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

014536

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: April 12, 2001

MEMORANDUM

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

FROM: David Nixon, Toxicologist. David Nixon 4/24/01
Registration Action Branch 1
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair Jess Rowland 4/12/01
and
Elizabeth Doyle, Co-Chair E.A. Doyle 4/20/01
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Sarah Levy, Risk Assessor
Registration Action Branch 1
Health Effects Division (7509C)

PC Code: 122990

On March 13, 2001, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for **MESOTRIONE** with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to **MESOTRIONE** was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996. The conclusions drawn at this meeting are presented in this report.

Committee Members in Attendance

Members present were: Bill Burnam, Beth Doyle, Jess Rowland, Pam Hurley, Elizabeth Mendez, David Nixon, Yung Yang, Jonathan Chen, Ayaad Assaad, Brenda Tarplee, (Executive Secretary)

Member(s) in absentia: Paula Deschamp

Data evaluation prepared by: David Nixon, RAB1

Also in attendance were: Bill Dykstra, Pauline Wagner, Bill hazel, Sara levy, Dana Vogel, Jim Stone (RD)

Data Evaluation / Report Presentation

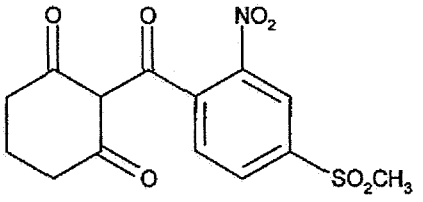
David Nixon
Toxicologist

1. INTRODUCTION

On March 13, 2001, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for mesotrione with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to mesotrione was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996.

The Health Effects Division (HED) Mechanism of Toxicity Science Assessment Review Committee convened on January 31, 2001 to evaluate the mechanism of toxicity of mesotrione in rats and mice in regards to tyrosine-mediated effects and assess the relevance of the two animal models to human health.

The Mechanism of Toxicity Committee determined that the petitioner has adequately demonstrated that for tyrosine-mediated toxicological effects, the mouse is a more appropriate model for assessing human risk than is the rat. This decision is based on comparative data on the activity of tyrosine aminotransferase (TAT) in the rat, mouse and human, and the similarities of the response to elevated plasma tyrosine levels in humans and the mouse (D272633; March 27, 2001).



Mesotrione

2. HAZARD IDENTIFICATION

2.1 Acute Reference Dose (RfD)

No appropriate endpoint was available to quantitate risk to the general population from a single-dose administration of mesotrione. The developmental effect, delayed ossification, was considered not to occur after a single dose in this instance since the weight-of-the-evidence suggests that the toxic effects caused by mesotrione occur after longer-term repeated exposures. No endpoint was chosen to quantitate risk to females 13-50 from a single-dose administration of mesotrione.

2.2 Chronic Reference Dose (RfD)

Study Selected: Multi-generation Reproduction Study - Mouse OPPTS 870.3800

MRID No.: 44505034

Executive Summary: In a 2-generation reproduction study (MRID 44505034), mesotrione (96.8% a.i., lot # P17) was administered in the diet continuously to Alpk:AP$_f$CD-1 mice (26 mice/sex/dose) at dose levels of 0, 10, 50, 350, 1500, or 7000 ppm (equivalent to 0, 2.1/2.4, 10.1/11.7, 71.4/82.5, 306.7/362.7, or 1455.5/1652.3 mg/kg/day [M/F] in the P and F_1 animals). The P animals were exposed to the test substance beginning at approximately 3 weeks of age and exposure lasted for approximately 8 weeks prior to mating. F_1 pups selected (26/sex/dose) to produce the F_2 generation were exposed to the same dosage as their parents beginning on postnatal day (PND) 29 and continuously throughout the rest of the study. F_1 animals were administered the test article for approximately 8 weeks prior to mating to produce the F_2 animals. Mating to produce an F_{2b} generation was not performed. Exposure of all animals to the test material was continuous throughout the study. The analytical data indicated that the mixing procedure was adequate and the variation between nominal and actual dosage to the study animals was acceptable.

There were no statistically significant treatment-related changes in mortality or reproductive performance observed in the P or F_1 adults. Adjusted (to LD 1) body weights were decreased during lactation in 7000 ppm P dams on LDs 5, 8, and 15 (↓3-13%). Body weights were decreased (↓9-12%) in 7000 ppm F_1 dams on LDs 1 (absolute) and 15 (adjusted). Food consumption was decreased in the 7000 ppm P dams throughout lactation (↓15-28%) and in 7000 ppm F_1 dams during weeks 2, 3, and 4 of lactation (↓10-33%).

An increase in the incidence of opaque eyes in 7000 ppm F_1 males (4/26 treated vs 0/26 controls) and females (6/26 treated vs 0/26 controls) was observed. Opaque eyes were also observed in a single animal from each of the following groups: 7000 ppm P males, 10 and 350 ppm F_1 males, and 1500 ppm F_1 females. At necropsy, an associated increase was observed in the incidence of grossly visible opaque/cloudy eyes (4 - 20% treated vs. 0 - 4% controls) in 7000 ppm P males, P females, F_1 males and F_1 females. In addition, upon histological examination, an increase was observed in the incidence of minimal to marked unilateral and bilateral ocular cataractous change in the 7000 ppm P males (unilateral - 3/26 treated vs 0/26 controls), F_1 males (unilateral - 7/25 treated, bilateral 1/25 treated vs 0/26 controls), and F_1 females (unilateral - 4/26 treated, bilateral 1/26 treated vs 0/26 controls). Retinal detachment with marked cataractous change was also observed in one of the 7000 ppm F_1 males and females; whether this lesion was a primary effect of mesotrione on the eye is unclear.

Absolute kidney weights were statistically significantly increased in F_1 males at 350 ppm and above. Percent increases above controls were 11, 8, and 13% for 350, 1500, and 7000 ppm, respectively. Relative kidney weights were also statistically significantly increased at 350 ppm and above in males. Percent increases above controls were 6, 8 and 17% for 350, 1500, and 7000 ppm, respectively. In females, relative kidney weights were statistically significantly increased at 50 ppm and above, but percent increases on ranged from 4 to 9% above controls.

The LOAEL for parental toxicity is 350 ppm (equivalent to 71.4/82.5 mg/kg/day [M/F]) based upon dose-related and statistically significant increases in kidney weights in F_1

males. The increased kidney weights may be associated with tyrosyluria since tyrosinemia was observed at all dose levels in F_1 animals (exposed in utero) in this study. Ocular lesions in both sexes and decreased body weights in females were observed at higher dose levels. The NOAEL for parental toxicity is 50 ppm (equivalent to 10.1/11.7 mg/kg/day [M/F]).

No treatment-related effects on mortality or viability were observed at any time in the F_1 and F_2 litters. An increase in the number of pups with opaque eyes (6 pups in 1 litter vs 0 controls) and pups with eye(s) shut (7 pups in 4 litters vs 0 controls) was observed in the 7000 ppm F_2 pups. An increase in the number of pups with ocular discharge was observed in the 7000 ppm dose groups (F_1 and F_2 - 11 pups in 5 litters each vs 0 controls). A slight increase in the number of pups with ocular discharge was also observed in the other F_1 (1-5 pups/group in 1-3 litters/group in the 10, 50, 350, and 1500 ppm groups) and F_2 litters (2-3 pups/group in 2-3 litters in the 10, 350, and 1500 ppm groups). At necropsy, an increase was observed in the incidence of opaque or cloudy eyes in 7000 ppm (10/33 treated vs 0/30 controls) and 1500 ppm (4/30 treated) F_2 males. Upon histological examination, an associated increase was observed in the incidence of microscopic minimal to marked unilateral and/or bilateral cataractous changes in all 7000 ppm groups, with the severity ranging from minimal to marked: F_1 males - 4/30 treated vs 0/37 controls; F_1 females - 2/30 treated vs 0/40 controls; F_2 males - 11/33 treated vs 0/30 controls; F_2 females - 2/31 treated vs 0/32 controls. Minimal unilateral cataractous change was also observed in the 1500 ppm F_2 males (2/18 treated vs 0/30 controls).

Body weights were decreased in all 7000 ppm groups: F_1 males from PND 8 to weaning (↓6-24%); F_1 females from PND 15 to weaning (↓14-22%); F_2 males from PND 15 to weaning (↓9-20%); F_2 females from PND 22 to weaning (↓12-17%). Body weights were also decreased in 1500 ppm groups on PND 22 and 29 (↓6-14%). Body weight gains, as calculated by the reviewers, were decreased in both generations in the 7000 ppm groups (↓16-24%).

