2,4-D/TOX

(69)

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Memorandum

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THRU: Robert B. Jaeger RBJ 8/7/84
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FROM: Henry Spencer, Ph.D. HS 8/7/84
Toxicology Branch
Hazard Evaluation Division (TS-769) for WHB 8/7/84

SUBJECT: Review of subchronic feeding studies in mice and rats for inclusion in 2, 4-D file.

Caswell No. 315

Conclusions and Recommendations:

1. Toxicology Branch concludes that NOEL's and LEL's in both 90 day rodent studies are based on the effects in the kidney. The LEL in the rat is equivocal at 1 mg/kg with no NOEL established. The LEL in the mouse is 5 mg/kg (LDT). A NOEL was not established in the study.

Study: Subchronic Toxicity Study in Rats by Hazleton Laboratories Inc. Report No. 2184-102, dated September 12, 1983, for Industry Task Force on 2,4-D Research Data.

Accession No. 251474

Material Tested: 2,4-Dichlorophenoxyacetic acid [97.5% pure].

Animal Tested: Fischer 344 rats.

261 young of both sexes were received and acclimated for 26 days. At start of the study the males weighed 147 to 189 g and females weighed 117 to 132 g. They were approximately 8 weeks of age.

Methods: The animals were individually caged and numbered. Cages were arranged and rotated biweekly.

Purina(R) rodent chow and tap water were allowed ad libitum.

Animals were chosen for groups by a computer generated table of randomization. Animals were withdrawn from feed prior to sacrifice or blood collections.

Cage areas were maintained at 75.7°F ± 1.1°F and 60% ± 9.3% relative humidity. A light-dark cycle of 12 hr was maintained.

Groups of 20/sex/dose were selected and dosages were 0, 1, 5, 15 or 45 mg/kg/bw in feed. Concentrations in the feed were based on the previous week's weights. Diets were prepared fresh weekly and presented on a weekly basis. Diets were analyzed for the test compound throughout the study. Clinical signs were recorded daily and body weights were obtained weekly. Palpation of rats for masses was conducted at the body weighings and food consumption was recorded weekly.

Blood samples from the orbital sinuses of 10/sex/dosage were analyzed for the following:

"Hematology

Hematocrit (HCT)
Hemoglobin (HGB)
Erythrocyte Count (RBC)
Total Leukocyte Count (WBC)
Platelet Count (PLATELET)
Reticulocyte Count (RETIC)
Differential Leukocyte Count
Erythrocyte and Leukocyte Morphology

Clinical Chemistry

Total Protein (T PROT)
Albumin
Globulin
Albumin/Globulin Ratio (ALB/GLOB RATIO)
Sodium
Potassium
Alkaline Phosphatase (ALK PHOS)

Total Bilirubin (T BILI)
Blood Urea Nitrogen (BUN)
Fasting Glucose (GLUCOSE)
Calcium
Serum Glutamic Oxaloacetic Transaminase (SGOT)
Serum Glutamic Pyruvic Transaminase (SGPT)
Lactate Dehydrogenase (LDH)
Thyroxine (T4)"

Hematological and clinical chemistry determinations were made at 7 weeks and at study termination. Urinalysis included the following parameters.

pH (PH)
Specific Gravity (SP GR)
Glucose
Ketones
Protein
Bilirubin (BILI)
Urobilinogen (UROBIL)

At termination, sacrifice was by exsanguination after anesthesia with sodium pentabarbital.

Necropsies were performed on all rats. Organ weights were determined in both fixed and fresh organs, and organ-to-body weight ratios as well as organ to brain weight ratios were determined. Histopathological evaluations were performed as noted below:

Prior to Fixation	After Fixation
Brain (including brainstem)	Thyroid
Heart	Ovaries (females)
Liver	Adrenals
Kidneys*	Pituitary
Testes with Epididymides (males)"	

* Kidneys were identified as left and right and were weighed separately.

