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Acute, Pharmacokinetic, and Subchronic Toxicological Studies of 2,4-Dichlorophenoxyacetic Acid[1,2]

S. J. GORZINSKI, R. J. KOCIBA, R. A. CAMPBELL, F. A. SMITH, R. J. NOLAN, AND D. L. EISENBRANDT

Mammalian and Environmental Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan 48674

Acute, Pharmacokinetic, and Subchronic Toxicological Studies of 2,4-Dichlorophenoxyacetic Acid. GORZINSKI, S. J., KOCIBA, R. J., CAMPBELL, R. A., SMITH, F. A., NOLAN, R. J., AND EISENBRANDT, D. L. (1987). *Fundam. Appl. Toxicol.* **9**, 423–435. The single-dose oral LD_{50} values in Fischer 344 rats for technical-grade 2,4-dichlorophenoxyacetic acid (2,4-D), esters, and salts ranged from 553 mg/kg (isobutyl ester in females) to 1090 mg/kg (dimethylamine salt in males). The LD_{50} values for the acid, esters, or salts, when expressed as acid equivalents, were consistent which suggests that the acute toxicity was due to 2,4-D per se. Acute dermal LD_{50} values in rabbits for the acid, esters, and salts were greater than 2000 mg/kg. Overall, these results indicate that the acute oral and dermal toxicity of 2,4-D are low. Pharmacokinetics were evaluated in male Fischer 344 rats given single oral doses of 10, 25, 50, 100, or 150 mg 2,4-[^{14}C]D/kg. The amount of 2,4-D in the plasma, kidney, and urine 6 hr postdosing indicated that the urinary elimination of 2,4-D was saturated in male rats given oral doses in excess of 50 mg/kg. Subchronic dietary studies in male and female Fischer 344 rats used dose levels of 0, 15, 60, 100, or 150 mg/kg/day of purified or technical-grade 2,4-D acid for 13 weeks. Body weight gains were decreased for both sexes at the higher dose levels of purified and technical-grade 2,4-D acid. Kidney weights were increased in all treated male rats and in females given the higher three dose levels of purified 2,4-D. Treatment-related cytoplasmic alterations were present in the renal proximal tubules of most rats given 60 mg/kg/day and higher of purified or technical-grade 2,4-D; a few females given 15 mg/kg/day also had slight alterations in the cytoplasm of the proximal tubules. A dose-related degenerative change was identified in the descending proximal renal tubules of all male rats given the highest three dose levels of either test material and some given 15 mg/kg/day. Dose levels of 100 or 150 mg/kg/day of either compound for both sexes produced minimal swelling and increased staining homogeneity in the liver cells and were associated with a slight elevation of liver weight and serum glutamic pyruvic transaminase activity. Higher dose levels of technical-grade and purified 2,4-D decreased total serum tetraiodothyronine levels in female rats, however, the morphology of the thyroid gland was normal. The no-observed-effect level (NOEL) was less than 15 mg/kg/day for both purified and technical-grade 2,4-D acid.

2,4-Dichlorophenoxyacetic acid (2,4-D) is a systemic herbicide available primarily in salt and ester formulations that are effective in the control of broadleaf weeds in cereal crops, sugarcane, turf, pastures, and noncrop land (Weed Science Society of America, 1974). Exposure of the general population to 2,4-D from any source is negligible (World Health Organization, 1984). The total daily intake of 2,4-D by people in a use area does not normally exceed 0.002 mg/kg body wt/day during application periods. Estimated short-term exposure of 2,4-D applicators is typically less

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[2] Acute studies conducted by Wil Research Laboratories, Cincinnati, OH, and International Research and Development Laboratories (IRDC), Mattawan, MI.

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than 0.1 mg/kg body wt/day (World Health Organization, 1984).

Several reviews on the toxicity of 2,4-D acid and formulations of 2,4-D have been published (Hill and Carlisle, 1947; Rowe and Hymas, 1954; IARC, 1977; National Research Council of Canada, 1978; Mullison, 1981; World Health Organization, 1984). These reviews primarily reflect studies that were conducted 15 to 40 years ago.

The single-dose oral LD_{50} of purified 2,4-D acid in rats was estimated to be 666 mg/kg (Hill and Carlisle, 1947). These investigators also fed 100, 200, or 400 mg 2,4-D/kg of diet (approximately 15, 30, or 60 mg/kg/day) to young male rats for 1 month and reported normal food intake and growth rate. Commercial-grade 2,4-D was fed (Rowe and Hymas, 1954) at approximate dose levels of 5, 15, 50, 150, and 500 mg/kg body wt/day for 113 days. Decreased body weights, increased liver weight, and microscopic hepatocellular swelling were noted at 50 mg/kg/day. The rats did not tolerate diets providing 150 or 500 mg/kg/day. A 2-year dietary chronic toxicity and carcinogenicity study of technical-grade 2,4-D acid in Osborne–Mendel rats was conducted (Hansen *et al.*, 1971) at dietary levels of 0, 5, 25, 125, 625, or 1250 ppm (1250 ppm was approximately 60 mg/kg/day). The authors concluded that the study did not show a carcinogenic response for 2,4-D.

The metabolism of radiolabeled 2,4-D at dose levels of approximately 3 to 300 mg/kg was investigated by Khanna and Fang (1966) in adult Wistar rats. Urinary elimination was dose-dependent in both sexes with maximum radioactivity detected in tissues 6 to 8 hr after dosing. The data suggested saturation of urinary excretion at approximately 60 mg/kg.

The acute, pharmacokinetic, and subchronic studies reported herein were conducted to evaluate the toxicity of 2,4-D with state-of-the art methodologies and current production material. Technical-grade and purified 2,4-D were both evaluated in subchronic studies to determine if possible impurities in the technical material alter the toxicity of 2,4-D. The pharmacokinetic and subchronic studies provided a basis for the selection of dose levels for a subsequent 2-year chronic toxicity–oncogenicity study.

