Caswell

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

JUL 14 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Review of the Chronic/oncogenicity studies in mice and rats on Trifluralin

To: Carol Gray/Richard Mountfort, PM-23
Registration Division, TS-767C

From: Marcia van Gemert, Ph.D. M. van Gemert 7/13/87
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CASWELL # 889

Two chronic/oncogenicity studies on Trifluralin were submitted to the Agency for review. Toxicology Branch sent both studies to the contractor Dynamac for review and the DERs are attached. The summary of the conclusions are presented below.

1. Oncogenicity Study with trifluralin Active ingredient technical (HOE 38474 0 H AT210) in mice. RCC 0008853, Feb. 7, 1986.
The conclusions were as follows: Under the conditions of the study, there was a significant increase ($p \leq 0.05$) in hepatocellular carcinomas in males receiving 200 ppm trifluralin but not in males at the higher dose (800 ppm) when compared to concurrent controls. The oncogenic potential of trifluralin cannot be fully assessed without laboratory historical control data for hepatocellular neoplasms in NMRI mice. No compound-related clinical symptoms or signs of toxicity were noted. No compound-related changes in body weights or food consumption were noted. Ophthalmoscopic examinations hearing tests, and examination of teeth and mucous membranes revealed no compound-related findings. Assessment of hematology data indicated a statistically significant increase in methemoglobin and Heinz bodies in low (50 ppm), and mid (200 ppm) and high (800 ppm) males and high (800 ppm) dose females at 52 weeks; these increases were not considered to be of biological significance. Methemoglobin was also significantly increased in high (800 ppm) dose males and Heinz bodies were significantly increased in mid and high dose males at 104 weeks. These increases were considered to be treatment-related. There were no changes of toxicological significance in clinical biochemistry data at 54 and 104 weeks. After 104 weeks, higher mean absolute and relative liver weights were noted for male mice that received 200 and 800 ppm trifluralin and for female mice that received 800 ppm trifluralin. Based on the organ weight changes, the NOEL for systemic toxicity is 50 ppm in male mice and 200 ppm in female mice. The Oncogenic NOEL is for the present reserved pending receipt of the historical control data.
The study is classified as Core Supplementary. This classification

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may be upgraded upon receipt of the laboratory's historical control data for the incidence of hepatocellular tumors in NMRI mice, and summary tabulation of gross pathology findings.

2. Trifluralin (code: Hoe 38474 OH AT208) chronic feeding study (24 months) in rats and trifluralin (code: Hoe 38474 OH AT208) carcinogenicity study in rats (28-month feeding study). 680 (chronic A32718, oncogenicity A32719) March 26 1985 and april 2, 1985.

When 200, 800 or 3200 ppm trifluralin was fed to Wistar rats for 24 months in a chronic toxicity study, there were no overt signs of toxicity or dose-related effects on mortality, clinical biochemistry or histopathology. Body weight gain and food consumption were decreased throughout the study in males and females receiving 3200 ppm. Body weights were also decreased at study termination in males receiving 800 ppm. There were significant ($p < 0.05$) decreases in red cell parameters in high dose males and females. There were also nonsignificant decreases in liver and thyroid weights in males and females receiving 3200 ppm, although there were no histologic findings that correlated with the organ weight changes.

In an oncogenicity study conducted simultaneously, 200, 800 and 3200 ppm trifluralin were not oncogenic when fed to male and female Wistar rats for 28 months. Tumor incidence was found to be age, sex or strain related and was not due to compound treatment. Body weight gain and food consumption were decreased throughout the study in males and females receiving 3200 pm. Body weights were decreased in the 800 ppm female group during the last 6 months of the study, and in males of this group at study termination. As in the chronic study, there were nonsignificant increases in liver and thyroid weights in males and females receiving 3200 ppm. Based on the body weight changes, the LOEL is 800 ppm and the NOEL is 200 ppm. The oncogenic NOEL is 3200 ppm (HDT).

The core classification for this study is guideline.

EPA: 68-02-4225
DYNAMAC No. 297-A
July 10, 1987

DATA EVALUATION RECORD

TRIFLURALIN

Oncogenicity Feeding Study in Mice

APPROVED BY:

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Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-10-87

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EPA: 68-02-4225
DYNAMAC No. 297-A
July 10, 1987

DATA EVALUATION RECORD

TRIFLURALIN

Oncogenicity Feeding Study in Mice

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DATA EVALUATION REPORT

MRID NO.:

STUDY TYPE: Oncogenicity feeding study in mice.

ACCESSION NUMBER: 262516-262520.

TEST MATERIAL: Trifluralin; 2,6-dinitro-N,N-dipropyl-4-trifluoromethyl-aniline.

SYNONYMS: HOE 38474 O H AT210.

STUDY NUMBER(S): RCC 008853.

SPONSOR: Hoechst Aktiengesellschaft, Frankfort, Federal Republic of Germany.

TESTING FACILITY: RCC, Research and Consulting Company, AG, Itingen, Switzerland.

TITLE OF REPORT: Oncogenicity Study with Trifluralin Active Ingredient Technical (HOE 38474 O H AT210) in Mice.

AUTHOR(S): Suter, D., Horst, K., Vogel, W., Luetkemeier, H., Schlotke, B., Terrier, Ch., and Sachsse, K.

REPORT ISSUED: February 7, 1986.

CONCLUSIONS: Under the conditions of the study, there was a significant increase ($p \leq 0.05$) in hepatocellular carcinomas in males receiving 200 ppm trifluralin but not in males at the higher dose (800 ppm) when compared to concurrent controls. The oncogenic potential of trifluralin cannot be fully assessed without laboratory historical control data for hepatocellular neoplasms in NMRI mice. No compound-related clinical symptoms or signs of toxicity were noted. No compound-related changes in body weights or food consumption were noted. Ophthalmoscopic examinations, hearing tests, and examination of teeth and mucous membranes revealed no compound-related findings. Assessment of hematology data indicated a statistically significant increase in methemoglobin and Heinz bodies in low (50 ppm)-, mid (200 ppm)-, and high (800 ppm)-dose males and high (800 ppm)-dose females at 52 weeks; these increases were not considered to be of biological significance. Methemoglobin was also significantly increased in high (800 ppm)-dose males and Heinz bodies were significantly increased in mid (200 ppm)- and high (800 ppm)-dose males at 104 weeks. These increases were considered to be treatment related. There were no changes of toxicological significance in clinical biochemistry data at 54 or 104 weeks. After 104 weeks, higher mean absolute and relative liver weights were noted for male mice that received 200 and 800 ppm trifluralin and for female mice that received 800 ppm trifluralin. Based on the organ weight changes, the NOEL for systemic toxicity is 50 ppm in male mice and 200 ppm in female mice. ONCOGENIC NOEL is reserved pending receipt of historical control data.

Classification: Core Supplementary. This classification may be upgraded upon receipt of the laboratory's historical control data for the incidence of hepatocellular tumors in NMRI mice, and summary tabulation of gross pathology findings.

A. MATERIALS:

1. Test Compound: Trifluralin active ingredient (technical), Code: HOE 38474 0 H AT210; description: red orange; purity: >99%.

2. Test Animals: Species: mouse; strain: NMRI, KFM-Han, outbred, SPF quality; age: 4 weeks; mean weights: males--15-23 g and females--14-22 g; source: KFM, Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland.

B. STUDY DESIGN:

1. Animal Assignment: After 8 days of acclimation, the animals were assigned to the following groups using a computer-generated random algorithm:

Test group	Dose in diet (ppm)	Oncogenicity[a] Study (24 months) Males	Oncogenicity[a] Study (24 months) Females	Laboratory Investigations and Interim Sacrifice[b] (12 months) Males	Laboratory Investigations and Interim Sacrifice[b] (12 months) Females
1 Control	0	50	50	10	10
2 Low (LDT)	50	50	50	10	10
3 Mid (MDT)	200	50	50	10	10
4 High (HDT)	800	50	50	10	10

[a]Laboratory investigations conducted at termination of the study on all remaining animals for hematology analysis and on 10 mice per sex per group with the highest identification numbers for clinical biochemistry analysis.

[b]Animals were assigned the 10 highest identification numbers in each group for each sex.

Dose Selection: The dose levels selected for this study were based on a 3-month subchronic toxicity study in mice in which a dose level of 1000 ppm caused "slight effects" (effects were not reported) on the liver and hematopoietic system. Considering that the severity of these effects could be cumulative during a longer study, 800 ppm was chosen as the highest dose level.

2. Diet Preparation: Pelletized diets were prepared at least twice monthly and stored at 4°C. Food and water were available to the animals ad libitum. Samples of food containing test material were analyzed for stability and homogeneity prior to study initiation. Samples were analyzed for concentration and homogeneity at 3-month intervals during the study.

   Results: During the study, diets were found to be homogeneous within +/-15% of target for each group, with the exception of group 3 in week 28. Mean concentrations were found to be >90% of the target at each level. In a previous study, dietary levels of 200 to 2000 ppm were shown to be stable over a period of 21 days at room temperature.

3. Animals received food (pelleted standard Kliba) and water ad libitum and were housed individually.

4. Statistics: The following methods were used to analyze body weight, food consumption, organ weight, and clinical laboratory data. Univariate one-way analysis of variance was used to assess the significance of intergroup differences. If the variables could be assumed to follow a normal distribution, the Dunnett test based on a pooled variance estimate was applied for the comparison between the treated groups and the control groups. The Steel test was applied when the data could not be assumed to follow a normal distribution. For the overall spontaneous mortality data, the Fisher's exact test for 2 x 2 tables was applied. Group means were calculated for discrete data in the summary tables. Individual values, means, standard deviations, and statistics were rounded off before printing. Significant departures from the control group values were reported at the $p < 0.05$ or $p < 0.01$ level.

5. A quality assurance statement and a statement of compliance were each signed and dated on March 5, 1986.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily, morning and afternoon, for signs of toxicity and mortality. In addition, each animal had a detailed weekly clinical examination that included a palpation for tissue masses. A description of any lesion or mass observed at any examination was recorded and the subsequent progress was monitored. Examinations of the teeth and mucous membranes were performed weekly during the first 3 months and twice monthly thereafter.

   Results: It was reported that no compound-related clinical signs of toxicity were noted. Individual animal data were not provided. Palpable mass observations were not provided.

   A total of 300 mice (132 males/168 females) died during the study or were killed in a moribund state. Overall mortality was reported to be significantly greater only for the males receiving 50 ppm but was considered not to be compound-related; there were no dose-related differences in the mortality rate of any group during the study. Mortality and survival data are presented in Table 1.

2. Body Weight: Mice were weighed weekly during months 1 to 3 and twice monthly thereafter.

   Results: The data on mean body weight gain for all compound-treated groups were reported to be comparable with controls; however, significantly increased mean body weights were noted sporadically for males receiving 200 ppm and females receiving 200 and 800 ppm trifluralin. Table 2 presents mean body weight data at selected intervals.

TABLE 1. Mortality and Percent Survival of Mice Fed Trifluralin for 24 Months

Dose Group (ppm)	Cumulative Mortality (percent survival)[a] at Days				
	180	360	540	720	736 (Termination)
			MALES		
0	2(96.7)	5(91.7)	12(80.0)	26(56.7)	28(53.3)
50	1(98.3)	6(90.0)	10(83.3)	38(36.7)	42(30.0)[b]
200	1(98.3)	3(95.0)	11(81.7)	28(53.3)	29(51.7)
800	0(100.0)	3(95.0)	8(86.7)	32(46.7)	33(45.0)
			FEMALES		
0	4(93.3)	7(88.3)	21(65.0)	37(38.3)	41(31.7)
50	2(96.7)	9(85.0)	24(60.0)	41(31.7)	42(30.0)
200	1(98.3)	3(95.0)	17(71.7)	42(30.0)	44(26.7)
800	0(100.0)	5(91.7)	15(75.0)	39(35.0)	41(31.7)

[a]Based on 60 mice/group.

