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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: *TACKLE; Sodium Acifluorfen; Evaluation of rat reproduction data; 359-TNI; Action Code 166; Record #165936; Caswell #818B. Registrant: Rhone-Poulenc*

TO: *Richard Mountfort, PM #23*
Registration Division (TS-769C)

FROM: *Alan C. Katz, M.S., D.A.B.T.* Alan C Katz 2/25/86
Toxicology Branch
HED (TS-769C)

THROUGH: *Clint Skinner, Ph.D., D.A.B.T.* Clint Skinner 2-25-86
Head, Review Section III
and
Theodore, M. Farber, Ph.D., D.A.B.T. Theodore M. Farber 2/26/86
Chief, Toxicology Branch

Action Requested:

Review 2-generation rat reproduction study to complete data base for registration.

Background:

This study replaces a previous 3-generation rat reproduction study which was classified as Invalid (see review by R. Zendzian, 10/10/84). The attached one-liners provide a brief outline of completed toxicology studies and overall conclusions.

Results:

The attached DER (EPA #68-02-4225; Dynamac #1-075-A; 2/21/86) has been reviewed and approved by Tox Branch. The study, "Reproductive effects of Tackle administered orally in feed to Crl:COBS CD(SD)BR rats for two generations," by Lochry et al (unpublished study No. 218-002 prepared by Argus Research Laboratories, Inc., and submitted by Rhone-Poulenc, Inc.; 1/20/86), is classified Core Minimum.

Dietary concentrations were 0, 25, 500 and 2500 ppm. No adverse effects on adult reproductive performance were apparent in any of the treated groups. Based primarily on treatment-related mortality and reduced body weight in the high dose groups and increased incidence of kidney lesions in mid and high dose females (P_1 and F_1) and high dose males (F_1), the LEL for parental toxicity is 500 ppm and the NOEL is 25 ppm. Increased mortality occurred in mid and high dose pups (F_1 and F_2), and body weights of high dose pups were significantly reduced at birth and throughout the lactation period. Dose-related renal pelvic dilatation was found in F_2 offspring. Thus, for offspring toxicity, the LOEL is 500 ppm and the NOEL is 25 ppm.

CONFIDENTIAL E NESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-02-4225
DYNAMAC No: 1-075-A
February 21, 1986

DATA EVALUATION RECORD

TACKLE

Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Lochry, E.A., Hoberman, A.M., and Christian, M.S. Reproductive effects of Tackle administered orally in feed to Crl:COBS CD(SD)BR rats for two generations. (Unpublished study No. 218-002 prepared by Argus Research Laboratories, Inc., Horsham, PA, and submitted by Rhone-Poulenc, Inc., Monmouth Junction, NJ; dated January 20, 1986.)

APPROVED BY:

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

Signature: Ira Cecil Felkner

Date: 2-21-86

1. CHEMICAL: The active ingredient in Tackle is sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate; sodium salt of acifluorfen.

2. ~~TEST MATERIAL: Technical grade Tackle, Lot No. 305164001, was described as~~ a dark yellow liquid.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Lochry, E.A., Hoberman, A.M., and Christian, M.S. Reproductive effects of Tackle administered orally in feed to Crl:COBS CD-(SD)BR rats for two generations. (Unpublished study No. 218-002, prepared by Argus Research Laboratories, Inc., Horsham, PA, and submitted by Rhone-Poulenc, Inc., Monmouth Junction, NJ; dated January 20, 1986.)

5. REVIEWED BY:

Robin B. Phipps, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Robin B. Phipps
Date: February 21, 1986

Guillermo Millicovsky, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: G Millicovsky
Date: 21 FEB 1986

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Teratogenicity and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Signature: Ira Cecil Felkner
Date: 2-21-86

Alan Katz, M.S., D.A.B.T.
EPA Reviewer

Signature: Alan C Katz
Date: 2/25/86

Clint Skinner, Ph.D., D.A.B.T.
EPA Section Head

Signature: Clint Skinner
Date: 2-25-86

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7. CONCLUSIONS:

   A. We assess the LOEL and NOEL for parental toxicity of Tackle in rats at 500 ppm and 25 ppm, respectively, based on compound-related mortalities and increased incidence of kidney lesions at the 500- and 2500-ppm doses and reduced body weights at the 2500-ppm dose. No adverse effects on reproductive performance were noted at any dose; therefore, 2500 ppm, the highest dose tested, is assessed as the NOEL for reproductive effects. The LOEL and NOEL for offspring toxicity are assessed at 500 and 25 ppm, respectively, based on decreased viability and increased incidence of kidney lesions at the 500- and 2500-ppm doses, and reduced body weights at the 2500-ppm dose.

   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: The test material, technical grade Tackle (Lot No. 305164001), was described as a dark yellow liquid. Fresh test diets were prepared weekly on a weight per weight basis to yield Tackle concentrations of 0, 25, 500, and 2500 ppm. To prepare each test diet, the required amount of test material was poured into 100 mL of distilled water contained in a spray gun. This mixture was sprayed into a rotary mixer containing the appropriate amount of basal diet, and the diet was mixed for 15 minutes. Diets were administered ad libitum to groups of 35 P_1 rats per sex beginning at 47 days of age, and continuing until sacrifice, and to groups of 40 F_1 rats from weaning until sacrifice.

