

Chronic Dietary Toxicity/Oncogenicity Studies on 2,4-Dichlorophenoxyacetic Acid in Rodents¹

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Forms of 2,4-dichlorophenoxyacetic acid (collectively known as 2,4-D) are herbicides used to control a wide variety of broadleaf and woody plants. Doses in the 2-year chronic/oncogenicity rat study were 0, 5, 75, and 150 mg/kg/day. The chronic toxicity paralleled subchronic findings, and a NOEL of 5 mg/kg/day was established. A slight increase in astrocytomas observed (in males only) at 45 mg/kg/day in a previously conducted chronic rat study was not confirmed in the present study at the high dose of 150 mg/kg/day. Doses in the 2-year mouse oncogenicity studies were 0, 5, 150, and 300 mg/kg/day for females and 0, 5, 62.5, and 125 mg/kg/day for males. No oncogenic effect was noted in the study. In summary, the findings of these studies indicate low chronic toxicity of 2,4-D and the lack of oncogenic response to 2,4-D following chronic dietary exposure of 2,4-D in the rat and mouse. © 1996

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Herbicides containing 2,4-dichlorophenoxyacetic acid (collectively known as 2,4-D) were some of the first 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 the leaves of most plants, especially broadleaf species (EPA, 1989). 2,4-D's structure is similar to that of the plant-specific hormone indole acetic acid and it thus acts as a plant growth regulator. The acid is the parent compound, but many 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 potential for mammalian toxicity (Munro *et al.*, 1992).

In August 1980, the EPA issued a Data Call-In notice to registrants of 2,4-D products requiring the submission of certain data to update the database for this chemical (EPA, 1980). The registrants of 2,4-D products entered into an agreement to jointly produce the requested data as the Industry Task Force on 2,4-D Research Data (ITF-I). Among the data required by the 1980 notice were oncogenicity tests on 2,4-D acid in the rat and mouse. The ITF-I submitted the results of the completed studies in 1986, and these studies were previously summarized by Munro *et al.* (1992).

EPA reviews of the 1986 studies questioned whether sufficiently high doses had been tested in both species (EPA, 1988). In addition, EPA requested further study of a possible increased incidence of astrocytomas in the highest dose male rats, a finding that was considered not to be treatment-related (Munro *et al.*, 1992). Consequently, the EPA required that oncogenicity testing in rats and mice be repeated at higher dose levels. The new studies were performed for the Industry Task Force II on 2,4-D Research Data (ITF-II) at the Toxicology Research Laboratory of the Dow Chemical Company. These studies were completed in 1995 and are described in this report.

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).

MATERIALS AND METHODS

Test chemicals and treatments. The test chemical was obtained from the Industry Task Force II on 2,4-D Research Data. 2,4-D acid was the technical grade with a purity of 96.4%. The analytical procedure utilized for the test chemical was high-pressure liquid chromatography. For all studies, the test chemical was admixed in fresh diets (weekly, biweekly, or monthly) and was available *ad libitum*. At each diet mixing, dietary levels were adjusted based on the most recent body weight and food consumption determinations in order to deliver a constant average dietary intake (mg/kg body wt/day). Commercially available Purina Certified Rodent Chow 5002 served as the control diet. 2,4-D acid was added directly to the Purina Chow without any vehicle.

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Homogeneity and stability of the test chemical in the diets were verified analytically by gas chromatography. Concentration analyses of the dietary formulations for each group were determined weekly for the first 13 weeks and quarterly thereafter until termination. These analyses indicated that all diets were homogeneous and within $\pm 10\%$ of targeted concentrations. Test material intakes were generally within $\pm 10\%$ of targeted doses, as calculated from the diet concentration analyses, the actual food consumption, and body weight measurements in the studies.

In the 1986 2-year chronic/oncogenicity study in rats (Munro *et al.*, 1992), diets were administered to give a constant average dietary intake of 2,4-D acid of 0, 1, 5, 15, and 45 mg/kg/day. Recent comparative subchronic toxicity studies indicated that the forms of 2,4-D have comparable toxicity (Charles *et al.*, 1996). Therefore, a chronic study on 2,4-D acid would also represent the other forms of 2,4-D. Due to limited chronic effects observed at 45 mg/kg/day, and based on the results of the subchronic studies, the dietary dose levels selected for the current 2-year chronic/oncogenicity study in rats were 0, 5, 75, and 150 mg/kg/day. This spread of doses was selected so that either of the two top doses would likely attain a maximum tolerated dose (MTD), while the low dose was intended to affirm a chronic rat no-observed-effect level (NOEL). Sixty rats per sex per group were utilized, and at 12 months, 10 rats per sex per group were terminated. The remaining 50 animals per sex per group were treated with 2,4-D acid for up to 2 years.

