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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D. C. 20460

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FEB 10 1989

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Picloram

FROM: John A. Quest, Ph.D., Section Head *J.A. Quest 1/5/89*  
Science Support Section  
Science Analysis and Coordination Branch  
Health Effects Division (TS-769C)

TO: Robert J. Taylor, PM 25  
Fungicide-Herbicide Branch  
Registration Division (TS-767C)

The Toxicology Branch Peer Review Committee met on May 26, 1988, to discuss and evaluate the weight-of-the-evidence on picloram with special reference to its oncogenic potential.

A. Individuals in Attendance

1. Peer Review Committee (Signature indicates concurrence with the peer review unless otherwise stated):

Robert Beliles

*Robert Beliles*

William Burnam

*W. Burnam*

Marion Copley

*Marion Copley*

Kerry Dearfield

*Kerry Dearfield*

Theodore M. Farber

*THEODORE M. FARBER*

Judith W. Hauswirth

*Judith W. Hauswirth*

Richard Levy

*Richard Levy*

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John A. Quest

John A. Quest

Lynnard Slaughter

Lynnard Slaughter

Reto Engler

Reto Engler

2. Scientific Reviewers (Non-committee members responsible for presentation of data; signature indicates technical accuracy of panel report):

Henry Spencer

Henry Spencer 11/15/89

Albin Kocialski

Albin Kocialski 11/15/89

Bernice Fisher

Bernice Fisher 11/9/89

3. Peer Review in Absentia (Committee members who were not able to attend the discussion; signatures indicate concurrence with overall conclusions of the Committee):

Anne L. Barton

Anne L. Barton

Diane Beal

Diane Beal

Richard Hall

Richard Hall

Esther Rinde

Esther Rinde

B. Material Reviewed

The material available for review consisted of DERs, one-liners, and other data summaries prepared by Dr. Spencer. Tables and statistical data analyses were provided by Ms. B. Fisher. The material reviewed is attached to the file copy of this report.

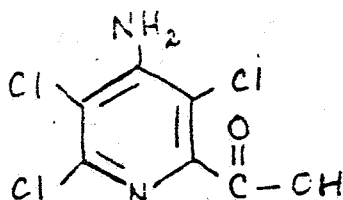
C. Background Information

Picloram (4-amino 3,5,6-trichloropicolinic acid) is a systemic herbicide manufactured by the Dow Chemical Company. The chemical is registered for both food and nonfood uses, the primary one being the control of broadleaf and woody plants. The mechanism of the herbicide action of picloram appears to be inhibition of protein synthesis in plants.

The Peer Review Committee considered oncogenicity data on picloram on from three rodent studies. These included an 80-week study in Osborne Mendel rats (NTP study) in which liver tumors occurred in females but not in males, a 2-year study in F344 rats (Dow Chemical study) in which no oncogenic response was observed, and a 2-year study in B6C3F<sub>1</sub> mice

(NTP study) in which no tumors were seen. The Committee was asked to evaluate these studies in view of the fact that they were associated with problems related to experimental design, GLP issues, dosing concerns (some of the studies appeared to be conducted using inadequately low doses), and the presence of a contaminant, hexachlorobenzene (HCB), in technical grade picloram. HCB is known to produce liver tumors in some strains of rodents.

Structure of Picloram



D. Evaluation of Oncogenicity Evidence for Picloram

1. Osborne Mendel Rat Study

Reference: Bicassay of Picloram for Possible Carcinogenicity in the Rat. NCI-CG Technical Report 23, 1978.

Picloram (Technical Grade 90% pure with 130 ppm HCB) was administered in the diet to groups of 50 male and 50 female Osborne Mendel rats at doses of 10,000 and 20,000 ppm for 39 weeks. The doses were reduced to 5000 and 10,000 ppm on study weeks 40 to 80 due to what was perceived to be a pattern of increased mortality, reduced weight gain, and poor appearance of the animals, after which all animals were allowed to recover for 33 more weeks on control diets. As shown in Table 1, the doses tested were 372 and 747 mg/kg. These doses corresponded to TWA doses of 7437 and 14,875 ppm, respectively. Ten animals/sex were used as a group of matched control animals, with 30 additional animals/sex/group comprising pooled controls. The study was conducted for the NTP by Gulf South Research Institute (GSRI). The following incidence pattern of liver tumors suggestive of a compound-related effect were observed in female rats. No compound-related tumors were observed in male rats.