Plasma tyrosine levels were elevated in a dose related manner in all F_1 treatment groups (exposed in utero). A statistical assessment of these data was not presented by the study investigators. However, increases ranged from 269-586% of controls in males and from 159-542% of controls in females. In addition, plasma tyrosine levels were even more dramatically elevated in F_2 pups. At the 10 ppm level, male tyrosine levels were 794% of controls and females levels were 665% of controls. At the 7000 ppm level, male tyrosine levels were 5009% of controls and females were 3945% of control levels.

The LOAEL for offspring toxicity for males and females is 10 ppm (LDT) (equivalent to 2.1/2.4 mg/kg/day [M/F]) based on tyrosinemia at all dose levels in F_{2a} pups (exposed in utero). In addition, ocular discharge was observed at all dose levels in F_1 pups (and in nearly all F_2 pup dose groups) with a 0 incidence in controls and cataractous changes were observed histologically at the high dose level. An offspring toxicity NOAEL was not observed.

Even though a NOAEL for effects observed in offspring was not determined, this reproductive

study in the mouse is determined to be **acceptable/guideline (§83-4(b), reproduction)** and satisfies the requirements for a multigenerational reproductive toxicity study in mice as per the Hazard Identification Assessment Review Committee (March 13, 2001).

Dose and Endpoint for Establishing RfD: 2.1 mg/kg/day (LOAEL), based upon tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups.

Uncertainty Factor(s): 300

Comments about Study/Endpoint/Uncertainty Factor: This study and endpoint are appropriate for the route and duration of exposure. A 3X is applied to the uncertainty factor due to the use of a LOAEL for this endpoint. There is concern about the relationship between elevated plasma tyrosine levels and neurotoxicity in children; however, since there is strong evidence that the sensitivity to mesotrione in humans is similar to the mouse, a 3X should be an adequate safety factor to protect sensitive populations. Also, the requested developmental neurotoxicity study should provide data to correlate plasma tyrosine levels to neurological effects to further characterize the risk to children.

$$\textbf{Chronic RfD} = \frac{2.1 \text{ mg/kg/day}}{300} = \textbf{0.007 mg/kg/day}$$

2.3 Occupational/Residential Exposure

2.3.1 Short-Term (1-7 days) Incidental Oral Exposure

Study Selected: Developmental Toxicity - Rat Guideline #: OPPTS 870.3700

MRID No.: 44920801

Executive Summary: In a developmental toxicity study (MRID 44920801), mesotrione (96.8% a.i., Lot#: P17) in deionized water was administered to pregnant Alpk:AP$_f$SD rats (24/dose) at dose levels of 0, 100, 300, or 1000 (limit dose) mg/kg/day by gavage on gestation days (GDs) 7 through 16. All dams were sacrificed on GD 22. No premature deaths occurred during the study.

Treatment-related toxicity was characterized by reduced body weight gains and food consumption at the mid- and high-dose levels. When compared to concurrent controls, no treatment-related changes in absolute body weight, gravid uterine weight, Cesarean section parameters, or gross pathology were noted at any dose level tested. Urine staining and colored feces were not considered adverse clinical effects.

At 1000 mg/kg, pink and/or purple colored feces (130 incidents in 22/24 animals vs 0/24 controls) and dry or wet staining with urine (29-39 observations in 6-8/24 animals vs 0/24 controls) were observed. Body weight gains, as calculated by reviewers, were reduced during the overall treatment interval (↓20%, GDs 8-16, not analyzed for statistical significance). In addition, decreases were noted in food consumption during GDs 7-16 (↓14-18%, $p \leq 0.01$). Body weights and food consumption increased in all dose groups including controls during the post-treatment interval. At 300 mg/kg, an increased incidence of pink and/or purple colored feces was noted (35 observations in 10/24 animals vs 0/24 controls). Body weight gains were reduced during the overall treatment interval (↓17%, GDs 8-16). Decreases were noted in food consumption during GDs 7 through 16 (↓8-13%, $p \leq 0.01$). At 100 mg/kg, no treatment-related adverse effects were noted.

The maternal LOAEL is 300 mg/kg/day, based upon decreased body weight gains during treatment and decreased food consumption. The maternal NOAEL is 100 mg/kg/day.

Developmental toxicity was characterized by decreased mean fetal weight (↓6%, $p \leq 0.01$) at the 1000 mg/kg/day level and a dose-dependent increase in incidences of decreased ossification of vertebral centra and of the *manus* and *pes* at all dose levels. Incidences of "non-ossification" of several cervical centra ranged from 2 - 87% (25 - 100%)[% fetal incidence (% litter incidence)] as compared to controls [0.2 - 22% (4.2 - 88%)]. Reduced ossification was seen in other skeletal structures such as cervical vertebra arches, vertebra transverse processes, otontoids, and calcaneum. When compared to mean scores for the controls, increased mean *manus* and *pes* scores/litter for all treatment groups were observed (↑4-11%). It should be noted that this shift toward reduced ossification was also observed in mice administered the test substance (MRID 44920802).

The developmental LOAEL is 100 mg/kg/day, based on delays in skeletal ossification and changes in *manus/pes* ossification assessments. The developmental NOAEL was not established.

Even though a developmental NOAEL was not established and no dose rationale was provided, this developmental toxicity study is classified **acceptable/guideline (§83-3[a])**, and satisfies the guideline requirements for a developmental toxicity study in the rat as per the Hazard Identification Assessment Review Committee (March 13, 2001).

Dose and Endpoint for Risk Assessment: Maternal NOAEL of 100 mg/kg/day, based upon decreased body weight gains during treatment and food consumption.

Comments about Study/Endpoint: The endpoint of concern is appropriate for the population of concern (infants and children). The effects seen in this rat study occurred in the absence of other tyrosine-mediated effects that are considered part of the

n

mechanism of toxicity of mesotrione. Since these effects were seen, along with the clinical signs observed at the high dose, without any ocular effects, it is presumed that the body weight effects were a result of a different mechanism of toxicity.

2.3.2 Intermediate-Term (7 Days to Several Months) Incidental Oral Exposure

Study Selected: Multi-generation Reproduction Study- Mouse
Guideline #: OPPTS 870.3800

MRID No.: 44505034

Executive Summary: See Chronic RfD (Section 2.2)

Dose and Endpoint for Risk Assessment: Offspring LOAEL of 2.1 mg/kg/day, based upon tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups.

Comments about Study/Endpoint: The endpoint of concern is appropriate for the population of concern (infants and children). There is concern about the relationship between elevated plasma tyrosine levels and neurotoxicity in children; however, since there is strong evidence that the sensitivity to mesotrione in humans is similar to the mouse, a 3X should be an adequate safety factor to protect sensitive populations. Also, the requested developmental neurotoxicity study should provide data to correlate plasma tyrosine levels to neurological effects to further characterize the risk to children.

2.3.3 Dermal Absorption

Dermal Absorption Factor: No dermal absorption study was submitted. Using a ratio of the maternal LOAEL from the developmental rabbit study and the NOAEL from the rabbit dermal toxicity study, one can derive a dermal absorption factor of 25% as an upper-bound estimate.

$$\frac{\text{Dev. Rabbit LOAEL}}{\text{Dermal Tox. NOAEL}} = \frac{250 \text{ mg/kg/day}}{1000 \text{ mg/kg/day}} = 25\%$$

2.3.4 Short-Term Dermal (1-7 days) Exposure

Study Selected: Developmental Toxicity - Rat Guideline #: OPPTS 870.3700

MRID No.: 44920801

8

Executive Summary: See Short-term Incidental Oral (Section 2.3.1)

Dose and Endpoint for Risk Assessment: Developmental LOAEL of 100 mg/kg/day, based upon delays in skeletal ossification and changes in manus/pes ossification assessments.

Comments about Study/Endpoint: A 21-day dermal study was submitted with no systemic effects noted; however, the dermal study did not evaluate developmental toxicity. Since the rat, mouse and rabbit developmental toxicity studies had developmental effects at doses below those at which maternal toxic effects were seen, it is appropriate to choose an oral developmental study for this risk assessment and do route-to-route extrapolation to adequately protect against potential developmental hazards via dermal exposure. The developmental effects seen in this rat study are not considered part of the tyrosine-mediated mechanism of toxicity of mesotrione. A dermal absorption factor of 25% should be applied.

