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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

No. 256619
No. ~~254708~~ 254572

SUBJECT: Accession No. 254708. Interim 52-Week Report on 2,4D Acid (2,4-dicloropheноxy acetic acid). Chronic Feeding/Oncogenicity Study in Rats Submitted by the 2,4-D Industry Task Force on Research Data. Cas. No. (315)

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Special Review Branch
Registration Division (TS-767C)

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Accession No.: 254708

Testing Facility: Hazelton Laboratories of America, Inc., Vienna, Virginia

Project No.: 2184-103

Testing Period: February 22, 1983 - February 21, 1984

Report Submitted to Sponsor: June 11, 1984

Conclusions and Comments:

1. The interim report is acceptable only as an interim report.

2. All individual animal data will be required for final evaluation of this study.

3. Histopathological observations consisting of frequency of occurrence and degree of severity will be required in the final report for all tissues. A summary incidence table of findings will need to be provided for the final report. This includes observations of gross necropsy.

4. Purity of the compound tested as well as the batch or lot number must be provided.

5. Data reflecting homogeneity, stability and concentration of the test chemical in feed will be required.

6. The presence of compounds other than the active ingredient needs to be qualified and quantified. The structure, the chemical name and any code number affiliated with each impurity should be reported as these data will be helpful.

7. Toxicology Branch considers it essential to further address the apparent T_4 increases by a) provide expert histopathological thyroid gland/morphometric evaluation, b) determine T_3/T_4 values with c) simultaneous TSH values (using rat TSH antibody). The need to determine the mode of action of 2,4-D or its metabolites on the gland directly or through the TSH route is noted.

Experimental Design: Five groups of 60 animals (Charles River rats) of each sex were placed on test. Animals were treated as follows:

Group	Dietary Level (mg/kg/day)	Males	Females
1 (Control)	0	60	60
2	1.0	60	60
3	5.0	60	60
4	15.0	60	60
5	45.0	60	60

Parameters Evaluated: All animals were observed twice daily for mortality and moribundity. Animals were monitored weekly for weeks 1-14 and then every 2 weeks for body weight, food consumption, compound consumption and clinical signs. Animals were also palpatated on this same schedule.

Hematology: Ten animals per sex per dose were monitored for changes in WBC, RBC, HGB, HCT, platelets, and reticulocytes. Blood samples were taken initially and at weeks 27 and 53.

Clinical Chemistry: Ten animals per sex per dose were monitored for changes in Na, K, Ca, total protein, albumin, globulin, A/G ratio, alkaline phosphatase, total bilirubin, glucose, SGOT (AST), SGPT (ALT), and LDH. Values were determined initially and at 27 and 53 weeks. T_4 values were determined at 27 and 53 weeks only.

004498

Urine was examined initially and at weeks 27 and 53 for pH, specific gravity, glucose, ketones, protein, bilirubin and urobilinogen.

Organ Weights were determined on ten animals per sex per dose at week 53. The following organs were weighed: brain, heart, liver, testes, kidney, adrenals, pituitary, ovaries, and thyroid (with parathyroid). Absolute organ weights were recorded and organ to body weight and organ to brain weight ratios calculated.

Pathology: Gross and Microscopic: Pathology was conducted on 10 animals/sex/dose at week 53. Approximately 40 tissues were examined microscopically including all major organs.

Results

Mortality: Survival was excellent for all groups with not more than 2 animals found dead in any one group by week 53.

Body Weights: Females: Mean growth rate and mean body weight at week 52 was statistically significantly decreased only for group 5 females when compared to controls. Males: Mean growth rate and mean body weight at 52 weeks was not statistically significantly different between treated and control groups.

Food Intake: Only Group 5 (45.0 mg/kg/day) females showed a statistically significant decrease in food consumption. All males receiving the test compound showed no statistically significant differences when compared to controls.

Clinical Signs: The following signs were reported but did not appear to be dose related - wheezing, rhinorrhea, chromodacryorrhea and exophthalmus. However, it is pointed out here that the incidence of chromodacryorrhea in the control females was at times comparable to treated groups.

Palpatation of Whole Animal: A tissue mass appeared at week 40 on the ventral front left side of one female in the high dose group (45 mg/kg/day). No other females in any other group manifested a tissue mass.

A "wart-like" lesion also appeared at week 32 on one male in the low dose (1.0 mg/kg/day) group. This lesion was not observed at higher dose levels.