[b]Reported to be statistically significant by the study authors; significance level not indicated by study authors.

TABLE 2. Representative Mean Body Weights of Mice Fed Trifluralin for 24 Months

Dose Group (ppm)	Mean Body Weights (g) ± S.D. at Week						
	0	1	13	27	53	79	104
				Males			
0	19±1.1(60)[a]	30±2.0(60)	46±5.8(60)	52±7.0(58)	55±6.8(47)	54±6.0(40)	48±6.6(24)
50	19±1.4(60)	30±2.0(60)	48±6.4(60)	54±7.7(59)	56±7.0(45)	53±7.1(41)	45±6.5(13)
200	19±1.9(60)*	30±2.6(60)	48±5.2(60)	54±5.9(59)	57±5.7(48)	54±7.4(40)	48±7.5(22)
800	20±1.9(60)**	30±2.0(60)	46±4.9(60)	51±6.4(60)	54±6.0(46)	52±5.9(40)	48±6.2(17)
				Females			
0	18±1.6(60)	23±1.5(60)	31±2.9(60)	35±3.8(56)	40±5.0(43)	41±4.7(30)	39±6.3(11)
50	19±1.7(60)	23±1.7(60)	30±3.1(60)	35±4.8(58)	40±6.6(43)	41±6.5(29)	40±5.1(12)
200	18±1.7(60)	24±1.9(60)**	32±3.4(60)	36±5.2(59)	40±6.4(47)	43±9.1(35)	40±5.3(8)
800	18±1.7(60)	24±2.1(60)	32±3.9(60)	36±6.1(60)	40±6.8(46)	41±7.6(33)	43±4.4(9)

[a]Number in parentheses represents number of animals weighed.

*Significantly different from control value ($p \leq 0.05$), as determined by the Dunnett test.

**Significantly different from control value ($p \leq 0.01$), as determined by the Dunnett test.

3. Food Consumption and Food Nominal Dosage Level: Food consumption was recorded for a 7-day period and mean daily food consumption was calculated. These data were recorded weekly during months 1 to 3 and twice monthly thereafter. The nominal dosage level in food was calculated for each cage (animal) using the following formula:

$$NDL = \frac{C \times ND}{AD \times BW}, \text{ where}$$

C = Measured cage food consumption (in g food/interval).

ND = Nominal dose (mg test material/g food).

AD = Total of consumption days over all animals in the cage during the consumption interval (the mortality during the consumption interval is therefore taken into account).

BW = Average body weight over all weighted animals in the cage during the consumption interval (in kg).

Group means were calculated thereafter.

Results: Significantly increased mean food consumption values were noted sporadically throughout the study in the compound-treated groups when compared to controls. However, the overall mean food consumption values over the entire treatment period were comparable to controls. Food efficiency was not determined. The overall mean food consumption values and the mean daily nominal test article consumption values reported for the 104 weeks of treatment are presented in Table 3.

4. Ophthalmoscopic Examinations and Hearing Tests: Ophthalmoscopic examinations were performed on 10 mice per sex per group with the lowest identification numbers at pretest and at 6, 8, 12, 18, and 24 months. Ten mice per sex per group with the lowest identification numbers were tested for hearing impairment using a tone generator (frequency of 10 kHz, volume of 80 dB, duration of 30 milliseconds, the test was repeated five times with 2-second pauses between each test) at pretest and at 6, 8, 12, 18, and 24 months. The evidence of Preyer's reflex constituted a positive reaction.

Results: No compound-related effects were reported for any animal of any group for either the ophthalmoscopic examinations or hearing tests. No data were provided.

TABLE 3. Mean Food Consumption and Food Nominal Dosage Level for Mice Fed Trifluralin for 24 Months[a]

Dose Group (ppm)	Overall Mean Food Consumption (g/animal/day)	Overall Food Nominal Dosage Level mg substance/kg body weight/day
	Males	
0	7	0.00
50	7	7.50
200	7	28.76
800	7	118.15
	Females	
0	7	0.00
50	7	10.48
200	7	41.30
800	7	164.63

[a]Number of animals/group for which data were analyzed and standard deviations of the mean values were not reported.

5. Hematology and Clinical Chemistry: Blood was collected from the retro-orbital plexus of animals under light ether anesthesia for hematology and clinical biochemistry parameters. Prior to sacrifice at 52 weeks, blood was collected from 10 mice per sex per group with the highest identification numbers. The animals were nonfasted for hematology analysis, whereas they were fasted for 18 hours (with water provided) before blood sampling for methemoglobin and clinical biochemistry analysis. Additionally, a differential blood count was made on all animals (nonfasted) from the oncogenicity groups at 52 weeks.

At termination of the study (104 weeks), blood was collected from all of the remaining animals (nonfasted) for hematology analysis and blood was collected from a minimum of nine mice (fasted) per sex per group with the highest identification numbers for clinical biochemistry analysis.

The CHECKED (X) parameters were examined:

a. Hematology

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
Platelet count†
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Nucleated erythrocytes - normoblasts
X Heinz bodies
X Methemoglobin
X Red cell morphology
  X Polychromatophilia
  X Anisocytosis
  X Microcytes
  X Poikolocytosis
  X Target cells
  X Hypochromia
  X Rouleaux formation of erythrocytes
  X Howell-Jolly bodies

†Recommended by Subdivision F (October 1982) Guidelines.

Results: Significantly ($p < 0.05$) increased methemoglobin values were reported by the study authors for males receiving 50 and 800 ppm at 52 weeks and 800 ppm at 104 weeks. Heinz bodies were also significantly ($p < 0.05$) increased at 104 weeks for males receiving 800 ppm. A significantly ($p < 0.05$) increased methemoglobin value was also reported by the study authors for females receiving 800 ppm at 52 weeks; methemoglobin was non-significantly elevated at 104 weeks for this group when compared to controls. The study authors considered these changes to be incidental and of no toxicological significance. The number of Heinz bodies was comparable among all female groups at both 52 and 104 weeks. There were no other noteworthy differences in the hematology values between groups. Selected hematology data are presented in Table 4. No standard deviations were provided with the mean hematology data, and the actual number of animals for which a blood sample was obtained and analyzed was not presented in the summary tables.

b. Clinical Chemistry

	Electrolytes		Other
	Calcium†		Albumin†
	Chloride†	X	Blood creatinine†
	Magnesium†	X	Blood urea nitrogen† (BUN)
	Phosphorus†		Cholesterol†
	Potassium†		Globulins
	Sodium†		Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase		Direct bilirubin
	Cholinesterase		Total protein†
	Creatinine phosphokinase†		Protein quotient (A/G ratio)
	Lactic acid dehydrogenase		Triglycerides
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
X	Gamma-glutamyl-transferase		

†Recommended by Subdivision F (October 1982) Guidelines.

TABLE 4. Selected Mean Hematology Values for Mice Fed Trifluralin for 24 Months

Dose Group (ppm)	Methemoglobin (%)		Heinz Bodies (%)	
	52 Weeks	104 Weeks	52 Weeks	104 Weeks
		Males		
0	1.0±0.2[a](7)[b]	1.9±0.7 (23)	14±14 (8)	15±10 (25)
50	1.6±0.6*(7)**	1.5±0.4 (9)	58±81 (9)**	17±12 (13)
200	1.5±0.3 (2)**	1.9±0.7 (21)	36±14 (9)**	25±18 (20)**
800	1.8±0.6*(6)**	2.7±1.0*(16)**	49±23 (10)**	33±24*(16)**
		Females		
0	1.2±0.3 (5)	2.3±1.8 (9)	26±18 (9)	5.2±3.3 (13)
50	1.1±0.2 (4)	1.8±0.7 (12)	18± 8 (6)	5.2±2.9 (12)
200	1.3±0.9 (4)	2.4±1.2 (8)	27±18 (9)	3.4±1.8 (8)
800	2.7±1.0*(4)	3.5±1.3 (9)**	35±18 (7)	8.3±9.2 (9)

[a]Standard deviations for mean values were calculated by our reviewers.

[b]Number in parentheses represents number of animals for which a sample was obtained and analyzed.

*Significantly different from control value ($p < 0.05$) by the Dunnett test based on pooled variance or the Steel test, as reported by study authors.

**Significantl, different from control value ($p < 0.05$) by Kruskal-Wallis nonparametric ANOVA, as calculated by our reviewers.

Results: No changes of toxicological significance were reported for the clinical biochemistry data at either 52 or 104 weeks. Significant changes in the levels of a few parameters were considered to be incidental and reported to be of normal biological variation. At 52 weeks, serum aspartate aminotransferase (ASAT) and alkaline phosphatase (ALP) levels were significantly ($p < 0.05$) elevated for the females receiving 800 ppm when compared to controls. At 104 weeks, serum alanine aminotransferase (ALAT) was significantly ($p < 0.05$) higher for the males receiving 50 ppm and gamma-glutamyl-transferase (G-GT) was significantly ($p < 0.05$) lower for the males receiving 50 and 200 ppm when compared to controls. Selected clinical biochemistry data are presented in Table 5. No standard deviations were provided with the mean clinical biochemistry data, and the actual number of animals for which a blood sample was obtained and analyzed was not presented in the summary tables.

6. Urinalyses: Urinalyses were not performed.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were weighed from all animals sacrificed on schedule (52 or 104 weeks).

	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta†	XX	Brain†
X	Salivary glands†	XX	Heart†	X	Peripheral nerve† (sciatic)
X	Esophagus†	X	Bone marrow†	X	Spinal cord
X	Stomach†		(sternum and femur)	X	Pituitary†
X	Small intestine†	X	Lymph nodes†	X	Eyes (optic nerve)†
	Cecum†	XX	Spleen†		Glandular
X	Colon†	X	Thymus†	X	Adrenals†
	Rectum†		Urogenital	X	Harderian gland
XX	Liver†	XX	Kidneys†	X	Mammary gland†
X	Gallbladder†	X	Urinary bladder†	X	Parathyroids†
XX	Liver†	XX	Testes†	X	Thyroids†
X	Pancreas†	X	Epididymides		Other
	Respiratory	X	Prostate	X	Bone (femur)†
X	Trachea†	X	Seminal vesicle	X	Skeletal muscle†
X	Lung†	XX	Ovaries	X	Skin
		X	Uterus	X	All gross lesions and masses

†Recommended by Subdivision F (October 1982) Guidelines.

TABLE 5. Selected Mean Clinical Biochemistry Values for Mice Fed Trifluralin for 24 Months[a]

Dose Group (ppm)	ASAT (ukat/L) 52 Weeks	ASAT (ukat/L) 104 Weeks	ALAT (ukat/L) 52 Weeks	ALAT (ukat/L) 104 Weeks	ALP (ukat/L) 52 Weeks	ALP (ukat/L) 104 Weeks	G-GT (nkat/L) 52 Weeks	G-GT (nkat/L) 104 Weeks
				MALES				
0	1.67 (8)[b]	1.84 (9)	0.77 (8)	1.69 (9)	2.07 (8)	2.72 (9)	42.15 (7)	211.99 (6)
50	1.41 (9)	2.80 (9)	0.99 (9)	4.76* (9)	1.90 (9)	4.37 (9)	57.15 (7)	146.97*(6)
200	1.19 (9)	2.00 (10)	1.07 (9)	1.95 (10)	1.95 (9)	4.37 (10)	41.67 (5)	136.46*(7)
800	1.54 (10)	2.52 (9)	1.25 (10)	3.04 (9)	2.64 (10)	3.98 (9)	35.72 (7)	170.99 (7)
				FEMALES				
0	1.46 (10)	3.96 (9)	0.80 (10)	3.27 (9)	2.72 (10)	5.03 (9)	58.16 (9)	186.37 (5)
50	1.53 (8)	3.35 (9)	0.57 (8)	2.62 (9)	3.52 (8)	5.04 (9)	50.29 (6)	189.56 (7)
200	1.62 (10)	2.67 (9)	0.89 (10)	1.34 (9)	2.80 (10)	4.13 (9)	58.34 (6)	161.22 (7)
800	2.32*(10)	2.39 (9)	1.36 (10)	1.89 (9)	5.00*(10)	4.18 (9)	47.93 (4)	162.25 (6)

[a] Standard deviations for mean values were not reported.