         All test diets were prepared and analyzed by Bio/dynamics, Inc., East Millstone, NJ, a subcontracted laboratory. Each test diet, including the control, was analyzed weekly to determine concentrations of the test material. In addition, homogeneity and stability analyses were performed at the beginning of the study. The technical material used to prepare the test diets was analyzed at 6, 9, and 12 months after study initiation.

      2. Test Animals and Study Design: One hundred sixty Crl:COBS CD(SD)BR rats per sex were supplied by Charles River Breeding Laboratories, Inc., Kingston, NY, for use in this study. The rats were 40 days of age when received, and their body weights ranged from 123-185 g for the males and 74-145 g for the females. Following an acclimation period of approximately 1 week, 35 rats per sex were selected for each study group on the basis of physical appearance and body weight.

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[1] Only items appropriate to this DER have been included.

Beginning at 7 weeks of age, P_1 rats were provided test diets ad libitum for 12 weeks prior to a 3-week cohabitation period. The rats were mated on a one to one basis; females were paired with new males if mating was not confirmed after 14 days. Day 0 of gestation was defined as the day sperm were observed in a vaginal smear and/or a copulatory plug was observed. The day of birth was defined as day 1 of lactation. On day 4 of lactation, the F_1 litters were culled to eight pups (four males and four females, when possible), and on day 21, 40 pups/sex/group[2] were randomly selected for continuation on study. Beginning at 4 weeks of age, these F_1 rats received test diets for 12 weeks prior to mating as described for the first generation. Second generation (F_2) litters were not culled and were sacrificed at weaning (day 21 of lactation).

Gross necropsies were performed on any rat that died, on culled pups that were not continued on study, and on all rats in both generations following scheduled sacrifice. All gross lesions, target organs (liver, kidney, and stomach), pituitary glands, and reproductive organs were preserved. The reproductive tissues, pituitary glands, and livers of 20 randomly selected control and high-dose rats/sex/generation were examined histopathologically. In addition, the stomachs, kidneys, and all gross lesions of all P_1 and F_1 adult rats were examined microscopically. Gross lesions were the only tissues from F_1 and F_2 pups that were examined microscopically.

3. Observations and Measurements: During the study period, all rats were observed at least twice daily for mortality and once daily for clinical signs of compound effects. Body weights and feed consumption were recorded at least once weekly for parental rats. Maternal body weights and feed consumption were recorded on days 0, 6, 10, 15, 20, and 25 (if required) of gestation and on days 1, 4, 7, 11[3], 14, 16[3], 18[3], and 21 of lactation.

During the cohabitation period, estrous cycling (vaginal cytology) was evaluated until the presumed day 0 of gestation was identified; mating performance was evaluated daily, and confirmed by the natural delivery of a litter or the presence of implantation sites *in utero* at time of sacrifice. Females that did not deliver litters by presumed day 25 of gestation were sacrificed and examined for implantation sites; uteri that appeared nongravid were stained with ammonium sulfide to confirm pregnancy status. Maternal behavior was observed when the pups were examined during the 21-day lactation period.

Both generations of female rats were evaluated for duration of gestation, litter size, and pup viability at birth.

Each litter was examined daily during lactation for viability and physical signs including nursing behavior and gross external anomalies. Pup body weights were recorded on days 1 (birth), 4, 7,

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[2]Additional pups were later added as replacements for rats that died soon after weaning.
[3]Only for F_1 adults.

14, and 21 postpartum. Except for the F_1 pups that were culled or selected as the next parental generation, all first and second generation pups were sacrificed at weaning (day 21 postpartum).

The heads were cross-sectioned by a single cut at the suture between the frontal and parietal bones, and the brains were examined for hydrocephaly.

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

12. REPORTED RESULTS:

A. Test Material Analyses: Homogeneity assays performed on test diets at the beginning of the study yielded mean recoveries of 99.2%, 89.5%, and 94.2% of nominal concentrations for the low-, mid-, and high-dose groups, respectively. The authors stated that technical grade Tackle was stable for at least 21 days at room temperature when mixed in rodent chow. The reported results on day 21 were 77.0%, 94.8%, and 84.8% of nominal levels for the low-, mid-, and high-dose groups, respectively. The authors stated that initially a limit of $\pm 10\%$ of target concentration was used to determine acceptability of the test diets for administration to the animals. However, because of difficulties in diet preparation, the limit was increased to $\pm 15\%$ of the nominal concentration. The mean percentage of the nominal concentrations for test diets were 96%, 93%, and 93% for the low-, mid-, and high-doses, respectively. The purity of the test material, when received for use on study, was not reported. Analyses of the technical material at months 6, 9, and 12 showed the percent purity to be 21.1%, 21.6%, and 21.3% of the nominal concentration, respectively.

B. Parental Effects:

1. Survival and Clinical Signs: In both generations, there was a low incidence of mortality attributable to effects of the high dose of Tackle (Table 1). The authors attributed the deaths of one P_1 male, one F_1 male, and three F_1 females in the high-dose group to compound administration; at necropsy, these rats had compound-related kidney lesions.

