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

APR 4 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE:

SUBJECT: Request For Review Of Final Reports For TACKLE 3-Generation Reproduction In Rats, 2-Year Feeding In Dogs And Miscellaneous Mutagenicity. Caswell No. 818B

TO: Richard Mountfort, PM #23
Registration Division (TS-767)

FROM: Carolyn Gregorio, Toxicologist CG 3-8-84
Toxicology Branch/ HED (TS-769)

THRU: William Butler, Section Head
and
William Burnam, Chief
Toxicology Branch/ HED (TS-769)

William H. Butler 3/30/84
WB 3/31/84

Petitioner: Rhone-Poulenc

Petition Number: 3F2811

Background: An oncogenic potential has been demonstrated in mice being fed TACKLE for 18 months (Gulf South Research Institute, Report No. 413-984-41, dated November 3, 1981). A statistically significant (Chi-square) increase in liver tumors was observed in the 625 ppm (lowest dose tested) and the 2500 ppm (Highest dose tested) treated males when compared to concurrent controls. The incidence of liver tumors was also statistically increased in the 2500 ppm females.

In addition, a dose related increase in rare stomach papillomas was observed in all treated female groups (625, 1250, and 2500 ppm) and in high dose males (2500 ppm) when compared to concurent controls. This type of tumor was not observed in the concurrent controls of either sex.

Weak mutagenic potential was obseved in Drosophilia melanogaster and fungus (see memo Gregorio to Mountfort, dated January 26, 1984).

Recommendations: The data submitted have some serious problems:

1.) 3-Generation Reproduction: As indicated in the extensive review (attached), reproductive performance of rats being fed TACKLE could not be evaluated based on the data provided (Gulf South Research Institute, GSRI Project No. 413-987-41, dated November 3, 1982).

Numerous reporting errors, inconsistencies, and omissions were noted throughout the report. In addition, reproductive performance of the control animals was so poor during the first two generations of the study there is no baseline data available for comparisons to the treated animals.

An extremely high incidence of cannibalization amoung control and treated animals were reported. This behavioral abnormality may reflect a generic problem during the study, such as stress. Stress may be a reflection of poor animal handling and/or husbandry.

These above mentioned problems, which were are more elaborately explained in the attached review, have an adverse impact on the interpretation of this study. Therefore, a definitive assessment of this study could not be made and the Registrant should be apprised that an additional reproduction study is required.

2.) Mutagenicity: Additional information is required in the DNA-binding study (SRI International, Project No. CSC-5573-4 and -5) and the unshceduled DNA synthesis study (SRI International, project No. LSU-83-16, dated May 23, 1983.) as explained in the attached reviews.

3.) 2-Year Feeding- Dogs: This study was adequate to fulfill testing requirements.

Study Test: 2-Year Dietary Study - Dogs

Accession Numbers: 251297, 251298

MRID Number: Not Assigned

Sponsor: Rhone-Poulenc

Contracting Lab: International Research and Development Corporation (Report No. 450-039)

Date: June 30, 1983

Test Substance: Tackle 2S (Acifluorfen Sodium, MC 10109) Purity unspecified

Purity of Technical Tackle: Assays of Tackle (Lot Numbers: LCM-266821, LCM-266821, LCM-26680) identify the purity as 74.5-82.8 percent.

Concentration of Tackle in Feed: Diets were analyzed once a week from week 1 through 26 (under sponsorship of Mobil Oil). Subsequently, diets were analyzed on weeks 36, 55, 59, 63 and 104 (under sponsorship of Rhone-Poulenc). The following table indicates mean actual concentrations:

Mean Diet Analysis for Dogs Fed Tackle

Target Dose ppm	Mean Actual Concentration (ppm)						Average ppm in Diet over 104 Wks.
	Wk 1 - 26	Wk 36	Wk 55	Wk 59	Wk 63	Wk 104	
0	ND	a	a	a	a	a	a
20	22.9	23.9	20.5	17.2	~~18.3~~	19	20.3
300	320	274	275	265	305	301	290
4500	5385	4455	4301	4191	4280	3980	4432

ND = None Detected
a = Not Analyzed

Stability of Tackle in Feed: As indicated in a letter (of Mobil Oil to J. Laneglia of IRDC, dated June 18, 1980), "although the reports are not yet available [on the dog study], we have completed stability and palatability trials in rats. The data indicate that Tackle is sufficiently stable in rat chow to insure at least a two-week shelf life at 4°C." The submitted report does not address the stability of Tackle in dog chow.

