1/3/86 006939

DATA EVALUATION REPORT

TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: Chronic toxicity/oncogenicity feeding study in rats.

ACCESSION NUMBER: 261129-261133.

TEST MATERIAL: Picloram; 4-amino-3,5,6-trichloropicolinic acid.

SYNONYMS: Not applicable.

STUDY NUMBER(S): K038323-034. MRID# 00155940

SPONSOR: Dow Chemical Co., Midland, MI.

TESTING FACILITY: Mammalian and Environmental Toxicology Research Laboratory, Dow Chemical U.S.A.

TITLE OF REPORT: Picloram: A two-year dietary chronic toxicity-oncogenicity study in Fisher 344 rats.

AUTHOR(S): Landry, T. D., Johnson, K. A., Cieszlak, F. S., Wolfe, E. L., and Kropscott, B. E.

REPORT ISSUED: January 3, 1986.

(1)

CONCLUSIONS:

When picloram was fed to Fischer 344 rats for 2 years in the diet at levels to give a daily intake of 20, 60, or 200 mg/kg, there were no overt signs of toxicity or dose-related effects on mortality, body weight, food consumption, or clinical laboratory findings. There was a significant ($p < 0.05$) dose-related increase in size and altered tinctorial properties of centrolobular hepatocytes in males and females dosed at 60 and 200 mg/kg/day. The change was graded slight or very slight and did not increase in severity in the course of the study. At the 6-month sacrifice, there was also an increase in absolute and/or relative liver weights at the mid and high doses. At 12 months, there was an increase in the size of hepatocytes and an increased liver weight only at the high dose. The histologic changes at study termination were accompanied by significant increases in liver weights in high-dose males only and the biological significance of the increased weight is of doubtful biologic importance since the livers of only 10 of 36 high-dose males at terminal sacrifice were weighed (see discussion). There were no increases in necrotic or hyperplastic changes in the liver at any dose. There was no oncogenic effect at any site. The LOEL, based on the histologic changes in the liver, is 60 mg/kg/day and the NOEL is 20 mg/kg/day.

Core Classification: Core Guideline.

2

A. MATERIALS:

1. Test Compound: Picloram, technical; description: a white powder, lot No. AGR 177446; purity: 33% by infrared spectrometry, 94.1% by gas chromatography; hexachlorobenzene, 197 ppm; [redacted]

2. Test Animals: Species: rat; strain: Fischer 344; mean weights: males 101-102 g, females--86 g on day 1 of study; source: Charles River Breeding Laboratories, Portage, MI.

B. STUDY DESIGN:

1. Animal Assignment: After 7 days of acclimation, animals were weighed and assigned to the following groups with a computerized randomization procedure designed to result in equivalent group mean body weights and homogeneous variance:

Test group	Dosage (mg/kg/day)	Main study (24 months) Males	Main study (24 months) Females	Interim sacrifice (6 and 12 months) Males	Interim sacrifice (6 and 12 months) Females
1 Control	0	50	50	20	20
2 Low (LDT)	20	50	50	20	20
3 Mid (MDT)	60	50	50	20	20
4 High (HDT)	200	50	50	20	20

The dose levels selected for this study were based on a 13-week feeding study in rats performed by Gorzinski et al.[1] Rats fed the test material at doses of 150, 300, or 500 mg/kg/day exhibited an increase in absolute and relative liver weights accompanied by slight histological changes (enlargement and altered cytoplasmic tinctorial properties of the centrilobular hepatocytes).

2. Diet Preparation: One percent premixes were prepared twice monthly during the study, and the diets were prepared weekly. The premixes and diets were found to be 90 percent stable over 75

---

[1] Gorzinski, S. J., Johnson, K. A., Park, C. N., Wade, C. E., Childs, R. C., Dittenber, S. A., Campbell, R. A., Phillips, J. E., and Chen, W. L. (1981). Picloram: A 13-Week Dietary Toxicity Study on Various Concentrations of Hexachlorobenzene in CDF Fischer 344 Rats. HET K-038323-(33). Unreported Data. Dow Chemical Company, Midland, MI.

3

[redacted]

days of storage and were homogeneous. The mean body weight (all animals/group) and food consumption values (20 animals/group) were used to calculate the amount of test material needed to maintain the targeted dose levels on a mg/kg body weight basis. Adjustments were made weekly for 13 weeks and monthly thereafter.

