



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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

TXR No: 0050924

October 2, 2003

Memorandum

SUBJECT: TRIFLURALIN (PC Code: 036101) Toxicology Disciplinary Chapter for the Tolerance Reassessment Eligibility Decision Document

FROM: Robert Fricke, Toxicologist
Reregistration Branch 2
Health Effects Division (7509C)

THRU: Alan Nielsen, Branch Senior Scientist
Reregistration Branch 2
Health Effects Division (7509C)

TO: Richard Griffin, Risk Assessor
Reregistration Branch 2
Health Effects Division (7509C)

DP Barcode: D284395

Submission No: S618837

Action Requested: Review toxicology studies submitted by the registrant and prepare the toxicology chapter to support Tolerance Reassessment Eligibility Decision (TRED) for trifluralin.

Attached is the updated toxicology chapter summarizing the findings of the toxicology studies.

TRIFLURALIN

PC Code: 036101

Toxicology Disciplinary Chapter for the Tolerance Reassessment Eligibility Decision Document

Date completed:
October 2, 2003

Prepared by:

Robert F. Fricke, Ph.D.
Reregistration Branch 2
Health Effects Division

Reviewed by:

Linda Taylor, Ph.D.
Reregistration Branch 1
Health Effects Division

form: FINAL June 21, 2000

1	HAZARD CHARACTERIZATION	1
2	REQUIREMENTS	3
3	DATA GAP(S)	4
4	HAZARD ASSESSMENT	4
4.1	Acute Toxicity	4
4.2	Subchronic Toxicity	6
4.2.1	Subchronic Oral Toxicity	6
4.2.1.1	870.3100 90-Day Oral Toxicity - Rat	6
4.2.1.2	870.3100 90-Day Oral Toxicity - Mouse	7
4.2.1.3	870.3150 90-Day Oral Toxicity - Dog	7
4.2.2	Subchronic Dermal Toxicity	9
4.2.2.1	870.3200 31-day Dermal Toxicity Study - Rat	9
4.2.2.2	870.3200 21-Day Dermal Toxicity - Rabbit	10
4.2.2.3	870.3200 21-Day Dermal Toxicity - Rabbit	10
4.2.3	870.3465 30-Day Inhalation - Rat	11
4.3	Prenatal Developmental Toxicity	12
4.3.1	870.3700a Prenatal Developmental Toxicity Study - Rat	12
4.3.2	870.3700a Prenatal Developmental Toxicity Study - Rat	13
4.3.3	870.3700b Prenatal Developmental Toxicity Study - Rabbit	14
4.3.4	870.3700b Prenatal Developmental Toxicity Study - Rabbit	15
4.4	Reproduction and Fertility Effects	16
4.4.1	870.3800 Reproduction and Fertility Effects - Rat	16
4.4.2	870.3800 Reproduction and Fertility Effects - Rat	17
4.4.3	870.3800 Reproduction and Fertility Effects - Rat	19
4.5	Chronic Toxicity	20
4.5.1	870.4300 Chronic Toxicity/Carcinogenicity - Rat	21
4.5.2	870.4100b Chronic Toxicity - Dog	22
4.5.3	870.4100b Chronic Toxicity - Dog	23
4.6	Carcinogenicity	23
4.6.1	870.4200a Carcinogenicity Study - Rat	24
4.6.4	870.4200a/b Carcinogenicity Study - Rat and Mouse	25
4.7	Mutagenicity	25
4.7.1	Gene Mutation	25
4.7.2	Cytogenetics	27
4.7.3	Other Genotoxicity	29
4.8	Neurotoxicity	29
4.8.1	870.6100 Delayed Neurotoxicity Study - Hen	30
4.8.2	870.6200 Acute Neurotoxicity Screening Battery	30
4.8.3	870.6200 Subchronic Neurotoxicity Screening Battery	30
4.8.4	870.6300 Developmental Neurotoxicity Study	30
4.9	Metabolism	30

4.9.1	Adequacy of data base for metabolism:	30
4.9.2	870.7485 Metabolism - Rat	31
4.9.3	870.7600 Dermal Absorption - Rat	31
4.10	Other Studies	32
4.10.1	Urinary Tract Effects (Range Finding) - Rat	32
4.10.2	Urinary Tract Effects in Male Rats	33
5	TOXICITY ENDPOINT SELECTION	33
5.1	Endpoint Selection	34
5.2	Acute Reference Dose (aRfD)	34
5.3	Chronic Reference Dose (cRfD)	34
5.4	Occupational/Residential Exposure	35
5.4.1	Incidental Oral Exposure	35
5.4.1.1	Short-Term (1 - 30 days) Incidental Oral Exposure	35
5.4.1.2	Incidental Oral Exposure: Intermediate-Term (1 - 6 Months)	35
5.4.2	Dermal Exposure	36
5.4.2.1	Dermal Exposure: Short Term (1 - 30 Days)	36
5.4.2.2	Dermal Exposure: Intermediate-Term (1 - 6 Months)	36
5.4.2.3	Dermal Exposure Long-Term (> 6 Months)	37
5.5	Dermal Absorption	38
5.6	Classification and Quantification of Carcinogenic Potential	38
5.6.1	Classification of Carcinogenic Potential	38
5.6.2	Quantification of Carcinogenic Potential	38
6	FQPA CONSIDERATIONS	39
6.1	Special Sensitivity to Infants and Children	39
6.1.1	Determination of Susceptibility	39
6.1.2	Degree of Concern Analysis and Residual Uncertainties	39
6.1.3	Special FQPA Safety Factor(s)	39
6.2	Recommendation for a Developmental Neurotoxicity Study	39
7	REFERENCES	39
8	APPENDICES	48
8.1	Toxicity Profile Summary Tables	49
8.1.1	Acute Toxicity Table	49
8.1.2	Subchronic, Chronic and Other Toxicity Tables	49
8.2	Summary of Toxicological Dose and Endpoints	62

1 HAZARD CHARACTERIZATION

The toxicity database for trifluralin is adequate for the selection of endpoints for use in risk assessment. The Health Effects Division [HED] Hazard Identification Assessment Review Committee [HIARC] evaluated all the available studies in the database and established an acute and a chronic reference dose [RfD], as well as doses and endpoints for incidental oral exposure, residential, and short-term, intermediate-term, and long-term dermal and inhalation exposure scenarios. The HIARC also evaluated available studies to determine if there is a special sensitivity for infants and children.

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 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.

In the subchronic oral rat toxicity study, 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. In a 6-month oral (capsule) study in the dog, increased absolute and relative (to body) liver weights, liver enlargement, discolored kidneys, decreased red cell indices, increased platelets in males; and increased alkaline phosphatase were observed. In dermal toxicity studies with technical trifluralin and a formulation, no systemic toxicity was observed at the limit dose; dermal effects included sub-epidermal inflammation and ulcerations. In a 30-day inhalation study in rats, increased methemoglobin and bilirubin, as well as dyspnea and ruffled fur were observed.

Chronic toxicity to trifluralin was evaluated in the rat, mouse, and dog. Systemic toxicity in rats included decreases in body weight and body weight gains; no systemic toxicity was observed at the highest dose tested in a 2-year oncogenicity study in the mouse. Two 12-month oral (capsule) toxicity studies were performed in the dog. In one study, increased frequency of abnormal stool, decreased body weights and body weight gains, decreased erythrocytes and hemoglobin, and increased thrombocytes in males were observed, while increased absolute liver weights were observed in the other.

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 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 were observed. Offspring toxicity consisted of decreased pup weight and increased number of runts. Decreased fetal, neonatal, and litter viability, and decreased lactation index were also observed.

Extensive testing showed trifluralin is neither mutagenic nor genotoxic. 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 Special Review, Carcinogenicity Peer Review Committee (CPRC) in 1986, as well as an IARC Monograph in 1991. Two NCI oncogenicity studies in the rat and mouse revealed hepatocellular carcinomas in both studies; these tumors were attributed to nitrosamine (N-dinitroso-di-n-propylamine, NDPA); contamination. 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 with a Q1* of 0.0077 (mg/kg/day)⁻¹. Recalculation of the Q1* with 3/4s interspecies scaling factor resulted in a Q1* of 0.00579 (mg/kg/day)⁻¹ (TXR 0051890).

The kidney appears to be a target organ for trifluralin. In a special urinalysis study in the rat tubular cytoplasmic hyaline droplets, increased total protein, aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) were observed in the urine. Following electrophoresis, albumin, α 1-globulin and α 2-globulin were identified in the urine. Histopathological findings included increased incidences of lesions of the renal proximal tubules, decreased corticomedullary mineralization, hyaline droplets in the tubular epithelium in the rat; in the dog, multifocal cortical tubular cytoplasmic pigment deposition was observed.

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 alanine aminotransferase (ALT), AST, gamma glutamyl transferase (GGT) and alkaline phosphatase (AP) activities were unaffected by treatment. In a 30-day inhalation study in the rat, liver weights were increased, but again, were considered to be adaptive since no changes in clinical chemistry or histopathological findings were observed.

In a rat metabolism study, ¹⁴C-trifluralin in the rat, many non-conjugated (20-30) and conjugated (10-20) urinary metabolites were observed, the majority of the metabolites were present at 1-2% of the total urinary radioactivity. One metabolite, found at 8.2-8.9% of the total urinary, was partially characterized as retaining the trifluoromethyl groups, the two equivalent aromatic protons, and the two nitro groups, but the propyl groups were lost. Another metabolite, identified as N-[(3-(acetylamino)-2-amino-5-(trifluoromethyl)phenyl] acetamide, was found at 4.0-5.2%. Based on the metabolic profile, four metabolic pathways were identified (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.

2 REQUIREMENTS

The requirements (CFR 158.340) for food and non food for trifluralin are in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Table 1: Data Requirements

Test	Technical	
	Required	Satisfied
870.1100 Acute Oral Toxicity	Yes	Yes
870.1200 Acute Dermal Toxicity	Yes	Yes
870.1300 Acute Inhalation Toxicity	Yes	Yes
870.2400 Primary Eye Irritation	Yes	Yes
870.2500 Primary Dermal Irritation	Yes	Yes
870.2600 Dermal Sensitization	Yes	Yes
870.3100 Oral Subchronic (rodent)	Yes	Yes
870.3150 Oral Subchronic (nonrodent)	Yes	Yes
870.3200 21-Day Dermal	Yes	Yes
870.3250 90-Day Dermal	Yes	Yes
870.3465 90-Day Inhalation	Yes	Yes
870.3700a Developmental Toxicity (rodent)	Yes	Yes
870.3700b Developmental Toxicity (nonrodent)	Yes	Yes
870.3800 Reproduction	Yes	Yes
870.4100a Chronic Toxicity (rodent)	Yes	Yes
870.4100b Chronic Toxicity (nonrodent)	Yes	Yes
870.4200a Oncogenicity (rat)	Yes	Yes
870.4200b Oncogenicity (mouse)	Yes	Yes
870.4300 Chronic/Oncogenicity	Yes	Yes
870.5xxx Mutagenicity—Gene Mutation - Bacterial	Yes	Yes
870.5xxx Mutagenicity—Gene Mutation - Mammalian	Yes	Yes
870.5xxx Mutagenicity—Structural Chromosomal Aberrations	Yes	Yes
870.5xxx Mutagenicity—Other Genotoxic Effects	Yes	Yes
870.6100a Acute Delayed Neurotoxicity (hen)	No	Yes
870.6100b 90-Day Neurotoxicity (hen)	No	-
870.6200a Acute Neurotoxicity Screening Battery (rat)	No	-
870.6200b 90 Day Neurotoxicity Screening Battery (rat)	No	-
870.6300 Developmental Neurotoxicity	No	-
870.7485 General Metabolism	Yes	Yes
870.7600 Dermal Penetration	Yes	No ^a
Special Studies for Ocular Effects Acute Oral (rat)	No	-
Subchronic Oral (rat)	No	-
Six-month Oral (dog)	No	--

^a The requirement for a dermal penetration study has been satisfied with a dermal absorption study with ¹⁴C-ethalfluralin (a close structural analog of trifluralin) in monkeys (MRID 132820, 92062028; TXR 004090, 004235).

3 DATA GAP(S)

None

4 HAZARD ASSESSMENT

4.1 Acute Toxicity

Adequacy of data base for acute toxicity: The data base for acute toxicity is considered complete; no additional studies are required at this time. The acute toxicity of trifluralin is summarized in Table 2a; acute toxicity of formulation is summarized in Tables 2b and 2c. Technical trifluralin shows low acute toxicity via the oral, dermal and inhalation routes of exposure, with toxicity categories III, IV, and IV, respectively. Technical trifluralin was found to be an eye irritant (Toxicity Category II) and showed some skin irritation (Toxicity Category III); 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

Table 2a: 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

Table 2 (b) Acute Toxicity of Trific 10G (10% Trifluralin)

Guideline No.	Study Type	MRID No.	Results	Toxicity Category
870.1100	Acute Oral (Rat)	43447301 (1994) TXR 5000582 Acceptable/Guideline	LD50 > 5050 mg/kg	IV
870.1200	Acute Dermal (Rat)	43447302 (1994) TXR5000582 Acceptable/Guideline	LD50 > 2020 mg/kg	III
870.1300	Acute Inhalation (Rat)	43447303 (1994) TXR 5000582 Acceptable/guideline	LC50 > 1.21 mg/L (analytical) > 5.60 mg/L (nom)	IV
870.2400	Primary Eye Irritation (Rabbit)	43447304 (1994) TXR 5000582 Acceptable/guideline	Conjunctival redness at 24hr, cleared by 4 d	III
870.2500	Primary Skin Irritation (Rabbit)	43447305 (1994) TXR 5000582 Acceptable/guideline	Not an irritant	IV
870.2600	Dermal Sensitization	43447306 (1994) TXR 5000582 Acceptable/guideline	Sensitizing agent	N/A

Table 2(c) Acute Toxicity of Trilin 5 (50.8% Trifluralin)

Guideline No.	Study Type	MRID No.	Results	Toxicity Category
870.1100	Acute Oral (Rat)	42972701 (1993) TXR5000675 Acceptable/Guideline	LD50 > 5000 mg/kg	IV
870.1200	Acute Dermal (Rat)	42972702 (1993) TXR 5000675 Acceptable/Guideline	LD50 > 5000 mg/kg (both sexes)	IV
870.1300	Acute Inhalation (Rat)	42972703 (1983) TXR 5000675 Acceptable/guideline	LC50 = 4.3 4.15-4.46) mg/L(male) 5.0 mg/L (female)	IV
870.2400	Primary Eye Irritation (Rabbit)	42972704 (1993) TXR 5000675 Acceptable/Guideline	Ocular opacity in 1/6 on day 21	II
870.2500	Primary Skin Irritation (Rabbit)	42972705 (1993) TXR 5000675 Acceptable/Guideline	Erythema at 72 hr Moderate to severe 1/6 Well-defined 3/6 Very slight 2/6	III

870.2600	Dermal Sensitization	42972706 (1993) TXR 5000675 Acceptable/Guideline	Sensitizing agent	N/A
----------	----------------------	--	-------------------	-----

4.2 Subchronic Toxicity

Adequacy of data base for subchronic toxicity: The data base for subchronic toxicity is considered complete. No additional studies are required at this time. In the subchronic oral rat toxicity study, minor decreases in overall body weight gains and food consumption in males and females, decreased hemoglobin and alkaline phosphatase in the males, and increased absolute and relative (to body) liver weights in males and females were observed. In a 6-month oral (capsule) study in the dog, increased absolute and relative (to body) liver weights, liver enlargement, discolored kidneys, decreased red cell indices, increased platelets in males; and increased alkaline phosphatase. In dermal toxicity studies with technical trifluralin and a formulation, no systemic toxicity was observed at the limit dose; dermal effects included sub-epidermal inflammation and ulcerations. In a 30-day inhalation study in rats, increased methemoglobin and bilirubin, as well as dyspnea and ruffled fur were observed.