Tissue Preservation

The following tissues from each animal were preserved in 10% neutral buffered formalin:

Brain (fore-, mid-, hind-)	Pancreas
Pituitary	Adrenals
Eyes (with Harderian gland)	Kidneys
Salivary Gland	Small Intestine
Trachea	Large Intestine
Esophagus	Urinary Bladder

Thyroid (with parathyroids)
Thymus

Sternum (with marrow)
Heart
Lung and Mainstem Bronchi
(two coronal sections including all lobes)
Liver (two lobes)
Stomach
Spleen

Testes (males) with Epididymides Prostate (males)
Ovaries (females)
Uterus (females)
Skeletal Muscle
Gross Lesions

All tissues preserved were examined microscopically in control and 45 mg/kg groups and only brain, liver, heart and kidneys in 5, 15 and 45 mg/kg groups.

Statistical Analysis:

Appropriate statistical anlayses of the data were performed. See report for details.

Results:

Four females died from the orbital sinus bleeding processes. Otherwise, no deaths occurred in the study.

Minimal weight reduction of 1-3% occurred in males and females in the 45 mg/kg treatment group when compared to controls.

Hematology:

A significant reduction in hemoglobin values is seen in males at 14 weeks in all treated groups and also in groups treated at 5 and 45 mg/kg/bw at 7 weeks. Spurious accounts of a reduction in hematocrit and reduced numbers of red blood cells were not considered treatment-related.

Reticulocyte values, however, are significantly reduced in males at 5, 15,and 45 mg/kg and in females treated 45 mg/kg at 7 weeks in the study. Normal occurrences of reticulocytes were noted at week 14 when compared to controls.

Other hematological parameters were variable and were not treatment related.

Clinical Chemistry:

Serum sodium and potassium values were increased in males at week 7 of the study. Slight hemolysis was present in control and treated group sera of both sexes. Serum potassium values were elevated in a dose-response relationship in treated

females. Serum calcium levels for males at 15 and 45 mg/kg was slightly thoughout statistically increased at 7 weeks. Female test animals exhibited a significant increase in plasma glucose values at 15 and 45 mg/kg treatment.

Plasma T_4 values did not show a significant response in females in any dosage group when comparing 7 week interval values and 14 week values.

However, T_4 values at 14 weeks in males were increased at both 5 and 15 mg/kg dosages when compared to controls.

LDH values were reduced in males at 7 weeks on a dose-related basis, but did not attain significance. The reduction in LDH values for females was also seen as a chemical dose-related trend but did reach significance at 15 and 45 mg/kg at the 7 week interval.

Alkaline phosphatase values at 7 weeks in male rats were depressed at 15 and 45 mg/kg dosages and at 14 weeks in only the 45 mg/kg group. This type depression was only noted in females after 14 weeks at 15 and 45 mg/kg.

Blood urea nitrogen levels were reduced in males and females only after 14 weeks at 15 mg/kg and 45 mg/kg.

SGOT values for males were significantly lower than controls at 14 weeks in the study at 15 and 45 mg/kg dosages. Females, on the other hand, exhibited significantly reduced SGPT values at 15 and 45 mg/kg at week 14.

Urinalysis:

Examination of urinalysis data revealed slight increases in ketones, protein and urobilinogen excretion in all groups at 14 weeks when compared to 7 weeks. Specific gravity values were seemingly increased but are also a function of protein excretion as well as concentrating capability. Urobilinogen excretion appeared slightly elevated. However, all increases seen in the urinalysis values were not considered to be dose or treatment related since control animals presented the same trends.

Changes in Absolute Organ Weight, Organ-to-Body Ratio and Organ-to-Brain Weight Ratios.

At termination of the study, body weights of treated male and female test animals were similar to their respective controls.

Mean Absolute Organ Weight Changes vs. Controls

Males

Organ	Dosage	1	5	15	45 mg/kg
Kidneys	1.938	1.896	1.948	1.937	+2.121
Thyroid	.0099	+.0115	+.0129	+.0122	+.0127
Brain	1.915	1.922	1.943	1.927	1.956
Pituitary	.0080	.0076	.0087	.0087	.0082

Females

Kidneys					
Pituitary	.0102	.0104	.0110	+.0116	.0104
Ovary	.0632	.0714	+.0749	.0697	+.0772
Thyroid	.0092	.0104	.0104	+.0125	.0101

Relative Organ to Body Weight Ratios Changes

Males

Organ	Dosage	1	5	15	45 mg/kg
Kidneys	.6895	.6912	.6746	.6969	+.7585
Thyroid	.0035	+.0042	+.0045	+.0044	+.0045

