascular/Hemo.System
x Liver[ac]	
x Pancreas[a]	x Aorta (thoracic)[a]
Gall bladder[ab]	x Heart[a]
	x Bone marrow[a]
Neurological System	x Lymph nodes[a]
	x Spleen[a]
x Brain[ac]	x Thymus[a]
x Pituitary[a]	
Peripheral nerve[ab]	Urinogenital System
Spinal cord (3 levels)[ab]	
x Eyes (optical nerve)[ab]	x Kidneys[ac]
	x Urinary bladder[a]
	x Testes[ac]
Glandular System	x Epididymides
	x Uterus[a]
x Adrenals[a]	x Ovaries[ac]
Lacrimal glands[c]	Others
x Parathyroids[ad]	
x Thyroids[ad]	x All gross lesions and masses
	x Skeletal muscle[a]

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

Mean absolute body weight, body weight change, mean weekly food consumption, total food consumption, clinical pathology data (with the exception of urinalyses and cell morphology), and organ weight data of the control group were compared statistically to the data from the compound-treated groups.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Labs, and the Study Protocol. A quality assurance statement was signed and dated April 9, 1991.

III. RESULTS

1. Analysis of Diet Mix

Concentration analyses summarized below showed that in general, the mean concentration was within 10% of target level except for the high dose mix at Week 12 (74%).

Group / Sex	Target Level (ppm)	Target Level (Mean%)					
		Week 1	Week 2	Week 3	Week 4	Week 8	Week 12
2 / M	16	103	105	92.1	91.5	100	107
2 / F	14	110	99.9	102	108	101	94.7
3 / M	241	97.5	100	97.4	104	102	102
3 / F	211	100	101	98.9	99.7	105	101
4 / M	1621	106	86.4	101	102	105	100
4 / F	1463	106	101	96.7	99.9	97.9	94.4
5 / M	5041	106	101	96.9	101	101	74.0
5 / F	4731	108	107	98.1	103	104	97.9

Homogeneity analysis indicated the diet mix to be homogeneous with target levels ranging from 77 to 124% (mean, 95.5%) in the top, middle, and bottom samples at the low dose and ranging from 88 to 94% (mean, 92%) in the top, middle, and bottom samples at the high dose.

Stability analyses of samples stored at room temperature demonstrated the test material to be stable with recovery of 98% and 99% at the low and high dose, respectively after 7 days, and 95% and 100%, respectively after 14 days.

2. Survival

Except for one female that died at the high dose (361 mg/kg/day) that died following the Week 6 blood sample collection, no mortality occurred during the study.

2. Clinical Observations

Treatment-related clinical signs of toxicity were limited to opaque eyes observed in 6/10 females at 361.0 mg/kg/day; other frequently seen signs (urine stains, alopecia, and lacrimation) did not appear to be dose- or compound-related.

3. Body Weights and Body Weight Changes

Mean body weight data are summarized in Tables 1 and 2 for males and females, respectively. Statistically significant ($p < 0.05$) reduction in mean body weight was observed for males and females at 361 mg/kg/day at Weeks 6 and 13, and for females at 120 mg/kg/day at Week 6. A significant monotonic negative trend was seen for the males at Week 13.

Table 1. Mean Body Weights and S.D (G) for Male Rats.

Dose Level (mg/kg/day)	Week: 0	Week: 6	Week: 10	Week: 13[a]
0	133.3 ± 4.1	250.2 ± 5.8	293.0 ± 11.2	305.9 ± 12.2
1.2	132.5 ± 5.0	242.3 ± 16.3	284 ± 21.5	301.0 ±21.0
18.1	128.9 ± 3.0	243.3 ± 10.4	285.0 ± 12.3	301.0 ±12.1
120.0	133.9 ± 3.8	248.3 ± 11.5	290.1 ± 13.0	307 ± 14.5
361.0	133.5 ± 5.1	**196.6* ± 12.8** 79	219.6 ±17.5 75	**234.0 ± 18.0*** 76

* Significantly different from control value at $p \leq 0.05$.
a Significant negative monotonic trend, $p \leq 0.05$

Table 2. Mean Body Weights and S.D (G) for Female Rats.

Dose Level (mg/kg/day)	Week: 0	Week: 6	Week: 10	Week: 13
0	99.2 ± 2.5	140.0 ± 5.5	162.1 ± 8.1	169.4 ± 9.1
1.2	99.1 ± 3.9	143.0 ± 9.8	167.0 ± 11.7	174.0 ± 12.5
18.1	99.5 ± 2.0	142.6 ± 1.9	165.1 ± 3.3	169.5 ± 6.9
120.0	101.1± 2.7	132.6 ± 5.6	154.4 ± 7.0	162.5 ± 6.6
361.0	98.9 ± 2.2	**104.5* ±8.7** 75	112.6 ± 13.0 69	**123.3 ± 15.2*** 73

* Significantly different from control value at $p \leq 0.05$

865

Mean body weight change data are presented in Tables 3 and 4 for males and females, respectively. Statistically significant ($p < 0.05$) decreases in mean body weight gains were seen in both males and females at 361 mg/kg/day during Weeks 0-6, 6-13, and 0-13 intervals, and in females at 120 mg/kg/day during Week 0-6 interval. No body weight loss was seen in males at 1.2, 18.1, and 120 mg/kg/day, or in the females at 1.2 and 18.1 mg/kg/day groups.

Table 3. Mean Body Weight Change (G) in Male Rats.

Dose Level (mg/kg/day)	Weeks: 0 - 6	Weeks: 6 - 13	Weeks: 0 - 13
0	116.8 ± 5.68	55.7 ± 7.4	172.6 ± 11.74
1.23	109.8 ± 13.36	58.7 ± 7.4	168.5 ± 18.3
18.1	113.4 ± 8.66	58.7 ± 6.3	172.1 ± 11.3
120.0	114.4 ± 11.3	58.7 ± 9.0	173.1 ± 15.3
361.0	**63.1* ± 8.9**	**37.4* ± 10.0**	**100.5* ± 15.2**

54 67 53

* Significantly different from control at $p \leq 0.05$.

Table 4. Mean Body Weight Change (G) in Female Rats.

Dose Level (mg/kg/day)	Weeks: 0 - 6	Weeks: 6 - 13	Weeks: 0 - 13
0	41.4 ± 5.99	28.8 ± 4.3	70.2 ± 9.0
1.2	43.9 ± 7.0	31.0 ± 5.3	74.9 ± 10.6
18.1	43.1 ± 2.3	27.0 ± 6.9	70.1 ± 8.1
120.0	**31.5* ± 4.7**	30.0 ± 3.4	61.4 ± 5.9
361.0	**5.4* ± 8.0**	**18.8* ± 8.5**	**24.2* ± 14.1**

13 34

* Significantly different from control at $p \leq 0.05$.

4. Food Consumption

The mean total food consumption was significantly ($p < 0.05$) decreased in males and females at 361 mg/kg/day during Weeks 1-6, 6-13, and 1-13, and in females at 120 mg/kg/day during Weeks 1-6. A significant negative trend was seen for males during Weeks 1-6 and 1-13. No treatment-related changes were seen in males at 1.2,18.1, and 120 mg/kg/day, and in females at 1.2 and 18.1 mg/kg/day groups. The average daily compound consumption for Weeks 1-13 was 1.13, 16.97, 112.87, and 340.97 mg/kg/day for males and 1.15, 17.34, 115.92, and 349.34 mg/kg/day for females.

5. Ophthalmology Examination

Ophthalmoscopic findings for the Week 11 and 13 examinations are summarized in Tables 5 and 6 for males and females respectively. The DMA salt of 2,4-D induced bilateral cataract formation and retinal degeneration in females administered the high dose (361 mg/kg/day). Retinal degeneration was observed in one male rat at this dose; however, this was not supported by collaborative histopathology. No other treatment-related ophthalmoscopic changes were seen in male rats.

Table 5. Ophthalmoscopic Observations In Male Rats.

Observations	Dose Level (mg/kg/day)				
No.of animals = 10/dose level	0	1.2	18.1	120	361
Conjunctiva - chromodacryorrhea	1	0	0	2	0
Cornea - corneal dystrophy	1	0	1	0	0
Fundus - retinal linear atrophy	1	0	1	0	0
Fundus - possible retinal degeneration	0	0	0	0	1
Coloboma of optic nerve or myopic degeneration	1	0	0	0	0

Table 6. Ophthalmoscopic Observations In Female Rats.

Observations	Dose Level (mg/kg/day)				
No. of animals=10/dose level	0	1.2	18.1	120	361
Conjunctiva - chromodacryorrhea	0	0	1	0	0
Cornea - corneal scar	1	0	0	0	0
Cornea - corneal dystrophy	0	0	2	0	0
Anterior chamber iris - anterior synechia	1	0	0	0	0
Fundus - retinal linear atrophy	0	0	1	0	0
Fundus - retinal degeneration	0	0	0	0	5
Fundus - possible retinal degeneration	0	0	0	0	1
Lens - complete cataract	0	0	0	0	5
Lens - posterior subscapular cataract	0	0	0	0	5
Vitreous/retinas - not visible/examineable	0	0	0	0	3

6. Hematology and Clinical Chemistry

Treatment-related, statistically significant ($p \leq 0.05$) decreases observed in hematology parameters are summarized below:

Dose Level / Sex	Parameters Affected	Study Weeks
120.0 mg/kg/day (F)	↓ in RBC	14
	↓ in Platelet	6,14
	↓ in SEG	14
361.0 mg/kg/day (M)	↓ in RBC	14
	↓ in HGB	14
	↓ in PLATELET	6,14
	↓ in WBC	6,14
	↓ in COR WBC	6,14
	↓ in LYMPH	6,14
361.0 mg/kg/day (F)	↓ in RBC	6,14
	↓ in HGB	6,14
	↓ in HCT	6,14
	↓ in PLATELET	6,14
	↓ in COR WBC	6,14
	↓ in SEG	6,14
	↓ in LYMPH	6

Alterations observed in hematology parameters correlated with histopathological changes observed in the bone marrow and spleen (see Histopathology).

Treatment-related, statistically significant ($p \leq 0.05$) differences observed in clinical chemistry parameters are summarized below:

Dose Level / Sex	Parameters Affected	Study Weeks
18.1 mg/kg/day (F)	↓in T_3	6
120.0 mg/kg/day (M)	↓ in Total protein	6
	↓ in Albumin	14
	↓ in Calcium	14
	↓ in T_4	6
120.0 mg/kg/day (F)	↓ in Glucose	6
	↑ in ALT	6,14
	↓ T_3	6,14
	↓ in T_4	6,14
361.0 mg/kg/day (M)	↓ in Glucose	6
	↓ in AST	6
	↓ in ALT	14
	↓ in Total protein	6
	↓ in Globulin	6,14
	↓ in Calcium	6,14
	↓ in T_4	6,14
361.0 mg/kg/day (F)	↓ in Glucose	6,14
	↑ in ALT	14
	↓ in Total protein	14
	↑ in Albumin	6
	↓ in Globulin	6,14
	↓ T_3	14
	↓ in T_4	6,14

Alterations seen in clinical chemistry parameters correlated well with histopathological changes observed in the liver, thyroids and adrenal glands (see Histopathology).

7. Gross Pathology

Gross necropsy revealed an increased incidence of small testes (7/10) and soft testes (3/10) in males and opaque eyes (6/10) in females at the high dose (361 mg/kg/day). Other gross necropsy findings were considered incidental in nature.

8. Organ Weights

Statistically significant differences in absolute and relative organ weight were primarily seen in male and/or female rats at the high dose. The absolute weights of adrenals (females), thymus (both sexes), brain (both sexes), heart (both sexes), kidneys (males), testes with epididymides, ovaries and pituitary (females) were decreased while absolute liver weights were increased in females. The relative weights of adrenals (males), brain (both sexes), heart (both sexes), kidneys (both sexes), liver (both sexes), thyroid/parathyroid (both sexes), and pituitary (males) were increased while the relative weights of testes with epididymides, ovaries, and pituitary (females) were decreased.

9. Histopathology

Treatment-related histopathological changes (Tables 7 and 8) were primarily observed in the high dose group which included:

1. Hypertrophy of the zona glomerulose of the adrenal cortex,
2. Bilateral retinal degeneration and cataract formation of the eye
3. Hypertrophy of thyroid follicular cells,
4. Centrilobular hepatocellular hypertrophy of the liver,
5. Brush border loss in proximal tubular cells of kidneys,
6. Atrophy of the testes,
7. Hypoplasia of bone marrow, and
8. Hypoplasia of spleen.

Histopathological evaluation of the eye revealed degenerative changes in the lens and retina in females at the high dose. Retinal degeneration was slight in one, and moderate in six animals. Cataractous change was moderate in one animal and moderately severe in five. In most cases, the histopathological changes correlated well with alterations observed in hematology and clinical chemistry parameters and organ weight data. The increase in adrenal gland weight can be correlated to hypertrophy of cells in zona glomerulose. Hypertrophy of follicular cells of the thyroid reflects a response to decreased circulating thyroid hormone levels. Increases in liver weight, ALT, and AST are associated with centrilobular hepatocellular hypertrophy of the liver. Increased kidney weights (females) correlated with brush border loss in the proximal tubules. Decreases in testes weight correlated with testicular atrophy, while decreased ovarian weights did not show any corresponding histological changes. Decreases in RBC, HGB and HCT are associated with bone marrow hypoplasia. Decreases in absolute and corrected leukocyte counts correlated with lymphoid of the spleen and bone marrow. Other microscopical changes were considered incidental and unrelated to treatment.

510

Table 7. Histopathological Findings in Male Rats At Sacrifice.

Organ / Lesion	Dose Level (mg/kg/day)				
No.of animals= 10/dose levels	0	1.2	18.1	120	361
Adrenal, cortex -Hypertrophy, zona glomerulose	0	0	0	0	10
Thyroid -Follicular cell hypertrophy	0	0	0	0	4
Kidney -Brush border loss, tubular	0	0	0	0	7
Testes -Atrophy, bilateral	0	0	0	0	10
Marrow, femur -Hypoplasia	0	0	0	0	7
Eye -Corneal dystrophy	2	1	5	3	6

Table 8. Histopathological Findings in Female Rats At Sacrifice.

Organ / Lesion	Dose Level (mg/kg/day)				
No.of animals= 10/dose levels	0	1.2	18.1	120	361
Adrenal, cortex -Hypertrophy, zona glomerulose	0	0	0	0	8
Thyroid -Follicular cell hypertrophy	0	0	0	0	6
Kidney -Brush border loss, tubular	0	0	0	0	8
Liver - Hypertrophy	0	0	0	0	7
Marrow, femur -Hypoplasia	0	0	0	0	9
Spleen - Hypoplasia	0	0	0	0	7
Eye -Corneal dystrophy -Degeneration, retinal, bilateral -Cataractous change, bilateral	 1 0 0	 1 0 0	 2 0 0	 3 0 0	 2 7 6

IV. DISCUSSION

Male and female Fischer-344 rats were fed diets containing DMA salt of 2,4-D at concentrations of 0, 1.2, 18.1, 120.0, or 361.0 mg/kg/day for 13 weeks.

Analytical data showed that the diet mixes were homogeneous, stable at room temperature for up to 14 days and the mean concentrations were within 10% of the target level.

Treatment had no effect on survival. No treatment-related effects were seen at the low- (1.2 mg/kg/day) or the mid-1- dose (18.1 mg/kg/day). Decreases in mean body weight, body weight gain, and reduction in food consumption were observed in rats fed the mid-2- (120 mg/kg/day) and the high-dose (361 mg/kg/day).

Ophthalmology examinations revealed bilateral cataract formation and retinal degeneration only in females at 361.0 mg/kg/day; no ocular changes were seen at the lower doses.

Treatment-related alterations in hematology parameters were confined to the 120 mg/kg/day and 360 mg/kg/day groups which included: decreased red cell mass, decreased hemoglobin, decreased platelet count, decreased absolute and corrected leukocyte counts, decreased lymphocyte counts, and decreased segmented neutrophil counts. Changes in cell morphology consisted of decreased incidence/grade for echinocytes and acanthocytes.

Also, treatment-related alterations in clinical chemistry parameters were primarily seen at 120 and 360 mg/kg/day groups which included: decreased glucose and globulin levels decreased thyroxin levels, decreased triiodothyronine, decreased total protein, increased ALT, and increased albumin levels. The significant decrease in thyroxine level seen in females at 18.1 mg/kg/day (2.3 µg/DL vs 3 µg/DL in controls) at Week 6 was within the reference range (2.2 to 5.6 µg/DL) and therefore, was not considered to be treatment related.

Treatment-related gross pathological changes were limited to small and soft testes (males) and opaque eyes (females) at the high dose. Compound-induced differences in absolute and relative organ weights were generally limited to the high-dose which included: decreased mean absolute brain weight and increased mean brain/body weight rations, increased mean relative thyroid weights, decreased mean absolute kidney weight and increased mean kidney/body weight ratio, and increased absolute and relative liver weights.

In most cases, alterations noted in hematology, clinical chemistry, gross pathology, and organ weights correlated well with histopathological changes. Treatment-related histopathological changes which were limited to the high-dose group included bilateral retinal degeneration and cataract formation (females), centrilobular hepatocellular hypertrophy (females), atrophy of the testes (males), hypertrophy of thyroid follicular cells, and brush border loss in proximal tubular cells in the kidney (males and females), and hypoplasia of the spleen (females) and bone marrow (males and females).