[b] Number in parentheses represents number of animals for which a sample was obtained and analyzed.

*Significantly different from control value ($p \leq 0.05$), as determined by the Dunnett test or Steel test.

Results:

a. Organ Weights: After 52 weeks, no compound-related changes were noted. After 104 weeks of treatment, a dose-related trend of higher absolute liver weights and higher liver weights relative to both body and brain weights was reported for males of the 200- and 800-ppm dose groups and for females of the 800-ppm dose group. Absolute and relative spleen weights were observed to be higher than control values for the females receiving 200 and 800 ppm, but these mean values appeared to be increased by one or two outliers in each group. These findings were also confirmed histopathologically as enlarged spleens due to malignant lymphoma or lymphoid hyperplasia.

Other significant changes in organ weights noted at 52 and 104 weeks were considered to be incidental in nature and unrelated to treatment. These included a significantly increased kidney/brain weight ratio for the 50-ppm males at 52 weeks, significantly increased heart/body and heart/brain weight ratios for the 50-ppm males at 104 weeks, and significantly decreased brain/body and kidney/body weight ratios for the 800-ppm females at 104 weeks.

The mean liver weight data at 104 weeks are presented in Table 6.

b. Gross Pathology: The gross lesions that were observed in the animals that died or were killed during the study and in those that were sacrificed on schedule were reported to correlate with their histological diagnoses and were not indicative of compound-related effects. No summary data for the incidences and types of gross lesions were provided.

c. Microscopic Pathology:

1. Nonneoplastic: A number of nonneoplastic lesions were reported in the organs examined; these mainly involved the endocrine, reproductive, and large parenchymatous organs. None were considered to be compound related. Selected nonneoplastic findings for the animals designated to be sacrificed at 52 weeks are presented in Table 7. Selected findings for the animals designated to be sacrificed at 104 weeks are presented in Table 8.

2. Neoplastic: Neoplastic lesions were observed primarily in the hemolymphoreticular system, lungs, liver, ovaries, and Harderian glands. Selected neoplastic findings for the animals sacrificed at 52 weeks are presented in Table 9. Selected findings for the animals sacrificed at 104 weeks are presented in Table 10. The authors concluded that the type and incidence of the neoplasms did not indicate an effect of the test article.

TABLE 6. Selected Mean Organ Weight Data for Mice Fed Trifluralin for 24 Months--104-Week Sacrifice

Dose Group (ppm)	Absolute Liver Weight (g)	Liver/ Body Weight (%)	Liver/ Brain Weight (%)
		MALES	
0	2.23±0.55 (24)[a]	4.86±1.06 (24)	458.83±125.06 (24)
50	2.31±0.45 (10)	5.36±1.48 (10)	490.57±114.38 (10)
200	2.95±1.40*(22)	6.30±2.51*(22)	599.61±271.23*(22)
800	2.89±0.88 (17)	6.32±1.81*(17)	603.54±183.54 (17)
		FEMALES	
0	2.56±1.29 (10)	6.21±2.10 (10)	505.35±248.50 (10)
50	2.35±0.42 (12)	5.93±0.96 (12)	463.80± 72.97 (12)
200	2.53±0.57 (8)	6.43±0.94 (8)	494.54±109.79 (8)
800	3.11±0.47 (9)	7.07±1.02 (9)	637.53±108.14 (9)

[a]Number in parentheses represents number of animals for which an organ weight was taken.

*Significantly different from control value ($p \leq 0.05$), as determined by the Dunnett test.

TABLE 7. Selected Nonneoplastic Findings for Mice Fed Trifluralin for 24 Months--52-Week Sacrifice[a]

Organ/Finding	Dose Group (ppm)							
	Males				Females			
	0	50	200	800	0	50	200	800
Kidneys								
No. examined	10	10	10	10	10	10	10	10
Tubular atrophy	5	6	7	7	2	4	0	5
Tubular dilation	8	9	10	9	10	9	10	10
Lymphoid cell infiltration	5	8	7	10	8	8	9	9
Mandibular Lymph Node								
No. examined	7	3	10	7	9	10	9	9
Lymphoid hyperplasia	2	1	0	3	1	1	2	6
Granulocytosis	1	1	0	0	0	2	1	4
Mesenteric Lymph Node								
No. examined	8	8	10	10	10	9	8	10
Lymphoid hyperplasia	2	2	7	6	0	4	1	5
Histiocytosis	3	5	6	4	6	3	2	1

[a]Includes 10 mice/sex/group designated to be sacrificed at 52 weeks, whether or not they died or were sacrificed moribund prior to that time.

TABLE 8. Selected Nonneoplastic Findings for Mice Fed Trifluralin for 24 Months—104-Week Sacrifice[a]

Organ/Finding	Dose Group (ppm)							
	Males				Females			
	0	50	200	800	0	50	200	800
Adrenal cortex								
No. examined	48	47	49	48	50	49	48	50
A-cell hyperplasia	31 (64.6)[b]	25 (53.2)	24 (49)	29 (60.4)	44 (88)	38 (77.6)	43 (89.6)	42 (84)
B-cell hyperplasia	26 (54.4)	16 (34)	20 (40.8)	24 (50)	1 (2)	1 (2)	1 (2.1)	0
Ceroid degeneration	16 (33.3)	11 (23.4)	7 (14.3)	8 (16.7)	43 (86)	48 (98)	45 (93.8)	45 (90)
Bone (femur)								
No. examined	50	49	50	50	48	49	50	48
Fibro-osseous lesion	0	0	2 (4)	0	2 (4.2)	4 (8.2)	4 (8)	7 (14.6)
Kidneys								
No. examined	50	49	50	50	50	50	50	50
Lymphoid cell infiltration	32 (64)	31 (63.3)	45 (90)	42 (84)	43 (86)	36 (72)	40 (80)	35 (70)
Interstitial fibrosis	2 (4)	6 (12.2)	7 (14)	6 (12)	3 (6)	2 (4)	1 (2)	9 (18)
Liver								
No. examined	50	50	50	50	50	50	50	50
Lymphoid cell infiltration	1 (2)	4 (8)	6 (12)	2 (4)	3 (6)	7 (14)	8 (16)	10 (20)
Vacuolation	14 (28)	10 (20)	3 (6)	6 (12)	1 (2)	2 (4)	3 (6)	6 (12)
Kupffer cell proliferation	4 (8)	6 (12)	10 (20)	9 (18)	7 (14)	12 (24)	3 (6)	5 (10)
Necrosis	8 (16)	18 (36)	13 (26)	16 (32)	12 (24)	7 (14)	6 (12)	8 (16)
Mandibular lymph node								
No. examined	42	38	33	39	31	43	36	35
Lymphoid hyperplasia	7 (16.7)	15 (39.5)	12 (36.4)	10 (25.6)	5 (16.1)	14 (32.6)	10 (27.8)	12 (34.3)
Granulocytosis	3 (7.1)	5 (13.2)	2 (6.1)	5 (12.8)	4 (12.9)	11 (25.6)	8 (22.2)	10 (28.6)
Spleen								
No. examined	49	50	50	50	50	50	50	49
Increased erythropoiesis	9 (18.4)	4 (8)	7 (14)	5 (10)	5 (10)	13 (26)	13 (26)	16 (32.7)
Lymphoid hyperplasia	2 (4.1)	0	1 (2)	1 (2)	0	2 (4)	5 (10)	5 (10.2)

[a] Includes 50 mice/sex/group designated to be sacrificed at 104 weeks, whether or not they died or were sacrificed moribund prior to that time.

[b] Number in parentheses represents percent (%) incidence.

TABLE 9. Selected Neoplastic Findings for Mice Fed Trifluralin for 24 Months—52-Week Sacrifice[a]

Organ/Finding	Dose Group (ppm)							
	Males				Females			
	0	50	200	800	0	50	200	800
Harderian glands								
No. examined	10	10	10	10	10	10	10	10
Adenoma	0	1 (10)[b]	0	0	0	0	1 (10)	0
Adenocarcinoma	0	0	0	0	0	0	1 (10)	0
Hemolymphoreticular system								
No. examined	10	10	10	10	10	10	10	10
Malignant lymphoma	0	0	0	0	0	0	0	1 (10)
Liver								
No. examined	10	10	10	10	10	10	10	10
Hepatocellular adenoma	1 (10)	0	1 (10)	0	0	0	0	0
Hepatocellular carcinoma	0	0	0	0	0	0	0	1 (10)
Lungs								
No. examined	10	10	10	10	10	10	10	10
Bronchioalveolar tumor	0	2 (20)	2 (20)	1 (10)	0	0	0	0

[a] Includes 10 mice/sex/group designated to be sacrificed at 52 weeks, whether or not they died or were sacrificed moribund prior to that time.

[b] Number in parentheses represents percent (%) incidence.

TABLE 10. Selected Neoplastic Findings for Mice Fed Trifluralin for 24 Months—104-Week Sacrifice[a]

Organ/Finding	Dose Group (ppm)							
	Males				Females			
	0	50	200	800	0	50	200	800
Harderian glands								
No. examined	48	49	50	48	47	49	47	49
Adenoma	6 (12.5)[b]	6 (12.2)	7 (14.0)	6 (12.5)	1 (2.1)	5 (10.2)	0	2 (4.1)
Adenocarcinoma	3 (6.3)	0	0	4 (8.3)	0	1 (2.0)	3 (6.4)	1 (2.0)
Hemolymphoreticular system								
No. examined	50	50	50	50	50	50	50	50
Malignant lymphoma	7 (14)	6 (12)	6 (12)	13 (26)	21 (42)	22 (44)	18 (36)	14 (28)
Kidneys								
No. examined	50	49	50	50	50	50	50	50
Adenoma	0	1 (2)	0	0	0	0	0	0
Carcinoma	0	0	0	1 (2)	0	1 (2)	0	0
Liver								
No. examined	50	50	50	50	50	50	50	50
Hepatocellular adenoma	5 (10)	8 (16)	7 (14)	6 (12)	0	0	1 (2)	1 (2)
Hepatocellular carcinoma	1 (2)	3 (6)	7 (14)*	4 (8)	0	0	0	0
Hemangioendothelioma	0	0	1 (2)	0	1 (2)	0	0	0
Metastasis/sarcoma	0	0	0	1 (2)	0	0	0	0
Metastasis/carcinoma	0	0	1 (2)	0	0	0	0	0
Hemangioma	0	0	1 (2)	0	0	0	0	0
Unclassified carcinoma	1 (2)	0	0	0	0	0	0	0
Lungs								
No. examined	50	50	50	50	50	50	50	50
Bronchioalveolar tumor	12 (24)	19 (38)	25 (50)	17 (34)	6 (12)	8 (16)	10 (20)	8 (16)
Metastasis/carcinoma	0	0	0	1 (2)	0	0	1 (2)	2 (4)
Ovaries								
No. examined					49	49	49	45
Theca/granulosa cell tumor					5 (10.2)	7 (19.3)	5 (10.2)	5 (11.1)
Sertoli cell tumor					0	2 (4.1)	1 (2.0)	1 (2.2)
Tubular adenoma					0	1 (2.0)	1 (2.0)	0
Luteoma					2 (4.1)	0	0	2 (4.4)
Papillary adenoma					0	1 (2.0)	0	0

[a] Includes 50 mice/sex/group designated to be sacrificed at 104 weeks, whether or not they died or were sacrificed moribund prior to that time.

[b] Number in parentheses represents percent (%) incidence.

*Significantly different from control value ($p < 0.05$) by Fisher's exact test, as calculated by our reviewers. The combined incidence of hepatocellular adenoma and carcinoma for the 200-ppm males (14 or 28%) was also found to be significantly ($p < 0.05$) greater than for control males (6 or 12%).