A slight (nonsignificant) increase in the incidence of chromodacryorrhea was reported for the P_1 high-dose males (213 occurrences in 9 rats) when compared with the control value (84 occurrences in 4 rats). In the second generation, the incidences of high-dose males with thin and/or emaciated appearance (52 occurrences in 3 rats)[4], and of high-dose females with thin appearance (11 occurrences in 4 rats)[4] were significantly increased ($p \leq 0.01$) over control values (zero incidence). The authors attributed these clinical signs to administration of Tackle at 2500 ppm. No other clinical signs were associated with the test material.

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[4]These values could not be verified from the data presented in the final report.

TABLE 1. Summary of Mortality in Parental Rats Fed Tackle for Two Generations

Dose Level ppm	Incidence of Mortality			
	First Generation (P_1)		Second Generation (F_1)	
	Males	Females	Males	Females
0	0/35	0/35	0/40	1/40
25	0/35	1/35	1/41	0/40
500	0/35	0/35	0/40	0/40
2500	1/35	0/35	1/40	5/43**a

**Significantly different from control value ($p \leq 0.01$).

[a]Two of these deaths were attributed to failure to thrive after weaning.

2. Body Weight and Food Consumption:

A. Premating: High-dose male and female rats of both generations had significant ($p \leq 0.05$ to $p \leq 0.01$) inhibition of premating body weight and/or body weight gain compared to control values (Table 2). Mean daily feed consumption (g/kg/day) was significantly ($p < 0.05$ to $p < 0.01$) inhibited for high-dose P_1 males and females when compared with controls for the first 1 or 2 weeks of diet administration (Table 3). Thereafter, values for all groups were generally comparable to controls. In the second generation, significant ($p \leq 0.01$) increases were reported in mean daily feed consumption for high-dose rats when compared to control values. Although differences diminished during the premating period, they remained significant. The authors considered this effect to be "interrelated with the relatively high concentration of the test substance in the diet and the nutritional requirements of the early and middle phases of the growth curve." The authors stated that feed consumption for low- and mid-dose F_1 males was comparable to control values whereas a dose-dependent increase was apparent for F_1 females during the premating period.

B. Gestation: During days 6-10 of gestation, body weight gain of mid-dose P_1 females was significantly inhibited ($p < 0.01$) when compared to control values (Table 4). This correlated with significantly lower ($p \leq 0.01$) feed consumption for the mid-dose females during gestation (Table 5).

Body weight gain was also inhibited at the high-dose level (days 0-6); however, feed consumption values for this group were comparable to control values. In the second generation, high-dose females had significantly ($p \leq 0.01$) lower mean body weights than the controls throughout gestation. Body weight gain for high-dose females was slightly lower than controls and feed consumption values were slightly increased. Body weight gains and feed consumption values for the low- and mid-dose F_1 females were comparable to controls.

C. Lactation: As during the premating and gestation intervals, body weights of high-dose females continued to be lower than controls during lactation for both generations (Table 6). In the first generation, feed consumption was significantly decreased for high-dose dams when compared to controls (Table 7). In the second generation, feed consumption values were comparable among all treatment groups.

3. Reproductive Effects: The authors stated that administration of Tackle at 25, 500, or 2500 ppm did not adversely affect the adult primary reproductive parameters for either generation. The mating incidence, the mean number of days in cohabitation, the fertility index (percentage of mated rats that were pregnant), and the gestation index (percentage of pregnancies resulting in litters) were comparable for all treatment groups (Table 8). In the first generation, the mean length of gestation was comparable for all

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TABLE 2. Mean Parental Body Weights (g+SD) in Rats Fed Tackle for Two Generations

Generation	Dose Level ppm	Day of Study				
		0	22	50	78	119[a]
P_1 Males	0	201.4+14.5	352.8+18.8	454.5+32.1	524.9+40.3	588.9+50.0
	25	201.0+13.5	348.7+20.3	450.5+34.4	515.2+44.7	574.5+55.1
	500	200.3+15.4	353.0+21.3	448.2+33.5	512.6+41.8	568.2+50.1
	2500	201.1+14.4	336.4+16.2**	428.6+23.4**	489.2+31.1**	545.2+42.0**
		1	22	50	78	123[a]
F_1 Males	0	155.6+16.7	336.3+24.3	472.0+36.6	548.2+43.2	620.4+52.0
	25	134.3+31.8	305.3+37.2	441.9+36.8	521.9+44.0	602.5+56.9
	500	139.2+26.9	314.9+33.4	457.3+42.0	536.0+53.4	615.3+66.9
	2500	117.5+21.5*	277.4+40.7**	418.4+34.2*	488.9+46.4*	558.5+58.0*
		0	22	50	78	82[a]
P_1 Females	0	146.1+10.8	212.1+18.3	259.2+22.2	287.0+25.3	291.6+26.5
	25	148.1+10.6	217.2+14.8	265.0+18.9	290.6+23.2	296.6+24.0
	500	146.4+10.9	216.8+19.6	263.4+24.9	289.8+29.4	295.4+29.3
	2500	146.8+10.7	206.6+15.1	247.1+18.9*	274.4+22.6*	278.0+22.4*
		1	22	50	78	82[a]
F_1 Females	0	126.3+13.2	204.2+14.6	253.5+16.2	282.7+21.0	287.9+21.4
	25	117.8+24.0	202.8+23.2	256.2+27.7	287.3+32.2	292.6+33.3
	500	112.0+19.4	201.2+17.2	259.7+23.0	294.9+28.0	299.4+30.4
	2500	96.1+18.4*	178.2+18.4*	232.6+23.6	262.0+26.2	264.0+26.9

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

[a]Rats were placed into cohabitation on day 82. Weights were not recorded during the 21-day mating period.