V. CONCLUSION

Under the conditions of this study, a NOEL of 18.1 mg/kg/day and a LOEL of 120.0 mg/kg/day is established for the 90-day oral toxicity of the Dimethylamine Salt of 2,4-Dichlorophenoxyacetic acid to male and female rat. The LOEL is based on decreases in mean body weight, body weight gain and food consumption, and alterations in hematology and clinical chemistry parameter observed at 120 mg/kg/day.

VI. **CORE CLASSIFICATION:** Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

[DIETHANOLAMINE SALT OF 2,4-D] 21-Day Dermal Toxicity Study - Rabbit [§82-2/OPPTS 870.3200]

Amendment to DER for MRID 41920905 - 21-Day Dermal Toxicity Study [Rabbit] [DIETHANOLAMINE SALT OF 2,4-D]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 009335].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/6/00
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 12/6/00
Reregistration Branch I, Health Effects Division (7509C)
TXR # 0051360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: 21-day dermal toxicity - rabbit § 82-2/OPPTS 870.3200
P.C. CODE: 030016 REREGISTRATION CASE NO.: 818713
CASWELL NO.: 315K CAS #: 5742-19-8
TEST MATERIAL (PURITY): Diethanolamine salt of 2,4-D [73.09%]
SYNONYMS: 2,4-D DEA; dichlorophenoxyacetic acid, diethanolamine salt
SPONSOR: PBI/Gordon Corporation, Kansas City, MO

CITATION: Siglin, J.C. (1991). 21-Day Dermal Toxicity Study in Rabbits with Diethanolamine Salt of 2,4-D. Springborn Laboratories, Inc.; Study No. SLS 3229.1, February 4, 1991. MRID 41920905. Document No. 009335. Unpublished.

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study [MRID 41920905], 5 New Zealand White rabbits/sex/group were administered the diethanolamine salt of 2,4-dichlorophenoxyaceticacid [73.09%] *via* dermal application in sterile distilled water [once a day for 6 hours, 7 days/week] for 21 days at concentrations of 0, 15, 148, or 443 mg/kg/day.

Treatment had no adverse effect on survival, clinical signs, hematology, urinalysis, or gross pathology of either sex, and body weights and food consumption were comparable among the female groups. Although there was wide variability [large standard deviations; statistical significance was not attained], males at the high-dose level displayed slightly lower body weights compared to the control [93%-95% of control], with the magnitude of the deficit increasing with time. Body-weight gains of the high-dose males were decreased compared to the control throughout the study [8%-75% of control; 34% of control overall], and the high-dose females displayed a negative body-weight gain during the last week of dosing and decreased body-weight gain overall [82% of control]. Food consumption of the high-dose males was decreased throughout the study compared to the controls, but females displayed comparable values among the groups.

There was a dose-related increase in the incidence and severity of the signs of dermal irritation. Although minor dermal irritation was observed at the low dose, histopathology revealed no treatment-related lesions at this dose level. At the mid- and high-dose levels, 2,4-D DEA induced dermal lesions, which included the increased incidence and/or severity of acanthosis, hyperkeratosis, and chronic dermatitis. Additionally, acute dermatitis, surface exudate, dermal hemorrhage, and vesiculation of the epidermis were seen at the high-dose level.

At the high-dose level, liver lesions [hypertrophy of hepatocytes and the presence of hyaline droplets within the

hepatocytes] were observed, and these correlated with the elevated serum enzyme levels [AST, ALT, and alkaline phosphatase] and increased absolute and relative liver weights observed in the rabbits at the high-dose level. No liver lesions were observed at the lower dose levels.

The NOAEL for systemic toxicity is 148 mg/kg/day, based on liver toxicity [increased AST, ALT, and AP levels, liver weight, liver lesions] in both sexes at the systemic toxicity LOAEL of 443 mg/kg/day. The dermal irritation NOAEL is 15 mg/kg/day, based on microscopic dermal lesions at the dermal irritation LOAEL of 148 mg/kg/day.

This 21-day dermal toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement [§82-2; 870.3200] for a 21-day dermal toxicity study.

875

[DIETHANOLAMINE SALT OF 2,4-D] 21-Day Dermal Toxicity Study - Rabbit [§82-2/OPPTS 870.3200]

Table 1.Body Weight/Body-Weight Gain [grams]				
Dose/Day	0 mg/kg/day	15 mg/kg/day	148 mg/kg/day	443 mg/kg/day
MALE body weight				
1	2983±199	3070±149	3022±221	2922±106
8	3102±221	3211±192	3198±263	2948±126 [95]
15	3174±265	3362±249	3346±283	2955±136 [93]
21	3252±300	3443±328	3408±327	3013±193 [93]
MALE body-weight gain				
1-8	119±83	140±106	176±76 [148]	26±82 [22]
8-15	72±62	151±82	148±83 [205]	6±81 [8]
15-21	77±42	82±105	62±60 [81]	58±79 [75]
1-21	268±177	373±244	386±126 [144]	91±202 [34]
FEMALE body weight				
1	2876±110	2914±124	2869±76	2851±216
8	2946±129	3103±162	2987±110	2945±207
15	2999±189	3151±158	3131±178	3020±167
21	3070±249	3211±109	3229±218	3010±128
FEMALE body-weight gain				
1-8	70±91	189±107	118±94	94±19
8-15	53±119	47±70	144±73	75±45
15-21	71±62	60±67	98±75	-9±82
1-21	194±244	297±149	360±220	160±129 [82]

√[% of control]; n=5; data from Tables 3-4, pages 30-33 of the report

87

Table 2. Food Consumption

Dose/Day	0 mg/kg/day	15 mg/kg/day	75 mg/kg/day	150 mg/kg/day
MALES grams/rat/day				
1-2	204±34	229±50	235±46	169±22 [83]√
2-3	196±37	207±49	206±24	135±35 [69]
4-5	203±35	220±39	210±22	123±45** [61]
5-6	203±40	211±45	201±30	135±42* [66]
15-16	166±41	191±42	187±37	120±22 [72]
20-21	166±29	178±46	175±30	136±21 [82]
MALES grams/kg/day				
1	68±10	74±15	77±10	58±8 [85]
2	65±10	67±15	68±6	46±12* [71]
3	67±9	68±13	63±12	43±5** [64]
4	68±9	72±13	69±5	42±16** [62]
10	58±10	62±8	63±7	45±10 [78]
15	52±10	57±10	55±7	41±7 [79]
20	52±5	53±10	52±6	46±6 [88]
FEMALES grams/rat/day				
1-2	157±38	218±52	195±45	195±25
2-3	151±32	182±52	160±37	167±26
4-5	149±41	201±32	182±31	155±17
5-6	154±32	206±31*	175±30	146±24
15-15	148±31	169±41	173±30	144±29
20-21	131±22	147±26	155±34	114±35 [87]
FEMALES grams/kg/day				
1	55±14	75±18	68±16	69±10
2	53±11	63±19	56±13	59±11
3	52±13	64±19	59±12	54±11
4	52±15	69±12	64±11	55±8
10	50±8	61±11	57±8	54±5
15	49±8	53±11	55±7	48±11
20	44±5	47±9	49±9	38±12 [86]

√[% of control]; * $p<0.05$; ** $p<0.01$; data from Tables 5-6, pages 34-49 of the report

Table 3. Liver Weights

Dose/Day	0 mg/kg/day	15 mg/kg/day	75 mg/kg/day	150 mg/kg/day
MALES				
absolute	85.38±8.35	92.88±20.82	109.81±21.80	119.24±20.66* [140]
relative	2.764±0.345	2.851±0.648	3.371±0.426	4.124±0.632** [149]√
FEMALES				
absolute	73.88±11.28	78.71±8.77	99.67±13.32** [135]	116.49±9.17** [225]
relative	2.508±0.232	2.541±0.237	3.223±0.367** [129]	4.012±0.185** [160]

√[% of control]; * $p<0.05$; ** $p<0.01$; n=5; data from Table 11-12, pages 83-86 of the report

Table 4. Liver Lesions

Dose/Day	0 mg/kg/day	15 mg/kg/day	75 mg/kg/day	150 mg/kg/day
MALES				
hepatocyte hypertrophy				
minimal	0	0	0	5
hyaline droplets				
minimal	0	0	0	1
mild	0	0	0	1
moderate	0	0	0	0
necrosis				
mild	1	0	0	0
moderate	0	0	0	1
FEMALES				
hepatocyte hypertrophy				
minimal	0	0	0	5
hyaline droplets				
minimal	0	0	0	3
mild	0	0	0	1
moderate	0	0	0	1

n=5; data from Table 1, page 390 of the report

Table 5. Microscopic Findings in Treated Skin

Dose/Day	0 mg/kg/day	15 mg/kg/day	75 mg/kg/day	150 mg/kg/day
MALES				
acanthosis				
minimal	2	0	3	1
mild	0	0	2	2
moderate	0	0	0	0
marked	0	0	0	1
dermatitis, acute				
mild	0	0	0	1
dermatitis, chronic				
minimal	2	0	1	1
mild	0	1	0	0
exudate, epidermal surface				
minimal	0	0	0	1
mild	0	0	0	0
moderate	0	0	0	0
hemorrhage				
minimal	0	0	0	1
mild	0	0	0	1
hyperkeratosis				
minimal	0	0	0	0
mild	0	0	4	3
moderate	0	0	1	2
FEMALES				
acanthosis				
minimal	0	1	1	0
mild	0	0	3	1
moderate	0	0	0	4
marked	0	0	0	0
dermatitis, acute				
mild	0	0	0	0
dermatitis, chronic				
minimal	1	1	4	2
mild	0	0	0	2
exudate, epidermal surface				
minimal	0	0	0	0
mild	0	0	0	1
moderate	0	0	0	1
hemorrhage				
minimal	0	0	0	2
mild	0	0	0	0
hyperkeratosis				
minimal	0	0	2	0
mild	0	0	3	1
moderate	0	0	0	4

n=5; data from Table 1, page 391 of the report

009335

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist Jess Rowland 2/19/92
Section II, Toxicology Branch II (H7509C)

SECONDARY REVIEWER: K. Clark Swentzel K. Clark Swentzel 2/21/92
Head, Section II,Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 21-Day Dermal Toxicity **GUIDELINE: 82-2**

Caswell No. 315 K MRID No. 419209-05 HED PROJECT No. 1-2160

TEST MATERIAL: Diethanolamine salt of 2,4-D.

REGISTRANT: PBI/Gordon Corporation, Kansas City, MO

TESTING LABORATORY: Springborn Laboratories,Inc., Spencerville, OH.

STUDY IDENTIFICATION: SLS Study No. 3229.1

TITLE OF REPORT: 21-Day Dermal Toxicity Study in Rabbits with Diethanolamine Salt of 2,4-D.

STUDY COMPLETION DATE: February 04, 1991 **AUTHOR:** J.C. Siglin

SUMMARY: Male and female rabbits received repeated dermal application of the Diethanolamine Salt of 2,4-Dichlorophenoxyacetic acid at doses of 0, 15, 148, or 443 mg/kg, 6 hours/day, 7 days/week for 21 days. Treatment had no adverse effect on survival, clinical signs, mean body weight, body weight gain, hematology, urinalysis or gross pathology. Food consumption at the high dose was decreased on several days during the first week of the study and was lower than controls on all other days. Signs of dermal irritation were observed in a dose-related manner with the severity ranging from mild at the low dose to severe at the high dose. Although minor dermal irritation was seen at the low dose, histopathology revealed no treatment-related lesions at this dose. At 148 and 443 mg/kg/day, 2,4-D DEA induced dermal lesions included increased incidence and/or severity of acanthosis, hyperkeratosis and chronic dermatitis. In addition, acute dermatitis, surface exudate, dermal hemorrhage and vesiculation of the epidermis were also seen at the high dose. 2,4-D DEA was also shown to be a hepatotoxin at a dose of 433 mg/kg/day, inducing liver lesions consisting of hypertrophy of hepatocytes and the presence of hyaline droplets within hepatocytes. These hepatic lesions correlated with the elevated serum enzyme levels [AST, ALT, and alkaline phosphatase] and increased absolute and relative liver weights in animals at the high dose. No liver lesions were seen in at 15 or 148 mg/kg/day groups.

The following NOEL's and LOEL's are established:

End Point	NOEL mg/kg/day	LOEL mg/kg/day	Basis for LOEL
Dermal toxicity	15	148	Microscopic dermal lesions
Systemic toxicity	148	443 [HDT]	Liver toxicity

VI. CORE CLASSIFICATION

Guideline; this study satisfies the requirements for a 21-day dermal toxicity study (82-3) in rabbits.

RANGE-FINDING STUDY

I. INTRODUCTION

A **range-finding study** was conducted with the Diethanolamine Salt of 2,4-D [2,4-D DEA] in rabbits to select dose levels for a full scale 21-day dermal toxicity study.

II. MATERIALS & METHODS

Test Material: **2,4-D DEA** (lot No.0017; Purity: 73.09%).
Test Animals: Male and female New Zealand White rabbits.
Dose Groups: 1 male and 1 female/dose level.
Dose Levels: 0, 15, 44, 148, 443, or 1476 mg/kg/day
Treatment: Test material (clear,light brown liquid) in deionized water was applied topically on the dorsal shaved area (approximately 10% of the total body surface). The test site was covered with gauze/tape/wrap.
Exposure Time: 6 hours/day, 7 days/week for 21-days
Observations: Mortality, moribundity, clinical signs, body weights (Days 1, 8, 15, and 22), and gross pathology.

III. RESULTS

Both rabbits treated at 1476 mg/kg/day died on study day 7. The male rabbit at 443 mg/kg/day was sacrificed on study day 6 due to a broken back. All other animals survived to termination. Clinical signs which preceded the animals that died [1476 mg/kg/day] included decreased activity, labored breathing, few feces, loss of fore and hind limb mobility, and wobbly gait. Treatment and dose-related dermal irritation was observed with the irritation being quite severe [leading to desquamation, thickening and/or eschar formation] at levels above 148 mg/kg/day. Dermal irritation at 15 mg/kg/day was limited to slight erythema. No treatment-related effects were observed in body weight gain or gross necropsy.

IV. CONCLUSION

Based on these findings, the dose levels selected for the full scale 21-day dermal toxicity study with **2,4-D DEA** were: 15, 148, and 443 mg/kg/day.

V. CORE CLASSIFICATION

Not applicable; range finding study to determine dose levels for a full scale study.

21-DAY DERMAL TOXICITY STUDY

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a 21-day dermal toxicity study in rabbits with **2,4-D DEA.**

II. MATERIALS AND METHODS

1. Test Material

Chemical Name: **Diethanolamine Salt of 2,4-D**
Active Ingredient: **73.09%**
Lot No.: 0017
Description: Clear, light brown liquid

2. Test Animals

Species: Rabbit
Strain: New Zealand White
Sex: Males and females
Age at Initiation: 22 weeks (M); 23 weeks (F)
Weight at Initiation: Males - 2.7 to 3.3 kg;
Females - 2.6 to 3.2 kg
Identification: Ear tags
Acclimation: 11 days
Health Status: Good
Housing: Individually in suspended wire mesh cages
Food: Certified Purina Rabbit Chow #5322 ad libitum
Water: Tap water ad libitum
Environment: Temperature - 67 ± 4°F; Humidity -40 to 60%
12 hr. light/dark cycle

3. Study Design

Group No.	No. of Animals Males	Females	Dose Level (mg/kg/day)[a]	Actual Test Material (mg/mL)
1 (vehicle control)	5	5	0	0
2 (Low-dose)	5	5	15	10.26
3 (Mid-dose)	5	5	148	101.25
4 (High-dose)	5	5	443	303.05

[a] Based on results of a 21-day pilot range-finding study.

4. Test Material Formulation

An appropriate amount of the test material for each group was weighed and sufficient amount of sterile distilled water was added to achieve the desired concentration. The concentration of each dosing solution was adjusted based on a test article activity of 73.09%. Dosing solutions were prepared fresh weekly and individual aliquots were stirred for approximately ten minutes prior to daily dispensation.

5. Analyses of Dosing Solutions

Concentration, homogeneity, and stability analyses of the dosing solution were performed prior to the initiation of the study.

6. Treatment

Approximately 24 hours prior to dosing, the fur was clipped dorsally and laterally from shoulder to rump to cover an area of approximately 10 % of the total body surface. Appropriate volume of the test material/vehicle mixture [dosing solution] was applied uniformly over the exposure area; the control group received distilled water. Each animal's dose was held in contact with the skin using a porous 8 ply 4" x 4" gauze dressing which secured in place using Blenderm tape. A stockinette sleeve was then place around the rabbit's torso and secured with athletic tape. To prevent possible ingestion of the test material, during the course of the 6 hour exposure period all animals wore flexible plastic restraint collars. At the end of the exposure period, the dressings and collars were removed and the treated area was wiped with clean gauze moistened with sterile water to remove any remaining test material. Rabbits were treated for 6 hours/day, 7 days/week for 21 days.