MATERIALS AND METHODS

Acute (Single-Dose) Toxicologic Studies

Test materials. The test materials for acute studies were supplied by the Industry Task Force on 2,4-D Research Data. Single-dose oral and dermal evaluations were conducted with the test materials listed in Table 1.

Single-dose oral LD_{50}. Young adult male and female Fischer 344 rats (Charles River Laboratory, Kingston, NY) were used for the acute oral LD_{50} studies. Dose groups consisted of five rats of each sex. Food was withheld overnight (approximately 18 hr) prior to dosing. Each test material was suspended in corn oil on a weight per volume basis such that the actual volume administered on a body weight basis was the same for all groups and did not exceed 4 ml. Animals were observed for overt signs of toxicity and mortality during the day of dosing and thereafter for 2 weeks.

Single-dose dermal LD_{50}. Young adult New Zealand white rabbits (Kuiper's Rabbit Ranch, Gary, IN, or Langshaw Farms, Augusta, MI) were used for the dermal studies. The stratum corneum of a 4-cm^2 area of abdominal skin of one-half of the animals was abraded with a hypodermic needle. The test materials were applied neat, occluded with a gauze bandage, and the trunk of the rabbit wrapped. After 24 hr, wrappings and residual test material were removed. All animals were observed thereafter for 2 weeks.

Single-Dose Oral Pharmacokinetics

Test material. Uniformly ^{14}C-ring-labeled 2,4-D was obtained from Pathfinder Labs (St. Louis, MO) and had a specific activity of 4.71 mCi/mmol and a radiochemical purity of 99% as determined by high-performance liquid chromatography (HPLC). Unlabeled 2,4-D acid with a purity of 99.9% was supplied by the Industry Task Force on 2,4-D research data and was used to adjust the specific activity of the 2,4-D in the dose solutions.

Experimental design. Adult male Fischer 344 rats had an average body wt of 260 ± 8 g. Groups of six rats were given single oral doses of 10, 25, 50, 100, or 150 mg/kg 2,4-[^{14}C]D. Food was withheld for approximately 12 hr prior to dosing and was returned 4 hr after dosing. The 2,4-[^{14}C]D was converted to its sodium salt and administered by gavage as an aqueous solution. The concentrations of labeled and unlabeled 2,4-D in the dose solutions

were adjusted such that the targeted dose was administered in 5 ml/kg body wt and that each rat received approximately 5 μCi of radioactivity.

Six hours after dosing, the rats were anesthetized with methoxyflurane and exsanguinated. Blood, kidneys, and all urine excreted during the first 6 hr were collected and analyzed for radioactivity. Two additional groups of two rats were given single oral doses of 10 or 150 mg/kg 2,4-[^{14}C]D; their urine was collected for 12 hr and analyzed by HPLC for 2,4-D.

Blood samples were collected in heparinized tubes and centrifuged to obtain the plasma. Urine samples were collected in dry-ice-cooled containers. Weighed aliquots of blood plasma and urine were added directly to ACS liquid scintillation fluid (Amersham/Searle Corporation, Arlington Heights, IL) and the radioactivity was quantified in a Searle Analytic, Inc., Mark III liquid scintillation spectrometer. Kidneys were oxidized in a Biological Materials oxidizer (Harvey Instruments, Hillsdale, NY) and the $^{14}CO_2$ produced was trapped in 5 M ethanolamine in 2-methoxyethanol and quantified in a liquid scintillation counter.

Urine samples were analyzed for 2,4-D by reverse-phase HPLC using both a uv detector (Milton Roy LDC Division, Riviera Beach, FL) and a Tri-Carb RAM 7500 flow-through radioactivity detector (United Technologies Packard, Downers Grove, IL). The urine was acidified (pH 3.5) and separated on a 10 × 0.8-cm 10-μm C-18 Radial-PAK column (Waters Associates, Inc., Milford, MA) using a linear solvent gradient. The flow rate was maintained at 1.5 ml/min with the mobile phase going from 20/80 (v/v) acetonitrile/0.01 M acetic acid to 100% acetonitrile in 25 min with a 5-min hold at final conditions. Under these conditions, 98% of the applied radioactivity was recovered in the column effluent.

Subchronic (13-Week) Dietary Studies

Test materials. Technical-grade 2,4-D acid was obtained from the manufacturing unit of The Dow Chemical Co. (Midland, MI). The technical-grade 2,4-D was recrystallized once from ethyl benzene and twice from perchloroethylene and vacuum-dried to produce the purified sample. Results of liquid chromatography (LC) analyses indicated purities of 97.3 and 100% for the technical-grade and purified materials, respectively. No 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was found at a detection limit of 1 ppb in either preparation.

Diet preparation and analysis. A 1% (w/w) concentrate of each 2,4-D material in Certified Purina Laboratory Chow No. 5002 (Ralston Purina Co., St. Louis, MO) was prepared weekly. The concentrate was diluted serially with the control feed using heavy-duty paddle mixers. The concentration of the test material for the diets was adjusted weekly according to group mean body weights and food consumption values to provide the designated doses on a mg/kg/day basis. Diet samples were analyzed for 2,4-D twice during each of the studies.

Animals. Male and female Fischer 344 rats, 4 weeks of age, were purchased from the Charles River Breeding Laboratories, Inc. (Wilmington, MA) and acclimated for 10 days to the laboratory environment. Animals of the same birthdate were used for both subchronic studies, however, the study with purified 2,4-D was initiated 6 days after the start of the study with technical-grade 2,4-D. Animals for each study were weighed and randomly assigned to dose groups the day prior to start of the respective study. The rats were housed singly in suspended, stainless-steel cages (Unifab Corp., Kalamazoo, MI). Food and tap water (municipal supply) were available *ad libitum* throughout the study. Animal rooms were maintained at approximately 22°C, a relative humidity of 50%, a 12-hr light/dark cycle, and 10 air changes/hr.