4.2.1 Subchronic Oral Toxicity

4.2.1.1 870.3100 90-Day Oral Toxicity - Rat

Executive Summary: In a 3-month oral toxicity study (MRID 00151906), HOE 38474 (trifluralin; purity not reported, Lot/Batch # AT204) was administered to 20 HOE: Wistar (SPF 71) rats/sex/group in the diet at dose levels of 0, 800, 2000, or 5000 ppm (0/0, 59/69, 154/168, and 392/421 mg/kg/day in males/females) for three months. Ten rats/sex/dose were sacrificed at the end of the 3-month treatment period, and the remaining 10 rats/sex/dose were sacrificed after a 15-day recovery period in which they were given control diets. In the main study, there were no effects of treatment on mortality, clinical observations, ophthalmology, gross pathology, or histopathology. There were no adverse effects on urinalysis. In the recovery group, urine color returned to normal; otherwise, findings (when reported) were similar to the main study.

Absolute and relative (to body) liver weights were dose-dependently increased in females dosed at 800 ppm and higher (11-48%) and in males dosed at 2000 ppm and higher (28-48%). On day 93, blood serum phosphate was dose-dependently increased in all female dose groups (21-29%) and in the 2000 and 5000 ppm males (35-41%).

Additionally at 5000 ppm, overall body weight gains were decreased 9% each in males and females compared to controls. Food consumption was decreased 16-18% on day 8 in males and females and remained decreased 10% on day 92 in females. Similarly, relative (to body) food consumption was decreased on day 8 in males and females (10.38-10.48% treated vs 11.64-12.08% controls) and remained decreased on day 92 in females (6.79% treated vs 7.10% controls). Additionally in the males in this group, hemoglobin was decreased 6 to 9% at weeks 7 and 13; alanine aminotransferase, on days 1, 44, and 93 (20-26%); and alkaline phosphatase, 26 to

30% on days 44 and 93.

The LOAEL for this study is 5000 ppm (392/421 mg/kg/day [M/F]) based on minor decreases in overall body weight gains and food consumption in males and females, decreased hemoglobin and alkaline phosphatase in the males, and increased absolute and relative (to body) liver weights in males and females. The NOAEL is 2000 ppm (154/168 mg/kg/day [M/F]).

The submitted study is classified as **acceptable/guideline** and satisfies the guideline requirements for a subchronic oral toxicity study in the rat (OPPTS 870.3100a; OECD 408).

4.2.1.2 870.3100 90-Day Oral Toxicity - Mouse

Executive Summary: In a 13-week oral toxicity study (MRID 00151905), trifluralin (>99% a.i., Batch # AZ01751) was administered to 10 NMRI KFM-Han mice/sex/dose in diet at dose levels of 0, 400, 1000, or 2500 ppm (approximately equivalent to 0, 60, 150, and 375 mg/kg/day as calculated by the reviewers).

Mortality, clinical signs, neurological status, body weights, food consumption and efficiency, ophthalmology, hearing, organ weights, and gross and histological pathology for both sexes at all doses were unaffected by treatment. There were no unequivocal adverse effects observed in either sex at any dose.

The following changes were equivocally treatment-related and may have been toxicologically adverse (historical control values were not reported). Platelets were increased by 23% each in the 2500 ppm males and females. Creatinine was increased by 33-64% in the 1000 ppm females and the 2500 ppm males and females. Urea was increased by 42% in the 2500 ppm males, but not in females. Total bilirubin and cholesterol were increased by 90 and 52%, respectively, in the 2500 ppm females, but not in the males. Although statistically significant increases were observed for methemoglobin concentrations at 1000 (0.8% females) and 2500 ppm (1.9% males, 1.2% females), this findings was not considered to be an adverse effect; control value was 0.5% for both sexes. Since the changes in clinical pathology do not fit a pattern consistent with a treatment-related effect and the negative histopathological results, these effects were not considered to be treatment related.

The LOAEL was not observed. The NOAEL is 2500 ppm (375 mg/kg/day), the highest dose tested.

This study is **unacceptable/not upgradable guideline** and does not satisfy the requirements of a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in mice. The animals could have tolerated a higher dose.

4.2.1.3 870.3150 90-Day Oral Toxicity - Dog

Executive Summary: In a 6-month oral toxicity study (MRID 00151907), HOE 38474

(trifluralin; 96.1% a.i., Lot/Batch # 8900) was administered to 6 Hoe: BEAK strain (Hoechst breed) beagle dogs/sex/group in the diet at dose levels of 0, 400, 1000, or 2500 ppm (equivalent to 0, 10, 25, and 63 mg/kg/day; based on 1 ppm = 0.025 mg/kg) for six months. Four dogs/sex/dose were sacrificed at the end of the 6-month treatment period, and the remaining 2 dogs/sex/dose were sacrificed after a 1-month recovery period in which they were given control diets.

There were no treatment-related effects on urinalyses or histopathology.

At 2500 ppm, one female and one male were sacrificed *in extremis* on days 39 and 85, respectively, due to marked reduction in food consumption, cachexia (general ill health due to rejection of food), and/or mild neurologic impairment. Overall body weight gains were decreased in males and females by 0.5 kg compared to increases of 1.0 kg in female and 1.6 kg in male controls. Food consumption was decreased 4-35% for individual males and females compared to controls.

Corneal vascularization was dose-dependently increased at the end of the main study, occurring in 3/12, 5/12, and 7/12 dogs in the 400, 1000, and 2500 ppm groups, respectively, vs 0/12 controls. Corneal necrosis and ulceration were observed in control (7/12 dogs), 400 ppm (7/12 dogs), 1000 ppm (8/12 dogs) and 2500 ppm (9/10 dogs) groups. These lesions were still present at the end of the recovery period. In the early part of the study, these lesions were thought to be due to introduction of sand from the dog run area into the animals eyes. However, the dose-relationship indicates an effect of treatment, either direct or indirect (e.g. behavioral alterations increased the likelihood of getting sand in their eyes). In another chronic dog study (MRID 00151908), no treatment-related effects on ophthalmology were observed at doses up to 750 ppm.

Dose-dependent decreases (7-29%) were observed in hemoglobin, erythrocytes, and hematocrit in the 400 ppm males and 1000 and 2500 ppm animals at week 8 and in the 2500 ppm animals (12-26%) at 6 months. At 6 months, leukocytes were increased 31-41% in the 1000 and 2500 ppm females. Platelets were increased at 400 ppm and higher in males (42-51%) and 2500 ppm females at week 8 (62%) and in the 2500 ppm animals at 6 months (82-98%). Alkaline phosphatase was increased in females at 400 ppm at 3 months (99%), at 1000 ppm at 2, 5, and 6 months (84-146%), and at 2500 ppm throughout the study (121-241%).

Absolute and relative to body liver weights were dose-dependently increased 22-81% in males and females dosed at 400 ppm and higher. At the schedule 6 month sacrifice, the following macroscopic findings were noted (vs 0 controls): (i) liver enlargement in all treated animals; (ii) discolored kidneys in all treated animals (except for one 2500 ppm male); (iii) small testes and prostate at 1000 (2/4) and 2500 (2/3) ppm; (iv) black gravelly contents in the gall bladder in the 2500 ppm males (2/3) and females (1/3); and (v) discolored (yellowish) fatty tissues in the 1000 (1/4) and 2500 (3/3) ppm females; this discoloration is characteristic of dinitroaniline treatment and not considered to be treatment related.

The LOAEL for this study is 400 ppm (10 mg/kg/day) based on increased absolute and relative (to body) liver weights, liver enlargement, discolored kidneys, and corneal necrosis and ulceration in males and females; decreased red cell indices and increased platelets in

males; and increased alkaline phosphatase in females. The NOAEL was not observed.

The submitted study is classified as **unacceptable/not upgradable** and does not satisfy the guideline requirements for a subchronic oral toxicity study in the dog (OPPTS 870.3150; OECD 409) because a NOAEL was not observed, and dietary analyses (homogeneity, stability, and concentration) were not provided in the report.

4.2.2 Subchronic Dermal Toxicity

4.2.2.1 31-day Dermal Toxicity Study - Rat

Executive Summary: In a 31-day dermal toxicity study (MRID 00153171), HOE 38474 (trifluralin; >99% a.i.; Lot/Batch #: OH AT 210) in 2% (w/v) carboxymethylcellulose was applied to the shaved intact skin of 10 Wistar rats/sex/dose at dose levels of 0, 40, 200, or 1000 mg/kg/day (limit dose), 6 hours/day, 5 days/week for a total of 23 applications. Additional recovery groups consisting of 5 rats/sex/dose were similarly treated and then observed for 13 days after the final dermal application. Dermal irritation was evaluated daily using the Draize method.

No compound-related effects on mortality, clinical signs, body weight, body weight gain, food consumption, food conversion, ophthalmoscopy, hematology, clinical chemistry, or gross pathology parameters were observed.

At ≥ 200 mg/kg, the following histopathological effects (# affected/10) were noted in the treated skin of the main study animals: (i) minimal to slight sub-epidermal inflammation (7-8 males and 4 females each *vs* 0 controls); (ii) minimal to slight ulcerations (1 treated male *vs* 0 controls and 1 treated female each *vs* 1 control); (iii) minimal to moderate acanthosis (8-9 treated males *vs* 6 minimal controls and 7-9 treated females *vs* 5 minimal to slight controls); and (iv) minimal to slight sebaceous gland hyperplasia (5-10 treated males *vs* 7 slight controls and 4-6 treated females *vs* 3 slight controls). In the recovery groups, minimal acanthosis was still observed in 2 treated females each (*vs* 0 controls).

The only findings at 40 mg/kg were minimal to slight sub-epidermal inflammation (4 males and 1 female *vs* 0 controls); minimal to slight acanthosis (3 treated males *vs* 6 minimal controls and 2 treated females *vs* 5 minimal to slight controls); and slight sebaceous gland hyperplasia (2 treated males *vs* 7 minimal controls). Additionally, minimal acanthosis was noted in one 40 mg/kg recovery group female (*vs* 0 controls).

The systemic LOAEL was not observed. The systemic NOAEL is 1000 mg/kg/day.

The dermal LOAEL is 200 mg/kg/day based on sub-epidermal inflammation and ulcerations in males and females. The dermal NOAEL is 40 mg/kg/day.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3200; OECD 410) for a dermal toxicity study in rats.

4.2.2.2 870.3200 21-Day Dermal Toxicity – Rabbit

Executive Summary: In a 21-day dermal toxicity study (MRID 00152888), trifluralin (96.45% a.i.; Lot/Batch #: 00554AP2) was applied undiluted to the shaved intact skin of 5 New Zealand White rabbits/sex/dose at dose levels of 0 or 1000 mg/kg/day (limit dose), 6 hours/day for 21 consecutive days. Dermal irritation was evaluated daily using the Draize method.

No compound-related effects on mortality, clinical signs, body weight, body weight gain, food consumption, ophthalmoscopic examinations, hematology, clinical chemistry, organ weights, or gross or histopathological parameters were observed in either sex. At 1000 mg/kg, all animals showed signs of dermal irritation, characterized by moderate to severe erythema and slight to moderate edema accompanied by coriaceous, cracked, and bleeding skin beginning at 6-12 days after initiation of treatment and persisting until the end of the study with desquamation being observed within 11-20 days after initiation of treatment.

At 1000 mg/kg, increased incidences of the following gross and histopathological lesions of the treated skin were observed: (i) thickened treatment area; (ii) minimal to slight acanthosis; (iii) slight hyperkeratosis; and (iv) minimal to slight inflammation of the dermis. Additionally, minimal to slight hyperplasia of the bone marrow was noted (2 males and 4 females vs 0 controls). These findings were considered secondary to the dermal irritation observed, rather than primary compound related effects.

The systemic LOAEL was not observed. The systemic NOAEL is 1000 mg/kg/day.

The dermal LOAEL was 1000 mg/kg/day (limit dose) based on erythema, edema, and desquamation of the treated skin. The dermal NOAEL was not established.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3200; OECD 410) for a 21-day dermal toxicity study in rabbits.

4.2.2.3 870.3200 21-Day Dermal Toxicity - Rabbit

Executive Summary: In a 21-day dermal toxicity study (MRID 41993810), XRM-5313 (a formulation containing 35.8% trifluralin and 2.6% XRD-498; Lot/Batch #: AGR 291670) was applied undiluted to the shaved intact skin of 5 New Zealand White rabbits/sex/dose at dose levels of 0, 100, 500, or 1000 mg/kg/day, 6 hours/day for 5 days/week over a 21 day period for a total of 15 applications.

No compound-related effects on mortality, clinical signs, body weight, body weight gain, hematology, clinical chemistry, organ weight, or gross or histopathology parameters were observed at any dose level in either sex. There were signs of dermal irritation (slight to moderate erythema, edema, and/or scaling and fissuring) at all doses. The dermal signs in the 100 mg/kg group reversed by study termination.

The systemic LOAEL was not observed. The systemic NOAEL is 1000 mg/kg/day (limit

dose).

The dermal LOAEL was 100 mg/kg/day based on erythema, edema, and/or scaling and fissuring. The dermal NOAEL was not established.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3200; OECD 410) for a 21-day dermal toxicity study in rabbits.

