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Guideline Series 83-2

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DATA EVALUATION REPORT

STUDY TYPE: Two-year dietary oncogenicity study in mice (Guideline Series 83-2)

TEST MATERIAL: 4-Amino-3,5,6-trichloropicolinic acid

TOX CHEM. NUMBER: 39

PC Code: 005101

MRID Number: 426193-01

SYNONYMS: Picloram, Tordon, Amdon, Borolin, K-Pin, Grazon

STUDY NUMBER: K-038323-058

SPONSOR: DowElanco, 9002 Purdue Road, Indianapolis, Indiana

TESTING FACILITY: The Toxicology Research Laboratory Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan

TITLE OF REPORT: Picloram: Two-year Dietary Oncogenicity Study in B6C3F1 Mice

AUTHORS: W.T. Stott, B.L. Yano, K.T. Haut, S.N. Shabrang

REPORT ISSUED: December 24, 1992

CONCLUSIONS: Doses of 0, 100, 500, or 1,000 mg/kg/day of Picloram were fed to B6C3F1 mice, 50/sex/group, for 2 years. Additional groups of mice (10/sex/dose) were sacrificed at 52 weeks.

NOEL(Systemic effects) = 1,000 mg/kg/day (HDT) (Limit dose). Although there was a significant increase in absolute and relative kidney weight in males no histopathological lesions were found to corroborate these changes. The target organ for Picloram toxicity was not identified.

LEL > 1,000 mg/kg/day

There was no evidence of an oncogenic potential of Picloram.

CORE CLASSIFICATION: Core Guideline. The study satisfies the requirements of EPA Guideline Series 83-2 for an oral oncogenicity study.

A. MATERIALS AND METHODS

1. Test Article Description

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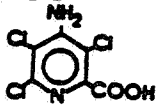
Name: 4-Amino-3,5,6-trichloropicolinic acid

Molecular formula: $C_6H_3Cl_3N_2O_2$

Structural formula:



Molecular weight: 241.5

Lot number: AGR 274601

Purity: 81.8% (average over eight determinations)

Physical property: Solid

Stability: Up to 2 years

2. Diet Preparation

The diets were prepared weekly for the first 13 weeks and at least once every 2 weeks thereafter by serially diluting test material-feed concentrate into Purina Certified Rodent Chow #5002. Initially, the concentration of the test material was calculated from the pretest body weights and food consumption data to achieve the desired intake on a mg/kg body weight basis. After that, the concentration of the test material in the diet was adjusted every 4 weeks to reflect the most recent body weight and food consumption. The purity of the test material was tested eight times during the study using HPLC and Karl Fisher water analysis. The stability of Picloram in the test diet was analyzed at all three dietary levels four times in the course of the study and once after study completion. The concentration of Picloram in the diets and the homogeneity (from the top, middle, and bottom of the sample) of the diets was determined during weeks 1, 13, 25, 39, 49, 63, 77, 91, and 103.

Results: The purity of the test material ranged from 80.3% to 82.8%. The stability of Picloram in Purina Certified Rodent Chow #5002 was at least 75 days and up to 92 days. The mean Picloram concentrations for diets analyzed at nine study intervals were 95%, 97%, and 98% of target and 92%, 96%, and 100% of target for males and females at the low, mid, and high-doses, respectively. The homogeneity of Picloram in the diets was 91.5% of the targeted concentration.

3. Animals

Species: Mouse

Strain: B6C3F1

Age: Approximately 5 weeks

Weight at initiation (day -2): Males, 20.1-25.8 g; females, 17.5-22.3 g

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Source: Charles River Breeding Laboratory, Portage, MI

Group assignment: Mice were acclimated to laboratory conditions for at least 7 days and their health status was assessed. After acclimation, animals were assigned to the following treatment groups using a randomization procedure based on body weights:

Test Group	Dose Level (mg/kg BW/day)	Satellite (1 year)		Main Study (2 years)	
		M	F	M	F
1 Control	0	10	10	50	50
2 Low dose (LDT)	100	10	10	50	50
3 Mid dose (MDT)	500	10	10	50	50
4 High dose (HDT)	1,000	10	10	50	50

Animals were housed one per cage in suspended, stainless steel cages in animal rooms designed to maintain adequate temperature, humidity, and photocycle for the species used. Food and water were available ad libitum during the prestudy and study periods.