Experimental design. Fifteen rats per sex were given diets formulated to provide 0 (control), 15, 60, 100, or 150 mg/kg/day of either purified or technical-grade 2,4-D acid for 13 weeks. Animals were observed daily for overt signs of toxicity or changes in demeanor.

Serum biochemistry determinations (CentrifiChem 400, Methods File, Union Carbide Corp., Diagnostic Division, Rye, NY) included urea nitrogen, glutamic pyruvic transaminase activity (SGPT), alkaline phosphatase activity (AP), glucose, total protein, albumin, globulin, and total thyroxine (tetraiodothyronine, T_4; Bio-Science Laboratories, Van Nuys, CA). Serum samples were obtained from cervical blood vessels of all animals at necropsy.

Hematological determinations (Microhematocrit Centrifuge, Clay Adams Co., NY; Coulter counter Model, ZBI and Hemoglobinometer, Coulter Electronics, Hialeah, FL) for packed cell volume, hemoglobin, red blood cell count, total white blood cell count, and white cell differential counts were performed on 10 male and 10 female rats from the control and 150 mg/kg/day dose levels. Samples were obtained from the orbital sinus of unanesthetized animals 2 weeks prior to study termination.

Urinalyses were conducted concurrently on the same rats used for hematological determinations and included specific gravity (Golberg Refractometer, American Optical Co., Instrument Division, Keene, NH) and a semiquantitative estimation (Multistix, Ames Co., Elkhart, IN) of pH, glucose, protein, ketones, bilirubin, occult blood, and urobilinogen.

The rats were fasted overnight at the termination of the respective studies. Animals were weighed, euthanized, and a complete necropsy was performed. Brain, heart, liver, kidneys, thymus, and testes were weighed. Representative sections of an extensive number of organs and tissues were preserved in neutral phosphate-buffered 10% formalin fixative. The eyes of the first five rats/sex/dose level were preserved in Zenker's fixative whereas the eyes of the other rats were preserved in 10% formalin. Tissues from all organ systems (Federal Regis-

TABLE 1

SINGLE-DOSE ORAL LD_{50} RESULTS FOR TECHNICAL-GRADE ACID, ESTERS, AND SALTS OF 2,4-D IN RATS

Material	Acid[a] equivalent	Percentage[b] active	LD_{50} ± 95% C.L. (mg/kg) Male: Material tested	Male: Acid equivalent[c]	Female: Material tested	Female: Acid equivalent[c]
2,4-D acid[d]		95.0	639 (555–736)	607	764 (663–879)	726
Isooctyl ester[d]	66.4	94.0	982 (865–1114)	612	>720–<864[e]	
Dimethylamine salt[d]	83.7	67.9	1090 (959–1241)	619	863 (816–913)	490
Isobutyl ester[f]	79.8	96.1	700 (652–751)	536	553 (513–597)	424
Sodium salt[f]	94.9	90.8	876 (671–1143)	754	975 (856–1111)	840
Butoxyethanol ester[f]	65.6	97.1	887 (810–951)	564	831 (688–1002)	565
Butyl ester[f]	79.8	98.6	732 (593–904)	575	600 (545–798)	519

[a] Ratio of 2,4-D molecular wt to test material molecular wt × 100.
[b] Value is percentage of active ingredient in sample.
[c] Acid equivalent LD_{50} = acid equivalent × percentage active × material tested ÷ 10,000.
[d] LD_{50} determined by method of Thompson and Weil (1952).
[e] Not calculable.
[f] LD_{50} determined by method of Litchfield and Wilcoxon (1949).

ter, 1978) of animals treated with technical-grade 2,4-D were examined microscopically from 10 rats of each sex from the control and high-dose groups. In addition, sections of liver, kidneys, lungs, thyroid, and trachea were examined from 10 rats of each sex of the lower-dose groups. Histopathological examination of tissues from rats on the purified 2,4-D study was confined to the liver and kidneys (target organs) from 10 rats/sex/group.

Statistical evaluation. Estimations of the single-dose oral and dermal LD_{50} values were performed by the method of Litchfield and Wilcoxon (1949), or by the method of Thompson and Weil (1952). Body weights, food consumption, clinical biochemical values, appropriate hematological parameters, urine-specific gravity, fasted body weights, and absolute and relative organ weights were evaluated by a one-way analysis of variance; differences between experimental groups and the corresponding controls were examined using Dunnett's test, $\alpha = 0.05$ (Steel and Torrie, 1960). Outlying values for body weights and food consumption data were identified using a sequential outlier method (Grubbs, 1969). Body weights, organ weights, clinical biochemical values, appropriate hematological parameters and urine-specific gravity also were analyzed by two-way analysis of variance to determine whether the pattern of effects in the target organs were similar between the two test materials. No significant or meaningful dose–compound interaction was found.

Statistical analyses were regarded as exploratory because there were no predefined hypotheses. The frequency of false-positive (Type I) errors was unknown because numerous measurements were statistically compared on the same set of animals; however, the frequency was greater than the nominal alpha level. The final toxicological interpretation of the data also considered factors such as dose-response relationships, biological plausibility and consistency, and historical values.