6. Experimental Procedures

Parameter	Time measured
Mortality and Moribundity	Twice daily
Dermal irritation	Daily prior to treatment
Body weight	Weekly
Food consumption	Daily
Hematology & Clinical Chemistry	Prior to initiation and at termination
Urinalysis	Termination

Hematology

Hematocrit	Leukocyte count (WBC)
Hemoglobin (HGB)	Platelet count
Erythrocyte count (RBC)	Leukocyte differential
Mean cell volume (MCV)	Mean cell hemoglobin concentration (MCHC)
Mean cell hemoglobin (MCH)	Reticulocyte count

Clinical Chemistry

Albumin	Globulin
Albumin/globulin (A/G) ratio	Alkaline Phosphatase
Blood Creatinine	Cholesterol
Triglycerides	Fasting Glucose
Aspartate aminotransferase (AST)	Alanine aminotransferase (ALT)
Total Bilirubin	Total Serum Protein
Sodium	Chloride
Potassium	Calcium
Blood urea nitrogen	

Urinalysis

Appearance	Bilirubin
Specific gravity	Occult blood
pH	Urobilinogen
Protein	Glucose
Ketone	Microscopic examination of sediment

885

7. Termination

At termination, animals were weighed, anesthetized with sodium pentobarbital, and sacrificed by exsanguination and were subjected to a complete necropsy. At necropsy the brain, kidneys, liver with drained gall bladder, thyroid, testes with epididymides and ovaries of all animals were weighed and organ-to-body weight ratios were calculated. Skin (treated and untreated), liver, kidneys, eyes, and target organs (those organs showing gross pathological changes) from all animals were fixed in 10% neutral-buffered formalin.

8. Histopathology

The tissues listed above from control and high-dose animals were trimmed and processed for histopathological evaluation.

9. Statistical Analyses

Statistical analyses were two-tailed with minimum significance level of 5%. One Way Analysis of Variance [ANOVA] was used for body weight, weight gain, food consumption and organ weight data. When significance was observed with ANOVA, control to treatment group comparisons were performed using Dunnett's Test.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Springborn Laboratories, and the study Protocol. A quality assurance statement was signed and dated on February 4, 1991.

III. RESULTS

1. Analytical Data

Concentration analyses showed an average recovery of 99%, 97.9% and 98.% for nominal concentrations of 10.26, 101.25, and 303.05 mg/mL, respectively. The pretest stability analyses indicated the dosing solution to be stable [recovery range:100.1 to 103.0%] for up to 8 days in room temperature. Homogeneity analyses showed the dosing solution to be homogeneous.

2. Survival

All animals survived to terminal sacrifice on day 22.

3. Clinical Signs

No treatment-related clinical signs of toxicity were observed during the study.

4. Dermal Observations

No dermal irritation was observed in rabbits treated with distilled water [vehicle control]. However, dermal irritation was observed at all dose levels with the severity being dose-related. At 15 mg/kg/day, irritation was mild and consisted primarily of slight erythema. At 148 mg/kg/day, the irritation was more severe and was characterized by slight to moderate erythema, edema, and desquamation. At 443 mg/kg/day, the irritation progressed to eschar formation in most rabbits with additional observations of fissuring, thickening, and exfoliation. The dermal observations are summarized below:

58

MALES [Total Incidence/No.of Animals]			
Dermal Lesions From Day 1 - Day 22	Dose [mg/kg/day]		
	15	148	443
Erythema - Slight	50/5	21/5	36/5
Erythema - Moderate	2/1	76/5	36/5
Edema - Slight	2/1	75/5	20/5
Edema - Moderate	0	6/3	18/3
Desquamation - Slight	0	29/5	14/3
Desquamation - Moderate	0	25/3	10/2
Fissuring - Slight	0	0	32/3
Eschar - Partial	0	0	14/3
Eschar - Complete	0	0	27/3
Eschar - Exfoliation	0	0	27/3
Thickening of skin	0	3/1	4/3
Areas of eschar within patch site	0	0	4/2
Blanching	0	0	3/1

FEMALES [Total Incidence/No.of Animals]			
Dermal Lesions From Day 1 - Day 22	Dose [mg/kg/day]		
	15	148	443
Erythema - Slight	71/5	24/5	28/5
Erythema - Moderate	11/1	76/5	18/5
Edema - Slight	17/1	63/5	9/5
Edema - Moderated	0	15/4	5/4
Desquamation - Slight	0	14/4	0/0
Desquamation - Moderate	0	47/5	0/0
Fissuring - Slight	0	0	63/5
Eschar - Partial	0	0	2/1
Eschar - Complete	0	5/1	24/5
Eschar - Exfoliation	0	0	42/5
Thickening of skin	0	2/1	46/5
Areas of eschar within patch site	0	4/1	5/3
Blanching	0	2/1	16/5

5. Body Weight

Mean body weight an body weight gain were comparable between the treated and control groups. However, the cumulative weight for the 443 mg/kg/day males [91 ± 201.5 g] was appreciably lower than controls [268 ± 117.1 g].

6. Food Consumption

Males at 443 mg/kg/day consumed significantly [p <0.01] less food [g/animal/day and g/kg/day] on several days during the first week of the study and also on other days when compared to controls. No other statistically significant differences in food consumption were noted..

7. Hematology, Clinical Chemistry, and Urinalysis

No biologically significant changes were seen in mean hematology values in treated groups when compared to appropriate corresponding control group values. The sporadic statistically significant differences seen in a few hematology data were not considered to be treatment-related due to the low magnitude of the change, presence of similar differences at the pretreatment intervals, and/or lack of dose response.

Clinical chemistry data exhibited elevated serum enzyme levels in both sexes of rabbits at the high dose [443 mg/kg/day] on day 22 as shown below:

MALES

Parameter	0 mg/kg/day	443 mg/kg/day
AST [IU/L]	30.4 ± 8.1	32.8 ± 10.8
ALT [IU/L]	50.8 ± 14.2	113.2 ± 60.1*
ALK.PHOSPHATASE [IU/L]	93.2 ± 19.5	157.4 ± 68.7*

FEMALES

Parameter	0 mg/kg/day	443 mg/kg/day
AST [IU/L]	28.0 ± 6.9	92.4 ± 75.7*
ALT [IU/L]	41.6 ± 7.5	293.0 ± 289.8*
ALK.PHOSPHATASE [IU/L]	97.2 ± 42.5	449.4 ± 193.1*

* significantly different from controls at p <0.01 or 0.05

Urinalyses revealed a possible increase in the occurrence of occult blood in both sexes at 148 and 443 mg/kg/day. Microscopic analyses revealed a high urine RBC content in only one animal at 443 mg/kg/day. No other apparent differences in urinalysis data were noted.

8. Gross Pathology

No treatment-related gross pathological changes were seen. Sporadic findings of low incidence included pitted and small kidneys, enlarged lymph nodes, mottled lungs, yellow-tan focus on the liver, reddened thyroid, and mass in the abdominal cavity.

9. Organ Weight

The mean absolute and relative liver weight values were increased in a dose-related manner with the difference reaching statistical significance [p <0.01] at 148 and 443 mg/kg/day. Other organ weight data were comparable between the treated and control groups.

10. Histopathology

Histopathology revealed no treatment-related microscopic changes in the treated skin of rabbits at 15 mg/kg/day. Treatment-related dermal lesions observed at 148 and 443 mg/kg/day levels consisted of an increased incidence and/or severity of acanthosis, hyperkeratosis and chronic dermatitis. Acute dermatitis, surface exudate, dermal hemorrhage and vesiculation of the epidermis were also seen in rabbits at the high dose. Skin lesions are summarized below:

No. of Animals with Lesions	Dose [mg/kg/day]							
	0		15		148		443	
Treated Skin	♂	♀	♂	♀	♂	♀	♂	♀
Acanthosis	2	0	0	1	5	4	4	5
Dermatitis, acute	0	0	0	0	0	0	1	0
Dermatitis, chronic	2	1	1	1	1	4	1	4
Exudate, epidermal surface	0	0	0	0	0	0	1	2
Hemorrhage	0	0	0	0	0	0	2	2
Hyperkeratosis	0	0	0	0	5	5	5	5
Necrosis, mild	0	0	0	1	0	0	0	0
Vesiculation, mild	0	0	0	0	0	0	0	1

910

Treatment-related liver lesions limited to the high dose were characterized by hepatocyte hypertrophy [5 ♂ and 5 ♀] and the presence of homogenous, faintly eosinophilic hyaline droplets within hepatocyte cytoplasm [2 ♂ and 5 ♀]. No treatment-related liver lesions were seen at 15 or 148 mg/kg/day. Other microscopic changes were typical of common spontaneous lesions in rabbits of this age.

IV. DISCUSSION

Male and female rabbits received repeated dermal applications of the Diethanolamine Salt of 2,4-Dichlorophenoxyacetic acid at doses of 0, 15, 148, or 443 mg/kg, 6 hours/day, 7 days/week for 21 days. Treatment had no adverse effect on survival, clinical signs, mean body weight, body weight gain, hematology, urinalysis or gross pathology. Food consumption at the high dose was decreased on several days during the first week of the study and was lower than controls on all other days. Signs of dermal irritation were observed in a dose-related manner with the severity ranging from mild at the low dose [15 mg/kg/day] to severe at the high dose. Although minor dermal irritation was seen at the low dose,histopathology revealed no treatment-related lesions at this dose. At 148 and 443 mg/kg/day, 2,4-D DEA induced dermal lesions included increased incidence and/or severity of acanthosis, hyperkeratosis and chronic dermatitis. In addition, acute dermatitis, surface exudate, dermal hemorrhage and vesiculation of the epidermis were also seen at the high dose. 2,4-D DEA was also shown to be a hepatotoxin at a dose of 433 mg/kg/day, inducing liver lesions consisting of hypertrophy of hepatocytes and the presence of hyaline droplets within hepatocytes. These hepatic lesions correlated with the elevated serum enzyme levels [AST, ALT, and alkaline phosphatase] and increased absolute and relative liver weights in animals at the high dose. No liver lesions were seen in at 15 or 148 mg/kg/day groups.

V. CONCLUSION

Based on the results of this study, the following NOEL's and LOEL's are established:

End Point	NOEL mg/kg/day	LOEL mg/kg/day	Basis for LOEL
Dermal toxicity	15	148	Microscopic dermal lesions
Systemic toxicity	148	443 [HDT]	Liver toxicity

VI. CORE CLASSIFICATION

Guideline; this study satisfies the requirements for a 21-day dermal toxicity study (82-3) in rabbits.

891

EPA Reviewer: Linda L. Taylor, Ph.D. **Signature:** [signature]
Reregistration Branch I, Health Effects Division (7509C) **Date** 7-29-03
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** [signature]
Reregistration Branch I, Health Effects Division (7509C) **Date** 7/29/03

Template version 11/01

TXR#: 0051340

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [capsule]-dog; OPPTS 870.3150 [§82-1b] ; OECD 409.

PC CODE: 030066 **DP BARCODE:** none **SUBMISSION NO.:** none

TEST MATERIAL (PURITY): 2,4-D Isopropyl Ester (97.1%)

SYNONYMS: isopropyl (2,4-dichlorophenoxy) acetate; 2,4-dichlorophenoxyaceticacid, isopropyl ester

CITATION: Tompkins, E. C. (1995) A 90-Day Oral (Capsule) Toxicity Study of 2,4-D-Isopropyl Ester in dogs. WIL Research Laboratories, Inc., Ashland, OH. Laboratory Study No. WIL-233001, January 9, 1995. MRID 43515501. Unpublished

SPONSOR: California Citrus Quality Control/John Wise & Associates, Ltd. [representative]

EXECUTIVE SUMMARY: In a 90-day oral (capsule) toxicity study (MRID 43515501), 2,4-D isopropyl ester (97.1% a.i., lot # 918009)] was administered to 5 outbred beagle dogs/sex/dose *via* capsule [after feeding period] at dose levels of 0, 0.3, 1.0, 3.0, and 10.0 mg/kg bw/day [2,4-D acid equivalent].

All dogs survived until study termination. Body weights and body-weight gains were comparable among the females. Males at the highest dose level displayed a gradual decrease in body weight over the course of the study, showing a 15% deficit at study termination. Males at the next higher dose also displayed a deficit [19%] in body weight at study termination. Only the highest dose males displayed decreased body-weight gains throughout the study [weeks 0-13, 60%]. Food consumption was not affected.

There was an increase in activated partial thromboplastin time at the high-dose level [both sexes; 10 mg/kg/day] at both the 3- and 12-week time points. Elevations in blood urea nitrogen [3 and 10 mg/kg/day; both sexes], creatinine [3 and 10 mg/kg/day; both sexes], aspartate aminotransferase [males; 10 mg/kg/day] and alanine aminotransferase [10 mg/kg/day; both sexes] were observed at week 3 and/or week 12; however, no corroborative histopathological changes were observed in the liver or kidneys. Similar alterations were observed in the other dog subchronic oral toxicity studies on 2,4-D and/or DMA and EHE.

892

Decreased absolute and relative testes and ovarian weights and decreased absolute adrenal (male) weights were observed at the highest dose level and, although statistical significance was not always attained [n=5], these findings are considered treatment-related.

The LOAEL is 3 mg/kg/day, based on increased blood urea nitrogen, creatinine, aspartate aminotransferase, and alanine aminotransferase values. The NOAEL is 1 mg/kg/day.

This 90-day oral toxicity study in the dog is acceptable/guideline and it satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in nonrodents.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: 2,4-D isopropyl ester
Description: combination amber liquid and off-white solid
Lot/Batch #: 918009
Purity: 97.1% a.i. [98.2%±0.33% analysis report]
Compound Stability: > 12 months
CAS # of TGAI: 94-11-1

2. Vehicle and/or positive control: gelatin capsules

3. Test animals: nonrodent
Species: dog
Strain: outbred beagle
Age/weight at study initiation: 7 months old; males 6.0-10.6 kg; females 6.3-8.6 kg
Source: Ridglan Farms, Incorporated, Mount Horeb, Wisconsin
Housing: individually in stainless steel cages suspended
Diet: ≈400 grams of basal ration [Purina® Certified Canine Chow® #5007
Water: municipal water *ad libitum*
Environmental conditions:
Temperature: 72±4°F [actual 64.0-72.6 °F]
Humidity: 30-70% [actual 41.4%-72.6%]
Air changes: not specified
Photoperiod: 12 hrs dark/12 hrs light

B. STUDY DESIGN:

1. In life dates - Start: June 13, 1994 End: September 12, 1994 (wk 13); November 13, 1994 (histo)

2. Animal assignment: Animals were assigned randomly *via* a computer-generated program, based on body weight stratification in a block design, to the test groups noted in Table 1.

TABLE 1: Study design

Test Group [Dose]	# Male	# Female
0 mg/kg/day	5	5
0.3 mg/kg/day	5	5
1.0 mg/kg/day	5	5
3.0 mg/kg/day	5	5
10.0 mg/kg/day	5	5

894

3. Dose selection rationale: There was no discussion of dose-level selection, but the doses are the acid equivalent of doses used in the rat subchronic oral toxicity studies performed on 2,4-D.

4. Capsule preparation, analysis, and administration: During the dosing period, the test material was placed in an incubator [≈50°C to liquefy]. The appropriate amount of test material was placed into a size 11 gelatin capsule. A correction factor of 0.98 was used for dose calculations, based on the acid equivalent (1.19) corrected for purity (97.1%) and adjusted for the specific gravity (1.252). Capsules were prepared daily for each dog, and the control dogs received empty capsules. Individual dosages were adjusted weekly, based on the most recently-recorded body weight to provide the correct mg/kg/day dose. According to the report, the prepared capsules were administered to the test dogs at approximately the same time each day after the daily food offering, 7 days/week for 91 or 92 consecutive days.

Results - Homogeneity Analysis: Not applicable.

Stability Analysis: The preparations were used on the day of preparation and the neat material [stable for at least 12 months] used was found to be stable for 8 days [range 96.1%-107% of day 0 value].

Concentration Analysis: The test material was administered *via* capsule; individual capsules were prepared for each dog daily. No analyses were performed.

5. Statistics - Body weight, body-weight change, food consumption, clinical laboratory, and organ weight data were subjected to a one-way analysis of variance followed by Dunnett's Test. Clinical laboratory values for cell types that occur at a low incidence were not subjected to statistical analysis. The statistical tests were performed by a Digital® MicroVAX® 3400 computer with appropriate programming.

C. METHODS:

1. Observations:

1a. Observations: The dogs were observed twice daily [am and pm], for mortality and moribundity, and at the time of dosing and approximately 1-2 hours post dose.

1b. Clinical Examinations: Weekly detailed physical examinations were performed beginning one week prior to study initiation.

1c. Neurological Evaluations: There was no specific neurological evaluation performed.

2. Body weight: Body weights were obtained weekly, beginning one week prior to dosing. Body-weight changes were calculated for various time intervals, and terminal body weights were obtained on the day of necropsy.

3. Food consumption and compound intake: Food consumption for each dog was recorded daily, and weekly averages were reported for the corresponding body-weight intervals. Food intake was calculated as grams/animal/day. Food efficiency was not determined.

4. Ophthalmoscopic examination: Eyes were examined prior to the start of dosing and during week 13 using a hand-held slit lamp and an indirect ophthalmoscope, preceded by mydriasis.

