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

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DATE: **July 8, 1998**

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: **TRALKOXYDIM-** Report of the Hazard Identification Assessment Review Committee.

FROM: James O. Peggins, Ph.D.
Pharmacologist
Toxicology Branch I
Health Effects Division (7509C)
and
Jess Rowland, Executive Secretary 7/8/98
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

THROUGH: K. Clark Swentzel, Chairman, 7/8/98
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)
and
Mike Metzger, Co-Chairman
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Alberto Protzel, Ph.D.
Branch Senior Scientist, Toxicology Branch I
Health Effects Division (7509C)

PC Code: 121000

On June 2, 1998 the Health Effects Division's Hazard Identification Assessment Review Committee evaluated the toxicology data base of **Tralkoxydim,** established a Reference Dose (RfD) and selected the toxicological endpoints for acute dietary as well as occupational exposure risk assessments. The HIARC also addressed the potential enhanced sensitivity of infants and children from exposure to **Tralkoxydim** as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report. This was the second meeting regarding this chemical, the first was held on March 31, 1998. At that time no RfD or endpoints were set for risk assessment pending a meeting with the registrant to clarify issues raised during the review process.

Committee Members in Attendance

Members present were: Clark Swentzel (Chairman), Jess Rowland (Executive Secretary), John Redden, Sue Makris, Melba Morrow, Karl Baetcke, Karen Hamernik, Mike Metzger and Bill Burnam. Data was presented by Jim Peggins of Toxicology Branch I.

In attendance were also Steve Dapson, Mike Ioannou, Alberto Protzel, Bill Hazel and Jose Morales who attended the meeting to discuss exposure etc.

Data Presentation:
and
Report Presentation

James O. Peggins, Ph.D.
Pharmacologist

Report Concurrence:

Jess Rowland
Executive Secretary

I. INTRODUCTION

Tralkoxydim (2-[1-(ethoxyimino)propyl]-3-hydroxy-5-(2,4,6-trimethylphenyl-2-cyclohexen-1-one) is a systemic 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 is proposed for use as a post-emergent herbicide in barley, wheat and grass seed crops. It is a Tox. Category III chemical with an oral LD_{50} in rats of between 934 and 1324 mg/kg and is formulated as water dispersible granules.

On June 2, 1998 the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology database for Tralkoxydim, established a Reference Dose (RfD) and selected the toxicological endpoints for acute dietary as well as occupational exposure risk assessments. The HIARC also addressed the potential for enhanced sensitivity of infants and children from exposure to Tralkoxydim as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report. This was the second meeting on this chemical, the first was held on March 31, 1998. At that time a decision was made not to select toxicity endpoints pending a meeting with the registrant to clarify issues regarding the use of the hamster in place of the mouse for chronic and oncogenicity studies. At the March 31 committee meeting the HIARC did evaluate the available animal carcinogenicity data and decided to forward the chemical to the Cancer Assessment Review Committee for evaluation of its carcinogenic potential.

II. HAZARD IDENTIFICATION

A 1. Acute Reference Dose (RfD) Females 13 +

Study Selected: Developmental Study in Rats §83-3a

MRID No.: 43339717

Executive Summary: Two developmental toxicity studies [43339717 (study 1); 43383805 (study 2)] were performed. Pregnant Wistar Alpk:AP rats received tralkoxydim (PP604) by gavage on gestation days (GD) 7-16 inclusive. Animals were randomly assigned to control or treatment groups (24 per group) and received doses of: (study 1) - 0, 3.0, 30.0, or 300 mg/kg/day; (study 2) - 0, 0.5, 1.0, 3.0, or 200 mg/kg/day. The second study was conducted because excessive maternal toxicity occurred at the highest dose in the first study. Cesarean section examinations were performed on all surviving dams on GD 22 followed by external, visceral, and skeletal examination of all fetuses.

No maternal toxicity was observed at ≤30 mg/kg/day. At 200 and 300 mg/kg/day 4 animals each were either dead or killed in moribund condition on GD 14-18. All other animals survived to terminal sacrifice. Piloerection and a hunched posture were observed in a few animals at lower doses but was common at 200 mg/kg (12 and 5 animals, respectively) and 300 mg/kg/day (16 and 16 animals, respectively). At the high dose staining around the mouth and nose was seen. Of animals sacrificed moribund or found

dead, three of four in the 200 mg/kg/day group and 4 of 4 in the 300 mg/kg group had stomachs distended with gas. Significantly ($p \leq 0.01$) reduced body weights occurred in the 200 and 300 mg/kg groups during the treatment period and to the end of the study; final body weights were 91% and 82% of control, respectively. Significantly ($p \leq 0.01$) reduced food intake occurred in the 200 and 300 mg/kg group animals from GD 7-10 to GD 16-19. Recovery was only seen in the high-dose animals after GD 19) but not in the 200 mg/kg group. **For maternal toxicity, the NOEL was 30 mg/kg and the LOEL was 200 mg/kg/day based on maternal mortality, reduced body weights, and reduced food consumption.**