3. Results: The analysis of diets was monthly for 6 months and quarterly thereafter. Values were within acceptable limits. Table 1 summarizes the results. Analysis of diets at 13, 35, and 43 weeks for the concentration of hexachlorobenzene showed that levels were close to those calculated based on picloram containing 197 ppm hexachlorobenzene; at the 200 mg/kg/day level, the diets contained 0.78 ppm hexachlorobenzene (average of six samples). Animals received food and water ad libitum.

4. Statistics: Statistical analyses were performed for mortality (Gehan-Wilcoxon test) and body weights (Bartlett's test). Descriptive statistics (mean and standard deviation) were calculated for food consumption and appropriate clinical laboratory data. Histopathology data from all rats at all dose levels were tested for linearity; if linear, a dose-response relationship was tested (Cochran Armitage test). Tissue data from all control and high-dose groups and selected tissues from mid- and low-dose groups were analyzed (chi-square) for pairwise comparison between control and high-dose groups.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of toxicity and mortality. All rats were individually examined for palpable masses prior to study initiation, at 6 and 12 months, and monthly thereafter.

   Results: It was reported that there were no overt signs of toxicity. Individual observation data or summary data of clinical observations were not presented. A summary of palpable mass observations was presented, and these results were correlated with histologic findings. There were no increases in the number of lesions or masses observed in-life that were related to dosing. Masses were examined at necropsy and histologically diagnosed; there was a good correlation between the findings.

   When compared to controls, mortality was increased in males receiving 20 mg/kg/day; however, there were no significant changes in males receiving 60 or 200 mg/kg/day. Survival was similar in all groups of females. Representative mortality and survival data are presented in Table 2.

2. Body Weight: Rats were weighed weekly for 3 months and monthly thereafter.

TABLE 1. Dietary Analyses Picloram, as Percent of Targeted Dose (± SD), Fed to Rats for 2 Years

Targeted Dosage (mg/kg/day)	Percent of Target[a]	
	Males	Females
20	103± 9.5	103± 8.1
60	100± 7.4	101± 7.9
200	97± 7.5	98± 7.9
Premix[b]	100± 7.2	

[a]Mean of quadruplicate analyses at 11 intervals: monthly for 6 months and quarterly thereafter.

[b]Males and females were combined.

5

COE

005721

TABLE 2. Representative Results of Mortality/Percent Survival of Rats Fed Picloram for 2 Years

Dosage Group (mg/kg/day)	Mortality (Percent Survival)[a] at Month				
	6	12	18	21	24
			Males		
0	0(100)	0(100)	1 (98)	4 (92)	8 (84)
20	0(100)	0(100)	1 (98)	5 (90)	17 (66)*
60	0(100)	0(100)	2 (96)	3 (94)	8 (84)
200	0(100)	0(100)	0(100)	5 (90)	14 (72)
			Females		
0	0(100)	1 (98)	1 (98)	4 (92)	12 (76)
20	0(100)	0(100)	5 (90)	8 (84)	13 (74)
60	0(100)	0(100)	3 (94)	8 (84)	14 (72)
200	0(100)	0(100)	2 (96)	5 (90)	13 (74)

*Significantly different from control incidence ($p \leq 0.05$) with the Gehan-Wilcoxon test.

[a]Based on 50 rats/group.

6

Results: There were no effects of dosing on mean body weights. During the second year of the study mean body weights of males receiving 200 mg/kg/day tended to be slightly lower than controls but only differed significantly ($p \leq 0.05$) at week 104. Table 3 presents mean body weight data at selected intervals.

3. Food Consumption and Compound Intake: Consumption was determined for 20 rats/sex/group and the mean daily test compound intake was calculated at weekly intervals for 3 months and 1 week per month thereafter. Food consumption and body weight data were used to adjust the concentration of the test compound in the diet to maintain the targeted dosage level on a mg/kg/day basis.

   Results: Food consumption values were similar in all groups of males and females. During the first year of the study, there were sporadic significant ($p \leq 0.05$) increases and decreases in food consumption in dosed males and females when compared to controls. However, these changes were not consistent with time and there were no dose-related changes. Therefore, they were not considered of toxicologic importance. The values during the second year of the study were not compared statistically. Food efficiency was not calculated.

4. Ophthalmological examinations were not performed, but eyes were examined in situ using a wet slide technique on all animals at scheduled sacrifice.