Table 1. Liver Tumor Rates<sup>†</sup> in Female Osborne Mendel Rats Administered Picloram in the Diet for 80 Weeks

Liver Tumor Type	Dose (mg/kg/day)		
	0 <sup>a</sup>	372 <sup>b</sup>	747 <sup>b</sup>
Adenoma	0/39 (0%)**	5/33 (15%)*	7/34 (21%)**
Carcinoma	0/39 (0%)	0/33 (0%)	1/34 (3%)
Combined	0/39 (0%)**	5/33 (15%)*	8/34 (24%)**

<sup>†</sup>Number of tumor bearing animals/number of animals examined (excluding those that died before the appearance of the first tumor).

<sup>a</sup>Matched controls plus pooled control.

<sup>b</sup>The 372 and 747 mg/kg/day doses of Picloram contained 0.0483 and 0.0967 mg/kg/day of HCB contaminant, respectively.

\*p < .05; \*\*p < 0.01; Significance of trend analysis denoted at control. Significance of pairwise comparison with control denoted at dose level.

Picloram was associated with statistically significant positive dose-related trends for liver adenomas/carcinomas combined, and for liver adenomas per se. In addition, the incidence of liver adenomas, and adenomas/carcinomas combined at both dose levels of picloram were significantly increased when compared to the controls by the Fisher Exact test. Historical control data related to hepatocellular tumors in female Osborne-Mendel rats was not available to the Committee. The NTP considered the above results in female rats to constitute "equivocal" evidence for carcinogenicity. That is, a marginal increase in neoplasms was observed that may be chemically related, but the findings were characterized as uncertain. In the case of male rats the findings of no chemically related increases in malignant or benign neoplasms was considered by the NTP to represent "no evidence" of carcinogenicity activity.

The doses of picloram selected for testing in the NTP chronic study were chosen from a 6-week feeding study in rats (doses ranging from 1250 to 20,000 ppm, or 62.5 to 1000 mg/kg/day) in which the only change observed was a "slight" reduction in the body weights of male rats (quantitative data was not provided in the NTP report). In the actual chronic study, there were "slight" reductions in mean body weight of treated rats only until week 16, and the appearance of various clinical signs of toxicity after 6 months of treatment (alopecia, dermatitis, diarrhea, tachypnea, dark urine, and some vaginal bleeding), but no increases in

mortality. Additional findings seen after histopathology evaluation included liver focal cell changes in females (10% controls; 16% low dose; 37% high dose), bile duct fibrosis in females (0% control; 4% low dose; 12% high dose), thyroid gland C-cell and/or follicular cell hyperplasia in females (0% control; 11 to 13% low dose; 13% high dose), and parathyroid gland hyperplasia in males (0% controls; 3% low dose; 20% high dose). None of the above findings in either the subchronic or chronic studies were considered by the Peer Review Committee to be overly toxic. As such, the dose levels of picloram selected for testing in chronic Osborne-Mendel rats were thought to be insufficient to fully evaluate the compound's oncogenic potential.

In addition to inadequate dose selection, the picloram chronic study was associated with other deficiencies. These included the use of a matched control group of insufficient size, a shorter than 2-year (lifetime) exposure period, probable GLP deficiencies due to the fact it was conducted at GSRI, and the presence of HCB as a contaminant in the technical grade picloram that was tested.

## 2. Fischer 344 Rat Study

Reference: Picloram: A 2-Year Dietary Chronic Toxicity-Oncogenicity Study in Fischer 344 Rats. Dow Chemical Company, Midland, MI, Report No. K038323-034, January 3, 1986.