No developmental toxicity was observed at ≤3 mg/kg/day. Significantly reduced ($p \leq 0.01$) gravid uterine weights occurred in the 300 mg/kg group. This correlated with significantly ($p \leq 0.01$) decreased numbers of live fetuses per dam and reduced fetal weights. Mean fetal weights were also significantly reduced in the 200 mg/kg/day group. The high-dose group had significantly ($p \leq 0.01$) more total, early, and late resorptions per dam as compared to controls and three animals had whole litter resorption. The 300 mg/kg group had a significantly ($p < 0.01$) greater number of litters containing fetuses with anasarca and/or edema of the neck or torso: 5 of 17 vs. 0 of 47 control. A significantly ($p < 0.01$) greater number of litters in the 300 mg/kg/day group contained fetuses with pale spleens with 35% of high-dose litters (6 of 17) affected compared to 2% of control litters (1 of 47). At 30 mg/kg/day, centrum bipartite and reduced ossification of the centrum in the thoracic vertebrae were observed ($p \leq 0.05$). Significant ($p \leq 0.01$), treatment-related effects on the vertebrae at 200 and 300 mg/kg included reduced ossification of the centrum and hemicentrum, centrum bipartite, misshapen centra, and fused centra. There was a dose-related statistical increase in the number of litters containing fetuses with sternebrae not ossified and misaligned. Significantly reduced ossification of the bones in the fore- and hind-paws was observed in fetuses from the 30 mg/kg/day ($p \leq 0.05$), 200 and 300 mg/kg/day ($p \leq 0.01$) groups as indicated by greater *manus* and *pes* scores. Given the intermittent nature of the delayed ossification observed at 30 mg/kg it was not considered a significant developmental endpoint. In addition, similar effects were not observed in the developmental toxicity study in rabbits or in the two-generation reproduction study in rats. Thus, it was determined that the 30 mg/kg/day is not appropriate for risk assessment. **. Therefore, the developmental toxicity NOEL is 30 mg/kg/day and the developmental toxicity LOEL is 200 mg/kg/day based on reduced ossification of the centrum and hemicentrum, centrum bipartite, misshapen centra, and fused centra.** Developmental toxicity did not occur at doses below those causing maternal toxicity.

Dose and Endpoint for Risk Assessment: Developmental toxicity NOEL = 30 mg/kg/day based on delayed ossification of the centrum and hemicentrum, centrum bipartite, misshapen centra and fused centra which were seen in the 200 and 300 mg/kg/day dose groups.

Comments about Study/Endpoint: The skeletal malformations are presumed to occurr after a single exposure (dose) and thus are appropriate for this (acute) risk assessment. Since the NOEL is based on a developmental endpoint, its applicable only to the

population subgroup, Females 13+. Also, the dose spacing between the NOEL (30 mg/kg/day) and the LOEL (200 mg/kg/day) and the signficant increase in treatment-realted effects seen between them, indicates that the LOEL is probably is lower than 200 mg/kg/day.

Uncertainty Factor (UF): 100 (10 x for inter-species extrapolation and 10 x for intra-species variability).

$$\textbf{Acute RfD} = \frac{30\ \text{mg/kg}}{100} = \textbf{0.3 mg/kg}$$

This Risk Assessment is required.

A 2. Acute Reference Dose (RfD) General Population including Infants & Children

Study Selected: None

MRID No.: None

Executive Summary: None

Dose and Endpoint for Risk Assessment: Not Applicable

Comments about Study/Endpoint: An endpoint attributable to a single expsoure (dose) was not identified in oral toxicity studies including the developmental toxicity studies in rats and rabbits.

This risk assesment is NOT required for this subpopulation.

B. Chronic RfD

Study Selected: One year feeding study in dogs §83-1b

MRID No.: 43339709

Executive Summary: In a chronic toxicity study (MRID 43339709), tralkoxydim technical (94.9% a.i.) was administered in capsules to four beagle dogs/sex/dose at dose levels of 0, 0.5, 5 or 50 mg/kg/day for 52 weeks.

Toxic effects were observed in the livers and adrenal glands of dogs treated at 50 mg/kg/day. Hepatotoxic effects included livers that were enlarged (1/4 males, 2/4 females); had an accentuated lobular pattern (2/4 males, 4/4 females); swollen lobes (2/4 males, 4/4 females); discoloration (1/4 males); mottling (2/4 males, 3/4 females); and

friable texture (3/4 males, 4/4 females). Diffuse or periportal fatty change of the liver 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. Both sexes had increased absolute and adjusted (for body weight) liver weights (51-69%), and elevated plasma alkaline phosphatase (313-349%) and plasma alanine transaminase (78-230%) activities. Toxic effects on the adrenal glands included, increased (62-77%) absolute and adjusted weights and moderate to marked vacuolation of the zona fasciculata and zona reticularis in both sexes. Increased absolute and adjusted (37-47%) thyroid weights in both sexes lacked associated histopathological changes. Both sexes had reduced levels of plasma albumin (20%) and total protein (29-48%), and males were slightly anemic. Reduced cholesterol (29-48%) and triglyceride (49-64%) levels in both sexes treated at 50 mg/kg/day indicated effects on lipid metabolism. In the 5 mg/kg/day treatment group, males had a slightly increased (8%) absolute liver weight, one male had moderate periportal fatty change and elevated plasma alanine transaminase activity, and one female had decreased (26-28%) cholesterol and triglyceride levels. Dogs in the 0.5 mg/kg/day treatment groups did not exhibit toxic responses to treatment. No animals died during the study, and no treatment-related effects in clinical signs or body weight gains were observed. Food consumption did not appear to be affected by treatment. The LOEL for this study is 5 mg/kg/day, based on changes in liver function and morphology in males. The NOEL is 0.5 mg/kg/day.