The most frequently observed neoplasm in the hemolymphoreticular system of all groups was malignant lymphoma. If the infiltration of a tissue by lymphoid cells was clearly neoplastic, the diagnosis "malignant lymphoma" was made. Otherwise, the term "lymphoid cell infiltration" was used. Malignant lymphoma, when disseminated, was counted as a single neoplasm. At the 52-week sacrifice, malignant lymphoma was noted in one female of the 800-ppm group. In the animals designated for the 104-week sacrifice (50/sex/group), the incidence of male mice with malignant lymphomas was 14% in controls, 12% in the 50- and 200-ppm groups, and 26% in the 800-ppm group. The incidence of female mice with malignant lymphomas was 42% in controls, 44% in the 50-ppm group, 36% in the 200-ppm group, and 28% in the 800-ppm group. The number of tumor-bearing mice was considered to be similar in control and compound-treated mice.

Primary neoplasms observed in the lungs were bronchioalveolar tumors. They were more frequent in males than in females. Because of their malignant biological potential (Stewart et al., 1979), all bronchioalveolar neoplasms were classified as malignant, even in cases where morphological criteria of malignancy (i.e., multiple or invasive growth, metastases) were absent. At the 52-week sacrifice, bronchioalveolar tumors were noted in two males in each of the 50- and 200-ppm groups and in one male in the 800-ppm group. In the animals designated for the 104-week sacrifice, the incidence of males with bronchioalveolar tumors was 24% in controls, 38% in the 50-ppm group, 50% in the 200-ppm group, and 34% in the 800-ppm group. The incidence of females with bronchioalveolar tumors was 12% in controls, 16% in the 50-ppm group, 20% in the 200-ppm group, and 16% in the 800-ppm group. The number of tumor-bearing mice was considered to be similar in control and compound-treated mice.

The hepatocellular neoplastic lesions were classified according to Frith (1985). Hepatocellular neoplasms (adenomas and carcinomas) were observed predominantly in males. At the 52-week sacrifice, one adenoma each was noted in one control male and one male in the 200-ppm group. One carcinoma was noted in one male in the 800-ppm group. The incidence of adenomas in males designated to be sacrificed at 104 weeks (50 mice/group) was 10% in the controls, 16% in the 50-ppm group, 14% in the 200-ppm group, and 12% in the 800-ppm group; whereas, in females only one adenoma each (2%) was noted in the 200- and 800-ppm groups. The incidence of hepatocellular carcinomas in males was 2% in controls, 6% in the 50 ppm group, 14% in the 200-ppm group, and 8% in the 800-ppm group. In females, no carcinomas were noted.

The authors concluded that neither the incidence of hepatocellular adenomas nor carcinomas or their combined incidence were compound or dose related (in males: 12, 22, 28 and 20% for controls and 50-, 200-, and 800 ppm groups, respectively; in females: 0, 0, 2, and 2% for controls and 50-, 200-, and 800-ppm groups, respectively).

Neoplasms in the ovaries were predominantly theca-granulosa cell tumors, Sertoli cell tumors, tubular adenomas, and luteomas. No ovarian neoplasms were noted in the females designated to be sacrificed at 52 weeks (10/group). In the females designated to be sacrificed at 104 weeks (50/group), the incidence of tumor-bearing mice was 14.3% in controls, 22.4% in the 50-ppm group, 14.2% in the 200-ppm group, and 17.7% in the 800-ppm group. No increase in ovarian neoplasms was observed in compound-treated mice as compared to controls.

The neoplasms of the Harderian glands were adenomas and adenocarcinomas. In the animals designated for the 52-week sacrifice (10/sex/group), neoplasms of the Harderian glands were noted in one male of the 50-ppm group and in two females of the 200-ppm group. In the animals designated for the 104-week sacrifice (50/sex/group), the incidence of tumor-bearing male mice was 18.8% in controls, 12.2% in the 50-ppm group, 14% in the 200-ppm group, and 20.8% in the 800-ppm group. The incidence of tumor-bearing female mice was 2.1% in controls, 12.2% in the 50-ppm group, 6.4% in the 200-ppm group, and 6.1% in the 800-ppm group. The number of tumor-bearing mice was considered to be similar in control and compound-treated mice.

The incidence of neoplasms in the other organs routinely examined was comparatively low, ranging to a maximum of 12%.

D. STUDY AUTHORS' CONCLUSIONS:

Based on the organ weight changes, the authors concluded that the NOEL of trifluralin for systemic toxicity in this study was 50 ppm in male NMRI mice and 200 ppm in female NMRI mice, which corresponds to a daily test material consumption of approximately 7.5 mg/kg body weight for males and 41 mg/kg body weight for females. No morphologic, toxic, or oncogenic effects were observed in the mice. The neoplastic and nonneoplastic lesions noted in this study were considered to be spontaneous and to reflect the normal spectrum of lesions encountered in aged NMRI mice.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and complete, and the conduct of the study was acceptable. Reporting of the data was incomplete. Clinical examination data, including results of examinations of the teeth and mucous membranes, and palpation for tissue masses and subsequent progress as well as results of the ophthalmoscopic examinations and hearing tests were not provided. Although the study authors stated that there were no compound-related changes in these data, we could not confirm these conclusions without the individual animal data.

Statistical analysis of selected hematology data by our reviewers indicated significantly greater than control methemoglobin values for the 50-, 200-, and 800-ppm males at 52 weeks and for the 800-ppm males and females at 104 weeks. Heinz bodies also were found by our reviewers to be significantly greater than control for the 50-, 200-, and 800-ppm males at 52 weeks and for the 200- and 800-ppm males at 104 weeks. These significant changes were not observed by the study authors using the Dunnett or Steel tests. However, since these increases in methemoglobin and Heinz bodies in 50 ppm males were not found at 104 weeks and this group contained values considered to be outliers for both parameters, these increases in methemoglobin and Heinz bodies for 50 ppm males were not considered to be of biological significance. Methemoglobin was examined on only two animals in the group of 200 ppm males at 52 weeks. In addition, the increased level of methemoglobin found at 52 weeks was not seen at 104 weeks; even though these parameters were found to be statistically significant, they are not considered to be biologically significant. Outliers were also found in the group of 800 ppm females with significantly increased methemoglobin. This group also had a limited number of individual values; this increase in methemoglobin was not considered to be of biological significance. The significant increase in methemoglobin in 800 ppm males and the increase in Heinz bodies in 200 and 800 ppm males were considered to be compound related. Aromatic amino and nitro compounds, such as aniline and nitrobenzene, are known to produce methemoglobin and Heinz bodies in many species (Doull et al., 1986).

Although clinical biochemistry laboratory reference values of untreated NMRI mice were presented in an attachment, standard values for creatinine, total bilirubin, or alkaline phosphatase could not be found for animals of comparable age at 52 weeks of study and gamma-glutamyl-transferase values could not be found for animals of comparable age at either 52 or 104 weeks of study.

Summary data for the incidences and types of gross lesions observed at necropsy were not provided. This may have been beneficial in evaluating the liver findings since after 104 weeks of compound administration, a dose-related trend of higher absolute liver weights and higher liver weights relative to both body and brain weights was reported for males of the 200- and 800-ppm dose groups and for females of the 800-ppm dose group. However, there were no increases in preneoplastic changes in the liver of the compound-treated animals.

The study authors did not consider the incidence of hepatocellular neoplasms to be different between control and compound-treated groups; however, our reviewers found the incidence of hepatocellular carcinomas taken separately, as well as the incidence of hepatocellular adenomas and carcinomas taken together, to be significantly ($p < 0.05$) increased for the 200-ppm males designated to be sacrificed after 104 weeks when compared to concurrent control males using Fisher's exact test.

There is a positive oncogenic response when compared to concurrent control data. The study authors did not present laboratory historical control data for the incidence of hepatocellular tumors in NMRI mice. This would have been helpful in evaluating the oncogenic potential of trifluralin in NMRI mice.

An apparent decrease in time-to-tumor was also observed for the compound-treated males, since 2 out of 6 control males, 7 out of 11 50-ppm males, 4 out of 14 200-ppm males, and 3 out of 10 800-ppm males that exhibited hepatocellular adenomas or carcinomas died or were sacrificed moribund prior to the 104-week sacrifice. The study authors combined the neoplastic incidence data for all animals scheduled to be sacrificed after 104 weeks of treatment regardless of the actual time of death or sacrifice of an animal.

Based on the changes in absolute and relative liver weights, we concur with the study authors and assess the NOEL for systemic toxicity to be 50 ppm trifluralin in male mice and 200 ppm trifluralin in female mice.

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EPA: 68-02-4225
DYNAMAC No. 208-B
February 5, 1987

DATA EVALUATION RECORD

TRIFLURALIN

Chronic Toxicity/Oncogenicity Feeding Study in Rats

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 2-5-87

EPA: 68-02-4225
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Date: 2/10/87

DATA EVALUATION REPORT

TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: Chronic toxicity/oncogenicity feeding study in rats.

ACCESSION NUMBER: 262521-262526.

TEST MATERIAL: Trifluralin; α,α,α-trifluoro-2,6-dinitro-N,N-dipropy p-toluidine.

SYNONYMS: Digermin, Ipersan, Treflan, Triflurex.

STUDY NUMBER(S): 680 (Chronic A32718, oncogenicity A32719).

SPONSOR: Hoechst Aktiengesellschaft, Frankfurt, Federal Republic o Germany.

TESTING FACILITY: Pharma Forschung Toxikologie, Hoechst Aktiengesell schaft, Frankfurt, Federal Republic of Germany.

TITLE OF REPORT: Trifluralin (code: Hoe 38474 OH AT208) Chronic Feedin Study (24 months) in Rats and Trifluralin (Code: Hoe 38474 OH AT208 Carcinogenicity Study in Rats (28-Month Feeding Study).

AUTHOR(S): Donaubauer, Schutz, Leist, and Kramer.

REPORT ISSUED: March 26, 1985, and April 2, 1985.

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CONCLUSIONS:

When 200, 800, or 3200 ppm trifluralin was fed to Wistar rats for 24 months in a chronic toxicity study, there were no overt signs of toxicity or dose-related effects on mortality, clinical biochemistry, or histopathology. Body weight gain and food consumption were decreased throughout the study in males and females receiving 3200 ppm. Body weights were also decreased at study termination in males receiving 800 ppm. There were significant ($p < 0.05$) decreases in red cell parameters in high-dose males and females. There were also nonsignificant decreases in liver and thyroid weights in males and females receiving 3200 ppm, although there were no histologic findings that correlated with the organ weight changes.

In an oncogenicity study conducted simultaneously, 200, 800, and 3200 ppm trifluralin were not oncogenic when fed to male and female Wistar rats for 28 months. Tumor incidence was found to be age, sex, or strain related and was not due to compound treatment. Body weight gain and food consumption were decreased throughout the study in males and females receiving 3200 ppm. Body weights were decreased in the 800 ppm female group during the last 6 months of the study, and in males of this group at study termination. As in the chronic study, there were nonsignificant increases in liver and thyroid weights in males and females receiving 3200 ppm. Based on the body weight changes, the LOEL is 800 ppm and the NOEL is 200 ppm. ONCOGENIC NOEL = 3200ppm (HDT)

Classification: Core Guideline.

A. MATERIALS:

1. Test Compound: Trifluralin, technical, Code: Hoe 38474 OH AT208, from December 1982, Code: Hoe 38474 OH AT210; description: orange powder from batch No. 10653 OP.112/80; purity: >99 percent.

2. Test Animals: Species: rat; strain: Wistar, Hoe: Wiskf (SPF71); age: 4 weeks; mean weights: males--123-130 g and females--117-119 g; source: Hoechst breeding colony.