5. Blood was collected from fasted animals by orbital sinus puncture at 6, 12, and 24 months for hematology and clinical analysis from 10 animals/sex/group. The CHECKED (X) parameters were examined:

   a. Hematology

X	Hematocrit (HCT)†	X	Total plasma protein (TP)
X	Hemoglobin (HGB)†	X	Leukocyte differential count[a]
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)†	X	Mean corpuscular HGB concentration (MCHC)
X	Platelet count†	X	Mean corpuscular volume (MCV)

[a]Control and high-dose animals.

Results: There were no toxicologically important effects on any hematologic parameter. At the 6-month interim sacrifice, mean HGB was significantly decreased ($p < 0.05$) in males receiving 60 and 200 mg/kg/day; however, there were no effects on any other parameter and no effects at 12 months. All hematologic parameters in females sacrificed at 6 and 12 months were similar in control

†Recommended by Subdivision F (October 1982) guidelines for chronic studies.

TABLE 3. Representative Results of Mean Body Weights (±SD) of Rats Fed Picloram for 2 Years

Dosage Group (mg/kg/day)	Mean Body Weights (g±SD) at Month					
	0	3	6	12	18	24
				Males		
0	101± 7	314±20	373±20	441±25	447±27	427±23
20	102± 6	324±14*	379±17	449±21	461±23	417±42
60	102± 6	320±13	375±18	440±21	450±28	417±37
200	101± 7	307±18	368±19	435±22	442±24	409±36*
				Females		
0	86± 5	182± 8	196± 8	225±14	257±21	283±21
20	86± 5	186± 8*	200± 9*	226±14	265±21	281±28
60	86± 5	184± 9	198± 9	228±19	263±24	287±21
200	86± 6	185± 8	199± 8	229±16	261±23	277±23

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

N = [illegible] all/group.

and dosed groups. At the terminal sacrifice, statistical outliers were identified but included in calculating means and standard deviations. No group statistical comparisons were made. Values for several parameters were abnormal for some leukemic rats: a male receiving 200 mg/kg/day, one female receiving 60 mg/kg/day and one female receiving 200 mg/kg/day. The leukemia resulted in an abnormally high WBC count and large standard deviations in these groups. Differential WBC counts were similar in control and high-dose groups; therefore, they were not performed on mid- and low-dose groups.

b. Clinical Chemistry

	Electrolytes		Other
	Calcium†	X	Albumin†
	Chloride†		Blood creatinine†
	Magnesium†	X	Blood urea nitrogen† (BUN)
	Phosphorus†		Cholesterol†
	Potassium†	X	Globulins
	Sodium†	X	Glucose†
	Osmolality		Total bilirubin†
	Enzymes		Total protein†
X	Alkaline phosphatase (ALP)		
	Cholinesterase		
	Creatinine phosphokinase†		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)†		
X	Serum aspartate amino transferase (also SGOT)†		

Results: It was reported that there were no toxicologically important effects on clinical chemistry parameters that were related to dosing. At 6 months, BUN was slightly but significantly decreased ($p \leq 0.05$) in females receiving 200 mg/kg/day but there were no effects in males. At 12 months, there was a significant decrease ($p \leq 0.05$) in SGPT and SGOT in males receiving 200 mg/kg/day. These values were generally within the normal range. At final sacrifice, the data were not statistically analyzed; however, statistical outliers were identified. There were abnormal values for ALP, SGOT, and SGPT in three animals that had leukemia; this caused an increase in the group mean value that was accompanied by a large standard deviation. These animals (82A2543, 200-mg/kg male; 82A2735, 60-mg/kg female; and 82A2805, 200-mg/kg female) had abnormal hematologic values also (see above).

6. Urinalyses: Urine was collected from fasted animals at the same intervals as blood. The CHECKED (X) parameters were examined.

	Appearance†	X	Glucose†
	Volume†	X	Ketones†
X	Specific gravity†	X	Bilirubin†
X	pH	X	Blood†
	Sediment (microscopic)†		Nitrite
X	Protein†	X	Urobilinogen

Results: There were no effects of dosing on any urinary parameter.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination. The CHECKED (X) tissues were collected for histological examination (these (XX) organs were also weighed):