Dose and Endpoint for Establishing RfD: NOEL = 0.5 mg/kg/day, based on changes in liver function and morphology in males at 5 mg/kg/day (LOEL)..

Uncertainty Factor(s): 100 (10 for inter-species extrapolation and 10 x for intra-species variability).

$$\textbf{Chronic RfD} = \frac{0.5 \text{ mg/kg/day}}{100} = \textbf{0.005 mg/kg/day}$$

Comments about Study/Endpoint/Uncertainty Factor: None

This risk assessment is required.

C. Occupational/Residential Exposure

There are no registered residential uses at the present time. Therefore, the following risk assessments are made for occupational exposures.

1. Dermal Absorption

Study: Dermal Absorption Study - Rats §85-2

MRID No.: 43339724

Executive Summary: In a dermal absorption study (MRID 43339724), 24 male Wistar rats/dose group received [^{14}C]-tralkoxydim (99% a.i.) as a suspension of a 25% dry flowable (DF) formulation in water at dose levels of 9.63, 0.963, or 0.066 mg/rat (0.963, 0.0963, or 0.0066 mg/cm^2). A non-occlusive protector was used on the shaved application site of the animals' backs. Four rats/dose were sacrificed for assessment of dermal absorption after 0.5, 1, 2, 4, 10, and 24 hours of exposure. Absorption was determined from the amount of administered radioactivity recovered. The majority of the applied doses remained unabsorbed throughout the 24 hour period at all dose levels. At 0.963 mg/cm^2 absorption ranged from 8% at 0.5 hours to 15% at 24 hours. In addition, at 24 hours the amount of tralkoxydim remaining in the skin plus the amount absorbed corresponded to 18% of the dose. At 0.0963 mg/cm^2 absorption ranged from 9% at 0.5 hours to 13% after 24 hours. At 24 hours the amount of tralkoxydim remaining in the skin plus the amount absorbed corresponded to 15% of the dose. At 0.0066 mg/cm^2 absorption ranged from 12% at 0.5 hours to 30% after 24 hours. Maximum absorption was noted at 4 hours with approximately 31% of the dose absorbed. At 24 hours the amount of tralkoxydim remaining in the skin plus the amount absorbed corresponded to 35% of the dose.

	Percent of Total Dose on Skin & Absorbed		
Average Dose	1 hour	10 hours	24 hours
0.0066 mg/cm^2	43%	26%	30%
0.0963 mg/cm^2	11%	15%	13%
0.963 mg/cm^2	9%	11%	15%

A high degree of inter-animal variability in the percent of tralkoxydim recovered at the low and high doses was noted. At the high dose, individual recoveries after 0.5 hours ranged from 42% to 103%, and after 1 hour, 37% to 82%. Similarly, at the low dose, individual recoveries after 0.5 hours ranged from 63% to 124% and after 24 hours, 73% to 130%.

Dermal Absorption Factor: **30%** at 10 hours

2. Short-Term Dermal - (1-7 days)

Study Selected: - Developmental -Rat §83-3a

MRID No.: 43339717

Executive Summary: See Acute Dietary

Dose and Endpoint for Risk Assessment: Developmental NOEL = 30 mg/kg/day based on delayed ossification and skeletal misalignments at 200 mg/kg/day (LOEL).

Comments about Study/Endpoint:. In a 21-day dermal toxicity study in rats, no dermal or systemic toxicity was seen following repeated dermal apllication at doses of 0, 10, 100 or 1000 mg/kg/day, 6 hours/day for 21 days. For dermal and systemic toxicity, the NOEL was 1000 mg/kg/day. The Committee did not use this study. Instead selected the developmental NOEL because: 1) developmental effects are considered to be acute effects and thus are appropriate for this exposure period (i.e., 1-7 days); 2) of the concern that the reproductive/fetal effects are not evaluated in the dermal toxicity study and thus these effects (reproductive/fetal) can not be ascertained for the dermal route of exposure and 3) the developmental endpoint will provide adequate protection for the subpopulation of pregnant (Females 13+) workers. Also, the dose spacing between the NOEL/LOEL and the significant increase in treatement-related effects noted between them, indicates that the LOEL was probably lower than 200 mg/kg/day. In addition, the use of a 30% dermal absorption factor inconjunction with the oral developmental NOEL of 30 mg/kg/day yields an equivalent dermal dose of 100 mg/kg/day (i.e., 30 ÷ 0.3 = 100) which is between the NOEL (30 mg/kg/day) and the LOEL (200 mg/kg/day) for maternal and developmental toxicity study in the developmental study.

Since an oral NOEL was selected a dermal absorption factor of 30% should be used for dermal risk assessment.

This risk assessment is required.

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3. Intermediate-Term Dermal (7 Days to Several Months)

Study Selected: - Developmental -Rat §83-3a

MRID No.: 43339717

Executive Summary: See Acute Dietary

Dose and Endpoint for Risk Assessment: Developmental NOEL = 30 mg/kg/day based on delayed ossification and skeletal misalignments at 200 mg/kg/day (LOEL).

