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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

TXR NO. 0051869

DATE: **May 2, 2003**

MEMORANDUM

SUBJECT: **TRIFLURALIN (P.C. Code 036101)** - Report of the Hazard Identification Assessment Review Committee.

FROM: Robert Fricke
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Richard Griffin, Risk Assessor
Health Effects Division (7509C)

PC Code: 036101

On April 17, 2003, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for trifluralin 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 trifluralin 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: Ayaad Assaad, Bill Burnam, Jonathan Chen, Beth Doyle, Pamela Hurley, Susan Makris, Elizabeth Mendez, P.V. Shah, Jess Rowland, Brenda Tarplee, Bill Dykstra

Member(s) in absentia: Paula Deschamp, John Liccione

Data evaluation prepared by: Robert Fricke

Also in attendance were: Ken Dockter, Shanna Recore, Linda Taylor, Mohsen Shafeyan, Pauline Wagner

Data Evaluation / Report Presentation

Robert F. Fricke

Robert F. Fricke
Toxicologist

1 INTRODUCTION

On April 17, 2003, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for trifluralin 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 trifluralin was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996.

2 FQPA HAZARD CONSIDERATIONS

2.1 Adequacy of the Toxicity Data Base

The HIARC concluded that the toxicology database for trifluralin is complete.

2.2 Evidence of Neurotoxicity

The HIARC concluded that there is not a concern for neurotoxicity resulting from exposure to trifluralin.

In a rat developmental study with trifluralin, clinical signs included marked drowsiness (decreased motor activity?), piloerection and alopecia, possible indicative of dose-related stress; these effects occurred at the highest-dose tested (500 mg/kg/day) and were not considered to be evidence of neurotoxicity.

2.3 Developmental Toxicity Study Conclusions

2.3.1 Developmental Toxicity in Rats

Executive Summary: In a prenatal developmental toxicity study (MRIDs 00151899, 00159620 and 40392310), HOE 38474 (trifluralin; 99.0% a.i., Lot/Batch #AT210) in sesame oil was administered to pregnant Wistar (Hoe: WISKf strain, SPF 71) rats (24/dose) via gavage at concentrations of 0, 20, 100, or 500 mg/kg/day on gestation days (GD) 7 through 16. All dams were sacrificed on GD 21 and their uterine contents examined. Additionally, a replicate study with animals dosed only at 500 mg/kg/day (with no concurrent control group) was conducted in order to clarify results from the main study.

At 500 mg/kg, one dam from the replicate group died after eight treatments; no clinical signs of toxicity were noted on the previous day, and advanced autolysis precluded a necropsy. Yellow or orange-yellow discolored urine was observed in all dams, and increased urination was noted in 16 dams of both groups. Yellow discoloration of fatty tissues was noted in most dams at this dose. This discoloration was not considered to be treatment related and is characteristic of dinitroaniline treatment. One dam in the main study group was markedly drowsy and had a blood-encrusted nose on GD 8-9.

Piloerection was noted in one dam in the main study and 2 dams in the replicate group during the second half of the treatment period. Maternal body weight gains were decreased 26% in the main study group and 52% in the replicate group during the treatment period. Food consumption was decreased 5-8% ($p<=0.05$) during GD 14-17 and increased ($p<=0.05$) 9-22% post-treatment in both groups. Increases ($p<=0.05$) in liver weights of 14-17% and in spleen weights of 27-36% were observed in both groups. The number of resorptions/dam was increased in the main study and replicate group (1.08-2.31 treated *vs* 0.25 controls), resulting in a decreased number of live fetuses/dam (10.8 each treated *vs* 11.8 controls). Distension of the renal pelves was observed in two dams in the main study group and two dams in the replicate group. The following other macroscopic renal findings were observed (1 each treated): (i) clear fluid in the renal pelvis; (ii) grey hollows on the kidney surface; and (iii) enlarged kidney with yellow calculi in the pelvis.

The only findings at 100 mg/kg/day included complete litter resorption in one dam of 20 and distension of the renal pelves in one dam. Yellow discoloration of fatty tissues was noted in most dams at this dose. This discoloration was not considered to be treatment related and is characteristic of dinitroaniline treatment

The only finding at 20 mg/kg/day was distension of the renal pelves in one dam.

The maternal LOAEL is 500 mg/kg/day based on clinical signs, decreased body weight gains, decreased food consumption, and increased liver and spleen weights. The maternal NOAEL is 100 mg/kg/day.

At 500 mg/kg/day, statistically significant $p <= 0.05$) increases were observed in the incidence [fetal % (litter %)] of reduced ossification of the vertebrae [7.6 (25); control 0 (0)] and ribs [10.9 (31.3), control 0 (0)], as well as thickened, wavy, or bent ribs [34.8 (81.3), control 3.3 (15); historical control 5 (21)].

The initial review of this study a NOAEL was not established, based on increased vascular fragility and reduced skeletal maturity at the LOAEL of 20 mg/kg/day. The initial review did not evaluate the historical control values for skeletal abnormalities. Except for the thickened/wavy/bent ribs observed at the high dose, the other skeletal abnormalities were within the historical control values. Further, the initial review inappropriately grouped blood in the abdominal cavity and hematomas for evaluation.

The developmental LOAEL was established at 500 mg/kg/day, based on reduced ossification of the vertebrae and ribs; thickened, wavy or bent ribs; and increased number of total litter resorptions. The developmental NOAEL was established at 100 mg/kg/day.

This developmental toxicity study is classified **acceptable/guideline (OPPTS 870.3700; §83-3a)** and satisfies the requirement for a developmental toxicity study in the rat.

2.3.2 Prenatal Developmental Toxicity Study - Rabbit

Executive Summary: In a developmental toxicity study (MRID 00152421), trifluralin (Lot/batch # 0554AP2; 96.7% a.i.) was administered at dose levels of 0, 100, 225, or 500 mg/kg/day in 10% (w/v) aqueous acacia (10 mL/kg) by gavage to 25 artificially inseminated female Dutch Belted rabbits/group on gestation days (GDs) 6 through 18. All surviving does were sacrificed on GD 28 and their fetuses removed by cesarean section and examined.

Abortions occurred between GD 17 and 28 at 225 (4/18) and 500 (5/17) mg/kg/day *vs* 0/16 controls. Following a protracted period of anorexia and cachexia, two 500 mg/kg/day does died on GD 13 and 15. The maternal mortalities and abortions resulted in a decreased number of litters at 225 (14 treated *vs* 16 controls) and 500 (10 treated) mg/kg/day. Orange-colored urine was observed at 225 and 500 mg/kg/day, and orange-colored pelage was noted at 500 mg/kg/day, this was due to trifluralin and/or its metabolites and not considered to be treatment related.

In the 500 mg/kg/day does, body weights were decreased 6-16% (not significant) beginning on GD 19, and body weight gains were decreased ($p<=0.05$) during the treatment and post-treatment intervals, resulting in decreased ($p<=0.05$) absolute and corrected (for gravid uterine weight) body weight gains for the overall study. Food consumption was dose-dependently decreased ($p<=0.05$) at 225 (28-40%) and 500 (43-67%) mg/kg/day during the treatment interval and remained decreased (53%; $p<=0.05$) at 500 mg/kg/day during the post-treatment interval.

At necropsy, dose-dependent increases (*vs* 0/25 controls) of hair in the stomach and empty intestines were noted in the 100 (1/25 treated), 225 (5/25 treated), and 500 (12/25 treated) mg/kg/day does. Liver that was pale, mottled, fatty, and/or had reticulated reddening was noted at 225 and 500 mg/kg/day (4/25 each treated *vs* 0/25 controls). Dark red lungs were observed at 225 (1/25 treated *vs* 0/25 controls) and 500 (3/25 treated) mg/kg/day. Additionally at 500 mg/kg/day, yellow adipose tissue was observed in 7/25 does (*vs* 0/25 controls). This discoloration was not considered to be treatment related and is characteristic of dinitroaniline treatment.

The maternal LOAEL is 225 mg/kg/day based on abortions, macroscopic changes in the liver and lungs, and decreased food consumption. The maternal NOAEL is 100 mg/kg/day. At the highest dose tested (500 mg/kg/day), abortions, mortalities, decreased body weight and body weight gains, and decreased food consumption were observed.