Picloram (Technical Grade 93% pure with 197 ppm HCB) was administered in the diet to groups of 50 male and 50 female Fischer 344 rats at doses of 0, 20, 60, and 200 mg/kg/day for 2 years. An additional 20 rats/sex/dose level underwent interim sacrifice at intervals of 6 and 12 months, respectively. The 20, 60, and 200 mg/kg/day doses of picloram contained 0.0039, 0.0118 and 0.0394 mg/kg/day of HCB contaminant, respectively. The study was conducted by the Dow Chemical Company. No dose-related oncogenic effects were observed in either male or female rats. Using the NTP's description of levels of evidence of carcinogenicity, the data in male and female Fischer 344 rats would be categorized as "no evidence" for carcinogenicity.

The doses of picloram selected for testing in the Dow chronic study were based on a 13-week feeding study in rats (doses ranging from 15 to 500 mg/kg/day) in which increased liver weights with accompanying histological effects (enlargement and altered cytoplasmic tinctorial properties of centrilobular hepatocytes) were observed at doses of 150 mg/kg/day or more. In addition, elevated kidney weights occurred in males at 300 mg/kg/day or more. In the Dow

chronic study, the principal compound-related change was a minimal increase in the size and tinctorial properties of centrilobular hepatocytes in male and female rats given doses of 60 and 200 mg/kg/day for 2 years; progression of severity with time did not occur. The changes were accompanied by increases in liver weights in high dose males and females (and also increases in liver size in females) throughout the study. The Peer Review Committee did not consider these findings to be overly toxic and thus concluded that the doses of picloram chronically tested in Fischer 344 rats were not sufficient to fully evaluate the compound's oncogenic potential. The Committee also noted that the highest dose of picloram tested in the Dow Chemical study (200 mg/kg/day) was about 3.7X less than that used in the NTP chronic study (747 mg/kg), and that the latter dose was too low for adequate testing in the NTP chronic study.

### 3. B6C3F<sub>1</sub> Mouse Study

Reference: Bioassay of Picloram for Possible  
Carcinogenicity in the Mouse.  
NCI-CB Technical Report 23, 1978.

Picloram (Technical Grade 90% pure with 130 ppm HCB) was administered in the diet to groups of 50 male and 50 female B6C3F<sub>1</sub> mice at doses of 2500 ppm (357 mg/kg/day) and 5000 ppm (714 mg/kg/day) for 79 weeks. The animals were allowed to recover for 10 weeks prior to sacrifice. The doses were initially set at 5000 and 10,000 ppm but were lowered after week 1 on the basis of data provided from a 6-week range-finding study, discussed below. Ten animals/sex were used as matched controls. The 357 and 714 mg/kg/day doses of picloram contained 0.0464 and 0.0928 mg/kg/day of HCB contaminant, respectively. The study was conducted for the NTP by GSRI. No dose-related oncogenic effects were observed in either male or female mice. The data in B6C3F<sub>1</sub> mice are best categorized as constituting "no evidence" of oncogenicity using the NTP's description of levels of evidence of carcinogenicity.

The doses of picloram selected for testing in the NTP chronic study were based on a 6-week feeding study in mice (doses ranging from 1250 to 30,000 ppm, or 17.8 to 4285.7 mg/kg/day) in which the only change observed was death in 4/5 males and 3/5 females at a dose of 20,000 ppm. A dose of 10,000 ppm was a NOEL in both sexes. This finding was used to set initial doses of 5000 and 10,000 ppm in the chronic study. These were then reduced to 2500 and 5000 ppm after 1 week, "since it was believed that excessive mortality would occur before termination of the study, based on the pattern of mortality, weight changes, and the general condition of mice used in similar studies on other chemicals at

Gulf South Research Institute." In the actual NTP chronic study, the only toxic effects seen at the 2500 and 5000 ppm doses were hyperactivity and rough hair coats after 1 year of treatment. The Peer Review Committee did not consider the findings excessively toxic and concluded that the doses of picloram tested in the chronic study were insufficient to fully evaluate the compound's oncogenic potential.