In the 1986 2-year oncogenicity study in mice (Munro *et al.*, 1992), diets were administered for 104 weeks to give a constant average dietary intake of 2,4-D acid of 0, 5, 15, and 45 mg/kg/day. Again, due to limited effects at 45 mg/kg/day, and based on rangefinding studies, the dietary dose levels selected for the new 2-year oncogenicity study in mice were 0, 5, 150, and 300 mg/kg/day. The spread of doses was intended to accomplish the same purpose as the rat study. Due to excessive toxicity to males at the mid and high dosages, all male mice were terminated at 1 year. A new male mouse study was initiated at dose levels of 0, 5, 62.5, and 125 mg/kg/day. Sixty mice per sex per group were utilized in each study, and at 12 months in each of the studies, 10 mice per sex per group were sacrificed. The remaining 50 animals per sex per group were treated with 2,4-D acid for up to 2 years.

Laboratory animals and care. Male and female Fischer 344 rats (4 weeks old) and B6C3F1 mice (5 to 6 weeks old) were obtained from Charles River Laboratories (Portage, MI). Rats and mice were selected for the studies based on examination by a veterinarian after an acclimation period of at least 7 days. A weight randomization computer program designed to ensure homogeneity of body weights was used to select and assign the animals to the experimental groups. The animals were individually housed in elevated stainless steel, wire-mesh cages. A 12-hr light/dark cycle was maintained in the room, and food and water were available *ad libitum*.

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 first 13 weeks and monthly thereafter until study termination. Ophthalmoscopic examinations on all rats and mice were conducted prior to treatment and at study termination (Weeks 52 and 104).

Clinical pathology. In the 2-year chronic/oncogenicity rat studies, clinical chemistry, hematology, and urinalyses were performed prior to treatment and during Weeks 26, 52, 78, and 104. For the 2-year mouse study, hematology was performed during Weeks 52, 78, and 104. Blood samples were collected by orbital sinus venipuncture under methoxyflurane anesthesia following an overnight fast. Urine samples were collected from similarly fasted animals by compression of the urinary bladder.

Hematology parameters evaluated included cell morphology, corrected leukocyte count, erythrocyte count, hematocrit, hemoglobin, leukocyte count, leukocyte differential, and platelet count. Serum chemistry parameters investigated included alanine aminotransferase (ALT), albumin, aspartate aminotransferase (AST), blood urea nitrogen (BUN), lactate dehydrogenase (LDH), alkaline phosphatase, calcium, chloride, creatinine, globulin, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, total cholesterol, total protein, triiodothyronine (T3), and thyroxine (T4). Urinalysis

measurements included bilirubin, glucose, ketones, pH, protein, specific gravity, and urobilinogen.

Anatomic pathology. All animals surviving to study termination (either Week 52 or 104) were anesthetized by methoxyflurane, 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, ovaries, pituitary, testes (with epididymides), thymus, and thyroid/parathyroids. The following tissues from each animal were preserved in 10% neutral-buffered Formalin: adrenals, aorta, bone marrow (sternum), brain with brain stem, cecum, esophagus, eyes (fixed in Bouin's and stored in 70% alcohol), femur, heart, kidneys, duodenum, jejunum, ileum, colon, rectum, lacrimal gland, liver, lung, mammary gland, mesenteric lymph nodes, ovaries, pancreas, pituitary, salivary gland, sciatic nerve, skeletal muscle, spinal cord (three levels), spleen, stomach, testes with epididymides, thymus, thyroid/parathyroids, trachea, urinary bladder, uterus, and any other tissues with gross lesions. Preserved tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Statistics. Rats and mice 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 analysis of variance (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

Rat Chronic/Oncogenicity Study

Salient results in rats are presented in Tables 1 through 3. For comparative purposes, selected data from the 1986 study are also presented since these data were previously reported only in review form (Munro *et al.*, 1992). No treatment-related effects on survival were noted in either study through 2 years of treatment (Table 1). Male rats fed 150 mg/kg/day gained 11.7% less weight than controls over the entire course of the study (Table 1). Across the two studies, females fed 45, 75, or 150 mg/kg/day gained 8.8, 19.4, and 42.9% less weight, respectively. Based on these results it was apparent that the high-dose males in the current study were at, or slightly below, the MTD. Females however, clearly exceeded the MTD at 150 mg/kg/day and possibly may have exceeded the MTD at 75 mg/kg/day.