B. STUDY DESIGN:

1. Animal Assignment: After 7 days of acclimation, the animals were weighed and assigned to the following groups with a computerized randomization procedure:

CHRONIC TOXICITY STUDY

Test Group	Dose in diet (ppm)	Main study (24 months) Males	Main study (24 months) Females	Residue Examination (6,12,18, & 24 months) Males	Residue Examination (6,12,18, & 24 months) Females	BSP/PSP Function Tests (25 months) Males	BSP/PSP Function Tests (25 months) Females
1 Control	0	20	20	10	10	6	6
2 Low (LDT)	200	20	20	10	10	6	6
3 Mid (MDT)	800	20	20	10	10	6	6
4 High (HDT)	3200	20	20	10	10	6	6

ONCOGENICITY STUDY

Test Group	Dose in diet (ppm)	Main Study (28 months) Males	Main Study (28 months) Females
1. Cont.	0	60	60
2. Low (LDT)	200	60	60
3. Mid (MDT)	800	60	60
4. High (HDT)	3200	60	60

Dose Selection: The dose levels selected for this study were based on a 3-month subchronic toxicity study in rats; as a result of these studies, 3200 ppm was expected to cause intoxication and impairment of body weight gains. Two hundred and 800 ppm were selected based on user exposure concentrations and residue limits in food.

2. Diet Preparation: One-kilogram premixes were prepared at 21-day intervals and stored at < 6°C. Diets were prepared weekly. Food and water were available to the animals ad libitum. Samples of treated food were analyzed for concentration and homogeneity at weekly intervals; stability of test compound in diet was analyzed at monthly intervals.

Results: The premixes and diets were found to be homogeneous and ≥ 90 percent stable over 21 days of storage. Recovery values of the diets were within acceptable limits, e.g., 93-108 percent of the calculated values.

3. Animals received food (Altromin 1321) and water ad libitum except during scheduled urine collection periods.

4. Statistics: The following procedures were utilized in analyzing the numerical data for the chronic toxicity and oncogenicity studies. Body weights, clinical chemistry, and appropriate hematologic data were analyzed by the tests of Dunnett, Sidak, Nemenyi/Dunnett, and Nemenyi/Sidak for the chronic toxicity study; body weights were analyzed by these procedures for the oncogenicity study. Organ weights were analyzed by the tests of Sidak and Nemenyi/Sidak for both studies. Water consumption during the chronic toxicity study was analyzed by the procedure of Shapiro and Wilk. Mortality patterns for both studies were tested with the Kaplan-Meier and log-rank procedures. All methods were tested at the $p=0.05$ level of significance. Data on the numbers of animals with tumors were analyzed by the IARC time-to-tumor method, including the tests for homogeneity, positive trend, nonlinearity, and pairwise comparison. Descriptive statistics (mean, standard deviation) were calculated for food consumption.

5. A quality assurance statement was signed and dated March 26, 1985, and April 2, 1985.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of toxicity and mortality. The animals were examined once a month for neurological disturbances, opacity of the eyes, impairment of dental growth, and changes in the oral mucosa. All rats were individually examined twice monthly (from 6 to 24 months) for palpable masses.

   Results: It was reported that there were no overt signs of toxicity. Palpation of the skin revealed a number of pathological findings in animals of all treatment groups; however, these pathological findings were reported to be unrelated to dosing. Individual observation data or summarized palpable mass observations were not reported.

   a. Chronic Toxicity Study - Survival was similar for all groups of males. Mortality was slightly but nonsignificantly increased in females receiving 3200 ppm relative to controls and slightly increased in females relative to males at 78 and 104 weeks. Mortality was also found earlier in the study in females receiving 3200 ppm (56 weeks) or 800 ppm (66 weeks). The first death in males was at week 78. Representative mortality and survival data are presented in Table 1.

TABLE 1. Representative Results of Mortality and Percent Survival of Rats Fed Trifluralin for 24 Months - Chronic Toxicity Study

Dose Group (ppm)	Mortality (Percent Survival)[a] at Week			
	26	52	78	104
		MALES		
0	0(100)	0(100)	0(100)	1(95)
200	0(100)	0(100)	0(100)	3(85)
800	0(100)	0(100)	1(95)	2(90)
3200	0(100)	0(100)	0(100)	3(85)
		FEMALES		
0	0(100)	0(100)	1(95)	3(85)
200	0(100)	0(100)	2(90)	5(75)
800	0(100)	0(100)	1(95)	4(80)
3200	0(100)	0(100)	3(85)	6(70)

[a]Based on 20 rats/group.

b. Oncogenicity Study - Mortality was slightly, but nonsignificantly, increased in males receiving 800 or 3200 ppm relative to controls at 104 and 121 weeks; however, mortality was decreased in females receiving 3200 ppm relative to controls. At 104 and 121 weeks, mortality was slightly increased among males receiving 3200 ppm relative to females in the same dose group. Representative mortality and survival data are presented in Table 2.

Mortality during the chronic and oncogenicity studies was considered unrelated to dosing.

TABLE 2. Representative Results of Mortality and Percent Survival of Rats Fed Trifluralin for 28 Months - Oncogenicity Study

Dose Group (ppm)	Mortality (Percent Survival)[a] at Week					
	13	26	52	78	104	121
			MALES			
0	0(100)	0(100)	0(100)	1(98)	4(93)	19(68)
200	0(100)	0(100)	1(98)	2(97)	8(87)	18(70)
800	0(100)	0(100)	1(98)	4(93)	11(82)	22(63)
3200	0(100)	0(100)	1(98)	4(93)	14(77)	26(57)
			FEMALES			
0	0(100)	0(100)	1(98)	2(97)	14(77)	26(57)
200	0(100)	1(98)	2(97)	5(92)	14(77)	23(62)
800	0(100)	0(100)	0(100)	1(98)	11(82)	25(58)
3200	0(100)	0(100)	1(98)	1(98)	10(83)	20(67)

[a]Based on 60 rats/group.

2. Body Weight: Rats were weighed weekly.

Results:

a. Chronic Study - There were no effects of dosing on mean bo weights at 200 ppm, whereas mean body weights of males a females receiving 800 ppm tended to be slightly lower th controls. There was no significant change among females this dose group; males only differed significantly ($p < 0.0$ at week 104. Mean body weights of males and females receivi 3200 ppm were decreased relative to controls throughout t study. Mean body weights of males receiving 3200 ppm we significantly ($p < 0.05$) decreased at week 61 and from week to study termination (Table 3). Mean body weights of femal receiving 3200 ppm were significantly ($p < 0.05$) decreased fr week 26 to study termination (Table 3).

b. Oncogenicity Study - There were no effects of dosing on me body weights of low- or mid-dose males and low-dose femal with the exception of a slight reduction in the body weigh of mid-dose males at the end of the second year of the stu [significantly ($p < 0.05$) decreased at week 121]. Mean bo weights of females receiving 800 ppm were slightly decreas from controls beginning at week 39; the decreases were signif cant ($p < 0.05$) from weeks 73 to 112. Mean body weights males and females receiving 3200 ppm were significant ($p < 0.05$) decreased throughout the study. Table 4 presen mean body weight data at selected intervals.

3. Food Consumption, Water Consumption, and Compound Intake: Fo consumption was determined weekly at the time of body weig determinations. Food consumption and body weight were used adjust the concentration of the test compound in the diet maintain the targeted dosage level on a mg/kg/day basis.

Results:

a. Chronic Study - The absolute food consumption of femal receiving 3200 ppm was found to be decreased relative to t controls throughout the study. The relative food consumpti was found to be slightly increased in males and femal receiving 3200 ppm, whereas the body weights were decreased these groups (Table 5). Food efficiency was not calculated.

TABLE 3. Representative Results of Mean Body Weights (± SD) of Trifluralin for 24 Months - Chronic Study

Dose Group (ppm)	Mean Body Weights (g ± SD)[a] at Week						
	0	13	26	52	65	78	104
				MALES			
0	125±8	410±32	453±37	508±41	531±44	535±47	544±
200	124±9	416±37	464±44	515±58	544±59	548±64	554±
800	120±12	406±41	449±48	492±55	511±57	511±60	493±
3200	121±10	388±41	439±47	478±47	487±49*	481±47*	449±
				FEMALES			
0	119±7	233±20	250±22	291±32	313±43	330±40	353±
200	114±8	240±26	258±31	299±35	320±41	333±57	351±
800	118±6	228±20	247±21	280±30	297±41	314±32	323±
3200	117±5	219±11	234±13*	253±19*	257±23*	264±18*	262±

[a]Based on 20 rats/group.

*Significantly different from control value ($p < 0.05$).

TABLE 4. Representative Results of Mean Body Weights (± SD) of Rats Fed Trifluralin for 28 Months - Oncogenicity Study

Dose Group (ppm)	Mean Body Weights (g ± SD)[a] at week							
	0	13	26	52	65	78	104	121
				MALES				
0	131±9	414±35	466±44	504±53	528±55	539±59	530±63	499±62
200	128±8	412±30	467±35	510±59	528±42	540±44	537±49	496±71
800	130±9	410±35	465±41	508±49	523±54	534±56	519±53	451±58*
3200	130±8	389±37*	440±41*	483±45*	495±47*	496±53*	449±42*	408±20*
				FEMALES				
0	121±8	229±18	251±22	288±29	309±35	328±38	347±42	323±59
200	120±6	228±15	250±18	287±30	303±35	320±38	334±44	321±52
800	118±7	231±16	251±18	280±28	295±35	310±39*	314±48*	300±45
3200	118±7	215±19*	230±23*	248±23*	255±24*	259±26*	252±30*	258±28*

[a]Based on 60 rats/group.

*Significantly different from control value ($p < 0.05$).

TABLE 5. Mean Food and Compound Consumption for Rats[a] Fed Trifluralin for 24 Months - Chronic Study

Dose Group (ppm)	Mean Food Consumption Absolute (g/day)	Mean Food Consumption Relative (g/100 g/day)	Mean Compound Consumptic (mg/kg/day)
		MALES	
0	24.2	5.25	--
200	24.5	5.20	10.39
800	23.6	5.26	42.11
3200	23.1	5.37	171.77
		FEMALES	
0	18.4	6.62	--
200	18.6	6.63	13.26
800	17.7	6.59	52.73
3200	16.4	6.77	216.79

[a]Based on 20 rats/group.

b. Oncogenicity Study - The absolute food consumption of males a females receiving 3200 ppm was found to be decreased relati to the controls, whereas the relative food consumption these animals was found to be slightly increased. Th increase in relative food consumption was associated with t decrease in body weights found in these groups (Table 6 Mean compound intake was higher in females at all dose leve in the chronic toxicity and oncogenicity studies (Tables 6). Food efficiency was not calculated.

TABLE 6. Mean Food and Compound Consumption for Rats[a] Fed Trifluralin for 28 Months - Oncogenicity Study

Dose Group (ppm)	Mean Food Consumption		Mean Compound Consumpti
	Absolute (g/day)	Relative (g/100 g/day)	(mg/kg/day)
		MALES	
0	24.1	5.17	--
200	23.6	5.01	10.03
800	23.3	5.04	40.33
3200	22.8	5.29	169.17
		FEMALES	
0	18.5	6.56	--
200	18.2	6.55	13.11
800	17.8	6.58	52.61
3200	16.3	6.83	218.72

[a]Based on 60 rats/group.

Water consumption was recorded over a 16-hour period for 10 animals/sex/group at 6, 12, and 24 months of the chronic toxicity study. The absolute water consumption varied sporadically; there were no definite indications of a dose-related effect. The relative water consumption, however, was significantly ($p < 0.05$) increased in males receiving 3200 ppm at 6 and 24 months and females receiving the same dose at 12 months; these increases in water consumption were associated with the reductions in body weight found in these groups (Table 7).

