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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Tralkoxydim - Report of the Cancer Assessment Review Committee

FROM: Jess Rowland 10/22/98
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Jim Tompkins, PM 25
Fungicide-Herbicide Branch
Registration Division (7505C)

The Cancer Assessment Review Committee met on July 8, 1998 to evaluate the carcinogenic potential of Tralkoxydim. Attached please find the Final Cancer Assessment Document.

cc: Karl Baetcke
Luke Brennecke
Lori Brunsman
Bill Burnam
Marion Copley
Kerry Dearfield
Virginia Dobozy
Mike Ioannou
Esther Rinde
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CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF
TRALKOXYDIM

FINAL REPORT

October 7, 1998

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: James Peggins, Toxicologist

DOCUMENT PREPARATION: Jess Rowland, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam, Chairman

Karl Baetcke

Marion Copley

Kerry Dearfield

Virginia Dobozy

Mike Ioannou

Esther Rinde

Joycelyn Stewart

Linda Taylor -

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

Luke Brennecke, Pathology Consultant

Lori Brunsman, Statistical Analysis

OTHER ATTENDEES: Elizabeth Mendez

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EXECUTIVE SUMMARY

On July 8, 1998, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of tralkoxydim. Dr. James Peggins of Toxicology Branch I introduced the chronic toxicity/carcinogenicity study in Wistar rats and the carcinogenicity study in the Syrian golden hamster by describing the following: experimental design; reporting on survival and body weight effects; treatment-related non-neoplastic and neoplastic lesions; statistical analysis of the tumor data; the adequacy of the dose levels tested; and presenting the weight of evidence for the carcinogenicity of tralkoxydim. Dr. Peggins also discussed the toxicology, metabolism, and mutagenicity studies as well as structure activity relationships.

Tralkoxydim (technical, 92.4%) was administered in the diet to male and female Wistar rats at 0, 50, 500, or 2500 ppm (equivalent to 0, 2.3, 23.1 or 117.9 mg/kg/day and 0, 3.0, 30.1 or 162.8 mg/kg/day, in males and females, respectively) for 104 weeks. In the study with Syrian golden hamsters, the animals received tralkoxydim (technical, 97.6%) in the diet at dose levels of 0, 250, 2500 or 7500 ppm (equivalent to 0, 14.9, 153 or 438.6 mg/kg/day and 0, 14.8, 148.3 or 427.9 mg/kg/day, in males and females, respectively) for 78 weeks.

A carcinogenicity study in the second species, the mouse (as recommended in Subdivision F Guidelines), was not conducted due to significant porphyria which occurred after dosing with Tralkoxydim which precluded selection of doses adequate to test for carcinogenicity. The data indicated that of all the species tested (rats, hamsters, Guinea pigs, marmosets, dogs, deer mice, white-footed mice, and meadow voles), only the single species of mouse (*Mus musculus*) was susceptible to porphyrin accumulation in the liver following treatment with tralkoxydim. This was considered to be an idiosyncratic response of the mouse (*Mus musculus*) and not relevant to human exposure.

The Committee concluded that the dose levels tested in the Wistar rat study were adequate and not excessive to assess the carcinogenic potential of tralkoxydim in both sexes. This was based on decreased body weight gains, decreased food consumption, and increased liver weights correlated with hepatic clear cell areas in both sexes at the high-dose, relative to the controls. In addition, decreased RBC count and hematocrit were noted in the high dose females which was reflected in hemosiderosis in the liver.

The Committee concluded that the dose levels tested in the hamster study were not adequate to assess the carcinogenic potential of Tralkoxydim since no systemic toxicity was seen even at the highest dose level. The Committee also concluded that the study in the hamster was unacceptable and inadequate to assess the carcinogenic potential of Tralkoxydim for the following reasons: 1) a rationale for doses tested was not provided; 2) very low survival among females in the control group compromised the study; 3) amyloid deposition in kidneys, liver, adrenals, spleen, and thyroid affected 20% of males and 70% of females across all groups; and 4) most of the

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intercurrent deaths were attributed to non-treatment-related reasons (such as enteropathy of the small intestine, generalized amyloidosis, and cardiovascular disorders) indicating that the poor survival rate was probably due to poor animal husbandry procedures at the testing laboratory or intercurrent disease. This could alter the results of the study.

Evidence of carcinogenicity was limited to the presence of benign testicular tumors in male Wistar rats. The occurrance of astrocytomas of the brain in males, benign stromal cell polyps in the cervix, and adenocarcinomas in the uterus of female rats were not attributed to treatment. The significance of adenomas of the adrenal glands seen in female hamsters could not be ascertained due to the poor survival of this sex which invalidated the study.

In male rats at the high dose (2500 ppm or 117.9 mg/kg/day), there was a significant trend ($p=0.002$) and a pair-wise significant ($p=0.006$) increase in benign Leydig cell tumors when compared to controls. Tumor incidences at the high dose (15/49, 31%) exceeded the concurrent controls (3/42, 7%) as well as the historical control range (3.8 to 19.2%) of the testing laboratory. In addition, there was an increase in the incidence of Leydig cell hyperplasia of the testes in male rats at the high dose (14/64, 22%) when compared to controls (4/64, 6%).

The Committee noted that in spite of a ten-fold increase in doses between the low-dose (50 ppm or 2.1 mg/kg/day) and the mid-dose (500 ppm or 23.1 mg/kg/day), there was no statistically significant increase in tumor incidences between the low-dose (5/40, 12%) and the mid-dose (6/47, 13%). Additionally, the incidences at these doses were within the historical control range (3.8 to 19.2%). The Committee, however, noted that the numerical increase of testicular tumors at the low-and mid-dose groups could be biologically significant and, therefore, cannot be discounted in spite of the lack of statistical significance.