At 500 mg/kg/day, the numbers of early, late, and complete resorptions were increased, resulting in an increased post-implantation loss (64.8% treated *vs* 15.5% controls) and a decreased number of live fetuses/doe (2.1 treated *vs* 5.3 controls). At 500 mg/kg/day, fetal weights were decreased 13-18% ($p<=0.05$), and the percent of fetal runts was significantly increased ($p<=0.05$) in the males (37.5% *vs* 0.0%, control) and increased

(not significant) in the females (40 % *vs* 3.3%, controls). Edema and hemorrhagic area(s) were noted at 225 mg/kg/day (1.4% fetuses; 8.3% litters) and 500 mg/kg/day (4.8-9.5% fetuses; 20.0% litters) compared to 0% in the concurrent controls. Additionally at 500 mg/kg/day, hypoplastic thymus, cardiomegaly, and hypoplastic lungs were observed among the fetal runts from one litter (9.5% fetuses and 20.0% litters, each finding) compared to 0% in the concurrent controls. Increased incidences of spade ribs (9.5% fetuses; 20.0% litters), fused vertebrae (4.8% fetuses; 20.0% litters), and incomplete ossification of the vertebrae (14.3% fetuses; 40.0% litters) were observed at 500 mg/kg/day compared to 0% in concurrent controls.

The developmental toxicity LOAEL is 225 mg/kg/day based on abortions. The developmental toxicity NOAEL is 100 mg/kg/day. At the highest dose tested (500 mg/kg/day), increased resorptions, decreased fetal body weights, decreased number of live fetuses, increased number of runts, incomplete ossification of vertebrae, and skeletal abnormalities were observed.

This study is classified **acceptable/guideline** (OPPTS 870.3700b; OECD 414) and satisfies the requirements for a developmental study in the rabbit

2.4 Reproductive Toxicity Study Conclusions

2.4.1 Executive Summary: In a multi-generation reproduction toxicity study (MRID 00151901, 00151902, 00151903, and 00159619), trifluralin (>99% a.i.; Lot/batch #AT 210) was administered continuously in the diet to outbred Wistar KFM-Man SPF quality rats at nominal dose levels of 0, 200, 650, or 2000 ppm (equivalent to approximately 0, 10, 32.5 and 100 mg/kg/day; calculated by reviewers using 1 ppm = 0.05 mg/kg). The P animals (30/sex/dose) were given test article diet formulations for 80 days prior to mating to produce the F1a litters. Ten days after weaning of the F1a litters, a second mating of the P generation (using different pairs) was conducted to produce the F1b litters. When possible, animals that were not fertile after the first breeding were subsequently paired with fertile animals. After weaning, 26 rats/sex/dose from the F1b litters were selected to be F1 parents and were given the same diet concentration as their dam for at least 100 days prior to mating. F1 parents were bred (siblings not paired) to produce F2a and F2b litters using the same procedures described for their parents.

Parental mortalities included one 650 ppm P generation male and one F1 generation female each from the control, 650 ppm, and 2000 ppm groups. The death in 650 ppm female was attributed to acute renal failure; no cause of death was stated for the other mortalities. Yellow discoloration of the urine was reportedly dose dependently increased; however, neither summary nor individual data were provided. Yellow discoloration of the adipose tissue was noted in the 650 ppm females and 2000 ppm males and females of both generations. This discoloration was not considered to be treatment related and is characteristic of dinitroaniline treatment. Relative (to body) liver weights were increased ($p<=0.01$) at 650 ppm in the P males and F1 males and females (6-10%) and at 2000 ppm in the P and F1 males and females (16-27%). In the parental males, relative kidney

weights were increased (p<=0.01) 8-14% at 650 and 2000 ppm in the both generations. Relative testes weights were increased 8-13% (p<=0.05) at 650 ppm in the F1 generation and at 2000 ppm in both generations. In the 650 and 2000 ppm F1 females, incidences of lesions of the renal proximal tubules were increased, and corticomedullary mineralization was decreased. Hyaline droplets in the tubular epithelium occurred in females of all dosed groups.

Additionally at 2000 ppm, body weights were decreased (p<=0.05) in the F1 females during pre-mating (6-15%), gestation (7-9%), and lactation (4-9%) for both litters. In the males, food consumption was decreased (p<=0.05) in the P generation during Week 1 of pre-mating (9%). Food consumption was decreased (p<=0.05) in the P females during pre-mating (12% each during Weeks 1 and 3) and lactation (7-10% after LD 4). Food consumption was decreased (p<=0.05) in the F1b females during gestation (7-10% during Weeks 1 and 3) and throughout lactation (10-13%). Relative thymus weights were decreased (p<=0.05) 15-16% in the F1 males and females.

The only finding at 200 ppm was an increase (p<=0.01) of 6% in relative kidney weight in the P generation males.

The LOAEL for parental toxicity is 650 ppm (equivalent to 32.5 mg/kg/day) based increased lesions of the renal proximal tubules in the F1 females and increased relative (to body) weights of the liver and kidney (males). The NOAEL is 200 ppm (equivalent to 10 mg/kg/day).

There was no adverse effect of treatment on pup mortality. Pup weights were decreased (p<=0.05) in the following litters: (i) both F1 litters at 650 and 2000 ppm on post-natal days (PND) 7 and 21 (5-12%); (ii) F2a litter at 2000 ppm on PND 1, 7, and 21 (3-8%); and (iii) F2b litters at 650 and 2000 ppm on PND 21 (2-7%). Litter size (the number of live pups) was decreased (p<=0.05) 13-16% at 2000 ppm in the F2a litter on PND 0 and in the F2b litter on PND 0 and 21. Relative liver weights were increased (p<=0.05) at 650 ppm in the F2b females and at 2000 ppm in the F2a males and females and F2b females (6-13%). Additionally at 2000 ppm, relative kidney weights were increased (p<=0.01) 5% in the F2b females, and relative testes weights were increased 8% (each) in both F1 litters.

There were no effects of treatment at 200 ppm.

The LOAEL for offspring toxicity is 650 ppm (equivalent to 32.5 mg/kg/day) based on decreased pup weights in both generations and increased relative to body liver weights in the F2b females. The NOAEL is 200 ppm (equivalent to 10 mg/kg/day).

There were no effects of treatment on precoital interval, gestation length, behavior of dams during parturition and lactation, pup mortality, or the proportion of parents that mated, became pregnant, delivered, or reared litters to weaning.

The LOAEL for reproductive toxicity was not observed. The NOAEL for reproductive toxicity is 2000 ppm (equivalent to 100 mg/kg/day).

This study is **acceptable/guideline** and satisfies the guideline requirements for a two-generation reproductive study in the rat (OPPTS 870.3800; OECD 416).

2.4.2 Executive Summary: In a multi-generation reproduction toxicity study (MRIDs 00162543 and 44135107), trifluralin (96.4% a.i.; Lot/batch #554AP2) was administered continuously in the diet to Crl:CD(SD) rats (25/sex/dose) at nominal dose levels of 0, 200, 630, or 2000 ppm (equivalent to 0, 15, 47, and 148 mg/kg/day). The P animals were given test article diet formulations for 70 days prior to mating to produce the F1a litters. After weaning, 25 rats/sex/dose from the F1a litters were given the same concentration test formulation as their dam for 69 days prior to mating to produce the F2a litters. A gross necropsy was performed on one pup/sex/litter from the weanlings not selected as parents. At 25 weeks of age, a second mating of the P animals was conducted to produce the F1b litters, and a second mating of the F1a animals was conducted to produce the F2b litters. After weaning, F1b and F2b animals were sacrificed and given a gross necropsy. Parental animals were sacrificed at 36 weeks and given a gross necropsy.

There were no effects of treatment on parental mortality, mating/fertility indices, number of females with live-born progeny, gestation length, gestation survival, live-born litter size, sex ratio, or pup survival.

At 630 ppm, body weights and food consumption were decreased ($p<=0.05$) in the F1 parental males at the end of pre-mating (6% each). Body weights were decreased ($p<=0.05$) in the P females during gestation from the F1b mating (7-9%).

At 2000 ppm, body weights and food consumption were decreased 6-13% ($p<=0.05$) during (14-20%, males;7-20%, females) and at the end (14%, males;11%, females) of pre-mating in the P and F1 parents. Body weights were also decreased 10% ($p<=0.05$) in the F1 females at the beginning of pre-mating. Body weight gains for the overall pre-mating period were decreased 9-23% ($p<=0.05$) in both generations. Food efficiency was decreased 14% ($p<=0.05$) during pre-mating in the P generation females. During the reproduction period, body weights were decreased 6-10% in the males in both generations. During gestation, body weights of the parental females were decreased 8-18% ($p<=0.05$) for both matings in both generations. During lactation, food consumption was comparable to controls, and body weight gains were dose-dependently increased in the P generation (both litters) and F1 generation (F2a litter). These increases attained significance ($p<=0.05$) at 2000 ppm (-5.6 to 5.9 g treated *vs* -25.6 to -11 g controls). Pale yellow adipose tissue was observed at necropsy in this group. This discoloration was not considered to be treatment related and is characteristic of dinitroaniline treatment. Microscopic examination revealed normal tissue.

The LOAEL for parental toxicity is 630 ppm (equivalent to 47 mg/kg/day) based on

decreased body weights and food consumption. The NOAEL is 200 ppm (equivalent to 15 mg/kg/day).