4.2.3 870.3465 30-Day Inhalation – Rat

Executive Summary: In a 30-day inhalation toxicity study (MRID 40392312, 00151904), 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/m³ (equivalent to 0, 27, 81 and 270 mg/kg/day). 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/m³, 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 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. At the end of the main study, absolute and relative (to body) liver weights were dose-dependently increased 9-32% in the 300 and 1000 mg/m³ animals. Relative (to brain) liver weights were dose-dependently increased 10-14% in the 100 and 300 mg/m³ females and 21-28% in the 1000 mg/m³ 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/m³ males (4-10/10 treated vs 0/10 each control group) and in the 1000 mg/m³ 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%;) in males at ≥100 mg/m³.

The LOAEL for this study is 1000 mg/m³ (270 mg/kg/day) based on increased methemoglobin and bilirubin in females and incidences of dyspnea and ruffled fur in males and females. The NOAEL is 300 mg/m³ (81 mg/kg/day).

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)

4.3 Prenatal Developmental Toxicity

Adequacy of Data Base for Prenatal Developmental Toxicity: The data base for developmental toxicity is considered complete. No additional studies are required at this time. In the rat, resorptions, decreased fetal body weight, reduced ossification of vertebrae and ribs, and thickened, wavy, or bent ribs were observed at the same dose levels where maternal toxicity was observed [mortality, clinical signs, decreased body-weight gains and food consumption, increased incidence of resorptions, increased liver and spleen weights]. In the rabbit, abortions were observed at the same dose levels where maternal toxicity was observed [abortions, macroscopic changes in the liver and lungs, and decreased food consumption]. More severe developmental toxicity [increased resorptions, decreased fetal body weights, decreased number of live fetuses, increased number of runts, incomplete ossification of vertebrae, and skeletal abnormalities] was observed in the rabbit at the next higher dose, at which more severe maternal toxicity also occurred [mortalities, decreased body weight and body-weight gain].

4.3.1 870.3700a Prenatal Developmental Toxicity Study - Rat

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 (no explanation given).

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% during GD 14-17 and increased 9-22% post-treatment in both groups. Increases 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 pelvis 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. Again, 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 mortality, clinical signs, decreased body weight gains, decreased food consumption, increased liver and spleen weights. The maternal NOAEL is 100 mg/kg/day.

At 500 mg/kg/day, statistically significant 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 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 incidences of 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.

4.3.2 870.3700a Prenatal Developmental Toxicity Study - Rat

Executive Summary: In a prenatal developmental toxicity study (MRID 00152419), trifluralin (96.7% a.i., Lot # 00554AP2) was administered (via gavage in 10% aqueous acacia) to pregnant Charles River Crl:COBS, CD (SD)BR rats (25/dose) at concentrations of 0, 100, 225, 470, or 1000 mg/kg/day on gestation days (GD) 7 through 17. All dams were sacrificed on GD 21 and their uterine contents examined.

All animals survived to terminal sacrifice without the appearance of any treatment-related clinical signs. Yellow or orange-yellow discoloration of the urine and fatty tissue was observed at all dose levels; this discoloration was not considered to be treatment related and is characteristic of dinitroaniline treatment.

Maternal body weights were significantly decreased 5 to 7% on GD 12 to 21 at 475 (5 to 7%) and 1000 (5 to 8%) mg/kg/day. Significant decreases in food consumption was observed at all dose levels of GD 7 - 12 and persisted from GD 12 - 17 in the two highest dose levels.

The maternal LOAEL was established at 1000 mg/kg/day based decreased body weights and decreased food consumption. The maternal NOAEL was established at 475 mg/kg/day.

At 1000 mg/kg/day, fetal body weights were significantly decreased by 7% in males and 6% in females. External, visceral and skeletal examinations of the fetuses did not reveal any treatment-

related effects.

The offspring LOAEL was established at 1000 mg/kg/day based on decreased fetal body weights. The offspring NOAEL was established at 475 mg/kg/day.

The developmental LOAEL was not established. The developmental NOAEL was established at 1000 mg/kg/day.

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

4.3.3 870.3700b 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. Orange-colored urine was observed at 225 and 500 mg/kg/day, and orange-colored pelage was noted at 500 mg/kg, 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 during the treatment and post-treatment intervals, resulting in decreased absolute and corrected (for gravid uterine weight) body weight gains for the overall study. Food consumption was dose-dependently decreased at 225 (28-40%) and 500 (43-67%) mg/kg/day during the treatment interval and remained decreased (53%;) 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. Additionally at 500 mg/kg, 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, 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, fetal weights were decreased 13-18%, and the percent of fetal runts was significantly increased 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, 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.

4.3.4 870.3700b Prenatal Developmental Toxicity Study - Rabbit

Executive Summary: In a prenatal developmental toxicity study (MRID 00151900), HOE 38474 (trifluralin; 98.4% a.i., Lot/Batch #AT210) in sesame oil was administered to pregnant hybrid chinchilla rabbits (16/dose) via gavage in a dosing volume of 2 mL/kg at concentrations of 0, 4, 16, or 60 mg/kg/day on gestation days (GD) 6 through 18. All does were sacrificed on GD 28 and their uterine contents examined.

All maternal animals survived until termination. There were no effects of treatment on clinical observations, body weight gains (absolute and corrected for gravid uterine weight), food consumption, gross pathology, the number of resorptions, post-implantation losses, the numbers of fetuses (live and dead), fetal weights, and sex ratios.

The maternal LOAEL was not observed. The maternal NOAEL is 60 mg/kg/day (the highest dose tested).

There were no treatment-related external, visceral, or skeletal findings in the fetuses.

The developmental LOAEL was not observed. The developmental NOAEL is 60 mg/kg/day (the highest dose tested).

This developmental toxicity study is classified **unacceptable/not upgradable** and does not satisfy the guideline requirements (OPPTS 870.3700; §83-3[b]) for a developmental toxicity study

in the rabbit because a LOAEL was not established for maternal or developmental effects, and animals were not dosed to the limit dose (1000 mg/kg/day).

4.4 Reproduction and Fertility Effects

Adequacy of data base for Prenatal Developmental Toxicity: The data base for reproductive toxicity is considered complete. No additional studies are required at this time. Reproductive toxicity was observed in the rat, as evidenced by a decrease in lactation index in the F1a pups and decreased number of implantation sites, newborn pups, litter size, and pup weights in both generations.

4.4.1 870.3800 Reproduction and Fertility Effects - Rat

Executive Summary: In a multi-generation reproduction toxicity study (MRIDs 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; 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 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 8-14% at 650 and 2000 ppm in the both generations. Relative testes weights were increased 8-13% 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 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 in the P generation during week 1 of pre-mating (9%). Food consumption was decreased 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 in the F1b females during gestation (7-10% during weeks 1 and 3) and throughout lactation (10-13%). Relative thymus weights were decreased 15-16% in the F1 males and females.

The only finding at 200 ppm was an increase of 6% in relative kidney weight in the P generation males.

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

There was no adverse effect of treatment on pup mortality. Pup weights were decreased 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 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 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 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 (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 (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 (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).

4.4.2 870.3800 Reproduction and Fertility Effects - Rat

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 (0, 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; and 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 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 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 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 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% 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 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 (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 (35/42 mg/kg/day, M/F).

At 4000 ppm, decreases 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 was decreased in the F1a (9%) and F2 (18%) litters, resulting in a decreased number of newborn pups (17-22%) and significantly reduced litter size on post-natal days (PND) 1 and 4 (17-25%). Litter size remained decreased (3-14%) throughout the remainder of lactation in both generations. Pre-implantation loss was increased (15.5% treated vs 9.8% controls;) in the F1b litters (mating of treated males with untreated females), resulting in a decreased (11%;) number of live fetuses.

At 4000 ppm pup weights were decreased 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 ovarian 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. 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).

4.4.3 870.3800 Reproduction and Fertility Effects - Rat

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 CrI:CD(SD) rats (25/sex/dose) at nominal dose levels of 0, 200, 630, or 2000 ppm (equivalent to approximately 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 in the F1 parental males at the end of pre-mating (6% each). Body weights were decreased in the P females during gestation from the F1b mating (7-9%).

At 2000 ppm, body weights and food consumption were decreased 6-13% 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% in the F1 females at the beginning of pre-mating.

Body weight gains for the overall pre-mating period were decreased 9-23% in both generations. Food efficiency was decreased 14% 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% 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 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 (47 mg/kg/day) based on decreased body weights and food consumption. The NOAEL is 200 ppm (15 mg/kg/day).

The LOAEL for reproductive toxicity was not observed. The NOAEL is 2000 ppm (148 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 (47 mg/kg/day) based on small pup size in 3 litters. The NOAEL is 200 ppm (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).

4.5 Chronic Toxicity

Adequacy of data base for chronic toxicity: The data base for chronic toxicity is considered complete. No additional studies are required at this time. Chronic toxicity to trifluralin was evaluated in the rat, mouse, and dog. Systemic toxicity in rats included decreases in body weight and body weight gains; no systemic toxicity was observed at the highest dose tested in a 2-year oncogenicity study in the mouse. Two 12-month oral (capsule) toxicity studies were performed in the dog. In one study, increased frequency of abnormal stool, decreased body weights and body weight gains, decreased erythrocytes and hemoglobin, and increased thrombocytes in males were observed, while increased absolute liver weights were observed in the other.

4.5.1 870.4300 Chronic Toxicity/Carcinogenicity – Rat

Executive Summary: In a chronic toxicity/carcinogenicity 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 (achieved intake: 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 bromosulphophthalein 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 (MRID 00162458), 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%. During the studies, decreased 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%; 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 (169/219 mg/kg/day in males/females) based on decreases in body weight and body weight gains. The NOAEL is 800 ppm (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 870.4100a, OECD 453) in rats.

4.5.2 870.4100b Chronic Toxicity - Dog

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%) 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%;) and females (41%). Increases were also noted in relative (to body) liver weights (45-62%;) and relative (to brain) liver weights (28-33%) 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 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 ALT in males on day 363 (44%); (v) decreased 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 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 are 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%) 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%,) in females on day 363.

The LOAEL for this study is 40 mg/kg/day based on increased frequency of abnormal stool and pigment deposition in the kidney and liver in males and females, 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).

4.5.3 870.4100b Chronic Toxicity - Dog

Executive Summary: In a 12-month oral toxicity study (MRID 00151908, 00159618), HOE

38474 (trifluralin; >99% a.i., Lot/Batch # AT210) was administered to 6 purebred beagle dogs/sex/group in the diet at dose levels of 0, 30, 150, or 750 ppm (0.0, 0.8, 3.8, 18.8 mg/kg/day; based on 1 ppm = 0.025 mg/kg/day) for 12 months. There were no effects of treatment on mortality, food consumption, ophthalmology, auditory tests, urinalyses, or histopathology.

At 150 ppm, absolute liver weights were increased by 9% in the males. Methemoglobin levels were increased in males at months 6 and 9 (1.2-1.5% treated vs 0.8-1.0% controls) and in females at month 12 (1.8% treated vs 0.7-1.1% controls).

At 750 ppm, one male sacrificed in a moribund condition on day 142 exhibited myocardial necrosis with fatty changes, pericarditis/epicarditis, arteritis in the urinary bladder and epididymis, and myeloid hyperplasia and erythroid hypoplasia in the bone marrow. However, it was stated that this death was considered unrelated to treatment. Occurrences of diarrhea were more frequent than controls in the males during weeks 1-41 and in the females throughout the study. Body weight gains were lower than controls in males from week 18 onward and in females during weeks 17-44. Overall body weight gains were decreased by 24-28% in males and females after one month. Methemoglobin was increased in males and females throughout the study (1.1-2.4% treated vs 0.7-1.1% controls); these effects were considered minor and not toxicologically significant. Erythrocytes and hemoglobin were intermittently decreased in males and females. Increases in total lipids (31-55%), total cholesterol (22-57%), and triglycerides (12-58%) were observed in males and females. With the exception of methemoglobin, all differences in hematology and clinical chemistry fell within the 95% confidence interval of the historical controls. Absolute, relative to body, and relative to brain liver weights were increased by 28-52% in males and females. Absolute and relative to body spleen weights were increased by 31-41% in females, and relative to body spleen weights were increased by 61% in males after 1 month. Hepatomegaly, splenomegaly, and yellow discoloration of the body fat were observed in one male. This discoloration was not considered to be treatment related and is characteristic of dinitroaniline treatment.

The LOAEL for this study is 150 ppm (3.8 mg/kg/day) based on increased absolute liver weights in males. The NOAEL is 30 ppm (0.8 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).

4.6 Carcinogenicity

Adequacy of data base for Carcinogenicity: The data base for carcinogenicity is considered complete. No additional studies are required at this time. The oncogenic potential of trifluralin was addressed by the Agency (PD1/2/3 and PD 4), CPRC (TXR 0005578, 0007362) in 1986, as well as an IARC Monograph in 1991. Oncogenicity studies with purified trifluralin revealed malignant neoplasms of the renal pelvis and benign urinary bladder neoplasms in the rat. After review of the cancer data by the CPRC, new rat and mouse oncogenicity studies were submitted to the Agency. However, the CPRC concluded that the results of these studies were insufficient to warrant reevaluation. The CPRC concluded that trifluralin is a "Group C" (limited evidence of

carcinogenicity) carcinogen, based on the results of a chronic feeding/oncogenicity study in the rat (MRID 00044337, TXR 0005535, 0005578) which showed renal pelvis carcinomas, follicular cell adenomas, papillary adenomas, and cystadenomas.

Trifluralin has a Q1* of 0.00579 (mg/kg/day)⁻¹ based on male rat follicular cell adenomas, papillary adenoma, cystadenoma, and carcinoma combined tumor rates. The Q1* was calculated using an interspecies scaling factor of 3/4.

4.6.1 870.4200a Carcinogenicity Study - Rat

Executive Summary: See section 4.5.1

4.6.2 870.4200a Carcinogenicity Study - Rat

Executive Summary: In a 2-year rat study (MRID 00044337) doses were 0, 813, 3250, or 6500 ppm (equivalent to 41, 163 and 325 mg/kg/day). The NOEL level for non-oncogenic effects was 813 ppm (41 mg/kg/day), marked body weight depression, and elevated BUN occurring at the higher doses. With respect to neoplastic changes, there was a significant increase in malignant neoplasms in the kidneys of all treated male rats, which was dose related. There was also a significant and dose related increase in benign bladder neoplasms in female rats. There also is a trend toward oncogenicity for thyroid follicular epithelial tumors in males when the Cochran-Armitage Test is applied, but not with the Fisher's Exact Test with a Bonferroni correction.