The statistical evaluation of mortality indicated a significant decreasing trend with increasing doses of Tralkoxydim in male rats. Female rats showed no significant incremental changes in mortality with increasing doses of Tralkoxydim. Because of this finding, the statistical analyses of the male rats were based upon Peto's Prevalence Test. The statistical analyses of the female rats were based upon the Exact Trend Test and the Fisher's Exact Test for pair-wise comparisons. The Committee did not attribute the numerical increase in astrocytomas of the brain seen in male rats at the high dose (3/50, 6%) to treatment because:

1) the increase did not show pair-wise significance ($p=0.140$) when compared to concurrent controls (2/50, 4%).

2) the incidence was within the general range (0-5.8%) of historical controls.

3) there were no treatment-related non-neoplastic lesions in the brain.

The Committee did not attribute the numerical increases in benign stromal cell polyps (3/48, 6%) and adenocarcinomas (3/49, 6%) of the uterus observed in female rats at the high dose (2500 ppm 162.8 mg/kg/day) to treatment because:

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1) of the lack of a dose response.

2) increases were not statistically significant in the pair-wise test.

3) there was no adenomas present in the uterus.

4) the incidence of adenocarcinomas was in the general range (0-5.8%) of historical controls.

The Committee concluded that the carcinogenicity study in hamster was unacceptable and inadequate to assess the carcinogenic potential of tralkoxydim for reasons stated earlier. The very low survival among females in the control (6%) and treated (0%, 9.7% and 16.7% at the low-, mid-, and high-dose) groups compromised the evaluation of the significance of the adenomas of the adrenal glands observed in this sex at the mid and high dose groups.

In mutagenicity testing, tralkoxydim was shown to be non-mutagenic in adequate *in vivo* and *in vitro* assays.

Clethodim and Sethoxydim, compounds structurally-related to tralkoxydim, were reported to be not carcinogenic or mutagenic. They were not however, evaluated by HED's Cancer Assessment Review Committee.

In accordance with the Agency's *Proposed Guidelines for Carcinogen Risk Assessment (April 10, 1996)*, the Committee classified Tralkoxydim as a **"likely"** human carcinogen. This classification is based on the following factors:

1) occurrence of benign Leydig cell tumors at all dose levels with the incidences at the high dose exceeding the concurrent and historical control range.

2) lack of an acceptable carcinogenicity study in a second species as required by Subdivision F Gudidelines.

3) the relevance of the testicular tumors to human exposure can not be discounted.

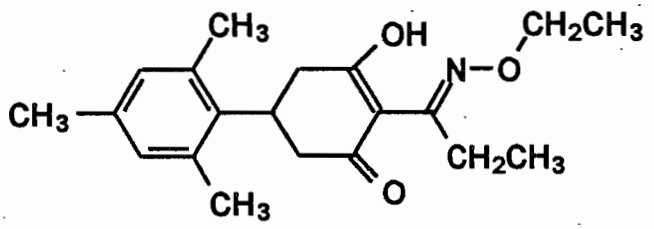
The Committee recommended that a linear low-dose approach (q_1*) for human risk characterization and extrapolation of risk should be based on the occurrence of benign Leydig cell tumors of the testes in male rats at all dose levels tested. At the present time, HED is continuing to use the multistage model which calculates the q_1* due to the inconsistencies and lack of consensus regarding the method of low dose linear extrapolation discussed in the *1996 Proposed Guideline for Carcinogen Risk Assessment.*

I. INTRODUCTION

On July 8, 1998, the Health Effects Division's Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of tralkoxydim. The Committee evaluated a combined chronic toxicity/carcinogenicity study in Wistar rats and a carcinogenicity study in Syrian golden hamsters. Dr. James Peggins of Toxicology Branch 1 presented the experimental design and results of these studies, statistical analysis of the tumor data, weight-of-evidence considerations, as well as the toxicology, metabolism, mutagenicity and structure-activity relationship data.

II. BACKGROUND INFORMATION:

Tralkoxydim (2-[1-(ethoxyimino)propyl]-3-hydroxy-5-(2,4,6-trimethylphenyl-2-cyclohexen-1-one) is a systemic, postemergence herbicide belonging to the cyclohexanedione class, which acts specifically on the inhibition of acetyl-CoA carboxylase in plants resulting in the inhibition of malonic acid biosynthesis. Tralkoxydim selectively controls annual grass weeds in cereal grain crops. The proposed use is for the control of annual grass weeds (wild oats, green and yellow foxtail, annual ryegrass, and Persian darnel) in wheat and barley. Tralkoxydim is currently registered for use on barley and wheat in Canada, where only the parent is regulated, with a MRL of 0.02 ppm (method loq). The PC Code: 121000.



Tralkoxydim

III. EVALUATION OF CARCINOGENICITY STUDIES

1 Combined Chronic/Oncogenicity in Wistar Rats

Reference: Stonard, M.D. (1994). First Revision to Tralkoxydim: Two-Year Feeding Study in the Rat. Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Chesite, UK. Laboratory Study No. PRO648. June 9, 1994. MRID 43339707.

A. Experimental Design

In a combined chronic toxicity/carcinogenicity study (MRID 43339707), tralkoxydim (92.4% a.i.) was administered to 64 Wistar rats/sex/dose in the diet at dose levels of 0, 50, 500, or 2,500 ppm for 104 weeks. These doses were equivalent to 0, 2.3, 23.1 or 117.9 mg/kg/day for males and 0, 3.0, 30.1, or 162.8 mg/kg/day for females. Twelve rats/sex/group were sacrificed at 52 weeks (interim sacrifice).