Test Diet Preparation: Tackle 2S is a 216 GRAMS/gallon solution of the soidum salt of the [redacted] to contain 240 g/L of Tackle. "Each mix of Tackle 2S solution was used for 2 weeks of diet preparation."

The Tackle 2S solution was mixed with acetone and water and added to the dog chow (Certified Canine Diet #5007) to obtain the required concentrations. Diets were prepared weekly and "stored under refrigeration."

PROTOCOL: Three-month-old purebred beagle dogs (38/sex) were obtained from Ridglan Farms (Mt. Horeb, Wisconsin) and acclimated to laboratory conditions for 4 weeks. The animals were randomized (8/sex/dose) into the test groups, in which the final concentrations of the test material were 0, 20, 300 and 4500 ppm. The mean body weights ranged from 5.5-11.9 for males and 5.1-10.5 kg for females.

"The dogs were individually housed in metabolism cages and maintained in a temperature, humidity and light (12-hour light/dark cycle) controlled environment. Controls or test diets... were available ad libitum during a 2-hour period each day. Water was available ad libitum."

Observations: "The dogs were observed daily for general physical appearance, behavior, pharmacologic and toxic signs, mortality and morbundity."

Ophthalmology: "Ophthalmoscopic examinations were conducted by a veterinary ophthalmologist, on all dogs pretest, monthly from 3-14 months and at 16, 18, 21 and 24 months of the study."

Hematology: "Hematological determinations included total and differential leukocyte count, erythrocyte count, hemoglobin, hematocrit, platelet count and reticulocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)." Hematology and clinical chemistry determinations were "run prior to study initiation, at monthly intervals through 14 months and at 19 and 24 months. Urinalysis

INFORMATION WHICH MAY REVEAL A PRODUCT MANUFACTURING PROCESS IS NOT INCLUDED

tests were run prior to study initiation and at 2, 4, 6, 8, 10, 12, 14, 19 and 24 months of study."

Clinical Chemistry: Clinical chemistries included "sodium, potassium, chloride, calcium, phosphorus, alkaline phosphatase, total bilirubin, gamma glutamyl transferase (GGT), aspartate aminotransferase (AST) formerly SGOT, alanine aminotransferase (ALT) formerly SGPT, lactic dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine, total protein, albumin, globulin (calculated), cholesterol, glucose and uric acid."

Urinalysis: "Urinalysis determinations included color, appearance, microscopic examination of sediment, specific gravity, volume, pH, protein, glucose, occult blood, nitrites, bilirubin, ketones and urobilinogen."

At 6 months, 2 dogs/sex/dose were sacrificed (6-month interim sacrifice). All surviving animals were sacrificed at 24 months (terminal sacrifice). At necropsy the following organ weights were recorded: heart, kidney, liver, lungs and mainstem bronchi, testes, ovary, adrenal, brain, pituitary, thyroid/parathyroid.

