FUNDAMENTAL AND APPLIED TOXICOLOGY **29,** 78–85 (1996)
Article No. 0008

# Comparative Subchronic and Chronic Dietary Toxicity Studies on 2,4-Dichlorophenoxyacetic Acid, Amine, and Ester in the Dog[1]

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Received December 15, 1994; accepted June 30, 1995

**Comparative Subchronic and Chronic Dietary Toxicity Studies of 2,4-Dichlorophenoxyacetic Acid, Amine, and Ester in the Dog. CHARLES, J. M., DALGARD, D. W., CUNNY, H. C., WILSON, R. D., AND BUS, J. S. (1996).** *Fundam. Appl. Toxicol.* **29, 78–85.**

**Forms of 2,4-dichlorophenoxyacetic acid (2,4-D) are herbicides used in the control of a wide variety of broadleaf and woody plants. Subchronic toxicity studies in dogs were conducted on three forms of 2,4-D: the parent form, 2,4-D acid (ACID); 2,4-D dimethylamine salt (DMA); and 2,4-D 2-ethylhexyl ester (2-EHE). The three studies were designed to allow for comparison of the toxicity of the three forms. Doses in the subchronic studies, on an acid equivalent basis, were 0, 0.5 (ACID only), 1.0, 3.75, and 7.5 mg/kg/day. Treatment related findings in the three studies included reductions in body weight gain, and food consumption, and minor increases in blood urea nitrogen, creatinine, and alanine aminotransferase. The data from the three subchronic studies demonstrated the comparable toxicity of ACID, DMA, and 2-EHE and support a subchronic no observed adverse effect level (NOAEL) of 1.0 mg/kg/day for all three forms. Due to the similarity in toxicity of the three forms of 2,4-D, a 1-year chronic toxicity study was performed on the parent ACID to fully characterize the potential toxicity of 2,4-D in the dog. ACID was well tolerated at doses of 0, 1.0, 5.0, and 7.5 mg/kg/day. The clinical pathology alterations were similar to those seen in the subchronic studies and were not progressive. The histopathology alterations observed were not severe in nature and the no observed effect level in the chronic study was determined to be 1.0 mg/kg/day. There was no indication of any immunotoxic or oncogenic response in the studies. In conclusion, the findings of these studies indicate comparable toxicity among representative forms of 2,4-D and their generally low toxicity following subchronic and chronic dietary exposure in the dog.**



Forms of 2,4-dichlorophenoxyacetic acid (2,4-D) were some of the first herbicides to be registered in the United States and have been widely used in the control of broadleaf and woody plants on rangelands, lawns, golf courses, forests, roadways, parks, and agricultural land. 2,4-D is used extensively because of its efficacy and low acute toxicity. The various forms of 2,4-D are absorbed through both the roots and leaves of most plants, especially broadleaf species (EPA, 1989). 2,4-D's structure is similar to that of the plant hormone indole acetic acid and acts as a plant growth regulator that can interfere with normal hormonal action and plant growth. The acid is the parent compound, but almost all of the 2,4-D formulations in use contain the amine salts, which are more water soluble than the acid, or the ester derivatives, which are readily dissolved in an organic solvent. 2,4-D is regarded as having low mammalian toxicity potential (Carlo *et al.,* 1992). Subchronic and chronic dog studies were conducted to further expand the toxicological data base that supports the safety of 2,4-D and were also designed to fulfill EPA's reregistration data requirements. In addition to being a suitable nonrodent test species, the dog is a domestic animal with potential for exposure to 2,4-D in areas such as treated lawns.

A previous 2-year study in the dog (Hansen *et al.* 1971) was conducted prior to the establishment of Good Laboratory Practice Standards and did not include all the parameters usually evaluated in current studies. To enhance the data base, subchronic toxicity studies in dogs were conducted on three forms of 2,4-D: 2,4-D acid (ACID); 2,4-D dimethylamine salt (DMA); and 2,4-D 2-ethylhexyl ester (2-EHE). The studies were intentionally designed to allow for comparison of the toxicity of three representative forms of 2,4-D. In addition, a 1-year chronic toxicity study was performed on ACID to fully characterize the potential toxicity of 2,4-D in the dog. All studies were conducted in accordance with Good Laboratory Practice regulations and applicable toxicology guidelines for pesticide testing (EPA, 1984; EPA-FIFRA, 1990; MAFF, 1985; EEC, 1987, 1988). The results of these studies are the subject of this report.

## MATERIALS AND METHODS

***Test articles and treatments.*** All test articles were obtained from the Industry Task Force II on 2,4-D Research Data. The ACID was the technical

[1] This work was supported by Industry Task Force II on 2,4-D Research Data.