4.6.3 870.4200b Carcinogenicity Study - Mouse

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 (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 (375 mg/kg/day), the highest dose tested.

4.6.4 870.4200a/b Carcinogenicity Study - Rat and Mouse

A NCI cancer study (MRID 00124928, TXR 0051428) in both rat and mouse is **unacceptable and not suitable for regulatory purposes**. Although this carcinogenicity study (MRID 00124928) was reviewed in 1978 and found to be acceptable/guideline, the trifluralin used in the study was subsequently found to be contaminated with **dipropylnitrosamine** at concentrations of 84 to 88 ppm. The liver tumors observed in both the rat and mouse were a result of this impurity. More recent studies in both the rat and mouse were found to be negative for hepatic carcinogenicity.

This study is **unacceptable/guideline**, not upgradable, and does not satisfy the guideline requirement for a carcinogenicity studies in either rats [OPPTS 870.4200a; OECD 451] or mice [OPPTS 870.4200b; OECD 451].

4.7 Mutagenicity

Adequacy of data base for Mutagenicity: The data base for mutagenicity is considered adequate based on pre-1991 guidelines. No mutagenic potential was seen in adequately conducted pre-1991 guideline mutagenicity studies with trifluralin. The mutagenicity tests requested in PD 4 were submitted to the Agency and found to be acceptable. There is no mutagenic concern.

4.7.1 Gene Mutation

<p>870.5100 Bacterial reverse gene mutation assay</p> <p>MRID 00148345 (1984) TXR 0051428</p> <p>Acceptable/Guideline</p>	<p>Trifluralin was tested up to the limit of solubility (400 $\mu\text{g}/\text{plate}$ -S9; 800 $\mu\text{g}/\text{plate}$ +S9). No cytotoxicity was observed in any strain at up to 800 (+S9) or 400 (-S9) $\mu\text{g}/\text{plate}$. No treatment-related increases in revertant colonies were observed at any dose in any strain (\pmS9). The positive controls induced the appropriate responses. There was no evidence of induced mutant colonies over background</p>
<p>870.5100 Bacterial reverse gene mutation assay</p> <p>MRID 40334707 (1987) TXR 0051428</p> <p>Acceptable/Guideline</p>	<p>In a reverse gene mutation assay in bacteria (MRID 40334707), Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to trifluralin (96.8% a.i., Lot/Batch #: 335/336) in dimethylsulfoxide (DMSO) at concentrations of 30, 100, 300, 1000, 3000, or 10,000 $\mu\text{g}/\text{plate}$ in the presence and absence of mammalian metabolic activation (S9). The standard plate incorporation method was used. Standard strain-specific mutagens served as positive controls.</p> <p>Trifluralin was tested up to the limit of solubility (3000 $\mu\text{g}/\text{plate}$, +/-S9). No cytotoxicity was observed in any strain at up to 3000 $\mu\text{g}/\text{plate}$ (+/-S9). No treatment-related increases in revertant colonies were observed at any dose in any strain (+/-S9). The positive controls induced the appropriate responses. There was no evidence of induced mutant colonies over background.</p>
<p>870.5100 Bacterial reverse gene mutation assay</p> <p>MRID 00153173 (1979) TXR 005898</p> <p>Acceptable/Guideline</p>	<p>In a reverse gene mutation assay in bacteria (MRID 00153173), Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to HOE 38474 (trifluralin; purity not reported; Lot/Batch #: OH AT204) in DMSO at concentrations of 0, 4, 20, 100, 500, 2500, or 10,000 $\mu\text{g}/\text{plate}$ (+/-S9). Standard strain-specific positive controls were used.</p> <p>HOE 38474 was tested up to the limit dose (10,000 $\mu\text{g}/\text{plate}$, +/-S9). No treatment-related increases in revertant colonies were observed at any dose in any strain (\pmS9). The positive controls induced the appropriate responses. There was no evidence of induced mutant colonies over background.</p>

<p>870.5250 Gene Mutation Assay - Yeast</p> <p>MRID 00151898 ((1982)</p> <p>Acceptable/Guideline</p>	<p>The test material was tested up to the limit of solubility (1000 mg/L); however, no solubility data were provided. No treatment-related increases in mutation frequency were observed at any dose with or without S9-activation. The positive controls induced the appropriate response. There was no concentration-related positive response of induced mutant colonies over background.</p>
<p>870.5300 <i>In vitro</i> mammalian cell gene mutation assay</p> <p>MRID 0040392306 126661</p> <p>Acceptable/Guideline</p>	<p>In a mammalian cell gene mutation assay at the thymidine kinase (TK) locus, mouse lymphoma L5178Y cells cultured <i>in vitro</i> were exposed to trifluralin (95.0% a.i.; Lot/Batch #: 00554AP2) in DMSO for 4 hours at 8 concentrations ranging from 0.5 to 20 $\mu\text{g}/\text{mL}$ (individual doses not reported) both in the presence and absence of S9-activation.</p> <p>Trifluralin was tested up to cytotoxic concentrations (20 $\mu\text{g}/\text{mL}$, +/-S9). No treatment-related increases in mutation frequency were observed at any dose compared to controls. The positive controls induced the appropriate response. There was no concentration-related positive response of induced mutant colonies over background.</p>
<p>870.5300 Forward Gene Mutation Assay</p> <p>MRID 00148318 (1984) TXR 0051428</p> <p>Acceptable/Guideline</p>	<p>In a mammalian cell gene mutation assay at the HGPRT locus (MRID 00148318), Chinese hamster ovary (CHO) cells cultured <i>in vitro</i> were exposed to triflurex technical (trifluralin; purity not reported; Lot/Batch #: not reported) in ethanol for 4 hours at concentrations of 10, 50, 100, 200, 300, 400, or 500 $\mu\text{g}/\text{mL}$ (-S9) and 50, 100, 200, 300, 400, 500, or 600 $\mu\text{g}/\text{mL}$ (+S9).</p> <p>Triflurex technical was tested up to cytotoxic concentrations (≥ 200 $\mu\text{g}/\text{mL}$, -S9 and ≥ 300 $\mu\text{g}/\text{mL}$, +S9) and the limit of solubility (≥ 100 $\mu\text{g}/\text{mL}$, +/-S9). No treatment-related increases in mutant frequency were observed in either trial in the presence or absence of S9. The positive controls induced the appropriate response. There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.</p>
<p>870.5300 Forward Gene Mutation Assay</p> <p>MRID 40765601 (1988) TXR 0051428</p> <p>Acceptable/Guideline</p>	<p>In a mammalian cell gene mutation assay at the HGPRT locus (MRID 40765601), Chinese hamster ovary (CHO) cells cultured <i>in vitro</i> were exposed to trifluralin (97.6% a.i.; Lot/Batch #: 39) in dimethyl sulfoxide for 4 hours at concentrations of 50, 100, 150, 200, 300, 400, or 500 $\mu\text{g}/\text{mL}$ (Trial 1, -S9); 50, 100, 200, 300, 500, 600, or 700 $\mu\text{g}/\text{mL}$ (Trial 2, -S9); 50, 100, 200, 250, 300, 400, or 500 $\mu\text{g}/\text{mL}$ (Trial 1, +S9); and 50, 100, 200, 300, 400, 500, or 600 $\mu\text{g}/\text{mL}$ (Trial 2, +S9).</p> <p>Trifluralin was tested up to cytotoxic concentrations (≥ 200 $\mu\text{g}/\text{mL}$, +/-S9) and the limit of solubility (≥ 100 $\mu\text{g}/\text{mL}$, +/-S9). No treatment-related increases in mutant frequency were observed in either trial in the presence or absence of S9. The positive controls induced the appropriate response. There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.</p>

4.7.2 Cytogenetics

<p>870.5385 <i>In Vivo</i> Mammalian Cytogenetics [Bone Marrow/Spermatogonial Aberration Test]</p> <p>MRID 40765603 (1988) TXR 0051428</p> <p>Acceptable/Guideline</p>	<p>In a bone marrow/spermatogonial chromosome aberration assay (MRID 40765603), ICR mice (10 males/dose, spermatogonial tissue; and 5/sex/dose, bone marrow) were dosed once daily via gavage (10 mL/kg) with trifluralin (97.6% a.i., Lot/BatchNo 39) in corn oil at doses of 0, 62.5, 208, or 625 mg/kg for 5 consecutive days. Bone marrow and spermatogonial cells were harvested at 4.5 hours after the last treatment.</p> <p>Mortalities were observed in the 625 mg/kg females (2/5 treated vs 1/5 controls), and at 62.5 mg/kg in the males (2/10 treated vs 1/10 controls) and females (2/5 treated vs 1/5 controls). Clinical signs of toxicity (lethargy, swollen neck, and yellow stains around the mouth and perianal area) were also observed at >=62.5 mg/kg. No statistically significant increases in the percent of aberrant cells were observed at any dose in either sex in the bone marrow assay or in the males in the spermatogonial assay. Trifluralin was tested at an adequate dose based on mortalities observed at >=62.5 mg/kg. The positive control induced the appropriate response. There was no evidence of chromosome aberration induced over background</p>
<p>870.5385 <i>In Vivo</i> Mammalian Cytogenetics [Bone Marrow Chromosome Aberration Test]</p> <p>MRID 00148320 TXR 0051428</p> <p>Acceptable/Guideline</p>	<p>In a bone marrow chromosome aberration assay (MRID 00148320), 5 Sprague-Dawley (HSD:[SD] BR) rats/sex/dose/sacrifice time were treated once via oral gavage with Triflurex technical (trifluralin; 97.3% a.i.; Batch #: 5320), in corn oil at doses of 0, 500, 1650, or 5000 mg/kg. Bone marrow cells were harvested at 6, 24, or 48 hours after treatment.</p> <p>Mortality was observed at 1650 (3/15 males and 5/15 females) and 5000 (2/15 males and 1/15 females) mg/kg upon initial dosing; however, these animals were replaced and only one replacement 1650 mg/kg female in the 48 hour group died after dosing. Triflurex technical induced minimal bone marrow toxicity (as indicated by decreased mitotic index) at >=500 mg/kg in males and >=1650 mg/kg in females. Dosing was considered adequate based on bone marrow toxicity and that the animals were dosed above the limit dose (2000 mg/kg). No statistically significant increases in the percent of aberrant cells were observed at any dose or sampling time compared to concurrent controls. The positive control induced the appropriate response. There was no evidence of chromosome aberration induced over background.</p>
<p>870.5395 <i>In vivo</i> Mouse Erythrocyte Micronucleus assay</p> <p>MRID 00151895 (1981) TXR</p> <p>Acceptable/Guideline</p>	<p>In a bone marrow micronucleus assay, 5 NMRI mice/sex/dose were treated via oral gavage with HOE 38474 (Trifluralin; 98.3% a.i., Lot/Batch #: OH AT208), in sesame oil at doses of 0, 25, 250, or 2500 mg/kg on two consecutive days (24 hours apart). Bone marrow cells were harvested at 6 hours after the last treatment.</p> <p>No unscheduled deaths occurred during the study. No clinical signs of toxicity were observed. No statistically significant differences in the number of micronucleated polychromatic erythrocytes (MPCE) or normocytes and no decrease in polychromatic erythrocyte to normocyte (PCE:NCE) ratios were noted in the treated animals compared to controls; however, only individual data were provided. Additionally, although no evidence of cytotoxicity (decreased PCE:NCE) was noted in the bone marrow, the animals were sufficiently dosed (the limit dose was given twice). The test material was absorbed as indicated by the presence of orange urine in the 2500 mg/kg animals. The positive control induced the appropriate response. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow compared to controls.</p>

<p>870.5450 Dominant Lethal - Mouse</p> <p>MRID 00151896 (1984)</p> <p>Acceptable/Guideline</p>	<p>In a dominant lethal assay (MRID 00151896), 30 male NMRI mice were dosed once daily for 5 consecutive days via oral gavage (5 mL/kg) with HOE 38474 (trifluralin; 98.3% a.i.; Lot/Batch #: OHZD99002), in sesame oil at concentrations of 0, 10, 100, or 1000 mg/kg. After the final treatment, each male was mated with 13 untreated females during separate 4-day intervals over a 52 day period.</p> <p>No treatment-related mortalities were noted during the study. No treatment-related effects on clinical signs, body weight, fertilization rate, and pre- or post-implantation loss were observed; however, no data were provided. The positive control (cyclophosphamide) increased the number of post-implantation fetal losses. There was no time-related positive response of increased pre- or post-implantation loss compared to controls.</p>
<p>870.5450 Dominant Lethal - Rat</p> <p>MRID 00148319 (1984) TXR 0051428</p> <p>Acceptable/Guideline</p>	<p>In a dominant lethal assay (MRID 00148319), groups of 20 male Sprague-Dawley (CD) rats/dose were treated once daily via gavage (1.0 mL/dose) with Triflurex technical (trifluralin; 97.3% a.i.; Batch #: 5320), in corn oil for 5 consecutive days at doses of 0, 100, 333, or 1000 mg/kg/day (total doses of 0, 500, 1665, or 5000 mg/kg). Beginning two days after the last exposure, each male was mated sequentially to two untreated female rats per week for seven weeks. At 14 days after the midpoint of each mating week, the females were killed, determined to be pregnant or not pregnant, and the number of corpora lutea, living, dead, and total implantations was determined. On the fifth day of dosing, the positive control males were given a single dose of triethylenemelamine (TEM; 0.3 mg/kg, i.p. in 0.9% saline).</p> <p>One 1000 mg/kg male (#8476) was found dead 72 hours after the last treatment. Triflurex technical was tested at the limit dose (5000 mg/kg = 1000 mg/kg/day X 5 days). There were no treatment-related effects on fertility, number of implants, pre-implantation losses, number of dead implants, number of females with ≥ 1 or ≥ 2 dead implants, or ratio of dead implants to total implants at any dose in the study. The positive control induced the appropriate response. There was no time-related positive response of increased pre- or post-implantation loss compared to controls.</p>