In the current study, decreased average daily food consumption was observed in males fed 150 mg/kg/day (4.7%) and females fed 75 or 150 mg/kg/day (3.9 and 11.6%) when compared to control food consumption over the 2-year period (data not shown). No effects on food consumption were seen at 2 years in the 1986 study.

Treatment-related effects in the eyes of male and female rats fed 150 mg/kg/day were observed at 2 years. Constricted

TABLE 1
Percentage of Survival and Body Weight Gains from the 2-Year Chronic/Oncogenicity Studies in F-344 Rats on 2,4-D

Sex	Month	Dose group (mg/kg body wt/day)								
		0 ^a	1 ^a	5 ^a	15 ^a	45 ^a	0 ^b	5 ^b	75 ^b	150 ^b
Percentage survival										
Males	12	98	100	100	97	100	100	100	96	98
	24	64	85	96	84	76	56	50	66	72
Females	12	97	100	100	97	100	100	100	100	100
	24	80	74	96	84	76	70	78	80	70
Cumulative body weight gains										
Males	0-12	229.6	225.4	227.1	232.3	227.9	212.2	217.9	202.4 ^c	175.0 ^c
	0-24	216.8	211.2	214.5	213.9	206.5	161.4	172.9	171.9	142.5
Females	0-12	113.4	114.1	116.7	113.5	105.2 ^c	88.3	89.7	79.0 ^c	64.5 ^c
	0-24	145.6	142.9	141.0	144.8	132.8 ^c	147.8	147.5	119.2 ^d	84.4 ^d

^a 1986 Hazleton rat study.

^b Current study.

^c Dunnett's $p \leq 0.05$.

^d Wilcoxon $p \leq 0.05$.

blood vessels and hyperreflectivity of the fundus were observed via the indirect ophthalmoscope in 18 and 14 of 36 high-dose males, respectively. Lens opacity was observed

in 34 of 35 high-dose females examined via the indirect ophthalmoscope, and these findings were confirmed during final necropsy. On histologic examination, retinal degenera-

TABLE 2
Terminal Sacrifice Organ Weight Data from the 2-Year Chronic/Oncogenicity Studies in F-344 Rats on 2,4-D

Sex	Organ ^a	Dose group (mg/kg body wt/day)								
		0 ^b	1 ^b	5 ^b	15 ^b	45 ^b	0 ^c	5 ^c	75 ^c	150 ^c
Males	Kidney weight	2.78	2.75	2.74	2.84	2.85	2.85	2.94	2.98	2.80
	Kidney to body wt	0.83	0.84	0.83	0.86	0.88	0.87	0.87	0.89	0.92
	Kidney to brain wt	1.35	1.34	1.33	1.39	1.38	1.40	1.43	1.47	1.39
	Liver weight	10.02	9.66	9.94	9.41	8.82	11.04	11.18	9.49 ^e	9.49 ^e
	Liver to body wt	3.00	2.96	2.99	2.84	2.73	3.34	3.29	2.85 ^d	3.12
	Liver to brain wt	4.85	4.70	4.83	4.61	4.28	5.42	5.46	4.68 ^d	4.72 ^d
	Thyroid weight	0.027	0.031	0.032	0.033 ^d	0.034	0.038	0.041	0.060	0.050 ^e
	Thyroid to body wt	0.008	0.0094	0.0097	0.0100 ^d	0.0106 ^d	0.0115	0.0120	0.0178	0.0161 ^e
	Thyroid to brain wt	0.0133	0.0150	0.0157	0.0163 ^d	0.0166	0.0186	0.0198	0.0292 ^e	0.0247 ^e
Females	Kidney weight	1.89	1.95	1.98	1.94	2.07 ^d	1.97	1.97	2.00	1.86 ^e
	Kidney to body wt	0.81	0.84	0.87 ^d	0.84	0.95 ^d	0.74	0.78	0.89 ^e	0.96 ^e
	Kidney to brain wt	1.01	1.04	1.06	1.03	1.10 ^d	1.04	1.05	1.06	1.00 ^e
	Liver weight	7.14	7.16	7.07	7.04	6.73	6.91	6.71	6.06 ^d	6.33 ^d
	Liver to body wt	3.07	3.10	3.10	3.06	3.07	2.60	2.66	2.69	3.27 ^d
	Liver to brain wt	3.81	3.81	3.80	3.76	3.58	3.67	3.55	3.21 ^d	3.41
	Thyroid weight	0.025	0.024	0.027	0.031 ^d	0.027	0.025	0.033	0.035 ^e	0.033 ^e
	Thyroid to body wt	0.0106	0.0105	0.0117	0.0134	0.0123	0.0094	0.0133	0.0156 ^e	0.0169 ^e
	Thyroid to brain wt	0.0131	0.0128	0.0143	0.0164 ^d	0.0144	0.0132	0.0174	0.0185 ^e	0.0176 ^e