	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta†	XX	Brain†
X	Salivary glands†	XX	Heart†	X	Peripheral nerves†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes†	X	Pituitary†
X	Duodenum†	X	Spleen†	X	Eyes (optic nerve)†
X	Jejunum†	X	Thymus†		
X	Ileum†		Urogenital		Glandular
X	Cecum†	XX	Kidneys†	X	Adrenals†
X	Colon†	X	Urinary bladder†		Lacrimal gland
	Rectum†	XX	Testes†	X	Mammary gland†
XX	Liver†	X	Epididymides	X	Parathyroids†
	Gall bladder†	X	Prostate	X	Thyroids†
X	Pancreas†	X	Seminal vesicle		Other
	Respiratory	X	Ovaries	X	Bone†
X	Trachea†	X	Uterus †	X	Skeletal muscle†
X	Lung†	X	Zymbal gland	X	Skin
X	Larynx	X	Nasal turbinates	X	All gross lesions and masses

Only the liver, kidney, gross lesions, and masses suggestive of neoplasm were processed at 6 and 12 months. For all rats that died or were sacrificed in extremis, all tissues were examined histologically.

Slides were prepared from the checked (X) tissues in control and high-dose groups at terminal sacrifice. For mid- and low-dose animals that survived to termination, adrenal, kidneys, larynx, liver, lungs, ovaries, pancreas, pituitary, spleen, testes, thyroid/parathyroid, esophagus, and trachea were examined.

Results:

a. Organ Weights: Mean liver weights and liver-to-body weight ratios were increased when compared to controls in both males and females receiving 200 mg/kg/day throughout the study (Table 4). The relative liver weight at 6 months and the absolute and relative weights of liver in high-dose males at 12 months and at termination were significantly ($p \leq 0.05$) increased over controls. Both absolute and relative liver weights at 6 months and the relative liver weights of high-dose females at 12 months were significantly ($p \leq 0.05$) increased over controls. In addition, liver weights in mid-dose males and both absolute and relative liver weights

TABLE 4. Mean Liver Weights (±SD) and Liver-to-Body Weight Ratios in Rats Fed Picloram for 2 Years

Dosage Level (mg/kg/day)	6 Months		12 Months		24 Months[b]	
	grams	percent	grams	percent	grams	percent
			Males			
0	8.507±0.293	2.392±0.097	9.984±0.757	2.461±0.102	10.822±0.985	2.711±0.278
20	8.312±0.582	2.354±0.112	10.359±0.879	2.404±0.189	11.817±1.261	2.961±0.358
60	9.057±0.471*	2.490±0.085	9.908±0.659	2.460±0.121	11.355±0.808	2.783±0.284
200	8.813±0.736	2.517±0.121*	10.809±0.720*	2.690±0.110*	12.636±1.340*	3.621±0.980*
			Females			
0	4.459±0.247	2.517±0.150	5.511±0.375	2.509±0.110	6.968±1.342	2.728±0.586
20	4.541±0.177	2.525±0.067	5.397±0.415	2.564±0.100	7.129±0.983	2.734±0.280
60	4.862±0.358*	2.681±0.144*	5.432±0.457	2.673±0.091	7.264±1.045	2.754±0.374
200	5.255±0.700*	2.703±0.140*	5.830±0.382	2.704±0.102*	7.991±1.388	3.183±0.752

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

[b]Analyzed by our reviewers using ANOVA and Duncan's test for multiple comparisons; statistical outliers were inc in the analysis.

N = 20/sex/dose.

in mid-dose females were significantly ($p < 0.05$) increased over control values at 6 months. Relative kidney weight in high-dose males and absolute kidney weight in high-dose females were slightly but significantly ($p \leq 0.05$) higher than in controls at the 6-month sacrifice; however, at 12 and 24 months data for kidney weights were similar in all groups. Weights of other organs were similar among dosed and control groups at all sacrifices.

b. Gross Pathology: At the 6- and 12-month sacrifices, the size of the liver in high-dose females was observed to be increased (4/10 and 5/10 females at 6 and 12 months, respectively). There were no increases in any gross finding in dosed rats at terminal sacrifice and all observations were those normally found in aging rats.

c. Histopathology:

1. Nonneoplastic: Table 5 summarizes histologic findings in the liver in the satellite and main groups. After 2 years of dosing with picloram, there was a statistically significant ($p \leq 0.05$) increase in the incidence of minimally increased size and altered tinctorial properties of the centrolobular hepatocytes in males and females receiving 60 or 200 mg/kg/day. There was also a significant dose-related trend. This effect was also seen at the 6-month sacrifice in both males and females at 60 and 200 mg/kg/day picloram but only at 200 mg/kg/day at the 12-month sacrifice. Progression of severity with time was not found. The incidence was lower at the terminal sacrifice and it was reported that this was probably due to the greater variability in histologic appearance of the liver in aged rats. The change was graded very slight or slight in affected males and very slight in affected females; incidence was also lower in high-dose females than in high-dose males. The incidence of very slight bile duct hyperplasia was increased in mid- and high-dose males at 24 months, but there was no increase in more severe grades of this finding; therefore, it was not considered to be compound related. Hepatocellular hyperplasia and necrosis were not increased in dosed groups. There was an increase in the incidence of foci of altered basophilic cells and the number of foci/cell over the course of the study; however, the incidence was similar in the dosed and control groups.

Nonneoplastic changes in other tissues (main groups) were considered to be incidental and not related to dosing. There was a significant ($p \leq 0.05$) increase in the severity of pancreatic acinar atrophy in mid- and high-dose males (8/50, each group) when compared to control males (1/50); however, there was no change in the number of rats in each group with some degree of acinar atrophy. Slight to

TABLE 5. Frequent Nonneoplastic Histologic Findings in the Livers of Rats Fed Picloram for 2 Years[a]

Finding/Interval	Dosage Level (mg/kg/day)							
	Males				Females			
	0	20	60	200	0	20	60	200
Finding: Increased size of centrolobular hepatocytes often accompanied by altered tinctorial properties—very slight or slight.								
6 mos.	2/10	3/10	4/10	10/10	1/10	2/10	5/10	8/10
12 mos.	1/10	3/10	2/10	7/10	2/10	0/10	3/10	10/10
24 mos.	2/50	6/50	11/50*	20/50*T	2/50	4/50	8/50*	10/50*T
Finding: Bile duct hyperplasia								
6 mos.								
Very slight	2/10	3/10	4/10	4/10	0/10	0/10	2/10	0/10
12 mos.								
Very slight	4/10	5/10	3/10	5/10	4/10	1/10	5/10	5/10
Slight/moderate	6/10	5/10	7/10	4/10	0/10	1/10	1/10	3/10
24 mos.[b]								
Very slight	3/50	2/50	12/50	9/50	25/50	30/50	32/50	25/50
Slight/moderate	46/50	45/50	37/50	40/50	20/50	15/50	12/50	18/50
Severe	1/50	2/50	1/50	1/50	0/50	0/50	0/50	0/50
Total	50/50	49/50	50/50	50/50	45/50	45/50	44/50	43/50
Finding: Hyperplasia, hepatocellular								
24 mos.[b]								
Nodular, focal	1/50	2/50	0/50	0/50	0/50	0/50	0/50	0/50
Nodular, multiple	1/50	1/50	0/50	2/50	0/50	0/50	0/50	0/50
Reactive	5/50	12/50	5/50	6/50	4/50	4/50	1/50	2/50

[a]No statistical notations were included in the report for the findings at the 6- and 12-month sacrifices.

[b]Includes animals that were sacrificed at termination and those that were sacrificed moribund or died.

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

TSignificant trend ($p \leq 0.05$).

very slight alveolar histiocytosis of the lung was significantly ($p \leq 0.05$) increased in high-dose females (24/50) when compared to controls (12/50); the report authors considered this due to chance variability and it was reported as a minor lesion of no toxicologic importance. Most males and females (all groups) had chronic progressive glomerulonephropathy. There was a slight increase in the incidence of increase in hematogenous pigment in the tubules of the kidney in the low-dose males and females, but there was no dose-related trend.

2. Neoplastic lesions: Table 6 summarizes neoplastic histologic findings in rats dosed with picloram in the main study. There were no neoplasms at the 6-month sacrifice. At the 12-month sacrifice there was one pituitary adenoma in a male receiving 60 mg/kg/day; 2/10 control males and 4/10 high-dose males had Leydig cell tumors of the testes; 2/10, 1/10, and 3/10 females at 0, 60, and 200 mg/kg/day, respectively, had endometrial stromal polyps of the uterus; and one male receiving 200 mg/kg/day had a squamous papilloma of the hard palate.