TABLE 3. Mean Parental Feed Consumption (g/kg/day±SD) in Rats Fed Tackle for Two Generations

Generation	Dose Level ppm	Day of Study				
		0-8	22-29	50-57	78-82[a]	106-119
P_1 Males	0	104.3±7.5	68.9±4.4	57.5±2.8	48.9±3.5	45.3±2.5
	25	104.4±6.7	71.2±3.2*	57.7±3.4	48.9±3.6	45.9±2.6
	500	105.6±6.2	68.9±2.6	57.3±3.9	48.6±2.3	45.5±2.6
	2500	94.0±7.4**	69.8±2.4	58.5±2.6	48.9±4.3	45.4±3.5
		1-8	22-29	50-57	78-82[a]	106-123
F_1 Males	0	129.3±7.6	81.3±4.0	60.0±2.9	55.2±3.5	48.5±2.6
	25	135.0±13.4	83.0±8.8	60.8±4.0	56.2±3.5	48.5±2.4
	500	134.3±11.1*	84.3±6.4*	61.2±4.1	56.9±3.8*	49.3±3.6
	2500	141.6±10.2**	86.9±5.6**	61.3±3.2**	59.3±2.4**	50.6±3.8**
		0-8	22-29	50-57	78-82[a]	
P_1 Females	0	113.2±7.0	84.6±4.3	69.8±3.0	61.9±4.2	
	25	111.9±6.7	83.2±4.3	67.7±5.1	59.4±9.9	
	500	112.7±6.1	83.1±5.8	68.4±5.0	61.4±4.7	
	2500	105.3±7.1**	82.7±5.1	70.5±5.0	61.0±4.2	
		1-8	22-29	50-57	78-82[a]	
F_1 Females	0	126.6±12.2	86.8±8.1	71.7±6.3	66.2±4.8	
	25	133.4±12.5**	90.9±12.2	72.7±5.8	66.4±5.0	
	500	136.8±14.2**	91.1±7.1*	75.4±4.7**	67.0±5.8	
	2500	139.2±15.0**	93.8±6.6**	77.2±5.8**	68.8±5.3	

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

[a]Rats were placed into cohabitation on day 82. Feed weights were not recorded during the 21-day mating period.

TABLE 4. Mean Maternal Body Weights and Weight Changes During Gestation in Rats Fed Tackle for Two Generations

Generation	Dose Level ppm	Body Weight (g±SD) on Gestation Day 0	6	10	15	20
P_1	0	286.0±23.3	312.4±23.5	326.4±25.3	349.9±26.2	403.5±33.1
	25	289.2±22.7	314.2±23.2	328.4±23.8	352.0±26.4	404.5±29.7
	500	290.8±33.4	314.6±31.3	323.7±30.8	344.0±31.5	399.4±39.7
	2500	274.3±24.2	294.7±24.5*	312.3±24.6	335.7±25.1	383.9±27.6
F_1	0	287.5±17.7	306.6±18.6	319.6±19.5	338.1±17.3	389.6±21.8
	25	284.0±23.9	306.3±27.7	319.7±28.7	338.9±28.6	381.6±24.7
	500	296.0±28.0	316.6±26.7	329.9±30.8	349.0±32.2	398.4±35.9
	2500	258.5±24.8**	278.5±25.2**	290.4±25.9**	307.6±26.2**	353.1±32.0**

Generation	Dose Level ppm	Body Weight Change (g±SD) for Gestation Days 0-6	6-10	10-15	15-20	0-20
P_1	0	26.5±9.6	14.0±4.4	23.5±8.0	53.6±13.1	117.6±22.6
	25	25.0±6.6	14.2±4.4	23.6±5.7	52.6±12.7	115.3±18.4
	500	23.8±9.9	9.0±7.0**	20.3±6.8	55.4±16.3	108.6±18.9
	2500	20.4±7.4*	17.6±6.1*	23.4±7.7	48.2±6.9	109.6±12.6
F_1	0	19.2±6.8	13.0±3.8	18.4±5.7	50.5±15.0	101.1±18.8
	25	22.4±6.9	13.4±4.9	19.1±4.3	46.5±15.1	100.7±18.4
	500	20.6±8.1	13.3±6.4	19.1±5.0	47.0±13.7	99.4±22.3
	2500	20.0±6.5	11.9±5.1	17.2±5.4	45.4±10.8	94.3±16.2

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

TABLE 5. Mean Maternal Feed Consumption During Gestation in Rats Fed Tackle for Two Generations