4.7.3 Other Genotoxicity

<p>870.5550 Unscheduled DNA synthesis in mammalian cell culture</p> <p>MRID 40765602 (1988) TXR 0051428</p> <p>Acceptable/Guideline</p>	<p>In an unscheduled DNA synthesis assay (MRID), primary rat hepatocyte cultures were exposed to trifluralin (97.6% a.i.; Lot/Batch #: 39) in DMSO for 18-19 hours at concentrations of 0, 0.032, 0.214, 0.404, 0.917, 2.22, 4.36, 8.52, 21.3, 42.9, 88.0, 448, or 898 $\mu\text{g}/\text{mL}$. Fifteen doses ranging from 0.032-898 $\mu\text{g}/\text{mL}$ were used in each assay; however, the three doses between 0.032 and 0.214 $\mu\text{g}/\text{mL}$ were not reported.</p> <p>Trifluralin was tested up to cytotoxic levels (determined by trypan blue exclusion), 88.0 $\mu\text{g}/\text{mL}$ in rat #1 and 42.9 $\mu\text{g}/\text{mL}$ in rat #2. There were no marked increases observed in the mean NNG or percent cells in repair at any dose in either trial. The positive controls induced marked increases in mean NNG and the percent of cells in repair. There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures (nuclear silver grain counts), was induced.</p>
---	--

<p>870.5550</p> <p>Unscheduled DNA synthesis in mammalian cell culture</p> <p>MRID 00151894 TXR 0051428</p> <p>Acceptable/Guideline</p>	<p>In an unscheduled DNA synthesis assay (MRID 00151894), HeLa cell cultures were exposed to HOE 38474 (trifluralin, 98.3% a.i.; Lot/Batch #: OH AT 208) in DMSO for 1 hour at concentrations of 0, 50, 100 or 500 $\mu\text{g/mL}$ both in the presence and absence of S9-activation.</p> <p>HOE 38474 was tested up to cytotoxic concentrations ($\geq 50 \mu\text{g/mL}$, +/-S9). No statistically significant increases in mean counts per minute of the test material with hydroxyurea were noted compared to concurrent solvent controls at any dose level, either in the presence or absence of S9-activation. The positive controls induced the appropriate response. There was no evidence that unscheduled DNA synthesis, as determined by liquid scintillation counting procedures, was induced.</p>
<p>870.5900</p> <p><i>In vivo</i> Sister Chromatid Exchange Assay</p> <p>MRID 00133426 (1983) TXR 005535, 051428</p> <p>Acceptable/Guideline</p>	<p>In a mammalian cell cytogenetics assay [SCE] using the BrdU tablet method, groups of 3 female Chinese hamsters/dose were exposed once via gavage (10 mL/kg) to trifluralin (95.0% a.i., Lot/batch No 00554AP2) in DMSO and 10% acacia at concentrations of 200, 300, 400, or 500 mg/kg at 5 hours following BrdU tablet implantation. Bone marrow was collected at 21 hours after treatment.</p> <p>Trifluralin was tested up to cytotoxic concentrations ($\geq 400 \text{ mg/kg}$). Cytotoxicity (as indicated by an increase in the number of first division metaphase figures) was observed in all animals at $\geq 400 \text{ mg/kg}$. No statistically significant increases in SCE frequency were observed at any dose compared to controls. The positive control induced the appropriate response. There was no evidence of SCE induced over background</p>

4.8 Neurotoxicity

Adequacy of data base for Neurotoxicity: The HIARC concluded that there were no signs of neurotoxicity in the trifluralin data base. None of the guideline neurotoxicity studies are required.

4.8.1 870.6100 Delayed Neurotoxicity Study - Hen

Executive Summary: In an acute delayed neurotoxicity study (MRID 00159616), White Leghorn hens were gavaged with trifluralin (98.4%, Batch No. 038474) at 0 (10 mL/kg sesame oil) or 5000 mg/kg; positive control hens received 500 mg/kg triorthocresyl phosphate (TOCP) trifluralin *via* capsule at doses of 800, 2000 or 5000 mg/kg.. The acute oral LD50 for trifluralin was greater than 5000 mg/kg.

Trifluralin-treated hens showed marginal disturbances in muscle coordination from days 9 to 11; from day 12 on, no further disturbances were noted. Histopathological evaluation of the brain, spinal cord and sciatic nerve did not show any evidence of neurotoxic effects.

Under the conditions of this study, trifluralin at 5000 mg/kg did not produce acute delayed

neurotoxicity.

This study is **acceptable/guideline** (OPPTS 870.6100) and satisfied requirements for an acute delayed neurotoxicity study in the hen.

4.8.2 870.6200 Acute Neurotoxicity Screening Battery

Not required

4.8.3 870.6200 Subchronic Neurotoxicity Screening Battery

Not required

4.8.4 870.6300 Developmental Neurotoxicity Study

Not required

4.9 Metabolism

4.9.1 Adequacy of data base for metabolism:

Adequacy of data base for metabolism: The data base for metabolism is considered to be complete. No additional studies are required at this time. In a rat metabolism study, ¹⁴C-trifluralin appears to be extensively metabolized, as evidenced by many non-conjugated (20-30) and conjugated (10-20) metabolites observed. Although the percent of the administered dose excreted in the urine is not known, the majority of the metabolites were present at 1 to 2% of the total urinary radioactivity. Based on the metabolic profile, four metabolic pathways were identified (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.

4.9.2 870.7485 Metabolism - Rat

Executive Summary: In a rat metabolism study (MRID 41218901), ¹⁴C-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: (i) oxidative N-dealkylation of one or both propyl groups and metabolites which were hydroxylated on the propyl side chain; (ii) reduction of one or both nitro groups to the corresponding amine; (iii) cyclization reactions to give a variety of substituted and unsubstituted benzimidazole metabolites; and (iv) conjugation reactions, including acetylation of the reduced nitro groups, sulfate, and glucuronic acid conjugates.

This study is classified as **acceptable/non-guideline** and satisfies the purpose for which it was intended.

4.9.3 870.7600 Dermal Absorption - Rat

Although there is a dermal absorption study with trifluralin (MRID 40673701), it was found to be unacceptable. However, there is an acceptable dermal absorption study with ethalfluralin in monkeys. Trifluralin and ethalfluralin have similar structures (differing only in the dialkyl groups) and physical constants (melting points, solubilities, and the log K_{ow}s). Using the dermal absorption percentage for ethalfluralin (3%) 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, the dermal absorption value of 3% is similar to the approximation from the ratio of the dermal and oral NOAELs (1000/154 = 6%).

Executive Summary (Ethalfluralin): 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 (MRID: 00132820, 92062028). 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.

4.10 Other Studies

Special studies were submitted to determine the NOAEL/LOAEL for nephrotoxicity in male rats. The findings included the presence of tubular cytoplasmic hyaline droplets, increased urinary volume, and increased total protein, AST, and LDH in the urine. Electrophoresis revealed albumin, α 1-globulin and α 2-globulin in the urine.

4.10.1 Urinary Tract Effects (Range Finding) - Rat

Executive Summary: In a 2-week oral toxicity study (MRID 00157154), trifluralin (96.4% a.i., Batch # 554AP2) was administered to 10 male Fischer 344 rats at 6500 ppm (approximately 346.47 mg/kg/day) or 5 rats at 0 ppm. The objective of this study was to determine the effects of trifluralin on the urinary tract of male rats.

All animals survived until the scheduled sacrifice. Food consumption was reduced by 27%, and may have been indicative of a lack in palatability. Additionally, decreases (not statistically significant) in body weight gain (5.4 g treated vs 15.6 g control) and food utilization (2.6 treated vs 6.3 controls) were observed.

The treated animals urinated orange-yellow fluid, indicative of the test compound and/or metabolites, resulting in soiled genital areas. During urinalysis, the following observations were made: (i) urine clarity was reduced in the treated animals on days 6 and 13; (ii) triple phosphate crystals were observed in all treated animals and were particularly abundant on days 6 and 13 (vs 3/5 control rats); (iii) amorphous material was found in the urine of treated animals; (iv) LDH and AST were increased at days 6 and 13; on day 13 the increase was 84% for LDH and 205% for AST; (v) mildly increased protein concentrations were observed on days 6 and 13. Cytoplasmic hyaline droplet formation in many renal cortical tubules of each treated animal indicated epithelial degeneration (vs 0/5 controls).

This study is classified as **acceptable/non-guideline** and satisfies the purpose for which it was intended.

4.10.2 Urinary Tract Effects in Male Rats

Executive Summary: The purpose of this special study (MRID 00157156, 40138301, 41086101) 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 $\alpha 1$ -globulin and $\alpha 2$ -globulin (all biochemical indicators of kidney toxicity).

In the ≥ 800 ppm groups, significant increases of 73-489% in urinary total protein, AST, and LDH and in the urinary volume (38-89%) were observed.

In the 6400 ppm group, increases 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 $\alpha 1$ -globulin and $\alpha 2$ -globulin observed by urine electrophoresis; and increased urinary volume. The NOAEL is 200 ppm (10.1mg/kg/day).

This study is classified as **acceptable/non-guideline** and satisfies the purpose for which it was intended

5 TOXICITY ENDPOINT SELECTION

5.1 Endpoint Selection

See Section 9.2 for Endpoint Selection Table.

5.2 Acute Reference Dose (aRfD)

5.2.1 General Population

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

5.2.2 Females 13-50

Study Selected: Developmental Toxicity Study - Rat

MRID No.: 00151899, 00159620 and 40392310

Executive Summary: See section 4.3.1

Dose and Endpoint for Establishing aRfD: **Developmental NOAEL = 100 mg/kg/day**, based on increased incidences of resorptions at the developmental LOAEL of 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 resorptions observed at 500 mg/kg/day could be due to a single dose.

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

5.3 Chronic Reference Dose (cRfD)

Study Selected: Chronic toxicity oral (capsule) - dog

MRID No.: 42447001

Executive Summary: See section 4.5.2

Dose and Endpoint for Establishing cRfD: **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.

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

5.4 Occupational/Residential Exposure

5.4.1 Incidental Oral Exposure

5.4.1.1 Short-Term (1 - 30 days) Incidental Oral Exposure

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

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

Executive Summary: See section 4.4.1

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 at the LOAEL of 32.5 mg/kg/day.

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 indicating 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.

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

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

MRID No.: 00157156, 40138301, 41086101

Executive Summary: See section 4.10.2

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 α 1-globulin and α 2-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 dose, endpoint and study, as well as the population of concern (infants and children) are appropriate for this exposure scenario.

5.4.2 Dermal Exposure

5.4.2.1 Dermal Exposure: Short Term (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. 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.

5.4.2.2 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 4.10.2

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 α 1-globulin and α 2-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 of concern.

5.4.2.3 Dermal Exposure Long-Term (> 6 Months)

Study Selected: Chronic toxicity oral (capsule) - dog

MRID No.: 42447001

Executive Summary: See section 4.5.2

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.

5.4.3 INHALATION EXPOSURE

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

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

MRID No.: 40392312, 00151904

Executive Summary: See section 4.2.3

Dose and Endpoint for Risk Assessment: **NOAEL = 300 mg/m³ (81 mg/kg/day)** based on increased bilirubin in females and incidences of dyspnea and ruffled fur in males and females at 1000 mg/m³ (270 mg/kg/day).

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

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

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

MRID No.: 00157156, 40138301, 41086101

Executive Summary: See section 4.10.2

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 α 1-globulin and α 2-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.

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

Study Selected: Chronic toxicity oral (capsule) - dog

MRID No.: 42447001

Executive Summary: See section 4.5.2

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.

5.5 Dermal Absorption

Selected Study: Percutaneous absorption ¹⁴C-**ethalfuralin** in monkeys. Guideline #:NG

MRID No.: 00132820, 92062028

Executive Summary: See section 4.9.3

Dermal Absorption Factor: 3%

5.6 Classification and Quantification of Carcinogenic Potential

5.6.1 Classification of Carcinogenic Potential

The CPRC concluded that trifluralin is a "Group C" (limited evidence of carcinogenicity) carcinogen, based on the results of a chronic feeding/oncogenicity study in the rat (MRID 00044337) which showed renal pelvis carcinomas, follicular cell adenomas, papillary adenomas, and cystadenomas.

5.6.2 Quantification of Carcinogenic Potential

$Q1^* = 0.00579 \text{ (mg/kg/day)}^{-1}$ based on male rat follicular cell adenomas, papillary adenoma, cystadenoma, and carcinoma combined tumor rates. The $Q1^*$ was calculated using an interspecies scaling factor of 3/4 (TXR 0051890).

6 FQPA CONSIDERATIONS

6.1 Special Sensitivity to Infants and Children

6.1.1 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).

6.1.2 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 were 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.

6.1.3 Special FQPA Safety Factor(s):

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

6.2 Recommendation for a Developmental Neurotoxicity Study

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

7 REFERENCES

- 00044337 Emmerson, J.L.; Pierce, E.C.; McGrath, J.P.; et al. (1980) The Chronic Toxicity of Compound 36352 (Trifluralin) Given as a Component of the Diet to Fischer 344 Rats for Two Years: Studies R-87 and R-97. (Unpublished study received Sep 18, 1980 under 1471-35; submitted by Elanco Products Co., Div. of Eli Lilly and Co., Indianapolis, Ind.; CDL:243289-A, 243290)
- 00044338 Emmerson, J.L.; Owen, N.V.; McGrath, J.D.; et al. (1980) The Chronic Toxicity of Compound 36352 (Trifluralin) Given as a Component of the Diet to the B6C3F1 Mouse for 24 Months: Studies M-9067 and M-9077. (Unpublished study received Sep 18, 1980 under 471-35; submitted by Elanco Products Co., Div. of Eli Lilly and Co., Indianapolis, Ind.; CDL:243291-A; 243292; 243293)
- 00126661 Oberly, T.; Emerson, J.; Bewsey, B.; et al. (1983) The Effect of Trifluralin (Compound 36352) on the Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells: Study 830201MLA2055. (Unpublished study received Apr 5, 1983 under 1471-70; submitted by Elanco Products Co., Div. of Eli Lilly and Co., Indianapolis, IN; CDL:249846-C)

- 00124928 Weisburger, J.; Weisburger, E.; Powers, M.; et al. (1977) Bioassay of Trifluralin for Possible Carcinogenicity: DHEW Publication No. (NIH) 78-834; Pre-RPAR Review Submissions #4 and #5. (Unpublished study received Nov 9, 1977 under 1471-35; prepared by U.S. National Institutes of Health, National Cancer Institute, Div. of Cancer Cause and Prevention, Carcinogenesis Testing Program and others, submitted by Elanco Products Co., Div. of Eli Lilly and Co., Indianapolis, IN; CDL:233235-A; 233234)
- 00132820 Bridge, T.; van Lier, R.; Adams, E.; et al. (1982) Percutaneous Absorption of 14C-Ethalfuralin (EL-161, Compound 94961) in Monkeys: Studies M-6162 and P03282. (Unpublished study received Dec 2, 1983 under 4F3006; submitted by Elanco Products Co., Div. of Eli Lilly and Co., Indianapolis, IN; CDL:072180-B)
- 00133426 Neal, S.; Emmerson, J.; Probst, G.; et al. (1983) The Effect of Trifluralin (Compound 36352) on the in vivo Induction of Sister Chromatid Exchange in Bone Marrow of Chinese Hamsters: Study 830207SCE2055. (Unpublished study received Dec 9, 1983 under unknown admin. no.; submitted by Elanco Products Co., Div. of Eli Lilly and Co., Indianapolis, IN; CDL:251921-K)
- 00148318 Young, R. (1984) Evaluation of Triflurex Technical (Trifluralin) in the CHO/HGPRT Forward Mutation Assay: Final Report: LBI Project No. 22207. Unpublished study prepared by Litton Bionetics, Inc. 27 p.
- 00148319 Moore, M. (1984) Evaluation of Triflurex Technical in the Rat Dominant Lethal Assay: Final Report: Project No. 22203. Unpublished study prepared by Litton Bionetics. 69 p.
- 00148320 Ivett, J. (1984) Mutagenicity Evaluation Triflurex Technical, Batch No. 5320 in the Bone Marrow: Cytogenic Assay: Final Report: LBI Project No. 22202. Unpublished study prepared by Litton Bionetics. 32 p.
- 00148345 Jagannath, D. (1984) Mutagenicity Evaluation of Triflurex Tech (Trifluralin) Batch 5230 in the Ames Salmonella/Microsome Plate Test: Final Report: LBI Project No. 20988. Unpublished study prepared by Litton Bionetics, Inc. 19 p.
- 00151894 Mellano, D. (1982) Study of the Capacity of the Test Article HOE 38474 OH at 208 to Induce "Unscheduled DNA Synthesis" in Cultured Hela Cells: Study No. M 372. Unpublished study prepared by Istituto Di Ricerche Biomediche "Antoine Marxer" S.p.A. 19 p.
- 00151895 Leist; Weigand; Kramer (1981) Testing of Hoe 38474 - Active Ingredient for Mutagenicity in the Micronucleus Test following Oral Administration to NMRI Mice: (Code: HOE 38474 OH AT 208): Report No. 285/81. Unpublished report prepared by Hoechst Aktiengesellschaft. 16 p.