Pup weights were decreased 6-12% in both generations at 2000 ppm starting at post-natal day 4. In the combined F1a, F1b, F2a, and F2b litters, small pups (approximately half the size of litter mates) were noted in the 630 (3 litters), and 2000 (4 litters) ppm groups compared to concurrent controls (0 litters). Additionally, a slight increase in microphthalmia was observed at 2000 ppm (4 pups treated *vs* 1 control); however, three of these pups were from the same litter. Yellow adipose tissue was observed in the 2000 ppm pups. Again, this discoloration was not considered to be treatment related and is characteristic of dinitroaniline treatment

The only finding at 200 ppm was small pups in one litter out of all combined F1a, F1b, F2a, and F2b litters.

The LOAEL for offspring toxicity is 630 ppm (equivalent to 47 mg/kg/day) based on small pup size in 3 litters. The NOAEL is 200 ppm (equivalent to 15 mg/kg/day).

This study is **acceptable/guideline** and satisfies the guideline requirements for a two-generation reproductive study in the rat (OPPTS 870.3800; OPP §83-4).

2.4.3 Executive Summary: In a multi-generation reproduction toxicity study (MRID 40405007), trifluralin (97.3% a.i.; Lot/batch #5320) was administered continuously in the diet to CD(CRL) rats (25/sex/dose) at nominal dose levels of 0, 50, 450, or 4000 ppm (equivalent to 3.9/4.7, 35/42, 295/337 mg/kg/day, M/F). The P animals were given test article diet formulations for 10 weeks prior to mating to produce the F1a litters. In a sub-study to evaluate dominant lethal effects, a second mating was conducted with treated males and untreated females of the P generation to produce the F1b litters. After weaning, 25 rats/sex/dose from the F1a litters were randomly-selected (one/sex/litter, when possible) to become the parents of the F2 generation and were given the same concentration test formulation as their dam for at least 10 weeks prior to mating. There were no effects of treatment on parental survival, clinical signs, or pup sex ratio.

At 4000 ppm, body weights were decreased ($p<=0.05$) during pre-mating in the P generation males and females (6-15%) and F1 males and females (15-25%) and during gestation and lactation in the P (7-19%) and F1 (18-24%) dams. Body weight gains were decreased ($p<=0.05$) consistently during pre-mating in the P males, during Week 1 in the P females and F1 males, and during Weeks 1, 5, 10 in the F1 females. Food consumption was decreased ($p<=0.05$) in the P generation males and females (8-20%) and F1 males and females during pre-mating (13-30%) and during gestation and lactation in the P (13-28%) and F1 (21-32%) dams. Food efficiency during pre-mating was decreased ($p<=0.05$) consistently in the P males, during Week 1 in the P females, and during Weeks 1 and 10 in the F1 females. Hematocrit, hemoglobin, and erythrocytes were reduced approximately 5-10% ($p<=0.05$) in the P and F1 males and females; increases in mean

corpuscular volume, platelets (10%), and reticulocytes (130-180%) were also noted in these animals. Absolute (20% each) and relative to body (10-11%) ovary weights were decreased ($p<=0.05$) in both generations. Yellow discolored adipose tissue was observed in the P and F1 males and females (23/25 to 24/25 treated *vs* 0/25 controls). This discoloration was not considered to be treatment related and is characteristic of dinitroaniline treatment. Colon distension was observed in the F1 males (4/25 treated *vs* 0/25 controls). Denser stroma of the endometrium were observed in the P and F1 females (14-15/25 treated *vs* 0/25 controls), indicating uterine atrophy.

The only findings at 450 ppm were minor and/or transient decreases in body weight and food consumption and colon distension in the F1 males (1/25 treated *vs* 0/25 controls). Yellow discolored adipose tissue in the P females (2/25 treated *vs* 0/25 controls),but was not considered to be treatment related and is characteristic of dinitroaniline treatment. There were no effects of treatment at 50 ppm.

The LOAEL for parental systemic toxicity is 4000 ppm (equivalent to 295/337 mg/kg/day, M/F) based on decreased body weights, body weight gains, food consumption, and food efficiency in males and females of both generations; decreased ovary weights in both generations; colon distension in the F1 males; and uterine atrophy in the females of both generations. The NOAEL is 450 ppm (equivalent to 35/42 mg/kg/day, M/F).

At 4000 ppm, decreases ($p<=0.05$) were observed in the P generation (F1a litters) in fetal viability (86% treated *vs* 94% controls), neonatal viability index (85% treated *vs* 97% controls), and litter viability (80% treated *vs* 100% controls). Lactation index was decreased (not significant) in these animals (62% treated *vs* 75% controls). The number of implantation sites were decreased ($p<=0.05$) in the F1a (9%) and F2 (18%) litters, resulting in a decreased ($p<=0.01$) number of newborn pups (17-22%) and significantly reduced ($p<=0.01$) litter size on post-natal days (PND) 1 and 4 (17-25%). Litter size remained decreased (3-14%; not significant) throughout the remainder of lactation in both generations. Pre-implantation loss was increased (15.5% treated *vs* 9.8% controls; $p<=0.05$) in the F1b litters (mating of treated males with untreated females), resulting in a decreased (11%; $p<=0.05$) number of live fetuses.

At 4000 ppm pup weights were decreased ($p<=0.05$) throughout lactation in the F1a litters (average 15%) and on PND 14 and 21 in the F1a litters (74-81%).

There were no offspring effects at 50 or 450 ppm.

The LOAEL for reproductive toxicity is 4000 ppm (equivalent to 295/337 mg/kg/day, M/F) based on decreased ovary weights in both generations; decreased lactation index in the F1a pups; and decreased number of implantation sites, newborn pups, litter size, and pup weights in both generations. The NOAEL is 450 ppm (equivalent to 35/42 mg/kg/day, M/F).

The LOAEL for offspring toxicity is 4000 ppm (295/337 mg/kg/day M/F), based on decreased fetal, neonatal, and litter viability; decreased pup weights in F1a litters; and decreased number of newborn pups, litter size, and pup weights in both generations; and decreased number of implantation sites. The NOAEL for offspring toxicity is 450 ppm (35/42 mg/kg/day, M/F).

This study is **acceptable/guideline** and satisfies the guideline requirements for a two-generation reproductive study in the rat (OPPTS 870.3800 OPP §83-4).

2.5 Additional Information from Literature and Other Sources

Even though the mode of action of trifluralin is the inhibition microtubule (mitotic spindle) polymerization, it does not appear to inhibit polymerization in mammalian cells. Specific tests with trifluralin [dominant lethal (MRID 00148319) and an *in vivo* cytogenetics assay (MRID 40765603)] recommended by the SAP to evaluate tubulin inhibition in mammalian cells were negative.

Ethalfuralin (TXR 013192), a structural analog of trifluralin, was evaluated for developmental toxicity in rats and rabbits. Pregnant rats and rabbits were dosed once daily by gavage on gestation days 6-15 and 6-18, respectively. Doses for rats were 0, 50, 250, and 1000 mg/kg/day; doses for rabbits were 0, 25, 75, 150, and 300 mg/kg. Cesarean sections were performed on rats and rabbits on gestation days 20 and 28, respectively. In rats, maternal toxicity was indicated at the 1000 mg/kg dose level by depression of body weight gain and food consumption. Fetal viability, weight, and morphology were not adversely affected at any dose level. The NOAELs for maternal and developmental toxicity in the rat were 250 and 1000 mg/kg, respectively. The A/D ratio in rats was less than 1. In rabbits, maternal toxicity was indicated at the 150- and 300-mg/kg/day dose levels by abortions in conjunction with depression of food consumption. Fetal viability, weight, and morphology were not adversely affected by ethalfluralin. The NOAELs for maternal and developmental toxicity in the rabbit were 75 and 300 mg/kg, respectively. The A/D ratio in rabbits was less than 1. Based on these data, ethalfluralin did not exhibit selective toxicity toward the developing conceptus

Benfluralin (TXR 014534) was evaluated for developmental toxicity in the rat and rabbit and reproductive toxicity in the rat . There were no effects on fetuses in either rats or rabbits in prenatal studies. Similar effects (weight decrement, liver and kidney toxicity) were seen in pups, adult offspring and parents at the two highest doses tested. No effects were seen at the lowest dose tested either in pups or parents. Litter size in the reproduction study was depressed at the HDT. In the reproductive study adult toxicity, comparable with offspring toxicity, were seen at the same dose, although at the next higher dose (5x the dose) decreased litter size was noted. Dead and missing pups at the mid dose were neither dose related nor statistically significant

2.6 Pre-and/or Postnatal Toxicity

The HIARC concluded that there is a concern for pre- and/or postnatal toxicity resulting from

exposure to trifluralin.