Along with inadequate dose selection, the picloram chronic study was also confounded by other problems including the use of a matched control group, of insufficient size probable GSRI-related GLP deficiencies, and the presence of HCB as a contaminant.

#### E. Additional Toxicology Information

##### 1. Oncogenicity of Hexachlorobenzene (HCB) in Rodents

HCB, the contaminant in picloram, has been shown to be carcinogenic in rats (Agus, Wistar, and Sprague Dawley strains), and mice (Swiss strain) (USEPA Health Effects Assessment for Hexachlorobenzene, 1984). Liver tumors were produced in rats with doses between 75 and 150 ppm (3.5 to 7.5 mg/kg/day). The incidence of liver tumors was as high as 100 percent in Agus rats (a liver tumor sensitive strain) but lower in the other strains (46 to 87%). Liver tumors were also produced in Swiss mice with doses of 100 to 300 ppm (14.2 to 42.8 mg/kg) at incidences of 4 to 34 percent. The Peer Review Committee noted from these data that HCB was a fairly potent liver oncogen in rodents (especially rats), producing high incidences of tumors at low doses. This information was felt to be consistent with the observation that picloram when contaminated with low doses of HCB (0.05 to 0.1 mg/kg) could produce a low threshold incidence of liver tumor in one strain of rat (Osborne Mendel) but not in another strain of rat (Fischer 344) or in B6C3F<sub>1</sub> mice. The Committee was also aware of another analogous situation in which rodent liver tumors attributable to paradichloronitrobenzene (PCNB) were actually due to the presence of a HCB contaminant (amount unspecified) in the technical grade PCNB product.

##### 2. Mutagenicity

Several studies have been performed with picloram. Submitted acceptable studies indicate that picloram does not induce mutations in Salmonella or Aspergillus nidulans nor nondisjunction or crossing over in Aspergillus. However, forward mutation was observed in Streptomyces coelicolor. In vivo studies, both of which probably should be repeated, indicated negative results in a mouse micronucleus assay at doses up to 1543 mg/kg (however, bone marrow was obtained

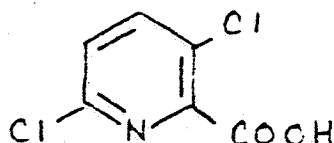


sacrifice) and in a rat bone marrow aberrations assay at doses up to 2000 mg/kg (however, little detail given; some aberrations noted, but not significant at 5% level and toxicity parameters not clear).

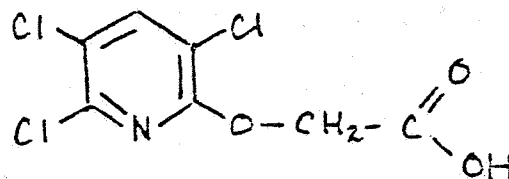
The NTP reports picloram to be negative in Salmonella and the Drosophila sex-linked recessive lethal assays; however, picloram was reported to induce aberrations and SCE in cultured CHO cells.

### 3. Structural Activity Relationships

Picloram is structurally similar to the herbicides Lontrel and Triclopyr. Chronic studies conducted with these compounds in rodents were considered to be negative for oncogenic activity.



LONTREL



TRICLOPYR

### 4. Reproduction and Teratology

No teratogenic effects were noted with picloram in rats at doses up to 1000 mg/kg. In a similar test in rabbits, the pups were not adequately evaluated for potential abnormalities. No evidence for adverse reproductive effects was found in a multigeneration study in rats.

### 5. Metabolism

Limited studies in rats and dogs (i.e., studies in which a minimal number of animals were examined) suggested that picloram is excreted in unchanged form in both the urine and feces over a few days. In one rat study, unchanged compound was found mainly in the urine (82%) as opposed to the feces (15%) over a 72-hour period.

### F. Weight of Biological Evidence Consideration

The Committee considered the following facts regarding the toxicology information on picloram to be of importance in a weight-of-the-evidence determination of oncogenic potential.