- 00151896 Horstmann (1984) Dominant-lethal Test for Determination of Mutagenic Effect in Male NMRI-mice after Oral Administration: Trifluralin: Code: Hoe 38474 OHZD99 0002: Report No. 84.0763. Un- published report prepared by Hoechst Aktiengesellschaft. 89 p.
- 00151898 Fumero, S. (1982) Study of the Mutagenic Activity "In Vitro" of the Compound HOE 38474 OH AT 208 with Schizosaccharomyces Pombe: Study No. M 374. Unpublished study prepared by Instituto Di Ricerche Biomediche "Antoine Marxer" S.p.A. 16 p.
- 00151899 Baeder, Weigand, Kramer (1983) Testing for Embryotoxicity in Wister Rats following Oral Administration: HOE 38474 - Active Ingredient: Report No. 83.0557: Study No.G2R0383. Unpublished study prepared by Hoechst Aktiengesellschaft. 51 p.
- 00151901 Becker, H. (1984) Multiple Generation Study in the Rat: Trifluralin Substance Technical Grade (Code : HOE 38474 OH AT210): Project No. 008875. Unpublished study prepared by Research & Consulting Co., AG. 604 p.
- 00151902 Ellgehausen, H. (1984) Determination of Trifluralin Substance Technical Grade (Code : HOE 38474 O H AT210) in Rodent Feed: Project No. 008875. Unpublished study prepared by Research & Consulting Co. 26 p.
- 00151903 Westen, H. (1984) Multiple Generation Study in Rat: Trifluralin Substance Technical Grade (Code: HOE 38474 O H AT210): Pathology Report Part II: Project No. 008875. Unpublished study prepared by Research & Consulting Co., AG. 704 p.
- 00151904 Ullmann, L. (1982) 30 Day Repeated Dose Inhalation Toxicity Study with HOE 38474 OH AT 210 Active Ingredient (Technical) in Rats: Part 1: Project No. 005488. Unpublished study prepared by Research & Consulting Co., Ltd. 400 p.
- 00151905 Suter, P. (1983) 13-Week Toxicity Study with Trifluralin (HOE 38474 O H AT210) in Mice following Dietary Administration: Report: Project No. 008842. Unpublished study prepared by Research & Consulting Co., AG. 223 p.
- 00151906 Schutz; Weigand; Kramer (1980) Repeated-dose (3 Months) Oral Toxicity Study of the Active Substance HOE 38474 (Code: HOE 38474 O H AT204) Administered in the Feed to Rats: Report No. 618/80. Unpublished study prepared by Hoechst Aktiengesellschaft. 422 p.
- 00151907 Brunk; Weigand; Kramer (1981) Toxicology Testing of Trifluralin (Hoe 38474 OH AT 204) by Repeated Oral Administration to Beagle Dogs for Six Months: Report No. 636/81. Unpublished report prepared by Hoechst Aktiengesellschaft. 742 p
- 00151908 Bathe, R. (1984) 12-Month Oral Toxicity (Feeding) Study in Beagle Dogs: Trifluralin

Substance Technical Grade (Code: HOE 38474 O H AT210): Project No. 008864:
Report Part 1. Unpublished study prepared by Research & Consulting Co. 439 p

- 00152419 Byrd, R. (1984) A Teratology Study of Trifluralin (EI-152, Compound 36352) Administered Orally to Charles River CD Rats: Study R08484. Unpublished study prepared by Lilly Research Labs. 308 p.
- 00152421 Byrd, R. (1984) A Teratology Study (II) of Trifluralin (EI-152, Compound 36352) Administered Orally to Dutch Belted Rabbits: Study B01784. Unpublished study prepared by Lilly Research Labs. 223 p.
- 00153171 Suter, P. (1982) 31 Days Dermal Toxicity Study with Hoe 38474 OH AT 210 Active Ingredient (Technical) in Rats: Project 005490. Unpublished study prepared by Research & Consulting Co., Ltd. 450 p.
- 00152888 Negilski, D. (1985) Subchronic (21-day) Dermal Toxicity Study in New Zealand White Rabbits with Technical Trifluralin: Study B00184. Unpublished study prepared by Lilly Research Laboratories. 222 p.
- 00153173 Engelbart (1979) Test for Mutagenicity in Bacteria Strains in the Absence and Presence of a Liver Preparation: Hoe 38474 OH AT204 (Active Ingredient) Trifluralin: Report No. 74/79. Unpublished study prepared by Hoechst AG. 7 p.
- 00155261 Leist, K. (1981) Four Hour LC50 Aerosol Inhalation Toxicity Study in Rats on HOE 38474 OH at 210 Active Ingredient (Technical): Report: Project 005477. Unpublished study prepared by Research & Consulting Co. Ltd. 37 p.
- 00157154 Jordan, W. (1983) A Preliminary Dietary Study of the Effects of Trifluralin on the Urinary Tract of Male Fischer 344 Rats: Study No. R15083: Report No. 1. Unpublished study prepared by Lilly Research Labs. 89 p.
- 00157156 Usher, R. (1985) A Special Urinalysis Study in Fischer 344 Rats Maintained on Diets Containing Trifluralin (Compound 36352) for Three Months: Study No. R04785. Unpublished study prepared by Lilly Research Labs. 711 p.
- 00157482 Vigna, E. (1985) Trifluralin Technical: Acute Dermal Toxicity Study in Rats: RBM Exp. No. 2159. Unpublished study prepared by *Istituto di Ricerche Biomediche "Antoine Marxer", RBM S.p.A.* 13 p.
- 00157483 Vigna, E. (1985) Trifluralin Technical: Acute Eye Irritation Study in Rabbits: RBM Exp. No. 2160. Unpublished study prepared by *Istituto di Ricerche Biomediche "Antoine Marxer", RBM S.p.A.* 15 p.
- 00157484 Bassi, L. (1985) Trifluralin Technical: Dermal Sensitization Study in Guinea Pigs: RBM

- Exp. No. I 892. Unpublished study prepared by Istituto di Ricerche Biomediche "Antoine Marxer", RBM S.p.A. 15 p.
- 00157485 Vigna, E. (1985) Trifluralin Technical: Acute Dermal Irritation Study in Rabbit: RBM Exp. No. 2161. Unpublished study prepared by Istituto di Ricerche Biomediche "Antoine Marxer", RBM S.p.A. 13 p.
- 00157486 Vigna, E. (1985) Trifluralin Technical: Acute Toxicity Study by Oral Route in Rats: RBM Exp. No. 2162. Unpublished study prepared by Istituto di Ricerche Biomediche "Antoine Marxer", RBM S.p.A. 13 p.
- 00158935 Suter, P. (1986) Oncogenicity Study with Trifluralin Active Ingredient Technical (HOE 38474 O H AT210) in Mice: Project No. 008853. Unpublished study prepared by Research & Consulting Company AG. 6478 p.
- 00159616 Ebert, E. (1985) Trifluralin-Active Ingredient Technical Testing for Acute Delayed Neurotoxicity in White Leghorn Hens: Report No. 85.0742. Unpublished study prepared by Hoechst AG. 38 p.
- 00159618 Bathe, R. (1985) Trifluralin, Substance Technical Grade: 12-Month Oral Toxicity (Feeding) Study in Beagle Dogs: Concentration of Trifluralin, Substance Technical Grade in Dog Feed; Project No. 008864: Report No. A32778. Unpublished study prepared by Research & Consulting Co. AG. 4 p.
- 00159619 Becker, H. (1985) Trifluralin, Substance Technical Grade: Multiple Generation Study in the Rat: Diet Preparation, Stability, and Analysis of the Test Article; 1st Amendment to Report: Project No. 008875: Report No. A32777. Unpublished study prepared by Research & Consulting Co. AG. 4 p.
- 00159620 Baeder; Mayer (1986) Testing for Embryotoxicity in Wistar Rats following Oral Administration of Hoe 38474: Incidence of Wavy Ribs in Control Studies and at Dose Levels Toxic to Either Dams or Embryos: Supplement to Report No.83.0557: Report No. 86.0166. Unpublished study prepared by Hoechst AG. 15 p.
- 00162458 Donaubaue (1986) Carcinogenicity Study in Rats (28-month Feeding Study): Trifluralin: Study No. 680: Report No. 85.0087. Unpublished study prepared by Hoechst Aktiengesellschaft, Pharma Forschung Toxikologie. 3126 p.
- 00162543 Hoyt, J. (1986) A One-year Two-generation Reproduction Study in CD Rats Maintained on Diets Containing Trifluralin (EL-152, Compound 36352): Studies R06384 and R13984. Unpublished study prepared by Lilly Research Laboratories. 778 p.
- 00162457 Donaubaue (1986) Chronic Feeding Study (24 Months) in Rats: Trifluralin: Study

- No. 680: Report No. 85.0302. Unpublished study prepared by Hoechst Aktiengesellschaft, Pharma Forschung Toxikologie. 1837 p.
- 40138301 Usher, R. (1986) A Supplementary Report of a Special Urinalysis Study in Fischer 344 Rats Maintained on Diets Containing Trifluralin (Compound 36352) for Three Months: Study R04785. Unpublished study prepared by Lilly Research Laboratories. 263 p.
- 40334707 Loveday, K. (1987) Evaluation of Trifluralin in the Ames Mutagenesis Assay: ADL Reference: 88720-68. Unpublished study prepared by Arthur D. Little, Inc. 28 p.
- 40392310 Leist, K.; Penseler, D.; Mayer (1987) Trifluralin Substance Technical Grade (Code: HOE 38474 OH AT210): Testing for Embryotoxicity in Wistar Rats following Oral Administration: Supplementary Data in Support of Previously Submitted Study Acc. No. 258993: Laboratory Project No. 87.0997, Report No. A 36041. Unpublished study prepared by Hoechst Aktiengesellschaft. 19 p.
- 40392312 Ullman, L. (1987) 30-Day Repeated Dose Inhalation Toxicity Study with HOE 38474 OH at 210 in Rats: Amended Version of Previously Submitted Study Acc. No. 258996: Laboratory Project No. 005488, Report No. A 22688 and A 36084. Unpublished study prepared by Research & Consulting Co. Ag. 711 p.
- 40392313 Suter, P.; Horst, K.; Vogel, W.; et al. (1987) Oncogenicity Study with Trifluralin Active Ingredient Technical (HOE 38474 OH AT210) in Mice: Supplementary Data in Support of Previously Submitted Study Acc. No. 262516-262520: Laboratory Project No. 008853, Report No. A 36197. Unpublished study prepared by Research & Consulting Co. AG. 47 p.
- 40405007 Rubin, Y.; et al. (1987) Triflurex: Two-generation Reproduction Study in the Rat: Laboratory Project ID AGN/125/TRI. Unpublished study prepared by Life Science Research Israel Ltd. 1664 p.
- 40673701 Adams, E.; Glass, S.; Van Lier, R. (1988) Percutaneous (Dermal) Absorption of Carbon 14-Trifluralin in Rhesus Monkeys: Laboratory Project ID: P07487 and P03087. Unpublished study prepared by Lilly Research Laboratories. 55 p.
- 40765601 Young, R. (1988) Mutagenicity Test on Trifluralin Technical Grade: Batch Number 39 in the CHO/HGPRT Forward Mutation Assay: Project ID. 10137-0-435. Unpublished study prepared by Hazleton Laboratories America, Inc. 46 p.
- 40765602 Cifone, M. (1988) Mutagenicity Test on Trifluralin, Technical Grade in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay: Project ID. 10137-0-447. Unpublished study prepared by Hazleton Laboratories America, Inc. 39 p.
- 40765603 Ivett, J. (1988) Mutagenicity Test on Trifluralin Technical Grade in the Mouse Bone

Marrow and Spermatogonial Cell Cytogenetics Assay: Project ID. 10137-0-473.
Unpublished study prepared by Hazleton Laboratories America, Inc. 51 p.