Comments about Study/Endpoint: See Short-Term.Since an oral NOEL was selected a dermal absorption factor of 30% should be used for dermal risk assessment.

This risk assessment is required.

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4. Long-Term Dermal (Several Months to Life-Time)

Study Selected: None

Comments about Study/Endpoint:. The expected pattern of use (once a year, post-emergence) indicate a minimal concern for potential long-term expsoure/risk..

This risk assessment is NOT required.

5. Inhalation Exposure (Short- and Intermediate-Term).

Study Selected: Developmental Study in Rats §83-3a

MRID No.: 43339717

Executive Summary: See Acute Dietary

Dose and Endpoint for Risk Assessment: Developmenatl NOEL = 30 mg/kg/day based on delayed ossification and skeletal misalignments at 200 mg/kg/day (LOEL).

Comments about Study/Endpoint: Except for an acute inhalation toxicity study, no inhalation toxicity studies are available in the database. Therefore, HIARC selected the oral NOEL for inhalation risk asessments and the risk assessment should follow the route-to-route extrapolation as outlined below:

Step I. The inhalation exposure component (i.e. µg a.i /day) using 100% absorption rate (default value) and application rate should be converted to an **equivalent oral dose** (mg/kg/day).

Step II. The dermal exposure component (mg/kg/day) using a 30% dermal absorption rate and application rate should be converted to an **equivalent oral dose**. This dose should then be combined with the oral dose in Step I.

Step III. The combined dose from Step II should then be compared to the oral NOEL of 30 mg/kg/day to calculate the Margins of Exposure (MOEs)

The use pattern does not indicated the need for Long-Term inhalation exposure risk assessment via this route.

This risk assessment is required.

D. Margins of Exposures for Occupational/Residential Exposure Risk Assessments

A MOE of 100 is adequate for Short-and Intermediate-Term exposures via the dermal and/or inhalation expsoures; a MOE for Long-Term expsure is not required. There are no registered residential uses.

E. Recommendation for Aggregate (Food, Water and Dermal) Exposure Risk Assessments

There are no registered residential uses. Therefore aggregate exposure risk assessment will be limited to Food + Water only.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID No. 43339707

Discussion of Tumor Data: In a combined chronic/oncogenicity 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 (actual 0, 2.3, 23.1, and 117.9 mg/kg/day for males, and 0, 3.0, 30.1, and 162.8 mg/kg/day for females) for 104 weeks. A total of 52 rats/sex/group were sacrificed at 104 weeks. In addition, 12 rats/sex/group were sacrificed at 52 weeks (interim sacrifice).

In high dose males there was a significant increase in benign Leydig cell testicular tumors. The incidence was 15/64 (23.4%) at 2,500 ppm tralkoxydim compared to 3/64 (4.7%) in controls. There was also an increased incidence of benign Leydig cell tumors in males dosed at 500 ppm (9.4%) and 50 ppm (7.8%) tralkoxydim, but these incidences were within the range observed in historical controls. The only malignant tumors observed were adenocarcinomas of the uterus in females and astrocytomas of the brain in males at 2,500 ppm tralkoxydim. While the control and low dose groups fell within the range of historical controls (0 - 3.8%) the incidence of uterine tumors in the 2500 ppm group (4.7%) was outside the range. There were no historical control data provided for

the astrocytoma findings which occurred in 3.1, 1.6, 3.1 and 3.6% of the control, 50, 500 and 2500 ppm groups respectively.

The incidence of benign Leydig cell tumors in the low and middle dose groups was within the range of the historical control data provided by the registrant (0 to 14.4%). However, it should be noted that the 95% C.I. for the data was 3.77 - 7.81%. Excluding the 14.4% study from the calculation (a 1979 study) gives a much smaller range of 0 - 4.8%. The concurrent controls (4.7%) from this study fall within that smaller range. It seems clear that the incidence of these tumors in the mid dose group (9.4%) is actually outside the reduced range for historical controls and it is likely that the dose related increases seen in this study for Leydig cell tumors are test article related and tralkoxydim has a positive oncogenic response.

In addition to the tumors mentioned in the summary above, benign stromal cell polyps were observed in the cervix of 3/64 (3.6%) high dose and 1/64 (1.6%) low dose females. Tumors were absent in the control and mid dose groups.

Adequacy of the Dose Levels Tested: Dosing was considered adequate 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.

2. Carcinogenicity Study in Hamster

The hamster was chosen for the study because of an idiosyncratic response in the mouse (MRID 43339715) not observed in other species or in cultured human hepatocytes.

MRID No. 43339710

Discussion of Tumor Data: In a hamster oncogenicity 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.

Survival among males was 40-49% at 78 weeks and was not affected by treatment. Among females, survival was very low, 6% in controls and 0% at the 250 ppm dose, but was slightly improved in the 2500 and 7500 ppm dose groups at 9.7 and 16.7%, respectively. Treatment with tralkoxydim had no effect on clinical signs, food consumption, body weight gains, hematology, clinical chemistry, organ weights and/or histopathology. The systemic NOEL = 7500 ppm (438.6 mg/kg/day for males and 427.9 mg/kg/day for females). The systemic toxicity LOEL > 7500 ppm.