Rationale for dose selection: The rationale for dose selection was based on data from two subchronic dietary studies in mice using up to 3,000 mg/kg/day of Picloram. The studies concluded that the NOEL for subchronic administration of Picloram to mice was less than 1,000 mg/kg/day based on increased liver weight, increased basophilia and "ground glass" appearance of centrilobular hepatocytes.

4. Statistics

Statistical analysis of food consumption, feed efficiency, and white blood cell differential counts were not performed; only means and standard deviations were reported for these parameters. Bartlett's test for equality of variances was used in analyses of body and organ weights, clinical chemistry data, and appropriate hematology data. Depending on the result of Bartlett's test, exploratory data analyses were performed by parametric or nonparametric variance analyses (ANOVA), followed by Dunnett's or Wilcoxon Rank-Sum tests with a Bonferroni correction for multiple comparisons. The incidences of specific histopathologic observations were tested for deviation from linearity, for linear trend using the Cohran-Armitage Trend test, and comparison to controls was done using a pairwise chi-square test with Yate's continuity correction. The mortality pattern differences were tested using the Gehan-Wilcoxon procedure.

5. Quality Assurance

A signed quality assurance statement, dated December 22, 1992, was provided. A GLP certification statement and a flagging statement were included; both were signed on December 21, 24, and 25, 1992, respectively.

B RESULTS

1. General Observations

All animals were examined at least once daily for morbidity, mortality, treatment-related effects, and availability of water and feed. Evaluations were also made of the skin, fur, mucous membranes, respiration, nervous system function, and behavior pattern. In addition, all animals were given detailed weekly clinical examinations including the record on progression or disappearance of palpable masses.

Results: There were no significant effects of dosing on survival in any of the groups. The survival was greater than 70% in both control and treatment groups over the 2 years. No treatment-related changes were observed in male or female mice treated with Piclorам during clinical examinations.

2. Body Weights/Food Consumption/Test Material Intake

Individual body weights were recorded before treatment, at weekly intervals for the first 13 weeks, and monthly thereafter. Food consumption was determined weekly for the first 13 weeks, and for 1 week every 4 weeks. Food efficiency (g food consumed/g body weight) was also calculated.

Results: Table 1 presents mean body weights at selected study intervals. No treatment-related differences in body weights were observed in mice throughout the study. Similarly, no toxicologically important differences in body weight gain were observed in any of the dosed groups at any point during the study.

No differences in food consumption were noted between treated and control animals throughout the study. The food efficiency data indicate that there were no significant changes during the growth phase of animals ingesting Piclorам and control animals.

3. Ophthalmoscopic Examination

Eyes were examined in all animals prior to the start of the study and at scheduled 12-month and 24-months necropsies.

Results: There were no ocular abnormalities in animals examined at the beginning of the study. In addition, no treatment-related ophthalmologic findings were noted in any dose groups during the 2 year study.

Table 1. Mean Body Weight and Percent (%) Control at Representative Intervals in Mice Fed Picloram For 2 Years[a]

Dose (mg/kg/day)	Mean Body Weight (g ± SD) and (%) Control on Day:						
	2	40	89	229	397	565	730
Males							
0	23.1 ± 1.1	28.4 ± 1.4	30.8 ± 1.8	35.3 ± 2.9	38.9 ± 4.2	36.2 ± 4.4	35.2 ± 4.0
100	23.1 ± 1.1	28.4 ± 1.4	30.3 ± 2.2	34.5 ± 3.5	39.0 ± 4.8	35.1 ± 4.7	35.0 ± 4.0
(%)	(100)	(100)	(98)	(98)	(100)	(97)	(99)
500	23.0 ± 1.1	28.2 ± 1.3	30.2 ± 1.7	34.8 ± 2.8	37.9 ± 3.8	35.7 ± 3.6	35.7 ± 3.2
(%)	(100)	(99)	(98)	(99)	(97)	(99)	(101)
1000	23.2 ± 1.1	28.4 ± 1.6	30.2 ± 1.8	34.5 ± 2.5	37.7 ± 3.0	34.7 ± 3.9	34.6 ± 2.8
(%)	(100)	(100)	(98)	(98)	(97)	(96)	(98)
Females							
0	19.9 ± 1.0	25.2 ± 1.1	26.9 ± 1.6	30.2 ± 2.9	32.5 ± 4.0	31.8 ± 5.0	33.4 ± 4.5
100	19.7 ± 1.1	24.8 ± 1.1	25.9* ± 1.2	29.7 ± 2.7	32.5 ± 4.1	31.2 ± 3.7	32.5 ± 3.8
(%)	(99)	(98)	(96)	(98)	(100)	(98)	(97)
500	19.5 ± 1.0	24.9 ± 1.2	26.1* ± 1.3	30.9 ± 3.0	32.2 ± 3.4	31.5 ± 3.0	32.2 ± 3.3
(%)	(98)	(99)	(97)	(102)	(99)	(99)	(96)
1000	19.5 ± 0.9	24.8 ± 1.1	26.6 ± 1.4	31.0 ± 2.9	34.5 ± 4.6	31.7 ± 4.8	32.3 ± 4.2
(%)	(98)	(98)	(99)	(103)	(106)	(100)	(97)