   There were no dose-related increases in tumors at any site. It was reported that the only statistically identified alteration in tumor incidence was a dose-related decrease in pituitary adenomas in males dosed with picloram. This was significantly different from control incidence ($\alpha = 0.05$, Yates chi-square test) in high-dose males and there was a significant linear trend ($\alpha = 0.02$, Cochran-Armitage test). It was reported that neoplastic nodules of the liver were split into two diagnostic categories: hepatocellular adenoma (true benign tumors) and hepatocellular hyperplasia. The latter was described as focal or multifocal nodular lesions, which are considered a regenerative change caused by liver damage due to a hepatoxin or a disease process such as leukemia in aging Fischer 344 rats. Hyperplasia occurred in 2/50, 3/50, 0/50, and 2/50 males fed 0, 20, 60, and 200 mg/kg/day picloram, respectively; no hepatocellular hyperplasia was reported in females.

D. <u>STUDY AUTHORS' CONCLUSIONS</u>:

The principal compound-related effect was increased size and altered tinctorial properties of the centrolobular hepatocytes in male and female Fischer 344 rats fed 60 and 200 mg/kg/day for 2 years. The severity of this finding did not increase over the course of the 2-year study. At the 6-month interim sacrifice, this finding was accompanied with increases in absolute or relative liver weights at a dosage level of 60 or 200 mg/kg/day; at 12 months, changes in liver weights and histologic findings were only found at 200 mg/kg/day and it was concluded that effects at 60 mg/kg/day were of minor

TABLE 6. Incidence of Neoplastic Lesions in Rats Fed Picloram for 2 Years[a]

Organ/Finding	Dosage Level (mg/kg/day)							
	Males				Females			
	0	20	60	200	0	20	60	200
Liver	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Hepatocellular carcinoma	0	1	1	1	0	0	0	0
Hepatocellular adenoma	1	1	4	2	2	1	0	2
Adenoma or carcinoma	1	2	5	3	2	1	0	2
Histiocytic sarcoma	0	2	0	0	0	0	0	0
Spleen	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Histiocytic sarcoma	2	0	0	1	0	0	0	0
Leukemia	13	19	13	11	6	11	8	5
Pituitary	(50)	(50)	(50)	(50)	(49)	(50)	(50)	(50)
Adenocarcinoma	1	2	4	1	2	3	3	5
Adenoma	24	17	18	9	23	23	25	23
Pancreas (islet cell)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Adenocarcinoma	3	2	2	0	0	0	0	0
Adenoma	10	6	5	9	1	1	2	0
Adrenal	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Cortical adenoma	0	0	1	2	0	0	0	1
Pheochromocytoma, benign	5	6	5	8	1	1	0	2
Pheochromocytoma, malignant	0	2	3	0	0	1	0	0
Pheochromocytoma, total	5	8	8	8	1	2	0	3
Testes	(50)	(50)	(50)	(50)				
Leydig cell tumor	50	48	49	49[c]				
Mesothelioma	1	2	2	2				
Bladder	(50)	(19)	(8)	(50)	(50)	(14)	(14)	(48)
Papillary adenoma	2	1	0	0	1	0	1	0
Uterus					(50)	(50)	(50)	(49)
Endometrial stromal polyp					19	16	23	18
Stromal cell sarcoma					1	1	2	0
Adenoma					1	0	0	0
Adenocarcinoma					0	0	0	1
Leiomyosarcoma					1	1	0	1
Thyroid	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Follicular adenocarcinoma	0	0	0	2	0	2	1	0
Follicular adenoma	1	1	0	1	1	1	1	0
Adenoma or carcinoma	1	1	0	3	1	3	2	0
Parafollicular adenocarcinoma	1	0	0	0	0	0	0	0
Parafollicular adenoma	8	16	12	7	3	10	4	4
Adenoma or carcinoma	9	16	12	7	3	10	4	4

(Continued)

TABLE 6. Incidence of Neoplastic Lesions in Rats Fed Picloram for 2 Years[a] (Continued)

Organ/Finding	Dosage Level (mg/kg/day)							
	Males				Females			
	0	20	60	200	0	20	60	200
Mammary gland	(50)	(19)	(17)	(50)	(50)	(17)	(23)	(50)
Adenocarcinoma	0	0	0	0	1	0	1	1
Adenoma	0	0	0	0	1	0	2	2
Fibroadenoma	4	1	4	3	5	7	7	5
Fibroma	6	2	5	3	1	0	0	2
Benign tumors	9	3	9	6	7	7	9	9
Preputial/clitoral gland	(1)	(2)	(1)	(4)	(0)	(1)	(4)	(2)
Adenoma	0	1	0	2	—	0	3	1
Tongue	(50)	(20)	(9)	(50)	(50)	(13)	(15)	(50)
Squamous papilloma	0	2	0	1	0	0	1	1
Sebaceous gland (auditory)	(2)	(2)	(0)	(3)	(0)	(0)	(0)	(0)
Carcinoma	2	1	—	3	—	—	—	—
Adenoma	0	1	—	0	—	—	—	—
Multiple organs	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Leukemia, lymphocytic granular	12	17	11	11	6	10	8	5
Histocytic sarcoma	1	2	0	1	0	0	0	0