5. Hematology & Clinical Chemistry: Clinical pathologic parameters were evaluated for all dogs once prior to the start of dosing [week -1] and during weeks 3 and 12. Blood was collected prior to the feeding period and test material administration, and samples were taken *via* the jugular vein for hematology and clinical chemistry assessment. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*	X	RBC morphology
X	(Activated Partial Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral nonrodent studies based on Guideline 870.3150

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Triiodothyronine [3]
X	Alanine aminotransferase (ALT/also SGPT)*		Thyroxine [T4]
X	Aspartate aminotransferase (AST/also SGOT)*		Thyroid stimulating hormone [TSH]
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral nonrodent studies based on Guideline 870.3150

896

6. Urinalysis

An overnight urine sample was collected from each dog [using a metabolism gage] once prior to the start of dosing and during weeks 3 and 12 prior to the feeding period and test material administration. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
X	Sediment (microscopic)	X	Nitrites
X	Protein*		Urobilinogen
		X	Color
		X	Leukocytes

* Recommended for 90-day oral nonrodent studies

7. Sacrifice and Pathology

At study termination, all dogs were anesthetized by an *i.v.* injection of sodium pentobarbital and euthanized by exsanguination. A complete necropsy, including examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities including contents, was performed on each dog. The CHECKED (X) tissues were collected for histological examination. The (XX) organs were weighed. Microscopic examinations were performed on all tissues listed below from all dogs [except the brain 1 low-dose female, 3 males at 3 mg/kg/day, and 1 male from the high-dose group, due to improper preparation of formalin solution].

	DIGESTIVE SYSTEM		**CARDIOVASC./HEMAT.**		**NEUROLOGIC**
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	X	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**		Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroid*+
X	Rectum*	X	Urinary bladder*	XX	Thyroid*+
XX	Liver*+	XX	Testes*+		**OTHER**
X	Gall bladder (not rat)*	X	Epididymides*	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*		Seminal vesicles	X	Skin*
	RESPIRATORY	XX	Ovaries*+ and oviducts	X	All gross lesions and masses*
X	Trachea*	X	Uterus*+		
X	Lung*	X	Mammary gland*		
	Nose*	X	Vagina		
	Pharynx*				
	Larynx*				

* Recommended for 90-day oral nonrodent studies based on Guideline 870.3150; Organ weights required for rodent studies.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity -Excreta-related findings [soft stool and mucoid feces] were observed in both sexes and all groups, with slightly higher incidences at the two highest dose levels.

Table . Clinical Findings [total occurrence/# of dogs

	0 mg/kg/day	0.3 mg/kg/day	1.0 mg/kg/day	3.0 mg/kg/day	10 mg/kg/day
MALES					
AT DOSING					
soft stool	0/0	12/2	6/2	7/2	13/3
yellow mucoid feces	1/1	1/1	3/3	0/0	13/3
mucoid feces	3/2	9/3	5/2	13/4	22/5
1 HR POST DOSE					
soft stool	0/0	0/0	2/1	0/0	3/2
yellow mucoid feces	0/0	0/0	2/1	1/1	4/2
mucoid feces	0/0	2/2	0/0	2/2	4/3
FEMALES					
AT DOSING					
soft stool	1/1	2/1	1/1	8/3	13/4
yellow mucoid feces	2/1	0/0	0/0	4/2	7/3
mucoid feces	7/2	2/2	9/4	23/4	21/4
1 HR POST DOSE					
soft stool	0/0	1/1	0/0	1/1	1/1
yellow mucoid feces	1/1	0/0	1/1	0/0	1/1
mucoid feces	0/0	0/0	1/1	5/1	4/2

Data from Table 3 and 4, pages 43-47 of the report

2. Mortality - All dogs survived until study termination.

B. BODY WEIGHT AND WEIGHT GAIN: MALES: At the high-dose level, a slight decrease [7%] in body weight was observed in males by the third week of treatment, and by study termination, the deficit had increased to 15%. Males at the high-dose level displayed decreased body-weight gains throughout the study, with a negative gain during weeks 2-3. During the initial week of treatment, males at all dose levels displayed a lower body-weight gain than control males, although there was no dose-response; i.e., the lowest dose level displayed a negative gain [-226 grams], whereas the gain [as a % of control] for the other dose groups was inversely related to dose [66%, 67%, and 88% with increasing dose]. The negative body-weight gain at the low-dose level was attributed to one male with swollen salivary glands and decreased food consumption during the initial week of dosing. **FEMALES**: Body weights were comparable among the female groups, although the high-dose females showed a slight decrease at study termination [4%]. Body-weight gains varied considerably among the female groups and within each group throughout the study. Prior to dosing [weeks -1-0], the control gained 22%-80% more weight than any of the other groups. During the initial week of dosing, the two lowest dose level groups displayed the lowest gain and the two highest dose groups displayed the highest gain in body weight. The high-dose females displayed a negative body-weight gain during weeks 8-9. The overall body-weight gain at the high-

dose level was 84% of control, but the 1 mg/kg/day dose group displayed the lowest overall body-weight gain [74% of control]. The body-weight changes in females did not show a consistent, dose-related effect and might not be treatment-related.

TABLE 2. Mean (±SD) Body Weight and Body-Weight Gain (g) a

Interval	Dose				
	0 mg/kg/day	0.3 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
MALES					
Week -1	7781±517	7436±713 [96]	7711±1315	7528±1132	7618±688 [98]
Week 0	8003±509	7715±693	8065±1584	7808±1152	7864±765
Week 1	8190±527	7489±1158 [91]	8190±1622	7934±1183	8029±810
Week 2	8349±509	7658±1137 [92]	8469±1660	8236±1195	8039±816
Week 3	8636±500	7908±1032 92]	8741±1780	8411±1264	8020±793 [93]
Week 4	8920±645	8217±1075	8989±1827	8700±1313	8246±859 [92]
Week 7	9469±634	8904±861	9484±1849	9091±1386	8572±803 [91]
Week 13	10339±633	10042±821 [97]	10190±1828	8411±1264 [81]	8791±648 [85]
Treatment:					
Weeks -1-0	222±56	279±190	354±325	280±53	246±269
Weeks 0-1	187±108	-226±577♪	124±60 [66]	126±206 [67]	165±157 [88]
Weeks 1-2	158±105	169±76	280±117	301±157	10±29 [6]
Weeks 2-3	287±25	249±157	272±163	176±139	-19±204*
Weeks 3-4	284±161	309±263	248±91	289±126	225±131 [79]
Weeks 4-5	162±137	142±236	238±95	157±232	17±131 [10]
Weeks 0-3	633±135	193±407 [30]	676±247	603±308	156±351 [25]
Weeks 0-5	1079±218146	643±396 [60]	1162±374	1049±506	398±353* [37]
Weeks 0-7	1466±293	1189±271	1418±321	1283±737	708±218* [48]
Weeks 0-13	2336±272	2327±515	2125±500	2220±759	927±188** [40]
FEMALES					
Week -1	7088±548	7124±563	7186±703	6931±473	7075±605
Week 0	7349±690	7176±589	7303±814	7135±646	7265±656
Week 1	7423±761	7218±612	7315±768	7318±724	7402±781
Week 2	7558±784	7374±571	7455±791	7498±732	7470±882
Week 3	7682±796	7603±598	7655±750	7712±833	7587±798
Week 4	7841±879	7769±538	7680±747	7815±879	7751±915
Week 7	8208±876	8071±551	7976±769	8203±927	8047±923
Week 13	8826±975	8749±488	8393±635	8680±1119	8502±1012 [96]
Treatment:					
Weeks -1-0	261±209	53±104	118±243	204±182	190±78
Weeks 0-1	74±159	41±63	12±71	183±123	137±173
Weeks 1-2	135±45	157±48	140±160	180±36	68±225 [50]
Weeks 2-3	125±73	228±69	200±97	214±119	118±171
Weeks 3-4	158±134	166±162	25±93 [16]	104±111 [66]	164±171
Weeks 8-9	35±60	43±114	43±107	15±106	-14±52
Weeks 0-3	334±258	426±125	351±227	557±233	322±203
Weeks 0-5	592±302	756±398	458±249	848±334	620±250
Weeks 0-7	859±413	894±262	672±424	1068±468	782±328
Weeks 0-13	1478±479	1572±479	1089±435 [74]	1545±694	1237±473 [84]

a Data obtained from Tables 5, 6, and 6A, pages 48-65 in the study report; * $p < 0.05$; ** $p < 0.01$; ♪ attributed to one male with swollen salivary glands/decreased food consumption during week 1; [% of control]

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption - There was no adverse effect of treatment on food consumption in either sex at any dose level. A slightly lower food consumption was observed for the low-dose male group during weeks 0-1 and 1-2, which was attributed to one male with swollen salivary glands that subsequently ruptured.

2. Compound consumption The test material was administered *via* capsule.

3. Food efficiency These data were not provided; however, there was no apparent effect on food consumption.

D. OPHTHALMOSCOPIC EXAMINATION: The treatment-related findings were reported.

E. BLOOD ANALYSES

1. Hematology - The only clear treatment-related parameter affected was an increase in activated partial thromboplastin time [APTT] at the high-dose level in both sexes at week 12 and in the high-dose males at week 3 also. Historical control data indicate that the values at the high-dose level for both sexes are slightly outside the laboratory ranges.

Table 5. Hematology Parameters

Parameter/Dose/Sex/Week	0 mg/kg/day	0.3 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
APTT (seconds) MALES					
week -1	9.9±0.53	9.7±0.34	10.7±0.65	10.9±1.57	10.2±0.54
week 3	10.6±0.36	10.7±0.32	11.6±0.54*	11.5±0.28	12.7±0.99**
week 12	10.1±0.54	10.0±0.37	10.6±0.80	11.0±0.54	12.8±0.86**
APTT (seconds) FEMALES					
week -1	10.3±0.72	10.0±0.49	10.1±0.59	10.4±0.91	10.2±0.62
week 3	11.1±1.04	10.6±0.60	10.6±0.95	11.0±0.80	12.1±0.99
week 12	10.7±0.78	10.3±0.65	10.4±0.52	11.3±0.74	12.7±0.90**
historical control males	mean 10.4±1.08 [range 8.24-12.56]				
historical control females	mean 10.5±0.90 [range 8.70-12.30]				

APTT activated partial thromboplastin time; [% of control]; * $p<0.05$; ** $p<0.01$; N=5; data from Table 8, pages 72 -9386 of the report

2. Clinical Chemistry - Increased creatinine levels were observed at the 100 and 300 mg/kg/day dose levels in both sexes at weeks 3 and 12, and the values were at the high end of the historical control range. Also at the two highest dose levels, blood urea nitrogen was elevated in both sexes [dose-response] and the values were above the historical control range at both the 3- and 12-week intervals. Both sexes at the high-dose level displayed increased ALT at both time points, and the values were above the historical control range.

90C

Table 6. Clinical Chemistry Parameters

Parameter/Dose/Sex/Week	0 mg/kg/day	0.3 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
creatinine [mg/dL]					
MALES					
-1	0.4±0.00	0.4±0.04	0.4±0.07	0.4±0.13	0.4±0.09
3	0.4±0.08	0.4±0.11	0.5±0.05	0.7±0.15*	0.8±0.13**
12	0.4±0.08	0.5±0.07	0.6±0.04	0.8±0.19**	0.9±0.17**
FEMALES					
-1	0.4±0.08	0.4±0.08	0.4±0.07	0.5±0.08	0.5±0.05
3	0.4±0.05	0.4±0.11	0.5±0.07	0.7±0.23**	0.8±0.04**
12	0.5±0.07	0.±0.07	0.6±0.15	0.9±0.11**	0.9±0.08**
blood urea nitrogen [mg/dL]					
MALES					
-1	13.9±2.37	15.2±2.61	14.5±1.93	16.4±1.88	17.2±3.96
3	12.3±1.99	13.3±1.07	13.4±0.49	19.4±1.94**	26.7±3.17**
12	14.0±1.32	15.6±1.10	15.3±1.51	21.7±1.64**	29.9±3.98**
FEMALES					
-1	13.8±2.27	16.7±4.10	13.3±2.15	17.0±2.04	13.9±2.71
3	13.8±0.58	15.2±1.93	15.5±2.50	19.9±3.36**	24.0±1.73**
12	13.1±1.52	16.2±2.63	15.8±1.42	22.2±2.33**	25.7±2.62**
AST [U/L]					
MALES					
-1	21±3	25±3	25±4	24±3	23±3
3	31±8	30±3	29±4	30±4	37±7
12	30±4	35±3	31±2	33±3	40±4**
FEMALES					
-1	24±4	26±3	23±3	25±2	26±3
3	28±1.1	30±4	29±4	37±5	36±10
12	31±2.6	33±3	32±5	35±4	36±7
ALT [U/L]					
MALES					
-1	28±4	39±26	26±7	26±7	30±3
3	31±4	41±18	29±8	48±24	99±31**
12	34±6	37±4	33±9	60±30	102±41**
FEMALES					
-1	35±11	30±5	31±8	35±8	26±5
3	35±8	31±7	31±4	45±14	118±48**
12	38±8	30±7	35±6	54±16	109±51**
cholesterol [MG/dL]					
MALES					
-1	189±27	182±25	178±9	199±28	184±34
3	159±15	172±6	160±19	193±18	187±35
12	159±19	159±13	167±10	204±14**	182±27
FEMALES					
-1	161±15	162±23	168±33	170±18	198±55
3	149±19	141±19	153±25	177±18	217±59*
12	138±11	149±21	169±37	204±18**	200±30**

Table 6. Clinical Chemistry Parameters

Parameter/Dose/Sex/Week	0 mg/kg/day	0.3 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
alkaline phosphatase [U/L]					
MALES					
-1	114±12	135±25	129±30	126±30	110±25
3	111±23	121±25	112±30	106±32	74±11
12	92±17	103±17	89±22	76±20	75±18
FEMALES					
-1	115±17	109±12	100±23	114±27	114±16
3	111±11	89±7	89±21	92±21	89±28
12	91±8	83±13	73±15	65±7**	67±13*

[% of control]; * p<0.05; p<0.01; N=10; data from Table 11 and 12, pages 87-96 of the report

Historical Control Data

parameter	mean ±s.d. [range]
blood urea nitrogen	
male	12±2.4 [7.2-16.8]
female	12±2.4 [7.2-16.8
cholesterol	
male	177±34.2 [108.6-245.4]
female	157±30.3 [96.4-217.6]
ALT	
male	35±7.7 [19.6-50.4]
female	37±11.3 [14.4-59.6]
AST	
male	40±6.2 [27.6-52.4]
female	39±8.1 [22.8-55.2]
alkaline phosphatase	
male	101±22.3 [56.4-145.6]
female	97±18.7 [59.6-134.4]
creatinine	
male	0.7±0.08 [0.54-0.86]
female	0.7±0.06 [0.58-0.82]

Data from pages 549-556 of the report

F. Urinalysis - The urinalysis parameters were comparable among the groups in both sexes.

G. SACRIFICE AND PATHOLOGY

1. Organ weight - There were dose-related decreases in absolute testes/epididymides and adrenal weights in males, and the decreases at the high-dose level were statistically significant [Table 7]. There were no statistically-significant changes in organ weights in females, but absolute ovarian weights were decreased at the 3 [11%] and 10 [16%] mg/kg/day dose levels. High-dose females displayed a slight increase [7%] in absolute liver weight, and both sexes displayed a slight decrease in kidney weight [males 13%; females 8%] at the high-dose level.

Table 7. Organ Weights [grams]

Parameter/Dose/Sex/Week	0 mg/kg/day	0.3 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
LIVER					
MALES					
absolute	301.9±31.8	291.3±29.3	289.8±17.7	283.3±44.2	278.3±42.0
relative/body	2.967±0.377	2.947±0.126	2.905±0.322	2.800±0.180	3.181±0.423
FEMALES					
absolute	258.4±19.0	260.6±34.9	249.7±28.3	249.4±21.6	275.3±17.5
relative/body	2.963±0.120	3.000±0.409	2.993±0.327	2.883±0.283	3.264±0.202 [110]
KIDNEY					
MALES					
absolute	50.28±3.41	47.17±6.67	51.08±6.07	45.38±3.22 [90]	43.55±2.71 [87]
relative/body	0.493±0.034	0.476±0.044	0.510±0.059	0.455±0.070	0.499±0.027
FEMALES					
absolute	42.99±5.74	41.30±3.01	42.11±5.04	40.11±5.53 [93]	39.74±4.58 [92]
relative/body	0.491±0.030	0.476±0.040	0.503±0.027	0.461±0.036	0.471±0.055
THYROID					
MALES					
absolute	1.157±0.347	1.049±0.212	1.535±1.102	1.117±0.337	1.168±0.175
relative/body	0.011±0.003	0.011±0.002	0.014±0.007	0.011±0.004	0.013±0.002
FEMALES					
absolute	1.060±0.285	1.042±0.278	0.978±0.096	1.002±0.164	0.919±0.147
relative/body	0.012±0.003	0.012±0.003	0.012±0.001	0.012±0.002	0.011±0.002
TESTES					
MALES					
absolute	19.39±3.94	17.96±1.87	17.95±3.87	17.12±1.57 [88]	11.40±1.91** [59]
relative/body	0.190±0.037	0.182±0.012	0.179±0.040 [94]	0.171±0.016 [90]	0.130±0.019** [68]
OVARIES					
FEMALES					
absolute	0.837±0.359	1.102±0.676	0.838±0.108	0.743±0.157 [89]	0.700±0.136 [84]
relative/body	0.010±0.004	0.013±0.008	0.010±0.002	0.009±0.003	0.008±0.001 [80]
ADRENAL					
MALES					
absolute	1.197±0.250	0.997±0.206 [83]	0.952±0.146 [80]	0.890±0.154 [74]	0.869±0.126* [73]
relative/body	0.012±0.002	0.010±0.002	0.010±0.001	0.009±0.002	0.010±0.002
FEMALES					
absolute	0.858±0.192	0.986±0.178	0.985±0.251	0.920±0.107	0.916±0.047
relative/body	0.010±0.002	0.011±0.003	0.012±0.001	0.012±0.002	0.011±0.002
TERMINAL BODY WEIGHT					
MALES n=5	10219±728	9885±868	10125±1788	10145±1649	8752±655 [86]
FEMALES n=5	8750±971	8701±448	8359±616	8715±1142	8478±959 [97]

[% of control]; * $p<0.05$; ** $p<0.01$; data from Tables 17 & 18, pages 122-129 of the report

2. **Gross pathology** - No treatment-related lesions were reported.