Generation	Dose Level ppm	Feed Consumption (g/kg/day±SD) on Gestation Days 0-6	6-10	10-15	15-20	0-20
P_1	0	66.4±7.3	69.4±9.2	65.4±6.6	59.1±6.4	64.0±6.0
	25	67.9±5.2	69.0±6.2	65.5±5.9	57.8±5.4	64.0±4.3
	500	66.2±8.0	61.3±9.0**	60.1±6.8**	61.9±6.0	61.9±5.3
	2500	68.1±14.6	72.3±7.7	68.3±7.2	59.3±5.1	65.8±6.0
F_1	0	66.7±5.7	68.6±9.5	66.5±7.5	59.3±6.8	64.0±5.1
	25	68.1±6.1	69.8±11.1	68.1±4.7	59.6±5.8	65.5±5.3
	500	68.0±6.6	69.0±8.9	68.2±6.9	57.6±6.9	64.0±5.7
	2500	70.4±6.5	71.9±7.4	70.3±7.8	62.2±9.7	67.6±7.1

**Significantly different from control value ($p \leq 0.01$).

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TABLE 6. Mean Maternal Body Weights and Weight Changes During Lactation in Rats Fed Tackle for Two Generations

Generation	Dose Level ppm	Body Weight (g±SD) on Lactation Day 1	4	7	14	21
P_1	0	319.0±26.1	319.7±20.3	325.0±19.3	320.0±25.7	316.4±27.1
	25	319.6±28.4	325.0±24.8	330.9±23.8	320.5±26.6	314.4±22.7
	500	316.3±30.6	320.4±30.6	327.7±30.3	323.5±30.7	328.5±32.4
	2500	302.9±27.0	305.1±24.8*	309.6±25.5	309.4±27.7	303.0±33.2
F_1	0	316.9±25.2	318.0±24.7	326.2±25.5	335.4±22.0	320.9±27.3
	25	314.6±30.6	310.9±26.2	322.5±22.4	338.4±25.0	326.4±20.0
	500	325.2±31.2	320.9±27.1	327.8±27.2	342.6±25.0	330.8±23.4
	2500	286.9±28.4**	282.6±31.8**	291.5±31.8**	298.0±33.6**	291.9±32.0**

Generation	Dose Level ppm	Body Weight Change (g±SD) for Lactation Days 1-4	4-7	7-14[a]	14-21[a]	1-21
P_1	0	0.8±11.3	5.3±6.9	-5.0±17.9	-3.6±20.7	-2.6±21.7
	25	5.5±8.7	5.9±6.8	-10.4±17.2	-6.0±24.1	-4.8±24.4
	500	5.7±9.0	7.3±7.6	-4.1±14.4	4.9±19.7	12.3±21.9*
	2500	2.1±8.1	4.5±6.6	-0.2±13.0	-6.4±18.9	0.1±18.3
F_1	0	1.0±10.6	8.3±10.1	9.1±14.3	-14.5±19.3	4.0±21.4
	25	-3.7±10.1	11.6±9.1	15.9±12.5	-12.0±15.0	11.8±21.4
	500	-2.8±13.2	7.0±14.7	14.9±14.0	-11.3±13.0	7.2±18.2
	2500	-4.2±12.4	6.5±7.8	7.0±12.2	-7.0±18.7	2.7±17.1

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

[a]Body weight changes for F_1 generation were calculated and analyzed by reviewers using Dunnett's t-test ($\alpha = 0.05$).

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TABLE 7. Mean Maternal Feed Consumption During Lactation in Rats Fed Tackle for Two Generations

Generation	Dose Level ppm	Feed Consumption (g/kg/day±SD) on Lactation Days			
		1-4	4-7	7-14[a]	
P_1	0	78.7±12.0	119.1±12.9	143.9±14.5	
	25	81.8±14.4	110.3±18.2	137.1±27.2	
	500	78.1±12.8	110.7±12.8	145.8±13.2	
	2500	69.9±13.0*	101.2±9.8*	126.8±10.6**	
		1-4	4-7	7-11	11-14[a]
F_1	0	69.0±18.7	115.0±22.5	149.1±28.4	168.5±31.0
	25	67.0±18.4	120.7±23.2	156.6±27.1	176.0±27.4
	500	71.5±23.2	114.9±27.0	148.1±30.1	165.5±27.1
	2500	72.1±21.0	115.8±27.9	141.0±24.5	156.6±22.9

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

[a]Maternal feed consumption was not tabulated beyond day 14 of lactation as feed consumption values are presumed to be confounded by pup consumption of maternal feed.