RESULTS

Acute Studies

The results of the single-dose oral LD_{50} determinations are presented in Table 1. Values for the test materials ranged from 553 mg/kg

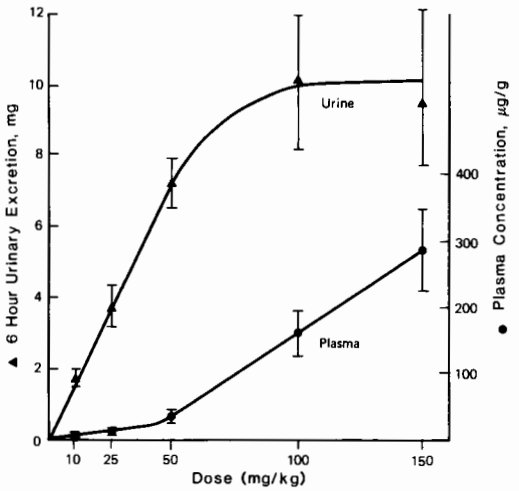

FIG. 1. Plasma ^{14}C concentrations and amounts of ^{14}C excreted in the urine 6 hr after various single oral doses of 2,4-[^{14}C]D. Each data point represents the means ± SD of six rats. Solid lines are drawn by inspection.

(isobutyl ester in females) to 1090 mg/kg (dimethylamine salt in males). The rabbit single-dose dermal LD_{50} for all compounds was greater than 2000 mg/kg. Only the dimethylamine salt of 2,4-D produced mortality when 2000 mg/kg was applied to the abraded skin of rabbits. The dermal LD_{50} for the dimethylamine salt on abraded skin subsequently was estimated at 2244 mg/kg.

Pharmacokinetic Study

Figure 1 depicts the concentration of ^{14}C in the plasma and the amount of ^{14}C in the urine of rats 6 hr after administration of single oral doses ranging from 10 to 150 mg 2,4-[^{14}C]D/kg of body wt. The concentration of ^{14}C in the plasma and the amount of ^{14}C excreted in the urine were proportional to the dose in the animals given 10, 25, or 50 mg 2,4-[^{14}C]D/kg. In rats given 100 or 150 mg 2,4-[^{14}C]D/kg, the concentration of ^{14}C in the plasma was greater than expected and the amount of ^{14}C in the urine was less than expected based on the data from the lower dose levels.

The concentration of ^{14}C in the kidney did not increase in proportion to the concentrations of ^{14}C in the plasma (Fig. 2). The kidneys from rats given the 10 or 25 mg/kg doses contained 6.6 and 5.5 times the concentration of ^{14}C found in the plasma. In contrast, the kidneys from rats given 50, 100, or 150 mg/kg contained 3.7, 2.1, and 1.6 times, respectively, the concentration of ^{14}C found in the plasma.

All ^{14}C in the urine excreted during the first 12 hr following oral administration of 10 or 150 mg 2,4-[^{14}C]D/kg eluted from the HPLC column as a single peak that had the same retention time as 2,4-D.

Subchronic Studies

Analytical results for 2,4-D concentrations in the diets ranged from 88–105% of targeted concentration. All animals survived until scheduled termination and no overt signs of

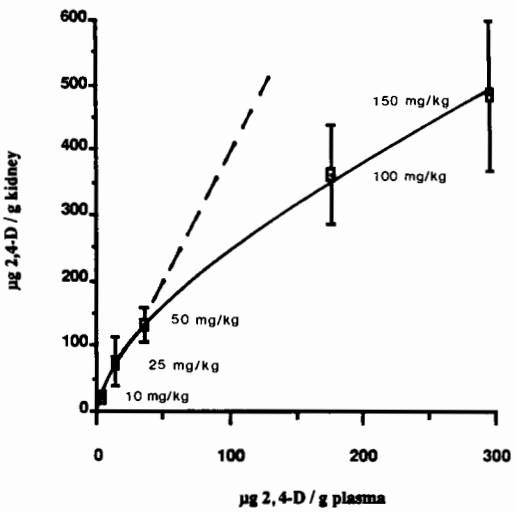

FIG. 2. Kidney concentrations versus plasma concentrations of ^{14}C 6 hr after rats were given various doses of 2,4-[^{14}C]D. Each data point represents the means ± SD kidney concentration versus the mean plasma concentration for six rats at each dose level. The solid line is drawn by inspection. The dashed line indicates the anticipated kidney concentrations if the kinetics of 2,4-D had remained first order.

TABLE 2

BODY WEIGHT GAIN AND FOOD CONSUMPTION FOR FISCHER 344 RATS FED PURIFIED OR TECHNICAL-GRADE 2,4-D FOR 13 WEEKS

		Dose level (mg/kg/day)				
	Sex	0	15	60	100	150
Purified						
Body-weight[a]	Males	174 ± 12	174 ± 10	166 ± 9	162 ± 11*	150 ± 13*
gain (g)	Females	73 ± 6	71 ± 7	65 ± 5*	65 ± 6*	57 ± 5*
Food consumption[b]	Males	17 ± 1.7	18 ± 1.4	17 ± 1.4	17 ± 1.3	16 ± 1.4
(g/rat/day)	Females	13 ± 2.7	13 ± 1.2	14 ± 1.6	12 ± 1.0	12 ± 1.2
Technical						
Body-weight[a]	Males	208 ± 15	207 ± 5	199 ± 13	197 ± 13	189 ± 9*
gain (g)	Females	82 ± 6	85 ± 5	80 ± 6	71 ± 19	65 ± 7*
Food consumption[b]	Males	18 ± 1.8	18 ± 2.3	18 ± 2.3	17 ± 1.5	17 ± 1.4
(g/rat/day)	Females	13 ± 1.7	13 ± 1.3	13 ± 1.2	12 ± 1.3	11 ± 1.1

[a] Body weight data are means ± SD for 15 rats/sex/group.
[b] Food consumption data are means ± SD of the weekly group means.
* Statistically identified difference from control, $\alpha = 0.05$, two sided.

toxicity, changes in demeanor, or scratching of feed were noted during routine daily observation. Food consumption was decreased slightly, although not statistically, for male and female rats given 150 mg/kg/day of either test material; these same animals had significantly decreased body weight gain (Table 2). Ingestion of purified 2,4-D also resulted in decreased body weight gain for both sexes given 100 mg/kg/day and females given 60 mg/kg/day.