3. **Microscopic pathology** - No treatment-related lesions were reported.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Clinical signs suggestive of a treatment-relatedeffect included soft stool and mucoid feces, which were observed in both sexes at 3 mg/kg/day and 10 mg/kg/day. Throughout the study, males at the high-dose level displayed mean body-weight changes that were either slightly lower than or similar to the control group values. Overall, the high-dose males gained 0.9 kg compared to the 2.3 kg gained by the control males, although food consumption was comparable among the groups. At study termination, body weights of the high-dose males was 15% lower than the control. Body weights and body-weight changes were comparable among the female groups.

Mean activated partial thromboplastin times [APTT] values were slightly above the concurrent control value in both sexes at the high-dose level, and changes in serum chemistry parameters [increased urea nitrogen, creatinine, alanine aminotransferase, cholesterol, bilirubin, and aspartate aminotransferase(males) , and decreases in sodium] suggestive of renal and/or hepatic damage were observed at the two highest dose levels at the week 3 and/or week 12 evaluations. The differences observed were not large, and no macroscopic or microscopic lesions were noted in the kidney or liver to account for these changes. Urinalysis parameters were not altered at any dose level, and there were no oculopathic lesion indicative of a toxic effect.

Mean testicular/epididymal weights were significantly below the control group mean on both an absolute (41%) and a percent of body weight (32%) basis. No treatment-related lesion was observed microscopically in either organ, and there was no evidence of seminiferous tubule degeneration to account for the individual decreases in absolute and relative organ weights at necropsy. No treatment-related lesions were observed at any dose level at the macroscopic or microscopic examination of tissues.

The NOEL was considered to be 1 mg/kg/day.

B. REVIEWER COMMENTS: All dogs survived until study termination. Body weights were unaffected in the females. Males at the highest dose level displayed a gradual decrease in body weight over the course of the study, showing a 15% deficit at study termination. Males at the next higher dose also displayed a deficit [19%] in body weight at study termination. Only the high-dose males displayed decreased body-weight gains throughout the study [weeks 0-13, 60%]. Food consumption was not affected.

There was an increase in activated partial thromboplastin time at the highest dose level [both sexes] at both the 3- and 12-week time points. Elevations in blood urea nitrogen [3 and 10 mg/kg/day; both sexes], creatinine [3 and 10 mg/kg/day; both sexes], aspartate aminotransferase [males; 10 mg/kg/day] and alanine aminotransferase [both sexes; 10 mg/kg/day] were observed at week 3 and/or week 12; however, no corroborative histopathological changes were observed in the liver or kidneys. Similar alterations were also observed in the other dog subchronic oral toxicity studies on 2,4-D and/or DMA and EHE.

Decreased absolute and relative testes and ovarian weights and decreased absolute adrenal (male) weights were observed at the high-dose level and, although statistical significance was not always attained [n=5], these findings are considered treatment-related.

The NOAEL is 1 mg/kg/day, and the LOAEL is 3 mg/kg/day, based on increased blood urea nitrogen, creatinine, aspartate aminotransferase, and alanine aminotransferase values.

C. STUDY DEFICIENCIES: None considered to impact on study interpretation.

DATA FOR ENTRY INTO ISIS

Subchronic (90 day) Oral Study - rodents (870.3100)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
030066	43515501	subchronic	dog	90 days	oral	capsule	0.3-10	0.3, 1, 3, 10	1 mg/kg/day	3 mg/kg/day	testes, ovaries,	

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 927 of 959

EPA Reviewer: Linda L. Taylor, Ph.D. **Signature:** Linda L. Taylor
Reregistration Branch I, Health Effects Division (7509C) **Date** 5-28-03
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** Whang Phang
Reregistration Branch I, Health Effects Division (7509C) **Date** 5/28/03

Template version 11/01

TXR#: 0051360

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [feeding]-rat; OPPTS 870.3100 [§82-1a] ; OECD 408.

PC CODE: 030066 **DP BARCODE:** none **SUBMISSION NO.:** none

TEST MATERIAL (PURITY): 2,4-D Isopropyl Ester (97.1%)

SYNONYMS: isopropyl (2,4-dichlorophenoxy) acetate; 2,4-dichlorophenoxyacetic acid, isopropyl ester

CITATION: Tompkins, E. C. (1995) A 90-Day Dietary Subchronic Toxicity Study of 2,4-D-Isopropyl Ester in Rats. WIL Research Laboratories, Inc., Ashland, OH. Laboratory Study No. WIL-233002, January 9, 1995. MRID 43515901. Unpublished

SPONSOR: California Citrus Quality Control/John Wise & Associates, Ltd. [representative]

EXECUTIVE SUMMARY: In a 90-day oral toxicity study (MRID 43515901), 2,4-D isopropyl ester (97.1% a.i., lot # 918009)] was administered to 10 Fischer 344 rats/sex/dose *via* the diet at dose levels of 0, 1, 15, 100, and 300 mg/kg bw/day [2,4-D acid equivalent].

There was one death [high-dose male; week 12], and clinical signs of toxicity included pale eyes, decreased defecation, and yellow and/or brown matting on the urogenital/anogenital area of females at 100 mg/kg/day and both sexes at 300 mg/kg/day. The body weight of both sexes at the highest dose level decreased significantly and progressively as treatment continued. Within the first week, females at the high-dose level displayed decreased body weight [91% of control] compared to the control and no body-weight gain. At study termination, the high-dose females showed no gain in body-weight from the start of the study; the high-dose males showed a 43% deficit in body weight and a 64% deficit in body-weight gain compared to the control. Throughout the study, both sexes at the high dose consumed less food than the controls.

Retinal degeneration was observed at the two highest dose levels, and all high-dose rats had cataracts. These findings are consistent with the other subchronic oral toxicity studies on 2,4-D and its amine salts and esters. Other consistent findings among the 2,4-D chemicals include changes in hematology [decreased RBCs, hemoglobin, platelets, WBCs, leukocyte counts] and clinical chemistry [decreased glucose, total protein, and T4, and increased TSH, triglycerides, alkaline

phosphatase, cholesterol (males), AST, and BUN] parameters at the two highest dose levels.

Decreased absolute and relative spleen, thymus, testes, ovaries, and adrenal (female) weights were observed at the high-dose level, and increased absolute and relative thyroid weights were observed in both sexes at the high-dose level. Decreased absolute brain weight was observed in both sexes at the high-dose level and, although the relative brain weight was increased reflecting the large decrease in body weight, due to the neurotoxic concern for 2,4-D, this finding is considered treatment-related.

There was a treatment-related increase in cataracts/retinal degeneration [eyes] in both sexes at the two highest dose levels, which is consistent with findings in studies on 2,4-D and its amine salts and esters. Other consistent histopathologic findings include aspermia [epididymides], atrophy and aspermatogenesis [testes], thymus atrophy, hepatocellular hypertrophy, and hypocellular bone marrow [femur].

The LOAEL is 100 mg/kg/day, based on a slight decrease in body weight/body-weight gain (females), an increased incidence of retinal degeneration, decreased T4 [both sexes], and increased TSH (females). The NOAEL is 15 mg/kg/day.

This 90-day oral toxicity study in the rat is acceptable/guideline and it satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rodents.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material**: 2,4-D isopropyl ester
   Description: combination amber liquid and off-white solid
   Lot/Batch #: 918009
   Purity: 97.1% a.i. [98.2%±0.33% analysis report]; a correction factor of 1.23 was used for 2,4-D acid equivalent calculation
   Compound Stability: > 12 months [MRID 43515901]
   CAS # of TGAI: 94-11-1

2. **Vehicle and/or positive control:** Purina ® Certified Rodent Chow® %5002 (meal form)

3. **Test animals**: rodent
   Species: rat
   Strain: Fischer 344
   Age/weight at study initiation: 6 weeks old; males 89-115 grams; females 80-95 grams
   Source: Charles River Breeding Laboratories, Inc. Kingston, NY
   Housing: individually in wire-mesh cages suspended above cage-board
   Diet: Purina ® Certified Rodent Chow® %5002 (meal form) *ad libitum*
   Water: municipal water ad libitum
   Environmental conditions:
   Temperature: 72±4° F [actual 71.3-74.4 ° F]
   Humidity: 30-70% [actual 32.7%-83.4%]
   Air changes: not specified
   Photoperiod: 12 hrs dark/12 hrs light

B. STUDY DESIGN:

1. **In life dates** - Start: February 17, 1994 End: May 20, 1994 (wk 13); August 26, 1994 (histo)

2. **Animal assignment**: Animals were assigned *via* a computer randomization procedure, based on body weight stratification in a block design, to the test groups noted in Table 1.

TABLE 1: Study design

Test Group [Dose]	Test Material Intake	# Male	# Female
0 mg/kg/day	0 mg/kg/day	10	10
1 mg/kg/day	1 mg/kg/day	10	10
15 mg/kg/day	15/16 mg/kg/day	10	10
100 mg/kg/day	102/102 mg/kg/day	10	10
300 mg/kg/day	300/291 mg/kg/day	10	10

3. Dose selection rationale: There was no discussion of dose-level selection, but the doses are the acid equivalent of doses used in the rat subchronic oral toxicity studies performed on 2,4-D. They were calculated using a conversion factor of 1.23, according to the report.

4. Diet preparation and analysis: Prior to preparation, the test material was placed in an incubator [≈50°C for 2 hours]. The appropriate amount of test material was weighed for each group, and acetone was used to dissolve the test material. The dissolved test material was added to a portion of rodent feed and mixed together. This premix was then added to a predetermined amount of feed and mixed for 15 minutes to achieve a total batch of homogeneous diet of the appropriate concentration per test group. The test diets were prepared weekly and stored at room temperature. A separate batch of diets was prepared for each sex for each dose concentration. Prior to the start of the study, samples were collected from the top, middle, and bottom of each diet preparation for the determination of homogeneity. Additionally, samples were taken from the middle of each diet preparation for stability analysis. Samples from the middle of each control and test diet were collected on the day of preparation for each week and stored frozen. Additional samples of each control and test diet, collected on day of preparation for study weeks 0, 1, 3, 7, and 12 were analyzed for concentration.

Results - Homogeneity Analysis: The diet preparations were found to be homogeneous, with the mean group concentration percent of target ranging from 92.4% to 97.5%

Stability Analysis: The preparations were found to be stable for 8 days [range 96.1%-107% of day 0 value].

Concentration Analysis: The results were consistent between the sexes, and the desired concentrations were attained [mean percent of the target ranged from 94.1% to 107%].

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics - Body weight, body-weight change, food consumption, clinical laboratory, and organ weight data were subjected to a one-way analysis of variance followed by Dunnett's Test. Clinical laboratory values for cell types that occur at a low incidence were not subjected to statistical analysis. The statistical tests were performed by a Digital® MicroVAX® 3400 computer with appropriate programming.

C. METHODS:

1. Observations:

1a. Cageside Observations: The rats were observed twice daily, for mortality and moribundity, and during the dosing period were observed daily, and significant findings were recorded.

1b. Clinical Examinations: Weekly detailed physical examinations were performed beginning one week prior to study initiation.

1c. Neurological Evaluations: There was no specific neurological evaluation performed.

2. Body weight: Body weights were obtained weekly, beginning one week prior to dosing. Body-weight changes were calculated for various time intervals, and terminal body weights were obtained on the day of necropsy.

3. Food consumption and compound intake: Food consumption for each rat was determined on a weekly basis, starting one week prior to dosing. Food intake was calculated and presented in the report as grams/animal/day for the corresponding body-weight intervals and not as mean daily diet consumption as g food/kg body weight/day. Food efficiency was not determined. Compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. Ophthalmoscopic examination: Eyes were examined prior to the start of dosing and during week 13 using a hand-held slit lamp and an indirect ophthalmoscope, preceded by mydriasis.

5. Hematology & Clinical Chemistry: Blood was collected from all surviving rats *via* the vena cava at necropsy [fasted overnight] for hematology and clinical chemistry assessment. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	RBC morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Triiodothyronine {3]
X	Alanine aminotransferase (ALT/also SGPT)*	X	Thyroxine [T4]
X	Aspartate aminotransferase (AST/also SGOT)*	X	Thyroid stimulating hormone [TSH]
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. Urinalysis[1]

An overnight urine sample was collected from each rat [fasted] during the week prior to necropsy [week 12] using a metabolism gage. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
X	Sediment (microscopic)	X	Nitrites
X	Protein*		Urobilinogen
		X	Color
		X	Leukocytes

* Recommended for 90-day oral rodent studies

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were weighed. Microscopic examinations were performed on all tissues listed below from the rat found dead and from all rats in the control and high-dose groups. The liver, lungs, kidneys, eyes, optic nerve, and thyroids with parathyroids were examined from all rats in the low- and two mid-dose groups. The stomach, testes, ovaries, spleen, and thymus were examined for all rats in the 100 mg/kg/day group.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	X	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroid*+
X	Rectum*	X	Urinary bladder*	XX	Thyroid*+
XX	Liver*+	XX	Testes*+		OTHER
	Gall bladder (not rat)*	X	Epididymides*	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin*
	RESPIRATORY	XX	Ovaries*+ and oviducts	X	All gross lesions and masses*
X	Trachea*	X	Uterus*+		
X	Lung*	X	Mammary gland*		
	Nose*	X	Vagina		
	Pharynx*				
	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity -At the high-dose level [both sexes], there was an increase in the incidence of pale eyes, and decreased defecation was noted. There was an increase in the occurrence of yellow matting on the urogenital area in females at 100 mg/kg/day and in both sexes at 300 mg/kg/day. At 300 mg/kg/day, the high-dose males displayed a brown matting on the anogenital area. There was a single occurrence of cold extremities and an increased incidence of bilateral ocular opacity in the high-dose females.

2. Mortality - There was one death [high-dose male during week 12]. The cause of death was not determined, although it was considered treatment-related, based on the large reduction in body weight.

B. BODY WEIGHT AND WEIGHT GAIN: At the high-dose level, decreased body weight was observed in males [82% of control] by week 4 and in females by week 3 [80% of control], and the magnitude of the deficits in both sexes increased over time [Table 2]. At week 13, mean body weight of the high-dose males was 58% of control, and mean body weight of the high-dose females was 52% of control. Females at the 100 mg/kg/day dose level displayed a slight decrease in body weight at week 13 [93% of control]. Decreased body-weight gains were observed in females at 100 mg/kg/day initially [week 0-1; 10% of control], and this group displayed a non-statistically-

significant decrease throughout the study [86%-88% of control]. Both sexes at the high dose displayed decreased body-weight gains during the first week of dosing [males 79% of control; females no gain] and throughout the study [weeks 1-13: males 29% of control; females no gain]. During the last half of the study [weeks 7-13], both sexes at the high-dose level displayed a negative body-weight gain.

