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January 23, 1987

DATA EVALUATION RECORD

TRIFLURALIN

Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Becker, H., Mueller, E., Ellgehausen, H., Westen, H., Schlotke, B., and Terrier, C. Multiple generation study with trifluralin substance technical grade (Code: HOE 38474 O H AT210) in the rat. (Unpublished project No. 008875 by Research & Consulting Company AG, Itingen, Switzerland, for Hoechst Aktiengesellschaft, Federal Republic of Germany; dated October 17, 1984.) Accession Nos. 258994-258995.

APPROVED BY:

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Department Manager
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1. CHEMICAL: Trifluralin; α,α,α -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine.
2. TEST MATERIAL: Trifluralin, technical grade, was described as a red solid containing greater than 99% active ingredient and stable at 25° or -5°C for 2 years.
3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.
4. STUDY IDENTIFICATION: Becker, H., Mueller, E., Ellgehausen, H., Westen, H., Schlotke, B., and Terrier, C. Multiple generation study with trifluralin substance technical grade (Code: HOE 38474 O H AT210) in the rat. (Unpublished project No. 008875 by Research & Consulting Company AG, Itingen, Switzerland, for Hoechst Aktiengesellschaft, Federal Republic of Germany; dated October 17, 1984.) Accession Nos. 258994-258995.

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7. CONCLUSIONS:

- A. The NOEL for parental toxicity of trifluralin in rats could not be determined due to increased relative kidney weights at all dose levels tested (i.e., 200, 650, and 2000 ppm), renal lesions of the proximal tubules and increased relative liver weights at 650 and 2000 ppm, one death due to acute renal failure at 650 ppm, and reduced body weights at 2000 ppm. The LOEL for this study was 200 ppm.

The NOEL and LOEL for reproductive and developmental toxicity were 200 and 650 ppm, respectively, based on reduced weanling body weights at 650 and 2000 ppm and reduced litter sizes at 2000 ppm.

- B. This study is classified Core Minimum.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

- A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Technical grade trifluralin was described as a red solid with greater than 99% purity. The test material was mixed with granulated food at least every 2 weeks to produce concentrations of 0 (control), 200, 650, and 2000 ppm. The diets were pelleted and refrigerated in paper bags until used. Food pellets were analyzed for concentration, homogeneity, and stability of the test material in the diet preparations. Animals were fed their respective test diets throughout the study.

2. Animals and Experimental Design: Male and female outbred Wistar KFM-Han, SPF quality, rats were obtained from KFM Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland, housed individually, and acclimated for 10 days. At the age of 6-7 weeks, 30 males and 30 females were randomly assigned to each of four study groups and designated F₀ parental animals.

After receiving the test diets for 80 days, F₀ animals were paired, one male to one female of the same group, for up to 20 days to produce F_{1a} litters. Litters were weaned at day 21 postpartum and approximately 10 days later the adults were rebred (using different pairs) to produce F_{1b} litters. Where possible, animals that were not fertile after the first breeding were subsequently paired with fertile animals.

¹Only items appropriate to this DER have been included.

Twenty-six male and 26 female F_{1b} weanlings per group were selected to be F₁ parental animals and received their respective diets for at least 100 days before breeding. F₁ adults were bred (siblings were not paired) to produce F_{2a} and F_{2b} litters using the same procedures described for their parents.

Body weights of parental animals were recorded weekly except during the breeding periods. Mated females were weighed on gestation days (GD) 0, 7, 14, and 21 and on days 1, 4, 7, 14, and 21 postpartum. Food consumption was recorded at the same intervals as body weights except the food consumption of dams was only recorded until day 14 postpartum.

Vaginal smears were examined daily during the breeding periods to detect mated females and estrous cycle anomalies. The day on which vaginal sperm or a vaginal plug were found was designated GD 0.