- 41086101 Adams, E.; Bendele, R.; Emmerson, J. (1989) Reanalysis and Discussion of Urine Protein Electrophoresis Data From a Special Urinalysis Study in Fischer 344 Rats with Trifluralin (Compound 36352). Unpublished supplemental submission prepared by Lilly Research Laboratories 63p.
- 41218901 Magnussen, J. (1989) Identification of the Urinary Metabolites of carbon 14Trifluralin in Rats: Project ID: Experiment ABC-0433. Unpublished study prepared by Lilly Research Laboratories. 53 p.
- 41993810 Vedula, U.; Kociba, R.; Bond, D. (1991) XRM-5313: A Formulation Containing Trifluralin and XRD-498--21 Day Dermal Study in New Zealand White Rabbits: Lab Project Number: M-005313-003A. Unpublished study prepared by The Dow Chemical Co., Tox. Research Lab. 166 p.
- 42447001 Adams, E.; Bernhard, N.; Jordon, W. (1992) A Chronic Toxicity Study of Trifluralin (Compound 036352) Administered Orally to Beagle Dogs for One Year (Supp.): Lab Project Number: D07190. Unpublished study prepared by Lilly Research Labs. 470 p.
- 42972701 Shapiro, R. (1993) EPA Acute Oral Toxicity-Defined LD50 in Rats: Trilin 5, Lot #227284: Lab Project Number: T--2527: 2527: P320. Unpublished study prepared by Product Safety Labs. 34 p.
- 42972702 Shapiro, R. (1993) EPA Acute Dermal Toxicity Limit Test: Rabbits: Trilin 5, Lot 227284: Lab Project Number: T--2530: 2530: P322. Unpublished study prepared by Product Safety Labs. 20 p.
- 42972703 Shapiro, R. (1993) EPA Acute Inhalation--Defined LC50: Rats: Trilin 5, Lot # 227284: Lab Project Number: T--2532. Unpublished study prepared by product Safety Labs. 54 p.
- 42972704 Shapiro, R. (1993) EPA Primary Eye Irritation: Rabbit: Trilin 5, Lot #227284: Lab Project Number: T--2528: P324: E30715-1. Unpublished study prepared by Product Safety Labs. 30
- 42972705 Shapiro, R. (1993) EPA Primary Dermal Irritation Test: Rabbits: trilin 5, Lot 227284: Lab Project Number: T--2529: 2529: P326. Unpublished study prepared by Product Safety Labs 23 p.
- 42972706 Shapiro, R. (1993) EPA Topical Skin Sensitization Test in Guinea Pigs (Buehler): Trilin 5, Lot #227284: Lab Project Number: T--2531: 2531. Unpublished study prepared by Product Safety Labs. 33 p.

- 43447301 Kuhn, J. (1994) Trific 10G: Acute Oral Toxicity Study in Rats: Lab Project Number: 0875-93. Unpublished study prepared by Stillmeadow, Inc. 11 p.
- 43447302 Kuhn, J. (1994) Trific 10G: Acute Dermal Toxicity Study in Rabbits: Lab Project Number: 0876-93. Unpublished study prepared by Stillmeadow, Inc. 11 p.
- 43447303 Holbert, M. (1994) Trific 10G: Acute Inhalation Toxicity Study in Rats: Lab Project Number: 0877-93. Unpublished study prepared by Stillmeadow, Inc. 20 p.
- 43447304 Kuhn, J. (1994) Trific 10G: Primary Eye Irritation in Rabbits: Lab Project Number: 0878-93. Unpublished study prepared by Stillmeadow, Inc. 18 p.
- 43447305 Kuhn, J. (1994) Trific 10G: Primary Dermal Irritation in Rabbits: Lab Project Number: 0879-93. Unpublished study prepared by Stillmeadow, Inc. 12 p.
- 43447306 Kuhn, J. (1994) Trific 10G: Dermal Sensitization Study in Guinea Pigs: Lab Project Number: 0880-93. Unpublished study prepared by Stillmeadow, Inc. 17 p.
- 44135107 Adams, E.; Markham, J. (1996) A One-Year Two-Generation Reproduction Study in CD Rats Maintained on Diets Containing Trifluralin: Supplement #1 to MRID No. 00162543: Lab Project Number: R06384: R13984-S1: R13984. Unpublished study prepared by Lilly Research Labs. 16 p
- 92062028 Environ Corp. (1990) Dowelanco Phase 3 Summary of MRID 00132820. Percutaneous Absorption of (Carbon-14) - Ethalfluralin in Monkeys: M-6162 and P03282. Prepared by LILLY RESEARCH LABS. 7 p
- IARC (1991) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Trifluralin, Vol 53, 1991
- USEPA (1979) Trifluralin (Treflan®) Position Document 1/2/3, 44 FR 50911, NTIS # PB80-213937, Aug 30, 1979.
- USEPA (1982) Alpha, alpha, alpha-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine, Trifluralin (Treflan®) Position Document 4, 47 FR 33777, NTIS# PB82-263252, Aug 4, 1982.

8 APPENDICES

Tables for Use in Risk Assessment

8.1 Toxicity Profile Summary Tables

8.1.1 Acute Toxicity Table - See Section 4.1

8.1.2 Subchronic, Chronic and Other Toxicity Tables

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.3100 2-Week R-F Feeding - Rats (male)	00157154 (1983) 0, 6500 ppm range-finding study for 00157156 (1985), 41038301 (1986) Acceptable/Nonguideline	NOAEL = not achieved LOAEL = 6500 ppm based on renal epith damage, urine triple phosphates crystals and urinary sediment
870.3100 90-Day Oral toxicity - Rat	00151906 (1980) 0, 800, 2000, or 5000 ppm M: 0, 59, 154, and 392 mg/kg/day F: 0, 69, 168, and 421 mg/kg/day Acceptable/Guideline	NOAEL = 2000 ppm (154/168 mg/kg/day, M/F) LOAEL = 5000 (392/421 mg/kg/day, M/F) Based on 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.
870.3200 21/28-Day dermal toxicity-rabbit	41993810 (1991) 0, 100, 500, or 1000 mg/kg /day, [formulation containing 35.8% trifluralin and 2.6% XRD-498] Acceptable/Guideline	Systemic NOAEL = 1000 mg/kg/day Systemic LOAEL = Not achieved Dermal NOAEL = Not achieved Dermal LOAEL = 100 mg/kg/day, edema, and/or scaling and fissuring 100 mg/kg/day based skin irritation
870.3200 31-Day dermal toxicity-rat	00153171 (1982) 0, 40, 200, or 1000 mg/kg/day Acceptable/Guideline	Systemic NOAEL = 1000 mg/kg/day (limit dose) Systemic LOAEL = not achieved Dermal NOAEL = 40 mg/kg/day. Dermal LOAEL = 200 mg/kg/day based on sub-epidermal inflammation and ulcerations in males and females.
870.3200 21/28-Day dermal toxicity-rat	00152888 (1985) 0, 1000 mg/kg/day (limit dose) Acceptable/Guideline	Systemic NOAEL = 1000 mg/kg/day. Systemic LOAEL = Not achieved Dermal NOAEL= Not achieved Dermal LOAEL = 1000 mg/kg/day (limit dose) based on erythema, edema, and desquamation of the treated skin.

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.3465 30-Day inhalation toxicity	40392312 (1987) reformat of 00151904 (1982) 0, 100, 301, 1006 mg/m ³ (6 hours/day 5 days/week for up to 30 days) Acceptable/Nonguideline	NOAEL = 301 mg/m ³ LOAEL = 1006 mg/m ³ based on increased bilirubin in females and incidences of dyspnea and ruffled fur in males and females.
870.3700 [83-3(a)] Developmental Toxicity Study - Rat	00151899 (1983), 159620 (1986), 40392310 (1987) 0, 20, 100, 500 mg/kg/day	Systemic Maternal NOAEL = 100 mg/kg/day Systemic Maternal LOAEL = 500 mg/kg/day based on mortality, clinical signs, decreased body weight gains, decreased food consumption, and increased liver and spleen weights, Developmental NOAEL =100 mg/kg/day. Developmental LOAEL = 500 mg/kg/day based on reduced ossification of the vertebrae and ribs and thickened, wavy or bent ribs and increased incidences of resorptions
870.3700 [83-3(a)] Developmental Toxicity Study - Rat	00152419 (1984) 0, 100, 225, 470, or 1000 mg/kg/day Acceptable/Guideline	Maternal NOAEL = 475 mg/kg/day Maternal LOAEL = 1000 mg/kg/day based decreased body weights and decreased food consumption. Offspring NOAEL = 475 mg/kg/day Offspring LOAEL = 1000 mg/kg/day based on decreased fetal body weights. Developmental NOAEL = 1000 mg/kg/day Developmental LOAEL was not established.
870.3700 [83-3(b)] Developmental Toxicity - Rabbit	00152421 (1984) 0, 100, 225, 500 mg/kg/day Acceptable/Guideline	Maternal NOAEL = 100 mg/kg/day Maternal LOAEL = 225 mg/kg/day based on abortions, macroscopic changes in the liver and lungs, and decreased food consumption. Developmental NOAEL = 100 mg/kg/day Developmental LOAEL = 225 mg/kg based on abortions.

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.3800 [83-4] 2-Gen Repro - Rat	00151901 (1984) 00151902 (1984) Feed analysis 00151903 (1984) Path 0, 200, 650, 2000 ppm 0, 20, 32.5, 200 mg/kg/day (1 ppm = 0.5 mg/kg/day) Acceptable/Guideline	Parental NOAEL = 200 ppm (10 mg/kg/day). Parental LOAEL = 650 ppm (32.5 mg/kg/day) based on mortality due to acute renal failure and increased lesions of the renal proximal tubules in the F1 females; increased relative (to body) weights of the liver, kidney (males), and testes in both generations. Offspring NOAEL = 200 ppm (10 mg/kg/day) Offspring LOAEL = 650 ppm (32.5 mg/kg/day) based on decreased pup weights in both generations and increased relative to body liver weights in the F2b females Repro NOAEL = 2000 ppm (100 mg/kg/day) Repro LOAEL = Not established.
870.3800 [83-4] 2-Gen Repro - Rat	00162543 (1986) 44135107 (1996) 0, 200, 630, 2000 ppm 0, 15, 47, 148 mg/kg/day Acceptable/Guideline	Parental NOAEL = 200 ppm (15 mg/kg/day) Parental LOAEL = 630 ppm (47 mg/kg/day), based on decreased BWG and food consumption Offspring NOAEL = 200 ppm (15 mg/kg/day) Offspring LOAEL = 630 ppm (47 mg/kg/day) based on small pup size in 3 litters Repro NOAEL = 2000 ppm (148 mg/kg/day) Repro LOAEL = Not established

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.3800 [83-4] 2-Gen Repro - Rat	40405007 (1987) 0, 50, 450, 4000 ppm M: 0, 3.9, 35, 295 mg/kg/day F: 0, 4.7, 42, 337 mg/kg/day Acceptable/Guideline	Parental NOAEL = 450 ppm [35/42 mg/kg/day M/F] Parental LOAEL = 4000 ppm [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. Offspring NOAEL = 450 ppm (35/42 mg/kg/day M/F) Offspring LOAEL = 4000 ppm [295/337mg/kg/day , M/F] based on decreased pup weight in F1a litters Repro NOAEL = 450 ppm (35/42 mg/kg/day) Repro LOAEL = 4000 ppm [295/337 mg/kg/day , M/F], based on decreased fetal, neonatal, and litter viability and decreased lactation index in the F1a pups; and decreased number of implantation sites, newborn pups, litter size, and pup weights in both generations.
870.4100 [83-1(b)] 1-Year Oral (capsule) Study - Dog	00151908(1984), 00159618 (1985) 0, 30, 150, or 750 ppm 0.0, 0.8, 3.8, 18.8 mg/kg /day Acceptable/guideline	NOAEL =30 ppm (0.8 mg/kg/day) LOAEL = 150 ppm (3.8 mg/kg/day) based on increased absolute liver weights in males
870.4100 [83-1(b)] 1-Year Oral (capsule) Study - Dog	42447001 (1992) 0, 0.75, 2.4, 40 mg/kg/day Acceptable/Guideline	Systemic NOAEL = 2.4 mg/kg/day Systemic LOAEL = 40 mg/kg/day, based on increased frequency of abnormal stool and pigment deposition in the kidney and liver in males and females, decreased body weights and body weight gains, and on decreased erythrocytes and hemoglobin and increased thrombocytes in males.

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.4300 [83-5(a)] 24- Month Chronic Toxicity/ Carcinogenicity Study - Rat	00162457 (1985), 00162458 (1985) 0, 200, 800, or 3200 ppm M: 0, 10, 40, and 169 mg/kg/day F: 0, 13, 53, and 219 mg/kg/day Acceptable/guideline	NOAEL = 800 ppm (40/53 mg/kg/day M/F). LOAEL = 3200 ppm (169/219 mg/kg/day M/F) based on decreases in body weight and body weight gains. 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
870.4300 [83-2(a)] 24- Month Carcinogenicity Study - Mouse	00158935 (1986), 40392313 (1987) 0, 50, 200, or 800 ppm M: 0, 7.5, 29, and 118 mg/kg/day F: 0, 10.5, 41, and 165 mg/kg/day Unacceptable/guideline	Sys NOAEL = 800 ppm (118/165 mg/kg/day in males/females) Sys LOAEL = Not achieved 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
870.5100 Bacterial reverse gene mutation assay	MRID 00148345 (1984) Acceptable/Guideline	Trifluralin was tested up to the limit of solubility (400 µg/plate -S9; 800 µg/plate +S9). No cytotoxicity was observed in any strain at up to 800 (+S9) or 400 (-S9) µg/plate. No treatment-related increases in revertant colonies were observed at any dose in any strain (±S9). The positive controls induced the appropriate responses. There was no evidence of induced mutant colonies over background
870.5100 Bacterial reverse gene mutation assay	MRID 40334707 (1987) Acceptable/Guideline	In a reverse gene mutation assay in bacteria (MRID 40334707), Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to trifluralin (96.8% a.i., Lot/Batch #: 335/336) in dimethylsulfoxide (DMSO) at concentrations of 30, 100, 300, 1000, 3000, or 10,000 µg/plate in the presence and absence of mammalian metabolic activation (S9). The standard plate incorporation method was used. Standard strain-specific mutagens served as positive controls. Trifluralin was tested up to the limit of solubility (3000 µg/plate, +/-S9). No cytotoxicity was observed in any strain at up to 3000 µg/plate (+/-S9). No treatment-related increases in revertant colonies were observed at any dose in any strain (+/-S9). The positive controls induced the appropriate responses. There was no evidence of induced mutant colonies over background.