Tissues were fixed in neutral formalin and stained with hemotoxylin-eosin. "Mobil Oil prepared slides from the dogs who died on study at 0-6 months and at 6-month interim sacrifice, and IRDC prepared slides from the deaths and unscheduled sacrifices 6-months to termination and terminal sacrifice." The following tissues were examined:

adrenal (2)
aorta
brain (3 levels - forebrain, midbrain and hindbrain)
eye (2)
gallbladder
heart
trachea
esophagus
stomach
duodenum
ileum
jejunum
cecum
colon
rectum
kidney (2)
liver
lungs with mainstem bronchi
mesenteric lymph node
skeletal muscle
skin
mammary gland
sciatic nerve
spleen
pancreas
pituitary
prostate/corpus and cervix uteri
rib junction (bone marrow)
salivary gland (submaxillary)
spinal cord (cervical and thoracic)
testis/ovary (2)
thymus
thyroid/parathyroid (2)
urinary bladder

and all other tissues with lesions

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Statistics: Statistics used by the registrant were: "Analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie (1960, Principles and Procedures of Statistics, McGraw-Hill Book Company, Inc., New York) and Ostle (1954, Statistics in Research, Iowa State College Press). Dunnett's multiple comparison tables were used to judge significance."

REPORTED RESULTS:

Clinical Observations: "Soft stool/diarrhea, emesis" and "relaxed nictitating membranes" were observed in all groups (males and females) throughout the study.

"Appears thin" was noted in high dose (4500 ppm) males and females throughout the study.

All treated male groups (not control) were noted to have "alopecia/flank/dorsal abdomen/front paw/thigh/side of face/chest area/general." "Lacrimation" was observed in all female groups, with the high dose females being more severely affected from week 66 until termination.

Mortality: The following deaths were reported:

Dose (ppm)	Male	Female
0	1 (wk 85)a/	0
20	1 (wk 85)b/	0
300	0	0
4500	0	1 (wk 13)b/

a/ Sacrificed in extremis

b/ Died

Body Weights: Body weight gains for the high dose (4500 ppm) males and females were consistently lower throughout the study when compared to concurrent control animals (Table 1).

Table 1. Mean Body Weights (kg) at Selected Intervals

Weeks on Study	Dose (ppm)			
	0	20	30	4500
Males				
0	9.5	8.8	9.5	9.6
13	11.6	11.8	11.8	10.7
52	13.4	12.8	13.9	11.0
104	13.8	13.3	14.7	11.1
% weight gain[a/]	31.2	33.8	35.4	13.5
Females				
0	6.9	7.7	7.6	7.7
13	9.1	9.8	9.3	8.3
52	9.5	10.9	10.3	8.6
104	10.1	11.7	10.6	9.1
% weight gain[a/]	31.7	34.1	28.3	15.4

a/ Percent (%) weight gain over 104 wks.

Food Consumption:

Males: Mean food consumption was lower for the 4500 ppm animals throughout the entire study when compared to controls. Scattered lower food consumption was seen in all dose groups at intermittent times, however no trend was established.

Females: Mean food consumption was higher for the 4500 ppm animals from week 27 until termination.

Ophthalmology: No apparent compound related eye pathology was observed in any dose group.

Hematology:

Males: Mean erthrocytes, mean hemoglobin and mean hematocrit were statistically decreased throughout the study in the 4500 ppm animals when compared to controls. Mean leukocyte counts (at 12 and 24 months) and mean platelets (at 6, 12

and 24 months) were increased in the 300 and 4500 ppm animals when compared to controls (Table 2).

Females: Mean erythrocytes, mean hemoglobin, and mean hematocrit were statistically decreased throughout the study in the 4500 ppm animals when compared to controls. Mean platelet counts and mean leukocytes were increased throughout the study in the 4500 ppm females (Table 2).

Biochemistry:

Males: Mean SGPT and mean LDH values were elevated at 24 months in the 4500 ppm animals when compared to controls. In the 4500 ppm group, mean creatinine was decreased from 6 months through 24 months and mean cholesterol was decreased from 3 through 24 months when compared to concurrent control values (Table 3).

Females: Mean calcium and mean cholesterol values were decreased throughout the study in the 300 and 4500 ppm groups. Mean creatinine values were consistently decreased in the 300 and 4500 ppm animals at 12 and 24 months of the study. In addition, mean glucose was lower for the 4500 ppm females throughout the study. Mean alkaline phosphatase and mean SGPT were elevated throughout the study for the 4500 ppm animals (Table 3).