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0272-0590/96 $12.00

grade with a purity of 96.7%. The DMA test substance was a 66.7% technical grade aqueous solution (95.3% dry weight basis), and the 2-EHE had a purity of 95.1%. For all studies, the test material was admixed in fresh diets weekly, fed to the appropriate groups, and was available *ad libitum.* Each week, dietary levels were adjusted based on the previous week's body weight and food consumption in order to attain a constant average dietary intake (mg/kg/day). Commercially available Purina Certified Canine Diet No. 5007 (meal form) served as the vehicle and control article. Test materials were added directly to the Purina Diet without any other vehicle.

In the subchronic studies, diets were administered for 13 weeks to give a constant average dietary intake of ACID, and the equivalent amount of 2,4-D present as the DMA and 2-EHE. Target doses in the studies (on an acid equivalent basis) were 0, 1.0, 3.75, and 7.5 mg/kg/day. An additional dose level of 0.5 mg/kg/day was included in the ACID study. There were 4 dogs/sex/group. These doses were selected based on the results from a 28-day rangefinding study on ACID at doses of up to 20 mg/kg/day.

In the chronic study on ACID there were 5 dogs/sex/group. The doses initially selected to be administered for 1 year were 0, 1.0, 5.0, and 10.0 mg/kg/day. Due to the absence of body weight gain at the high dose level, the dose was changed from 10.0 to 7.5 mg/kg body weight/day during Week 8 of the study.

Homogeneity and stability of the test articles in the diets were verified analytically by gas chromatography prior to the initiation of the studies. Concentration analyses of the dietary formulations for each group were performed once weekly for the first 4 weeks of each study and every fourth week thereafter until the studies were terminated. These analyses indicated that all diets were homogeneous and within ±10% of targeted concentrations. Test material intake was within ±10% of targeted doses, as calculated from the diet concentration analyses, the actual food consumption, and body weight measurements in the studies.

***Laboratory animals and care.*** Male and female purebred beagle dogs (4–6 months old) were obtained from Hazleton Research Products, Inc. Prior to receipt, all dogs were vaccinated against distemper, hepatitis, leptospirosis, parainfluenza, parvovirus, and rabies. Dogs were selected for the studies based on pretreatment physical and ophthalmoscopic examinations and clinical laboratory tests. Each dog was individually housed in an elevated stainless-steel cage. A 12-hr light/dark cycle was maintained in the room and food and water were available *ad libitum.* The dogs were quarantined for at least 2 weeks prior to the initiation of dosing and were exercised in compliance with the Animal Welfare Act requirements by placing the animal in an exercise pen for approximately 15 min per day, three times weekly.

***In-life observations.*** For all studies, animals were observed for overt toxicity, moribundity, and mortality at least twice daily. Animal weights, detailed clinical observations, and food consumption were determined weekly. For the subchronic studies, clinical hematology and chemistry tests were performed prior to treatment and during Weeks 4 and 13. Urinalysis tests were performed once prior to treatment and during Week 13. In the chronic study, clinical hematology and chemistry tests were performed prior to treatment and during Weeks 4, 13, 26, 39, and 52. Urinalysis tests were performed once prior to treatment and during Weeks 26 and 52. Ophthalmoscopic examinations on all dogs were conducted prior to treatment and at study termination (either Week 13 or 52).

***Clinical pathology.*** Blood samples were collected from the jugular vein following an overnight fast. Urine samples were collected by catheterization. Hematology parameters evaluated included cell morphology, corrected leukocyte count, erythrocyte count, hematocrit, hemoglobin, leukocyte count, leukocyte differential, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, platelet count and morphology, and reticulocyte count.

Serum chemistry parameters investigated included alanine aminotransferase (ALT), albumin, alkaline phosphatase, aspartate aminotransferase, urea nitrogen (BUN), calcium, chloride, creatine kinase, creatinine, globulin, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, total cholesterol, and total protein. Urinalysis measurements included appearance, bilirubin, glucose, ketones, occult blood, pH, protein, specific gravity, and urobilinogen.

***Anatomic pathology.*** All animals surviving to study termination (either Week 13 or 52) were anesthetized by intravenous sodium thiamylal, exsanguinated, and subjected to gross and microscopic examinations. A complete necropsy was performed on all animals. The following organs were weighed: adrenals, brain, heart, kidneys, liver (with gallbladder removed), ovaries, pituitary, testes (without epididymides), and thyroid/parathyroids. The following tissues from each animal were preserved in 10% neutral-buffered formalin: adrenals, aorta, bone marrow (sternum), brain, cecum, epididymides, esophagus, eyes with optic nerve, femur, gallbladder, heart, kidneys, intestines (5 levels), lacrimal gland, liver, lung, mammary gland with skin, mesenteric lymph nodes, ovaries, pancreas, pituitary, prostate, retropharyngeal lymph node, salivary gland, sciatic nerve with adjacent muscle, spinal cord (3 levels), spleen, stomach, testes, thymus, thyroid/parathyroids, tongue, trachea, urinary bladder, uterus with vagina, and any other tissues with gross lesions. All preserved tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