^a Organ weights are presented in grams, organ to body weight are in grams/100 g body weight, and organ to brain weight are in grams/gram brain weight.

^b 1986 Hazleton rat study.

^c Current study.

^d Dunnett's $p \leq 0.05$.

^e Wilcoxon $p \leq 0.05$.

TABLE 3
Selected Clinical and Histopathology Data from the 1995 2-Year Chronic/Oncogenicity Study in F-344 Rats on 2,4-D

Sex	Parameter	Dose group (mg/kg body wt/day)			
		0	5	75	150
Males	ALT (mu/ml)				
	Month 12	66	61	91 ^a	68
	Month 24	38	38	68 ^b	67 ^b
	AST (mu/ml)				
	Month 12	106	95	132	103
	Month 24	78	92	115 ^b	129
	Alkaline phosphatase (mu/ml)				
	Month 12	57	57	58	55
	Month 24	53	68	74 ^b	98 ^b
	BUN (mg/dl)				
	Month 12	15	13	14	13
	Month 24	23	21	18 ^a	18 ^a
	Creatinine (mg/dl)				
	Month 12	0.6	0.6	0.7 ^a	0.7 ^a
	Month 24	0.9	0.9	0.9	0.9
	T4 (mcg/dl)				
	Month 12	3.0	3.1	2.6 ^a	0.9 ^a
	Month 24	2.2	2.2	1.5 ^a	0.8 ^a
	Serum globulin (g/dl)				
	Month 12	3.2	3.1	3.1	3.0
Month 24	4.1	4.2	4.2	3.9	
Severe bilateral retinal degeneration	0/50	0/50	0/50	15/10 ^a	
Unilateral and bilateral cataracts	1/50	3/50	3/50	8/50 ^a	
Females	ALT (mu/ml)				
	Month 12	37	42	37	33
	Month 24	48	50	61	55
	AST (mu/ml)				
	Month 12	71	80	63	68
	Month 24	92	105	136	134
	Alkaline phosphatase (mu/ml)				
	Month 12	32	32	47 ^a	54 ^a
	Month 24	47	63	84 ^b	99 ^b
	BUN (mg/dl)				
	Month 12	14	14	15	13
	Month 24	20	16	20	25
	Creatinine (mg/dl)				
	Month 12	0.7	0.7	0.8 ^a	0.7 ^a
	Month 24	0.7	0.7	0.8 ^b	0.8 ^b
	T4 (mcg/dl)				
	Month 12	2.3	2.2	0.8 ^a	0.7 ^a
	Month 24	1.9	2.2	1.3 ^a	1.1 ^a
	Serum globulin (g/dl)				
	Month 12	3.3	3.3	3.0	2.8 ^a
Month 24	4.4	4.4	4.1	4.1	
Severe bilateral retinal degeneration	0/50	0/50	0/50	42/50 ^a	
Unilateral and bilateral cataracts	3/50	2/50	4/50	39/50 ^a	

^a Dunnett's $p \leq 0.05$.