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.5100 Bacterial reverse gene mutation assay	MRID 00153173 (1979) Acceptable/Guideline	<p>In a reverse gene mutation assay in bacteria (MRID 00153173), <i>Salmonella typhimurium</i> strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to HOE 38474 (trifluralin; purity not reported; Lot/Batch #: OH AT204) in DMSO at concentrations of 0, 4, 20, 100, 500, 2500, or 10,000 $\mu\text{g}/\text{plate}$ (+/-S9). Standard strain-specific positive controls were used.</p> <p>HOE 38474 was tested up to the limit dose (10,000 $\mu\text{g}/\text{plate}$, +/-S9). No treatment-related increases in revertant colonies were observed at any dose in any strain (\pmS9). The positive controls induced the appropriate responses. There was no evidence of induced mutant colonies over background.</p>
870.5250 Gene Mutation Assay - Yeast	MRID 00151898 ((1982) Acceptable/Guideline	<p>The test material was tested up to the limit of solubility (1000 mg/L); however, no solubility data were provided. No treatment-related increases in mutation frequency were observed at any dose with or without S9-activation. The positive controls induced the appropriate response. There was no concentration-related positive response of induced mutant colonies over background.</p>
870.5300 <i>In vitro</i> mammalian cell gene mutation assay	MRID 00126661 Acceptable/Guideline	<p>In a mammalian cell gene mutation assay at the thymidine kinase (TK) locus, mouse lymphoma L5178Y cells cultured <i>in vitro</i> were exposed to trifluralin (95.0% a.i.; Lot/Batch #: 00554AP2) in DMSO for 4 hours at 8 concentrations ranging from 0.5 to 20 $\mu\text{g}/\text{mL}$ (individual doses not reported) both in the presence and absence of S9-activation.</p> <p>Trifluralin was tested up to cytotoxic concentrations (20 $\mu\text{g}/\text{mL}$, +/-S9). No treatment-related increases in mutation frequency were observed at any dose compared to controls. The positive controls induced the appropriate response. There was no concentration-related positive response of induced mutant colonies over background.</p>

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.5450 Dominant Lethal - Rat	MRID 00148319 (1984) Acceptable/Guideline	<p>In a dominant lethal assay (MRID 00148319), groups of 20 male Sprague-Dawley (CD) rats/dose were treated once daily via gavage (1.0 mL/dose) with Triflurex technical (trifluralin; 97.3% a.i.; Batch #: 5320), in corn oil for 5 consecutive days at doses of 0, 100, 333, or 1000 mg/kg/day (total doses of 0, 500, 1665, or 5000 mg/kg). Beginning two days after the last exposure, each male was mated sequentially to two untreated female rats per week for seven weeks. At 14 days after the midpoint of each mating week, the females were killed, determined to be pregnant or not pregnant, and the number of corpora lutea, living, dead, and total implantations was determined. On the fifth day of dosing, the positive control males were given a single dose of triethylenemelamine (TEM; 0.3 mg/kg, i.p. in 0.9% saline).</p> <p>One 1000 mg/kg male (#8476) was found dead 72 hours after the last treatment. Triflurex technical was tested at the limit dose (5000 mg/kg = 1000 mg/kg/day X 5 days). There were no treatment-related effects on fertility, number of implants, pre-implantation losses, number of dead implants, number of females with ≥ 1 or ≥ 2 dead implants, or ratio of dead implants to total implants at any dose in the study. The positive control induced the appropriate response. There was no time-related positive response of increased pre- or post-implantation loss compared to controls.</p>

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.5300 Forward Gene Mutation Assay	MRID 40765601 (1988) Acceptable/Guideline	<p>In a mammalian cell gene mutation assay at the HGPRT locus (MRID 40765601), Chinese hamster ovary (CHO) cells cultured in vitro were exposed to trifluralin (97.6% a.i.; Lot/Batch #: 39) in dimethyl sulfoxide for 4 hours at concentrations of 50, 100, 150, 200, 300, 400, or 500 $\mu\text{g}/\text{mL}$ (Trial 1, -S9); 50, 100, 200, 300, 500, 600, or 700 $\mu\text{g}/\text{mL}$ (Trial 2, -S9); 50, 100, 200, 250, 300, 400, or 500 $\mu\text{g}/\text{mL}$ (Trial 1, +S9); and 50, 100, 200, 300, 400, 500, or 600 $\mu\text{g}/\text{mL}$ (Trial 2, +S9).</p> <p>Trifluralin was tested up to cytotoxic concentrations ($\geq 200 \mu\text{g}/\text{mL}$, +/-S9) and the limit of solubility ($\geq 100 \mu\text{g}/\text{mL}$, +/-S9). No treatment-related increases in mutant frequency were observed in either trial in the presence or absence of S9. The positive controls induced the appropriate response. There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.</p>
870.5300 Forward Gene Mutation Assay	MRID 00148318 (1984) Acceptable/Guideline	<p>In a mammalian cell gene mutation assay at the HGPRT locus (MRID 00148318), Chinese hamster ovary (CHO) cells cultured in vitro were exposed to triflurex technical (trifluralin; purity not reported; Lot/Batch #: not reported) in ethanol for 4 hours at concentrations of 10, 50, 100, 200, 300, 400, or 500 $\mu\text{g}/\text{mL}$ (-S9) and 50, 100, 200, 300, 400, 500, or 600 $\mu\text{g}/\text{mL}$ (+S9).</p> <p>Triflurex technical was tested up to cytotoxic concentrations ($\geq 200 \mu\text{g}/\text{mL}$, -S9 and $\geq 300 \mu\text{g}/\text{mL}$, +S9) and the limit of solubility ($\geq 100 \mu\text{g}/\text{mL}$, +/-S9). No treatment-related increases in mutant frequency were observed in either trial in the presence or absence of S9. The positive controls induced the appropriate response. There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.</p>

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.5385 <i>In Vivo</i> Mammalian Cytogenetics [Bone Marrow/Spermatogonial Aberration Test]	MRID 40765603 (1988) Acceptable/Guideline	<p>In a bone marrow/spermatogonial chromosome aberration assay (MRID 40765603), ICR mice (10 males/dose, spermatogonial tissue; and 5/sex/dose, bone marrow) were dosed once daily via gavage (10 mL/kg) with trifluralin (97.6% a.i., Lot/BatchNo 39) in corn oil at doses of 0, 62.5, 208, or 625 mg/kg for 5 consecutive days. Bone marrow and spermatogonial cells were harvested at 4.5 hours after the last treatment.</p> <p>Mortalities were observed in the 625 mg/kg females (2/5 treated vs 1/5 controls), and at 62.5 mg/kg in the males (2/10 treated vs 1/10 controls) and females (2/5 treated vs 1/5 controls). Clinical signs of toxicity (lethargy, swollen neck, and yellow stains around the mouth and perianal area) were also observed at ≥ 62.5 mg/kg. No statistically significant increases in the percent of aberrant cells were observed at any dose in either sex in the bone marrow assay or in the males in the spermatogonial assay. Trifluralin was tested at an adequate dose based on mortalities observed at ≥ 62.5 mg/kg. The positive control induced the appropriate response. There was no evidence of chromosome aberration induced over background</p>

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.5385 <i>In Vivo</i> Mammalian Cytogenetics [Bone Marrow Chromosome Aberration Test]	MRID 00148320 Acceptable/Guideline	<p>In a bone marrow chromosome aberration assay (MRID 00148320), 5 Sprague-Dawley (HSD:([SD] BR) rats/sex/dose/sacrifice time were treated once via oral gavage with Triflurex technical (trifluralin; 97.3% a.i.; Batch #: 5320), in corn oil at doses of 0, 500, 1650, or 5000 mg/kg. Bone marrow cells were harvested at 6, 24, or 48 hours after treatment.</p> <p>Mortality was observed at 1650 (3/15 males and 5/15 females) and 5000 (2/15 males and 1/15 females) mg/kg upon initial dosing; however, these animals were replaced and only one replacement 1650 mg/kg female in the 48 hour group died after dosing. Triflurex technical induced minimal bone marrow toxicity (as indicated by decreased mitotic index) at ≥ 500 mg/kg in males and ≥ 1650 mg/kg in females. Dosing was considered adequate based on bone marrow toxicity and that the animals were dosed above the limit dose (2000 mg/kg). No statistically significant increases in the percent of aberrant cells were observed at any dose or sampling time compared to concurrent controls. The positive control induced the appropriate response. There was no evidence of chromosome aberration induced over background.</p>

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.5395 <i>In vivo</i> Mouse Erythrocyte Micronucleus assay	MRID 00151895 (1981) Acceptable/Guideline	<p>In a bone marrow micronucleus assay, 5 NMRI mice/sex/dose were treated via oral gavage with HOE 38474 (Trifluralin; 98.3% a.i., Lot/Batch #: OH AT208), in sesame oil at doses of 0, 25, 250, or 2500 mg/kg on two consecutive days (24 hours apart). Bone marrow cells were harvested at 6 hours after the last treatment.</p> <p>No unscheduled deaths occurred during the study. No clinical signs of toxicity were observed. No statistically significant differences in the number of micronucleated polychromatic erythrocytes (MPCE) or normocytes and no decrease in polychromatic erythrocyte to normocyte (PCE:NCE) ratios were noted in the treated animals compared to controls; however, only individual data were provided. Additionally, although no evidence of cytotoxicity (decreased PCE:NCE) was noted in the bone marrow, the animals were sufficiently dosed (the limit dose was given twice). The test material was absorbed as indicated by the presence of orange urine in the 2500 mg/kg animals. The positive control induced the appropriate response. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow compared to controls.</p>
870.5450 Dominant Lethal - Mouse	MRID 00151896 (1984) Acceptable/Guideline	<p>In a dominant lethal assay (MRID 00151896), 30 male NMRI mice were dosed once daily for 5 consecutive days via oral gavage (5 mL/kg) with HOE 38474 (trifluralin; 98.3% a.i.; Lot/Batch #: OHZD99002), in sesame oil at concentrations of 0, 10, 100, or 1000 mg/kg. After the final treatment, each male was mated with 13 untreated females during separate 4-day intervals over a 52 day period.</p> <p>No treatment-related mortalities were noted during the study. No treatment-related effects on clinical signs, body weight, fertilization rate, and pre- or post-implantation loss were observed; however, no data were provided. The positive control (cyclophosphamide) increased the number of post-implantation fetal losses. There was no time-related positive response of increased pre- or post-implantation loss compared to controls.</p>

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.5550 Unscheduled DNA synthesis in mammalian cell culture	MRID 40765602 (1988) Acceptable/Guideline	<p>In an unscheduled DNA synthesis assay (MRID), primary rat hepatocyte cultures were exposed to trifluralin (97.6% a.i.; Lot/Batch #: 39) in DMSO for 18-19 hours at concentrations of 0, 0.032, 0.214, 0.404, 0.917, 2.22, 4.36, 8.52, 21.3, 42.9, 88.0, 448, or 898 $\mu\text{g}/\text{mL}$. Fifteen doses ranging from 0.032-898 $\mu\text{g}/\text{mL}$ were used in each assay; however, the three doses between 0.032 and 0.214 $\mu\text{g}/\text{mL}$ were not reported.</p> <p>Trifluralin was tested up to cytotoxic levels (determined by trypan blue exclusion), 88.0 $\mu\text{g}/\text{mL}$ in rat #1 and 42.9 $\mu\text{g}/\text{mL}$ in rat #2. There were no marked increases observed in the mean NNG or percent cells in repair at any dose in either trial. The positive controls induced marked increases in mean NNG and the percent of cells in repair. There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures (nuclear silver grain counts), was induced.</p>
870.5550 Unscheduled DNA synthesis in mammalian cell culture	MRID 00151894 (1982) Acceptable/Guideline	<p>In an unscheduled DNA synthesis assay (MRID 00151894), HeLa cell cultures were exposed to HOE 38474 (trifluralin, 98.3% a.i.; Lot/Batch #: OH AT 208) in DMSO for 1 hour at concentrations of 0, 50, 100 or 500 $\mu\text{g}/\text{mL}$ both in the presence and absence of S9-activation.</p> <p>HOE 38474 was tested up to cytotoxic concentrations ($\geq 50 \mu\text{g}/\text{mL}$, +/-S9). No statistically significant increases in mean counts per minute of the test material with hydroxyurea were noted compared to concurrent solvent controls at any dose level, either in the presence or absence of S9-activation. The positive controls induced the appropriate response. There was no evidence that unscheduled DNA synthesis, as determined by liquid scintillation counting procedures, was induced.</p>

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.5900 <i>In vivo</i> Sister Chromatid Exchange Assay	MRID 00133426 (1983) Acceptable/Guideline	<p>In a mammalian cell cytogenetics assay [SCE] using the BrdU tablet method , groups of 3 female Chinese hamsters/dose were exposed once via gavage (10 mL/kg) to trifluralin (95.0% a.i., Lot/batch No 00554AP2) in DMSO and 10% acacia at concentrations of 200, 300, 400, or 500 mg/kg at 5 hours following BrdU tablet implantation. Bone marrow was collected at 21 hours after treatment.</p> <p>Trifluralin was tested up to cytotoxic concentrations (≥ 400 mg/kg). Cytotoxicity (as indicated by an increase in the number of first division metaphase figures) was observed in all animals at ≥ 400 mg/kg. No statistically significant increases in SCE frequency were observed at any dose compared to controls. The positive control induced the appropriate response. There was no evidence of SCE induced over background</p>

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Comments
870.7845 (85-1) Metabolism - Rat Urinary metabolites	41218901 (1989) Acceptable/Guideline	<p>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).</p> <p>Four metabolic pathways were identified as follows: (i) oxidative N-dealkylation of one or both propyl groups and metabolites which were hydroxylated on the propyl side chain; (ii) reduction of one or both nitro groups to the corresponding amine; (iii) cyclization reactions to give a variety of substituted and unsubstituted benzimidazole metabolites; and (iv) conjugation reactions, including acetylation of the reduced nitro groups, sulfate, and glucuronic acid conjugates.</p>
Special study 3-Mo Feeding - Rat with Urinalysis study	00157156 (1985), 40138301(1986) 41086101 (1989) 0, 50, 200, 800, 3200, and 6400 ppm 0, 2.6, 10.7, 42.2, 170.2, and 342.1 mg/kg/day Acceptable/Nonguideline	NOAEL = 200 ppm (10.7 mg/kg/day) LOAEL for nephrotoxicity = 800 ppm (42.2 mg/kg/day), based on the presence of cortical tubular cytoplasmic hyaline droplets; increased total protein, AST, and LDH in the urine; and increased urinary volume upon protein electrophoresis and urinalysis.

8.2 Summary of Toxicological Dose and Endpoints

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 = <u>acute RfD</u> FQPA SF = 1.0 mg/kg/day	Developmental Toxicity Study - Rat LOAEL = 500 mg/kg/day based on increased total litter 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 = <u>chronic RfD</u> 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 α 1-globulin and α 2-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 α 1-globulin and α 2-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 = 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

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 α 1-globulin and α 2-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 = 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
Cancer (oral, dermal, inhalation)	Q1* = 0.00579 (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.