***Statistics.*** Dogs selected for the studies were stratified by weight and randomly assigned to treatment groups using a computerized randomization program. Bartlett's test, performed following randomization, ensured homogeneity of body weight variances and means (Bartlett, 1937). Body weight changes, total feed consumption, clinical pathology data (except cell morphology findings and routine urinalysis data), and organ weight data of the control group were compared statistically to the data from the same sex and interval of the treated groups using ANOVA (Winer, 1971). If variances of untransformed data were heterogeneous, a series of transformations was performed in an effort to achieve variance homogeneity using Levene's test (Draper and Hunter, 1969; Levene, 1960). When the series of transformations was not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were routinely performed using Dunnett's *t* test at the 5% two-tailed probability level (Dunnett, 1955, 1964).

## RESULTS

### *Subchronic Studies*

Comparison of the results from the three subchronic dog studies are presented qualitatively in Table 1. The results are discussed in detail below.

A low incidence of clinical observations typical for beagle dogs such as dis-colored feces, mucoid or soft feces, diarrhea, few or no feces, emesis, lacrimation, alopecia, and/or sores occurred sporadically in all groups of all three studies. These observations were considered typical for beagle dogs and could not be attributed to the administration of any of the three test materials.

In all three studies the high dose group of both sexes showed lower body weight gains compared to the controls (Table 2). In two of the studies (ACID and 2-EHE), these effects were also noted in the next lowest dose. In the ACID study, all groups of both sexes showed gradual increases in body weight, but the mean total body weight gain during the study was lowest from 1 to 13 weeks in the 3.75 and 7.5 mg/kg/day groups. Dogs on 7.5 mg acid eq/kg/day of DMA had consistently lower body weights than controls and

**TABLE 1**
**Comparison of the Results from the Subchronic Studies**

| Parameter | 2,4-D administered in the diet as: Acid | Dimethylamine salt (DMA) | 2 Ethylhexyl ester (2-EHE) |
|---|---|---|---|
| Clinical observations and mortalities | — | — | — |
| Body weight gains | ↓ | ↓ | ↓ |
| Food intake | ↓ | ↓ | ↓ |
| Ophthalmic observations | — | — | — |
| Hematology | — | — | — |
| Serum chemistry | | | |
| Alanine aminotransferase | ↑ | ↑ | ↑ |
| Albumin | — | — | — |
| Alkaline phosphatase | ↓ | ↓ | ↓ |
| Aspartate aminotransferase | — | ↑ | — |
| Blood urea nitrogen | ↑ | ↑ | ↑ |
| Creatinine | ↑ | ↑ | ↑ |
| All other parameters measured | — | — | — |
| Urinalysis | — | — | — |
| Organ weights | | | |
| Adrenals | — | — | — |
| Brain | — | — | — |
| Heart | ↓ | — | — |
| Kidneys | — | — | — |
| Liver (w/o gallbladder) | — | — | — |
| Ovaries | — | — | — |
| Pituitaries | — | — | — |
| Testes (w/o epididymides) | ↓ | ↓ | ↓ |
| Thyroid (with parathyroids) | ↑ | — | ↑ |
| Gross pathology | — | — | — |
| Liver histopathology | ↑ | ↑ | ↑ |

**TABLE 3**
**NOAELs and LOELs for Various Parameters from the Subchronic Dog Studies[a]**

| Parameter | Acid | DMA | 2-EHE |
|---|---|---|---|
| Body weight gain | | | |
| LOEL | 3.75 | 7.50 | 3.75 |
| NOAEL | 1.00 | 3.75 | 1.00 |
| Alanine aminotransferase | | | |
| LOEL | 1.00 | 1.00 | 1.00 |
| NOAEL | 0.50 | — | — |
| Blood urea nitrogen | | | |
| LOEL | 3.75 | 1.00 | 1.00 |
| NOAEL | 1.00 | — | — |
| Creatinine | | | |
| LOEL | 1.00 | 1.00 | 1.00 |
| NOAEL | 0.50 | — | — |
| Testis weight | | | |
| LOEL | 3.75 | 7.50 | 7.50 |
| NOAEL | 1.00 | 3.75 | 3.75 |
| Liver histology | | | |
| LOEL | 7.50 | 7.50 | 7.50 |
| NOAEL | 3.75 | 3.75 | 3.75 |
| Overall NOAEL | 1.00 | 1.00 | 1.00 |

*Note.* Values are expressed as mg acid eq/kg/day.