Urinalysis:

Males: Mean specific gravity was elevated at 12 and 24 months in the 4500 ppm animals when compared to controls. Mean volume values were increased from 6 months through 24 months for the 300 and 4500 ppm animals (Table 4).

Females: Mean volume values were increased from 6 months through 24 months for the 4500 ppm animals (Table 4).

Organ Weights: Mean liver and kidney weights and % body weight ratio were increased at 6 and 24 months (Table 5) for males and females. No other consistent changes were observed in any other organ examined.

Gross Pathology: No apparent compound related gross pathology was observed in any dose group.

Histopathology: Microscopic changes in the liver were observed at 6 and 24 months in males and females at 4500 ppm which are representative of the increased organ weights observed previously (Table 6).

Other noted histopathological changes were not considered to be highly relevant.

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Table 2. Mean Selected Hematology Values Over Selected Time Intervals

Dose (ppm)	Males					Females				
	Time Interval (Month)					Time Interval (Month)				
	Pretest	3	6	12	24	Pretest	3	6	12	24
Erythrocytes (10^6/cmm)										
0	6.18	6.37	6.10	6.19	6.08	6.12	6.52	6.29	6.26	6.19
20	5.92	6.38	5.96	6.06	6.15	6.32	6.69	6.06	6.25	6.02
300	6.13	6.43	6.19	6.40	6.27	6.38	6.63	6.20	6.15	5.91
4500	6.08	5.65*	5.31**	5.47*	5.43*	6.34	5.47**	5.06**	5.24**	5.08**
Hemoglobin (g/dl)										
0	16.2	14.6	16.0	16.5	16.1	16.0	14.9	16.6	16.5	16.1
20	15.6	14.6	15.8	16.0	16.1	16.6	15.3	16.1	16.9	16.1
300	16.1	14.6	16.2	16.5	16.3	16.8	15.2	16.2	16.0	15.5
4500	16.2	12.7**	13.9*	14.5*	14.3**	16.7	12.3**	13.2**	13.4**	13.1**
Hematocrit(%)										
0	41.2	49.7	44.9	45.7	44.0	40.0	50.6	46.3	46.1	44.6
20	39.5	49.7	43.9	44.8	44.3	41.4	52.0	44.9	46.7	44.1
300	41.0	49.4	45.2	46.4	44.6	42.0	51.0	45.4	45.1	42.5
4500	41.1	44.6*	38.8**	41.0*	39.7*	42.0	42.5*	37.1**	38.3**	37.3**
Platelets (10^3/cmm)										
0	324	329	321	267	243	352	347	358	317	309
20	378	341	328	279	255	312	317	372	318	343
300	343	332	343	294	272	355	353	382	373	313
4500	353	413*	404*	344*	342**	340	457	420	374	361
Leukocytes (10^3/cmm)										
0	12.4	13.2	10.4	8.5	8.0	10.6	12.1	10.1	7.7	8.9
20	10.2	12.9	9.9	8.9	7.7	11.0	12.3	10.2	9.1	7.9
300	11.2	12.9	10.5	9.3	9.7	10.8	12.3	9.4	8.3	7.5
4500	9.7	17.2*	11.1	9.4	11.6*	11.6	16.9*	13.5*	12.7**	10.5

* $P < 0.05$
** $P < 0.01$

Note: Numerical values reproduced from registrant's submitted data.