Females

Ovaries	.0393	.0451	+.0466	.0430	+.0490
Pituitary	.0063	.0066	.0068	+.0073	.0066
Thyroid	.0058	.0066	.0065	+.0079	.0064
Kidney	.7521	.7606	.7449	.7413	.7921

Mean Organ to Brain Weight Ratio Changes

Males

Organ	Dosage	1	5	15	45 mg/kg
Kidneys	1.0111	.9866	1.0033	1.0040	+1.0876
Thyroid	.0052	+.0060	+.0066	+.0063	+.0065

Females

Kidney	.6835	.6916	.6757	.6542	.7100
Ovaries	.0362	.0412	+.0425	.0385	+.0442
Thyroid	.0053	.0060	.0059	+.0069	.0058

Note: + Statistically significant change at $P < 0.05$

Several organ to body or brain weight ratios were reduced, but not to statistically significant levels in organs not shown in the above chart.

Lungs and Bronchi:

Perivascular and/or peribronchial lymphoid hyperplasia occurrences were similar in the 45 mg/kg group and controls in both sexes.

Chronic myocarditis occurred in all groups of females and were sporadic in males exhibiting no treatment-related response.

Renal changes were not evident in control male or female histopathology evaluations. Only a minor number (1) of males and (1) females exhibited ~~no~~ degenerative changes in the renal cortical tissues in Group II (1 mg/kg).

Dose related increases in degenerative changes are reported in the higher dosage levels.

NOEL (1)

Males

Grades	Controls	Group II	III	IV	V
1	0	1	10	9	1
2	0	0	0	4	4
3	0	0	0	1	15

Females

1	0	0	1	0	0
2	0	0	3	2	3
3	0	1	3	4	7
4	0	0	0	0	1

There were no other histopathological changes noted.

Discussion:

Feed intake reduction can account for the equivocal (1-3%) body wt. increase in both sexes at 45 mg/kg.

Hemoglobin variations noted in treated animals were not considered to be compound related since no dose-related decrease is seen. Other hematological effects such as reduced RBC and Hct values were spurious and not considered to be biologically significant.

The test groups appear to have compensated for the reduced reticulocyte output seen at 7 weeks with a normal occurrence of these immature RBC forms seen at 14 weeks.

Clinical chemistry values of potassium are of questionable value since hemolyzed sera do present spurious values in analyses. As a result, evaluation of the overall effect on serum K levels can not be made. In addition, this reviewer considers some of the potassium levels to be markedly elevated and could represent laboratory error in sample contamination.

Thyroid tissues were not histologically evaluated in groups which showed dose related, significant increases in both absolute and relative organ-to-body weights and organ-to-brain weight ratios in males.

Increases in thyroid weights are consistent in females with those noted in males excepting that all groups are not significantly increased.

Histological evaluation in both sexes in all test groups is necessary to evaluate this parameter.

Conclusion:

A. An LEL of 5 mg/kg exists for reduced reticulocytes in males at 7 weeks in the study. A NOEL = 1 mg/kg.

B. An LEL for decreased SGOT, SGPT, alkaline phosphatase exists at 15 mg/kg. A NOEL = 5 mg/kg.

C. Plasma T_4 values in males were increased at 5 and 15 mg/kg, though not statistically, but are supported by increases in both absolute organ weight and relative organ weight ratios as low as 1 mg/kg (no NOEL). Organ-to-brain weight ratios for thyroids were also significantly increased in all groups of treated males.

   This reviewer considers an equivocal LEL of 1 mg/kg for effects on the thyroid to exist in treated males. Further morphologic examinations are required.

D. Significant changes in renal cortical tissue were noted in treated animal groups 3 through 5 in both males and females. LEL = 5 mg/kg. NOEL = 1 mg/kg.

E. An LEL of 5 mg/kg exists for kidney effects with a NOEL = 1 mg/kg.

F. Thyroid weight ratios are increased in all dosed groups. Histopathological examination of these tissues are incomplete and are necessary for evaluation. Phenoxy-like compounds are known to affect thyroid function. Therefore, morphologic evaluation of these organs are required.