During late gestation, females were examined twice daily for signs of parturition; gestation lengths were recorded. The day that parturition was completed was considered day 0 postpartum. As soon as possible after parturition, litters were examined for live and dead pups and gross anomalies. Sex ratios were determined on days 0 and 21. Pups were identified individually by tattoos on day 1 and subsequently by color spots on the hair. Pups were individually weighed on days 1, 4, 7, 14, and 21.

3. Observations and Measurements: Animals were examined twice daily for mortality and clinical signs of toxicity. Animals found dead or euthanized were necropsied and tissues were saved for histological examination. Surviving parental animals were killed after 'b' litters were weaned and necropsies were performed. Selected organs were weighed and tissues were saved for possible histological examination. Kidneys of F₁ adults were examined histologically in all groups; other tissues were examined only in the control and high-dose groups.

The uteri of apparently nonpregnant females were stained with ammonium sulfide to detect implantation sites. The testes, prostate, and seminal vesicles of all F₁ males that were not proven fertile were also weighed and examined histologically.

Pups found dead were necropsied and/or preserved for possible further examination. Weanlings not selected to be F₁ parental animals were killed and examined macroscopically. One pup/sex/litter was selected for necropsy; organs were weighed and tissues were preserved for possible histological

examination. Tissues from the selected control and high-dose F_{2b} progeny were examined histologically. Organ weights for the F_{1a} and F_{1b} progeny were not compared since these litters were killed at various times between days 21 and 32 postpartum.

4. Statistical Methods: Univariate one-way analysis of variance (ANOVA) was used to assess body weight, food consumption, organ weight, and reproduction data "if the variables could be assumed to follow a normal distribution." Student's t-test, based on a pooled variance estimate, was used to identify significant differences from the control group. A univariate one-way ANOVA, based on Wilcoxon ranks, together with the Kruskal-Wallis test was used to assess the litter population data. Sex ratios were analyzed using a 2 x 2 chi-square test.

B. Protocol: A protocol was not included in the study report.

12. REPORTED RESULTS:

- A. Test Material Analyses: Chemical analyses of the test diets revealed mean concentrations (\pm S.D.) of 93.3 ± 6.6 , 95.0 ± 8.5 , and $91.0 \pm 4.3\%$ of the nominal values for the low-, mid-, and high-dose levels. Homogeneity samples were within 8.5% for all groups. Stability assays indicated that the test material concentrations generally remained within 10% of initial values for 3 weeks; however, diets prepared on two of the eight dates tested showed greater than 20% declines in concentration over the 3-week period.
- B. Parental Data: Deaths occurred in one mid-dose male of the F₀ generation and in one female from each of the control and mid- and high-dose groups of the F₁ generation. Acute renal failure was diagnosed as the cause of death for the F₁ mid-dose female; the study authors did not report diagnoses for the other deaths.

Yellow discoloration of the urine was the only clinical finding associated with the compound. Although this finding was reportedly dose related, no summary tables or individual clinical findings were included in the study report.

Body weights of F₀ parental animals revealed marginally significant ($p < 0.10$) reductions in low- and high-dose males (Table 1). No other significant weight reductions were noted in the F₀ generation. In the F₁ parental animals, however, high-dose female body weights were significantly ($p < 0.05$) reduced during all phases of the generation (Tables 1 and 2).

TABLE 1. Mean Body Weights (g) of Rats Fed Trifluralin Prior to Breeding

	Dose Level (ppm)	Pretest	Week			
			1	4	8	12
F ₀ Males	0	118	163	275	354	398
	200	121	164	269	342	383
	650	119	166	272	350	391
	2000	118	164	268	340	382
F ₀ Females	0	99	126	174	206	222
	200	98	125	174	206	222
	650	101	128	172	202	217
	2000	100	126	168	197	212
F ₁ Males	0		98	220	331	369
	200		98	226	338	378
	650		90	215	329	364
	2000		87	208	314	348
F ₁ Females	0		91	156	202	221
	200		87	155	203	220
	650		82	150	198	218
	2000		77*	146*	190*	207*