TABLE 2. Mean (±SD) Body Weight and Body-Weight Gain (g) a

Interval	Dose				
	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
MALES					
Body Weight [grams]					
Week -1	75±7.3	74±4.6	73±6.5	74±6.2	75±5.0
Week 0	104±7.7	103±6.8	103±7.4	103±7.3	103±7.2
Week 1	127±10.0	129±10.2	128±9.4	126±10.6	122±8.4
Week 4	199±17.4	204±22.3	207±17.9	201±18.7	164±15.5** [82]
Week 7	243±20.6	243±23.4	248±19.0	241±20.3	177±20.1** [73]
Week 13	291±20.9	286±21.3	292±18.5	285±19.7	170±23.7** [58]
Body-Weight Gain [grams]					
Treatment:					
Weeks -1-0	29±1.9	30√3.1	30±2.1	29±1.8	29±3.2
Weeks 0-1	24±3.6	26√3.6	25±2.9	22±4.7	19±3.0* [79]
Weeks 1-2	35±4.5	33√5.2	36±4.6	34±5.5	25±3.0** [71]
Weeks 2-3	8.0±24.4	22√8.0	20±17.8	20±19.6	6±19.0 [75]
Weeks 0-7√	139	140	145	138	74 [53]
Weeks 7-13√	48	43	44	44	-7
Weeks 0-13√	187	183	189	182	67 [36]
Weeks 1-13√	164	157	164	159	48 [29]
FEMALES					
Body Weight [grams]					
Week -1	67±3.7	67±3.0	68±3.7	67±4.6	66±3.8
Week 0	88±3.8	88±3.4	88±3.8	88±3.6	88±3.4
Week 1	97±6.3	95±7.6	97±9.7	89±8.6	88±5.5* [91]
Week 4	134±6.3	133±5.0	132|9.9	128|5.2	95|7.8** [71]
Week 7	152±7.4	148±5.0	146|10.6	143|4.4	96|9.4** [63]
Week 13	169±7.9	167±3.6	164±11.6	158±4.0* [93]	88±8.6** [52]
Body-Weight Gain [grams]					
Treatment:					
Weeks -1-0	20±2.5	21±1.6	21±1.1	21±2.1	21±2.6
Weeks 0-1	10±5.4	7±5.8	9±7.6	1±9.3* [10]	0±5.5*
Weeks 1-2	16±1.9	17±2.9	16±3.1	18±5.2	3±3.3** [19]
Weeks 2-3	11±3.2	13±1.8	12±2.2	10±2.4	2±2.3** [18]
Weeks 0-7√	64	60	58	55 [86]	8 [13]
Weeks 7-13√	17	19	18	15 [88]	-8
Weeks 0-13√	81	79	76	70 [86]	0
Weeks 1-13√	72	72	67	69	0

a Data obtained from Tables 4-5, pages 55-66 of study report; * $p < 0.05$; ** $p < 0.01$; [% of control]; √ calculated by reviewer

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption - Prior to treatment, all groups had comparable food consumption values [Table 3]. Both sexes at the high-dose level consumed less food than the control throughout the treatment period, with the magnitude of the deficit remaining about the same [males 10-12 grams/rat/day *vs* 13-16 grams/rat/day (control); females 6-7 grams/rat/day *vs* 10-13 grams/rat/day (control)].

TABLE 3. Mean (±SD) Food Consumption (g/rat/day) a

Interval	Dose				
	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
MALES					
Weeks -1-0	12±0.6	12±0.5	13±0.5	13±0.7*	13±0.5
Weeks 0-1	13±0.7	14±1.1	14±0.8	13±0.9	12±0.8*
Weeks 1-2	15±0.8	15±1.3	15±1.2	15±1.5	12±0.8**
Weeks 2-3	14±3.7	16±1.7	16±3.1	15±2.8	12±2.3
Weeks 6-7	15±1.2	15±1.4	16±3.6	15±1.4	10±2.0**
Weeks 12-13	16±1.4	16±1.1	16±0.9	15±0.9	10±1.7**
FEMALES					
Weeks -1-0	11±0.9	11±0.7	11±0.5	11±0.6	11±0.7
Weeks 0-1	10±1.6	10±1.8	10±1.8	9±2.2	7±0.8**
Weeks 1-2	11±0.7	11±0.8	11±0.9	11±0.7	7±0.8**
Weeks 2-3	12±0.8	12±0.7	12±1.2	11±0.6	7±0.6**
Weeks 6-7	11±0.7	11±0.5	11±1.0	11±0.7	6±1.1**
Weeks 12-13	13±1.3	12±0.7	12±1.0	12±0.8	7±0.7**

a Data obtained from Table 6, pages 67-72 in the study report; * $p < 0.05$; ** $p < 0.01$

2. Compound consumption The calculated, average, compound consumption values are listed in Table 1 and they are close to the targeted values.

3. Food efficiency These data were not provided in the report, but a calculation [by reviewer; Table 4] of the first two days of dosing shows the there was reduced food efficiency at the high-dose level for both sexes, and the females at 100 mg/kg/day displayed lower food efficiency during the initial week [weeks 0-1]. Based on the body-weight data, as the treatment progressed, body weight decrement became more pronounced, so one would expect the food efficiency value to continue to decrease.

Table 4. Food Efficiency [body-weight gain (grams) ÷ grams consumed]

treatment day	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
males					
0-1	2	1.79	1.79	1.69	1.58
1-2	2.3	2.2	2.4	2.27	2.08

Table 4. Food Efficiency [body-weight gain (grams) ÷ grams consumed]					
treatment day	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
females					
0-1	0.77	0.5	0.64	0.08	0
1-2	1.07	1.13	1.07	1.2	0.25

D. OPHTHALMOSCOPIC EXAMINATION: Retinal degeneration was observed in both sexes, with the increased incidences at the 100 and 300 mg/kg/day being considered treatment-related [Table 5]. All high-dose rats had cataracts. It was noted that the incidence of retinal degeneration might have been higher, but the cataracts blocked the view of the retina. These eye observations are consistent with the findings in other rat studies on 2,4-D and its amine salts and esters.

Table 5. Ophthalmoscopic Findings					
Observation/Dose	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
MALES n=	10	10	10	10	9
retinal degeneration	2	0	1	4	3
[unilateral]	0	0	0	0	3
retinal degeneration [bilateral]	0	0	1	3	6
cataract unilateral	0	0	0	0	5
cataract bilateral					
FEMALES n=	10	10	10	10	10
retinal degeneration	0	1	1	2	0
[unilateral]	0	0	0	0	2
retinal degeneration [bilateral]	0	0	0	0	0
cataract unilateral	0	0	0	0	10
cataract bilateral					

Data from Table 16, page 102.

E. BLOOD ANALYSES

1. Hematology - RBCs, hemoglobin, platelets, WBCs, leukocyte count, and MCHC were decreased in both sexes at the high-dose level at study termination [Table 6]. Decreased platelet counts were also observed in both sexes at 100 mg/kg/day [dose-related], although only the female value attained statistical significance.

Table 6. Hematology Parameters					
Parameter/Dose/Sex/Week	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
red blood cells [MI/UL]					
MALES	9.96±0.49	9.51±0.84	9.75±0.48	9.73±0.71	7.36±0.24** [74]
FEMALES	8.94±0.42	8.97±0.46	9.11±0.52	8.63±0.45	7.84±0.83** [88]
hemoglobin [g/dL]					
MALES	17.2±0.7	16.4±1.0	16.7±0.8	16.8±0.8	14.2±0.6** [83]
FEMALES	16.6±0.7	16.8±0.6	17.0±0.8	16.2±0.8	14.9±0.9** [90]

9\

Table 6. Hematology Parameters

Parameter/Dose/Sex/Week	0 mg/kg/day	1 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
hematocrit [%]					
MALES	53.2±2.7	51.8±2.8	52.0±2.1	52.9±2.8	50.9±2.6
FEMALES	51.0±2.2	50.2±3.0	51.1±3.2	52.6±2.1	52.0±2.1
platelets [TH/UL]					
MALES	663±65.5	711±130.9	713±79.0	566±181.0 [85]	495±54.1** [75]
FEMALES	754±58.1	763±67.0	786±47.6	553±45.2** [73]	466±116.0** [62]
white blood cells [TH/UL]					
MALES	10.9±1.5	9.6±2.1	10.5±1.7	10.3±1.7	5.5±1.0** [50]
FEMALES	7.8±0.9	8.4±1.1	7.2±1.5	8.0±1.0	4.4±2.1** [56]
leukocyte differential count (%)					
MALES					
segmental	17±7.2	24±10.4	14±5.3	16±3.7	22±4.7
lymphocyte	80±7.2	73±10.4	84±6.2	79±5.0	75±6.1
FEMALES					
segmental	13±3.5	16±5.5	16±4.1	12±6.2	29±15.9**
lymphocyte	84±4.7	80±6.3	82±3.9	86±6.4	70±16.6** [83]
leukocyte count *[TH/UL]*					
MALES					
segmental	1.8±0.8	2.1±0.6	1.5±0.7	1.7±0.4	1.2±0.3 [67]
lymphocyte	8.7±1.6	7.2±2.1	8.8±1.5	8.2±1.5	4.1±0.9** [47]
FEMALES					
segmented	1.0±0.3	1.4±0.5	1.1±0.4	1.0±0.6	1.3±1.1
lymphocyte	6.6±0.9	6.7±0.9	5.9±1.3	6.9±1.0	3.0±1.8** [45]
MCV					
MALES	53.4±0.5	54.7±3.8	53.3±0.8	54.5±1.8	69.2±1.7**
FEMALES	57.2±2.3	56.0±1.3	56.1±1.2	61.0±2.3*	66.9±6.5**
MCH [uug]					
MALES	17.3±0.4	17.3±0.9	17.2±0.3	17.4±0.6	19.3±0.3**
FEMALES	18.6±0.4	18.8±0.4	18.6±0.3	18.8±0.3	19.1±0.9
MCHC [g/dL]					
MALES	32.2±0.8	31.7±1.1	32.2±0.7	31.8±0.6	27.9±0.4**
FEMALES	32.5±1.2	33.6±1.1	33.2±0.9	30.9±0.9*	28.7±1.8**

[% of control]; * $p<0.05$; N=8-10; data from Tables 8, , and 109, pages 79 - 86 of the report

2. <u>Clinical Chemistry</u> - There was a dose-related decrease in T4 in both sexes at study termination [Table 7], with significant decrements in females at 100 mg/kg/day [33% of control] and both sexes at 300 mg/kg/day [males 16%/females 17% of control]. Similarly, TSH was elevated in these same dose groups [females at 100 mg/kg/day (219% of control); high-dose males (234%)/females (319%) of control]. Other parameters altered include decreased glucose [both sexes at 300 mg/kg/day and females at 100 mg/kg/day], decreased total protein [both sexes at 300 mg/kg/day], increased alkaline phosphatase [both sexes at 300 mg/kg/day], and increased triglycerides [females at 100 and 300 mg/kg/day].

Table 7. Clinical Chemistry Parameters

Parameter/Dose/Sex/Week	0 mg/kg/day	1. mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
glucose [mg/dL]					
MALES	215±36	194±35	221±37	237±62	114±18** [53]
FEMALES	147±22	156±14	167±31	173±25* [118]	105±13** [71]
blood urea nitrogen [mg/dL]					
MALES	14.5±0.9	13.8±0.0	14.0±1.6	13.5±1.8	20.3±2.5
FEMALES	15.1±1.2	17.4±2.3	18.0±3.6* [119]	17.4±2.1	23.9±2.1** [158]
AST [U/L]					
MALES	131±30	120±36	128±32	134±33	170±46 [130]
FEMALES	101±20	101±28	121±60	146±48	205±53** [203]
ALT [U/L]					
MALES	110±36	95±42	111±45	113±33	78±15
FEMALES	59±18	59±16	87±62 [147]	119±50** [202]	90±32 [153]
total protein [G/dL]					
MALES	7.2±0.3	7.2±0.3	7.3±0.4	7.0±0.4	5.9±0.2** [82]
FEMALES	6.9±0.4	6.9±0.2	6.9±0.3	7.0±0.2	5.9±0.4** [86]
cholesterol [MG/dL]					
MALES	55±5	51±6	53±6	44±5** [80]	69±10** [109]
FEMALES	88±7	85±6	88±9	63±5** [72]	87±14
alkaline phosphatase [U/L]					
MALES	102±11	100±8	98±9	96±15	121±16** [119]
FEMALES	81±9	88±5	89±11	98±37	136±13** [168]
triglycerides [MG/dL]					
MALES	51±15	45±13	60±30	69±57 [135]	69±10 [135]
FEMALES	42±8	46±7	46±12	55±22** [157]	68±15** [162]
T3 [ng/dL]					
MALES	96.3±13.3	92.6±25.0	94.1±20.8	76.3±15.9	94.0±8.88
FEMALES	89.0±13.0	100.1±9.6	93.7±13.0	72.4±19.2	95.2±16.9
T4 [µg/dL]					
MALES	5.14±0.52	5.11±0.97	5.00±0.48	4.42±0.60	0.82±0.27** [16]
FEMALES	3.45±0.90	3.83±0.83	3.61±0.82	1.13±0.43** [33]	0.60±0.40** [17]
TSH [ng/mL]					
MALES	1.34±0.58	1.17±0.63	1.41±0.43	1.16±0.49	3.14±0.97** [234]
FEMALES	0.91±0.34	0.97±0.59	1.55±0.52	1.99±0.80** [219]	2.90±1.20** [319]

[% of control]; * p<0.05; p<0.01; N=10; data from Table 11 and 12, pages 87-96 of the report

F. Urinalysis - The urinalysis parameters were comparable among the groups in both sexes.

G. SACRIFICE AND PATHOLOGY

1. Organ weight - Given the magnitude of the decrement in terminal body weight at the high-dose level in both sexes, the decreases observed in several organ weights may be attributed to the decreased body weight [Table 8]. Decreases were observed in absolute brain weight, kidney weight,

spleen weight, thymus weight, and adrenal weight in both sexes, with corresponding increases in relative weight at the 300 mg/kg/day dose level [except female adrenal]. Decreased absolute and relative testes and ovarian weights were observed at the high-dose level also. Both sexes at the high-dose level displayed increased absolute and relative liver and thyroid weights, although the male liver weight did not attain statistical significance. Since decreased brain weight was observed in the rat oral toxicity study on 2,4-D at a comparable dose level and decreased body weight was not as severe as in the current study, plus the fact that there is a neurotoxicity concern for the dichlorophenoxyacetic acid compounds, the finding in the brain is considered treatment-related.

Table 8. Organ Weights [grams]

Parameter/Dose/Sex/Week	0 mg/kg/day	12 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
LIVER					
MALES					
absolute	8.34±0.91	7.94±0.61	8.34±1.10	7.97±1.04	8.72±1.27
relative/body	3.07±0.17	2.96±0.13	3.03±0.26	2.97±0.26	5.63±0.37** [183]
FEMALES					
absolute	4.68±0.34	4.75±0.27	4.76±0.60	4.71±0.51	5.33±0.26** [114]
relative/body	3.00±0.15	3.09±0.15	3.14±0.27	3.21±0.28	6.77±0.68** [226]
KIDNEY					
MALES					
absolute	2.08±0.21	2.04±0.16	2.17±0.19	2.28±0.21	1.38±0.17** [66]
relative/body	0.77±0.03	0.76±0.03	0.79±0.03	0.85±0.04	0.89±0.06
FEMALES					
absolute	1.24±0.07	1.26±0.05	1.25±0.10	1.28±0.09	0.85±0.06** [69]
relative/body	0.80±0.03	0.82±0.02	0.82±0.03	0.87±0.05** [110]	1.08±0.05** [135]
BRAIN					
MALES					
absolute	1.80±0.09	1.82±0.04	1.78±0.08	1.79±0.08	1.54±0.06** [86]
relative	0.66±0.04	0.68±0.05	0.65±0.04	0.67±0.03	1.011±0.14** [152]
FEMALES					
absolute	1.68±0.04	1.71±0.04	1.69±0.04	1.69±0.04	1.39±0.05** [83]
relative	1.08±0.03	1.11±0.03	1.12±0.09	1.15±0.05	1.76±0.12** [163]
THYROID					
MALES					
absolute	0.022±0.006	0.021±0.005	0.018±0.004	0.021±0.005	0.029±0.004* [133]
relative/body	0.008±0.002	0.008±0.002	0.007±0.002	0.008±0.002	0.019±0.003** [238]
FEMALES					
absolute	0.014±0.002	0.016±0.004	0.014±0.002	0.017±0.004	0.018±0.003** [135]
relative/body	0.009±0.001	0.011±0.003	0.010±0.001	0.011±0.003	0.024±0.004** [267]
SPLEEN					
MALES					
absolute	0.62±0.06	0.62±0.05	0.62±0.04	0.62±0.10	0.29±0.05** [47]
relative/body	0.23±0.01	0.23±0.01	0.23±0.01	0.23±0.03	0.19±0.02** [82]
FEMALES					
absolute	0.43±0.03	0.44±0.02	0.42±0.04	0.39±0.03* [91]	0.14±0.02** [33]
relative/body	0.27±0.01	0.29±0.01	0.28±0.02	0.27±0.02	0.18±0.02** [67]

Table 8. Organ Weights [grams]

Parameter/Dose/Sex/Week	0 mg/kg/day	12 mg/kg/day	15 mg/kg/day	100 mg/kg/day	300 mg/kg/day
THYMUS					
MALES					
absolute	0.218±0.043	0.220±0.017	0.218±0.018	0.200±0.017	0.051±0.017** [23]
relative/body	0.080±0.012	0.082±0.008	0.080±0.008	0.075±0.008	0.032±0.008** [40]
FEMALES					
absolute	0.188±0.021	0.194±0.025	0.176±0.045	0.174±0.021	0.028±0.013** [15]
relative/body	0.120±0.012	0.124±0.016	0.115±0.025	0.118±0.014	0.035±0.016** [29]
TESTES					
MALES					
absolute	2.97±12	3.00±0.08	3.05±0.12	2.95±0.10	0.91±0.13** [31]
relative/body	1.098±0.055	1.124±0.078	1.112±0.028	1.104±0.057	0.595±0.112** [54]
OVARIES					
FEMALES					
absolute	0.100±0.016	0.099±0.013	0.101±0.022	0.088±0.009	0.036±0.004** [36]
relative/body	0.064±0.010	0.064±0.008	0.066±0.012	0.060±0.007	0.045±0.005** [70]
ADRENAL					
MALES					
absolute	0.048±0.005	0.052±0.004	0.047±0.004	0.047±0.006	0.033±0.004** [69]
relative/body	0.018±0.002	0.019±0.002	0.017±0.002	0.018±0.003	0.022±0.002** [122]
FEMALES					
absolute	0.052±0.005	0.056±0.004	0.055±0.007	0.050±0.005	0.025±0.002** [48]
relative/body	0.034±0.004	0.036±0.003	0.036±0.004	0.034±0.004	0.032±0.003 [94]
TERMINAL BODY WEIGHT					
MALES n=9	272±19	268±20	274±16	268±18	155±23** [57]
FEMALES n=10	156±7	154±3	152±11	147±7* [94]	79±7** [51]

[% of control]; * p<0.05; ** p<0.01; data from Tables 19 & 20, pages 107-114 of the report

2. <u>Gross pathology</u> - In the high-dose males, there was an increased incidence of small seminal vesicles and soft and/or small testes. Four high-dose males and two high-dose females had a small thymus gland, and 9 high-dose females had small spleens. There was an increased incidence of white cortico-medullary junctions in the kidney of 3 of the 9 males and 8 of the 10 females at the high-dose level, and white areas were observed on the lungs of all rats at the high-dose level.