Hematology: No treatment related effects were observed in males at 27 or 53 weeks. Findings with respect to females are reflected in the following table. The changes were observed only at the 27 week reading. Reported values were comparable to control at week 53.

004498

Females at 27 Weeks

Parameter / Dose (mg/kg)	$x10^3/mm^3$ WBC (7.5)*	$x10^6/mm^3$ RBC (7.73)	g/dl HGB (16.8)	% HCT (46.5)	$x10^3/mm^3$ Plate. (776)	% Ret. (2.5)
0	6.0	8.84	16.7	48.9	707	0.8
1.0						
5.0						
15.0		8.39(S-)	15.9(S-)	46.3(S-)	629(S-)	
45.0	7.3(S+)	8.31(S-)	15.9(S-)	45.6(S-)		

NOTE: (S+); indicates a statistically significant increase $p<0.05$

(S-); indicates statistically significant decrease $p<0.05$

*Values in parenthesis indicate pretreatment values for controls.

Clinical Chemistry: Males and females showed the following changes in clinical parameters at 27 weeks. Changes reported for the readings taken at week 53 are indicated by the asterisk.

Males: Serum sodium values for males were increased to statistically significant levels at week 27 and appeared to be dose responsive at 5.0, 15.0 and 45.0 mg/kg. Values were reported as normal at week 53.

Other clinical chemistry changes reported for males did not appear to be biologically meaningful.

Females: Clinical chemistry values for females were generally not biologically meaningful with the possible exception of values reported for T4. The mean values show statistically significant increases in T4 values in females at 27 weeks treatment, but not at 53 weeks. Indications of increased secretion of thyroxine (T4) are seen from the presence of increased thyroid gland weights, elevated T4 values in the female rats and no real reduction in food intake with a slight concomitant reduction in body weight. Wasting of the muscle mass to any degree should produce an elevation in serum potassium. There is only a slight (N.S.) elevation in serum potassium concentrations in the females. However, mobilization of bone protein should liberate Ca^{++} into the serum. The serum calcium values are elevated in the study.

Therefore, the important question of whether all these factors are related to thyroid activity and whether the T4 values are real and derive from either a TSH stimulation or from a direct action by 2,4-D or metabolites upon the gland

004498

itself requires addressing by the registrant. In order to further evaluate these values, the Agency would propose that the histopathology be evaluated by an expert on histological changes in that gland. Accompanying TSH values using the rat TSH antibody should be submitted for animals with T4 and T3 values.

	Males		Females	
	Week 27	Week 53	Week 27	Week 53
	Microgram/dl		Microgram/dl	
Control	5.0	4.5	3.1	2.5
1 mg/kg	4.5	4.2	3.6	2.8
5 mg/kg	5.3	4.3	3.6	2.8
15 mg/kg	5.3	4.1	3.8*	3.3
45 mg/kg	5.2	4.1	3.8*	2.3

* Significant at $p \leq .05$.

Parameters Dose (mg/kg)	Na	Albumin	A/G	Glu.	27 Weeks 53 Week* SGPT(ALT)	Ca	Tot.Bili.	SGOT(AST)	LDH	*BUN	*T4
		Males									
0	143	3.8	1.56	93	46					15.7	
1.0	143	3.8	1.54	82(S-)	53					16.3	
5.0	144(S+)	3.8	1.52	95	46					15.9	
15.0	145(S+)	3.7	1.45(S-)	93	57					15.1	
45.0	147(S+)	3.6(S-)	1.51	93	66(S+)					14.3(S-)	
		Females									
0					52	10.5	.27	83	319		2.5
1.0					49	10.4	.23	76	268		2.8
5.0					39(S-)	10.8(S+)	.26	76	282		2.8
15.0					40(S-)	10.8	.19(S-)	70(S-)	211(S-)		3.3(S+)
45.0					47	10.8(S+)	.21	79	250		2.3

(Note: Only those parameters reported as having a statistically significant change are reported here.)

Urinalysis: No statistically significant or biologically meaningful changes were reported.

Necropsy: No biologically meaningful changes were reported.

Organ Weights: Organ weights were determined for brain, heart, liver, testes, kidney, adrenals, pituitary, ovaries and thyroid/parathyroid at week 53 for 10 animals per sex per dose group. Results of the findings are noted below. Statistically significant changes were observed in males only and only for the organs indicated.

It is also pointed out here that terminal body weight in the male high dose group though slightly decreased from controls (cf. 351 gms. vs. 341 gms.) was not statistically significant. Separately absolute brain weights between control and treated groups were nearly identical. [NOTE: (S+) = statistically significant increase.]