[a] LOEL is defined as the lowest observed effect level with a statistically significant increase versus the controls. NOAEL is defined as the no observed adverse effect level where no biologically significant increase versus the controls was observed.

their total body weight gains from 1 to 13 weeks were less. Mean total body weight change for the 3.75 and 7.5 mg acid eq/kg/day of 2-EHE males and females were significantly lower than the controls from 1 to 13 weeks. At the dose of 1.0 mg acid eq/kg/day 2-EHE, mean total body weight change data were slightly lower for both sexes when compared to the concurrent controls, but the differences were not statistically significant.

**TABLE 2**
**Cumulative Body Weight Gains in the Subchronic Dog Studies (as Percentage of Control Body Weight Gain for 1–13 Weeks)**

| Study | 1 mg/kg | 3.75 mg/kg | 7.5 mg/kg |
|---|---|---|---|
| | | Males | |
| ACID | 107 | 52 | 55 |
| DMA | 132 | 132 | 68 |
| 2-EHE | 67 | 52* | 15* |
| | | Females | |
| ACID | 79 | 53 | 58 |
| DMA | 78 | 61 | 33* |
| 2-EHE | 75 | 38* | 50* |

* Body weight gains significantly different from control value, $p < 0.05$. SD's ranged between 0.1 and 1.0 kg for body weight gains.

Lower food consumption (approximately 15%) compared to the controls was observed in the high dose groups in all three studies. Slightly lower food consumption was also noted in the next lowest dose of the ACID and 2-EHE studies and was consistent with the body weight gain.

There were no ophthalmological abnormalities related to compound administration in any of the three studies. In all three studies, there were no apparent effects of compound administration on any of the hematology parameters examined during the treatment period.

Of the 17 clinical chemistry parameters evaluated: 12 were unaffected by treatment; 3 were consistently increased in the three studies (BUN, creatinine and alanine aminotransferase); 1 was decreased in all three studies (alkaline phosphatase); and 1 parameter (aspartate aminotransferase) was increased in only the DMA study. These changes generally followed a dose response with only minor effects, if any, seen at 1.0 mg/kg/day. As will be discussed under the histopathology below, these minor clinical pathology effects are not considered to be toxicologically significant due to the lack of gross pathology or histological observations in the kidney and only occasional minor histopathologic findings in the liver.

There were no clear compound-related effects in any of the three studies for albumin, calcium, chloride, creatine

**TABLE 4**
**Cumulative Body Weight Gains in the 1-Year Chronic Toxicity Study with 2,4-D Acid in Dogs**

| | | 2,4-D acid treatment group | | | |
|---|---|---|---|---|---|
| | Weeks | Control | 1 mg/kg | 5 mg/kg | 7.5 mg/kg |
| Cummulative body weight gain (kg)[a] | | | | | |
| Males | 1–13 | 2.7 ± 0.42 | 2.7 ± 1.00 | 1.9 ± 0.76 | 2.0 ± 1.00 |
| | 1–26 | 4.1 ± 1.00 | 3.5 ± 1.20 | 2.9 ± 1.40 | 2.8 ± 1.40 |
| | 1–39 | 4.4 ± 0.97 | 3.7 ± 1.20 | 3.0 ± 1.20 | 3.0 ± 1.30 |
| | 1–52 | 4.9 ± 1.10 | 3.8 ± 1.40 | 3.1 ± 1.20 | 3.3 ± 1.50 |
| Females | 1–13 | 2.0 ± 0.43 | 1.5 ± 0.53 | 1.4 ± 0.48 | 0.7 ± 0.46* |
| | 1–26 | 2.8 ± 0.81 | 2.0 ± 0.52 | 1.8 ± 0.66 | 1.3 ± 0.39* |
| | 1–39 | 3.1 ± 0.80 | 1.9 ± 0.19* | 2.0 ± 0.63* | 1.4 ± 0.42* |
| | 1–52 | 3.3 ± 1.30 | 2.4 ± 0.29 | 2.1 ± 0.62 | 1.2 ± 0.71* |

[a] Mean and standard deviation.
* Significantly different from control value, $p \leq 0.05$.

kinase, globulin, glucose, inorganic phosphate, potassium, sodium, total bilirubin, total cholesterol, and total protein. No treatment-related effects were noted on any of the urinalyses parameters in any of the three studies. There were no gross pathology findings attributed to treatment with any of the three test materials.

In the ACID study, testicular weights, both absolute and relative, showed a dose-related declining trend in the males at 3.75 and 7.5 mg/kg/day. For example, organ weight to body weight ratios at 3.75 and 7.5 mg/kg/day were 0.13 and 0.12, respectively, while for controls it was 0.16. In the DMA study, testicular weight ratios (testes-to-body weight and testes-to-brain weight) were somewhat lower versus the concurrent control at the dose level of 7.5 mg acid eq/kg/day. In the 2-EHE study, the absolute and relative testes weights were lowest in the males at 7.5 mg acid eq/kg/day but the differences were not significant.