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REVISED:DCR-44902:Spencer/Littel:mar:7/3/84:Del. 7/16/84
REVISED:DCR-44904:Spencer/Littel:KIM:7/16/84:Del. 7/26/84

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Study:

Subchronic Toxicity Study in Mice using 2,4-D, by Hazleton Laboratories, America, Inc. Lab. No. 2184-100, dated September 12, 1983 for Industry Task Force on 2,4-D Research Data. Acc. No. 251473.

Material Tested:

2,4-Dichlorophenoxyacetic acid as a beige powder, 97.5% purity.

Animal Tested:

$B_6C_3F_1$ mice from Charles River Breeding Labs. Inc. Portage, Michigan.

Methods:

295 mice consisting of 152 females and 143 males after a 23 day acclimation period were placed on study. Males weighed from 14.3 to 21.9 grams while females weighed from 13.6 to 22 grams. At start of study the animals were approximately 7 and one-half weeks of age. After randomization, housing was on an individual basis. Racks of cages were rotated biweekly. Commercial rodent chow (Purina®) and tap water was supplied ad libitum. Feed was removed prior to blood sampling and sacrifice. Room temperatures and relative humidity were mantained at 74.6 ± 0.76° F and 62.5 ± 9.3% respectively. A 12 hour light dark cycle was provided and air flow exceeded 10 changes per hour.

Groups of 20 per sex in each dosage were assigned from the initial 295 animal. Dosages included 0, 5, 15, 45, or 90 mg/kg. The compound was mixed into the diet at amounts calculated from the previous weeks body weights. Laboratory chow compound was sampled on weeks 1, 2, 3, 4, 8 and 12 for concentrations of test material.

Observations:

Eyes were observed by a veterinarian on all animals at week 13 of the study.

All animals were observed for mortality and morbidity 2X daily. Clinical signs and symptoms were recorded weekly. Body wts. and food consumption were also recorded weekly as well as palpation for masses. Blood samples were collected at termination of the study following a 12 hour fast.

Organ wts. were recorded for fresh tissues and included the following:

Brain, heart, liver, kidneys, testes and epididymides. Fixed organs were weighed and included: thyroid, ovaries, adrenals and pituitary.

Necropsies were also performed on mice found dead. Tissues examined histopathologically included the following (list excerpted from the report).

Tissue Preservation:

The following tissues from each animal were preserved in 10% neutral buffered formalin:

- Brain (fore-, mid-, hind-
- Pituitary
- Spinal Cord (three levels)
- Eyes (with Harderian Gland)
- Salivary Gland
- Trachea
- Esophagus
- Thyroid (with Parathyroids)
- Thymus
- Heart
- Lung and mainstem bronchi (two coronal sections including all lobes)
- Liver (two lobes)
- Gallbladder
- Stomach
- Spleen
- Pancreas
- Adrenals
- Kidneys
- Small Intestine
- Large Intestine
- Mesenteric Lymph Nodes
- Urinary Bladder
- Testes (with Epididymides) (males)
- Prostate (males)
- Ovaries (females)
- Uterus (females)
- Sternum (with marrow)
- Skeletal Muscle
- Sciatic Nerve
- Gross Lesions

Histopathology:

Two sections of the spinal cord and the preserved tissues (including gross lesions) from all control and high-dose animals as well as kidneys from the Groups 2, 3, and 4 animals were examined histopathologically. Each tissue was embedded in Paraplast®, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Statistical Analyses:

Appropriate statistical analyses of the data were performed. See report for details.

Results:

Analytical methods used by the laboratory allowed an assay of 2,4-D values in the feed. Values closely approximated 0, 20, 50, 150, and 300 ppm in the feed.

Ocular lesions were not found at the end of the study. Feed intake in all groups was not significantly varied from one another. No changes in body wts. were seen in females. Only a slight but nonsignificant reduction in final wts. at 13 weeks was seen in males when compared to controls. Weights were 26.7 ± 1.14g in controls and 25.9 ± 1.40g in the 90 mg/kg group males. This effect is only a 3% reduction and is not considered a significant change. All groups of animals in both sexes increased their food wastage by 10 weeks into the study. Alopecia was significantly increased in females in groups receiving 15 mg/kg and more of test material. Males exhibited similar rates of alopecia in all groups.