2.3.5 Intermediate-Term Dermal (7 Days to Several Months) Exposure

Study Selected: Multi-generation Reproduction Study- Mouse
Guideline #: OPPTS 870.3800

MRID No.: 44505034

Executive Summary: See Chronic RfD (Section 2.2)

Dose/Endpoint for Risk Assessment: Offspring LOAEL of 2.1 mg/kg/day, based upon tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups.

Comments about Study/Endpoint: A 21-day dermal study was submitted with no systemic effects noted; however, the dermal study did not evaluate offspring and reproductive effects. Since there are definitive effects in offspring at doses below those where effects are seen in adults, it is appropriate to choose an oral study for this risk assessment and do route-to-route extrapolation to adequately protect against reproductive hazards via dermal exposure. A dermal absorption factor of 25% should be applied. See comments in Section 2.3.2.

2.3.6 Long-Term Dermal (Several Months to Life-Time) Exposure

Study Selected: Multi-generation Reproduction Study- Mouse
Guideline #: OPPTS 870.3800
MRID No.: 44505034

Executive Summary: See Chronic RfD (Section 2.2)

Dose and Endpoint for Risk Assessment: Offspring LOAEL of 2.1 mg/kg/day, based upon tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups.

Comments about Study/Endpoint: No long-term dermal study was submitted. The chosen endpoint is from a study of the appropriate duration of exposure. A dermal absorption factor of 25% should be applied. See comments in Section 2.3.2.

2.3.7 Short-Term Inhalation (1-7 days) Exposure

Study Selected: Developmental Toxicity - Rat Guideline #: OPPTS 870.3700

MRID No.: 44920801

Executive Summary: See Short-term Incidental Oral (Section 2.3.1)

Dose and Endpoint for Risk Assessment: Developmental LOAEL of 100 mg/kg/day, based upon delays in skeletal ossification and changes in manus/pes ossification assessments.

Comments about Study/Endpoint: No inhalation study was submitted. The chosen endpoint is of the appropriate duration of exposure. The developmental effects seen in this rat study are not considered part of the tyrosine-mediated mechanism of toxicity of mesotrione. An inhalation absorption factor of 100% should be applied.

2.3.8 Intermediate-Term Inhalation (7 Days to Several Months) Exposure

Study Selected: Multi-generation Reproduction Study- Mouse
Guideline #: OPPTS 870.3800

MRID No.: 44505034

Executive Summary: See Chronic RfD (Section 2.2)

Dose/Endpoint for Risk Assessment: Offspring LOAEL of 2.1 mg/kg/day, based upon tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups.

Comments about Study/Endpoint: No inhalation study was submitted. The chosen

endpoint is of the appropriate duration of exposure. An inhalation absorption factor of 100% should be applied. See comments in Section 2.3.2.

2.3.9 Long-Term Inhalation (Several Months to Life-Time) Exposure

Study Selected: Multi-generation Reproduction Study- Mouse
Guideline #: OPPTS 870.3800

MRID No.: 44505034

Executive Summary: See Chronic RfD (Section 2.2)

Dose/Endpoint for Risk Assessment: Offspring LOAEL of 2.1 mg/kg/day, based upon tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups.

Comments about Study/Endpoint: No inhalation study was submitted. The chosen endpoint is of the appropriate duration of exposure. An inhalation absorption factor of 100% should be applied. See comments in Section 2.3.2.

2.3.10 Margins of Exposure for Occupational/Residential Risk Assessments

A MOE of 300 is required for short-, intermediate-, and long-term occupational risk assessments for both dermal and inhalation routes of exposure. This includes the conventional 100 and and additional 3x for the use of a LOAEL.

The acceptable MOEs for residential exposure will be determined by the FQPA SF committee.

2.4 Recommendation for Aggregate Exposure Risk Assessments

For short-term exposure, dermal and inhalation endpoints can be aggregated because of the use of oral equivalents and a common endpoint (developmental toxicity); however, incidental oral cannot be combined due to the lack of a common toxicological endpoint. For intermediate-term and long-term exposure, dermal, oral, and inhalation endpoints can be aggregated because of a common toxicological endpoint (tyrosinemia).

3 CLASSIFICATION OF CARCINOGENIC POTENTIAL

3.1 Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID No.: 44505035

Executive Summary: In a combined chronic/oncogenicity study (MRID 44505035, 44505036), mesotrione (96.8% a.i.) was administered via the diet to 64 Alpk:AP$_f$SD rats/sex/group at 0, 7.5, 100, or 2500 ppm (equivalent to 0, 0.48, 6.48 or 159.89 mg/kg/day in males and 0, 0.57, 7.68, or 189.48 mg/kg/day in females) for up to 104 weeks. To assess ocular toxicity, an additional 20 rats/sex/dose were dosed at 1 or 2.5 ppm (equivalent to 0.06 and 0.16 mg/kg/day in males and 0.08 and 0.19 in females). Twelve main study rats/sex/dose were terminated after 52 weeks.

No treatment-related adverse effects were observed on mortality, food consumption, food efficiency, or hematology, clinical chemistry, and urinalysis parameters for either sex at any treatment level. All male groups were terminated when survival dropped to approximately 25% during weeks 92/93 and 97/98. Chronic progressive glomerulonephropathy was the major contributory factor involved in the intercurrent deaths. There was no evidence of a trend in male or female Kaplan-Meier survival rates. Female groups were terminated as scheduled in week 104.

The major target organ was the eye. In the 7.5-, 100-, and 2500-ppm males and in the 100- and 2500-ppm females ocular lesions consisting of cloudy eyes, corneal lesions consisting of opacity/hazy opacity/vascularization/ghost vascularization, and/or corneal keratitis were observed. No treatment-related ocular lesions were observed in the 1- and 2.5-ppm animals or in females dosed at 7.5 ppm.

Reductions ($p<0.05$ or 0.01) in mean body weight were observed throughout the study in the 7.5-ppm males (↓2-13%) and the 100- and 2500-ppm males and females (↓1-17%). Body weight reductions ($p<0.05$ or 0.01) were also observed in the 2.5- and 1-ppm male satellite groups. Body weight decreases of over 10% in the 2.5- and 7.5-ppm males were noted only from week 75 to termination after body weights had stabilized in all groups. Since there were random fluctuations of the weights among all groups during this time period, body weight changes at 7.5 ppm and below were not considered biologically relevant. Decreases in body weight gain from weeks 1 to 47 were only biologically significant in males at 100 and 2500 ppm and, in females, at 2500 ppm. There was no dose relationship with the decrease in body weight gain in either sex among all treatment groups from week 47 to termination.

Increases ($p<0.01$) in absolute kidney weights in the 7.5-ppm and the 2500-ppm males (↑12-15%) and adjusted (for body weight) kidney weights in the 7.5-, 100-, and 2500-ppm males (↑13-22%) were observed at the interim sacrifice (week 53). These differences in kidney weights were not observed at the terminal sacrifice.

Increases ($p<0.05$ or 0.01) in absolute and adjusted liver weights in the 7.5-ppm males (↑18 and 17%, respectively), adjusted liver weights in the 100- and 2500-ppm males (↑15-18%), and adjusted liver weights in the 100- and 2500-ppm females (↑11-14%) were observed at the interim sacrifice. At the terminal sacrifice, increases ($p<0.05$ or 0.01) in absolute and adjusted liver weights in the 100- and 2500-ppm males (↑7-20%) were observed. During gross examination of the 7.5-, 100-, and 2500-ppm dose groups, pale liver was observed in the males (25-27/treated group vs. 9 controls) and females (3-10/treated group vs. 2 controls). During histopathological examination, minimal to marked hepatocyte fat vacuolation was observed in the 7.5-, 100-, and 2500-ppm males (36-39/treated group vs. 17 controls) and the 100- and 2500-ppm females (14-16/treated group vs. 8 controls).

Decreased absolute adrenal weights in the 100- and 2500-ppm males (↓21 and 22%, respectively) and decreased adjusted adrenal weights in the 7.5-, 100-, and 2500-ppm males (↓19, 25, and 29%, respectively) showed a slight dose-dependent trend, but no histopathological abnormalities were observed.

The administration of mesotrione to rats up to 2500 ppm (159.9 mg/kg/day for males, 189.5 mg/kg/day for females) in the diet did not result in an overall treatment-related increase in incidence of tumor formation.