Under the conditions of this study, there is a suggestion of a carcinogenic response in the adrenal gland of the female hamster, although the study is not adequate to definitively determine carcinogenicity due to low survival. For total tumor incidence of the adreno-cortical adenoma there was a positive trend at p=0.016. At the 2500 and 7500 ppm doses, incidences of adreno-cortical adenoma in females were significantly greater than in controls ($p<0.05$). The interval before appearance of the first tumor decreased from 65 and 72 weeks in controls to 55 weeks at the 2500 ppm dose and 42 weeks at 7500 ppm.

The submitted study is classified as **Unacceptable (Guideline)** and does not satisfy requirements for an oncogenicity study (**§83-2b**) owing to unacceptably high mortality in the females.

Adequacy of the Dose Levels Tested: The doses employed in this hamster oncogenicity study were not adequate to test the carcinogenic potential of tralkoxydim in Syrian hamster. Systemic toxicity was not observed in this study.

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3. Classification of Carcinogenic Potential

The HIARC recommended that the carcinogenic potential of tralkoxydim be assessed by the Cancer Assessment Review Committee based on the increased incidence of Leydig cell tumors in male rats at the highest dose tested. In high dose males there was a significant increase in benign Leydig cell testicular tumors (15/64 (23.4%) at 2,500 ppm tralkoxydim compared to 3/64 (4.7%) in controls).

IV. MUTAGENICITY

(i) Mutagenicity-gene mutation in bacteria (Ames Assay) §84-2a

The Ames battery of TA (mutant) strains of *Salmonella tyimurium* were 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 revertant 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).

(ii) Mutagenicity-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.

(iii) Mutagenicity-chromosome damage *in vitro* (HLC/CA) §84-2b

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.

(iv) Mutagenicity-DNA damage/repair *in vivo* (rat hepatocytes) §84-4

Groups of rats were treated with test article with a single oral dose of 250, 500 or 1000 mg/kg, and sacrifices either 4 or 12 hours later. No increase in unscheduled DNA synthesis (UDS) was found for any dose up to the cytotoxic HDT (1000 mg/kg).

(v) Mutagenicity-chromosome damage *in vivo* (mouse MT) §84-2b

Male and female mice were injected once ip with 300 or 480 mg/kg test article, and samples of bone marrow cells 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).

Tralkoxydim is not genotoxic under the conditions these assays were performed.

V. FQPA CONSIDERATIONS

1. Neurotoxicity:

No neurotoxicity studies are available in the database. However, the available studies (i.e., subchronic and chronic toxicity) do not show any evidence of Tralkoxydim exhibiting signs of neurotoxicity.

2. Developmental Toxicity

Rat

This study is discussed in detail in Section II.A.1. Acute Dietary. For maternal toxicity, the NOEL was 30 mg/kg and the LOEL was 200 mg/kg/day based on maternal mortality, reduced body weights, and reduced food consumption. For developmental toxicity, the NOEL was 30 mg/kg/day and the LOEL was 200 mg/kg/day based on skeletal malformations. Developmental toxicity occurred at doses causing maternal toxicity.

Rabbit

In a developmental toxicity study (MRID 43339718) tralkoxydim (97.8% a.i.) was administered to 18 New Zealand White rabbits/dose in corn oil by gavage at dose levels of 0, 2.5, 20, or 100 mg/kg/day from days 7 through 19 of gestation.

Maternal toxicity was noted at 100 mg/kg/day by a significant ($p \leq 0.05$) reduction in feed consumption during treatment. No treatment-related changes in body weight gain or feed consumption were noted at 2.5 or 20 mg/kg/day compared to controls. No treatment-related deaths occurred at 2.5, 20, or 100 mg/kg/day, however 8 dams in the 100 mg/kg/day treatment group aborted and were sacrificed and 1 additional dam was sacrificed in extremis. Necropsy revealed stomach hemorrhages in 7/9 sacrificed dams. Except for the high-dose abortions, no treatment-related clinical signs of toxicity were noted in any treatment group. Sporadic alopecia occurred in dams of all treatment groups. **The maternal LOEL is 100 mg/kg/day, based on reduced feed consumption. The maternal NOEL is 20 mg/kg/day.**

Treatment of pregnant rabbits with tralkoxydim at 100 mg/kg/day during gestational days 7-20, resulted in 8/18 litters being aborted. A significantly reduced ($p \leq 0.01$) number of live fetuses/dam compared to controls (6.6 vs 8.8) at 100 mg/kg/day was due to a significant ($p \leq 0.05$) increase in the proportion of late resorptions compared to controls (40.5% vs 23.3%). No evidence of teratogenicity was observed at treatment levels up to 100 mg/kg/day of tralkoxydim. **The developmental LOEL is 100 mg/kg/day, based on abortions and increases in late resorptions. The developmental NOEL is 20 mg/kg/day.**

The developmental toxicity study in the rabbit is classified **Acceptable** and satisfies the guideline requirements for a developmental toxicity study **(83-3b)** in rabbits.