Two females died during the study which included a single control animal and 1 female at 90 mg/kg.

Hematology:

Hematocrit, hemoglobin, red blood cell, platelet and reticulocyte parameters were not significantly different from controls in all male dosage groups. Slight increases in WBC values which were statistically significant were seen in males treated with 15 or 90 mg/kg. Females exhibited changes from control group values in WBC's at only 15 or 45 mg/kg and in platelets and reticulocyte values at 90 and 45 mg/kg respectively. These increased, changes, are not considered biologically significant nor chemically related since other hematologic parametes show no changes coinciding with these noted increases. Red cell morphology is a subjective parameter in which there was a significant increase in the severity or grading scores in males at 15 and 45 mg/kg. However, a reduction in scores to control values at the HDT (90 mg/kg) is noted. The same type activity is noted for treated females.

Ratios of Organ to Body Wts.:
Males:

Males treated with 15 mg/kg and 90 mg/kg exhibited an increase in pituitary wt. ratios compared to controls.

Kidney wt. ratios in males of both 45 and 90 mg/kg treatment groups were reduced and liver wt. ratios were reduced only at the 90 mg/kg treatment level while only at 15 mg/kg were adrenal wt. ratios increased.

Females:

Absolute pituitary and adrenal gland wts. were increased at 5 and 90 mg/kg.

Ovarian weight to body weight ratios were decreased only in females treated with 15 mg/kg. Brain wt. ratios were less than control group values in groups of females treated with 5, 45 and 90 mg/kg.

Organ to Brain Wt. Ratio:
Males:

Pituitary wt. to brain wt. ratios were increased at 15, 45 and 90 mg/kg. Adrenal wt. ratios were increased at 15 mg/kg.

Females:

Pituitary wt. to brain ratios were increased in groups treated at 5 and 90 mg/kg. In addition, kidney wt. ratios were increased at 90 mg/kg. Adrenal wt. ratios were also increased in females treated at 5 and 90 mg/kg.

Histopathology:
Males:

Kidneys showed an increased homogeneity and altered tinctorial properties of the cytoplasm and decreased intracellular/intruluminal vacuolization in the cortex in 3 male animals at 5 mg/kg. Only 1 control male exhibited the above kidney alterations. 9 males at 15 mg/kg, 18 males at 45 mg/kg and 20 males at 90 mg/kg exhibited renal changes with progressively more severe changes with increased dosage.

Females:

Renal changes were noted in 1, 4, 6, 12 and 14 animals in the control, 5, 15, 45 and 90 mg/kg dosage groups respectively.

Other changes noted included adrenal, fusiform cell hyperplasia, lung hyperplasia of pulmonary arteries, urinary bladder changes and alterations in ureters. These alterations did not exhibit increased numbers compared to controls.

Discussion:

The authors noted the increase in pituitary, adrenal and kidneys wts. were found in one or both sexes, but that only the kidney wt. changes were correlated histopathologically.

No compound related effects were evident in the hematology data. Spurious changes were noted in the white blood cell, reticulocyte and platelet counts were considered to be non-related to treatment.

The increases in organ to brain and or body wt. in the pituitary and adrenal gands were considered to be "possibly compound-related". Other alterations seen in the histological evaluation were considered to be of an incidental nature.

This reviewer finds that a pituitary wt. increase is noted and includes the 15 mg/kg dosage group in males. A pituitary wt increase is also noted in females at the high (90 mg/kg) dose. An increased adrenal wt. is recognized at the high dose for females only.

Kidney wt. ratios were reduced in males at both 45 and 90 mg/kg and increased in females at 90 mg/kg.

This reviewer considers a NOEL to exist for pituitary organ wt. changes as 5 mg/kg in males and 45 mg/kg in females. Male kidney wts. exhibited a NOEL of 15 mg/kg and for females of 45 mg/kg. Female adrenal wts. were increased (LEL) at 90 mg/kg and have a 45 mg/kg NOEL. A NOEL or LEL cannot be determined for adrenal wts. in males. However, renal microscopic (histopathological) alterations in either male or female mouse do not exhibit a NOEL at 5 mg/kg (LDT).

Core Evaluation: Supplementary

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