Under the conditions of this study, dosing is considered adequate to assess the carcinogenic potential of mesotrione based on the ocular and hepatic lesions and increased liver and kidney weights noted at 7.5 ppm and above and body weight effects at 100 ppm and above in males and increased liver weights and ocular and hepatic lesions at 100 ppm and above in females.

The LOAEL for this combined chronic toxicity/ carcinogenicity rat feeding study is 7.5 ppm (0.48 mg/kg/day for males, 0.57 for females) based on ocular lesions, increases in kidney and liver weights, and hepatocyte fat vacuolation in males. No NOAEL was determined for kidney and liver weights or hepatocyte fat vacuolation in males. The NOAEL for ocular lesions in the special study is 2.5 ppm (0.16 mg/kg/day for males, 0.19 mg/kg/day for females).

The submitted study is classified as **acceptable (§83-5)** and does satisfy the guideline requirements for a chronic toxicity study (§83-1) and a carcinogenicity study (§83-2) in rats.

Discussion of Tumor Data: The administration of mesotrione to rats up to 2500 ppm (159.9 mg/kg/day for males, 189.5 mg/kg/day for females) in the diet did not result in an overall treatment-related increase in incidence of tumor formation.

Adequacy of the Dose Levels Tested: Under the conditions of this study, dosing is considered adequate to assess the carcinogenic potential of mesotrione based on the ocular and hepatic lesions and increased liver and kidney weights noted at 7.5 ppm and above and body weight

effects at 100 ppm and above in males and increased liver weights and ocular and hepatic lesions at 100 ppm and above in females.

3.2 Carcinogenicity Study in Mice

MRID No.: 44505028

Executive Summary: In a mouse oncogenicity study (MRID 44505028), mesotrione (96.8% a.i., Lot/Batch # P17) was administered in the diet to C57BL/10J$_f$CD-1 Alpk mice (55/sex/group) for up to 80 weeks at 0, 10, 350, or 7000 ppm (equivalent to 0/0, 1.4/1.8, 49.7/63.5, and 897.7/1102.9 mg/kg/day [M/F], respectively). The high-dose animals received 3500 ppm of mesotrione for the first 7 weeks of the study and then received 7000 ppm for the remainder of the dosing interval.

The doses were selected on the basis of a 90-day feeding study in mouse carried out in the performing laboratory; no further information was provided. In a subchronic oral toxicity study (MRID 44505022) reviewed with the current submission, mesotrione (96.8% a.i) was administered for 13 weeks to 20 mice/sex/dose at dietary concentrations of 10, 50, 350, or 7000 ppm. All the parameters were unaffected. The NOAEL was 7000 ppm and no LOAEL was observed.

Mortality, clinical signs, food consumption, hematology, organ weights, and macroscopic and histopathological findings for both sexes at all doses were unaffected by treatment with mesotrione. In the 7000 ppm females and in both sexes of the 10 and 350 ppm dose groups, body weights, body weight gains, and food efficiency were also unaffected.

In the 7000 ppm males, slight, but consistent mean body weight reductions (↓2-9%; $p<0.05$ or 0.01) were observed during weeks 13 to 81. Overall (weeks 1 to 81) body weight gain (calculated by the reviewers) was reduced by approximately 20% compared to controls. Mean food consumption was consistently increased (↑4-10%; $p<0.01$) compared to controls during the first 12 weeks of the study and generally similar to controls thereafter, but food efficiency was reduced ($p<0.05$ or 0.01) during weeks 1-4 (↓12%), 9-12 (↓40%), and overall (weeks 1-12; ↓16%).

The LOAEL is 7000 ppm (equivalent to 897.7/1102.9 mg/kg/day M/F) based on minimal but, consistently reduced body weights, and reduced body weight gains and food efficiency in males. The NOAEL is 350 ppm (equivalent to 49.7/63.5 mg/kg/day M/F).

In this study no treatment-related neoplastic changes were observed.
The submitted study is classified as **acceptable/guideline (§83-2b)** and does satisfy the guideline requirements for a carcinogenicity study in mice.

Discussion of Tumor Data: The administration of mesotrione to mice up to 7000 ppm (897.7 mg/kg/day for males, 1102.9 mg/kg/day for females) in the diet did not result in an overall treatment-related increase in incidence of tumor formation.

Adequacy of the Dose Levels Tested: Under conditions of this study, dosing is considered adequate to assess the carcinogenic potential of mesotrione based upon decreases in body weight gains and food efficiency in males at 7000 ppm. No effects were noted in females; however, females received dosages above the limit dose.

3.3 Classification of Carcinogenic Potential

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the HIARC classified mesotrione as "not likely to be carcinogenic to humans" by all routes of exposure based upon lack of evidence of carcinogenicity in rats and mice.

4 MUTAGENICITY

Four acceptable genetic toxicology studies were available for review. The results from these studies indicate that mesotrione was not mutagenic in *Salmonella typhimurium*, *Escherichia coli* or in cultured mouse lymphoma cells. There was also no evidence of clastogenicity *in vitro*, and mesotrione gave a negative response for the induction of micronucleated polychromatic erythrocytes in bone marrow. Overall, the data suggest that mesotrione is negative for mutagenicity *in vitro* and *in vivo*. The acceptable studies satisfy the 1991 mutagenicity guideline requirements.

REVERSE GENE MUTATION ASSAY

In a microbial reverse gene mutation assay (MRID 44373526), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strains WP2P and WP2P *uvrA* were exposed to ZA 1296 (98.1% a.i.) in sterile deionized water in repeat tests at concentrations of 0, 100, 200, 500, 1000, 2500, or 5000 µg/plate in the presence and absence of mammalian metabolic activation (±S9). Both the standard plate incorporation test (±S9) and the liquid preincubation modification (+S9) were performed. S9 homogenates for metabolic activation were prepared from phenobarbital/ß-naphthoflavone-induced rat livers. The standard strain-specific mutagens served as positive controls.

ZA 1296 was tested to the limit dose. ZA 1296 was not mutagenic in any tester strain under all conditions. The positive control substances induced marked increases in revertant colonies in their respective strains with or without activation.
This study is classified as **acceptable (§84-2)** and satisfies the FIFRA Test Guideline requirements for an *in vitro* mutagenicity (reverse gene mutation) test.

FORWARD GENE MUTATION ASSAY

In a mammalian cell forward gene mutation assay at the thymidine kinase locus (MRID 44373525), L5178Y TK +/- mouse lymphoma cells cultured in vitro were exposed in repeat (independent) assays to ZA 1296 (98.1% a.i.) in dimethylsulfoxide (DMSO) at concentrations ranging from 125 to 1000 µg/mL with or without S9 activation provided by phenobarbital/beta naphthoflavone-mix. ZA 1296 was tested up to a dose slightly in excess of the limit of solubility. The highest concentrations evaluated for mutagenicity were based on a cell survival of ≥10% compared to the vehicle control. There were no treatment-related increases in mutant frequency in the presence or absence of S9 activation. The positive controls gave the appropriate responses in both trials. **ZA 1296 was negative for inducing forward mutations at the TK locus in mouse L5178Y cells with or without S9 activation.**

This study is classified as **acceptable (§84-2)**, and satisfies the FIFRA Test Guideline requirements for *in vitro* mammalian forward gene mutation data.

CHROMOSOME ABERRATION ASSAY

In two independent *in vitro* mammalian cell chromosome aberration assays (MRID 44353724), primary human lymphocyte cell cultures from one male and one female donor were exposed to culture medium or dimethylsulfoxide (DMSO) alone or to ZA 1296 (98.1% a.i.) in dimethylsulfoxide (DMSO) at concentrations of 250, 1000, and 1500 or 2000 µg/mL without metabolic activation (-S9) and 250, 1000, and 2000 µg/mL with metabolic activation (+S9) provided by phenobarbitol/beta naphthaflavone-mix. Cultures were treated for 20 hours without S9 activation and harvested immediately or 24 hours after cessation of treatment, or 3 hours with S9 activation and harvested 17 hours after cessation of treatment. Microscopic slides of metaphase-arrested lymphocytes were prepared and 150-200 metaphases per donor per dose level ±S9 were examined for chromosomal aberrations.