3. Reproductive Toxicity:

In a 3-generation reproduction study (MRID 43339719) tralkoxydim (94.9% a.i.) was administered to Alpk:APfSD (Wistar derived) rats at dose levels of 0, 50, 200, or 1000 ppm in the diet (calculated by the reviewer to be the equivalent of 0, 5, 20, or 100 mg/kg/day, respectively for young rats). Exposure to parental (P generation) animals (15 males and 30 females/dose level) began at 4 weeks of age and lasted for 12 weeks before they were mated to produce the F_{1a} litters. At 36 days of age, F_{1a} pups (15 males and 30 females/dose level) were selected to become the F_1 parents of the F_{2a} generation. Pups from the F_{2a} generation (15 males and 30 females/dose level) were selected, also at 36 days of age, to become the F_2 parents of the F_{3a} generation. F_1 and F_2 parents were given the same concentration test diets as their dam for 11 and 12 weeks prior to mating, respectively. Exposure of the test material to all animals was continuous in the diet throughout the study.

Parental toxicity was noted at 1000 ppm and was characterized as treatment-related reductions in body weights and body weight gains in females. For the high-dose P females, weekly body weight gains (↓5-22%) and cumulative body weight gains were reduced (↓4-5%, $p \leq 0.05$) while mean body weights at the end of the premating interval were comparable to the controls. More consistent reductions in cumulative body weight gains (↓6-11%, $p \leq 0.05$ or 0.01) and in weekly body weight gains (↓6-24%) were noted for the F_1 and F_2 females at 1000 ppm. In addition for the high-dose F_1 and F_2 females, mean body weights at the end of pre-mating were reduced relative to the controls (↓6%, $p \leq 0.01$). Decreased maternal body weights were also observed at the start of gestation for the high-dose F_1 dams (↓8%, $p \leq 0.05$).

At 1000 ppm, food consumption was reduced in the P generation males (↓5-9%, weeks 3-12, $p \leq 0.05$ or 0.01). Statistically significant reductions in food consumption were observed only sporadically in the high-dose F_1 and F_2 males (↓6-8%, weeks 1, 2, and/or 3, $p \leq 0.01$ or 0.05). Therefore, the decreases in food consumption for the P generation males at 1000 ppm cannot unequivocally be related to treatment with tralkoxydim because the differences from control were marginal and similar trends were not noted in the F_1 and F_2 high-dose males. Marginal, statistically significant ($p \leq 0.01$ or 0.05) reductions in food consumption were also noted in the P, F_1, and F_2 females (↓4-8%). There were no treatment-related effects noted in food utilization efficiency (g growth/100g food) at any dose level in any of the generations throughout the premating interval. There were no treatment-related clinical signs of toxicity, increases in mortality, gross pathology, or histopathology noted in the P, F_1, or F_2 parental animals at any dose level. No treatment-related effects were observed in the reproductive function or performance of the P, F_1, or F_2 adults at any dose level.

The LOEL for systemic toxicity is 1000 ppm (100 mg/kg/day) based on reduced body weights and body weight gains in P, F1 and F2 females. The systemic NOEL is 200 ppm (20 mg/kg/day).

No reproductive toxicity was observed. The developmental toxicity was demonstrated at 1000 ppm as treatment-related reductions in the mean pup weights for the F_{1a} (↓7-13%) and F_{3a} (↓6-11%) generations and reductions in the pup body weight gains for the F_{2a} litters (↓11-14%, $p \leq 0.0.05$). The reductions in mean pup weights noted for the F_{1a} and F_{3a} litters at the high-dose cannot unequivocally be considered treatment-related findings because similar reductions were not noted during lactation for the F_{2a} litter. The reductions in pup body weight gains for the F_{2a} litters at 1000 ppm are considered equivocally treatment-related because there were no related reductions in mean pup body weights. There were no treatment-related clinical signs of toxicity or changes in pup viability, litter sizes, or liver weights noted in the pups at any dose level for F_{1a}, F_{2a}, or F_{3a} generations. No treatment-related macroscopic or microscopic findings in the F_{1a}, F_{2a}, or F_{3a} pups were observed at any dose level.

The results of the present study are supported by the findings of an earlier range-finding study in which a decrease in pup weights was noted at 350 and 2,500 ppm. Therefore, the threshold LOEL for developmental toxicity is 1000 ppm based on decreased mean pup weights (F_{1a} and F_{3a}) and pup body weight gains (F_{2a}). The developmental toxicity NOEL is 200 ppm. There is an indication that tralkoxydim may induce male reproductive toxic effects related to endocrine disruption. Increased benign testicular Leydig cell tumors and decreased epididymal sperm, were observed at termination in the two-year rat study (MRID 43339707) at a dietary dose of 117.9 mg/kg/day. These tumors were first observed at study week 82. It was additionally noted that a decrease in epididymal weight was observed in the subchronic dog study (MRID 43339706) at 50 mg/kg/day, and an increase in testicular weight was observed in the chronic/oncogenicity study in hamsters (MRID 43339710) at a dietary dose of 438.6 mg/kg/day, although these findings were not confirmed histopathologically. It was noted that effects on male reproductive function were not observed in the three-generation reproduction study in rats at a high-dose level of approximately 100 mg/kg/day; however, the animals in the multi generation study did not attain the age at which testicular tumors were first observed in the chronic /oncogenicity study. Additionally, there were no measures of sperm count, motility, or morphology in the reproduction study. It was recommended that the need for a two-generation reproduction study in rats with tralkoxydim, conducted under the OPPTS 870.3800 guideline (which includes enhanced evaluation of the reproductive system, including sperm measures) be reevaluated following the issuance of final conclusions and recommendations of EDSTAC (estimated completion in 1999). Should a two-generation reproduction study be required at that time, consideration should be given to 1) maintaining the P and F1 parental animals on study for a longer duration, in order to assess the testicular and epididymal effects across generations, and 2) including a study segment to assess effects on functional development of the offspring (see below).