Heart weights (absolute and relative) in the ACID study showed a declining trend in the males at 3.75 and 7.5 mg/kg/day and a decreased heart-to-body weight ratio was noted in females at 7.5 mg/kg/day. There was also an increase in the thyroid-to-body weight ratio noted in females at 7.5 mg/kg/day. The heart and thyroid effects were considered to be within the range of normal variation.

In the 2-EHE study, thyroid/parathyroid weights were consistently higher in the treated animals than in the controls, although none of the values were significant. Due to a total lack of a dose response, the relationship of this effect to treatment was questionable. No other differences in organ weights in any of the three studies were considered to be treatment related.

Various spontaneous disease lesions and incidental lesions were noted histologically in the studies. They were generally of low frequency and sporadic incidence and expected for dogs of the age used in the three studies.

In the ACID, DMA, and 2-EHE studies, there was a minimal increase in the average severity of perivascular chronic active inflammation in the liver of the dogs at 7.5 mg/kg/day when compared to control dogs. This finding was observed in both sexes in the ACID and DMA studies, and only in males in the 2-EHE study. The severity increase resulted from observations of slight-to-moderate severity gradings in one to three dogs.

No microscopic change was noted in the kidneys from dogs in the three studies that correlated with increases in the mean values for blood urea nitrogen and creatinine concentration. An increased incidence of inactive/juvenile prostate was observed in several top dose males compared to control males in all three studies. This effect likely reflected delayed maturation due to reduced nutrition and was not considered to be a toxic effect of the test materials.

The lowest observed effect levels (LOEL) and NOAEL for the various parameters in the three studies are presented in Table 3.

### *Chronic Study*

There were no clinical signs or mortality that were clearly attributable to ACID during the one year of treatment. Cumulative body weight gains from 1 to 52 weeks were retarded only slightly in the treated groups; statistical significance was noted only in the females and was most pronounced in the animals at 7.5 mg/kg/day (Table 4). However, the trend for body weight gain depression in Weeks 1–13 was consistent with that observed in the subchronic studies. There were occasional periods of poor weight progression and decreased feed consumption in 1 to 2 dogs within mid- and high-dose groups throughout the study.

There were no alterations noted in the hematology or urinalysis data. Selected clinical chemistry parameters are pre-