ZA 1296 was tested to cytotoxic levels. The dose levels selected for the chromosome aberration assay were based on pH of the culture medium, the mitotic index, and cytotoxic effects on chromosome structure. With cell harvest immediately after a 20-hour treatment period in the absence of metabolic activation, dose-related, statistically significant increases ($p<0.05$ or $p<0.01$) in the percent of metaphases with aberrations excluding gaps were observed in male donor lymphocyte cultures treated with ZA 1296 at 1000 or 1500 µg/mL. However, the percent of metaphases with aberrations in the concurrent negative control was lower than expected. A dose-related increase in percent metaphases with aberrations was also observed in corresponding female donor lymphocyte cultures; however, the increases were not statistically significant. There were no increases in percent metaphases with aberrations in S9-activated cultures or in non-activated cultures sampled 24 hours after cessation of treatment. Under the given experimental conditions, ZA 1296 was not clastogenic with S9 activation and was equivocal for clastogenic activity without S9 activation. The sensitivity of the assay system to detect structural chromosome alterations was adequately demonstrated by the results obtained with the positive controls (cyclophosphamide, +S9; mitomycin C, -S9).

This study is classified as **acceptable (§84-2).** It satisfies the requirement for FIFRA Test Guideline for *in vitro* cytogenetic mutagenicity data.

MICRONUCLEUS ASSAY

In a bone marrow micronucleus assay (MRID 44373527), groups of 5 CD-1 mice/sex were treated by gavage with ZA 1296 (mesotrione, 98.1% a.i.) in deionized water at a single dose of 500 mg/kg. Five mice/sex received the vehicle alone. Mice were observed for mortality and clinical signs of toxicity at 24 and 48 hours post-dosing. Five mice/sex each from the vehicle and ZA 1296-treated groups were sacrificed at 24 or 48 hours after treatment; bone marrow cells were harvested at each sacrifice time and scored for micronucleated polychromatic erythrocytes (MPCEs). Five mice per sex received a single gavage dose of cyclophosphamide (65 mg/kg) as the positive control and bone marrow was harvested at 24 hours post-treatment.

The selected dose, 500 mg/kg, was based on a preliminary toxicity test at 320-2000 mg/kg that showed deaths occurring at ≥800 mg/kg. In the micronucleus test, one ZA 1296-treated male was found dead 4 hours after treatment. ZA 1296 was not toxic to the bone marrow (PCE:NCE ratio), and **ZA 1296 gave a negative response for the induction of micronucleated polychromatic erythrocytes in bone marrow at both sampling times.** The sensitivity of this test to detect a genotoxic response was demonstrated by the significant ($p<0.01$) increase in MPCEs induced by the positive control (cyclophosphamide).

This study is classified as **acceptable (§84-2)** and does satisfy the FIFRA Test Guideline requirements for an *in vivo* cytogenetic mutagenicity test.

5 FQPA CONSIDERATIONS

5.1 Adequacy of the Data Base

The following studies are available:

-- Acute and subchronic neurotoxicity studies in rat

-- Developmental toxicity studies in rat, mouse & rabbit

-- Multi-generation reproduction studies in rat & mouse

All studies are acceptable except the rabbit developmental study; however, there is adequate information for an FQPA evaluation. The HIARC determined that another rabbit study was not needed since an increased susceptibility of fetuses *in utero* was demonstrated and another study would not provide additional information for FQPA and hazard assessment.

5.2 Neurotoxicity

Acute Delayed Neurotoxicity - Hen N/A

Acute Neurotoxicity -§81-8 : In this acute oral neurotoxicity study (MRID 44505017), mesotrione (ZA1296; 97.6% a.i., Lot/batch # P22) was administered in a single dose by gavage to 10 Alpk:AP$_f$SD rats/sex/dose at doses of 0, 20, 200, or 2000 mg/kg. After two weeks, five animals/sex/group were perfused for neurohistological examination and animals from the control and high-dose groups were examined microscopically. Functional observational battery (FOB) and motor activity were evaluated during week -1 and on days 1 (approximately 2 hours post-dosing), 8, and 15. No treatment-related deaths occurred. Clinical signs, FOB parameters, motor activity, body weights, body weight gains, food consumption, gross pathology, histopathology, and brain weights and dimensions were unaffected by the test substance.

The LOAEL is >2000 mg/kg, based upon lack of any systemic effects. The NOAEL for this study is 2000 mg/kg.

Although no effects were noted at the high dose, the substance was tested at the limit dose, so the high dose is acceptable. This study is classified as **acceptable/guideline** and satisfies the requirements for an acute neurotoxicity screening battery in rats (§81-8a; 870.6200).
Subchronic Neurotoxicity -§82-5 : In this subchronic neurotoxicity screening battery, mesotrione (ZA1296, 97.6% a.i., Lot/Batch P22) (MRID 44505025) was administered continuously in the diet for 90 days to 12 Alpk:AP$_f$SD rats/sex/group at doses of 0, 2.5, 100, or 5000 ppm (equivalent to [M/F] 0/0, 0.20/0.23, 8.25/9.29, or 402.8/466.6 mg/kg/day). Five animals/sex/group were perfused for neurohistological examination and animals from the control and high-dose groups were examined microscopically. The functional observational battery (FOB) and motor activity were evaluated during weeks -1, 5, 9, and 14.

No treatment-related deaths occurred. Food consumption and utilization, FOB parameters, motor activity, brain dimensions, and neuropathology were unaffected by the test substance.

Corneal opacities and/or vascularization of the cornea were seen in 3/11 mid-dose males and 1/12 mid-dose females. Corneal opacities and/or vascularization of the cornea were seen in 10/12 high dose males, and 7/12 high dose females. Overall (weeks 1-14) body weight gains were decreased in the high-dose females (↓18%). High-dose females displayed decreased body weights (adjusted for initial body weight) from week 2 until study termination (↓5-9%).in the high-dose females at week 14 (↑37%). No treatment-related findings were observed in the 2.5 ppm group.

The LOAEL for this study is 100 ppm (equivalent to 8.25 mg/kg/day in males and 9.29 mg/kg/day in females) based on corneal opacities and/or vascularization of the cornea of the eye. The NOAEL for this study is 2.5 ppm (equivalent to 0.20 mg/kg/day in males and 0.23 mg/kg/day in females).

The submitted study is classified as **acceptable/guideline (§82-7[a])** and satisfies the guideline requirements for a subchronic neurotoxicity screening battery in rats.

-- EVIDENCE OF NEUROTOXICITY FROM OTHER ORAL TOXICITY STUDIES

Multi-generation mouse reproduction study (MRID 44505034) - One F_1 male and one F_1 female had retinal detachment with marked cataractous changes at the highest dose tested (>1000 mg/kg/day).

Subchronic toxicity dog study (MRID 44505023) - High-dose females had decreased absolute and relative brain weights; however, no microscopic abnormalities were noted in any brain tissue from the high-dose group and the effect was not observed in the chronic toxicity dog study.

5.3 Developmental Toxicity

Developmental Rat - See Short-term Incidental Oral (Section 2.3.1) for executive summary.

Developmental Mouse

In a developmental toxicity study (MRID 44920802), mesotrione (96.8% a.i., Lot #: P17) in water was administered to pregnant Alpk:AP$_f$CD-1 mice (26/dose) at dose levels of 0, 10, 60, 150, or 600 mg/kg/day by gavage on gestation days (GDs) 5 through 18. All dams were sacrificed on GD 19. One 60 mg/kg female was sacrificed *in extremis* on GD 12 following the observation of a subcutaneous mass on the left anterior thorax on GD 9 and subsequent reduced body weight and food consumption. No other premature deaths occurred during the study.

When compared to concurrent controls, no treatment-related clinical signs, changes in body weight or adjusted body weights (using GD 5 body weight as a covariant), gravid uterine weight, food consumption, gross pathology, or reproductive parameters were noted at any dose level tested.

The maternal LOAEL was not observed. The maternal NOAEL was ≥ 600 mg/kg/day.

At 600 mg/kg, a treatment-related pattern toward decreased ossification of the cervical vertebrae centra was observed. In addition, a number of other delays in ossification or variations were apparent at the high dose level (see Table 4c).

The developmental LOAEL is 600 mg/kg/day based on the pattern toward decreased ossification of the cervical vertebrae centra (fetuses and litters). The developmental NOAEL is 150 mg/kg/day.

This developmental toxicity study is classified **acceptable (§83-3[b])** and does satisfy the guideline requirement for a developmental toxicity study in the mouse.