T_4 values for females given 100 or 150 mg/kg/day of either test material and 60 mg/kg/day of purified 2,4-D were decreased significantly (Fig. 3). On the other hand, T_4 levels were increased in some treatment groups, especially the lower dose levels of females fed technical-grade 2,4-D and males fed the purified material. Several other clinical chemistry parameters were identified statistically, but were only slightly different from control values. These included slight increases in SGPT values in males and females fed technical-grade 2,4-D at 150 mg/kg/day and females at 100 mg/kg/day; slight increases in SGPT values also were present in males fed 150 mg/kg/day and females fed 100 mg/kg/day of purified 2,4-D. Females fed 150 mg/kg/day of purified or technical-grade 2,4-D had minor decreases in AP. Glucose was decreased slightly in males and females fed 100 or 150 mg/kg/

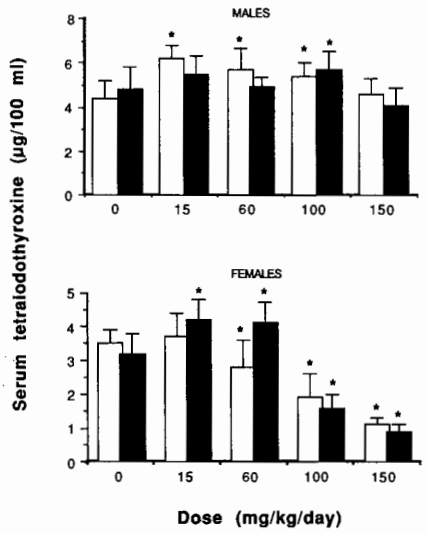

FIG. 3. Serum tetraidothyronine results for male and female rats fed purified (open bars) or technical-grade (solid bars) 2,4-D for 13 weeks. Data represent mean and SD for 15 rats/group; *statistically identified difference from control value, $\alpha = 0.05$.

day of purified 2,4-D, however there was no change in animals fed technical-grade 2,4-D. Minimal elevations in total protein values were present in females fed 150 mg/kg/day of technical-grade 2,4-D and slight increases in albumin were present in females fed 100 or 150 mg/kg/day technical-grade 2,4-D as well as females fed 150 mg/kg/day of the purified material. No treatment-related effects were detected in hematological or urinary parameters.

Fasted body weights were significantly decreased in all groups given 150 mg/kg/day of either test material and females at 60 mg/kg/day of purified 2,4-D (Table 3). Relative liver weights were increased in female rats given the highest three dose levels of purified 2,4-D, or 150 mg/kg/day of technical-grade 2,4-D. Male rats given 150 mg/kg/day of either purified or technical-grade 2,4-D had decreased liver weights associated with body weight decreases. Light microscopy of the liver (Table 4) revealed minimal swelling and increased cytoplasmic homogeneity of hepatocytes primarily in male and female rats in the highest two dose levels of both purified and technical-grade 2,4-D. This hepatocellular response was considered nonspecific and minor.

Relative kidney weights generally were increased in all treated males of both studies and in females given 60, 100, or 150 mg/kg/day of purified 2,4-D. Females treated with 150 mg/kg/day of technical-grade 2,4-D had significantly increased relative kidney weights, however this may have been due to the lower mean body weight of this group rather than being a direct, treatment-related effect.

Light microscopic examination of the kidneys revealed treatment-related changes in the proximal tubules of male and female rats (Table 4); these changes were noted with both test materials. The observations in the proximal convoluted tubules were characterized as increased epithelial cytoplasmic homogeneity in male rats and increased cytoplasmic vacuolization in female rats. Also, multifocal degeneration in the descending part of the proximal tubules was present in all male rats given the highest three dose levels, and some at 15 mg/kg/day (Figs. 4 and 5). This lesion consisted of basophilic epithelial cells that were crowded because of decreased cytoplasm. Yellow-brown pigment (probably lipofuscin) was present in the cytoplasm of some cells. The degenerative changes were accompanied by thickened basement membranes and interstitial fibrosis. Typically, the degeneration involved only a portion of the circumference of an affected tubule.

Detailed histologic examination of an extensive set of tissues did not reveal treatment-related changes in tissues other than the kidney and liver. There were no treatment-related microscopic changes in the brain, spinal cord, or peripheral nerve of rats treated with as much as 150 mg/kg/day of technical-grade 2,4-D. Also, there was no histologic correlate to the decreased T_4 values for the female; thyroid tissues were normal.

DISCUSSION

Acute oral LD_{50} values generally were similar between male and female rats that received technical-grade 2,4-D acid, esters, or salts. Correction for the percentage active ingredient and acid equivalent of 2,4-D in the test materials revealed remarkably consistent oral LD_{50} values which suggest that the acute toxicity was due to the acid. Acute dermal LD_{50} values in male and female rabbits for the acid, esters, or salts also were similar. Overall, the acute oral and dermal toxicity of 2,4-D are low.

Smith *et al.* (1980) reported that orally administered 2,4-D was rapidly absorbed ($t_{1/2}$ = 0.5 hr) and over 90% of the dose was absorbed in 6 hr. In agreement with previous investigators (Khanna and Fang, 1966; Sauerhoff *et al.*, 1977; Schulze *et al.*, 1985), we found that 2,4-D was eliminated primarily as unchanged 2,4-D in the urine. Therefore, the ^{14}C found in the plasma and kidneys