3. <u>Microscopic pathology</u> - In the high-dose male that died, microscopic findings included tubular mineralization of the kidney, aspermia [graded severe] in the epididymides, and atrophy of the testes, all of which were observed in the rats that survived [Table 9]. Cataracts were observed in 6 males and 9 females at 300 mg/kg/day. Retinal degeneration was found in one female at 100 mg/kg/day and 2 males and 8 females at 300 mg/kg/day. In the liver, bile duct hyperplasia and hepatocellular hypertrophy were observed in both sexes only at the 300 mg/kg/day dose level. Thymus atrophy was observed in 2. high-dose males and 4 high-dose females. Lesions in the epididymides [aspermia; graded severe], prostate [lymphocyte cell infiltrate], and testes [atrophy] occurred only at the high-dose level. Hypocellular bone marrow [consisting of depletion of erythroid-type cells] was observed in the femur of one male and 5 females at the high-dose level.

TABLE 9. Microscopic Findings [survivors]

Tissue/Lesion	Dose [mg/kg/day]				
	0	1	15	100	300
MALES					
femur					
marrow-hypocellular	0	NA	NA	NA	1
epididymides					
aspermia	0	0	NA	NA	9
eyes/optic nerve					
cataract	0	0	0	0	6
degeneration, retinal	0	0	0	1	2
kidneys					
mineralization, tubular	6	10	9	9	9 [1}♪
liver					
hyperplasia, bile duct	0	0	0	0	4
hypertrophy, hepatocellular	0	0	0	0	8
prostate					
infiltrate cell, lymphocyte	0	NA	NA	NA	3
inflammation, suppurative	0	NA	NA	NA	1
testes					
atrophy	0	NA	NA	0	9
aspermatogenesis	0	NA	NA	0	9
thymus					
atrophy	0	0	NA	0	2
FEMALES					
femur					
marrow-hypocellular	0	NA	NA	NA	5
eyes/optic nerve					
cataract	0	0	0	1	9
degeneration, retinal	0	0	0	1	8
kidneys					
mineralization, tubular	8	10	9	9	8
liver					
hyperplasia, bile duct	0	0	0	0	2
hypertrophy, hepatocellular	0	0	0	0	5
spleen					
depletion, lymphoid, germinal center "B" cells	0	NA	0	0	4
thymus					
atrophy	0	NA	NA	0	4

♪ also found in high-dose male that died; Data obtained from Table 22, pages 123-140 in the report

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Effects observed at 100 and 300 mg/kg/day included clinical signs, reduced body weight, hematologic and serum chemistry changes, retinal degeneration and cataract formation. Additional effects at 300 mg/kg/day were lethality, reduced food consumption, histopathologic alterations in the testes, liver, thymus and spleen and organ weight changes in the testes, ovaries, liver, thyroids, thymus, and spleen. In general, females were more affected than males. The NOAEL was considered to be 15 mg/kg/day.

B. REVIEWER COMMENTS: There was one death [high-dose male], and clinical signs of toxicity included pale eyes, decreased defecation, and yellow and/or brown matting on the urogenital/anogenital area of females at 100 mg/kg/day and both sexes at 300 mg/kg/day. Within the first week, females at the high-dose level displayed decreased body weight [91% of control] compared to the control and no body-weight gain. By the second week of dosing, the high-dose females displayed a 20% deficit in body weight. At study termination, the high-dose females showed no body-weight gain. Males at the high-dose level displayed a 17% deficit in body-weight gain during the first week of dosing and an 18% deficit in body weight by week 4. At study termination, the high-dose males showed a 43% deficit in body weight and a 64% deficit in body-weight gain compared to the control. Throughout the study, both sexes at the high dose consumed less food than the controls. During the first week of dosing, females at the 100 mg/kg/day dose level displayed a body-weight deficit of 90% [1 gram *vs* 10 grams for control], and the overall deficit in body-weight gain was 14%. With the exception of the first week [90% of control], food consumption was comparable to the control for this female group and terminal body weight was slightly lower than control [93% of control]. Retinal degeneration was observed at the two highest dose levels, and all high-dose rats had cataracts. These findings are consistent with the other subchronic oral toxicity studies on 2,4-D and its amine salts and esters. Other consistent findings among the 2,4-D chemicals include changes in hematology [decreased RBCs, hemoglobin, platelets, WBCs, leukocyte counts] and clinical chemistry [decreased glucose, total protein, and T4, and increased TSH, triglycerides, alkaline phosphatase, cholesterol (males), AST, and BUN] parameters at the two highest dose levels.

Decreased absolute and relative spleen, thymus, testes, ovaries, adrenal (female) weights were observed at the high-dose level, and increased absolute and relative thyroid weights were observed in both sexes at the high-dose level. Decreased absolute brain weight was observed in both sexes at the high-dose level and, although the relative brain weight was increased reflecting the large decrease in body weight, due to the neurotoxic concern for 2,4-D, this finding is considered treatment-related.

There was a treatment-related increase in cataracts/retinal degeneration [eyes] in both sexes at the two highest dose levels, which is consistent with findings in studies on 2,4-D and its amine salts and esters. Other consistent histopathologic findings include aspermia [epididymides], atrophy and aspermatogenesis [testes], thymus atrophy, hepatocellular hypertrophy, and hypocellular bone marrow [femur]. The NOAEL is 15 mg/kg/day, and the LOAEL is 100 mg/kg/day, based on a slight decrease in body weight/body-weight gain (females), an increased incidence of retinal degeneration,

decreased T4 [both sexes], and increased TSH (females).

C. STUDY DEFICIENCIES: None considered to impact on study interpretation.

DATA FOR ENTRY INTO ISIS

Subchronic (90 day) Oral Study - rodents (870.3100)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
030066	43515901	subchronic	rat	90 days	oral	dietary	1-300	1, 15, 100, 300	15 mg/kg/day	100 mg/kg/day	thyroid, liver, thymus, eyes, testes, ovaries	

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R157476 - Page 945 of 959

Reviewer: Linda L. Taylor, Ph.D. _____
Reregistration Branch II, Health Effects Division (7509C)
Secondary Reviewer: Whang Phang, Ph.D. _____ 5/27/03
Branch Senior Scientist, Reregistration Branch II, Health Effects Division(7509C)

TXR#: 0051360

DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity rats
OPPTS Number: 870.3700 OPP Guideline Number: §83-3 (b)
DP BARCODE: D SUBMISSION CODE: S
P.C. CODE: 030066
TEST MATERIAL (PURITY): 2,4-D isopropyl ester (97.12% a.i.)
CHEMICAL: isopropyl (2,4-dichlorophenoxy) acetate; 2,4-dichlorophenoxyacetic acid, isopropyl ester
SYNONYM: 2,4-D IPE
CITATION(s): Nemec, M. D., (1994) A Developmental Toxicity of 2,4-D-Isopropyl Ester in Rats. WIL Research Laboratories, Inc., Ashland, OH. Laboratory Study No. WIL-233004, January 11, 1995. MRID 43523101. Unpublished

Nemec, M. D., (1994) A Dose Range-Finding Developmental Toxicity of 2,4-D-Isopropyl Ester in Rats. WIL Research Laboratories, Inc., Ashland, OH. Laboratory Study No. WIL-233003, October 26, 1994. MRID 43523001. Unpublished

SPONSOR: California Citrus Quality Control/John Wise & Associates, Ltd. [representative]

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 43523101 +43523001], pregnant female Sprague-Dawley Crl:CD®BR rats [25/group] were administered the isopropyl ester of 2,4-dichlorophenoxyacetic acid [99.4%] *via* gavage at dose levels of 0 [100% corn oil], 10 mg/kg/day, 30 mg/kg/day, and 100 mg/kg/day [acid equivalents] from gestation day 6 through gestation day 15.

There were no abortions or premature deliveries. There was one death; a high-dose dam died on gestation day [GD] 17. Clinical signs [rocking, lurching, or swaying, decreased defecation, clear matting on the ventral abdominal and thoracic areas, red material on the urogenital area, and ptosis] were observed in this dam one to five days prior to death and in two other high-dose dams between GD 11 and GD 14. There was a slight decrease in body weight [93%-96% of control] from GD 7 through GD 20 at the high-dose level, and terminal body weights were 95% of control at this dose. There was a dose-related decrease in body-weight gain during the dosing period, with the mid- [81% of control] and high- [62% of control] dose deficits attaining statistical significance. Corrected body-weight gains were significantly lower at the high-dose level [83% of control] compared to the controls. During the dosing period, there was a decrease in food consumption at the mid- [91% of

control] and high- [77% of control] dose levels.

Pregnancy rate was comparable among the groups, although the high-dose dams [88%] displayed the lowest rate [control (100%), low (96%), and mid (100%)]. The numbers of corpora lutea/dam, implantation/dam, and live fetuses/dam were comparable among the groups. There were no dead fetuses. One high-dose dam totally resorbed a litter of 11[10 early resorptions; 1 late resorption]. Fetal body weights at the high-dose level were lower than the control [89% of control]. There was a significant decrease in the percent of female fetuses [40.8%] at the high-dose level compared to the concurrent control [49%] and historical control [43.3%-58.4%]. There was no increased incidence of external or visceral malformations or variations. There was a significant increase in the incidence of skeletal malformations [7th cervical ribs and 14th rudimentary ribs] at the high-dose level compared to the control.

The maternal toxicity NOAEL is 10 mg/kg/day, and the maternal toxicity LOAEL is 30 mg/kg/day, based on decreased body-weight gain and food consumption during the dosing period. At the highest dose tested [100 mg/kg/day], one death, clinical signs [rocking, lurching, or swaying, decreased defecation, clear matting on the ventral abdominal and thoracic areas, red material on the urogenital area, and ptosis], and one litter with 100% resorptions were observed.

The NOAEL for developmental toxicity is 30 mg/kg/day, and the developmental toxicity LOAEL is 100 mg/kg/day, based on a decreased fetal body weight, an increased incidence of skeletal malformations [7th cervical ribs and 14th rudimentary ribs], and a decreased percent of female fetuses.

This guideline developmental toxicity study is classified Acceptable/Guideline, and it satisfies the guideline requirement [OPPTS 870.3700; §83-3(a)] for a developmental toxicity study in the rodent.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: 2,4-D isopropyl ester
   Description: combination amber liquid and off-white solid
   Lot/Batch #: 918009
   Purity: 99.4% a.i.[bulk material: 97.12% pure; correction factor: 1.23 (adjust for purity & acid equivalents)]
   CAS #: 94-11-1

2. Vehicle: Mazola® 100% corn oil
   Description: yellow viscous liquid
   Lot/Batch #: Not provided

3. Test animals: Species: Rat
   Strain: Sprague-Dawley Crl:CD®BR
   Age at mating: ≈12 weeks old (86 days old)
   Weight at mating: 209-274 grams on gestation day 0; [216-275 grams (range-finding)]
   Source: Charles River Breeding Laboratories, Inc., Portage, Michigan
   Housing: Individually in suspended wire mesh cages.
   Diet: Purina® Certified Rodent Chow® #5002, ad libitum
   Water: municipal water, ad libitum
   Environmental conditions:
   - Temperature: 72±4° F
   - Humidity: 30-70%
   - Air changes: 10/hr
   - Photoperiod: 12 hrs dark/12 hrs light

   Acclimation period (P):11 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 4/12/94 end: 5/6/94; range-finding: start: 3/1/94 end: 3/24/94

2. Mating: Females were placed in a suspended wiremesh cage with a resident male [untreated, sexually mature] from the same strain and source for breeding. Positive evidence of mating was confirmed by the presence of a copulatory plug or the presence of sperm in a vaginal smear. The day on which evidence of mating was identified was termed day 0 of gestation , and the animals were separated.

3. Animal Assignment: The randomization procedure by which the bred rats were consecutively assigned in a block design to groups containing 25 rats each was as follows: the first mated

female and the appropriate gestation day 0 designation were recorded and the female was assigned to group 1, the second mated female was assigned to group 2 and the third to group 3, etc. This process was continued daily until 25 females were placed into each group (Table 1).

TABLE 1. Animal Assignment

Dose (mg/kg bw/day)♪	0	10	30	100
# Females	25	25	25	25

♪ acid equivalents

4. Dose selection rationale: The dose levels selected for the definitive developmental toxicity study (Table 1) were based on the results of the range-finding study [MRID 43523001] in which 2,4-D isopropyl ester was administered orally via gavage to 8 pregnant female rats/dose at dosages of 0, 25, 50, 100, 200, 400 mg/kg/day on gestational days [GD] 6 through 15. There was no adverse effect on pregnancy rate at any dose level. There was severe maternal toxicity at the 200 [mortality on GD 9 and 11 (2 dams), gait alterations (rocking, lurching, or swaying)] and 400 [mortality between GD 7 and 11 (all dams); body-weight loss (-9 grams between GD 6-7), decreased food consumption, gait alterations (rocking, lurching, or swaying), impaired use of hindlimbs, prostration; reddened adrenals and cortico-medullary junctions in kidneys] mg/kg/day dose levels. Postimplantation loss was severely increased at 200 mg/kg/day, and mean fetal body weight was reduced. One 200 mg/kg/day fetus had an omphalocele, and two fetuses in one litter had localized fetal edema of the neck and thorax. Based of the results of this study, dose levels of 10, 30, and 100 mg/kg/day were selected for the definitive study.

5. Dosage preparation and analysis

Prior to preparation, the test material was placed in an incubator [≈50°C for 2 hours]. The appropriate amount of test material was weighed for each group, and a sufficient amount of vehicle [Mazola® corn oil] was added. The preparations were stirred [magnetic stir plate] throughout the sampling and dosing procedures. Preparations for all dose groups were made twice [4/15/94 and 4/24/94] and were stored at room temperature. Prior to the start of the study, three sets of duplicate samples were collected from the top, middle, and bottom of a representative series of solutions for the four groups. One set of samples was analyzed for homogeneity, and the remaining sets were combined and analyzed for 8-day stability. On dose -preparations days, samples were collected from the middle stratum of each dosing formulation, and these were analyzed for concentration.

Results - Homogeneity Analysis: All groups met with WIL SOP requirements for homogeneity; i.e., the group mean differed by less than 15% from the target dose

concentration for that group and the location means for each group differed by less than 10% from the overall mean for that group.

Stability Analysis: After eight days of storage, there was no apparent degradation. The mean concentrations were 101% to 102% of the target concentrations.

Concentration Analysis: All dose formulations were 95.3%-104% of the target concentration.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered once daily by gavage, on gestation days 6 through 15, in a volume of 5 mL/kg of body weight. Dosing was based on the most recent body weight.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked for moribundity and mortality twice daily and daily for clinical signs [GD 0-20]. On the first dosing day, the animals were observed for signs of toxicity ≈ 1, 2, and 4 hours post dose. Since no remarkable clinical signs were observed at 2 and 4 hours post dose, only the 1-hour post dose observation period continued throughout dosing. Body weight data were recorded on gestation days 0, 6 through 16, and 20, and body-weight changes were calculated for various intervals. Gravid uterine weight was determined and net body weight and net body-weight change were calculated. Food consumption data were recorded on gestation days 0, 6-16, and 20. All surviving females were euthanized by carbon dioxide inhalation on gestation day 20. The thoracic, abdominal, and pelvis cavities were opened and the contents examined. The liver was excised and weighed, and sections from each liver were preserved for possible future histopathological examination. The uterus and ovaries were excised, and the number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened, and the number and location of all fetuses, early and late resorptions, and the total number of implantation sites were recorded. Uteri with no macroscopic evidence of nidation were excised, opened, and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss. Intrauterine data were summarized using two methods of calculation:

Group Mean Litter Basis:

$$\textbf{Post-implantation Loss/Litter} = \frac{\text{\# dead fetuses, resorptions (early/late)/group}}{\text{\# gravid females/group}}$$

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Proportional Litter Basis:

$$\textbf{Summation per Group} = \frac{\text{Post-implantation loss/litter (\%)}\,♪}{\text{\# of litters/group}}$$

$$♪ = \frac{\text{\# dead fetuses, resorptions (early/late)/litter}}{\text{\# implantation sites/litter}} \times 100$$

2. Fetal Evaluations - The fetuses were examined in the following manner: Each fetus was sexed, weighed, and tagged [WIL study #, dam #, and fetus #]. A detailed external examination of each fetus was conducted to include the eyes, palate and external orifices. Crown-rump measurements were recorded for late resorptions. Each fetus was examined viscerally by a modification of the Stuckhardt and Poppe√ fresh dissection technique to include the heart and major vessels. The sex of each fetus was verified by an internal examination. Fetal kidneys were examined and graded for renal papillae development by a method by Woo and Hoar. Heads from approximately one-half of the fetuses in each litter were placed in Bouin's fixative for subsequent soft-tissue examination by the Wilson sectioning technique. The heads from the remaining fetuses were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol. Following fixation, each fetus was macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described in Dawson. The skeletal examination was conducted utilizing low power magnification provided by a stereo microscope. External, visceral, and skeletal findings were recorded as developmental variations or malformations. Fetal developmental findings were summarized by (1) presenting the incidence of a given finding both as a percentage of the number of fetuses and the number of litters available for examination in the group; and (2) considering the litter as the basic unit for comparison and calculating the number of affected fetuses in a litter on a proportional basis as follows:

$$\text{Summation per group (\%)} = \frac{\text{Viable fetuses affected/litter (\%)}\,√}{\text{\# of litters/group}}$$

$$√ = \frac{\text{\# viable fetuses affected/litter}}{\text{\# viable fetuses/litter}} \times 100$$

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures. The statistical tests were performed by a Digital® MicroVAX® 3400 computer with appropriate programming.