2.6.1 Determination of Susceptibility: There was qualitative evidence of increased susceptibility in the rat developmental toxicity study, where fetal developmental effects (increased resorptions and wavy ribs) occurred in the presence of less severe maternal effects (decreases in body weight gain, clinical signs, and changes in organ weights). Qualitatively, there is an indication of increased sensitivity in the 2-generation reproduction study in the rat in that offspring effects (decreased fetal, neonatal and litter viability) were observed at a dose level where there was less severe maternal toxicity (decreased body weight, body weight gain and food consumption).

2.6.2 Degree of Concern Analysis and Residual Uncertainties: The concern is low for the qualitative susceptibility seen in the developmental rat study because the dose response was well characterized, the developmental effects sere seen in the presence of maternal toxicity, and clear NOAELs/LOAELs were established for maternal and developmental toxicities. There is low concern for the qualitative susceptibility observed in the rat reproduction study since the dose-response was well characterized; there was a clear NOAEL/LOAEL for maternal and developmental toxicities; and the effects were seen at a high-dose level (295/337 mg/kg/day). Offspring viability was not adversely affected in the two other 2-generation studies with trifluralin at dose levels up to 100 and 148 mg/kg/day. There are no residual uncertainties for pre- and postnatal toxicities since the doses selected for overall risk assessments will address the concerns seen in these studies.

2.6.3 Special FQPA Safety Factor(s):

Based on the above data, no Special FQPA Safety Factor is needed (1X) since there are no residual uncertainties for pre- and/or post-natal toxicity.

2.7 Recommendation for a Developmental Neurotoxicity Study

The HIARC concluded that there is not a concern for developmental neurotoxicity resulting from exposure to trifluralin.

2.7.1 Evidence that suggest requiring a Developmental Neurotoxicity study:

Benfluralin, a structural analog of trifluralin, showed sciatic nerve degeneration, muscle atrophy and brain weight decrease in a chronic rat study only at termination and only at the two highest doses, which were considered excessive.

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

There were no signs of neurotoxicity in the trifluralin data base.

3 HAZARD IDENTIFICATION

3.1 Acute Reference Dose (aRfD)

3.1.1 General Population

There was no appropriate single dose endpoint for this population sub-group.

3.1.2 Females 13-50

Study Selected: Developmental Toxicity Study - Rat

MRID No.: 00151899, 00159620 and 40392310

Executive Summary: See 2.3.1.

Dose and Endpoint for Establishing RfD: **Developmental NOAEL = 100 mg/kg/day,** based on increased incidences of resorptions at 500 mg/kg/day.

Uncertainty Factors: 100X (10x for interspecies extrapolation and 10x for intraspecies variation).

Comments about Study/Endpoint/Uncertainty Factor(s): This study and endpoint are appropriate for the acute RfD. Increased early absorptions observed at 500 mg/kg/day could be due to a single dose.

$$\textbf{Acute RfD} = \frac{\textbf{100 mg/kg/day (NOAEL)}}{\textbf{100 (UF)}} = \textbf{1.0 mg/kg}$$

3.2 Chronic Reference Dose (cRfD)

Study Selected: Chronic toxicity oral (capsule) - dog

MRID No.: 42447001

Executive Summary: In a chronic oral toxicity study (MRID 42447001), trifluralin (99.86% a.i., Lot/batch #326EF8) was administered in gelatin capsules to 4 beagle dogs/sex/group at dose levels of 0, 0.75, 2.4, or 40 mg/kg/day for one year. There were no adverse effects of treatment on mortality, food consumption, ophthalmology, urinalysis, or gross pathology.

At 40 mg/kg, the frequency of abnormal stool (mucoid, soft, runny, and/or containing white flakes), calculated by the reviewers, was increased over controls in males and females. Relative

to controls, body weights were decreased (7-18%; not significant) in females during the last six months of the study. Cumulative body weight change for the overall study was lower than controls (87.5% treated *vs* 103.4% controls). Absolute liver weights were increased in males (40%; p<=0.05) and females (41%; not significant). Increases were also noted in relative (to body) liver weights (45-62%; p<=0.05) and relative (to brain) liver weights (28-33%; not significant) in these animals. Minimal to slight multifocal pigment deposition was observed in the liver in males (1/4 treated *vs* 0 controls) and females (2/4 treated *vs* 0/4 controls). Minimal focal inflammation of the liver was observed in females (1/4 treated *vs* 0/4 controls). Treatment-related hematological and clinical chemistry differences (p<=0.05) included the following: (i) decreased erythrocytes in males on Days 92 and 363 (9-12%); (ii) decreased hemoglobin in males on Days 33, 92, and 363 (7-11%); (iii) increased thrombocytes in males on Days 92, 180, and 363 (42-53%); (iv) decreased alanine aminotransferase (ALT) in males on Day 363 (44%); (v) decreased aspartate aminotransferase (AST) in males on Day 363 (31%); (vi) increased cholesterol in males on Days 180 and 363 (51-65%); (vii) increased methemoglobin in females on Days 180 (1.225% treated *vs* 0.250% controls) and 363 (1.23% treated *vs* 0.18% controls) however, these differences were deemed not treatment-related or toxicologically important because they were minor, transient and/or not dose-related.; (viii) decreased gamma glutamyl transferase (GGT) in females on Day 363 (47%); and (ix) decreased ALT in females on Days 180 and 363 (40-56%). The toxicological significance of decreases in ALT, AST, and GGT is not known. Slight yellow adipose tissue was observed in males and females (1/4 each treated). This discoloration was not considered to be treatment related; this discoloration is characteristic of dinitroaniline treatment Microscopic examination revealed normal tissues. Minimal to slight multifocal cortical tubular cytoplasmic pigment deposition was noted in the kidneys in males and females (1/4 each treated *vs* 0/4 controls).

The only findings at 2.4 mg/kg/day were decreased (41-44%; p<=0.05) ALT and GGT in females on Day 363 and minimal multifocal cortical tubular pigment deposition in the kidneys in males and females (1/4 each treated *vs* 0/4 controls).

The only finding at 0.75 mg/kg/day was decreased GGT (31%, p<=0.05) in females on Day 363.

The LOAEL for this study is 40 mg/kg/day based on increased frequency of abnormal stool, decreased body weights and body weight gains, and on decreased erythrocytes and hemoglobin and increased thrombocytes in males. The NOAEL is 2.4 mg/kg/day.

The submitted study is classified as **acceptable/guideline** and satisfies the guideline requirements for a chronic oral toxicity study in the dog (OPPTS 870.4100b; OECD 452).

Dose and Endpoint for Establishing RfD: **NOAEL = 2.4 mg/kg/day**, based on increased frequency of abnormal stool, decreased body weights and body weight gains, and on decreased erythrocytes and hemoglobin and increased thrombocytes in males at the LOAEL of 40 mg/kg/day.

Uncertainty Factors: 100X (10x for interspecies extrapolation and 10x for intraspecies variation).

Comments about Study/Endpoint/Uncertainty Factor(s): The HIARC reaffirmed the previously established chronic RfD. This study is of the appropriate duration of exposure for this risk assessment. The lower NOAEL of 0.75 mg/kg/day established in the other dog study (MRID 00151908) was not selected since the endpoint (increase in liver weights) was not accompanied by any other corroborative changes such as alterations in clinical chemistry parameters or histopathological changes in the liver.

$$\textbf{Chronic RfD} = \frac{\textbf{2.4 mg/kg/day (NOAEL)}}{\textbf{100 (UF)}} = \textbf{0.024 mg/kg/day}$$

3.3 INCIDENTAL ORAL EXPOSURE

3.3.1 Short-Term Oral Exposure (1-30 days)

Selected Study: Two-Generation Reproduction Study - Rat § 870.3800

MRID No.: 00151901, 00151902, 00151903, and 00159619

Executive Summary: See 2.4.1

Dose and Endpoint for Risk Assessment: **Offspring NOAEL = 10 mg/kg/day,** based on decreased pup body weight in the F1a and F1b generations on post-natal days 7 and 21.

Comments about Study/Endpoint/Margin of Exposure: This study and endpoint are appropriate for the population (infants and children) and duration (1 to 30 days). On post-natal day 1, pups in the 32.5 mg/kg/day group had mean body weights comparable to the control group indication that there was no *in utero* effect on pup body weight. On post-natal days 7 and 21, however, there were significant decreases in pup weights.