TABLE 3

FINAL BODY AND ORGAN WEIGHTS FOR FISCHER 344 RATS FED PURIFIED OR TECHNICAL-GRADE 2,4-D FOR 13 WEEKS

Dose level (mg/kg/day)	Fasted body weight (g)	Liver		Kidney	
		(g)	(g/100g)	(g)	(g/100g)
Purified 2,4-D					
Males					
0	300 ± 16	7.94 ± 0.54	2.65 ± 0.08	2.13 ± 0.16	0.71 ± 0.04
15	301 ± 13	8.13 ± 0.45	2.70 ± 0.06	2.25 ± 0.13	0.75 ± 0.03*
60	296 ± 9	7.74 ± 0.40	2.62 ± 0.08	2.30 ± 0.09*	0.78 ± 0.02*
100	288 ± 14	7.54 ± 0.53	2.61 ± 0.08	2.34 ± 0.16*	0.81 ± 0.02*
150	279 ± 14*	7.15 ± 0.46*	2.56 ± 0.06*	2.19 ± 0.14	0.79 ± 0.02*
Females					
0	169 ± 9	4.37 ± 0.28	2.58 ± 0.07	1.25 ± 0.06	0.74 ± 0.03
15	170 ± 7	4.39 ± 0.21	2.58 ± 0.08	1.27 ± 0.05	0.75 ± 0.02
60	161 ± 10*	4.34 ± 0.36	2.70 ± 0.10*	1.28 ± 0.08	0.80 ± 0.03*
100	163 ± 9	4.40 ± 0.22	2.70 ± 0.09*	1.34 ± 0.05*	0.82 ± 0.03*
150	155 ± 7*	4.33 ± 0.18	2.79 ± 0.06*	1.31 ± 0.07	0.84 ± 0.02*
Technical-grade 2,4-D					
Males					
0	310 ± 18	8.37 ± 0.78	2.70 ± 0.19	2.18 ± 0.17	0.70 ± 0.04
15	311 ± 13	8.38 ± 0.46	2.69 ± 0.08	2.29 ± 0.09	0.74 ± 0.02*
60	302 ± 14	8.08 ± 0.55	2.68 ± 0.16	2.34 ± 0.14*	0.77 ± 0.04*
100	299 ± 16	8.20 ± 0.45	2.75 ± 0.11	2.39 ± 0.13*	0.80 ± 0.02*
150	292 ± 9*	7.72 ± 0.33*	2.64 ± 0.07	2.31 ± 0.10*	0.79 ± 0.02*
Females					
0	167 ± 8	4.33 ± 0.28	2.59 ± 0.09	1.28 ± 0.08	0.77 ± 0.04
15	168 ± 8	4.42 ± 0.27	2.63 ± 0.10	1.30 ± 0.09	0.77 ± 0.03
60	166 ± 8	4.35 ± 0.25	2.63 ± 0.07	1.31 ± 0.08	0.79 ± 0.03
100	161 ± 6	4.22 ± 0.18	2.63 ± 0.06	1.30 ± 0.05	0.81 ± 0.03
150	148 ± 8*	4.29 ± 0.26	2.90 ± 0.10*	1.25 ± 0.07	0.85 ± 0.02*

Note. Values are means ± SD for 15 rats/group.
* Statistically identified difference from control using Dunnett's test, $\alpha = 0.05$.

represents primarily unchanged 2,4-D. Berndt and Koschier (1973) showed that phenoxy acids are substrates for the organic acid transport system in the kidney. The decrease in kidney/plasma 2,4-D concentrations in our studies suggests that the organic acid transport system was saturated in rats given 50 mg 2,4-D/kg or more. Saturation of this transport system would explain the decreased urinary excretion of 2,4-D, expressed as a fraction of the dose, observed in rats given more than 50 mg 2,4-D/kg. The disproportionately large increase in plasma 2,4-D concentrations following the 100 and 150 mg/kg dose levels provides additional evidence that the elimination of 2,4-D was saturated in rats given more than 50 mg 2,4-D/kg. Collectively, these data indicate that the elimination of 2,4-D in the rat was saturated following single oral doses in excess of 50 mg/kg and that the amount of 2,4-D in the rat will increase disproportionately at doses above 50 mg/kg. As a consequence, the dose–response curve for 2,4-D would be expected to increase

TABLE 4

HISTOPATHOLOGICAL RESULTS[a] IN THE KIDNEY AND THE LIVER OF FISCHER 344 RATS FED PURIFIED OR TECHNICAL-GRADE 2,4-D FOR 13 WEEKS

	Dose level (mg/kg/day)									
	0		15		60		100		150	
	P[b]	T	P	T	P	T	P	T	P	T
Kidney										
Males										
Increased epithelial cytoplasmic homogeneity, proximal convoluted tubules										
Diffuse	1	0	0	0	0	0	4	1	10	10
Multifocal to focal	3	0	2	0	6	8	6	9	0	0
Degeneration, descending proximal tubules										
Multifocal, moderate	0	0	0	0	1	2	7	9	10	10
Multifocal, slight	0	0	1	2	9	8	3	1	0	0
Females										
Epithelial cytoplasmic vacuolization, proximal convoluted tubules										
Multifocal, slight	1	0	4	0	10	7	10	9	10	10
Liver										
Males										
Swelling and increased homogeneity, hepatocytes										
Diffuse, slight	0	0	0	0	0	0	0	0	0	8
Diffuse, very slight	0	0	0	0	0	0	3	3	4	1
Females										
Swelling and increased homogeneity, hepatocytes										
Diffuse, slight	0	0	2	0	1	0	2	0	4	8
Diffuse, very slight	1	0	1	0	1	0	5	3	5	0

[a] Pathology data presented as number of rats affected out of a group size of 10 per sex.
[b] P indicates purified material; T indicates technical-grade material.

disproportionately at dose levels above 50 mg/kg.