B. Discussion of Tumor Data

As shown in Table 1, benign Leydig cell testicular tumors were seen in all treated groups including the concurrent controls. However, the incidence at the high dose (2500 ppm or 117.9 mg/kg/day) had a significant increasing trend, and a significant difference in the pair-wise comparison (both at $p < 0.01$) when compared to concurrent controls. The statistical analyses of the male rats were based upon Peto's Prevalence Test since there was a statistically significant negative trend for mortality with increasing doses of Tralkoxydim in male rats.

Table 1. Male Rat Testicular Tumor Rates[+] and Peto's Prevalence Test Results.

Benign Leydig cell Tumors	0 ppm (0 mg/kg/day)	50 ppm (2.3 mg/kg/day)	500 ppm (23.1 mg/kg/day)	2500 ppm (117.9 mg/kg/day)
Incidence	3/42	5/40	6/47	15/49[a]
Percentage	7	12	13	31
P=	0.002**	0.249	0.341	**0.006****

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (censored data).

[a] First benign Leydig cell tumor observed at week 82, dose 2500 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

When compared to historical control data (uncensored data), the incidence of benign Leydig cell tumors in the low (5/40, 12%) and middle (6/47, 13%) dose groups was within the range (3.8 to 19.9%) while the incidence at the high dose (15/49, 31%) exceeded the range.

As shown in Table 2, there were also numerical increases in brain astrocytomas with increasing doses in male rats but there were no statistically significant increases in trend or pair-wise comparisons of the dosed groups with the concurrent controls and the incidences were within the general range (0 to 5.8%) of historical controls.

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Table 2. Male Rat Brain Tumor Rates[+] and Peto's Prevalence Test Results.

Brain Astrocytomas	0 ppm (0 mg/kg/day)	50 ppm (2.3 mg/kg/day)	500 ppm (23.1 mg/kg/day)	2500 ppm (117.9 mg/kg/day)
Incidence (%)	2/50 (4%)	1[a]/49 (2%)	2/51 (4%)	3/50 (6%)
P=	0.071	-	0.461	0.140

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a] First astrocytoma observed at week 56, dose 50 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

As shown in Table 3, among female rats, there was a statistically significant increasing trend ($p<0.05$) for cervical benign stromal poyps. There was, however, no significant differences in the pair-wise comparisons of the treated groups with the controls. There was a numerical increase in uterine adenocarcinoma at the high dose, but no statistically significant increases in trend or pair-wise comparisons and no uterine adenomas.

Table 3. Female Rat Uterine Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results

Tumor Type	0 ppm (0 mg/kg/day)	50 ppm (3.0 mg/kg/day)	500 ppm (30.1 mg/kg/day)	2500 ppm (162.8 mg/kg/day)
Benign stromal cell poyps (%)	0/49 (0)	1[a]/51 (2)	0/46 (0)	3/48 (6)
p=	0.033	0.510	1.000	0.117
Adenocarcinomas (%)	1/50 (2)	1[b]/51 (2)	0/48 (0)	3/49 (6)
p=	0.088	0.748	0.510	0.301

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a] First benign stromal cell polyp observed at week 64, dose 50 ppm.
[b] First adenocarcinoma observed at week 87, dose 50 ppm

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

When compared to historical control data, the incidence of benign stromal cell polyps of the cervix at high dose (6%) exceeded the range (0 to 2%). The incidence of uterine adenocarcinomas in all groups was in the general range of historical controls (0 - 5.8%).

C. Non-neoplastic Lesions

As shown in Table 4, there was an increase in the incidence of Leydig cell hyperplasia of the testes in male rats at the high dose.

Table 4. Leydig Cell Hyperplasia in Male Rats Fed Tralkoxydim for 104 Weeks (including interim sacrifice).

Leydig Cell Hyperplasia	0 ppm (0 mg/kg/day)	50 ppm (2.3 mg/kg/day)	500 ppm (23.1 mg/kg/day)	2500 ppm (117.9 mg/kg/day)
Unilateral	2/64	3/64	3/64	4/64
Bilateral	2/64	2/64	1/64	10/64
Combined	4/64 (6%)	5/64 (8%)	4/64 (6%)	14/64 (22%)

In additon, increased liver clear cell areas were observed upon histopathological examination (35/64 in males vs. 17/64 in controls and 12/64 in females vs. 1/64 in controls). Females also showed significantly increased hemosiderin in the Kupffer cells and elevated plasma alanine transaminase activity at the 2,500 ppm dose. Gross pathological changes, observed at the high dose, were increased numbers of discolored testes and testes with white areas compared to controls. At termination, treatment-related microscopic eye lesions, observed as bilateral retinal atrophy, occurred in 26/49 females at 2,500 ppm tralkoxydim.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

The doses tested were considered to be adequate and not excessive in both sexes. This was based on: significantly ($p<0.01$) decreased body weight gains (males, - 6 to 15% and females, - 18-25%); decreased food consumption (- 5 to 14%), and significantly increased liver weights (males, $p<0.05$ and females, $p<0.01$) which correlated with hepatic clear cell areas in both sexes at the high-dose, relative to the controls. In addition, decreased RBC count and hematocrit were noted in the high dose females which was reflected in hemosiderosis in the liver.

2. Oncogenicity Study in the Hamster

Reference: Stonard, M. D. (1994) Tralkoxydim: Lifetime Feeding Study in the Hamster. Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Lab ID: Report No. CTL/P/2362, Study No. PX0671, 6/8/94. MRID 43339710.