3.3.2 Incidental Oral Exposure: Intermediate-Term (1 - 6 Months)

Selected Study: Special Urinalysis Study [feeding] - rat § Non-guideline

MRID No.: 00157156, 40138301, 41086101

Executive Summary: The purpose of this special study was to provide additional information to establish a NOAEL for nephrotoxicity, which was observed in a chronic feeding study in rats at the lowest dose tested. Trifluralin (96-97% a.i.; Lot/Batch # 00554AP2) was administered continuously via the diet to Fischer 344 male rats at nominal doses of 0, 50, 200, 800, 3200, and 6400 ppm (time-weighted daily averages through month 4: 0, 2.5, 10.1, 40.1, 164, and 330 mg/kg/day) for up to 4 months. The rats (n=10, except n=15 in controls and 0.05% group) were sacrificed at 30, 60, and 90 days. At 0 and

50 ppm, 45 rats were treated, and 30 rats were treated in each of the other doses. The rats (n=10-15) were sacrificed at 30, 60, and 90 days. Additionally, a satellite group of 15 rats at 0 and 50 ppm and 10 rats in each of the other doses was treated for 4 months, followed by 6 weeks of maintenance on the control diet. All satellite study animals were sacrificed on day 171. Body weight, body weight gain, food consumption, food efficiency, gross pathology, and histopathology of the kidney and urinary bladder were evaluated. Measurements of numerous parameters were made during urine chemistry and protein electrophoresis of the urine.

No treatment-related adverse effects were observed on mortality, clinical signs, body weight, or body weight gain through month 3; after 4 months of treatment, there was a significant decrease in body weight, body weight gain and food utilization at >= 3200 ppm. Urine was discolored by the dinitroaniline compound at 3200 and 6400 ppm. No effect was observed in the 50 ppm group. Recovery was evident following 6 weeks of maintenance on the control diet in all groups, but was incomplete in the 6400 ppm group.

There was a dose related increase (>= 200 ppm) in the incidence of intra-cytoplasmic hyaline droplet formation in the renal cortical tubular epithelial cells (minimal to moderate severity) and an increase in urine α1-globulin and α2-globulin (all biochemical indicators of kidney toxicity).

In the >=800 ppm groups, significant increases of 73-489% in increases in urinary total protein, aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) and in the urinary volume (38-89%) was observed.

In the 6400 ppm group, additional toxic effects were observed. Increases ($p<=0.05$) of 27-169% in urinary calcium and sodium were observed.

The LOAEL for nephrotoxicity is 800 ppm (40.1 mg/kg/day), based on the presence of tubular cytoplasmic hyaline droplets; increased total protein, AST, and LDH in the urine; albumin α1-globulin and α2-globulin observed by urine electrophoresis; and increased urinary volume. The NOAEL is 200 ppm (10.1mg/kg/day).

Dose and Endpoint for Risk Assessment: **NOAEL: 10.1 mg/kg/day,** based on cortical tubular cytoplasmic hyaline droplets; increased total protein, AST, and LDH in the urine; and albumin α1-globulin and α2-globulin observed by urine electrophoresis; and increased urinary volume observed at the LOAEL of 10.1 mg/kg/day.

Comments about Study/Endpoint/Margin of Exposure: The dose, endpoint and study, as well as the population of concern (infants and children) are appropriate for this exposure scenario.

3.4 DERMAL ABSORPTION

Selected Study: Percutaneous absorption ^{14}C-**ethalfluralin** in monkeys. Guideline #:NG

MRID No.: 132820, 92062028; TXR 004090, 004235

Executive Summary: Four monkeys (2 males and 2 females) were administered 2 mg/kg radio-labeled ethalfluralin in ethanol intravenously or topically to the forearm and the plasma level determined for 120 hours to determine an area under the curve for both types of applications. Two compartments were noted with one-half lives of 1.71 hours for the plasma distributive phase and 79.1 hours for the terminal plasma disappearance phase. After 120 hours label was not detectable in 2 (1 male and 1 female) of the 4 animals studied. Since the 2 animals with undetectable plasma levels at 120 hour yielded the most consistent data, data from these animals were used to calculate the AUCs. The dermal absorption was determined by ratio of the area under the plasma curve AUC; [(AUC-dermal/(AUC-i.v.)] x 100 = 2.84%.

Percentage (%) Dermal Absorption: 3% for ethalfluralin as a surrogate for trifluralin.

Comments about Dermal Absorption: There is no acceptable dermal absorption study for trifluralin. However, there was an acceptable study conducted in monkeys for ethalfluralin. Trifluralin and ethalfluralin have similar structures (differing only in the dialkyl groups) and physical constants (melting points, solubilities, and the log Kows). Using the dermal absorption percentage for ethalfluralin is a more accurate estimation for trifluralin than assuming a value of 100% for conversion of an oral study to a dermal endpoint for intermediate and long-term occupational exposure. Further, this dermal absorption value is similar to the approximation from the ratio of the dermal and oral NOAELs (1000/154 = 6%).

3.5 DERMAL EXPOSURE

3.5.1 Dermal Exposure: Short-Term Exposure (1- 30 days)

Quantification of dermal risk for this exposure period is not required since no systemic toxicity was observed at 1000 mg/kg/day (limit dose) in the 21-day dermal toxicity studies. There is no concern for developmental toxicity. Also no nephrotoxicity was seen in the dermal studies.

The technical grade trifluralin caused typical delayed hypersensitivity in guinea pigs. Repeated dermal applications to rats resulted in skin lesions that progressed in severity and therefore may have the potential for adverse effects. Because risk can not be quantified, the HIARC also recommends that the products containing trifluralin should be labeled as SENSITIZER and should avoid human contact. A Local Lymph Node Assay in rats may be used to define the NOAEL for dermal sensitization and allow quantification.

3.5.2 Dermal Exposure: Intermediate-Term (1 - 6 Months)

Selected Study: Special Urinalysis Study [feeding] - rat § Non-guideline

MRID No.: 00157156, 40138301, 41086101

Executive Summary: See section 3.3.2

Dose and Endpoint for Risk Assessment: **NOAEL = 10.1 mg/kg/day corrected for 3% absorption by the dermal route relative to oral absorption,** based on cortical tubular cytoplasmic hyaline droplets; increased total protein, AST, and LDH in the urine; albumin α1-globulin and α2-globulin observed by urine electrophoresis; and increased urinary volume observed at the LOAEL of 40.1 mg/kg/day.

Comments about Study/Endpoint/Margin of Exposure: The HIARC selected this study to address the nephrotoxic concerns seen after subchronic exposure which is appropriate for this exposure period concern.

3.5.3 Dermal Exposure Long-Term (> 6 Months)

Study Selected: Chronic toxicity oral (capsule) - dog

MRID No.: 42447001

Executive Summary: See section 3.2

Dose and Endpoint for Risk Assessment: **NOAEL = 2.4 mg/kg/day corrected for 3% absorption by the dermal route relative to oral absorption,** based on increased frequency of abnormal stool, decreased body weights and body weight gains, and on decreased erythrocytes and hemoglobin and increased thrombocytes in males at the LOAEL of 40 mg/kg/day.

Comments about Study/Endpoint/Uncertainty Factor(s): This dose, endpoint and study were selected to establish the chronic RfD.

3.6 INHALATION EXPOSURE

3.6.1 Short-term Inhalation Exposure (1 to 30 days)

Selected Study: 30-Day Inhalation Study in the Rat § 870.3465

MRID No.: 40392312

Executive Summary: In a 30-day inhalation toxicity study (MRID 40392312), HOE

38474 (trifluralin; >99% a.i.; Lot/batch # AZ 01751) was dissolved in 70% acetone and administered via nose-only inhalation to Wistar KFM-Han (outbred, SPF-quality) rats (15/sex/dose) for 6 hours/day, 5 days/week for up to 30 days at nominal dose levels of 0 (air control), 0 (acetone control), 100, 300, or 1000 mg/m^3. Ten rats/sex/dose were sacrificed after 30 days, and the remaining 5 rats/sex/dose were sacrificed after a 14-day recovery period. There were no adverse effects of treatment on mortality, body weights, body weight gains, food consumption, food conversion ratio, water consumption, ophthalmology, or gross pathology.

At 1000 mg/m^3, all rats showed slight dyspnea and ruffled fur; these symptoms were seen daily just after exposure starting with Day 1 and were not observed on non-exposure days (i.e. weekends) or during the recovery period. In the females at this dose, increases ($p<=0.01$) in methemoglobin (1.59% treated *vs* 0.79% acetone control) and total bilirubin (62%) were observed at the end of the main study but were comparable to controls at the end of the recovery period. Direct bilirubin was increased in females; however, data were not provided.

Treatment-related effects on the liver were observed; however, these changes were limited to increased liver weights and centrilobular hypertrophy and were considered adaptive since there was no increase in liver enzymes levels in the blood. At the end of the main study, absolute and relative (to body) liver weights were dose-dependently increased 9-32% ($p<=0.05$) in the 300 and 1000 mg/m^3 animals. Relative (to brain) liver weights were dose-dependently increased ($p<=0.05$) 10-14% in the 100 and 300 mg/m^3 females and 21-28% in the 1000 mg/m^3 animals. Incidence of minimal to slight centrilobular hypertrophy, characterized by increased homogeneity and reduced basophilia of the cytoplasm in enlarged hepatocytes, was dose-dependently increased in the ≥100 mg/m^3 males (4-10/10 treated *vs* 0/10 each control group) and in the 1000 mg/m^3 females (7/10 treated *vs* 0/10 each control group). At the end of the recovery period, liver weights (absolute, relative to body, and relative to brain) remained dose-dependently increased (19-26%; $p<=0.05$) in males at ≥100 mg/m^3.