Table 3. Mean Selected Biochemistry Values Over Selected Time Intervals

	Males						Females				
	Time Interval (Month)						Time Interval (Month)				
Dose (ppm)	Pretest	3	6	12	24	Dose (ppm)	Pretest	3	6	12	24
SGPT (IU/l)[a/]						Calcium (mg/dl)					
0	19	34	36	35	39	0	10.9	11.9	10.6	11.0	10.7
20	17	33	34	28	31	20	10.9	11.9	10.5	11.0	10.7
300	18	33	36	30	35	300	10.9	11.5	10.3	10.8	10.2*
4500	19	44	41	39	74	4500	10.9	11.5	9.8**	10.3*	10.1*
LDH (IU/l)						Alkaline Phosphatase (IU/l)[a/]					
0	80	120	78	73	76	0	56	38	32	26	24
20	72	100	82	83	75	20	56	35	31	26	26
300	71	97	85	60	52	300	64	35	32	29	25
4500	85	149	127*	91	92	4500	61	14	57	41	38
Creatinine (mg/dl)						SGPT (IU/l)[a/]					
0	0.8	0.9	1.1	1.1	1.0	0	19	30	31	27	34
20	0.7	0.9	1.0*	1.0*	1.0	20	21	35	33	27	33
300	0.8	0.9	0.9**	1.0*	1.0	300	23	42	34	29	29
4500	0.8	0.9	0.9**	0.8**	0.9	4500	20	54	44	39	54
Cholesterol (mg/dl)						Creatinine (mg/dl)[a/]					
0	208	196	197	183	153	0	0.8	0.9	1.0	1.0	1.0
20	193	198	210	173	154	20	0.8	0.8	1.0	0.9	1.0
300	227	192	210	171	155	300	0.8	0.9	1.0	0.9	0.9
4500	236	145**	161*	149	140	4500	0.7	1.0	0.8	0.9	0.9

Note: Table continued on next page.

Table 3. Mean Selected Biochemistry Values Over Selected Time Intervals (contd.)

Dose (ppm)	Females: Time Interval (Month) Pretest	3	6	12	24
Albumin (g/dl)					
0	2.8	3.2	3.5	3.2	3.2
20	2.8	3.2	3.4	3.2	3.2
300	2.9	3.2	3.5	3.2	3.2
4500	2.9	3.0	3.1**	2.9**	3.0
Cholesterol (mg/dl)					
0	198	200	216	197	202
20	186	177	213	183	191
300	197	165	195	204	160
4500	191	122**	136**	101*	96**

a/ Variability of individual values preclude statistical analysis of mean values.

* $p < 0.05$

** $p < 0.01$

Note: Numerical values reproduced from registrant's submitted data.

Table 4. Mean Selected Urinalysis Values Over Selected Time Intervals

	Males					Females			
	Time Interval (Month)					Time Interval (Month)			
Dose (ppm)	Pretest	6	12	24	Dose (ppm)	Pretest	6	12	24
Specific Gravity					Specific Gravity				
0	1.044	1.045	1.044	1.045	0	1.044	1.045	1.045	1.045
20	1.044	1.044	1.045	1.045	20	1.045	1.042	1.043	1.044
300	1.045	1.044	1.045	1.045	300	1.045	1.044	1.044	1.044
4500	1.045	1.042	1.040*	1.037*	4500	1.045	1.037	1.042	1.039
Volume (ml)					Volume (ml)				
0	61	55	52	33	0	59	63	59	56
20	53	71	41	27	20	60	79	61	40
300	58	61	75	73*	300	56	61	90	57
4500	49	123	122**	153*	4500	58	144**	104*	136*

* $P < 0.05$

** $P < 0.01$

Note: Numerical values reproduced from registrant's submitted data.

Table 5. Mean Selected Organ Weights

Dose (ppm)	Body Wt. (kg)	Liver Wt. (G)	Liver/ Body Wt. %	Kidney Wt. (G)	Kidney/ Body Wt. %
6 Month - Males					
0	10.4	292	2.81	47.5	4.57
20	12.8	306	2.43	50.5	3.97
300	9.7	258	2.73	53.5	5.66
4500	11.5	389	3.39	69.5	6.08
24 Month - Males					
0	13.3	333	2.50	54.8	4.13
20	12.7	319	2.55	59.1	4.67
300	14.3	334	2.34	60.9	4.31
4500	10.5	373	3.62	63.9	6.17
6 Month - Females					
0	9.4	265	2.90	45.1	4.91
20	8.3	202	2.43	33.0	3.96
300	8.6	254	2.97	40.4	4.98
4500	8.1	339	4.19	49.6	6.17
24 Month - Males					
0	9.7	265	2.75	40.3	4.23
20	11.2	301	2.75	44.2	4.03
300	10.2	269	2.64	47.2	4.67
4500	8.7	367	4.22	62.1	7.05

Note: Numerical values reproduced from registrant's submitted data.