^b Wilcoxon $p \leq 0.05$.

tion and cataracts were observed in both sexes at the highest dose tested, 150 mg/kg/day. These lesions were not observed at a significant level in either study at doses of 75 mg/kg/

day and below. In the 1986 study, ophthalmic examination revealed no ocular toxicity that could be associated with 2,4-D administration at any dose up to 45 mg/kg/day.

Statistically significant decreases were identified in platelet counts at 6, 12, 18, and 24 months for males fed 150 mg/kg/day and at 24 months for males fed 75 mg/kg/day. Females fed 75 and 150 mg/kg/day exhibited decreases in erythrocyte count, platelet count, and hematocrit at most time points. These differences were usually statistically significant at 6, 12, and 18 months. The differences, although still apparent, were not as great at 24 months. There were no treatment-related effects on any of the hematological parameters at any dose level up to 45 mg/kg/day in the 1986 study.

In both studies, a number of clinical chemistry parameters were statistically identified as different from control values at one or more time points in males and/or females fed 45, 75, or 150 mg/kg/day. Alanine aminotransferase was increased in males and females at doses of 45, 75, and 150 mg/kg/day. Serum globulin was slightly decreased in males at doses of 45, 75, and 150 mg/kg/day. Serum T₄ was depressed in females at 45, 75, and 150 mg/kg/day and in males at doses of 75 and 150 mg/kg/day. Additional clinical chemistry parameters, including aspartate aminotransferase, alkaline phosphatase, creatinine, and BUN, were statistically identified as treatment-related only at the 75 and 150 mg/kg/day dose levels in this study.

No clear treatment-related effects were observed on kidney weights or histopathology of rats treated with 75 or 150 mg/kg/day. A slight but statistically significant increase in kidney weights accompanied by accumulation of brown tubular cell pigment was observed in females at 45 mg/kg/day in the 1986 study. Thyroid weights were increased in both sexes at 150 mg/kg/day and in females at 75 mg/kg/day, an observation also noted in both sexes at doses of 15 and 45 mg/kg/day in the 1986 study. However, histologic evaluation revealed nonsignificant increases in parafollicular cell nodular hyperplasia only in the female 150 mg/kg/day dose group. There was no evidence of treatment-related effects at dose levels below 75 mg/kg/day. While decreases in liver weights were statistically identified at 75 and 150 mg/kg/day, they were not considered toxicologically significant since a concomitant effect on the liver enzymes was absent. Upon histologic examination of the liver, only minimal panlobular tinctorial properties were noted in both sexes at 150 mg/kg/day. The incidence of this observation versus controls was not elevated in either study at dose levels of 75 mg/kg/day and below.

In the 1986 study, an apparent increase in astrocytomas was observed in males. The incidence in males was 1, 0, 0, 2, and 6 in the 0, 1, 5, 15, and 45 mg/kg/day groups, respectively. There was no increased incidence in females (0, 1, 2, 1, and 1 for the same dose groups, respectively). In both studies eight to nine sections of brain were evaluated histologically. The incidence of astrocytomas in the current study in males was 0 and 1 and in females 1 and 1 for the control and high-dose groups, respectively. Since no treatment-related effect was evident at an MTD dose in males, brain

sections in the low- and mid-dose animals were not evaluated. The results at 150 mg/kg/day clearly do not support an oncogenic response in rats in any organ (including brain) for 2,4-D acid.

Mouse Oncogenicity Studies

Selected results from the 2-year oncogenicity studies in mice are presented in Table 4. Mortality rates for female mice ingesting 0, 5, 150, and 300 mg/kg/day at the end of the study were 22, 16, 16, and 30%, respectively. Mortality rates for males ingesting 0, 5, 62.5, and 125 mg/kg/day were 24, 14, 16, and 14%, respectively. No treatment-related changes in clinical appearance or behavior were noted for either sex during the dosing period.

A nonstatistically significant depression in cumulative body weight gain was noted during the first 18 months in the 300 mg/kg/day female mice. Body weight gains were initially as much as 20–23% lower relative to controls; this difference appeared to stabilize around Test Day 50 and ranged between 5 and 15% until 20 months of dosing. No significant differences in body weights of treated females were observed over roughly the last 6 months of dosing. For males, a slight body weight gain decrement was noted at 125 mg/kg/day for the first 9 months of the study. No difference was noted during the remainder of the study.

No treatment-related findings were evident during the scheduled ophthalmoscopic evaluations. No statistically significant treatment-related changes in the hematology parameters evaluated in the studies were noted in either sex.