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

TABLE 2. Mean Maternal Body Weights (g) of Rats Fed Trifluralin

	Dose Level (ppm)	Gestation Day			Lactation Day			
		0	14	21	1	4	14	21
<u>F₀ Females</u>								
F _{1a} Interval	0	225	268	327	247	259	284	270
	200	223	264	347	242	258	283	272
	650	219	257	344	240	253	278	268
	2000	212	251	352	228	246	269	261
F _{1b} Interval	0	252	292	361	271	285	311	294
	200	251	288	354	266	285	310	302
	650	245	283	352	260	277	303	294
	2000	236	268	329	249	263	290	284

<u>F₁ Females</u>								
F _{2a} Interval	0	235	276	340	237	260	292	284
	200	226	265	325	236	254	288	278
	650	227	266	329	230	252	284	276
	2000	219*	255*	310*	218*	241*	270*	272*
F _{2b} Interval	0	262	301	367	279	294	320	305
	200	261	299	365	277	293	323	307
	650	253	291	357	271	288	313	299
	2000	240*	274*	334*	255*	271*	294*	289*

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

Food consumption was significantly reduced during the first week of the study for both F₀ males and females of the high-dose group (Table 3). Significantly reduced food consumption was also noted during week 3 and both lactation periods (Table 4) for the F₀ females. In the F₁ generation, food consumption of high-dose males was significantly reduced when compared to controls for a 2-week interval after the second breeding period. In general, high-dose females had significantly reduced food consumption during the second gestation and lactation periods. Food conversion ratios were similar in all groups for both sexes of both generations.

Gross necropsies of parental animals revealed yellow discoloration of adipose tissue in mid-dose females and high-dose males and females of both generations; high-dose females showed the greatest incidences. Histological examinations of F₁ adults also revealed dose-related increased incidences of lesions of the renal proximal tubules in mid- and high-dose females. In addition, increased incidences of hyaline droplets in the tubular epithelium occurred in females of all dosed groups and reduced incidences of corticomedullary mineralization occurred in mid- and high-dose females. All other gross and microscopic lesions were considered incidental.

Dose-related significant increases in relative liver weights occurred in mid- and high-dose F₀ males, F₁ males, and F₁ females and in high-dose F₀ females (Table 5). Significantly increased relative kidney weights occurred at all dose levels of F₀ males and in mid- and high-dose F₁ males. High-dose males of both generations and mid-dose males of the F₁ generation also had significantly increased relative testicular weights. Significantly reduced thymus weights occurred in high-dose males and females of the F₁ generation. Other significant differences in organ weights occurred inconsistently across generations or in nondose-related patterns.

Reproductive and Developmental Data: The proportions of females mating, becoming pregnant, delivering, and rearing their litters to weaning were generally comparable for all groups at all breeding intervals (Table 6). In addition, precoital intervals and gestation lengths were comparable for all groups and the behavior of dams during parturition and nursing were also reportedly similar for all groups.

High-dose litter sizes on day 0 were slightly reduced in the F_{1b} interval and significantly reduced in the F_{2a} and F_{2b} litters when compared to controls (Table 7). The F_{2b} high-dose litters were also significantly smaller on day 21. Pup mortality and pup weights at birth did not indicate any compound effects; however, mid-dose weanling weights were significantly reduced at all but the F_{2a} interval, and high-dose weights were significantly reduced at all litter intervals. External examinations of

TABLE 3. Mean Food Consumption (g/rat/day) of Rats Fed Trifluralin Prior to Breeding

	Dose Level (ppm)	Pretest	Week				
			1	3	7	10	11
F ₀ Males	0	20	23	24	24	23	26
	200	20	23	24	23	23	22
	650	21	23	24	25	23	23
	2000	20	21*	23	24	23	23
F ₀ Females	0	16	17	17	17	16	18
	200	16	16	17	16	16	16
	650	17	16	16	17	16	15
	2000	16	15*	15*	16	16	16
F ₁ Males	0		19	25	26	28	25
	200		18	23	25	26	24
	650		18	23	26	26	24
	2000		19	22	24	27	24
F ₁ Females	0		16	17	18	21	19
	200		15	17	18	19	17
	650		14	16	18	19	17
	2000		17	16	17	19	17