TABLE 8. Selected Reproductive Indices in Rats Fed Tackle for Two Generations

Generation	Dose Level (ppm) 0	25	500	2500
P1				
No. males cohabited	35	34	35	35
No. males that mated (%)	32 (91.4)	30 (88.2)	33 (94.3)	35 (100.0)
Pregnant rats/mated by males (%)	30 (93.8)	28 (93.3)	31 (93.9)	32 (91.4)
Males with confirmed dates of mating:	31	29	30	33
Day 1 (%)	7 (22.6)	5 (17.2)	4 (13.3)	4 (12.1)
Days 2-7 (%)	23 (74.2)	23 (79.3)	24 (80.0)	27 (81.8)
Days 8-14 (%)	1 (3.2)	1 (3.4)	2 (6.7)	2 (6.1)
No. females cohabited	35	34	35	35
No. females that mated (%)	32 (91.4)	33 (97.0)	34 (97.1)	35 (100.0)
No. mated females that were pregnant (%)	30 (93.8)	29 (87.9)	31 (91.2)	32 (91.4)
Females with confirmed dates of mating:	31	32	31	33
Day 1 (%)	7 (22.6)	5 (15.6)	4 (12.9)	4 (12.1)
Days 2-7 (%)	31 (74.2)	23 (71.9)	24 (77.4)	27 (81.8)
Days 8-14 (%)	1 (3.2)	1 (3.1)	2 (6.4)	2 (6.1)
Days 15-21[a] (%)	0	3 (9.4)	1 (3.2)	0
Duration of gestation in days (mean±SD)	23.2±0.4	23.5±0.5*	23.5±0.6	23.2±0.5
Implantation sites per delivered litter (mean±SD)	14.6±1.8	14.1±3.4	14.2±1.8	13.1±2.0**

(Continued)

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

[a]Mated by second male.

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TABLE 8. Selected Reproductive Indices in Rats Fed Tackle for Two Generations

Generation	Dose Level (ppm)			
	0	25	500	2500
F_1				
No. males cohabited	39	40	40	39
No. males that mated (%)	31 (79.5)	35 (87.5)	33 (82.5)	37 (94.9)
Pregnant rats/mated by males (%)	24 (77.4)	28 (80.0)	23 (69.7)	33 (89.2)
Males with confirmed dates of mating:	29	35	31	35
Day 1 (%)	3 (10.3)	7 (20.0)	5 (16.1)	7 (20.0)
Days 2-7 (%)	22 (75.9)	27 (77.1)	25 (80.6)	26 (74.3)
Days 8-14 (%)	4 (13.8)	1 (2.8)	1 (3.2)	2 (5.7)
No. females cohabited	39	40	40	39
No. females that mated (%)	35 (89.7)	36 (90.0)	37 (92.5)	39 (100.0)
No. mated females that were pregnant (%)	28 (80.0)	29 (80.6)	27 (73.0)	35 (89.7)
Females with confirmed dates of mating:	32	36	35	37
Day 1 (%)	3 (9.4)	7 (19.4)	5 (14.3)	7 (18.9)
Days 2-7 (%)	22 (68.8)	27 (75.0)	25 (71.4)	24 (64.9)
Days 8-14 (%)	4 (12.5)	1 (2.8)	1 (2.8)	4 (10.8)
Days 15-21[a] (%)	3 (9.4)	1 (2.8)	4 (11.4)	2 (5.4)
Duration of gestation in days (mean±SD)	23.3±0.6	23.0±0.4	23.1±0.7	22.9±0.5*
Implantation sites per delivered litter (mean±SD)	12.3±4.1	12.3±3.1	12.2±3.8	12.5±2.8

(Concluded)

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

[a]Mated by second male.

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groups (Table 8). In the second generation, the mean length of gestation for high-dose dams (22.9 days) was significantly ($p \leq 0.05$) decreased when compared to the control value of 23.3 days. However, the authors did not attribute this to compound administration because a clear dose response was not evident, there was no evidence of dystocia or other delivery complications, and the value was within the range of historical control data.

The mean number of implantation sites per dam was significantly less for high-dose P_1 females (13.1) when compared with the control value (14.6) (Table 8). The difference was not attributed to Tackle because the value was within the range of historical control data for this strain of rat, and a similar effect was not evident in the second generation.

4. Gross and Microscopic Pathology: No dose of Tackle resulted in gross or microscopic changes of the reproductive tissues of either sex for either generation.

Necropsy and subsequent histopathological evaluation of first generation males did not reveal any compound-related findings.

In high-dose second generation males there was a significant increase in the incidence of pelvic dilatation (hydronephrosis) when compared to the controls (Table 9). In both generations, administration of Tackle to females at 500 and 2500 ppm resulted in kidney lesions, characterized predominantly by dilatation of tubules in the outer medulla.

C. Offspring Effects:

1. Litter Size, Viability, and Clinical Observations: Total litter size (live and dead pups) was comparable for all groups in both generations (Table 10). The authors stated that litter survival indices were comparable among all groups for both generations (Table 10). In the second generation, the incidence of pups dying between days 1 and 4 of lactation was significantly increased ($p \leq 0.01$) for the mid- and high-dose groups when compared to the control group. This correlated with the deaths of all pups in one mid-dose litter by day 2 of lactation and in one high-dose litter by day 5 of lactation; pups from the high-dose litter appeared thin and weak prior to death. The authors considered the death of the high-dose litter to be possibly compound-related, in view of effects on maternal and pup body weights at 2500 ppm. The authors stated that the death of the mid-dose litter was probably spontaneous. The incidence of pups surviving from days 4 to 21 of lactation (lactation index) was comparable for all groups.

No compound-related clinical observations were noted in pups of either generation.