TABLE 7. Relative Water Consumption for Rats Fed Trifluralin for 24 Months - Chronic Study

Dose Group (ppm)	Mean Water Consumption (g ± SD)[a] at Month 6	12	24
		MALES	
0	28.3±2.8	26.0±8.5	21.3±3.3
200	26.9±4.9	22.2±4.7	25.1±8.8
800	25.4±5.3	23.3±7.4	20.1±6.8
3200	32.7±5.9*	30.7±6.9	26.1±6.2*
		FEMALES	
0	26.2±3.7	23.5±8.6	23.5±8.8
200	29.2±5.0	30.3±7.4	27.0±7.6
800	25.4±4.2	26.3±3.8	27.9±7.5
3200	27.0±5.1	29.7±4.3*	23.3±9.9

[a]Based on 10 rats/group.

*Significantly different from control value ($p=<0.05$)

4. Ophthalmological examinations, consisting of examination of the opacity of the refracting media of the eyes, were performed once per month on all animals. There were no changes reported.

5. Blood was collected by orbital sinus puncture before treatment a at 26, 52, and 78 weeks for hematologic and clinical analysis fr 10 animals/sex/dose of the chronic toxicity study and from animals/sex/dose of this study at 104 weeks.

The CHECKED (X) parameters were examined:

a. Hematology

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†

Total plasma protein (TP)
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentrati (MCHC)
X Mean corpuscular volume (MCV)
X Reticulocytes
X Heinz bodies
X Coagulation time
X Howell-Jolly bodies

Methemoglobin was determined before treatment and at 6, 1 and 18 months for 10 males and 10 females receiving 3200 p and at 24 months for all high-dose survivors of the chron study. Methemoglobin was not determined for control animals.

Results: The mean erythrocyte counts (RBC) for high-dose mal and females were significantly ($p < 0.05$) decreased at 26, 5 and 78 weeks (Tables 8A and 8B). Mean hemoglobin (HGB) a hematocrit (HCT) concentrations were similarly decreased 26, 52, 78, and 104 weeks. HGB and HCT were found to significantly ($p < 0.05$) reduced in high-dose females at 5 78 and 104 weeks. HGB was found to be significantly ($p < 0.0$ reduced in high-dose males at 26 and 104 weeks but not at or 78 weeks; HCT was significantly ($p < 0.05$) reduced in th group at 78 and 104 weeks but not at 26 or 52 weeks (Tables and 8B). There was a corresponding increase in reticulocyt at all examination intervals, and was significant ($p < 0.05$) females receiving 3200 ppm at 52 and 78 weeks. No Heinz bodi or Howell-Jolly bodies were found in erythrocytes and methemoglobin formation was detected. Significant differenc occurred sporadically among leukocyte, coagulation time, a platelet parameters; these values were considered random a not of toxicologic significance.

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†Recommended by Subdivision F.

TABLE 8A. Representative Mean Hematology Data for Male Rats Fed Trifluralin for 24 Months - Chronic Study

Dose Group (ppm)	RBC (10^{12}/L)	HGB (g/L)	HCT (unity)	Reticulocytes (unity)
	Mean Hematology Value (±SD)[a] at Pretest			
0	5.82±0.46	122±6	0.35±0.02	0.017±0.005
200	6.11±0.44	127±10	0.35±0.03	0.018±0.006
800	5.99±0.46	126±9	0.35±0.03	0.019±0.013
3200	5.77±0.45	123±9	0.34±0.03	0.021±0.008
	26 Weeks			
0	8.57±0.31	160±6	0.44±0.02	0.012±0.004
200	8.59±0.36	159±8	0.44±0.03	0.013±0.008
800	8.19±0.36*	153±6	0.42±0.03	0.014±0.006
3200	7.96±0.32*	150±8*	0.41±0.03	0.018±0.006
	52 Weeks			
0	8.87±0.34	169±8	0.47±0.02	0.022±0.007
200	8.85±0.32	171±7	0.47±0.03	0.022±0.007
800	8.60±0.32	164±7	0.45±0.03	0.025±0.007
3200	8.32±0.43*	162±8	0.44±0.03	0.026±0.010
	78 Weeks			
0	8.62±0.36	166±8	0.45±0.02	0.030±0.010
200	8.60±0.43	166±9	0.46±0.03	0.028±0.010
800	8.36±0.30	164±7	0.44±0.03	0.023±0.008
3200	7.74±1.09*	153±21	0.41±0.07*	0.033±0.012
	104 Weeks			
0	7.95±0.41	155±7	0.43±0.02	0.039±0.012
200	7.67±0.89	151±15	0.41±0.05	0.043±0.027
800	8.03±0.43	156±9	0.44±0.03	0.039±0.009
3200	7.59±0.92	145±11*	0.39±0.06*	0.039±0.013

[a]Based on 10 rats/group except the terminal blood analysis in which 20 rats/group were examined.

*Significantly different from control value ($p < 0.05$).

TABLE 8B. Representative Mean Hematology Data for Female Rats Fed Trifluralin for 24 Months - Chronic Study

Dose Group (ppm)	RBC (10^{12}/L)	HGB (g/L)	HCT (unity)	Reticulocytes (unity)
	Mean Hematology Value (±SD)[a] at Pretest			
0	6.17±0.39	126±4	0.36±0.02	0.039±0.011
200	5.95±0.34	124±6	0.35±0.02	0.027±0.009
800	6.12±0.39	127±4	0.36±0.02	0.033±0.007
3200	6.35±0.35	134±8*	0.37±0.02	0.035±0.013
	26 Weeks			
0	7.87±0.51	151±9	0.43±0.02	0.014±0.007
200	7.64±0.20	148±4	0.42±0.01	0.020±0.007
800	7.69±0.39	149±6	0.42±0.02	0.021±0.006
3200	7.26±0.33*	143±7	0.41±0.02	0.021±0.006
	52 Weeks			
0	7.95±0.50	160±6	0.45±0.03	0.017±0.007
200	7.53±0.54	157±5	0.43±0.03	0.027±0.008
800	7.89±0.35	159±3	0.45±0.01	0.024±0.009
3200	6.68±1.51*	140±24*	0.39±0.07*	0.064±0.107*
	78 Weeks			
0	7.87±0.63	158±10	0.45±0.02	0.027±0.010
200	7.53±0.30	155±5	0.44±0.01	0.029±0.008
800	7.76±0.44	158±8	0.44±0.02	0.032±0.007
3200	7.09±0.58*	146±10*	0.41±03*	0.041±0.010
	104 Weeks			
0	7.19±0.59	148±10	0.42±0.03	0.032±0.016
200	6.80±0.53	139±8	0.39±0.02	0.037±0.016
800	7.25±0.45	144±7	0.41±0.03	0.032±0.015
3200	6.76±0.50	134±7*	0.39±0.02*	0.040±0.019

[a]Based on 10 rats/group except the terminal blood analysis in which 20 rats/group were examined.

*Significantly different from control value ($p < 0.05$).

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium†		Albumin†
X	Chloride†	X	Blood creatinine†
	Magnesium†	X	Blood urea nitrogen† (BUN)
X	Phosphorus†	X	Cholesterol†
X	Potassium†		Globulins
X	Sodium†	X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)	X	Direct bilirubin
	Cholinesterase	X	Total protein†
	Creatinine phosphokinase†		Protein quotient (A/G ratio)
X	Lactic acid dehydrogenase		Triglycerides
X	Serum alanine aminotransferase (also SGPT)†	X	Uric acid
		X	Electrophoresis
X	Serum aspartate aminotransferase (also SGOT)†		

Bromosulfophthalein (BSP) and phenosulfonphthalein (PSP) w determined on satellite groups of six rats/sex/dose dosed 25 months. These determinations were made at 6, 12, 18, 24 months.

Results: The authors stated that there were no changes toxicologic importance in the biochemical data. There w significant changes in the levels for 23 parameters in males 19 parameters in females when compared to controls; howe these were sporadic changes and were within the range of age- strain-matched historical laboratory controls. The hepatic (B and renal (PSP) function tests were similar in control and d groups. Histological examination of the liver and kidr correlated with these results.

6. Urinalyses: Urine was collected from fasted animals of chronic study at the same intervals as blood. The CHECKED parameters were examined.

X	Appearance†	X	Glucose†
	Volume†	X	Ketones†
X	Specific gravity†	X	Bilirubin†
X	pH	X	Blood†
X	Sediment (microscopic)†		Nitrate
X	Protein†	X	Urobilinogen
X	Color	X	Ascorbic acid**

†Recommended by Subdivision F.

** Ascorbic acid was determined in controls and animals recei 3200 ppm at 52, 78, and 105 weeks and in animals receiving 200 800 ppm at 78 and 105 weeks.

Results: The urine of dosed animals showed a dark yellow yellowish-orange discoloration which was dependent on t concentration of dose. This was reported to be attributable excretion of trifluralin or its metabolites. Ascorbic acid v detected in the urine of males and females fed 3200 ppm.

7. Sacrifice and Pathology: All animals that died and that we sacrificed on schedule were subject to gross pathologic examination and the CHECKED (X) tissues were collected f histological examination. The (XX) organs were also weighed.

	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Nasal septum	X	Aorta†	XX	Brain†
X	Tongue	XX	Heart†		Peripheral nerve
X	Salivary glands†	XX	Bone marrow†	X	(sciatic nerve)†
X	Esophagus†	X	Spinal marrow†		Spinal cord (3 leve
X	Stomach†	X	Lymph nodes†	XX	Pituitary†
X	Duodenum†	XX	Spleen†	X	Eyes (optic nerve)†
X	Jejunum†	XX	Thymus†		Glandular
X	Ileum†		Urogenital	XX	Adrenals†
X	Cecum†	XX	Kidneys†		Lacrimal gland
X	Colon†	X	Urinary bladder†	X	Mammary gland†
X	Rectum†	XX	Testes†		Parathyroids†
XX	Liver†	X	Epididymides	XX	Thyroids†
	Gall bladder†	XX	Prostate		Other
X	Pancreas†	X	Seminal vesicle	X	Bone (sternum)†
	Respiratory	XX	Ovaries	X	Skeletal muscle†
X	Trachea†	X	Uterus	X	Skin
XX	Lung†			X	All gross lesions a masses

The aorta was not collected for histological examination dur the oncogenicity study; all other organs collected and weig were similar for both studies.

Results:

a. Organ Weights:

1. Chronic Toxicity Study - The absolute mean liver a thyroid weights were found to be slightly increased males and females receiving 3200 ppm; these increas were reported to be compound related (Table 9). T increases were not statistically significant ($p \leq 0.0$ when compared to control values. Absolute mean prosta weight in males fed 3200 ppm and absolute heart weight females fed 800 or 3200 ppm were found to be signi cantly ($p < 0.05$) decreased; relative prostate weight v not significantly decreased.

†Recommended by Subdivision F.

TABLE 9. Selected Mean Organ Weights (±SD) and Organ-To-Body Weight Ratios of Rats Fed Trifluralin for 24 Months - Chronic Study

Dose Level (ppm)	Organ Weight (g)				Organ/Body Weight (%)			
	Liver	Thyroid	Heart	Prostate	Liver	Thyroid	Heart	Prost
				MALES				
0	16.73 ±2.20 (23)[a]	0.026 ±0.006 (22)	1.63 ±0.18 (23)	0.81 ±0.22 (23)	3.18 ±0.31 (23)	0.005 ±0.001 (22)	0.31 ±0.04 (23)	0.15 ±0.0 (23)
200	17.52 ±2.19 (21)	0.029 ±0.006 (20)	1.67 ±0.16 (21)	0.96 ±0.28 (21)	3.22 ±0.32 (21)	0.005 ±0.001 (20)	0.31 ±0.03 (21)	0.1 ±0.0 (21)
800	16.18 ±2.24 (24)	0.030 ±0.006 (24)	1.55 ±0.15 (24)	0.71 ±0.22 (24)	3.29 ±0.28 (24)	0.006* ±0.001 (24)	0.32 ±0.04 (24)	0.1 ±0.0 (24)
3200	17.07 ±2.87 (23)	0.030 ±0.006 (23)	1.51 ±0.17 (23)	0.65* ±0.15 (23)	3.90* ±0.56 (23)	0.007* ±0.001 (23)	0.35* ±0.30 (23)	0.1 ±0.0 (23)
				FEMALES				
0	10.85 ±2.35 (21)	0.024 ±0.005 (20)	1.25 ±0.21 (21)		3.19 ±0.41 (21)	0.007 ±0.001 (20)	0.38 ±0.09 (21)	
200	10.94 ±1.70 (20)	0.023 ±0.005 (18)	1.16 ±0.13 (20)		3.24 ±0.31 (20)	0.007 ±0.001 (18)	0.35 ±0.04 (20)	
800	11.31 ±1.25 (21)	0.024 ±0.005 (21)	1.14* ±0.11 (21)		3.47 ±0.37 (21)	0.008 ±0.002 (21)	0.35 ±0.05 (21)	
3200	11.25 ±1.27 (19)	0.024 ±0.006 (18)	1.07 ±0.09* (19)		4.33* ±0.47 (19)	0.009* ±0.002 (18)	0.41* ±0.04 (19)	

[a] The numbers in parentheses are the numbers of animals/sex/group; this included a sate group of 6 animals/sex/group tested for BSP/PSP function analyses.