1. Administration of picloram in the diet of female Osborne Mendel rats for 80 weeks (1978, NCI study) was associated with statistically significant ( $p < 0.05$ ) positive trends for liver adenomas and for liver adenomas/carcinomas combined. In addition, the incidence of liver adenomas, and adenomas/carcinomas combined produced by both doses (372 and 747 mg/kg/day) of picloram were significantly ( $p < 0.05$ ) elevated in female rats. No histological control information was available on liver tumors in female rats of this strain.
2. The NTP described the data in female Osborne Mendel rats as "equivocal" evidence for carcinogenicity. Problems with the study involved testing at dose levels insufficient to adequately evaluate oncogenic activity, testing for only 80 weeks rather than the normal 2-year period, the use of matched control groups of small size, possible GLP deficiencies, and a suspicion that the observed liver tumors were due to the presence of HCB as a contaminant in the technical grade picloram that was tested.
3. Picloram was also tested by the NCI for oncogenicity in male Osborne Mendel rats, and in male and female B6C3F<sub>1</sub> mice. The findings were judged by the NTP as providing "no evidence" of carcinogenicity for each of these tests. The Peer Review Committee noted that many of the deficiencies described above for female rats (Section F.2.) also apply to these tests as well, and therefore considered them to be inadequate biological assays for oncogenicity.
4. No evidence for an oncogenic effect of picloram was found in male or female Fischer 344 rats in a study conducted by the Dow Chemical Company. The doses of picloram tested in this study did not appear to be sufficiently high enough to adequately evaluate oncogenic activity.
5. Picloram is structurally related to Lontrel and Triclopyr; these herbicides were not found to be oncogenic in adequately performed studies in rodents.
6. Mutagenicity studies did not provide sufficient evidence to either upgrade or downgrade the oncogenicity classification. While picloram was reported positive in one bacterial forward mutation assay, it was found to be negative in several other gene mutation assays, Salmonella, Aspergillus and Drosophila. Clastogenic activity was indicated by reported NTP results for aberrations and SCE in CHO

cells. This was supported by clastogenic activity reported for 2-methyl- and 4-methyl-pyridines (e.g., Raicu et al., 1984, Stud Cercet Ser Biol Anim 36:143-147; Hertzog and Chita, 1985, Stud Cercet Ser Biol Anim 37:75-78). As there are some questions about the submitted in vivo studies for clastogenicity, these studies may bear repeating.

7. No evidence for teratogenic or adverse reproductive effects of picloram was found in studies in rats.

#### G. Classification of Oncogenic Potential

The Committee classified picloram as a Category D oncogen (not classifiable as to human carcinogenicity) based on the EPA Guidelines and the above weight of the biological evidence considerations, because:

- o An oncogenic effect considered to be "equivocal evidence" of carcinogenicity by the NTP was seen at both dose levels tested in one sex of one species (liver adenomas/carcinomas combined in female Osborne Mendel rats). The presence of relatively high levels of HCB in technical grade picloram was suspected to be responsible for the occurrence of the liver tumors. Of interest in this regard, the Committee was informed that the levels of HCB contaminant used in the NTP study were actually much greater than those found in commercially marketed picloram. The study was further confounded because of inadequate dosage levels, a shortened duration of exposure, an insufficient control group, and possible GLP deficiencies.
- o Bioassays in B6C3F<sub>1</sub> mice and in a second strain of rat (Fischer 344) were negative for oncogenicity, but were considered inadequate for determining the potential oncogenicity of picloram because of similar confounding factors.

The Committee believed that the chronic rodent oncogenicity studies of picloram contained major qualitative/quantitative limitations and could not be accurately interpreted as showing either the presence or absence of an oncogenic effect. As part of their evaluation, the group also noted the absence of positive oncogenic correlation from two structurally similar pesticides.

Because of the deficiencies in the picloram oncogenicity studies, the Committee recommended that long-term studies be repeated in rats and mice of both sexes according to EPA Subpart F Guidelines and our Position Paper on the MTD. The studies should be conducted using commercially available technical grade picloram uncontaminated with potentially tumorigenic levels of HCB. The registrant is invited to consult with members of the Toxicology Branch in establishing appropriate dose levels for chronic testing.