Developmental Rabbit

In a developmental toxicity study with rangefinding (MRIDs 44901707 and 44505032), mesotrione (96.8% a.i., Lot #: P17) in deionized water was administered to pregnant New Zealand White rabbits (20/dose) at dose levels of 0, 100, 250, or 500 mg/kg/day by gavage on gestation days (GDs) 8 through 20. All does were sacrificed on GD 30.

A single 100 mg/kg animal was found dead on GD 4 and abnormal GI tract contents were observed at necropsy. One 250 mg/kg animal was sacrificed *in extremis* on GD 22 after displaying clinical signs including diarrhea, subdued behavior, thin appearance, and severe weight loss. Five does were sacrificed after showing signs of abortion as follows: one 100 mg/kg on GD 29; two 250 mg/kg on GD 25 or 28; and two 500 mg/kg on GD 23 or 25.

At 500 mg/kg/day, observations included the following: blood on the tray (6 observations in 4/20 animals vs 1 incidence in 1/20 controls); few feces on the tray (25 incidences in 7/20 animals vs 3 observations in 2/20 controls); no feces on the tray (6 observations in 3/20 animals vs 0/20 controls); and red to brown colored urine (41 observations in 12/20 animals vs 1 incident in 1/20 controls); decreases (NS) in food consumption during GDs 14-20 (↓12-24%) and increases during post-treatment (↑39%, GDs 26-30, $p \leq 0.01$); a decrease (NS) in gravid uterine weight (↓15%); and a decrease (NS) in the number of implantations/doe (↓13%). Food consumption was also reduced at both the 500 and 250 mg/kg/day dose levels with rebounds observed during the post dosing period.

At 250 mg/kg/day, findings observed included the following: few feces on the tray (11 observations in 4/20 animals vs 3 observations in 2/20 controls); no feces on the tray (4 observations in 3/20 animals vs 0/20 controls); red to brown colored urine (26 incidents in 12/20 animals vs 1 incident in 1/20 controls); and decreases (NS) in food consumption during GDs 14-20 (↓14-15%) and increases (NS) during the post-treatment interval (↑12%, GDs 26-30).

At 100 mg/kg/day, findings observed that were considered to be possibly treatment-related included few feces on the tray (6 observations in 5/20 animals vs 3 observations in 2/20 controls) and red to brown colored urine (14 incidents in 6/20 animals vs 1 incident in 1/20 controls).

When compared to concurrent controls, no treatment-related changes in body weight, adjusted body weight, or gross pathology were noted at any dose level tested.

NOTE: Due to the increase in preimplantation loss noted in the mid and high dose levels, these groups may have received test material prior to the completion of implantation. This would be a confounding factor which may interfere with the proper assessment of maternal toxicity in this study.

The maternal LOAEL is 250 mg/kg/day, based on abortions and clinical signs of toxicity. The maternal NOAEL is 100 mg/kg/day.

20

At 100, 250, and 500 mg/kg/day, skeletal examination (reported as [% fetal incidence (% litter incidence)]) revealed a shift toward a decreased degree of ossification of the 7th cervical vertebra transverse process, when compared to concurrent controls, as evidenced by i) a decreased ($p \leq 0.05$ or 0.01, fetal incidences only) incidence of a "partially ossified" transverse process at the low- [0.8 (7.1)], mid- [0.7 (5.9)], and high-dose levels [0 (0)] vs controls [6.7 (22.2)] and ii) a decreased (NS) incidence of a "fully ossified" transverse process in the 7th cervical vertebra in all treated groups [0 (0)] vs controls [3.3 (11.1)]. In addition, statistically significant decreases in the ossification (fetal incidence only) of the odontoid was observed in all dose groups as compared to controls. Conversely, an unexpected trend toward more complete ossification of the 5th sternebra was observed and was demonstrated by i) a dose-dependent decrease ($p \leq 0.01$, fetal incidences only) in the number of animals exhibiting a "partially ossified" 5th sternebra at the low- [32.3 (92.9)], mid- [28.9 (76.5)], and high-dose levels [24.6 (62.5)] vs controls [52.0 (94.4)] and ii) a decrease ($p \leq 0.05$ or 0.01, fetal incidences only) in the number of animals exhibiting a "nonossified" 5th sternebra at the low- [3.2 (14.3)], mid- [3.4 (11.8)], and high-dose levels [4.2 (25.0)] vs controls [12.7 (33.3)]. In addition, statistically significant ($p \leq 0.01$) increases in the fetal incidence of 13 full ribs and 27 pre-sacral vertebra were noted at all dose levels.

An increase in preimplantation loss ($p \leq 0.01$) was noted at the high-dose level in the submitted report, but errors were observed in the calculation, and therefore, the percent losses were recalculated by reviewers. As a result of the Agency contractor re-calculation, increases were observed at both the mid- (↑42%) and high-dose (↑152%) levels. The observation of preimplantion loss in several dose groups would suggest dosing began in these groups prior to the completion of the implantation process. This introduces a confounding factor into study interpretation.

At 500 mg/kg, a decrease (NS) in the number of live fetuses/doe (↓11%) was observed. This may be associated with the preimplantation loss noted in this dose group.

There were no treatment-related external or visceral effects noted at any dose level. Treatment-related changes observed in the *manus* ossification data included statistically significant increases in the proportion of fetuses with scores of 5 at the mid and high dose levels.

The developmental LOAEL is 100 mg/kg/day, based on delayed ossification of the 7th cervical transverse process and odontoid and increases in extra full 13th ribs and 27 pre-sacral vertebra. Hence, a developmental NOAEL was not established.

Since a developmental NOAEL was not established and because dosing was apparently initiated in several dose groups prior to the completion of implantation (resulting in increased preimplantation loss), this developmental toxicity study is classified **unacceptable/ not upgradeable** (§83-3[b]) and does not satisfy the guideline requirement for a developmental toxicity study in the rabbit.

5.4 Reproductive Toxicity

Reproduction Rat

In a 3-generation reproduction study (MRID 44505033), mesotrione (96.8% a.i., lot # P17) was administered in the diet continuously to 3 generations of Alpk:AP$_f$SD (Sprague-Dawley) rats (26 rats/sex/dose) at dose levels of 0, 2.5, 10, 100, or 2500 ppm (equivalent to 0, 0.3/0.3, 1.1/1.2, 11.7/12.4, or 287.7/311.4 mg/kg/day [M/F] in the P and F_1 animals). The P and F_1 animals were exposed to the test substance for approximately 10 weeks prior to mating. At approximately 14 weeks after selection, the F_2 animals were subdivided into a continuous treatment group (12 animals/sex/dose) and a recovery group (14 animals/sex/dose), which received control ration only. At approximately 4 weeks after subdivision, these groups were mated to produce the F_3 generation. Excluding the recovery group, exposure of all animals to the test material was continuous throughout the study.

There was no evidence of treatment-related changes in mortality, body weight gains, food efficiency, or reproductive performance observed in the P or F_1 adults at any dose. Decreased food consumption during lactation, increased incidences of ocular opacity, cloudiness, keratitis, and increased corneal vascularization, and increased bilateral hydronephrosis were observed in the100 and 2500 ppm groups. The severity of these effects was greater than the 10 ppm groups in a dose-dependent manner. At 10 ppm, differences in food consumption were observed in F_1 dams (↓15-17%, LWs 3 and 4).

Ophthalmologic findings were observed during clinical observation, at necropsy, and at histological examination in the treatment groups, but not in controls, except as noted. During clinical observation, cloudy/opaque eyes were observed in the following groups: F_1 males (1/26); F_2 males before subdivision (1/26); and F_2 males in the recovery group (1/14).

At necropsy, opaque/cloudy eyes were observed in the F_1 males (1/26 treated) and F_2 recovery males (1/14 treated). At histological examination, keratitis was observed in F_1 males (5/26 treated). Corneal vascularization was observed in F_1 males (5/26 treated). Bilateral hydronephrosis at histological exam and under 2X magnification was observed in F_1 males (10/26) and F_2 continuous treatment males (2/12). No incidences were noted in the controls for any of these groups.