[a]Data extracted from Study No. K-038323-058, Tables 8 and 9.

*Significantly different from control mean by Dunnett's test, α=0.05.

4. Clinical Pathology

Blood was collected for hematological determinations by orbital sinus puncture from 10/sex/group at the 12-month necropsy and on the first 20/sex/dose at the 24-month necropsy. In addition, blood smears were prepared for all animals from which blood samples were collected for differential leukocyte count and for assessment of erythrocyte, leukocyte, and platelet morphology. Morphologic examinations were also done on the blood collected from the tail vein from 10/sex/group of mice surviving 18 months of Picloram treatment. The parameters (marked X) below were examined:

Hematology

X Packed cell volume (PCV)*	X Leukocyte differential count*
X Hemoglobin (HGB)*	Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)*	Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)*	Mean corpuscular volume (MCV)
X Platelet count*	Coagulation: thromboplastin time (PT)
Reticulocyte count (RETIC)	X Leukocyte morphology
X Red cell morphology	
X Platelet morphology	

*Recommended by Subdivision F (November 1984) Guidelines

Results: No treatment-related effects were observed in any of the hematological parameters in male or female mice administered Picloram for 12 or 24 months. Similarly, no changes in the differential counts were observed at 18 months.

5. Sacrifice and Pathology

All animals that died, or were sacrificed moribund or by design were necropsied after 12 months (10/sex/dose) and 24 months. Terminal body weights were recorded and a complete gross examination of tissues was performed on all animals. The necropsy included examination of external tissues and orifices, cranial cavity, brain, pituitary gland and adjacent cervical tissues, the eyes, the nasal cavity; the thoracic and abdominal cavities were exposed and the viscera were examined in situ. Terminal body weights were not recorded in moribund mice or in mice that died spontaneously. The tissues checked (X) below were preserved and stained for histopathology and the double-checked (XX) organs were also weighed.

Digestive System	Cardiovascular/Hematologic	Neurologic
X Tongue	X Aorta*	XX Brain*
X Salivary glands*	XX Heart*	Peripheral nerve* (sciatic nerve)
X Esophagus	X Bone marrow*	X Spinal cord* (three levels)
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen*	X Eyes* (Optic nerve)
X Jejunum*	X Thymus*	
X Ileum*		Glandular
X Cecum*	Urogenital	
X Colon*	XX Kidneys*	XX Adrenals*
X Rectum*	X Urinary bladder*	X Lacrimal gland
XX Liver*	XX Testes*	X Mammary gland
X Gallbladder*	X Epididymides	X Thyroids*
X Pancreas*	X Prostate*	X Parathyroids*
X Oral tissue	X Seminal vesicle	X Harderian glands
	X Ovaries*	X Auditory sebaceous gland
Respiratory	X Uterus*	X Coagulating glands
X Trachea*	X Vagina	
X Larynx	X Oviduct	
X Lungs*		
X Nasal tissues		

Other

X Bone (sternum, femur, and joint)*
X Skeletal muscle*
X Skin*
X All gross lesions and masses*

*Recommended by Subdivision F (December 1982) Guidelines

All the above checked (X) tissues were examined microscopically in control and high-dose mice at the end of the 2 years. In mice given 100 or 500 mg/kg/day for 1 year, the following tissues were preserved for microscopic examination: lungs, liver, kidneys, ovaries, oviducts, uterus, cervix, vagina, urinary bladder, masses, and gross lesions. The histopathologic changes were graded as slight (minimal severity and <10% involvement of the parenchyma), moderate (up to 50% involvement of the parenchyma), and severe (significant organ/tissue dysfunction/failure).