(Concluded)

[a] Includes animals sacrificed at study termination and those of the main group that died or were sacrificed moribund in the course of the study. If a neoplasm occurred with an incidence of only 1/50, it was not tabulated unless it could be combined with other tumors in the same tissue for statistical analysis.

[b] The numbers in parentheses are the numbers of tissues examined histologically.

[c] One was malignant.

biological significance. There were no other liver changes of toxicologic importance. There were no increases in tumor incidence at any site at dietary levels of 20, 60, or 200 mg/kg/day.

There were no overt signs of toxicity or dose-related effects on mortality, body weight, food consumption, or clinical laboratory parameters. The LOEL was considered to be 60 mg/kg/day and the NOEL 20 mg/kg/day picloram.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were adequate; the summary data were supported by individual data. Recalculation of several mean values by our reviewers failed to identify any discrepancies. Histologic examination was adequate, and only a few tissues were missing or autolyzed. We agree with the authors' assessment that there was no oncogenic response. The control incidence of tumors at specific sites was generally in accordance to that found for Fischer 344 rats in other laboratories.[2] The incidences of parafollicular adenoma of the thyroid and adenoma of the islet cells of the pancreas in control males were 18 percent and 20 percent, respectively. This appears to be high for this strain of rat when compared to the average incidence historically found in several NTP bioassays where the average incidence of thyroid C-cell adenoma for over 2200 controls was 5.1 percent and for pancreatic islet cell adenoma was 3.8 percent. However, the concurrent control incidences in the present study were reasonably within the range of single bioassays conducted on 50 rats.[3]

The toxicologic importance of the histologic changes in the liver of rats receiving 60 and 200 mg/kg/day was not assessed in the final report. These changes decreased rather than increased in percent incidence over the course of the study and were not accompanied by necrotic changes or hepatocellular hyperplasia. In the interim report, it was the authors' assessment that the effects on the liver at 60 mg/kg/day were "considered of minor biological significance at most." However, there was statistical significance ($p \leq 0.05$) for hepatocyte swelling at 60 and 200 mg/kg/day at 24 months and a clear dose-related trend. It is possible that the liver changes were the result of induction of mixed function oxidases; however, enzyme induction was not monitored.

---

[2] Haseman, J.K., Huff, J. and Boorman, G.A. Use of historical control data in carcinogenicity studies in rodents. Toxicol. Pathol. 12:126-135, 1984.

[3] Ibid.

It is expected that hexachlorobenzene would cause a similar histologic change in the liver. We assess that the low levels of hexachlorobenzene contaminating the test material (less than 1 ppm at the high dose) were not responsible for the effect; however, it cannot be ruled out that the hexachlorobenzene impurity acted synergistically with picloram.

The increase in absolute and relative liver weights in males receiving 200 ppm at terminal sacrifice may be a somewhat biased finding since the organ weights were recorded only on 10 rats/sex/group at the terminal sacrifice (first 10 numerically) rather than on all rats sacrificed. It was noted that a statistical outlier in the group of males receiving 200 mg/kg was a rat with leukemia, which had resulted in reactive hyperplasia of the liver. None of the controls that had livers weighed had leukemia, although the histologic incidence was 12/50 compared to 11/50 for high-dose males. If the outlier was included, there was a significant ($p \leq 0.05$) increase in relative liver weight in high-dose (males), whereas if the outlier was not included in the analysis, the mean (3.35 percent) value was not significant. In female control and high-dose groups, one control and one high-dose rat used for organ weight measurements had leukemia and reactive hyperplasia of the liver (and were statistical outliers); no increase in mean liver weight was found when the high dose was compared to the controls. The increased liver weight in high-dose males may also have been due to an increase in fluid since there was cystic dilatation of the liver sinusoids in 30 percent of the high-dose males compared to 14 percent of controls.

Although it is possible that the rats may have tolerated a higher dose, the rationale for dose selection seems to be adequate.