The Registrant did not conduct a carcinogenicity study in the second species, the mouse,

(as recommended by Subdivision F Guidelines), due to significant porphyria which occurred after dosing with tralkoxydim and which precluded selection of doses adequate to test for carcinogenicity. The data indicated that of all the species tested (rats, hamsters, Guinea pigs, marmosets, dogs, deer mice, white-footed mice, and meadow voles), only the single species of mouse (*Mus musculus*) was susceptible to porphyrin accumulation in the liver following treatment with tralkoxydim. This was considered to be an idiosyncratic response of the mouse (*Mus musculus*) and not relevant to human exposure.

A. Experimental Design

In a carcinogenicity study (MRID 43339710), tralkoxydim (97.6% a.i.) was administered to 72 Syrian hamsters (strain Lak LVG (SYR))/sex/dose in the diet at 0 (three control groups of 72 animals each), 250, 2500, or 7500 ppm (equivalent to 0, 14.9, 153, and 438.6 mg/kg/day for males and 0, 14.8, 148.3, and 427.9 mg/kg/day for females) for 78 weeks.

B. Discussion of Tumor Data

Table 5. Adrenal Gland Tumors in Female Hamsters Fed Tralkoxydim for up to 79 weeks

Dose Group (ppm)	0	0	0	250	2500	7500
Adenoma	0/72 (0%)	1/72 (1%)	1/72 (1%)	2/72 (3%)	4/72 (6%)	4/72 (6%)
Week to first observation:	NA	72	65	66	55	42
Carcinoma	0/72 (0%)	1/72 (1%)	0/72 (0%)	0/72 (0%)	2/72 (3%)	0/72 (0%)
Adenoma/ carcinoma combined	0/72 (0%) p = 0.016	2/72 (3%)	1/72 (1%)	2/72 (3%)	6/72 (8%)	4/72 (6%)

As shown in Table 5, adenomas of the adrenal gland were observed in the three control groups as well as in the treated groups. Under the conditions of this study, there is a suggestion of a carcinogenic response in the adrenal gland of the female hamster. A positive trend at p=0.016 was shown for total tumor incidence. Historical control data for this tumor type/strain were not available. Among males the spontaneous occurrence of adrenocortical adenomas was more frequent than in females. Among control males in each of three groups, the tumor was observed in 8-12/72; the incidence in treated groups was 7-11/72. In addition, as shown in Table 6, due to the low survival of the females (6% in controls), a definitive conclusion regarding carcinogenicity of the chemical cannot be drawn from this study .

Table 6. Survival (%) of Hamsters Fed Tralkoxydim for 79 weeks (Kaplan-Meier estimate)[a]

	Males				Females			
Week	0 [b]	250	2500	7500	0	250	2500	7500
N	72	72	72	72	72	72	72	72
1	100	100	100	100	100	100	100	100
13	100	100	98.6	100	99.5	100	100	100
26	99.1	97.2	94.4	98.6	89.4	95.8	91.7	97.2
39	93.1	87.5	90.3	88.9	73.6	69.4	76.4	80.6
52	84.3	80.6	76.4	76.4	51.7	45.8	58.3	59.7
58	78.7	75.0	72.2	70.8	40.5	31.9	47.2	50.0
66	66.7	62.5	63.9	59.7	24.2	19.4	31.9	41.7
74	54.6	51.4	52.8	48.6	12.6	4.2	11.1	23.6
78	48.6	43.1	47.2	40.3	6.1	0	9.7	16.7

N = Number of animals

a Data from pp. 48-49 of the study report. b Control values are the mean of three control groups.

C. Non-neoplastic Lesions

A dose-related increase in lipofuscin accumulation was observed in the hepatocytes of males and females. The incidence was 85% in high dose males and 83% in mid-dose males (vs 58% of controls) and 71% in high dose females and 36% in mid-dose females (vs 10% of controls). This is not considered to be of toxicological significance. The number of animals with cortical pigmentation of the adrenals showed a slight increase with dose in females (11% at 7500 ppm vs 3% in controls), but not in males (33-50% were affected in all groups). The pigment accumulation in adrenals is not considered related to treatment.

Other lesions were notable because they were thought to be responsible for intercurrent deaths in all groups. Chronic enteropathy of the small intestine was considered to be a contributory factor in the deaths of 55% of both sexes. The findings indicated that this condition was progressive, appearing as Stage 1 (chronic enteritis) in the majority of animals, with Stages 2 (chronic ulcerative enteritis) and 3 (glandular dysplasia) occurring less frequently and still fewer animals showing Stages 4 and 5.

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Amyloid deposition in kidneys, liver, adrenals, spleen, and thyroid affected 20% of male and 70% of female hamsters across all groups, and was severe enough to have contributed to the unscheduled deaths of 11% of males and 50% of females. Cardiovascular disease was observed in 10% of males and 40% of females, and most of the affected animals had considerable amyloid deposition in major organs. Cardiovascular changes were thought to have contributed to the deaths of approximately 7% of the affected males and 30% of the affected females.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

The doses tested in this study were not adequate for testing the carcinogenic potential of tralkoxydim in Syrian hamsters. Treatment-related systemic toxicity was not observed at any dose level tested, including the highest dose. In addition, the low survival rates in both control (6%) and treated females (0% at low dose, 9.7% at mid-dose and 16.7% at high-dose) at termination further decreased the confidence of this study and deemed the study to be inadequate to assess the carcinogenic potential of tralkoxydim.