2. Indices: None provided.

3. Historical control data: Historical control data were submitted [APPENDIX B (pages 212-

219) and APPENDIX C (pages 220-298).

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and Clinical Observations**: One high-dose dam died on gestation day 17, and clinical signs [rocking, lurching, or swaying, decreased defecation, clear matting on the ventral abdominal and thoracic areas, red material on the urogenital area, and ptosis] were observed in the dam at the daily and/or one-hour post dose observation one to five days prior to death. Two additional high-dose dams displayed similar clinical signs [rocking, lurching, or swaying] between gestation days 11 and 14. There were no treatment-related effects noted in mortality or clinical signs in the other treated dams. Deaths and similar clinical signs were observed in the range-finding study at 200 mg/kg/day and 400 mg/kg/day but not at 25, 50, or 100 mg/kg/day.

2. **Body Weight** - At the high-dose level, a statistically-significant [slight (93%-96% of control)] decrease was observed in body weight from gestation day [GD] 7 through GD 20. At termination, body weight of the high-dose dams was 95% of the control value. All groups initially lost weight [GD 6-7], and the losses were statistically-significantly greater than the control [-1 gram] at all dose levels [-6, -6, and -9 grams with increasing dose]. Thereafter, only the high-dose group displayed a significant reduction in body-weight gain [50%-85% of control] compared to the control [Table 2]. There was a dose-related decrease in body-weight gain during the dosing period [days 6-15], with the mid- [81% of control] and high- [62% of control] dose deficits attaining statistical significance. At the high-dose level, body-weight gains during the post-treatment phase [days 16-20] were only slightly lower [95% of control] than the control. Corrected body-weight gains at the high-dose level were significantly lower [83% of control] than control.

TABLE 2. Mean (±SD) Maternal Body Weight Gain (g) [a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	Control 0 (25)	10 (24)	30 (25)	100 (22)
Pretreatment:	(25)	(24)	(25)	(22)
Days 0-6	36.0±8.5	33.0±5.0	34.0±5.9	33.0±5.6
Treatment:	(25)	(24)	(25)	(22)
Days 6-7	-1±4.2	-6**±5.9	-6**±5.0	-9**±7.6
Days 7-8	4±5.4	6±5.8	4±4.7	3±6.3 [75]
Days 8-9	2±5.0	3±4.4	4±5.2	3±6.4
Days 9-10	6±4.1	6±6.6	4±4.4	3±7.0 [50]
Days 12-13	5±4.8	5±5.0	5±4.6	3±5.7 [60]
Days 13-14	5±4.0	5±3.9	4±5.9	4±5.2 [80]
Days 6-9	6±6.4	4±7.6 [67]♪	2±4.6 [33]	-3**±10.8
Days 6-16	53±8.0	49±8.7	43*±7.2 [81]	33**±22.8 [62]
Days 0-20	147±18.1	143±15.0	135±14.6 [92]	125**±26.6 [85]
Posttreatment:	(25)	(24)	(25)	(21)
Days 16-20	58±11.1	61±9.2	58±8.4	55±18.0 [95]
Corrected BW Gain	70.7±13.2	66.5±9.0	64.9±11.8 [92]	58.9**±9.8 [83]

a Data obtained from Tables 6-7, pages 47-49 in the study report; * Statistically different (p <0.05) from the control.
** Statistically different (p <0.01) from the control; ♪ [% of control]

3. <u>Food Consumption</u> - There was a dose-related, statistically-significant, decrease [Table 3] in food consumption initially [GD 6 to 7; all dose levels on a g/rat/day basis] and throughout the dosing period [GD 6-16; mid- and high-dose levels].

Table 3. Mean maternal food consumption (g/rat/day ± SD).[a]

Interval	Dose in mg/kg/day			
	0	10	30	100
Pretreatment:				
Days 0-6	22±2.0	22±1.6	22±1.9	21±1.8
Treatment:				
Days 6-7	20±2.1	17*±3.6 [85]	16**±4.1 [80]	14**±4.0 [70]
Days 7-8	19±4.0	19±4.2	18±3.4	15**±3.5 [79]
Days 8-9	21±3.9	20±4.3	19±2.6	16±**±5.2 [76]
Days 9-10	21±2.6	22±3.0	20±2.2	17**±5.1 [81]
Days 12-13	23±3.6	24±3.3	21±1.8	19**±4.8 [83]
Days 13-14	22±2.4	22±2.2	20±4.2	18**±5.4 [82]
Days 6-9	20±2.6	19±3.5	18±2.6	15**±3.4 [75]
Days 6-16	22±2.0	21±1.9	20*±1.4 [91]	17**±3.6 [77]
Days 0-20	23±1.8	23±1.6	22*±1.5	21**±1.7 [91]
Posttreatment				
Days 16-20	29±2.2	29±1.8	29±2.2	28±2.4

a Data extracted from study report, Tables 8 and 9, pages 50-53; * p<0.05; ** p<0.01

4. **Gross Pathology** - There were no treatment-related gross pathologic findings at necropsy.

5. **Cesarean Section Data** - The mean numbers of corpora lutea, implantation sites, and viable fetuses were comparable among the groups [Table 4]. There were no dead fetuses. The mean number of early resorptions was comparable among the groups, and only the mid- [2] and high- [1] dose groups had late resorptions. Preimplantation loss was comparable among the groups, and there was only a slight increase in postimplantation loss at the mid- [1.3] and high-[1.5] dose levels compared to the control [1.0] and low-dose [0.9] groups. One high-dose dam [4.6%] had 100% resorptions compared to none in the control or other dose groups. The historical control incidence of total litter resorptions is 8 out of 2676 litters [0.3%]. There was a significant decrease in the number of female fetuses at the high-dose level [5.3] compared to the control [6.8], and the % females at the high-dose level [40%] was below the historical control range [43.3%-58.4%]. Fetal body weight was significantly lower [89% of control] at the high-dose level compared to the control.

TABLE 4 Cesarean Section Observations [a]

Observation	Dose (mg/kg bw/day)			
	0	10	30	100
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant	25	24	25	22
Pregnancy Rate (%)	(100)	(96)	(100)	(88)
# Nonpregnant	0	1	0	3
Maternal Wastage				
# Died	0	0	0	1
# Died Pregnant	0	0	0	1
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	402	394	407	345
Corpora Lutea/Dam	16.8±2.6	16.4±1.8	16.3±2.6	16.43.9
Total # Implantations	364	351	351	303
(Implantations/Dam)	14.6±3.2	14.6±1.8	14.0±2.1	14.4±3.4
Total # Litters	25	24	25	21
Total # Live Fetuses	339	329	319	272
(Live Fetuses/Dam)	13.6±3.6	13.7±2.5	12.8±2.5	13.0±5.0
Viable Fetuses (%)	91.7±11.3	93.2±8.8	90.7±11.3	89.0±26.9
Total # Dead Fetuses	0	0	0	0
(Dead Fetuses/Dam)	0	0	0	0
Total # Resorptions	25	22	32	31
Early	25	22	30	30
Late	0	0	2	1
Resorptions/Dam				
Early [%]	1.0±1.0 [8±11]	0.9±1.1 [7±9]	1.2±1.3 [9±9]	1.4±3.3 [11±25]
Late [%]	0	0	0.1±0.4 [1±4]	0.0±0.2 [0.4±2]
Litters with Total Resorptions	0	0	0	1 [4.6%]

Observation	Dose (mg/kg bw/day)			
	0	10	30	100
Mean Fetal Weight (g)	3.6±0.2	3.6±0.2	3.4±0.3	3.2**±0.3
Males	3.7±0.2	3.7±0.2	3.5±0.3	3.3**±0.3
Females	3.5±0.2	3.4±0.2	3.4±0.3	3.1**±0.2
Sex Ratio (% Male) # males/# females	(51) 168/171	(56) 184/145	(52) 164/155	(60) 161/111
Mean # males/# females	6.7/6.8	7.7/6.0	6.6/6.2	7.7/5.3*
Preimplantation Loss (%)	52 [2.3±2.7]	43 [1.8±1.3]	56 [2.2±2.6]	42 [2.0±1.9]
Postimplantation Loss (%)	25 [1.0±1.0]	22 [0.9±1.1]	32 [1.3±1.5]	31 [1.5±3.4]
mean (%)	8.3±11.3	6.8±8.8	9.3±11.3	11.0±26.9

a Data obtained from Tables 11-12, pages 55-58, Table 29, pages 133-136, and Table 30, pages 137-140] in the study report.
* p <0.05); ** p <0.01.

B. DEVELOPMENTAL TOXICITY

1. External Examination - There were no treatment-related external malformations or variations.

2. Visceral Examination -There were no treatment-related visceral malformations or variations.

3. Skeletal Examination -According to the study report, the only skeletal malformation observed at the high-dose level was one fetus/one litter with vertebral anomaly with or without associated rib anomaly. However, the presence of 7th cervical rib(s) and 14th rudimentary rib(s) are considered skeletal malformations also, and there was an increased [statistically-significant] incidence of both at the high-dose level compared to the control incidence [Table 5]. Skeletal variations [hyoid unossified, 25 presacral vertebrae] were slightly increased at the high-dose level compared to the control, and bent ribs were increased at the mid- and high-dose levels compared to the control, although there was no dose-response. The incidence of 7th cervical rib(s) is outside the historical control data [Table 6] on both a fetal and litter basis.

Table 5. Malformations and Variations

Dose/Finding	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
# fetuses/# litters examined	339/25	329/24	319/25	272/20
skeletal malformation	# fetuses/# litters	# fetuses/# litters	# fetuses/#	# fetuses/# litters
filamentous tail	1/1	0	litters	0
vertebral anomaly with or without associated rib anomaly	0	1/1	0	1/1
bent limb bone	0	1/1	0	0
only 12 full pairs of ribs	0	1/1	0	0
total skeletal malformations [reported]	1/1	2/2	0	1/1
presence of 7th cervical ribs	3 [0.9]✖/3 [12]	9 [2.7]/6 [25]	1/1	15 [5.5]/8* [40]
presence of 14th rudimentary ribs	3 [0.9]/2 [8]	4 [1.2]/3 [13]	5 [1.6]/3 [12] 6 [1.9]/5 [20]	26 [9.6]/13* [65]

Table 5. Malformations and Variations				
Dose/Finding	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
# fetuses/# litters examined	339/25	329/24	319/25	272/20
skeletal variations *hyoid unossified* *25 presacral vertebrae* *bent ribs*	# fetuses/# litters 4 [1.2]/2 [8.0] 0/0 2 [0.6]/2 [8.0]	# fetuses/# litters 4 [1.2]/3 [12.5] 0/0 3 [0.9]/2 [8.3]	# fetuses/# litters 0/0 0/0 9 [2.8]/8 [32]	# fetuses/# litters 4 [1.5]/3 [15] 1 [0.4]/1 [5.0] 5 [1.8]/5 [25]

✖[%]; data from Tables 13-17, pages 59-66 of the report

Table 6. Historical Control Data		
	# fetuses [24950]	# litters [2403]
skeletal malformation vertebral anomaly with or without associated rib anomaly presence of 7th cervical ribs presence of 14th rudimentary ribs	 12 (0-0.8)✖ 116 (0-3.3) 1349 (0-38.9)	 12 (0-5.0) 94 (0-27.3) 634 (0-94.7)
skeletal variations *hyoid unossified* *25 presacral vertebrae* *bent ribs*	 175 (0-38.9) 45 (0-4.0) 178 (0-4.2)	 117 (0-50) 32 (0-22.7) 120 (0-30)

✖ (%) data from pages 216 and 219 of the report

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: Maternal toxicity was exhibited at the 100 mg/kg/day dose level, where one death, clinical signs [rocking, lurching, or swaying, decreased defecation, clear matting on the ventral abdominal and thoracic areas, red material on the urogenital area, and ptosis], and decreased body-weight gains and food consumption were observed throughout the dosing period. Decreased body-weight gains and food consumption were also observed at the mid-dose level [30 mg/kg/day].

Developmental toxicity was observed at 30 and 100 mg/kg/day, where increased postimplantation losses and/or decreased mean fetal body weights were observed. At the high-dose level, an increased incidence of two fetal skeletal developmental variations [7th cervical ribs and 14th rudimentary ribs] was considered a possible effect on fetal development. The only skeletal malformation reported at the high-dose level was one fetus/one litter with vertebral anomaly with or without associated rib anomaly. The NOAEL for both maternal and developmental toxicity was considered to be 10 mg/kg/day.

B. REVIEWER'S DISCUSSION: 1.MATERNAL TOXICITY: There were no abortions or premature deliveries. There was one death; a high-dose dam died on gestation day 17. Clinical signs observed in this dam included rocking, lurching, or swaying, decreased defecation, clear matting on the ventral abdominal and thoracic areas and ptosis. Treatment-

related clinical signs were also observed in two other high-dose dams and included rocking, lurching, or swaying, which were observed at the one-hour post-dose examination period between gestation days 11 and 14. At the high-dose level, a slight, statistically-significant [93%-96% of control], decrease was observed in body weight from gestation day [GD] 7 through GD 20. At termination, body weight of the high-dose dams was 95% of the control value. All groups initially lost weight [GD 6-7]. Thereafter, only the high-dose group displayed a significant reduction in body weight [50%-75% of control] compared to the control. There was a dose-related decrease in body-weight gain during the dosing period, with the mid- [81% of control] and high- [62% of control] dose deficits attaining statistical significance. Corrected body-weight gains at the high-dose level were significantly lower [83% of control] than control. Decreased food consumption was noted at all dose levels initially [GD 6-7], but thereafter only the high-dose displayed decreased food consumption [70%-83% of control] during the dosing period.

The mean numbers of corpora lutea, implantation sites, and viable fetuses were comparable among the groups. There were no dead fetuses. The mean number of early resorptions was comparable among the groups, and only the mid- [2] and high- [1] dose groups had late resorptions. Preimplantation loss was comparable among the groups, and there was only a slight increase in postimplantation loss at the mid- [1.3] and high-[1.5] dose levels compared to the control [1.0] and low-dose [0.9] groups. One high-dose dam [4.6%] had 100% resorptions [10 early, 1 late] compared to none in the control or other dose groups. The historical control incidence of total litter resorptions is 8 out of 2676 litters [0.3%].

2. DEVELOPMENTAL TOXICITY: Fetal body weight was significantly lower [89% of control] at the high-dose level compared to the control. There was a significant decrease in the number of female fetuses at the high-dose level [5.3] compared to the control [6.8], and the % females at the high-dose level [40%] was below the historical control range [43.3%-58.4%].

There were no treatment-related external or visceral malformations or variations. The presence of 7th cervical rib(s) and 14th rudimentary rib(s) are considered skeletal malformations, and there was an increased [statistically-significant] incidence of both at the high-dose level compared to the control incidence. The incidence of 7th cervical rib(s) is outside the historical control data on both a fetal and litter basis. Skeletal variations [hyoid unossified, 25 presacral vertebrae] were slightly increased at the high-dose level compared to the control, and bent ribs were increased at the mid- and high-dose levels compared to the control, although there was no dose-response. Similar skeletal findings were observed in the rat developmental toxicity studies on the parent compound and amine salts and other esters of 2,4-D.

The maternal toxicity NOAEL is 10 mg/kg/day, and the maternal toxicity LOAEL is 30 mg/kg/day, based on decreased body-weight gain and food consumption during the dosing period. At the highest dose tested [100 mg/kg/day], one death, clinical signs [rocking, lurching, or swaying, decreased defecation, clear matting on the ventral

abdominal and thoracic areas, red material on the urogenital area, and ptosis], and one litter with 100% resorptions were observed.

The NOAEL for developmental toxicity is 30 mg/kg/day, and the developmental toxicity LOAEL is 100 mg/kg/day, based on a decreased fetal body weight, an increased incidence of skeletal malformations, and a decreased number of female fetuses.

This guideline developmental toxicity study is classified Acceptable/Guideline, and it satisfies the guideline [OPPTS 870.3700; §83-3(a)] requirement for a developmental toxicity study in the rodent.

C. STUDY DEFICIENCIES: None that would adversely affect study interpretation.

13544

R157476

Chemical: 2-4,D

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030001
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