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

TABLE 4. Mean Maternal Food Consumption (g/rat/day) of Rats Fed Trifluralin

	Dose Level (ppm)	Gestation Days			Lactation Days		
		0-7	7-14	14-21	1-4	4-7	7-14
<hr/>							
F₀ Females							
F _{1a} Interval	0	19.5	21.1	22.2	29.4	40.0	55.0
	200	18.6	20.3	25.1	29.6	40.7	55.5
	650	17.8	19.7	26.0	33.3	39.4	55.7
	2000	18.7	20.1	27.5	28.5	37.1*	50.8*
F _{1b} Interval	0	19.5	21.4	21.7	36.5	47.0	62.1
	200	18.4	20.5	21.6	36.2	46.3	62.0
	650	18.4	20.6	22.2	38.8	48.7	63.0
	2000	18.5	20.8	20.7	38.5	42.4*	56.6*
<hr/>							
F₁ Females							
F _{2a} Interval	0	19.0	21.4	22.8	31.9	47.7	58.3
	200	18.8	21.1	21.5	32.7	48.3	61.2
	650	19.6	21.3	22.1	33.7	49.9	60.0
	2000	19.4	20.8	21.5	32.5	45.8	57.5
F _{2b} Interval	0	20.8	23.3	24.0	38.4	49.7	66.0
	200	19.6	22.0	22.3	35.8	49.7	67.6
	650	21.0	22.0	21.2	37.5	46.3	63.9
	2000	19.3*	21.7	21.7*	33.4*	44.9*	59.1*

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

TABLE 5. Mean Relative Organ Weights (% Body Weight)
of Rats Fed Trifluralin

	Dose Level (ppm)	Body Weight (g)	Liver	Kidneys	Thymus	Gonads
F ₀ Males	0	481	3.08	0.51	0.044	0.79
	200	465	3.17	0.54**	0.048	0.81
	650	473	3.27**	0.55**	0.045	0.80
	2000	459	3.73**	0.58**	0.042	0.85*
F ₀ Females	0	271	4.42	0.70	0.043	0.034
	200	273	4.55	0.69	0.044	0.035
	650	264	4.62	0.69	0.049	0.037
	2000	264	5.60**	0.72	0.045	0.032
F ₁ Males	0	472	3.34	0.49	0.061	0.77
	200	486	3.44	0.50	0.059	0.80
	650	465	3.67**	0.54**	0.062	0.84*
	2000	446*	3.86**	0.54**	0.052*	0.87*
F ₁ Females	0	269	4.04	0.64	0.094	0.040
	200	268	4.32	0.64	0.089	0.040
	650	265	4.42**	0.63	0.082	0.042
	2000	253**	4.90**	0.63	0.079*	0.040

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

**Significantly different from control value (p <0.01).

TABLE 6. Summary of Reproductive Performance of Rats Fed Trifluralin

	Dose Level (ppm)	No. Paired	No. Mated	<u>Pregnant</u>		<u>Delivering</u>		<u>Weaning</u>	
				No.	%	No.	%	No.	%
<u>F₀ Females</u>									
F _{1a} Interval	0	30	29	29	100	29	100	28	97
	200	30	30	28	93	28	100	27	96
	650	30	30	30	100	30	100	30	100
	2000	30	30	29	97	29	100	29	100
F _{1b} Interval	0	30	30	28	93	28	100	28	100
	200	30	30	27	90	27	100	27	100
	650	30	30	23	77	23	100	23	100
	2000	30	30	29	97	29	100	29	100

<u>F₁ Females</u>									
F _{2a} Interval	0	26	26	24	92	24	100	24	100
	200	26	26	20	77	20	100	20	100
	650	26	26	21	81	21	100	20	95
	2000	26	26	23	88	23	100	23	100
F _{2b} Interval	0	26	26	21	81	21	100	20	95
	200	26	26	25	96	25	100	25	100
	650	26	26	25	96	23	92	22	96
	2000	26	26	26	100	16	100	26	100