Table 6. Selected Histopathological Observations

	Males				Females			
Dose (ppm)	0	20	300	4500	0	20	300	4500
6-Month								
LIVER								
- Congestion	0/2	0/2	0/2	1/2	-	-	-	-
- Necrosis	0/2	0/2	0/2	1/2	0/2	0/2	0/2	1/3
- Pigment, brown	0/2	0/2	0/2	1/2	0/2	0/2	0/2	2/3
24-Month								
LIVER								
- Congestion	1/6	1/6	0/0	5/6	0/6	0/6	0/6	2/5
- Fatty Vacular	0/6	0/6	0/0	4/6	0/6	0/6	0/6	1/5
- Inflammation	0/6	0/6	0/0	6/6	0/6	0/6	0/6	5/5
- Pigment, brown	0/6	0/6	0/0	4/6	0/6	0/6	0/6	4/5
KIDNEY								
- Pyelitis	0/6	0/5	0/6	2/6	-	-	-	-
- Calulus	-	-	-	-	0/6	0/6	0/6	1/5
GALL BLADDER								
- Macrophages, pigmented	0/0	0/0	0/0	6/6	0/6	0/6	1/6	5/5
TESTIS								
- Atrophy	0/6	0/6	2/6	1/6	-	-	-	-

CONCLUSION: Body weight gains, hematology (i.e., erythrocytes, hemoglobin, hematocrit, leukocyte counts and platelets), biochemistry (SGPT, LDH, creatinine, cholesterol), urinalysis and pathology (organ weights and histopathology) showed consistent changes in the high dose (4500 ppm) males and females when compared to controls throughout the 24 month study.

Hematology (leukocyte counts, platelets) were increased in males (only) at the 300 ppm treatment level. Biochemistry parameters (calcium, cholesterol, creatinine) were consistently different in the 300 ppm females throughout the study. Therefore, the following no observable effect level is established:

NOEL = 20 ppm (0.5 mg/kg/day)
LEL = 300 ppm (7.5 mg/kg/day)

Classification: Supplementary (Test substance purity not adequately identified in the text of the report and stability of the test substance in dog chow not reported.)

Revised:HED/TOX:DCR-32629:C.Gregorio:LM:Raven:557-2226:HED-15:3/6/84:Del.3/23/84
was 18033

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Study Type: 3 Generation Reproduction Study in Rats

Accession Number: ~~Not specified~~ 071276, 251414-251416

MRID Number: Not assigned

Sponsor: Rohne-Poulenc Inc.

Contracting Lab: Gulf South Research Institute
7600 GSRI Avenue
P.O. Box 14787
Baton Rouge, LA

Date: April 29, 1983

Test Material: Tackle (acifluorfen) purity approximately 77%

Materials and Methods

The test substance, Tackle [acifluorfen; sodium salt of sodium 5-(2-chloro-4(trifluoromethyl)phenoxy-2-nitrobenzoate)], was supplied as a gray/white powder received in 3 separate shipments from Mobil Oil or Rohne-Poulenc. The compound was designated MC10109 with lot numbers LCM266821-3, LCM266830-2, LCM266830-4, or RJH276096. The purity of only the last lot was provided in the report. That purity was approximately 77% active ingredient. The compound was incorporated into the diet each week at nominal concentrations of 0 (control), 25, 500, and 2500 ppm.