4. Additional information from the literature

None available

5. Determination of Susceptibility

The Committee concluded that the data indicated no special susceptibility to the offspring of rats or rabbits following pre- and/or postnatal exposure to tralkoxydim. In the prenatal developmental toxicity studies in rats and rabbits, and in the three-generation reproduction study in rats, offspring toxicity was observed only at dose levels which were also toxic to the parental animals. In the developmental toxicity study in rats, at the LOEL of 200 mg/kg/day, decreased fetal body weight and delays/alterations in fetal skeletal development occurred in the presence of significant maternal toxicity (decreased body weight, piloerection, hunched posture, moribundity). Similarly, at the LOEL of 100 mg/kg/day in the three-generation reproduction study in rats, both parental and offspring body weight decreases were observed.

In the prenatal developmental toxicity study in rabbits, both fetal and maternal toxicity were observed at the LOEL of 100 mg/kg/day. At this dose, reduced food consumption, accompanied by non-significant decreases in body weight, were observed in the maternal animals. Offspring observations consisted of abortions and late resorptions. It was noted by the Committee that hemorrhages of the stomach were noted at necropsy for the majority of the does that aborted; this was considered evidence of severe maternal toxicity at the same dose level that produced the fetal lethality and was supportive of the conclusion that there was no increased susceptibility of the offspring.

6. Recommendation for a Developmental Neurotoxicity Study

The Committee did not recommend that a developmental neurotoxicity study be required for tralkoxydim. The following information was considered in the weight-of-evidence evaluation:

(i) No evidence of anomalies of the developing nervous system were observed in the prenatal developmental toxicity studies in either fetal rats or rabbits, at maternally and developmentally toxic oral doses up to and including 300 and 100 mg/kg/day, respectively.

(ii) No evidence of offspring behavioral abnormalities, that might be indicative of an effect on functional development, was observed in a three-generation reproduction study in rats (MRID 43339719) at dietary doses up to and including 100 mg/kg/day.

(iii) In the chronic/oncogenicity study in rats (MRID 43339707), bilateral retinal atrophy was observed in females at the highest dose tested (162.8 mg/kg/day). However, this finding was not observed in male rats in the same study, nor was it confirmed in other studies, including chronic studies in hamsters and dogs. No other treatment-related effects on histopathology of non-perfused tissues of the central or peripheral nervous system were observed in studies that measured these endpoints. No acute or 90-day neurotoxicity studies in rats or delayed neuropathy studies in chickens were required or available for review so there was no evaluation of the nervous system following perfusion.

(iv) In the subchronic dog study (MRID 43339706), an apparent dose-related trend in decreasing brain weight was observed, with statistical significance attained for high-dose (50 mg/kg/day) females. This finding was, however, not observed in the chronic (1-year) dog study (MRID 43339709) at an equivalent dose level. In addition, no other evidence of treatment related effects on brain weight were observed in the subchronic or chronic studies in rats or hamsters.

(v) Effects on the male reproductive system, consisting of increased benign Leydig cell tumors and decreased epididymal sperm, were observed at termination in the two-year rat study. These findings are suggestive of a treatment-related endocrine-mediated effect. It was also noted that a decrease in epididymal weight was observed in the subchronic dog study (MRID 43339706) at 50 mg/kg/day, and an increase in testicular weight was observed in the chronic/oncogenicity study in hamsters (MRID 43339710) at a dietary dose of 438.6 mg/kg/day, although these findings were not confirmed histopathologically. Further discussion of these findings is addressed in the section on reproductive toxicity (above). Following reassessment of this chemical to determine the need for a reproduction study conducted under the OPPTS 870.3800 guideline, consideration should be given to including a study segment to screen for effects on functional development.

7. Determination of the FQPA Safety Factor:

The HIARC, based on hazard assessment, recommends to the FQPA Safety Committee that the additional 10x safety factor should be removed because:

(i) Developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.

(ii) A three generation reproduction study in rats showed no increased susceptibility in pups compared to adults.

(iii) There was no evidence of abnormalities in the development of the fetal nervous system in the pre/post natal studies.

(iv) The toxicology database is complete and there are no data gaps. There is no evidence that would require a developmental neurotoxicity study.

The final recommendation on the FQPA Safety Factor will be made during risk characterization by the FQPA Safety Committee.

VI. HAZARD CHARACTERIZATION

Tralkoxydim is in the cyclohexandione class of chemicals which includes the previously registered chemicals clethodim and sethoxydim. These products are post-emergent herbicides whose primary mechanism of action involves inhibition of lipid biosynthesis and interference with growth regulation. Tralkoxydim is proposed for use as a post-emergent herbicide in barley, wheat and grass seed crops. All the required guideline studies have been submitted. All are acceptable with the exception of an oncogenicity study in hamsters which is awaiting review by the Cancer Risk Assessment Committee. The data base is considered complete; there are no

studies missing which are critical to assessment of potential hazard to human, including special sensitivity of infants and children. No published studies on tralkoxydim were available for review.