TABLE 9. Incidence of Primary Kidney Lesions[a] in Adult Rats Fed Tackle for Two Generations

	First Generation (P_1)							
	Males				Females			
	Dose Level (ppm)				Dose Level (ppm)			
Kidneys	0	25	500	2500	0	25	500	2500
No. examined microscopically	35	35	35	35	35	35	35	35
No. normal	20	20	18	23	28	29	2	1
Outer medulla:								
- tubular dilatation	0	0	0	0	0	0	33	33
- tubular epithelial necrosis	0	0	0	0	0	0	1	17
Pelvic dilatation:								
- unilateral	4	6	2	5	2	0	3	6
- bilateral	1	2	0	1	1	1	0	0

	Second Generation (F_1)							
	Males				Females			
	Dose Level (ppm)				Dose Level (ppm)			
Kidneys	0	25	500	2500	0	25	500	2500
No. examined microscopically	40	41	40	40	41	40	40	43
No. normal	19	22	20	14	32	26	6	1
Outer medulla:								
- tubular dilatation	0	0	0	0	0	0	28	33
- tubular epithelial necrosis	0	0	0	0	0	0	0	6
Pelvic dilatation:								
- unilateral	5	6	6	8	4	8	3	5
- bilateral	2	2	3	8	2	1	3	3

[a] Histopathologically confirmed.

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TABLE 10. Selected Litter Data for Rats Fed Tackle for Two Generations

Generation	Dose Level (ppm) 0	25	500	2500
F_1				
Total pups delivered	381	373	395	391
Mean litter size (±SD)	13.1±2.0	12.9±3.3	12.7±2.1	12.2±2.0
Liveborn	373	369	393	390
Stillborn (%)	7 (1.8)	3 (0.8)*	1 (0.2)**	1 (0.2)**
Uncertain	1	1	1	0
Percent male pups on day 1 (±SD)	50.7±12.6	48.6±15.5	50.2±16.4	50.0±14.1
Pups dying:				
Day 1 (%)	1 (0.3)	1 (0.3)	4 (1.0)	2 (0.5)
Days 1-4 (%)	0	4 (1.1)	6 (1.5)	2 (0.5)
Days 4-7 (%)	0	0	0	1 (0.2)
Days 7-14 (%)	0	0	1 (0.2)	0
Days 14-21 (%)	0	0	1 (0.2)	0
Pups surviving 4 days/total liveborn pups (%)	99.7	98.6*	97.4**	99.0
Pups surviving 21 days/pups selected on day 4 (%)	100.0	100.0	99.2	99.6
F_2				
Total pups delivered	324	337	307	411
Mean litter size (±SD)	11.6±3.9	12.0±3.0	11.4±3.9	11.7±2.8
Liveborn	320	333	299	410
Stillborn (%)	4 (1.2)	4 (1.2)	5 (1.6)	1 (0.2)
Uncertain	0	0	3	0
Percent male pups on day 1 (±SD)	53.2±18.8	49.1±15.1	52.3±22.2	52.0±16.4
Pups dying:				
Day 1 (%)	1 (0.3)	3 (0.9)	1 (0.3)	1 (0.2)
Days 1-4 (%)	3 (0.9)	2 (0.6)	9 (3.0)**	14 (3.4)**
Days 4-7 (%)	1 (0.3)	2 (0.6)	0	5 (1.2)
Days 7-14 (%)	2 (0.6)	0	1 (0.3)	1 (0.2)
Days 14-21 (%)	0	1 (0.3)	1 (0.3)	3 (0.7)
Pups surviving 4 days/total liveborn pups (%)	98.8	98.5	96.6	96.3
Pups surviving 21 days/pups selected on day 4 (%)	99.0	99.1	99.3	97.9

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

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2. Body Weight: In both generations of offspring, the mean body weight was significantly less for high-dose litters at birth ($p \leq 0.05$ to $p \leq 0.01$) and on days 7, 14, and 21 ($p \leq 0.01$) of lactation (Table 11). Body weights of the low- and mid-dose groups were comparable to control values.

3. Gross and Microscopic Pathology: Lesions observed at necropsy of F_1 pups were infrequent and/or not compound related. A low incidence of renal pelvic dilation was seen grossly and microscopically in control and compound-treated pups, but the incidence and severity of the lesions were comparable for all groups (Table 12). In the F_2 generation, the incidence of grossly observed kidney lesions, primarily dilation of the pelvis(es), was significantly increased ($p \leq 0.01$) at the high-dose level when compared to controls. The authors suggested that the absence of a dose-related incidence of kidney lesions in F_1 pups was probably due to a somewhat shorter exposure to the test compound. The F_2 pups were possibly exposed to Tackle from preconception through lactation, with intentional dosing of their parents beginning at 4 weeks of age rather than 7 weeks of age as for the first generation. In addition, feed consumption was increased for the second generation high-dose group during the early weeks of exposure, resulting in higher mg/kg/day dosages of the compound than for the first generation.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Based on mortality, inhibited body weight gain and feed consumption, and increased incidences of kidney lesions, the authors concluded that administration of Tackle to rats for two generations produced toxic effects in adults at 500 and 2500 ppm. The NOEL for adult toxicity was assessed as 25 ppm. No adverse effects on adult reproductive performance were observed at doses up to and including 2500 ppm. The authors assessed the LOEL for developmental offspring effects at 2500 ppm based on inhibited body weights and an increased incidence of kidney pelvic dilatation. The NOEL for offspring toxicity was assessed to be 500 ppm.