The LOAEL for this study is 1000 mg/m^3 based on increased methemoglobin and bilirubin in females and incidences of dyspnea and ruffled fur in males and females. The NOAEL is 300 mg/m^3.

This 30-day inhalation toxicity study in the rat is classified as **acceptable/non-guideline** and is satisfactory as a range-finding study. Due to its duration (30 days), this study does not meet the guideline requirements for a 90-day inhalation toxicity study [OPPTS 870.3465 (§82-4); OECD 413]

Dose and Endpoint for Risk Assessment: **NOAEL = 300 mg/m^3 (81 mg/kg/day)** based on increased bilirubin in females and incidences of dyspnea and ruffled fur in males and females at 1000 mg/m^3 (270 mg/kg/day).

Comment about the Study/Endpoint/Margin of Exposure: This is the appropriate route of administration and duration of exposure.

3.6.2 Inhalation Exposure: Intermediate-Term (1- 6Months)

Selected Study: Special Urinalysis Study [feeding] - rat § Non-guideline

MRID No.: 00157156, 40138301, 41086101

Executive Summary: See section 3.3.2

Dose and Endpoint for Risk Assessment: **NOAEL = 10.1 mg/kg/day inhalation absorption assumed to be equivalent to oral absorption.** Based on cortical tubular cytoplasmic hyaline droplets; increased total protein, AST, and LDH in the urine; and albumin α1-globulin and α2-globulin observed by urine electrophoresis; and increased urinary volume observed at the LOAEL of 40.1 mg/kg/day.

Comments about Study/Endpoint/Margin of Exposure: The inhalation study was not selected since the NOAEL (81 mg/kg/day) would not address the nephrotoxicity seen at 40.1 mg/kg/day in the special urinalysis study. Since an oral dose was selected absorption via inhalation is presumed to be equivalent to oral absorption.

3.6.3 Inhalation Exposure: Long-Term (> 6 Months)

Study Selected: Chronic toxicity oral (capsule) - dog

MRID No.: 42447001

Executive Summary: See section 3.2

Dose and Endpoint for Establishing RfD: **NOAEL = 2.4 mg/kg/day with inhalation absorption assumed to be equivalent to oral absorption.** Based on increased frequency of abnormal stool, decreased body weights and body weight gains, and on decreased erythrocytes and hemoglobin and increased thrombocytes in males at the LOAEL of 40 mg/kg/day.

Comments about Study/Endpoint/Uncertainty Factor(s): Since an oral dose was selected, absorption via inhalation is presumed to be equivalent to oral absorption.

4 MARGINS OF EXPOSURE

Summary of target Margins of Exposure (MOEs) for risk assessment.

Route / Duration	Short-Term (1-30 Days)	Intermediate-Term (1 - 6 Months)	Long-Term (> 6 Months)
Occupational (Worker) Exposure			
Dermal	N/A	100	100
Inhalation	100	100	100
Residential (Non-Dietary) Exposure			
Oral	100	100	N/A
Dermal	N/A	100	100
Inhalation	100	100	100

* Since the dermal risk can not be quantified, the HIARC recommends that products containing trifluralin should be labeled **SENSITIZER** and that dermal contact should be avoided

For Occupational exposure: This is based on the conventional uncertainty factor of 100X (10X for intraspecies extrapolation and 10X for interspecies variation)

For Residential exposure: This is based on the conventional uncertainty factor of 100X (10X for intraspecies extrapolation and 10X for interspecies variation).

5 RECOMMENDATION FOR AGGREGATE EXPOSURE RISK ASSESSMENTS

As per FQPA, 1996, when there are potential residential exposures to the pesticide, aggregate risk assessment must consider exposures from three major sources: oral, dermal and inhalation exposures. The toxicity endpoints selected for these routes of exposure may be aggregated as follows: Intermediate- and long-term exposures (incidental oral, dermal, and inhalation exposures) can be aggregated because of the use of a common endpoint for oral, dermal (oral equivalent) and inhalation (oral equivalent) routes of exposure.

6 CLASSIFICATION OF CARCINOGENIC POTENTIAL

6.1 Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID No.: 00162457, 00162458

Executive Summary: In a combined chronic toxicity/oncogenicity study (MRID 00162457,

00162458), trifluralin (>99% a.i., Lot No. 10653 OP. 112/80) was administered daily in the diet to 36 Hoe:WISKf (SPF71) rats/sex/dose for up to 25 months at nominal doses of 0, 200, 800, or 3200 ppm (equivalent to an achieved intake of 0/0, 10/13, 40/53, and 169/219 mg/kg/day in males/females). In the chronic toxicity test, 20 rats/sex/dose were sacrificed at 24 months. For trifluralin residue analysis in the tissues of 10 rats/sex/dose, 2 rats/sex/dose were sacrificed at Months 6, 12, and 18, and the remaining animals were sacrificed at Month 24. The bromosulfophthalein hepatic function test and phenolsulfonaphthalein kidney function test were performed in the survivors of a group of 6 rats/sex/dose at Months 6, 12, 18, and 24. In a carcinogenicity study, an additional 60 rats/sex were treated at the same dosages for 28 months.

Mortality, clinical signs, food consumption, ophthalmoscopic findings, hematology, clinical chemistry, urinalysis, hepatic and renal function tests, organ weights, and gross pathology for both sexes at all doses were unaffected by treatment. No treatment-related adverse differences in any parameter were observed in the 200 and 800 ppm groups.

At 3200 ppm, the terminal body weights were decreased by 16-23% ($p<=0.05$). During the studies, decreased ($p<=0.05$) body weights were frequently observed (4-28%). Overall body weight gains (calculated by the reviewers) were decreased by 24-39%. Relative water consumption (as % body weight) was increased (11-54%; $p<=0.05$ or NS) throughout the study; however, the biological significance was unclear.

During Months 6, 12, 18, and 24, trifluralin residues were found concentrated in tissues of the 3200 ppm group, including the fatty tissue, kidney, and skeletal muscle (females only), and in the fatty tissue of the 800 ppm females. A generally time-dependent accumulation of trifluralin residue was observed in the remaining carcass of the 3200 ppm group, but was not observed in any other tissue. Tissue residues were generally higher in females than in males.

The LOAEL is 3200 ppm (equivalent to 169/219 mg/kg/day in males/females) based on decreases in body weight and body weight gains. The NOAEL is 800 ppm (equivalent to 40/53 mg/kg/day in males/females).

At the doses tested, the carcinogenic potential of trifluralin was negative. Dosing was considered adequate based on differences in body weight and body weight gains.

This study is **acceptable/guideline** and satisfies the guideline requirement for a chronic/ carcinogenicity study (OPPTS 870.4300; OECD 453) in rats.

Discussion of Tumor Data: No tumors were observed in this study.

Adequacy of Dose Levels Tested: The doses were adequate for evaluating the carcinogenic potential of trifluralin.

6.2 Carcinogenicity Study in Mice

MRID: 00158935, 40392313

Executive Summary: In a carcinogenicity study (MRIDs 00158935 and 40392313), trifluralin (>99% a.i., Batch No. HOE 38474 O H AT210) was administered daily in the diet to 60 NMRI, KFM-Han, outbred (SPF) mice/sex/dose for up to 104 weeks at nominal doses of 0, 50, 200, or 800 ppm (equivalent to 0/0, 7.5/10.5, 29/41, and 118/165 mg/kg/day in males/females). Ten mice/sex/dose were sacrificed at 52 weeks, and the remaining survivors were sacrificed at 104 weeks.

Mortality, clinical signs, food consumption, body weights, body weight gains, ophthalmology, audiology, teeth and mucous membrane, clinical chemistry, organ weights, gross pathology, and histology for both sexes at all doses were unaffected by treatment.

The LOAEL was not observed. The NOAEL for this study is 800 ppm (118/165 mg/kg/day in males/females), the highest dose tested.

At the doses tested, the carcinogenic potential of trifluralin was negative. Dosing was considered inadequate as a toxic effect was not observed, and the limit dose was not tested.

This study is **unacceptable/guideline** and does not satisfy the guideline requirement for a carcinogenicity study [OPPTS 870.4200b; OECD 451] in mice. The LOAEL was not observed, the limit dose was not tested, and the range-finding study indicated that dosing was inadequate. The NOAEL for the range finder was 2500 ppm (equivalent to 375 mg/kg/day), the highest dose

Discussion of Tumor Data: No tumors were observed in this study

Adequacy of Dose Levels Tested: The doses were not adequate for evaluating the carcinogenic potential of trifluralin.