Test diets were prepared in the following manner. A quantity of the Tackle stock solution was prepared by adding 297 g of the Tackle test material to 500 ml of freshly prepared 1.65 M sodium hydroxide

and allowing the solution to mix on a magnetic stirrer for approximately two hours. The pH of the final solution was adjusted to pH of 8.0 with either sodium hydroxide or hydrochloric acid and the resulting solution was made up to a final volume of 1000 ml with deionized, distilled water. The stock solution was utilized to prepare all three dosages of Tackle. The appropriate amount of stock solution was mixed with acetone according to a table which was provided in the original document. The feed (Ziegler Brothers NIH 07 Open Formula Mash) was then weighed out and one half of the feed was spread upon a pan. The mixture of Tackle and acetone was poured over the feed and allowed to dry under a hood for approximately 1 1/2 to 2 hours. The feed was then tossed or mixed in a Hobart mixer for 45 minutes to 2 hours (depending upon the dosage of the material and the amount of feed to be mixed). Batches of the test diets were prepared on a weekly basis and were analyzed prior to presentation to the animals. If a batch was not found to be within 10% of the target concentration, that batch was discarded and a new batch was mixed in its place.

Weanling Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Portage, Michigan on 7 October 1980 when they were approximately three weeks of age. A total of 136 female and 74 male rats were delivered by air and were quarantined for 17 days.

The rats were 38 days of age at the initiation of treatment. Throughout the study, they were housed individually in suspended wire-bottom cages, except as noted below. During mating, males and females were caged together in polycarbonate shoe-box cages with hardwood bedding (Absorb Dri). Following mating, the males were returned to the wire-bottom suspended cages; whereas, the females were housed individually in the polycarbonate shoe-box cages throughout gestation; during the period of lactation they remained in those cages with their litters. The vivarium was maintained at an approximate ambient temperature of 74°F. The rats were exposed to a 12-hour light/dark photoperiod in a room provided with 10-15 changes of air per hour. The humidity was maintained between 30 and 70%. The rats were allowed free access to feed and tap water.

At the initiation of treatment, 52 males and 104 females were designated the F_0 generation. Thirteen males and 26 females were assigned to each treatment group by a randomization procedure which utilized computer-generated random numbers and a weight class distribution of animals. Figure 1 is a flowchart which summarizes the dosing and breeding schedules and other milestones of the study.

The F_0 generation rats were identified by individual ear tags and cage cards. Animals for the F_1 and F_2 generations were identified by toeclips and cage cards. The means for identifying the F_{3b} animals were not reported.