Hill and Carlisle (1947) found no significant difference in the acute toxicity of technical-grade and purified 2,4-D samples. Our comparison of the subchronic toxicity of technical-grade and purified 2,4-D extends the results of the previous studies. The subchronic toxicity of purified and technical-grade 2,4-D materials was comparable. Decrements in body weight gain and food consumption were similar in rats given the higher dose levels of the purified or technical-grade 2,4-D. The kidney was the primary target organ and the effects on kidney weight and morphology were similar for both compounds. Mild microscopic cytoplasmic alterations were present in the renal proximal convoluted tubules of male and female rats at dose levels of 15 through 150 mg/kg/day. More substantial degenerative changes also were observed in the desending part of the proximal tubules of male rats. Both categories of renal tubular changes were dose related and were more extensive at 60 mg/kg/day and higher doses where saturation of elimination was noted in pharmacokinetic studies. Renal morphologic changes were not associated with alterations in urea nitrogen values or urinalysis data. Higher dose levels of both purified and technical-grade 2,4-D resulted in minimal light microscopic changes in the

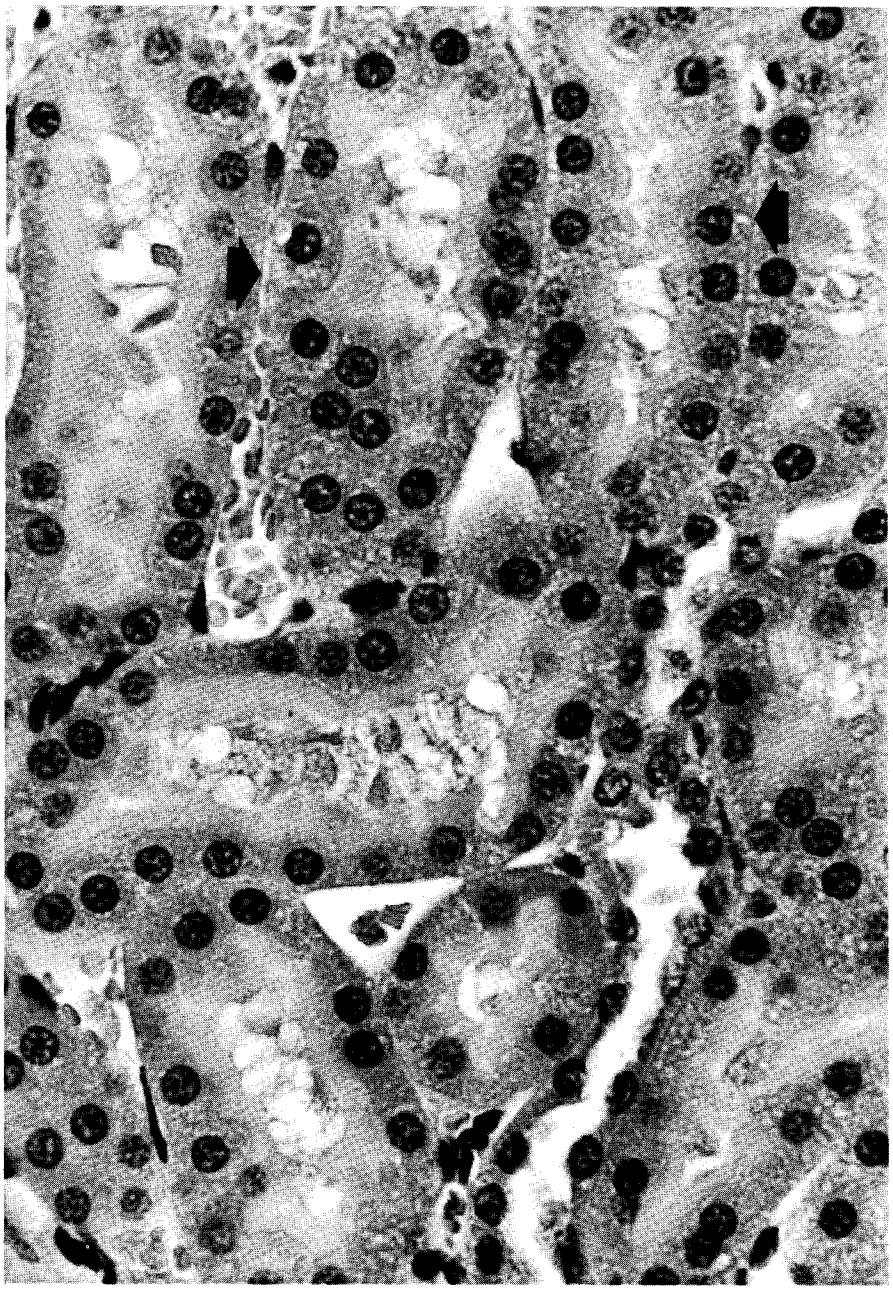

FIG. 4. Microscopic appearance of the descending part of the proximal renal tubules of a control, male rat. Note normal basement membranes and minimal interstitial connective tissue (arrows). H&E, 640×.

liver and associated elevations in liver weight and serum enzymes. The effects on the liver were considered nonspecific and minor.

Our studies revealed that T_4 was decreased in female, but not male, Fischer 344 rats treated with 100 or 150 mg/kg/day of either