TABLE 7. Summary of Litter Data of Rats Fed Trifluralin

	Dose Level (ppm)	Mean No. Live Pups ^a		% Mortality ^a Days 1-21	Mean Pup Weight (g)		
		Day 0	Day 21		Day 1	Day 7	Day 21
F _{1a} Litters	0	10.4	10.1	2.7	6.2	12.9	41
	200	10.7	10.4	2.8	6.0	12.5	40
	650	11.0	10.7	2.7	6.1	12.1*	37*
	2000	10.2	9.8	3.7	6.1	11.9*	36*
F _{1b} Litters	0	11.9	11.5	3.3	6.2	12.7	38
	200	11.4	11.2	1.3	6.4	13.0	39
	650	12.0	11.8	1.4	6.2	12.1*	35*
	2000	10.7	10.2	4.8	6.0	12.0*	34*
F _{2a} Litters	0	11.8	11.3	4.2	5.8	12.1	39
	200	11.8	11.5	2.6	5.8	12.3	40
	650	11.7	11.4	2.1	5.9	12.2	38
	2000	10.3*	10.1	1.7	5.6*	11.6*	36*
F _{2b} Litters	0	12.0	11.8	1.7	6.4	13.2	41
	200	11.9	11.7	1.3	6.3	13.2	41
	650	11.4	11.3	0.8	6.5	13.1	40*
	2000	10.1*	10.1*	0.0	6.4	12.9	38*

^aDoes not include litters that did not survive to day 21.

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

the pups revealed no anomalies attributable to the compound. Sex ratios of the pups also did not suggest a compound effect.

Organ weight comparisons of the F_{2a} and F_{2b} weanlings revealed significantly increased relative liver weights in the high-dose group at both litter intervals (Table 8). Significantly increased relative kidney weights were also noted by the study authors for F_{2b} females. Significantly increased relative testicular weights occurred at both intervals. Other significant changes did not appear to indicate an effect. No histological abnormalities attributable to the compound were noted.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

- A. The study authors cited the NOEL of this study to be 200 ppm. They noted that there were slight parental effects at 650 and 2000 ppm, reduced litter sizes at 2000 ppm, and reduced pup weight gains at 650 and 2000 ppm.
- B. A quality assurance statement was signed and dated November 14, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Test Material Analyses: Chemical analyses of the test diets indicated that they were accurately and properly prepared. Although diets prepared on two dates showed greater than 20% declines in concentration after 21 days, these were isolated occurrences and were not regarded to affect the integrity of the study.

Parental Data: Deaths were infrequent and their overall incidence did not suggest a compound effect; however, we regard the death of a mid-dose F₁ dam (attributed to acute renal failure) to be compound related. The renal lesions observed in this animal were consistent with the microscopic changes seen in mid- and high-dose F₁ kidneys. We consider the occurrence of lesions of the renal proximal tubules to be toxic effects in females at 650 and 2000 ppm. Hyaline droplets in the tubular epithelium in females of all dose groups and the reduced incidences of corticomedullary mineralization in mid- and high-dose females were considered to be physiological responses rather than toxic effects.

We consider the following significant changes in relative organ weights to be compound related: increased liver weights in males and females at 650 and 2000 ppm, increased kidney weights in males at all dose levels, reduced thymus weights in females at 2000 ppm, and increased testicular weights at 650 and 2000 ppm.