IV. TOXICOLOGY

1. Metabolism

In a series of related rat metabolism studies (MRIDs 43339720, 43339721, 43339723, and 43339747), [phenyl-U-^{14}C] or [2,4-^{14}C-cyclohexene] tralkoxydim (>95% a.i.) was administered to Crl:CD BR strain male and female rats by gavage as a single dose at 1 or 40 mg/kg or as a single dose at 1 mg/kg following a 14-day pretreatment with unlabeled tralkoxydim at 1 mg/kg.

[^{14}C]Tralkoxydim was excreted from male rats primarily via the urine, whereas female rats excreted approximately equal amounts via the urine and feces. The amount of radioactivity absorbed was rapidly excreted, and was independent of sex, dose level, and preconditioning. Within 48 hours of dosing to non-cannulated rats, radioactivity in the urine totaled 59-66% of the dose in males and 42-46% in females, and radioactivity in feces totaled 31-36% in males and 37-48% in females. In similarly-dosed bile-duct cannulated rats, radioactivity was primarily excreted via the bile (males, 78%; females, 64%), with lesser amounts excreted in the urine (both sexes, 10-13%), indicating enterohepatic circulation of tralkoxydim. When administered at 1 mg/kg (with or without pretreatment) or 40 mg/kg, 96-106% of the administered dose was recovered after 168 hours. Urinary excretion totaled 60-68% of the administered dose in males, and 45-51% in females. Fecal excretion totaled 33-38% of the dose in males and 43-53% in females. Of the remaining radioactivity, ≤0.7% was recovered in cage washes, and ≤0.5% was in tissues/carcasses. Within 48 hours of dosing at 40 mg/kg to bile-duct cannulated rats, biliary excretion accounted for 76-79% of the dose in males and 63.46-64.33% in females, and for both sexes, 9-12% was excreted in the urine and 1-2% was excreted in feces.

Levels of radioactivity in tissues were low for all dose groups, comprising ≤0.03% of the administered dose. For the single high dose group, residue levels in blood were 4x higher in males (0.20 μg/g) compared to females (0.05 μg/g).

The metabolite profiles in urine were similar between sexes and dose groups, although the levels of metabolites differed. No unchanged tralkoxydim was detected in urine of rats from any of the test groups, except for trace amounts in urine from the preconditioned low dose group, and <5% in bile from bile-cannulated rats. The data indicate that the major metabolic pathway for tralkoxydim involves oxidation of the methyl groups of the trimethylphenyl moiety, yielding tralkoxydim alcohol which is further oxidized to tralkoxydim acid or tralkoxydim diol.

In a hamster metabolism study [phenyl-U-^{14}C]tralkoxydim (96% a.i.) was administered to five Syrian hamsters/sex by gavage as a single dose at 1 mg/kg (MRID 43339722),.

[^{14}C]Tralkoxydim was readily absorbed by male and female hamsters following oral dosing, as evidenced by the high percentage of renal excretion. Peak urinary elimination (~57-59%) occurred within 24 hours of dosing. Within 7 days of dosing, ~82-88% of the administered dose was recovered from males and females, of which ~67-69% was in the urine, 14-18% was in the feces, and 0.7-1.0% was in the cage washes. In the tissues/organs of both sexes, levels of radioactivity were highest in the liver (0.009-0.010 μg/g), followed by the kidney (0.004-0.005 μg/g) and blood (0.003 μg/g), and were below detection limits in fat, heart, lung, brain, muscle, spleen, bone and gonads. The carcass accounted for the highest percentage of the administered dose in both sexes (males, 0.14%; females, 0.17%) (MRID 43339722),.

The metabolic profile in urine was similar for males and females; no unchanged tralkoxydim was detected, and two major degradates were tentatively identified: 4-(2-[1-(ethoxyimino)propyl]-3-hydroxy-2-cyclohexen-1-one-5-yl)-3,5-dimethylbenzoic acid (tralkoxydim acid) and 4-(3-ethyl-4,5,6,7-tetrahydro-4-oxo-1,3-benzoxazol-6-yl)-3,5-dimethylbenzoic acid (tralkoxydim acid oxazole) (MRID 43339722).

A comparison of these results with the previous rat metabolism study shows similarities as well as differences between the species. Based on the results of the rat study, tralkoxydim was readily absorbed with most the radioactivity being excreted with 48 hours of dosing. Similarly in hamsters, residues in tissues were low and no unchanged tralkoxydim was isolated in the urine, and the metabolites, tralkoxydim acid and tralkoxydim acid oxazole, were detected in hamster urine fractions. Two additional metabolites in rat urine were tralkoxydim alcohol and in males, tralkoxydim diol; neither metabolite was isolated in the hamster urine fractions.

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2. Mutagenicity

When tested in several *in vivo* and *in vitro* mutagenic assays, tralkoxydim was not shown to be mutagenic under the conditions of the assays. These assays satisfy the Subdivision F Guideline requirements for mutagenicity testing.

(i). Gene mutation in bacteria (Ames Assay)

The Ames battery of TA (mutant) strains of *Salmonella typhimurium* was exposed for 64-68 hours to test article (94.9% a.i.) at six concentrations (1.6 thru 5000 ug/plate). No significant increases over solvent in revertent colonies (*his*+) controls were evident in any bacterial strain at any dose up to the HDT (5000 ug/plate) either in the absence or presence of mammalian metabolic activation (rat S-9) (MRID No. 43301706).

(ii) Forward gene mutation in mammalian cells in culture (L5178Y/TK+/-)
L5178Y (mouse lymphoma) cells were exposed in two independent trials to test article at concentrations up to the limit of solubility. No consistent increase in forward gene mutation (TK+ to TK-) was found in either trial up to precipitating levels (400 ug/ml). At 400 ug/ml moderate cytotoxicity (relative survival 32% of solvent) was manifest (MRID Nos. 43355709 and 43222025).