* Significantly different from control value ($p < 0.05$).

Organ-to-body weight ratios of the heart, lungs, liver kidneys, spleen, testes, ovaries, adrenals, brain, an thyroid were found to be significantly ($p < 0.05$ increased in high-dose males and females; however, thes values are considered to be a reflection of the decrease body weights of these animals and are therefore no considered to be compound related.

2. Oncogenicity Study - The absolute mean liver and thyroi weights of males receiving 800 and 3200 ppm were found t be slightly increased relative to controls (Table 10) These increases were reported to be compound related however, the values did not differ significantl ($p \leq 0.05$) when compared to controls and there were n histological changes to correlate with these increase weights. The absolute mean lung weight of females fe 3200 ppm was found to be slightly increased while th absolute kidney weight of this same group was found to b slightly decreased.

Organ-to-body weight ratios of the heart, lungs, liver kidneys, spleen, adrenals, thyroid, and brain of male fed 800 or 3200 ppm were found to be significantl ($p < 0.05$) increased relative to controls, whereas th liver of females fed 800 ppm and the heart, lungs, liver spleen, ovaries, thyroid, and brain of females fed 320 ppm were found to be significantly ($p < 0.05$) increased As in the chronic toxicity study, these increased value are considered to be a reflection of the decreased bod weights of these animals and are therefore not considere to be compound related.

A slight dose-related decrease in absolute and relativ prostate weights in males was found with a significan ($p < 0.05$) decrease reported in the relative weights o high-dose males. However, this was reported to b unrelated to dosing since the absolute values were withi the normal range of historical controls and histologica examination revealed no change in the prostates of thes animals.

A significant ($p < 0.05$) dose-related decrease in th absolute and relative pituitary weights was found i females fed 200, 800, or 3200 ppm; only the relativ pituitary weights of females fed 3200 ppm were reporte to be significantly ($p < 0.05$) decreased by the stud authors. This decreased trend was the result of a marke increase in the absolute pituitary weights of femal rats; the increase was most pronounced in contro animals. This was reported to be the result of a rando increase in the incidence of pathological pituitar changes in female controls. Histological examinatio indicated more than a 50 percent incidence of combine adenomas (30/60) and carcinomas (2/60) in femal

TABLE 10. Selected Mean Organ Weights (±SD) and Organ-to-Body Weight Ratios of Rats Fed Trifluralin for 28 Months - Oncogenicity Study

Dose Level (ppm)	Organ Weight (g)			Organ/Body Weight (%)		
	Liver	Thyroid	Pituitary	Liver	Thyroid	Pituitar
			MALES			
0	15.65 ±2.47 (41)[a]	0.027 ±0.009 (40)	0.015 ±0.009 (41)	3.22 ±0.44 (41)	0.006 ±0.002 (40)	0.003 ±0.002 (41)
200	15.67 ±2.02 (42)	0.030 ±0.009 (40)	0.016 ±0.013 (42)	3.22 ±0.43 (42)	0.006 ±0.002 (40)	0.003 ±0.003 (42)
800	16.15 ±3.34 (38)	0.031 ±0.007 (33)	0.024 ±0.034 (38)	3.64* ±0.64 (38)	0.007* ±0.002 (33)	0.006 ±0.010 (38)
3200	17.52 ±5.85 (34)	0.030 ±0.007 (33)	0.014 ±0.005 (34)	4.28* ±1.40 (34)	0.007* ±0.002 (33)	0.003 ±0.001 (34)
			FEMALES			
0	10.66 ±1.75 (34)	0.022 ±0.005 (31)	0.096 ±0.11 (34)	3.39 ±0.40 (34)	0.007 ±0.002 (31)	0.038 ±0.054 (34)
200	11.58 ±2.69 (37)	0.023 ±0.006 (37)	0.058** ±0.08 (37)	3.59 ±0.51 (37)	0.007 ±0.002 (37)	0.021** ±0.034 (37)
800	10.95 ±1.76 (35)	0.023 ±0.005 (34)	0.036** ±0.056 (35)	3.72* ±0.50 (35)	0.008 ±0.002 (34)	0.013** ±0.024 (35)
3200	10.80 ±1.51 (40)	0.025 ±0.006 (37)	0.025** ±0.043 (40)	4.24* ±0.46 (40)	0.010* ±0.002 (37)	0.010 ±0.018** (40)

[a]Numbers in parentheses are the numbers of animals/sex/group.

[b]Not reported as significant by authors using the method of Nemenyi/Sida

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$) as calculated by reviewers using ANOVA followed by Duncans' test for multiple comparison

controls, a 48 percent incidence at 200 ppm (28/60 adenomas and 1/60 carcinomas), a 37 percent incidence at 800 ppm (20/60 adenomas) and a 17 percent incidence at 3200 ppm (10/60 adenomas). Hyperplasia of the pituitary was also found in all treatment groups. The decreased trend in pituitary weight is therefore not considered to be compound related. Absolute and relative pituitary weights in males were consistent between dose levels with the exception of the 800-ppm dosed group, which was found to be slightly increased relative to controls. Histological examination indicated hyperplasia (11/60) and an 11% incidence of adenomas (7/60) in this group. There were no corresponding effects on pituitary weights or histologic changes in the chronic study. The increased incidence of adenomas and carcinomas of the thyroid found in females was not considered to be of biological importance due to the age of the animals.

b. Gross Pathology: Males and females fed 800 and 3200 ppm trifluralin during the chronic toxicity and oncogenicity studies were reported to have yellow discoloration of the fatty tissue, especially prominent in the abdominal region. This was considered to be a result of compound residues. Other findings occurred randomly and were not considered to be compound related.

c. Microscopic Pathology:

1. Nonneoplastic:

a. Chronic Toxicity Study - There were no compound-related histopathological findings. The discolored fatty tissue was considered to be a histologically undetectable deposition of trifluralin. Table 11 summarizes histologic findings; the type and frequency of these findings were reported to be common for the age, strain, and sex of the animal. Alveolar histiocytosis of the lung was increased in high-dose males and females relative to controls; this was reported due to chance variability and of no toxicologic importance. Many males and females (all groups) had chronic progressive glomerulonephropathy. In most cases, the incidence of the histologic change was markedly increased among the controls, e.g., pituitary adenomas in female rats.

b. Oncogenicity Study - There were no compound-related histopathological findings. Table 12 summarizes nonneoplastic findings; these were considered to be incidental age-related changes and were not related to dosing. Many of the findings were similar to those identified in the chronic toxicity study. Pneumonitis of the lung was increased in high-dose

TABLE 11. Selected Histologic Findings of Rats Fed Trifluralin for 24 Months - Chronic Study[a]

Organ/Finding	Dose Level (ppm)							
	Males				Females			
	0	200	800	3200	0	200	800	3
Number of tissues examined	26[b]	26	26	26	26	26	26	2
Lung								
Histiocytosis	2	8	1	7	6	3	3	1
Kidney								
Pelvic distention	2	2	4	3	1	5	5	
Urinary gravel	3	4	2	6	5	8	9	
Chronic glomerulo-nephropathy	8	11	12	10	3	2	3	
Tubular dilatation with hyaline cysts	10	10	6	4	4	0	2	
Liver								
Bile duct proliferation	14	15	15	1	6	2	7	
Cholangiofibrosis	14	12	5	4	2	5	8	
Biliary cysts	5	0	0	1	0	2	3	
Pancreas								
Focal atrophy	3	6	2	1	5	3	2	
Stomach								
Cystic dilatation of fundus glands	13	7	10	3	14	10	11	11
Thyroid								
Colloid cyst	2	1	6	3	0	2	0	4
Papillary adenoma	0	0	0	1	0	0	0	0
Follicular adenoma	0	1	0	0	0	0	1	1

(Contin

TABLE 11. Selected Histologic Findings of Rats Fed Trifluralin for 24 Months - Chronic Study[a] (Continued)

Organ/Finding	Dose Level (ppm)						
	Males				Females		
	0	200	800	3200	0	200	800
Pituitary							
Adenoma of anterior lobe	2	5	2	1	12	14	9
Focal hyperplasia of anterior lobe	0	0	2	1	0	0	3
Brain							
Granular cell tumor	0	1	0	0	0	0	0
Mammary gland							
Adenocarcinoma					2	6	1
Testes							
Leydig cell tumor	1	2	6	3			
Hyperplasia of Leydig cells	3	2	3	2			
Ovary							
Cyst					5	5	4
Uterus							
Endometrial cysts					4	4	1
Eye							
Retinal atrophy	2	4	3	9	8	5	8
Bulbar trauma	1	4	4	5	0	2	2
Sciatic Nerve							
Degeneration of nerve fibers	9	15	17	10	10	11	15
Skeletal Muscle							
Atrophy	4	7	7	10	1	2	7

(Conc

[a] Pathology conducted at Hoechst Aktiengesellschaft.

[b] Includes animals from the 24-month chronic study, satellite groups of a tested for BSP/PSP function analyses, animals that were sacrificed at te tion and animals that were sacrificed moribund or died.

TABLE 12. Selected Nonneoplastic Histologic Findings of Rats Fed Trifluralin for 28 Months - Oncogenicity Study[a]

Organ/Finding	Dose Level (ppm) Males 0	200	800	3200	Females 0	200	800	3200
Number of tissues examined	60[b]	60	60	60	60	60	60	60
Lung								
Pneumonitis	2	5	5	5	4	2	2	13
Kidneys								
Glomerulonephrosis	24	19	23	27	8	8	3	10
Basophilic/dilated tubules	10	13	12	4	0	3	0	0
Pelvic dilatation	3	6	12	17	14	17	19	13
Urothelial hyperplasia	2	4	3	7	13	1	8	21
Pelvic calculi	3	6	10	9	19	22	26	24
Spleen								
Hemosiderosis	7	10	14	11	24	28	18	26
Liver								
Vacuolated hepatocytes	10	16	16	9	4	3	11	2
Eosinophilic hepatocytes	2	2	9	5	2	6	5	8
Bile duct hyperplasia	21	15	21	4	12	13	19	11
Lymph nodes								
Dilated sinuses	13	16	21	16	12	15	9	9
Histiocytosis	13	20	28	18	15	17	16	19
Adrenals								
Cortical vacuolation	31	16	20	23	1	3	7	1
Congested	0	2	3	5	18	19	16	11
Cystic degeneration	0	0	0	1	7	9	10	5
Pituitary								
Hyperplasia	7	11	11	10	6	7	10	11
Mammary gland								
Hyperplasia	1	1	0	2	10	7	7	6
Testes								
Atrophy	8	10	10	20				
Uterus								
Dilated gland					11	5	4	20
Sciatic nerve								
Nerve fiber degeneration	42	38	45	40	26	35	26	28
Skeletal muscle								
Atrophy	24	30	34	40	19	11	17	21

[a] Pathology conducted at Huntingdon Research Center, Huntingdon, England.