Dose-related increases of 12 and 18% for absolute and 10 and 17% for relative kidney/body weights were noted in females dosed at 150 and 300 mg/kg/day. For males, an increase of 5 and 10% for absolute and 5 and 7% for relative kidney/body weights were seen at 62.5 and 150 mg/kg body wt/day, respectively.

Treatment-related microscopic changes included minimal degeneration with regeneration of the descending portion of the proximal tubules in males at 62.5 and 125 mg/kg/day and in females at 150 and 300 mg/kg/day. In males, decreased vacuolation of proximal tubules was observed at 62.5 and 125 mg/kg/day; however, the biological significance of this finding is unclear since it was frequently associated with decreased body weights. No neoplasms were statistically identified as a result of the ingestion of 2,4-D acid. Although not statistically significant, primary hepatocellular adenomas were elevated in females.

DISCUSSION

Rat Chronic/Oncogenicity Studies

Coupled with the findings of the 1986 studies, the chronic/ oncogenicity studies in rats reported here collectively characterize the oncogenic and chronic toxicity potential of 2,4-

TABLE 4
Selected Results of the 2-Year Oncogenicity Study in B6C3F1 Mice on 2,4-D^a

	Mg/kg body wt/day			
	Control	5	62.5	125
Males				
Survival	38/50	43/50	42/50	43/50
Cumulative body weight gain (g)				
0-9 months	14.8 ± 5.8	13.1 ± 2.5	14.1 ± 2.9	13.9 ± 2.9
0-24 months	12.2 ± 2.9	12.4 ± 3.7	12.7 ± 3.3	11.6 ± 3.0
RBC counts (millions/ μ l)				
12 months	9.39 ± 0.34	9.42 ± 0.41	9.50 ± 0.56	9.40 ± 0.56
18 months	10.06 ± 1.77	9.41 ± 0.37	9.31 ± 0.36	9.31 ± 0.31
24 months	9.14 ± 0.34	9.07 ± 0.45	9.34 ± 0.94	9.25 ± 1.26
Hemoglobin (g/dl)				
12 months	14.8 ± 0.3	14.7 ± 0.6	14.7 ± 1.0	14.7 ± 0.8
18 months	15.4 ± 1.9	14.8 ± 0.5	15.9 ± 0.5	14.8 ± 0.4
24 months	14.4 ± 0.5	14.1 ± 0.7	14.4 ± 1.2	14.2 ± 1.3
Kidney/body weight ratio	2.123 ± 0.216	2.085 ± 0.183	2.239 ± 0.216 ^b	2.343 ± 0.183 ^b
Degeneration/regeneration, descending limb proximal tubules	0/50	0/50	25/50 ^b	48/50 ^b
Vacuolation of proximal tubules	0/50	2/50	39/50 ^b	48/50 ^b
Hepatocellular adenomas	12/50	9/50	13/50	16/50
Hepatocellular carcinomas	6/50	3/50	7/50	4/50
Females				
Survival	39/50	42/50	42/50	35/50
Cumulative body weight gain (g)				
0-18 months	15.6 ± 3.6	16.2 ± 2.9	15.6 ± 4.1	14.3 ± 3.2
0-24 months	13.2 ± 2.8	14.7 ± 2.7	14.4 ± 4.5	13.9 ± 3.0
RBC counts (millions/ μ l)				
12 months	9.63 ± 0.49	9.70 ± 0.53	9.44 ± 0.66	9.08 ± 0.55
18 months	9.28 ± 0.48	9.20 ± 0.45	9.16 ± 0.21	8.96 ± 0.38
24 months	9.11 ± 0.37	9.14 ± 0.62	8.86 ± 1.49	8.92 ± 0.72
Hemoglobin (g/dl)				
12 months	15.1 ± 0.7	15.1 ± 0.7	15.0 ± 1.0	14.4 ± 0.9
18 months	14.9 ± 0.5	14.7 ± 0.4	14.7 ± 0.3	14.7 ± 0.3
24 months	14.0 ± 0.8	14.2 ± 0.8	13.7 ± 2.1	14.0 ± 1.2
Kidney/body weight ratio	1.560 ± 0.187	1.487 ± 0.127	1.740 ± 0.179 ^b	1.872 ± 0.217 ^b
Degeneration/regeneration, descending limb proximal tubules	7/50	3/50	38/50 ^b	34/50 ^b
Primary hepatocellular adenomas	5/50	11/50	8/50	10/50
Hepatocellular carcinomas	1/50	2/50	0/50	1/50

^a Values reported are at the interval before study termination unless otherwise noted.