**TABLE 5**
**Selected Clinical Chemistry Parameters from the 1-Year Chronic Toxicity Study with 2,4-D Acid in Dogs**

| | | 2,4-D acid treatment group | | | |
|---|---|---|---|---|---|
| | Week | Control | 1 mg/kg | 5 mg/kg | 7.5 mg/kg |
| Urea nitrogen (mg/dl) | | | | | |
| Males | −1 | 9.0 ± 3.0 | 9.0 ± 2.8 | 10.0 ± 1.1 | 8.0 ± 1.5 |
| | 4 | 11.0 ± 2.1 | 14.0 ± 2.2 | 22.0 ± 6.1* | 24.0 ± 5.4* |
| | 13 | 12.0 ± 2.3 | 16.0 ± 3.0 | 25.0 ± 5.4* | 25.0 ± 5.9* |
| | 26 | 12.0 ± 2.8 | 15.0 ± 1.9 | 26.0 ± 10.5* | 23.0 ± 4.4* |
| | 39 | 13.0 ± 2.7 | 15.0 ± 1.3 | 28.0 ± 10.0* | 22.0 ± 4.2* |
| | 52 | 12.0 ± 1.8 | 15.0 ± 2.9 | 26.0 ± 9.9* | 24.0 ± 7.7* |
| Females | −1 | 10.0 ± 1.6 | 11.0 ± 3.0 | 9.0 ± 1.9 | 11.0 ± 2.6 |
| | 4 | 12.0 ± 2.1 | 16.0 ± 3.1 | 18.0 ± 2.9* | 30.0 ± 5.0* |
| | 13 | 14.0 ± 1.6 | 16.0 ± 5.7 | 20.0 ± 1.4* | 27.0 ± 3.5* |
| | 26 | 15.0 ± 2.9 | 17.0 ± 3.3 | 20.0 ± 1.9 | 28.0 ± 7.0* |
| | 39 | 15.0 ± 2.2 | 16.0 ± 2.5 | 22.0 ± 0.8* | 27.0 ± 4.9* |
| | 52 | 13.0 ± 1.7 | 16.0 ± 3.7 | 20.0 ± 2.6* | 30.0 ± 4.3* |
| Creatinine (mg/dl) | | | | | |
| Males | −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* | 1.2 ± 0.18* |
| | 13 | 0.8 ± 0.04 | 0.9 ± 0.05 | 1.2 ± 0.11* | 1.2 ± 0.13* |
| | 26 | 0.9 ± 0.05 | 1.0 ± 0.00 | 1.4 ± 0.09* | 1.3 ± 0.10* |
| | 39 | 0.9 ± 0.04 | 1.0 ± 0.10 | 1.5 ± 0.16* | 1.3 ± 0.18* |
| | 52 | 1.0 ± 0.04 | 1.0 ± 0.11 | 1.5 ± 0.19* | 1.4 ± 0.12* |
| Females | −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* | 1.1 ± 0.08* | 1.4 ± 0.13* |
| | 13 | 0.9 ± 0.08 | 0.9 ± 0.17 | 1.2 ± 0.11* | 1.4 ± 0.22* |
| | 26 | 1.0 ± 0.08 | 1.0 ± 0.05 | 1.3 ± 0.15* | 1.5 ± 0.28* |
| | 39 | 0.9 ± 0.08 | 0.9 ± 0.10 | 1.3 ± 0.07* | 1.5 ± 0.30* |
| | 52 | 0.9 ± 0.12 | 0.9 ± 0.06 | 1.5 ± 0.05* | 1.7 ± 0.27* |
| Total cholesterol (mg/dl) | | | | | |
| Males | −1 | 186 ± 31.4 | 168 ± 42.8 | 190 ± 22.9 | 191 ± 37.1 |
| | 4 | 158 ± 21.0 | 169 ± 29.9 | 201 ± 17.7* | 193 ± 17.8 |
| | 13 | 153 ± 14.6 | 157 ± 22.1 | 205 ± 25.1* | 190 ± 26.2 |
| | 26 | 130 ± 19.0 | 143 ± 16.4 | 194 ± 26.5* | 171 ± 22.2* |
| | 39 | 126 ± 7.4 | 133 ± 17.4 | 175 ± 28.7* | 164 ± 14.9* |
| | 52 | 127 ± 10.5 | 129 ± 16.6 | 174 ± 36.7* | 167 ± 23.8* |
| Females | −1 | 154 ± 27.3 | 184 ± 59.4 | 181 ± 27.4 | 167 ± 7.5 |
| | 4 | 137 ± 9.8 | 172 ± 10.9* | 189 ± 15.3* | 194 ± 48.8 |
| | 13 | 139 ± 19.3 | 187 ± 59.9 | 192 ± 19.6 | 177 ± 36.7 |
| | 26 | 159 ± 33.0 | 146 ± 20.9 | 198 ± 23.5 | 182 ± 34.5 |
| | 39 | 132 ± 22.3 | 133 ± 11.7 | 193 ± 43.8* | 168 ± 39.2 |
| | 52 | 178 ± 99.5 | 200 ± 86.5 | 187 ± 15.7 | 160 ± 41.5 |
| Glucose (mg/dl) | | | | | |
| Males | −1 | 114.0 ± 7.9 | 118.0 ± 6.8 | 112.0 ± 7.6 | 116.0 ± 8.4 |
| | 4 | 101.0 ± 3.9 | 106.0 ± 3.8 | 102.0 ± 5.4 | 94.0 ± 5.4 |
| | 13 | 100.0 ± 6.2 | 97.0 ± 8.5 | 89.0 ± 4.9 | 91.0 ± 8.4 |
| | 26 | 104.0 ± 5.4 | 101.0 ± 6.7 | 94.0 ± 8.3 | 93.0 ± 4.3* |
| | 39 | 96.0 ± 0.9 | 96.0 ± 5.1 | 85.0 ± 4.6* | 81.0 ± 5.5* |
| | 52 | 94.0 ± 3.0 | 92.0 ± 9.4 | 82.0 ± 3.2* | 80.0 ± 3.4* |
| Females | −1 | 114.0 ± 1.5 | 110.0 ± 5.0 | 109.0 ± 3.0 | 111.0 ± 4.9 |
| | 4 | 107.0 ± 4.1 | 108.0 ± 5.0 | 104.0 ± 6.3 | 90.0 ± 11.5* |
| | 13 | 98.0 ± 6.1 | 99.0 ± 3.0 | 91.0 ± 7.7 | 90.0 ± 4.8 |
| | 26 | 107.0 ± 2.7 | 105.0 ± 5.9 | 97.0 ± 8.7 | 94.0 ± 9.6* |
| | 39 | 97.0 ± 4.2 | 94.0 ± 6.6 | 88.0 ± 7.0 | 81.0 ± 5.7* |
| | 52 | 94.0 ± 6.3 | 97.0 ± 2.1 | 81.0 ± 6.0* | 79.0 ± 2.6* |