(a) Organ Weights

There were no significant organ weight changes in male or female mice at the 52-week sacrifice. The same is true for the 104-week sacrifice, except for increased kidney weights in high-dose males. Table 2 summarizes absolute and relative kidney weight data. Histopathologic findings in the kidney did not substantiate the significant absolute and relative kidney weight increase observed in male, but not female, mice administered 1,000 mg/kg/day of Picloram. Therefore, this change was not considered to be toxicologically important.

Table 2. Summary of Absolute and Relative Kidney Weights in Mice Fed Picloram for 2 Years[a,b]

	Dose (mg/kg/day)			
	0	100	500	1000
Males				
12 months				
Absolute	0.783	0.726 (103)[c]	0.734 (104)	0.727 (103)
Relative	1.878	1.979 (105)	1.958 (104)	1.969 (105)
24 months				
Absolute	0.771	0.771 (100)	0.788 (102)	0.815* (106)
Relative	2.264	2.292 (101)	2.301 (102)	2.431* (107)
Females				
12 months				
Absolute	0.445	0.444 (100)	0.467 (105)	0.464 (104)
Relative	1.438	1.391 (97)	1.392 (97)	1.416 (98)
24 months				
Absolute	0.541	0.512 (95)	0.509 (94)	0.494 (91)
Relative	1.665	1.637 (98)	1.622 (97)	1.595 (96)

[a]Data extracted from study No. K-018323 .58, Tables 22 and 23.
[b]Relative organ weight refers to organ-to-body weight ratios.
[c]Number in parenthesis indicates % control.
*Significantly different from control values, $p<0.05$

(b) Macroscopic Pathology

Gross examination of the tissues, cavities, and organs from treated mice showed that there were no treatment-related effects after either 12 or 24 months of exposure to Picloram At the 24-month necropsy there was an increase in the lacrimal gland mass in males given 500 or 1,000 mg/kg/day Picloram, but the histopathological examination did not substantiate the occurrence of neoplasms. Similar observations were made regarding uterine masses in female mice treated with 100, 500, or 1,000 mg/kg/day where histopathology did not confirm the potential neoplasms. All the other changes were considered to be spontaneous events characteristic of the species and age of mice used.

(c) Microscopic Pathology

Histopathological examinations at 12 months did not reveal any lesions considered to be treatment related, and no target organ of Picloram toxicity was identified.

Analysis of histopathological observations at 24 months did not reveal treatment-related effects in any of the tissues examined. There were, however, some statistically significant findings that were unrelated to the treatment. These findings include incidences of slight mononuclear cell aggregates in the kidneys and other organs and a lower incidence of oral tissue inflammation as compared to controls. The results were interpreted as a variability in spontaneously occurring disease processes unrelated to the treatment.

There was an increased incidence of slight, bilateral kidney tubular degeneration/regeneration in female mice given 500 or 1,000 mg/kg/day. In the 500- and 1,000 mg/kg/day treated females, the incidences were 8/50 and 9/50, respectively, compared to 3/50 and 2/50 in controls and 100 mg/kg/day mice. These kidney changes were also considered not to be treatment related.

The incidence of tumors observed in treated animals was not different from that in controls. The tumors were considered to be spontaneous in occurrence and characteristic of the species and age of mice used in the study. (Summary Tumor incidence tables abstracted from the Hazleton report are attached)

C. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS

The study was adequately conducted and reported. The 1,000 mg/kg/day of Picloram is the limit dose for a chronic dietary study in mice. The mean data were supported by individual animal data. Survival was excellent in the study. The decreases in body weight were not considered of biologic importance in the high-dose males. There were no significant differences in food consumption between the dosed and control groups. The 1,000 mg/kg/day dose is considered to be a limit dose for chronic exposure to Picloram.

There was no evidence of carcinogenic potential of Picloram in B6C3F1 mice orally treated for two years. The incidence of tumors in treated mice was not different from that observed in the controls.

There were no treatment-related effects during ophthalmoscopic examinations or in any of the hematological parameters.

The gross pathology changes observed in the sizes of lacrimal glands (in males dosed at 500 or 1,000 mg/kg/day) and uteri (in females dosed at 100, 500, or 1,000 mg/kg/day) were not substantiated by histopathological findings. Therefore, they were considered to be spontaneous changes seen in that species of mice at that particular age. Similarly, the significant kidney weight increase observed in the 1,000-mg/kg/day treated male mice was not supported by histopathological findings and was considered to be unrelated to Picloram treatment.

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RIN 1901-99

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