6.3 Classification of Carcinogenic Potential

The oncogenic potential of trifluralin was extensively addressed in the two Position Documents, issued in 1987 and 1982, by the CPRC (TXR 007362) in 1986, as well as an IARC Monograph in 1991. In an NCI cancer study in rats and mice (MRID 00124928, TXR 002468), the technical trifluralin used was found to be contaminated with NDPA. The hepatocellular carcinomas observed in a mouse oncogenicity study were attributed to NDPA contamination of the technical trifluralin. Two other studies in the rat (MRID 0044337) and mouse (MRID 0044338) were performed with purified trifluralin. The rat study showed malignant neoplasms of the renal pelvis and benign urinary bladder neoplasms. Based on the available data, the CPRC concluded that trifluralin is a "Group C" (limited evidence of carcinogenicity) carcinogen. The Q1* for

trifluralin is 5.79 X 10^{-3} $(mg/kg/day)^{-1}$ based on male rat thyroid follicular cell tumors combined. Two additional cancer studies (summarized below) in the mouse (MRID 00158935) and rat (MRID 00162457, 00162458) were submitted to the Agency, however, the data were insufficient to return trifluralin to the CPRC(EPA memo July 23, 1991, Whang Phang to Ester Rinde).

7 MUTAGENICITY

The HIARC concluded that there is not a concern for mutagenicity resulting from exposure to trifluralin.

8 HAZARD CHARACTERIZATION

Acute toxicity studies are available for technical trifluralin, as well as, the manufactured products. Technical trifluralin shows low acute toxicity via the oral, dermal and inhalation routes of exposure, toxicity categories IV, III, and IV, respectively. Technical trifluralin showed some irritation in the eye (toxicity category III), but not in the skin (toxicity category IV). In the dermal sensitization assay trifluralin was found to be a dermal sensitizer. Although not required, an acute delayed neurotoxicity study was also performed with negative results.

The subchronic toxicity data base is complete. Trifluralin was evaluated in rat and mouse oral studies, in rat and rabbit dermal studies, in a rat inhalation study, and in a 6-month oral (capsule) toxicity study in the dog. In the subchronic oral rat toxicity study (MRID 00151906), minor decreases in overall body weight gains and food consumption in males and females, decreased hemoglobin, alkaline phosphatase, and alanine aminotransferase in the males, and increased absolute and relative (to body) liver weights in males and females were observed at the LOAEL of 5000 ppm (391 mg/kg/day). In the mouse subchronic oral toxicity study (MRID 00151905), no toxicity was observed at the highest dose tested of 2500 ppm (375 mg/kg/day). In a 6-month oral (capsule) study in the dog (MRID 00151907), increased absolute and relative (to body) liver weights, liver enlargement, discolored kidneys, decreased red cell indices, increased platelets in males; and increased alkaline phosphatase at the LOAEL of 400 ppm (10 mg/kg/day). A 21-day dermal toxicity study (MRID 00152888)in the rat showed no systemic toxicity at the limit dose of 1000 mg/kg/day (only dose tested). In a 31-day dermal toxicity study in the rat (MRID 00153171) showed no systemic toxicity at 1000 mg/kg/day; dermal effects included sub-epidermal inflamation and ulcerations at 200 mg/kg/day. A rabbit 21-day dermal toxicity study (MRID 41993810) with a formulation (35.8% trifluralin and 2.6% XRD-498) also did not show any systemic toxicity at1000 mg/kg/day; dermal effects observed at the LOAEL (100 mg/kg/day) included erythema, edema, and/or scaling and fissuring. The systemic NOAELS (1000 mg/kg/day) observed in the dermal toxicity studies are consistent with the dermal absorption factor of 3%. A 30-day inhalation exposure to rats (MRID 00151904) with trifluralin at 1000 mg/m^3 resulted in increased methemoglobin and bilirubin, as well as dyspnea and ruffled fur.

Chronic toxicity to trifluralin was evaluated in the rat, mouse, and dog. Systemic toxicity in rats (MRID 00162457) exposed to 3200 ppm (169/219 mg/kg/day) included decreases in body weight and The NOAEL is 800 ppm (equivalent to 40/53 mg/kg/day in males/females). In a 2-year mouse study (MRID 00158935, 40392313) no systemic toxicity was observed at the highest dose tested of 800 ppm (118 mg/kg/day). Two 12-month oral (capsule) toxicity studies were performed in the dog. In one study

(MRID 42447001), increased frequency of abnormal stool, decreased body weights and body weight gains, decreased erythrocytes and hemoglobin, and increased thrombocytes in males were observed at the LOAEL of 40 mg/kg/day. In the other study (MRID 00151908) increased absolute liver weights in males were observed at the LOAEL of 3.8 mg/kg/day.

The developmental toxicity of trifluralin in the rat and rabbit, as well as three 2-generation reproduction studies was evaluated. In all of these studies the NOAEL/LOAEL for parental toxicity were the same as or lower than the NOAEL/LOAEL for reproductive and developmental toxicity. In the developmental toxicity studies, maternal toxicity consisted of decreased body weight gain and food consumption, increased liver and spleen weights, increased incidence of resorptions and litters with total resorptions were observed in the rat; and increased number of abortions, macroscopic changes in the liver and lungs, and decreased food consumption, in the rabbit. Reduced ossification of vertebrae and ribs were observed in both the rat and rabbit. In the reproduction studies kidney toxicity (acute renal failure, lesions of renal proximal tubule, increased relative liver) and uterine atrophy in females. Offspring toxicity consisted of decreased pup weight including an increase in the number of runts. Decreased fetal, neonatal, and litter viability, and decreased lactation index were also observed.

Extensive testing showed the trifluralin is neither mutagenic nor genotoxic. There was no evidence of mutagenicity for trifluralin in rat dominant lethal, L5178Y mouse lymphoma, *Salmonella typhimurium*, *Saccharomyces cerevisiae*, and DNA repair assays, nor did it induce sister chromatid exchange in Chinese hamster ovary cells. These tests showed that trifluralin does not inhibit the polymerization of microtubules in mammalian cells.

The oncogenic potential of trifluralin was addressed by the Agency (PD1/2/3 and PD 4), CPRC (TXR 007362) in 1986, as well as an IARC Monograph in 1991. Two oncogenicity studies carried our for NCI in the rat (TXR 00044337) and mouse (TXR 0044338) revealed hepatocellular carcinomas in both studies. Subsequent analysis of the trifluralin used in these studies showed high concentrations of nitrosamine [N-dinitroso-di-n-propylamine NDPA]; the hepatocellular carcinomas were attributed to this contaminant. Oncogenicity studies with purified trifluralin revealed malignant neoplasms of the renal pelvis and benign urinary bladder neoplasms in the rat. Based on the available data, the CPRC concluded that trifluralin is a "Group C" (limited evidence of carcinogenicity) carcinogen. The Q1* for trifluralin is 5.79×10^{-3} $(mg/kg/day)^{-1}$ based on male rat thyroid follicular cell tumors combined.

After review of the cancer data by the Carcinogenicity Peer Review Committee (CPRC), new rat and mouse oncogenicity studies were submitted to the Agency. The CPRC concluded, however, that the results of these studies were insufficient to warrant reevaluation. In both of these studies no oncogenicity was observed in either the rat (MRID 00162457, 00162458) at 3200 ppm (169 mg/kg/day) or the mouse (MRID 00158935, 40392313) at 800 ppm (118 mg/kg/day). The doses used in the rat study was adequate to evaluate the oncogenic potential of trifluralin, however, the doses used in the mouse study were not.

The kidney appears to be a target organ for trifluralin. These findings are summarized in a peer review of trifluralin (Apr 11, 1986) and include the following observations: Kidney and bladder tumors, decreased kidney weights, increased BUN, increases in total protein, aspartate aminotransferase and lactate dehydrogenase in the urine, protein electrophoresis of urine samples showed α1-globulin and α2-

globulin, tubular hyaline casts in the kidneys and minimal cortical tubular epithelial regeneration observed microscopically, and increased incidence of progressive glomerulonephritis.

The following renal effects were observed in

A special urinalysis study in the rat included the presence of tubular cytoplasmic hyaline droplets, increased total protein, AST, and LDH in the urine, albumin α1-globulin and α2-globulin observed by urine electrophoresis, and increased urinary volume.

A two-generation reproduction: Increased incidences of lesions of the renal proximal tubules, decreased corticomedullary mineralization, hyaline droplets in the tubular epithelium, and acute renal failure

A developmental toxicity study in the rat: Clear fluid in the renal pelvis, grey hollows on the kidney surface, and enlarged kidney with yellow calculi in the pelvis.