(iii) Chromosome damage *in vitro* (HLC/CA)

Human lymphocytes from two healthy donors (one male; one female) were exposed *in vitro* to test article, and analyzed for structural chromosomal aberrations. No increased incidence of chromosome damage was found in cultures treated up to the limit of solubility and cytotoxicity (250 ug/ml), in either the presence or absence of metabolic activation (MRID No. 43355711).

(iv) DNA damage/repair *in vivo* (rat hepatocytes)

Groups of rats received a single oral dose at 250, 500 or 1000 mg/kg, and were sacrificed 4 or 12 hours later. No increase in unscheduled DNA synthesis was found for any dose up to the cytotoxic HDT (1000 mg/kg) (MRID No.43355710).

(v) Chromosome damage *in vivo* (mouse MT)

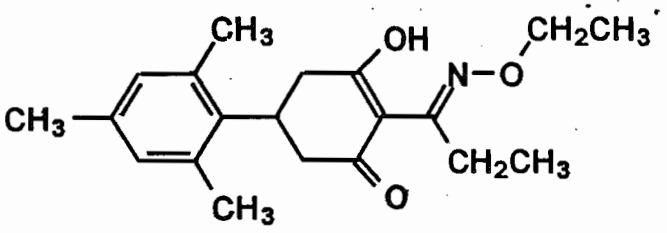
Male and female mice received a single intraperitoneal injection at 300 or 480 mg/kg, and samples of bone marrow cells were assessed for the presence of polychromatic erythrocyte (PCE) containing micronuclei at 24, 48 and 72 hours post dose. There was no reproducible induction of micronuclei at doses up to lethal levels (480 mg/kg) (MRID No.43355712).

3. Structure-Activity Correlations

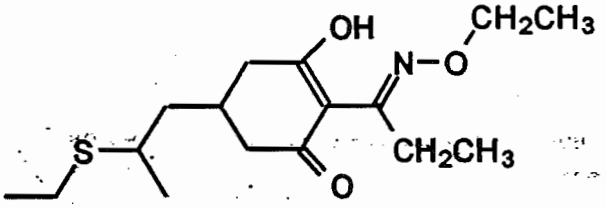
Tralkoxydim is structurally related to Clethodim and Sethoxydim. Both of these chemicals are registered for use by the Agency as post-emergence herbicides. Both were non-carcinogenic and non-mutagenic.

There was no evidence of carcinogenicity following dietary administration of clethodim to male and female mice at 0, 20, 200, 1000 or 3000 ppm (0, 3, 30, 150, or 450 mg/kg/day, respectively) for 78 weeks or to male and female rats at 0, 50, 20, 500 or 2500 ppm (0, 0.25, 1, 25, or 125 mg/kg/day, respectively) for 104 weeks (MRID Nos. 41030112 and 41030121).

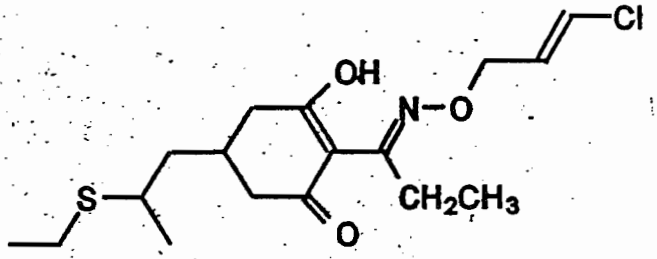
There was no evidence of carcinogenicity following dietary administration of sethoxydim to male and female mice at0, 40, 120, 360 or 1080 ppm (0, 6, 18, 54, or 162 mg/kg/day, respectively) or to male and female rats at 0, 40, 120, 360 ppm (0, 2, 6, 18 mg/kg/day, respectively) for 104 weeks (MRID Nos. 070817, 070819, 070815, 070816).



Tralkoxydim



Sethoxdim



Clethodim

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4. Subchronic, and Chronic Toxicity Studies

i. Subchronic

An initial 28 day feeding study examining groups of C57BL/10JfCD-1/Alpk mice dosed with tralkoxydim at 2 to 5000 ppm (MRID No.43339715) identified the liver as the target organ. Increases of 20-93% ($p \leq 0.05$) in relative (to body) liver weights were observed in animals dosed at ≥50 ppm. Biliary hyperplasia and fibrosis with accumulation of brown pigmentation (suggestive of porphyrin accumulation) in the bile ducts and Kupffer cells were observed in mice fed ≥25 ppm. Crystalline aggregates were observed in the lumen of bile ducts, macrophages, and hepatocytes in livers of mice dosed at 1250 and 5000 ppm. A slight vesiculation and increased ($p<0.01$) proliferation of the smooth endoplasmic reticulum was observed in the liver of the 5000 ppm mice. No treatment-related findings were observed in the livers of Alpk:AP (Wistar derived) rats that had been dosed via the diet for 28 days with tralkoxydim (99.2% a.i.) at 50 to 5000 ppm (MRID No. 43339708).

Additional studies (MRID No's 44104812 through 44104814) indicated that dosing mice with tralkoxydim led to the formation of N-methylprotoporphyrin IX (N-CH_3 PPIX), which caused an inhibition of the liver enzyme ferrochelatase resulting in an over stimulation of heme synthesis and accumulation of porphyrins in the liver. When rats were exposed to tralkoxydim, total porphyrin content was similar to controls and ferrochelatase activity was unchanged. An *in vitro* study (MRID No. 44104811) using mouse and rat hepatocytes confirmed these findings. An additional *in vitro* study using cultured human hepatocytes (MRID No.44104817) indicated that tralkoxydim will not cause porphyria in humans.