Males-Absolute Organ Weight Change

Organ / Dose(mg/kg)	Adrenal	Pituitary	Thy/Parathy.	Kidney(L)	Kidney(R)	Kidney(L+R)
0				1.21	1.22	2.44
1.0	(S+)	(S+)	(S+)	1.23	1.19	2.43
5.0				1.24	1.22	2.46
15.0				1.33 (S+)	1.29	2.61
45.0				1.34 (S+)	1.31	2.66 (S+)

Males: Organ Weight/Body Weight Ratio

Organ / Dose(mg/kg)	Adrenal	Pituitary	Thy/Parathy.	Kidney(L)	Kidney(R)	Kidney(L+R)
0				.345	.348	.693
1.0	(S+)	(S+)	(S+)	.347	.336	.684
5.0				.352	.346	.698
15.0				.374 (S+)	.364	.738
45.0				.394 (S+)	.386(S+)	.780(S+)

Males: Organ Weight/Brain Weight Ratio

Organ Dose(mg/kg)	Adrenal	Pituitary	Thy/Parathy.	Kidney(L)	Kidney(R)	Kidney(L+R)
0				.611	.616	1.225
1.0	(S+)	(S+)	(S+)	.616	.597	1.214
5.0				.617	.608	1.225
15.0				.657(S+)	.638	1.295
45.0				.679(S+)	.664(S+)	1.344(S+)

Histopathology: The only two apparently salient findings were: 1) an increase in the tubular cell pigment of the kidney and 2) a fine vacuolization of the cytoplasm in the renal cortex of the females. The frequency with which the former observation was noted is as follows:

INCREASED TUBULAR CELL (BROWN) PIGMENT

Dose (mg/kg)	Males	Females
0	2/10	3/10
1.0	2/10	3/10
5.0	9/10	5/10
15.0	10/10	6/10
45.0	10/10	7/10

The frequency and severity with which the latter observation was reported for females was as follows: (NOTE: fine vacuolization of the cytoplasm in the renal cortex was not observed in males.)

Fine Vacuolization of the Cytoplasm in the Renal Cortex Observed in Females Only

Severity Dose (mg/kg)	Minimal	Slight	Moderate	Total
0	2/10	3/10	0/10	5/10
1.0	0/10	3/10	0/10	3/10
5.0	1/10	1/10	3/10	5/10
15.0	0/10	1/10	4/10	5/10
45.0	2/10	1/10	5/10	8/10

004498

Discussion

The toxicological evaluation of this interim report has indicated that the kidney is a target organ for toxicity for 2,4D at doses higher than 1.0 mg/kg. The Toxicology Branch and the Task Force on 2,4D are in agreement on this point. Histomorphologic changes were observed in both males and females for an increased frequency of an increase in a brown tubular cell pigment at dose levels higher than 1.0 mg/kg. Additionally, for females alone, there was observed an increased incidence and severity of fine vacuolization of the cytoplasm in the renal cortex at 45.0 mg/kg (HDT) and an increased severity of the same effect at 5.0 and 15.0 mg/kg when results were compared to control values. Absolute increased kidney weights and increased kidney weight ratios (body weight and brain weight) also appear to support the histopathological findings particularly at the high mid-dose (15.0 mg/kg) and high dose (45.0 mg/kg) for males. Absolute kidney weight and kidney body weight brain weight ratios were not increased for females.

Serum sodium ion values were also statistically significantly increased and dose responsive at 27 weeks but not at 52 weeks. The significance of this change is not known based upon the experimental evidence contained within the report. However, its regulation by the kidneys should not be ignored.

Separately, we can say that the examination of values with respect to urinalysis findings did not reveal any overtly toxic effects.

Gross necropsy and histopathology were negative for skin tissue. This may indicate that those animals identified as having a "tissue mass" and a "wart-like lesion" were not among the animals sacrificed at the interim kill. However, we also point out that both of these observations were singular incidences and dose responses were not evident. These events cannot at this time be considered biologically meaningful.

Chromodacryorrhea was noted as reoccurring in treated and female control groups. Although the incidence of observations were small in controls (maximum 4/59 animals) it may suggest the possibility of some cross-contamination.

Conclusion: NOEL: (Based on interim report) = 1.0 mg/kg/day

LEL (based on interim report) = 5.0 mg/kg/day
kidney effects. Increased frequency (both sexes) of an increase in a brown tubular cell pigment. Increased severity (females only) of a fine vacuolization of the cytoplasm in the renal cortex.

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