A chronic dog study: Minimal to slight multifocal cortical tubular cytoplasmic pigment deposition was noted in the kidneys in males and females.

And a two-week range-finding study in the rat:: Urinary triple phosphates

Trifluralin does not appear to be an immunotoxicant. Effects suggestive of immunotoxicity include thymic hypoplasia and decreased relative thymus weights in the rabbit developmental toxicity study and rat reproduction study, respectively, and increased spleen weights in a rat developmental toxicity study. No other indications of possible immunotoxicity were observed in the trifluralin data base.

In the rat and dog absolute and relative liver weights were increased, but this response was considered to be adaptive since serum ALT, AST, GGT and alkaline phosphatase activities were unaffected by treatment.

In a rat metabolism study (MRID 41218901), ^{14}C-trifluralin (Lot no. 553-VE9-116, >98% radiochemical purity) in corn oil was administered by gavage at 300 mg/kg/day to 5 Fischer 344 rats/sex on three consecutive days. Metabolite characterization of the 24-48 hour urinary samples (pooled by sex) and quantitation of urinary samples collected at 0-24, 24-48, and 48-54 hours and pooled by sex were performed using liquid scintillation counting, silica gel column chromatography, TLC, HPLC, NMR, and mass spectroscopy. The objective of this study was to identify the urinary metabolites of trifluralin.

There was no sex-dependent effect on metabolic profiles. A minimum of 20-30 non-conjugated metabolites and an additional 10-20 conjugated metabolites were present in the urine, but no parent compound was detected. Information on the percentage of the administered dose excreted in the urine was not provided. However, no single metabolite accounted for more than 8-10% of the total urinary radioactivity, and the majority of the metabolites were present at 1-2% of the total urinary radioactivity. Thus, almost all of the metabolites were minor (<5% of the total radioactive dose). Metabolite F1B was found at 8.2-8.9% of the total urinary radioactivity in both sexes, and Metabolite F2, N-[(3-

(acetylamino)-2-amino-5-(trifluoromethyl)phenyl] acetamide, was found at 4.0-5.2%. Metabolite F1B was partially characterized as retaining the trifluoromethyl groups, the two equivalent aromatic protons, and the two nitro groups, but the propyl groups were lost. Ten other metabolites were identified (<0.1-3.7% of total urinary radioactivity, each compound in each sex). Two additional metabolites were partially characterized (0.1-2.6% of total urinary radioactivity, each compound in each sex).

Four metabolic pathways were identified as follows:

(1) oxidative N-dealkylation of one or both propyl groups and metabolites which were hydroxylated on the propyl side chain

(2) reduction of one or both nitro groups to the corresponding amine

(3) cyclization reactions to give a variety of substituted and unsubstituted benzimidazole metabolites; and

(4) conjugation reactions, including acetylation of the reduced nitro groups, sulfate, and glucuronic acid conjugates.

9 DATA GAPS / REQUIREMENTS

None

10 ACUTE TOXICITY

Acute Toxicity of Trifluralin, Technical

Guideline No.	Study Type	MRID No.	Results	Toxicity Category
870.1100	Acute Oral (Rat)	00157486 (1985) TXR 006174 Acceptable/Guideline	LD50 > 5000 mg/kg	IV
870.1200	Acute Dermal (Rat)	00157482 (1985) TXR 006174 Acceptable/Guideline	LD50 > 2000 mg/kg	III
870.1300	Acute Inhalation (Rat)	00155261 (1982) TXR 006174 Acceptable/guideline	LC50 > 4660 mg/m^3, 4.66 mg/L	IV
870.2400	Primary Eye Irritation (Rabbit)	00157483 (1985) TXR 006174 Acceptable/Guideline	Conjunctival redness at 24hr, cleared by 4 d	III
870.2500	Primary Skin Irritation	00157485 (1985) TXR 006174 Acceptable/Guideline	Not an irritant	IV
870.2600	Dermal Sensitization	00157484 (1985) TXR 006174 Acceptable/Guideline	Sensitizing agent	N/A

11 SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

Summary of Toxicological Dose and Endpoints for Trifluralin (PC Code 036101)

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary (Females 13-50 years of age)	NOAEL = 100 mg/kg/day UF = **100** **Acute RfD** = 1.0 mg/kg/day	FQPA SF = 1 **aPAD** = acute RfD / FQPA SF = 1.0 mg/kg/day	**Developmental Toxicity Study - Rat** LOAEL = 500 mg/kg/day based on increased total liter resorptions.
Acute Dietary (General population including infants and children)	No appropriate single dose endpoint was found for this population sub group		
Chronic Dietary (All populations)	NOAEL= 2.4 mg/kg/day UF = 100 **Chronic RfD** = **0.024** mg/kg/day	FQPA SF = 1 **cPAD** = chronic RfD / FQPA SF = 0.024 mg/kg/day	**Chronic Toxicity (capsule) - Dog** LOAEL = 40 mg/kg/day based on based on increased frequency of abnormal stool, decreased body weights and body weight gains, and on decreased erythrocytes and hemoglobin and increased thrombocytes in males
Short-Term Incidental Oral (1-30 days)	NOAEL= 10 mg/kg/day	**Residential** LOC for MOE = 100 **Occupational** = NA	**Two-generation Reproduction Study - Rat** LOAEL = 32.5 mg/kg/day based on decreased pup weights in both generations

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Intermediate-Term Incidental Oral (1- 6 months)	NOAEL= 10 mg/kg/day	**Residential** LOC for MOE = 100 **Occupational** =NA	**Special Urinalysis Study - Rat** LOAEL = 40 mg/kg/day based on based on the presence of tubular cytoplasmic hyaline droplets; increased total protein, AST, and LDH in the urine; albumin α1-globulin and α2-globulin observed by urine electrophoresis; and increased urinary volume
Short-Term Dermal (1 to 30 days)	No quantification required. since there was no systemic toxicity at the limit dose in the dermal toxicity study. There are no developmental toxicity concerns. Also, because risk can not be quantified, the HIARC also recommends that the products containing trifluralin should be labeled as SENSITIZER and should avoid human contact		
Intermediate-Term Dermal (1 to 6 months)	Oral study NOAEL = 10 mg/kg/day (dermal absorption rate = 3 %	**Residential** LOC for MOE = 100 **Occupational** LOC for MOE = 100	**Special Urinalysis Study - Rat** LOAEL = 40 mg/kg/day based on based on the presence of tubular cytoplasmic hyaline droplets; increased total protein, AST, and LDH in the urine; albumin α1-globulin and α2-globulin observed by urine electrophoresis; and increased urinary volume
Long-Term Dermal (>6 months)	Oral study NOAEL= 2.4 mg/kg/day (dermal absorption rate = 3 % when appropriate)	**Residential** LOC for MOE = 100 **Occupational** LOC for MOE = 100	**Chronic Toxicity (capsule) - Dog** LOAEL = mg/kg/day based on based on increased frequency of abnormal stool, decreased body weights and body weight gains, and on decreased erythrocytes and hemoglobin and increased thrombocytes in males

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Short-Term Inhalation (1 to 30 days)	Inhalation study NOAEL= 81 mg/kg/day	**Residential** LOC for MOE = 100 **Occupational** LOC for MOE = 100	**30-Day Inhalation Study - Rat** LOAEL = 270 mg/kg/day based on increased methemoglobin and bilirubin in females and incidences of dyspnea and ruffled fur in males and females
Intermediate-Term Inhalation (1 to 6 months)	Oral study NOAEL = 10 mg/kg/day (inhalation absorption rate = 100%)	**Residential** LOC for MOE = 100 **Occupational** LOC for MOE = 100	**Special Urinalysis Study - Rat** LOAEL = **40** mg/kg/day based on based on the presence of tubular cytoplasmic hyaline droplets; increased total protein, AST, and LDH in the urine; albumin α1-globulin and α2-globulin observed by urine electrophoresis; and increased urinary volume
Long-Term Inhalation (>6 months)	Oral study NOAEL= 2.4 mg/kg/day (inhalation absorption rate = 100%)	**Residential** LOC for MOE = 100 **Occupational** LOC for MOE = 100	**Chronic Toxicity (capsule) - Dog** LOAEL = mg/kg/day based on based on increased frequency of abnormal stool, decreased body weights and body weight gains, and on decreased erythrocytes and hemoglobin and increased thrombocytes in males
Cancer (oral, dermal, inhalation)	$Q1^* = 5.79 \times 10^{-3}$ $(mg/kg/day)^{-1}$ "Group C" (limited evidence of carcinogenicity)		

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable

NOTE: The Special FQPA Safety Factor recommended by the HIARC **assumes** that the exposure databases (dietary food, drinking water, and residential) are complete and that the risk assessment for each potential exposure scenario includes all metabolites and/or degradates of concern and does not underestimate the potential risk for infants and children.