In subchronic studies in rats, dogs and hamsters (MRID No's 43339705, 43339706 and 43339712) the major toxicity appeared to be hepatic. In the dog (MRID No. 43339706), this occurred primarily in the high dose group (50 mg/kg/day) and was reflected in significant changes in essentially all parameters measured. However, minimal histopathology was observed at this dose level. Minimal systemic toxicity was observed in rats (MRID No.43339705) administered 2500 ppm in the diet for 90 days, although plasma cholesterol and triglyceride levels were significantly decreased and ALT activity was significantly increased. In the hamster (MRID No. 43339712) tralkoxydim had a clear effect on liver morphology and function at dose levels of 5,000; 10,000 and 20,000 ppm.

ii. Chronic

In chronic toxicity studies in dogs and rats the primary target organ of tralkoxydim toxicity was also the liver.

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In a chronic dog study male and female beagles were administered tralkoxydim at 0, 0.5, 5 and 50 mg/kg/day in their feed. Tralkoxydim did not appear to be toxic to dogs treated at 0.5 mg/kg/day, while one male dog in the 5 mg/kg/day treatment group had elevated plasma alanine transaminase activity and moderate periportal fatty changes in the liver. A slightly increased (8%) absolute liver weight in males further indicated some hepatotoxicity at this dose level. Lipid metabolism was affected in the 5 mg/kg/day females, as demonstrated by cholesterol and triglyceride levels which were 26-28% lower than the control values during week 52 (MRID No.43339709).

Dogs in the 50 mg/kg/day treatment groups showed clear evidence of liver toxicity due to treatment. Both sexes exhibited increased plasma alkaline phosphatase (ALK) and plasma alanine transaminase (ALAT) activities compared to the controls. The increases were most pronounced during week 52, when ALK activities were elevated 313-349%, and ALAT activities were elevated 78% in males and 230% in females compared to the controls. Both sexes had increased absolute (51-69%) and adjusted (54-65%) liver weights. Liver abnormalities included enlargement in 1/4 males and 2/4 females; an accentuated lobular pattern in 2/4 males and 4/4 females; swollen lobes in 2/4 males and 4/4 females; discoloration in 1/4 males; mottling in 2/4 males and 3/4 females; and friable livers in 3/4 males and 4/4 females. Microscopic examination revealed diffuse or periportal fatty change in the livers of all 50 mg/kg/day dogs. The severity was slight in 1/4 females, moderate in 2/4 males and 1/4 females, and marked in 2/4 males and 2/4 females (MRID No.43339709).

Additional findings observed in the 50 mg/kg/day treatment groups appeared to be related to treatment. Males were slightly anemic, based in decreased red blood cell counts, hematocrit, and hemoglobin (8-11%) during weeks 13, 26 and/or 52 compared to the controls. Plasma albumin levels were depressed 20% in both sexes, and total protein levels were 29% lower in males and 48% lower in females compared to the corresponding controls. Lipid metabolism was affected in both sexes, as demonstrated by reduced cholesterol (29-48%) and triglyceride (49-64%) levels. Changes to the adrenal glands in both sexes indicated a toxic response to treatment. Increased absolute (62-77%) and adjusted (70-75%) adrenal gland weights were accompanied by vacuolation of the zona fasciculata and zona reticularis that was moderate in males and marked in females. The severity of vacuolation in females was concentration-dependent; minimal vacuolation was observed in 3/4 females treated at 5 mg/kg/day, and in 1/4 females in each of the control and 0.5 mg/kg/day treatment groups. The toxicological significance of increased absolute (37-42%) and adjusted (42-47%) thyroid weights in both sexes is equivocal since no associated histopathological changes were observed (MRID No.43339709).

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In a chronic toxicity /carcinogenicity study in rats, animals were administered tralkoxydim at 0, 50, 500 or 2500 ppm in the diet for 104 weeks. None exhibited treatment-related toxicity in the 50 or 500 ppm groups. There were no treatment-related effects on mortality. In the high-dose animals, mean body weight gains were significantly lower ($p \leq 0.01$) than controls for males at weeks 4-64 (↓6-15%) and for females ($p \leq 0.01$) throughout the feeding period (↓18-25%). Feed consumption was significantly lower ($p \leq 0.01$) during the first 24 weeks of feeding (↓5-14%) and sporadically significant ($p \leq 0.05$) through 104 weeks. Food conversion efficiency was also significantly lower ($p \leq 0.01$) during weeks 1-12 in males and females of the 2,500 ppm treatment group, compared to controls. Clinical signs of toxicity observed were urinary incontinence in males, thin appearance in females, and thickened eyelids in both sexes. Decreases in hemoglobin related to decreased red cell counts, hematocrit, and mean cell volumes were consistent with signs of microcytic anemia in both males and females. Plasma alanine transaminase was significantly elevated in females throughout the treatment period (↑32-56%) and in males at 13 weeks only (↑31%). Relative liver weights were significantly increased in both males and females ($p \leq 0.05$ and 0.01, respectively) at 52 weeks and still increased, but not significantly so, at 104 weeks. Increased liver clear cell areas were observed upon histopathological examination (35/64 in males vs. 17/64 in controls and 12/64 in females vs. 1/64 in controls). Females also showed significantly increased hemosiderin in the Kupffer cells and elevated plasma alanine transaminase activity at 2,500 ppm tralkoxydim. Gross pathological changes, observed at the high dose, were increased numbers of discolored testes and testes with white areas compared to controls. At termination, treatment-related microscopic eye lesions, observed as bilateral retinal atrophy, occurred in 26/49 females at 2,500 ppm tralkoxydim (MRID 43339070).