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

**TABLE 6**
**Selected Immunotoxicological Parameters from the 1-Year Chronic Toxicity Study with 2,4-D Acid in Dogs**

| | | 2,4-D acid treatment group | | | |
|---|---|---|---|---|---|
| | Week | Control | 1 mg/kg | 5 mg/kg | 7.5 mg/kg |
| Leukocyte count (TH/UL)[a] | | | | | |
| Males | −1 | 11.8 ± 3.68 | 11.0 ± 3.79 | 9.3 ± 1.88 | 10.5 ± 0.82 |
| | 4 | 9.2 ± 1.18 | 9.8 ± 2.02 | 9.3 ± 2.07 | 10.7 ± 2.26 |
| | 13 | 10.5 ± 1.11 | 8.9 ± 0.88 | 9.0 ± 1.55 | 9.1 ± 2.10 |
| | 26 | 10.3 ± 0.64 | 9.6 ± 2.60 | 8.8 ± 0.99 | 10.4 ± 2.74 |
| | 39 | 8.8 ± 1.15 | 9.4 ± 3.32 | 9.8 ± 1.64 | 10.5 ± 2.80 |
| | 52 | 10.8 ± 0.95 | 9.2 ± 2.09 | 10.9 ± 2.65 | 11.7 ± 2.32 |
| Females | −1 | 11.5 ± 2.08 | 11.1 ± 2.91 | 11.3 ± 2.70 | 10.7 ± 2.95 |
| | 4 | 10.4 ± 2.20 | 10.8 ± 1.41 | 10.6 ± 0.97 | 10.4 ± 3.42 |
| | 13 | 10.1 ± 2.20 | 12.2 ± 5.50 | 10.5 ± 1.07 | 9.8 ± 1.34 |
| | 26 | 10.5 ± 1.99 | 9.9 ± 1.20 | 9.9 ± 1.19 | 8.9 ± 1.29 |
| | 39 | 9.6 ± 1.55 | 11.0 ± 2.47 | 11.0 ± 1.85 | 10.6 ± 4.30 |
| | 52 | 11.2 ± 3.96 | 9.2 ± 1.32 | 9.2 ± 2.31 | 8.3 ± 1.73 |
| Bone marrow (sternum) histopathology | | | | | |
| Males | | | | | |
| Not remarkable | | 5/5 | 5/5 | 5/5 | 5/5 |
| Females | | | | | |
| Not remarkable | | 5/5 | 4/5 | 5/5 | 5/5 |
| Myeloid hyperplasia | | 0/5 | 1/5 | 0/5 | 0/5 |
| Mesenteric lymph node histopathology | | | | | |
| Males | | | | | |
| Not remarkable | | 5/5 | 5/5 | 5/5 | 5/5 |
| Females | | | | | |
| Not remarkable | | 5/5 | 5/5 | 5/5 | 5/5 |
| Spleen histopathology | | | | | |
| Males | | | | | |
| Not remarkable | | 5/5 | 5/5 | 5/5 | 5/5 |
| Females | | | | | |
| Not remarkable | | 5/5 | 5/5 | 5/5 | 5/5 |
| Thymus histopathology | | | | | |
| Males | | | | | |
| Not remarkable | | 5/5 | 5/5 | 5/5 | 5/5 |
| Females | | | | | |
| Not remarkable | | 5/5 | 4/5 | 5/5 | 5/5 |
| Inflammation, suppurative, parathymic | | 5/5 | 1/5 | 5/5 | 5/5 |

[a] Thousands per microliter.

sented in Table 5. Serum chemistry alterations which were considered to be treatment-related consisted of increases in urea nitrogen, creatinine, cholesterol, and alanine aminotransferase activity in the groups at 5.0 and 7.5 mg/kg/day. Glucose levels also tended to be significantly lower in these groups during the treatment period. ACID exposure had no adverse effects on any serum or biochemical measure of immune status. Selected samples of lymphoid tissue showed no gross pathological or histological changes indicative of immune system toxicity (Table 6).

There were no gross necropsy, organ weight, or ophthalmoscopic findings which were considered to be related to treatment. Histopathologic alterations included perivascular chronic active inflammation of the liver in both sexes at 5.0 and 7.5 mg/kg/day and pigment in the sinusoidal lining cells in the females of these groups. In the kidney, an increase of pigment in tubular epithelium was noted in both sexes at 5.0 and 7.5 mg/kg/day (Table 7).

## DISCUSSION

### *Subchronic Studies*

The data from the three studies builds a strong case for comparable toxicity of ACID, DMA, and 2-EHE. The three test materials were relatively well tolerated at all dose levels. There were no treatment-related deaths or illnesses, and all groups in the three studies gained weight during the experimental time periods. No treatment-related effects were noted in clinical signs, the ophthalmoscopic evaluations, hematol-