TABLE 8. Mean Relative Organ Weights (% body weight)
of Weanlings of Rats Fed Trifluralin

	Dose Level (ppm)	Body Weight (g)	Liver	Kidneys	Gonads	Uterus
F _{2a} Males	0	41	4.02	1.04	0.50	--
	200	42	4.08	1.04	0.52	--
	650	39	4.12	1.04	0.51	--
	2000	38	4.44**	1.05	0.54**	--

F _{2a} Females	0	38	3.88	1.06	0.044	0.107
	200	40	4.00	1.06	0.037**	0.103
	650	38	4.03	1.06	0.039*	0.101
	2000	37	4.38**	1.09	0.041	0.093

F _{2b} Males	0	43	3.98	1.00	0.48	--
	200	43	4.05	1.05*	0.49	--
	650	43	4.08	1.00	0.48	--
	2000	40	4.31	1.04	0.52*	--

F _{2b} Females	0	41	3.90	1.05	0.044	0.098
	200	41	3.98	1.07	0.041	0.101
	650	40	4.12*	1.08	0.044	0.102
	2000	38	4.26**	1.10**	0.042	0.100

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

**Significantly different from control value (p <0.01).

We regard the increased relative kidney weights to be indicative of a toxic effect in F₀ males at 200, 650, and 2000 ppm and in F₁ males at 650 and 2000 ppm. In addition, increased relative liver weights indicated a toxic effect in parental animals of both generations at 650 and 2000 ppm. We consider the yellow discoloration of adipose tissue and urine to be compound related, but not to reflect overt toxicity.

We consider the reduced body weights of males and females to indicate a toxic effect at 2000 ppm. We assess the F₀ male low-dose weight reductions to be incidental since the weights of mid-dose males and low-dose females were comparable to controls.

We consider the significantly reduced F₀ high-dose food consumption of both males and females during the first week of the study to indicate reduced palatability of the test article rather than a toxic effect. Except for gestation and lactation values, subsequent food intake data were generally comparable for all groups and did not suggest a compound effect. We attribute the reduced food consumption of high-dose F₀ dams during both lactations and F₁ dams during the F_{2b} gestation and lactation periods to developmental effects (i.e., smaller litters, reduced pup weights), rather than parental toxicity.

Reproductive and Developmental Data: The mating data, pregnancy rates, and the numbers of females delivering and weaning their litters did not indicate any reproductive effects of the compound. Significantly increased relative testicular weights in high-dose males of both generations and mid-dose F₁ males were not associated with reduced F₀ fertility and were therefore not considered a reproductive effect.

We regard the reduced F_{1b}, F_{2a}, and F_{2b} litter sizes at birth to indicate developmental toxicity at 2000 ppm. We also consider the significantly reduced weanling weights in the high-dose group at all litter intervals and in the mid-dose group at the F_{1a}, F_{1b}, and F_{2b} intervals to be toxic effects at 650 and 2000 ppm. Although we consider the significantly increased relative kidney, liver, and testicular weights of high-dose F_{2a} and F_{2b} weanlings to be compound related, their biological meaning in terms of developmental toxicity is unclear.

- B. The only major difference between the reviewers and the study authors in the interpretation of the results concerns parental relative organ weights. The study authors did not regard significant changes in relative organ weights to be compound related; however, we assess that increased relative kidney weights reflected toxicity at all dose levels and that increased relative liver weights indicated an effect at 650 and 2000 ppm.

The only other difference in interpretation concerns the food consumption data. Although the study authors noted significantly reduced food intake at 2000 ppm, we regard the differences for dams to be due to developmental effects; other differences were infrequent and did not suggest a toxic effect.

- C. The study report did not include summary tables or individual data of clinical findings. This deficiency precluded the correlation of individual clinical data with other parental data and with litter data.

Item 15--see footnote 1.

- 16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 21-35.

APPENDIX A

Materials and Methods

Page _____ is not included in this copy.

Pages 19 through 32 are not included.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients.
- ☐ Identity of product impurities.
- ☐ Description of the product manufacturing process.
- ☐ Description of quality control procedures.
- ☐ Identity of the source of product ingredients.
- ☐ Sales or other commercial/financial information.
- ☐ A draft product label.
- ☐ The product confidential statement of formula.
- ☐ Information about a pending registration action.
- ☒ FIFRA registration data.
- ☐ The document is a duplicate of page(s) _____.
- ☐ The document is not responsive to the request.

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