Increases in absolute and adjusted (to body weight) liver weights were observed. Liver weights were increased in the P, F_1, and F_2 groups as follows: In the 10, 100, and 2500 ppm P males and 100 and 2500 ppm P females, in the 10, 100, and 2500 ppm males, in the 2.5, 10, 100, and 2500 ppm F_2 continuous treatment males, and in the 2500 ppm F_2 recovery group females. Absolute and/or adjusted (to body weight) kidney weights were increased in all 2500, 100, and 10 ppm male groups, including the recovery groups. Increased kidney weights were only observed in 2500 ppm P and recovery females. The incidence of bilateral hydronephrosis at terminal

22

necropsy as determined under 2X magnification was increased in 2500 and 100 ppm males and females and F_2 continuous treatment males and in the 10 ppm males. At histological examination, the incidence of minimal to marked bilateral hydronephrosis was increased in 2500 ppm F_1, F_2 continuous treatment, and F_2 recovery males and females; in 100 ppm F_1 males and females, F_2 continuous treatment and recovery males; and in 10 ppm F_1 males. The nephrotoxicity was apparently not reversed in the recovery groups.

Plasma tyrosine levels were significantly increased in F_2 adult males under continuous treatment at all treatment doses during the pre-mating interval and at termination (↑569 - 2478%). Levels were significantly increased in F_2 adult females under continuous treatment at 10 ppm and above during the pre-mating interval and at termination (↑289 - 285%). Animals of both sexes in the recovery groups had similar plasma tyrosine levels as the controls at all doses.

The LOAEL for systemic parental toxicity is 2.5 ppm (equivalent to 0.3 mg/kg/day for both sexes) based on significantly increased plasma tyrosine levels and increased liver weights in F_2 males. No NOAEL was determined.

There was no evidence of treatment-related changes in body weights or body weight gains in the F_1 or F_2 litters at any dose.

A pattern of ocular toxicity consisting of macroscopic and microscopic ocular opacity/cloudiness was observed in all offspring generations. No opaque/cloudy eyes were observed in control animals at any time. An increase in the number of pups with cloudy eyes was observed in all 2500 groups and in the 100 ppm F_2 group (% pups[% litters]) with a range of 26-72 (36-61). An increase in the number of pups with ocular discharge was observed in the 2500 ppm F_1 (11[26] treated vs 0.4[4] controls) and F_2 litters (8[15] treated vs 0.5[5] controls), and 100 ppm F_2 litters (4[16]) but the effect was not observed in the F_3 litters.

At gross necropsy, an increase in the incidence of opaque or cloudy eyes was observed in the 2500 ppm F_1 and F_2 males and females and 100 ppm F_2 males. Closed eyelids were also observed in 2500 ppm F_1 males (18/54 treated vs 0/44 controls) and females (7/42 treated vs 1/49 controls). An increase in the incidence of minimal to marked ocular keratitis was observed at histological examination in the 2500 ppm P and F_1 males and females (18-26/26 treated vs 0/26 controls). Keratitis was also observed in 100 ppm P males and females (8-11/26 treated) and F_1 males and females (23-26/26 treated). Increased minimal to moderate corneal vascularization was observed in 2500 ppm P and F_1 males and females (16-26/26 treated vs 0/26 controls). Corneal vascularization was also observed in 100 ppm P males and females (7-8/26 treated), F_1 males and females (12-26/26 treated).

A pattern of nephrotoxicity consisting of increased kidney weights and increased macroscopic and microscopic renal hydronephrosis was observed in the pups. There was an increase in the relative kidney weights in F_2 males (↑11%) and relative and absolute kidney weights (↑15% each) in F_2 females. At gross necropsy, the incidence of bilateral renal pelvic dilatation was

increased in the 100 and 2500 ppm F_3 continuous treatment males (15-18% in treated vs. 0% in controls). At histological examination, minimal to marked bilateral hydronephrosis was increased in the 100 and 2500 ppm F_1 and F_2 males and females (8-15% treated vs 1-4% controls), in the 10 ppm F_1 and F_2 males and females (5-7% treated) and in the 100 and 2500 ppm F_3 continuous treatment males and females (12-33% treated vs 2-4% controls). In the recovery animals, the incidences of bilateral hydronephrosis were low and similar to controls. The incidence of bilateral hydronephrosis at terminal necropsy, as determined under 2X magnification, was increased as follows: 100 and 2500 ppm F_1 , F_2, and F_3 continuous treatment males and females; 10 ppm F_1 males and females; and 10 ppm F_2 males.

Plasma tyrosine levels were significantly increased in F_3 male pups under continuous treatment at all treatment doses (↑979 - 2374%). Levels were significantly increased in F_3 female pups under continuous treatment at 100 and 2500 ppm (↑633 - 960%). Animals of both sexes in the recovery groups had similar plasma tyrosine levels as the controls at all doses.

The LOAEL for systemic offspring toxicity is 2.5 ppm (equivalent to 0.3 mg/kg/day for both sexes) based on significantly increased plasma tyrosine levels in F_3 male pups. No NOAEL was determined.

Mean litter size was decreased 20-45% compared to the controls throughout lactation in all 2500 ppm groups, including F_3 recovery litters. Mean litter size was also decreased in the 10 ppm (↓19-23%, PND 8-29) and 100 ppm (↓20-22%, PND 5-29) F_2 litters. At 2500 ppm, the livebirth index was decreased in the F_2 (↓6%) and F_3 continuous treatment (↓12%) litters and the day 22 viability index was decreased in the F_1 and F_2 litters (↓16% each). The proportion of litters with whole litter losses was increased in the 2500 ppm F_2 litters (7/20 treated vs 1/21 controls). There was no significant difference in the F_1 and F_3 continuous treatment litters. Whole litter weights were decreased throughout lactation in the 2500 ppm F_1, F_2, and F_3 continuous treatment litters (↓19-49%, PND 1-29); at PND 1 in 2500 ppm F_3 recovery litters (↓29%); beginning at approximately the first week of lactation in the 100 ppm F_1 and F_2 litters (↓13-21); and at PND 11, 15, and 29 in the 10 ppm F_2 litters (↓18%).

The LOAEL for reproductive toxicity is 10 ppm (equivalent to 1.1/1.2 mg/kg/day [M/F]) based on decreased F_2 mean litter size. The reproductive NOAEL is 2.5 ppm (equivalent to 0.3 mg/kg/day for both sexes).

Even though no systemic NOAEL was determined for parents or offspring, the reproductive study is determined to be **acceptable/guideline (§83-4[a])** and satisfies the guideline requirement for a multigenerational reproductive toxicity study in rats as per the Hazard Identification Assessment Review Committee (March 13, 2001).

Reproduction Mouse - See Chronic RfD (Section 2.2) for executive summary.

5.5 Additional Information from Literature Sources

Ruetschi, U., Cerone, R., Perez-Cerda, C., Schiaffino, M.C., Standing, S., Ugarte, M., and Holme, E. 2000. Mutations in the 4-hydroxyphenylpyruvate dioxygenase gene (HPD) in patients with tyrosinemia type III. Hum. Genet. 106(6): 654-662.

Reported that some patients with tyrosinemia III (an autosomal recessive disorder in which 4-hydroxyphenylpyruvate dioxygenase is deficient) were presented with mental retardation or neurological symptoms. No correlation of the severity of the mutation and enzyme deficiency and mental function has been found. Also, tyrosine levels do not correlate with the clinical phenotype.

5.6 Determination of Susceptibility

There is quantitative and qualitative evidence of increased susceptibility of rat, mouse and rabbit fetuses *in utero* in developmental studies.

There is quantitative and qualitative evidence of increased susceptibility of mouse offspring in the multi-generation reproduction study. The rat multi-generation reproduction study did not establish a NOAEL for parental or offspring systemic toxicity, but there was qualitative evidence of increased susceptibility in the offspring; increased plasma tyrosine levels were seen in the parental animals and the F_2 pups.

5.7 Recommendation for a Developmental Neurotoxicity Study

5.7.1 Evidence that suggest requiring a Developmental Neurotoxicity study:

-- Neurotoxicity in mouse reproduction study at high doses.
-- Evidence of increased susceptibility of fetuses and offspring in more than one species in developmental and reproduction studies.
-- Possible link between neurological symptoms and tyrosinemia in the open literature.

5.7.2 Evidence that **do not** support a need for a Developmental Neurotoxicity study:

-- No neurotoxicity was seen in the acute and subchronic neurotoxicity studies.

The HIARC recommended that a developmental neurotoxicity study in the mouse be required in order to better characterize the effects of tyrosinemia on the developing nervous system and to correlate plasma tyrosine levels to neurotoxic effects.