B. A signed quality assurance statement, dated January 20, 1986, was presented in the final report.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The test material was described only as a dark yellow liquid. Information on the active ingredient in the test compound, including purity, was not provided. The authors discussion of test diet preparation and analysis results indicated difficulties in both mixing and analyzing the test diets. As a result, the acceptable limits for concentration levels (percent of nominal concentration) were increased to $\pm 15\%$. It is unclear whether the encountered difficulties were due to the nature of the test material or to laboratory procedures, or both. Therefore, the validity of the analytical data appears somewhat questionable. However, we do not consider this severe enough to preclude interpretation of the results.

TABLE 11. Mean Pup Body Weights (g±SD) During Lactation in Rats Fed Tackle for Two Generations

Generation	Dose Level ppm	Day of Lactation 1	4[a]	4[b]	7	14	21
F_1	0	6.4±0.5	9.2±1.0	9.2±1.1	14.8±1.4	30.8±2.5	46.2±3.3
	25	6.5±0.7	9.4±1.4	9.4±1.4	15.3±2.0	31.2±2.9	46.3±4.2
	500	6.2±0.7	9.0±1.0	9.0±1.0	14.5±1.4	30.4±2.6	46.2±4.1
	2500	6.0±0.4*	8.6±1.0	8.6±0.9	13.5±1.2**	26.6±2.5**	37.5±3.4**
F_2	0	6.5±0.7	9.4±1.4	–[c]	13.8±2.3	27.2±4.9	42.6±7.7
	25	6.2±0.6	9.1±1.3		13.2±2.0	26.4±3.6	40.9±6.2
	500	6.3±0.6	9.4±1.1		13.9±2.0	27.4±4.4	42.0±7.3
	2500	5.8±0.6**	8.2±1.3**		11.9±1.7**	22.0±3.5**	31.5±6.5**

*Significantly different from control value ($p < 0.05$).
**Significantly different from control value ($p < 0.01$).

[a]Preculling.
[b]Postculling.
[c]F_2 litters were not culled.

TABLE 12. Incidence of Primary Kidney Lesions[a] in Offspring of Rats Fed Tackle for Two Generations

	First Generation (F_1) Dose Level (ppm)			
	0	25	500	2500
Kidneys				
No. examined microscopically	2	1	1	0
No. normal	0	0	0	-
Pelvic dilatation:				
- unilateral	1	1	1	-
- bilateral	1	0	0	-

	Second Generation (F_2) Dose Level (ppm)			
	0	25	500	2500
Kidneys				
No. examined microscopically	2	4	8	29
No. normal	0	1	1	0
Pelvic dilatation:				
- unilateral	2	2	7	24
- bilateral	0	1	0	5

[a] Histopathologically confirmed.

We concur with the study authors' assessment of toxic effects of Tackle in the adult rats. Compound-related deaths occurred at the high-dose level in both generations of parental rats. Body weights were significantly lower for high-dose rats when compared to control values throughout the premating, gestation, and lactation intervals of both generations. The interrelationship of body weight with feed consumption was unclear because no consistent dose-related or cross-generational patterns were evident in feed consumption data. Necropsy and subsequent histopathological evaluation revealed compound-related kidney lesions in mid- and high-dose females of both generations (primarily dilatation of tubules of the outer medulla) and high-dose males of the second generation (primarily pelvic dilatation). Based on these effects, we assess 500 ppm, the mid-dose level, as the LOEL for parental toxicity, and 25 ppm, the low-dose level, as the NOEL.

We agree with the study authors' conclusion that adverse effects on reproductive performance were not evident at any dose tested in this study. Therefore, the NOEL for reproductive effects is assessed as 2500 ppm.

We do not agree with the study authors' conclusion that offspring toxicity was evident only at the high-dose level. The number of pups dying during lactation, predominantly during days 1-4, was increased at the 500- and 2500-ppm levels for both generations when compared to control values. Increases were significant ($p \leq 0.01$) for mid- and high-dose second generation offspring. Corresponding viability indices (percentage of liveborn pups that survived to day 4 postpartum) were lower than control values for the mid- and high-dose groups of both generations. High-dose pup body weights were significantly lower than controls at birth and throughout lactation for both generations. Body weights of the low- and mid-dose groups were comparable to control values. Necropsy and subsequent histopathological examination of F_2 offspring revealed a dose-related incidence of kidney lesions. Pelvic dilatation was confirmed for 2 (0.6%), 3 (0.9%), 7 (2.4%), and 29 (7.5%) pups from the control, low-, mid-, and high-dose groups, respectively. Based on these effects, we assess the LOEL and NOEL for offspring toxicity at 500 ppm and 25 ppm, respectively. This differs from the study authors' assessment of 2500 ppm as the LOEL for offspring toxicity.

Item 15 — see footnote 1.

16. <u>CBI APPENDIX</u>: Appendix A, Materials and Methods, CBI pp. III-1 - III-20.