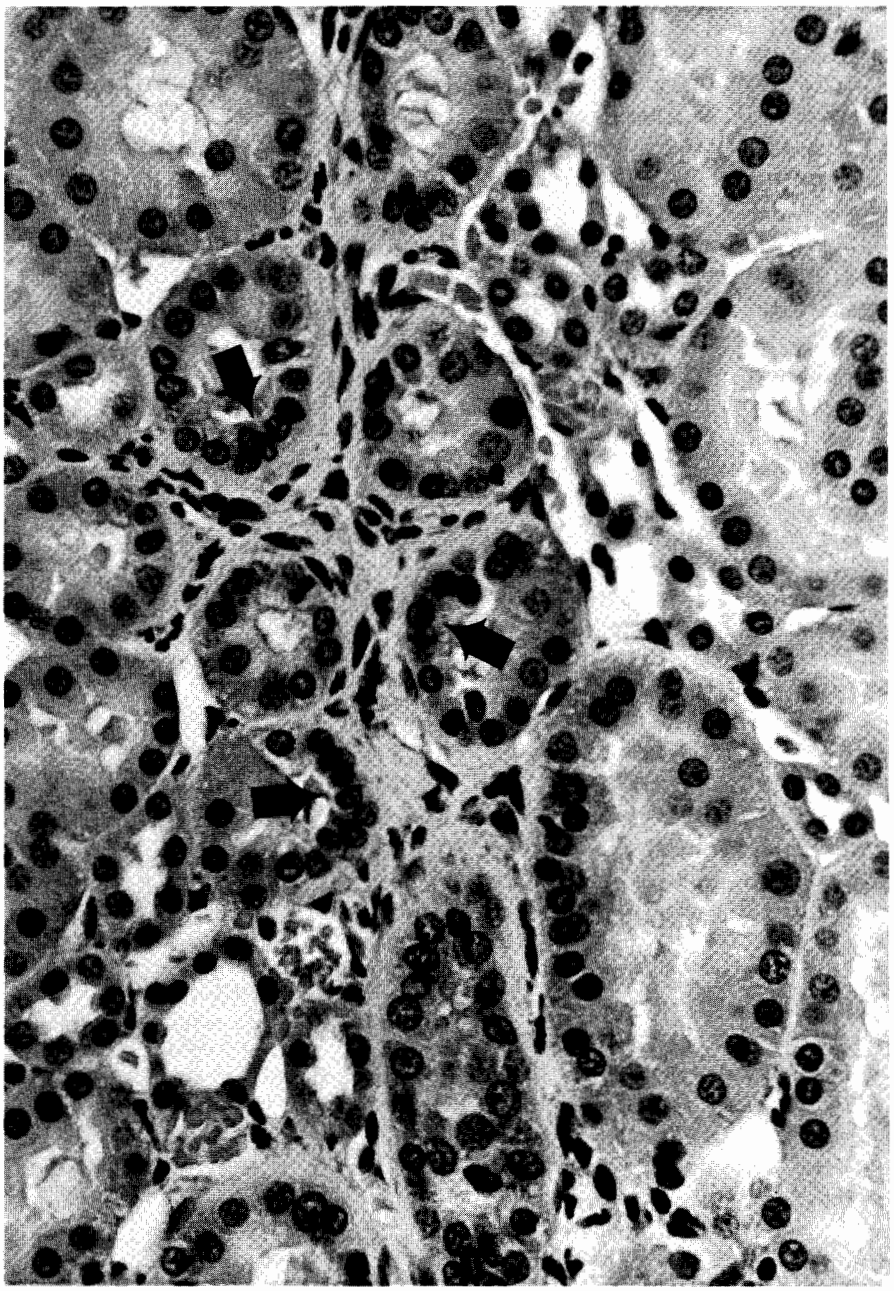

FIG. 5. Microscopic appearance of the descending part of the renal proximal tubules of a male rat treated with 150 mg/kg/day of 2,4-D for 13 weeks. Note the foci of epithelial cell degeneration (arrows) with adjacent thick basement membranes and interstitial fibrosis. H&E, 640×.

the purified or technical-grade 2,4-D, and 60 mg/kg/day of purified 2,4-D. A 50% decrease in protein-bound iodine (PBI) was reported previously (Florsheim *et al.*, 1963; Florsheim and Velcoff, 1962) in male Sprague–Dawley rats that were given seven daily subcutaneous

injections of 80 mg 2,4-D/kg. There were no effects in the Sprague–Dawley rats on the weight or histology of the thyroid gland and pituitary thyrotropic hormone was unaffected. Florsheim and co-workers concluded that the decrease in PBI was due to a competition between T_4 and 2,4-D for binding sites on serum proteins. The results from the current studies, as well as Florsheim *et al.* (1963) and Florsheim and Velcoff (1962), indicate that relatively high doses of 2,4-D may result in decreased serum levels of thyroid hormone in the absence of morphologic effects on the thyroid gland. The slight increases in T_4 levels in some dose groups were not considered related to treatment because control values were lower than historical control data and the increases were not repeatable in subsequent 13-week (Serota, 1983) and 2-year (Serota, 1986) studies with 2,4-D in rats.

A few human case reports have associated circumstantially the use of 2,4-D to a polyneuropathy syndrome (Goldstein *et al.*, 1959; Monarca and diVito, 1961; Todd, 1962; Berkley and Magee, 1963). Daily cage-side observations of the rats during the 13-week dietary studies did not reveal any overt toxicity, impaired locomotion, or other findings that would suggest a neurologic effect of either purified or technical-grade 2,4-D. Furthermore, histologic evaluation of the central and peripheral nervous systems was unremarkable. These observations are consistent with recent literature (Toyoshima *et al.*, 1985) which indicates that motor nerve conduction velocity and distal motor latency were unaffected in the tibial nerve of male Fischer 344 rats given intraperitoneal injections of 80 mg/kg of 2,4-D, 3 days per week, for 12 weeks. In addition, neurotoxicologic studies by Mattsson *et al.*, (1986a,b) revealed no untoward effects on the nervous system of male rats after dermal application of a 12% solution of the dimethylamine salt for 2 hr/day, 5 days/week, for 3 weeks. Parameters evaluated in the dermal studies were grip strength, performance on the accelerating rod, peripheral nerve electrophysiology, extensive light microscopy of the nervous system, and electron microscopy of peripheral nerves. The data from these animal studies, thus, do not support a relationship between 2,4-D exposure and polyneuropathy.

Results from the current studies indicate that 2,4-D has low oral and dermal acute toxicity. Urinary excretion of 2,4-D is saturated in rats at single oral doses in excess of 50 mg/kg. The kidney and liver were identified as target organs in the 13-week dietary studies of both technical-grade and purified 2,4-D acid. The no-observable-effect level (NOEL) for both 13-week studies was less than 15 mg/kg/day for the Fischer 344 rat.

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