**TABLE 7**
**Selected Histopathology from the 1 Year Chronic Toxicity Study with 2,4-D Acid in Dogs**

| | 2,4-D acid treatment group (mg/kg/day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | | | | Females | | | |
| | Control | 1 | 5 | 7.5 | Control | 1 | 5 | 7.5 |
| Kidney | | | | | | | | |
| Not remarkable | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Tubule, microconcretion | 5/5 | 5/5 | 4/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| Pigment, tubular epithelium | 2/5 | 4/5 | 5/5 | 5/5 | 1/5 | 1/5 | 5/5 | 5/5 |
| Liver | | | | | | | | |
| Not remarkable | 3/5 | 4/5 | 2/5 | 1/5 | 4/5 | 2/5 | 0/5 | 1/5 |
| Inflammation, chronic active, perivascular | 1/5 | 1/5 | 3/5 | 4/5 | 0/5 | 0/5 | 4/5 | 3/5 |
| Pigment, sinusoidal lining cell | 1/5 | 0/5 | 1/5 | 1/5 | 1/5 | 2/5 | 5/5 | 4/5 |
| Leukocytosis | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 |

ogy parameters, or gross pathology. The overall NOAEL for the combined subchronic studies was 1.0 mg/kg/day (Table 3). In addition, the similarity in the toxicity responses seen in the ACID and 2-EHE studies are in excellent agreement with findings from rat pharmacokinetic studies which predicted similar toxicity of these two forms of 2,4-D due to the demonstrated rapid conversion of 2-EHE to ACID on oral absorption (Frantz and Kropscott, 1993).

The rate of body weight gain and food consumption was retarded in certain groups of treated animals of both sexes when compared with controls in all three studies. The NOAEL for body weight and feed consumption effects for the ACID and 2-EHE studies was considered to be 1.0 mg/kg/day. A NOAEL for these parameters of 3.75 mg/kg/day was established for the DMA study.

Changes in blood urea nitrogen, creatinine, and ALT were seen consistently among the three studies. The LOEL for ALT in all three studies was 1.0 mg/kg/day. A clear NOAEL of 0.5 mg/kg/day was established in the ACID study. For BUN, the LOEL was 1.0 mg/kg/day in the DMA and 2-EHE studies. The LOEL and NOAEL in the ACID study for BUN were 3.75 and 1.0 mg/kg/day, respectively. For creatinine levels, the LOEL was 1.0 mg/kg/day in all three studies. Due to the lack of histological correlates to the minimal clinical chemistry findings, an overall NOAEL of 1.0 mg/kg/day was established in the three subchronic studies. The minor nature of the clinical chemistry findings was also supported by laboratory historical control data, a lack of progression in the chronic study, and an absence of biologically relevant histology in either the liver or kidney at 1.0 mg/kg/day at 1 year.

With respect to organ weights, the only effect considered to be treatment-related was that observed in the testes. For testes, a LOEL of 3.75 mg/kg/day and a NOAEL of 1.0 mg/kg/day were established in the ACID study. In the other two studies, the LOEL was 7.5 mg/kg/day and the NOAEL was 3.75 mg/kg/day.

There were no consistent histomorphologic alterations in the three studies. There were slight to minimal increases in the average severity of perivascular chronic active inflammation in the liver in all three studies at 7.5 mg/kg/day. These increases were due to the observations of increased severity in a few animals tested. In general there was not a correlation between the severity of these liver lesions and the observed increase in ALT activity. No microscopic observations were observed in any other tissue, including the kidney, that were considered to be treatment related.

*Chronic Study*

ACID was reasonably well tolerated at doses of 7.5 mg/kg/day or less with the exception of slight decreases in body weight gains, especially in the high-dose females. The clinical pathology alterations were comparable to those seen in the subchronic studies and did not appear to be progressive in nature. The histopathology alterations observed were relatively mild. Based on the findings in the chronic study, the NOEL was determined to be 1.0 mg/kg/day.

A recent epidemiological study reported an increased risk of malignant lymphoma in pet dogs associated with assumed 2,4-D use by occupants or lawn care services (Hayes *et al.*, 1991). This report is unique in its lymphoma hypothesis, and its conclusions have been directly challenged on further analysis of the study (Carlo *et al.*, 1992). An earlier 2-year dog study (Hansen *et al.*, 1971) did not provide any evidence of preneoplastic or carcinogenic responses induced by 2,4-D. Importantly, no evidence of an immunotoxic response was noted in the current studies, which further supports the

conclusion of Munro and co-workers (1992) that 2,4-D is unlikely to stimulate development of lymphatic system tumors through compromise of the immune system.

In conclusion, the findings of these studies indicate comparable toxicity among representative forms of 2,4-D and their general lack of toxic response following subchronic and chronic dietary exposure in the dog.

## ACKNOWLEDGMENTS

The authors acknowledge the following personnel at Hazleton Washington, Inc. for their involvement in the conduct of the studies: Chino G. Anyanwu, Jeffery A. Bartley, Brenda D. Hembrey, Ronald E. James, Anthony E. Nzesi, and Elizabeth R. May. The authors also acknowledge William M. Mahlburg, liaison with the Industry Task Force II on 2,4-D Research Data, Technical Committee.

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