In subchronic studies in rats, dogs and hamsters (MRIDs 43339705, 43339706 and 43339712) the major toxicity appeared to be hepatic. In the dog (MRID 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 observe at this dose level. Minimal systemic toxicity was observed in rats (MRID 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 43339712) tralkoxydim had a clear effect on liver morphology and function at dose levels of 5,000; 10,000 and 20,000 ppm.

In chronic toxicity studies in dogs and rats the primary target organ of tralkoxydim toxicity was also the liver. In a chronic dog study (MRID 43339709), male and female beagle 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.

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.

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 observed associated histopathological changes were observed.

Findings were similar in the high dose group from a Chronic/Onco study in rats (MRID 43339070). 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.

There was no special susceptibility to the offspring of rats or rabbits following pre- and/or postnatal exposure to tralkoxydim. In the prenatal developmental toxicity studies in rats and rabbits, and in the three-generation reproduction study in rats, offspring toxicity was observed only at dose levels which were also toxic to the parental animals.

In the developmental toxicity study in rats, at the LOEL of 200 mg/kg/day, decreased fetal body weight and delays/alterations in fetal skeletal development occurred in the presence of significant maternal toxicity (decreased body weight, piloerection, hunched posture, moribundity). Similarly, at the LOEL of 100 mg/kg/day in the three-generation reproduction study in rats, both parental and offspring body weight decreases were observed.

In the prenatal developmental toxicity study in rabbits, both fetal and maternal toxicity were observed at the LOEL of 100 mg/kg/day. At this dose, reduced food consumption, accompanied by non-significant decreases in body weight, were observed in the maternal animals. Offspring observations consisted of abortions and late resorptions. It was noted by the Committee that hemorrhages of the stomach were noted at necropsy for the majority of the does that aborted; this was considered evidence of severe maternal toxicity at the same dose level that produced the fetal lethality and was supportive of the conclusion that there was no increased susceptibility of the offspring.

There is an indication that tralkoxydim may induce male reproductive toxic effects related to endocrine disruption. Increased benign testicular Leydig cell tumors and decreased epididymal sperm, were observed at termination in the two-year rat study (MRID 43339707) at a dietary dose of 117.9 mg/kg/day. These tumors were first observed at study week 82. It was additionally noted that a decrease in epididymal weight was observed in the subchronic dog study (MRID 43339706) at 50 mg/kg/day, and an increase in testicular weight was observed in the chronic/oncogenicity study in hamsters (MRID 43339710) at a dietary dose of 438.6 mg/kg/day, although these findings were not confirmed histopathologically. It was noted that effects on male reproductive function were not observed in the three-generation reproduction study in rats at a high-dose level of approximately 100 mg/kg/day; however, the animals in the multi generation study did not attain the age at which testicular tumors were first observed in the chronic/oncogenicity study. There were no measures of sperm count, motility, or morphology in the reproduction study.

VII. DATA GAPS

There are no data gaps for the standard Subsection F guideline requirements for a food use chemical by CFR Part 158.

VIII. ACUTE TOXICITY

Acute Toxicity of Tralkoxydim

Guideline No.	Study Type	MRID #(S).	Results	Toxicity Category
81-1	Acute Oral	43355701	LD_{50} >500 mg/kg	III
81-2	Acute Dermal	43355703	LD_{50} > 2000 mg/kg	III
81-3	Acute Inhalation	43301703 & 43787702	LC_{50} > 0.9 m/L	III
81-4	Primary Eye Irritation	43301705	Moderate irritant	III
81-5	Primary Skin Irritation	43301705	Slight irritation at 72 hr	IV
81-6	Dermal Sensitization	43222023 & 43355707	No dermal sensitization	

IX SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (Females 13+)	NOEL=30 UF = 100	Delayed ossification and skeletal misalignments	Developmental toxicity- Rats
	Acute RfD = 0.3 mg/kg		
Acute Dietary (General Population including Infants & Children)	None	An appropriate endpoint attributable to a single dose was not identified in oral toxicity studies including the developmental toxcity studies in rats and rabbits.	
Chronic Dietary	NOEL =0.5	Changes in liver morphology and function	Chronic toxicity - Dogs
	UF = 100	**Chronic RfD = 0.005 mg/kg/day**	
Short-Term (Dermal)	Oral NOEL=30*	Delayed Ossification and skeletal misalignments	Developmental toxicity -Rats
Intermediate-Term (Dermal)	Oral NOEL=30*	Delayed Ossification and skeletal misalignments	Developmental toxicity -Rats
Long-Term (Dermal)	None	The use pattern (once/year, post emergence) dose not indicate the need for this risk assessment.	
Inhalation (Short & Intermediate)	Oral NOEL=30*	Delayed Ossification and skeletal misalignments	Developmental toxicity -Rats
Long Term (Inhalation)	None	**The use pattern does not indicate the need for this risk assessment.**	

* Since an oral NOEL was selected a dermal absorption factor of 30% and inhalation absorption factor of 100% should be used during route-to-route extrapolation.

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Chemical:	**2-Cyclohexen-1-one, 2-{1-(ethoxyimino)pr**
PC Code:	**121000**
HED File Code	**21100 HIARC**
Memo Date:	**07/08/1998**
File ID:	**TX012680**
Accession Number:	**412-01-0082**

HED Records Reference Center
01/10/2001