[b] Includes animals sacrificed at termination and those that were sacrificed

females relative to controls; this was reported due to chance variability and of no toxicologic importance. Many males and females (all groups) had dilated sinuses and histiocytosis of the lymph nodes, bile duct hyperplasia, hemosiderosis of the spleen, hyperplasia of the pituitary, and chronic glomerulonephropathy. Renal pelvic dilatation and urothelial hyperplasia found in males and females were reported to be associated with renal calculi, which were prevalent in all animals. These findings were reported to be common for the age, strain, and sex of the animals. As in the chronic study, the incidence of the finding was at least as prevalent among the controls as the dosed animals.

2. Neoplastic - Oncogenicity Study: Table 13 summarizes neoplastic histopathologic findings in rats dosed with trifluralin during the oncogenicity study. There were no compound-related increases in tumors at any site. The only statistically identified alteration in tumor incidence was reported to be an increase in granular cell meningiomas of the brain in males fed 3200 ppm trifluralin. This was significantly different from control incidence ($p < 0.05$) in high-dose males and there was a significant linear trend ($p < 0.001$). However, granular cell meningiomas are benign neoplasms found to occur spontaneously in older rats of various strains.[1] Since the Hoe WISKf(SPF71) Wistar rat strain was not referenced, the study laboratory compiled data regarding the incidence of granular cell tumors in past studies of 25-30 months (CBI pages 1111-1116). The results were found to be comparable to the referenced data. The incidence of granular cell tumors in the Hoe WISKf (SPF71) Wistar rat strain was found to be variable, ranging from 0-12 percent in collectives of 50-60 control animals of comparable ages; male rats displayed a higher incidence relative to females. In the trifluralin oncogenicity study, the granular cell tumors were found principally in high-dose males and were reported to be small in size; the incidence did not show any apparent dose relationship (0/60 in control males, 1/60 in 200-ppm males, 0/60 in 800-ppm males, and 7/60 in 3200-ppm males). In addition, the incidence of this tumor type was only minimal in females and animals of the chronic study fed trifluralin for 24 months (Table 11). Therefore, this finding was considered random and was not considered to be compound related.

A significant ($p < 0.05$) linear trend was found in the incidence of benign liver cell tumors in males receiving

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[1]Burek, J.D. 1978. Pathology of Aging Rats. CRC Press, p. 145.

TABLE 13. Incidence of Neoplastic Lesions in Rats Fed Trifluralin for 28 Months[a]

Organ/Finding	Dose Level (ppm) Males 0	200	800	3200	Females 0	200	800	3200
Liver	(60)[b]	(60)	(60)	(60)	(60)	(59)	(60)	(59)
Malignant liver cell tumor	1	2	1	1	0	0	1	0
Benign liver cell tumor	0	0	0	3[T]	0	0	1	1
Thyroid	(60)	(58)	(60)	(60)	(59)	(58)	(60)	(59)
Parafollicular cell carcinoma	3	4	3	0	1	0	1	0
Follicular adenoma	4	3	3	6	4	1	3	6[T]
Follicular adenocarcinoma	0	0	2	0	0	1	0	2[T]
Pituitary	(53)	(52)	(59)	(56)	(54)	(59)	(60)	(58)
Adenoma	9	9	7	4	30	28	20	10
Carcinoma	-	-	-	-	2	1	2	0
Brain	(60)	(59)	(60)	(59)	(60)	(60)	(60)	(59)
Granular cell meningioma	0	1	0	7*[T]	2	0	0	1
Testes	(60)	(60)	(60)	(60)				
Interstitial cell tumor	9	8	7	4				
Mammary gland					(17)	(19)	(17)	(15)
Fibroadenoma					9	8	6	6
Adenocarcinoma					8	8	8	6
Uterus					(60)	(59)	(60)	(59)
Adenocarcinoma					2	0	3	5[c]

[a] Includes animals sacrificed at study termination and those that died or were sacrificed moribund in the course of the study. If a neoplasm occurred with an incidence of only 1/60 or if a higher incidence was found only in control animals, it was not tabulated.

[b] The numbers in parentheses are the numbers of tissues examined histologically.

[c] Reported to be a significant trend by the study authors; when recalculated by our reviewers using the Cochran-Armitage test, this trend was not found to be significant.

*Significantly different from control value ($p \leq 0.05$).

[T]Significant trend ($p \leq 0.05$).

3200 ppm and in the incidence of follicular adenoma and adenocarcinoma of the thyroid of females receiving 3200 ppm. However, the incidence of these tumors was not found to be statistically significant ($p \leq 0.05$) on pairwise comparison. Since the total incidence was low, these variations were considered to be of no toxicologic significance. Pituitary adenomas and carcinomas found in the males and females (total tumor incidence in males was 13% and in females, 40%) were principally found in controls and low-dose animals. Mammary tumors in females were also more prevalent in controls and females receiving 200 or 800 ppm. These variations were considered to be age, sex, and strain related, and of no toxicologic significance.

3. Residue Analysis - Chronic Toxicity Study - Table 14 summarizes the trifluralin residues found in organs and tissues of rats tested at 6, 12, 18, and 24 months. The residues were found to be dose related in organs and tissues and were not found to be accumulative over 24 months, with the exception of the carcass residue, where a time-related increase was reported. Females showed higher residue levels in all tissues examined relative to males.

D. STUDY AUTHORS' CONCLUSIONS:

The authors concluded that the NOEL for the studies was 800 ppm for male and female rats, which corresponded to a compound intake of 42.1 mg/kg/day in males and 52.7 mg/kg/day in females of the chronic toxicity study and 40 mg/kg/day in males and 53 mg/kg/day in females of the oncogenicity study. The occurrence of granular cell meningiomas in male rats of the oncogenicity study was reported to be age related and random. Trifluralin was reported to have no carcinogenic effect in rats.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and complete and the conduct of the study and reporting of data were acceptable. However, a histopathology incidence table indicating grade of neoplasia or severity of finding as well as the results of the statistical calculation of absolute organ weights for females of the oncogenicity study were not provided. The histopathology for the chronic toxicity and oncogenicity studies was conducted in two separate pathology laboratories; this may have caused a problem if discrepancies had been found in the results of the two studies. There was some discrepancy between the study authors and the reviewers in determining the statistical significance of relative pituitary weights and establishing a positive trend in the incidence of adenocarcinomas of the uterus in females of the oncogenicity study. These differences are noted in Tables 10 and 13.

TABLE 14. Representative Results of Residue (mg/kg) Found in Organs and Tissues of Rats Fed Trifluralin for 24 Months - Chronic Study[a]

Organ or Tissue	Months	Dose Level (mg/kg)							
		Males				Females			
		0	200	800	3200	0	200	800	3200
Liver[b]	6	<0.01	ND	ND	0.05	<0.01	ND	0.04	0.8
	12	<0.01	0.04	ND	0.07	<0.03	ND	0.04	1.6
	18	<0.02	ND	ND	ND	<0.01	ND	0.04	0.3
	24	—	—	—	0.1	—	—	—	0.5
Kidney[c]	6	<0.01	ND	0.1	7.5	<0.03	0.07	0.3	5.5
	12	<0.02	ND	0.7	3.4	<0.04	ND	0.2	8.4
	18	<0.03	ND	0.2	0.6	<0.04	0.06	0.08	2.5
	24	<0.01	ND	0.06	0.4	<0.01	ND	0.3	1.7
Heart[c]	6	<0.02	ND	ND	0.9	<0.03	ND	ND	2.9
	12	<0.02	ND	ND	0.7	<0.02	ND	0.2	3.3
	18	<0.03	ND	ND	1.6	<0.02	ND	0.2	0.7
	24	<0.01	ND	0.4	0.9	<0.01	ND	0.2	1.6
Spleen[d]	6	<0.04	ND	ND	0.4	<0.05	ND	0.8	ND
	12	<0.03	ND	ND	0.2	<0.06	ND	0.2	0.7
	18	<0.05	ND	ND	0.3	<0.04	ND	ND	1.5
	24	<0.01	ND	ND	0.3	<0.01	ND	0.1	1.4
Brain[e]	6	<0.01	ND	ND	0.1	<0.01	ND	0.02	1.0
	12	<0.01	ND	0.02	0.2	<0.01	ND	0.02	1.0
	18	<0.01	ND	0.02	0.05	<0.01	ND	0.02	0.06
	24	<0.01	ND	0.01	0.08	<0.01	0.01	0.06	0.3
Intestine[f]	6	<0.02	0.04	0.9	11	<0.01	0.04	2.8	18
	12	<0.01	0.03	1.8	8.2	<0.01	0.03	2.3	14
	18	<0.02	ND	0.9	19	<0.01	ND	2.6	32
	24	<0.01	0.08	2.2	9.1	<0.01	0.3	3.3	27
Fatty Tissue[b]	6	<0.02	0.1	1.7	43	<0.03	0.1	16	190
	12	<0.01	ND	2.2	23	<0.01	0.07	6.9	100
	18	<0.01	0.1	3.4	10	<0.02	0.2	4.1	140
	24	<0.02	ND	1.9	51	<0.01	0.1	20	190

(Continued)

TABLE 14. Representative Results of Residue (mg/kg) Found in Organs and Tissues of Rats Fed Trifluralin for 24 Months - Chronic Study (Continued)[a]

Organ or Tissue	Months	Dose Level (mg/kg)							
		Males				Females			
		0	200	800	3200	0	200	800	3200
Muscle[c]	6	<0.04	ND	0.2	0.9	<0.03	ND	0.3	8.6
	12	<0.02	ND	ND	0.1	<0.03	ND	0.1	0.5
	18	<0.03	ND	ND	0.1	<0.02	ND	ND	7.8
	24	<0.01	ND	0.1	0.4	<0.01	0.08	0.6	1.4
Blood[e]	6	<0.01	ND	—	0.01	<0.01	ND	ND	0.04
	12	—	ND	ND	0.02	<0.01	ND	ND	0.07
	18	<0.01	ND	ND	ND	<0.01	ND	—	0.06
	24	<0.01	ND	0.01	0.09	<0.01	ND	0.03	0.2
Carcass[e]	6	<0.01	0.01	ND	0.4	<0.01	ND	0.2	3.0
	12	<0.01	0.01	0.2	0.5	<0.01	ND	0.3	0.6
	18	<0.01	ND	0.3	1.4	<0.01	0.05	0.3	9.3
	24	<0.01	0.02	0.3	6.3	<0.01	ND	1.5	22

(Concluded)

[a]Based on two rats/group.

[b]Based on a detection limit of 0.04 mg/kg.

[c]Based on a detection limit of 0.06 mg/kg.

[d]Based on a detection limit of 0.09 mg/kg.

[e]Based on a detection limit of 0.01 mg/kg.

[f]Based on a detection limit of 0.03 mg/kg.

ND = Not detectable; <detection limit.

— = Samples destroyed during analysis.

We agree with the authors' assessment that there was no oncogenic response. The control incidence of tumors at specific sites was at least as large as the tumor incidence of dosed animals; these findings were generally in accord to that found for the age and sex of similar strains of rats in other laboratories. The incidence of pituitary adenomas in control and low-dose females (50 and 48 percent, respectively) was high, but was not unusual considering the age of the animals. These data were compared to an average of several NTP bioassays conducted for 24 months with Fischer 344 rats.[2]

Absolute liver and thyroid weights were found to be slightly but non-significantly increased in males and females of the chronic toxicity and oncogenicity studies. However, there were no histologic changes in either study that correlated with these increased weights. These changes are therefore not considered to be of biological significance. There were no other absolute organ weights which consistently showed a compound-related change in the two studies.

Based on the body weight changes at 800 ppm, we assess that the chronic toxicity LOEL for the study is 800 ppm and the NOEL is 200 ppm trifluralin. The study authors set the NOEL at 800 ppm.

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[2] Haseman, J. K., Huff, J. and G.A. Boorman. 1984. Use of historical control data in carcinogenicity studies in rodents. Toxicol. Pathol. 12:126-135.