6 HAZARD CHARACTERIZATION

Mesotrione is a triketone herbicide with a primary mode of action that inhibits the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), an enzyme that is integral in the catabolism of tyrosine in animals and humans. The toxicology database for mesotrione is not complete. The HIARC recommended that a developmental neurotoxicity study be required in order to better characterize the effects of tyrosinemia on the developing nervous system and to correlate plasma tyrosine levels to neurotoxic effects. The HIARC also requested a 28-day inhalation study to characterize the direct effects of mesotrione on the pulmonary system and systemic effects via the inhalation route. Mesotrione has low acute toxicity via the oral, dermal, and inhalation routes. It is a mild eye irritant, but is not a dermal irritant or a dermal sensitizer.

The eye, liver, and kidney are the primary target organs of mesotrione. Ocular effects such as corneal opacity, corneal vascularization, and keratitis were observed in rat in subchronic, chronic, and reproduction studies and in the dog in a chronic study. Lenticular opacity was also noted in the chronic dog study. Ocular effects were observed in mice in the reproduction study. Liver effects included increased liver weights seen in the rat subchronic, chronic, and reproduction studies and hepatocyte fat vacuolation noted in the rat chronic study. Kidney effects included increased kidney weights observed in the rat subchronic and chronic studies and in the rat and mouse reproduction studies and hydronephrosis noted in the rat subchronic and reproduction studies.

Plasma tyrosine levels were increased in the rat, mouse and dog in the chronic and reproduction studies in which levels were measured. The ocular, liver and kidney effects are believed to be mediated by the high tyrosine levels in the blood caused by inhibition of the enzyme HPPD. The rat is the most sensitive species to this effect compared to the dog and the mouse. The Mechanism of Toxicity Science Assessment Review Committee determined that for tyrosine-mediated toxicological effects, the mouse is a more appropriate model for assessing human risk than is the rat. This decision was based on comparative data on the activity of TAT in the rat, mouse and human, and the similarities of the response to elevated plasma tyrosine levels in humans and the mouse (D272633; March 27, 2001).

Long-term dietary administration of mesotrione did not result in an overall treatment-related increase in incidence of tumor formation in rats or mice. The HIARC classified mesotrione as "not likely to be carcinogenic to humans" by all routes of exposure based upon lack of evidence of carcinogenicity in rats and mice.

Mesotrione did not show evidence of mutagenicity in *in vitro* or *in vivo* studies.

Oral rat, mouse, and rabbit developmental studies showed an increased susceptibility of the fetus to mesotrione *in utero*. Delayed ossification was seen in the fetuses at doses below those at which maternal toxic effects were noted. Maternal toxic effects in the rat were decreased body weight gain during treatment and decreased food consumption and in the rabbit, abortions and GI effects. No maternal toxic effects were noted in the mouse. Multi-generation reproduction studies also showed an increased

susceptibility of the young to mesotrione. In the mouse, the young exhibited significant tyrosinemia and ocular discharge at doses below those at which parental toxic effects were noted. The parental effects were tyrosinemia and increased kidney weights. In the rat, no NOAEL was determined for parental effects (tyrosinemia, increased liver weights) or offspring systemic effects (tyrosinemia); however, the tyrosinemia was much more severe in the young than in the adults. Decreased litter size was noted at the next highest dose.

No evidence of neurotoxicity or neuropathology was seen in the acute and subchronic neurotoxicity studies. In the multi-generation mouse reproduction study, one F_1 male and one F_1 female had retinal detachment with marked cataractous changes at the highest dose tested (>1000 mg/kg/day). In the subchronic toxicity dog study, the high-dose females had decreased absolute and relative brain weights; however, no microscopic abnormalities were noted in any brain tissue from the high-dose group and the effect was not observed in the chronic toxicity dog study. There is some concern about the effects of elevated plasma tyrosine levels on the developing nervous system in children due to a report by Ruetschi *et al* that some patients with tyrosinemia III (an autosomal recessive disorder in which 4-hydroxyphenylpyruvate dioxygenase is deficient) were presented with mental retardation or neurological symptoms and that no correlation of the severity of the mutation and enzyme deficiency and mental function has been found. Also, tyrosine levels did not correlate with the clinical phenotype.

A series of rat metabolism studies with [^{14}C-aromatic]mesotrione indicated that mesotrione was readily absorbed and distributed in the body. Tissue distribution was about the same in both sexes, although one study showed higher residues in the kidneys in females, with the highest residues of the test compound in the liver and kidney. Higher doses resulted in higher residues in the liver and kidney, while repeated doses resulted in reduced accumulation of residues in all tissues. Levels of radioactivity in tissues of iv-dosed animals were essentially the same as in orally-dosed animals. Over 50% of the administered dose was excreted in the urine in both sexes and around 25% was excreted in the feces within 72 hours. Females exhibited slightly higher total urinary excretion than males, but total fecal excretion was about the same in both sexes. Increasing the dose or repeated doses had little effect on the pattern of excretion in both sexes. The overall pattern of excretion was similar between orally-dosed and iv-dosed rats. The metabolite profile was similar between the sexes in each group and between the single-dosed and repeated-dosed animals. The parent compound, mesotrione, was the major component identified in the urine accounting for 47-64% of the dose. In addition, the following minor metabolites were identified: MNBA (1-4% of the dose), AMBA (3-12%), 5-hydroxymesotrione ($\leq$ 2%), and 4-hydroxymesotrione (3-6%). In bile cannulated rats administered [^{14}C-aromatic]mesotrione or [^{14}C-dione]mesotrione, the major component in fecal excreta and bile was the parent compound. Analysis of the bile identified mesotrione and 4-hydroxymesotrione as two minor components. Another minor component in the feces was 5-hydroxymesotrione. Metabolism in the mouse was very similar to the rat except that males had slightly increased total fecal excretion when compared to females and, females in the low-dose group excreted higher (1.5x) levels of parent compound in the urine than males. Free mesotrione was the major component in the urine and feces ($\geq$ 50% of the dose). Minor components in the fecal extracts included AMBA (1-4%) and MNBA ($\leq$ 2%).

7 DATA GAPS

28-day inhalation study
Developmental neurotoxicity study in mice with plasma tyrosine level measurements

8 ACUTE TOXICITY

Acute Toxicity of Mesotrione

Guideline No.	Study Type	MRID #(S).	Results	Toxicity Category
81-1	Acute Oral	44373512	$LD_{50} > 5000$ mg/kg	IV
81-2	Acute Dermal	44373514	$LD_{50} > 2000$ mg/kg	III
81-3	Acute Inhalation	44373516	$LC_{50} > 4.75$ mg/L	IV
81-4	Primary Eye Irritation	44373518	Mild eye irritant	IV
81-5	Primary Skin Irritation	44373520	Not a dermal irritant	IV
81-6	Dermal Sensitization	44373522	Not a dermal sensitizer	N/A

9 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	No appropriate endpoint was available to determine the Acute RfD for the general population or for the females 13-50 subpopulation.		
Chronic Dietary	LOAEL = 2.1 UF = 300	Tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups	Multi-generation Reproduction Study Mouse
		Chronic RfD = 0.007 mg/kg/day	
Cancer	No quantification needed	"Not likely" - No evidence of carcinogenicity in rats and mice	Combined Chronic Toxicity/Carcinog. Study - Rat Carcinogenicity Study - Mouse
Incidental Oral, Short-Term	NOAEL = 100	Decreased maternal body weight gains during treatment and food consumption	Developmental Toxicity - Rat
Incidental Oral, Intermediate-Term	LOAEL = 2.1	Tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups	Multi-generation Reproduction Study Mouse
Dermal, Short-Term[a]	LOAEL = 100	Delays in skeletal ossification and changes in *manus/pes* ossification assessments	Developmental Toxicity - Rat
Dermal, Intermediate-/ Long-Term[a]	LOAEL = 2.1	Tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups	Multi-generation Reproduction Study Mouse
Inhalation, Short-Term[b]	LOAEL = 100	Delays in skeletal ossification and changes in *manus/pes* ossification assessments	Developmental Toxicity - Rat
Inhalation, Intermediate-/ Long-Term[b]	LOAEL = 2.1	Tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups	Multi-generation Reproduction Study Mouse

a Since an oral endpoint was selected, a dermal absorption factor of 25% should be used in route-to-route extrapolation.
b Since an oral endpoint was selected, an inhalation factor of 100% should be used in route-to-route extrapolation.