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

D over a wide range of dose levels. These studies clearly established both a NOEL and an MTD for chronic effects of 2,4-D acid in the rat. In fact, in the case of females dosed at 150 mg/kg/day, a body weight gain depression of 42.9% indicated that the MTD had been exceeded. Astrocytomas were a concern for males in the 1986 study (see Munro *et al.*, 1992, for review). This suggestive finding was not supported in the current study at an MTD dose (a three-fold higher dose level). Therefore, the current study supports the conclusion that 2,4-D does not produce astrocytomas in the rat at a dose of 150 mg/kg/day, a dose equaling the MTD.

The data from this study are also consistent with those from an earlier rat oncogenicity study performed on 2,4-D

acid under non-GLP conditions (Hansen *et al.*, 1971). In that study, doses as high as 1250 ppm (approximately 60 mg/kg/day) were administered for up to 2 years. Tumors identified were randomly distributed among types normally found in aging rats. Importantly, this study also did not find any evidence of an increased incidence in astrocytomas. The data support the interpretation that a carcinogenic effect of 2,4-D was not demonstrated.

The histopathological findings in the Hansen *et al.* (1971) study were reanalyzed by Reuber (1983). This reanalysis suggested a possible increase in lymphosarcomas associated with 2,4-D administration in both sexes. This conclusion, however, was not supported by the findings in either the 1986 or current

studies which showed no evidence of 2,4-D-induced lymphosarcomas at doses as high as 150 mg/kg/day.

The chronic toxicity observed in eye, kidney, thyroid, and liver in the 2-year rat study reported here parallels the findings noted in the subchronic studies conducted with 2,4-D acid and two of its forms (Charles *et al.*, 1996). Ocular toxicity was restricted to the 150 mg/kg/day dose group and was consistent with similar findings at 300 mg/kg/day in the subchronic studies. The occurrence of ocular toxicity at a lower dose level in the chronic versus the subchronic study suggests that the eye lesions are late developing in nature and are restricted to high-dose administration.

Since minimal low-dose kidney effects observed at 15 mg/kg/day in the 1986 study were not replicated at doses of the current study (5, 150, and 300 mg/kg/day), and findings in eye, thyroid, and liver were restricted only to high-dose treatment, a chronic toxicity NOEL of 5 mg/kg/day is affirmed in both sexes of the rat. A combined evaluation of body weight effects from the two studies demonstrates that the MTD in males is 150 mg/kg/day. In females, the MTD is 75 mg/kg/day due to significant body weight effects and supported by extreme body weight gain depression (>40%) observed at 150 mg/kg/day.

Mouse Oncogenicity Studies

There was no clear evidence of an oncogenic potential for 2,4-D acid in mice, even under conditions in which an MTD (based on body weight changes) was attained. Although a slight elevation in the incidence of primary hepatocellular adenomas was observed in females, this finding is not considered related to treatment since there was no dose response and the highest incidence was in the low-dose group. The incidence also fell within the historical control range of the laboratory. In addition, there were no increases in hepatocellular carcinomas. This is consistent with a carcinogenic screening study (Innes *et al.*, 1969) in which neonate mice were intubated with 2,4-D acid and at weaning were continued on diets containing 2,4-D for approximately 18 months. In that study, 2,4-D acid did not produce a significant increase in tumors.

The only toxic effects on B6C3F1 mice ascribed to ingestion of the 2,4-D acid were a possible slight depression of RBC parameters, minor organ weight effects, and histopathologic effects upon the kidneys at the top two dose levels of both sexes. These data are also consistent with those from an earlier mouse oncogenicity study conducted at a top dose of 45 mg/kg/day (Serota, 1987).

In conclusion, 2,4-D acid has been tested in rodents over a wide range of dose levels, including high doses at and above the MTD. The findings of these studies indicate 2,4-D's general lack of toxicity and absence of oncogenicity following chronic dietary exposure in the rat and mouse. An overall NOEL of 5 mg/kg/day for chronic effects was established in the rat.

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