V. COMMITTEE'S ASSESSMENT OF WEIGHT-OF-EVIDENCE:

The Committee considered the following weight-of-evidence determination on the carcinogenic potential of tralkoxydim:

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reatment because 1) of the lack of a
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1. Carcinogenicity

Evidence of carcinogenicity was limited to the presence of benign testicular tumors in male Wistar rats. The occurrence of astrocytomas of the brain in males, benign stromal cell polyps in the cervix, and adenocarcinomas in the uterus of female rats were not attributed to treatment. The significance of the adenomas of the adrenal glands, seen in female hamsters, could not be ascertained due to the poor survival (intercurrent disease) of this sex which invalidated the study.

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els used were not adequate to a
ic toxicity was seen even at the hig
mg/kg day in

In males at the high dose (2500 ppm or 117.9 mg/kg/day), there was a significant trend (p=0.002) and a pair-wise significance (p=0.006) in benign Leydig cell tumors when compared to controls. Tumor incidences at the high dose (15/49, 31%) exceeded the concurrent controls (3/42, 7%) as well as the historical control range (3.8 to 19.2%) of the testing laboratory. In addition, there was an increase in the incidence of Leydig cell hyperplasia of the testes in male rats at the high dose (4/64, 6%) when compared to controls (14/64, 22%).

The Committee noted that in spite of a ten-fold increase in doses between the low-dose (50 ppm or 2.1 mg/kg/day) and the mid-dose (500 ppm or 23.1 mg/kg/day), there was no statistically significant increase in tumor incidences between the low-dose (5/40, 12%) and the mid-dose (6/47, 13%). Additionally, the incidences at these doses were within the historical control range (3.8 to 19.2%). The Committee, however, noted that the numerical increase of testicular tumors at the low-and mid-dose groups could be biologically significant and therefore can not be discounted in spite of the lack of statistical significance.

The statistical evaluation of mortality indicated a significant decreasing trend with increasing doses of tralkoxydim in male rats. Female rats showed no significant incremental changes in mortality with increasing doses of tralkoxydim. Because of this finding, the statistical analyses of the male rats were based upon Peto's Prevalence Test. The statistical analyses of the female rats were based upon the Exact Trend Test and the Fisher's Exact Test for pair-wise comparisons.

The Committee did not attribute the numerical increase in astrocytomas of the brain seen in male rats at the high dose (3/50, 6%) to treatment because:1) the increase did not show pair wise significance (p=0.140) when compared to concurrent controls (2/50, 4%); 2) the incidence was within the general range of historical controls (0-5.8%); and 3) there were no treatment-related non-neoplastic lesions in the brain.

The Committee did not attribute the numerical increases in benign stromal cell polyps (3/48, 6%) and uterine adenocarcinomas (3/49, 6%) observed in female rats at the high dose to treatment because: 1) of the lack of a dose response; 2) increases were not statistically significant in the pair-wise test; and 3) the incidence of adenocarcinomas was in the general range (0-5.8%) of historical controls. Also, there were no adenomas present in the uterus. The stromal cell polyps which occur spontaneously within the uterus, are considered benign, and there is no evidence to indicate that they would transform into a more aggressive form with time.

The Committee concluded that the carcinogenicity study in hamster was unacceptable and inadequate to assess the carcinogenic potential of tralkoxydim for the following reasons: 1) the dose levels tested were not adequate to assess carcinogenicity since no treatment-related systemic toxicity was seen even at the highest dose tested (7500 ppm; 438.6 mg/kg/day in males and 427.9 mg/kg/day in females); 2) a rationale for doses tested was

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not provided; 3) very low (6%) survival among females in the control groups compromised the evaluation of the significance of the adenomas of the adrenal glands (observed in this sex at the mid and high dose groups); 4) amyloid deposition in kidneys, liver, adrenals, spleen and thyroid affected 20% of males and 70% of females across all groups and 5) most of the intercurrent deaths were attributed to non-treatment-related reasons (such enteropathy of the small intestine, generalized amyloidosis, and cardiovascular disorders) indicating that the poor survival rate was due to intercurrent disease or the animal husbandry procedures at the testing laboratory.

2. Mutagenicity

In mutagenicity testing, tralkoxydim was shown to be non-mutagenic in adequate *in vivo* and *in vitro* assays.

3. Structure Activity Relationship

Clethodim and Sethoxydim, compounds structurally-related to tralkoxydim did not exhibit any carcinogenic activity in mice and rats. They were not however, evaluated by HED's Cancer Assessment Review Committee.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the Agency's *Proposed Guidelines for Carcinogen Risk Assessment (April 10, 1996)*, the Committee classified Tralkoxydim as a **"likely"** human carcinogen. This classification is based on the following factors:

(a) occurrence of benign Leydig cell tumors at all dose levels with the incidence at the high dose exceeding concurrent and historical control range.

(b) lack of an acceptable carcinogenicity study in a second species as required by Subdivision F Guidelines.

(c) the relevance of the testicular tumors to human exposure can not be discounted.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended that a linear low-dose approach (q_1^*) for human risk characterization and extrapolation of risk should be based on the occurrence of benign Leydig cell tumors of the testes in male rats at all dose levels tested. At the present time, HED is continuing to use the multistage model which calculates the q_1^* due to the inconsistencies and lack of consensus regarding the method of low dose linear extrapolation discussed in the *1996 Proposed Guideline for Carcinogen Risk Assessment.*

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