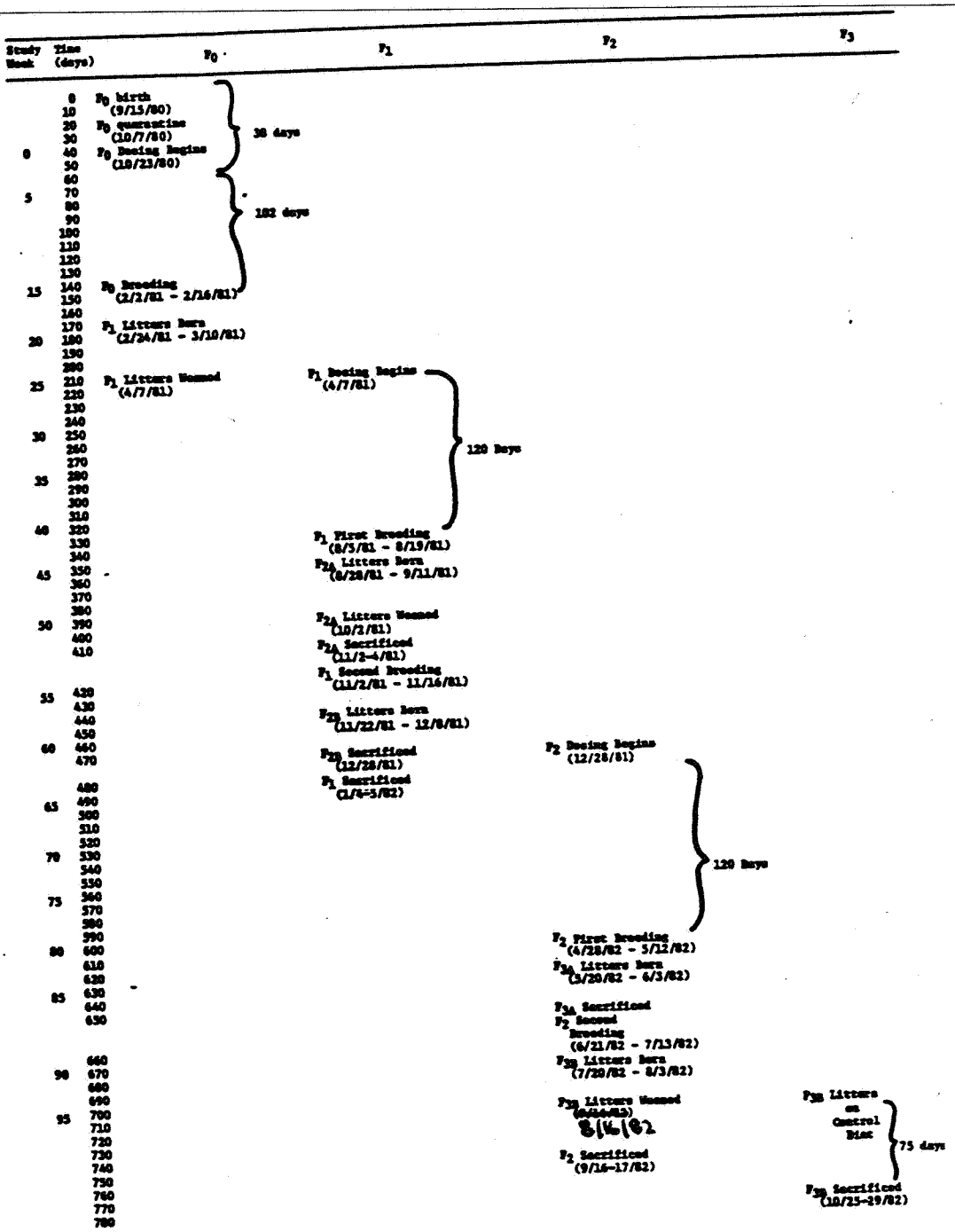

FIGURE 1

DOSING AND BREEDING SCHEDULES FOR A REPRODUCTION STUDY IN RATS WHICH RECEIVED DIETARY TACKLE OVER 3 GENERATIONS

Throughout the study, adult rats were weighed once each week for the first 13 weeks after initiation of dietary dosing. After that time, they were weighed at monthly intervals. The pups were weighed at birth and on days 4, 14, and 21 days postnatally. The F_{3b} pups were weighed on a weekly basis during the postweaning dosing period. Throughout the study, all animals were checked twice each day for signs of health and/or mortality. Male rats and unbred female rats were examined for toxic signs on a weekly basis. Observations for "general condition and behavior" were made on mated females during presumed days of gestation 0, 6, 15, and 21 and on days 0, 4, 14, and 21 of lactation. The difference between these observations and the twice daily observations for all animals was not specified. Pups were observed at birth and on a daily basis throughout the period of lactation. Animals found in extremis were sacrificed and promptly necropsied; likewise, animals found dead were also necropsied. Spermatogenesis was evaluated in all sires among the F_0 parental generation. Spermatogenesis was determined by microscopic examination of Bouin's-fixed testes.

When the rats of the F_0 generation were approximately 140 days old and had received the test diet for 102 days, males and females were placed together in polycarbonate shoe-box cages for mating. Mating was accomplished by allowing one male to cohabit with two females over a two-week period. Each morning during the mating

period, the females were checked for the presence of sperm in a vaginal lavage. The females were considered to be mated if either sperm were observed microscopically or if a vaginal plug was observed. That day was designated day 0 of gestation. Apparently, mated females remained with the males for the entire 2-week period. All females were subjected to a matinal examination each day whether or not they were mated; in the event that a dam was found to be sperm positive on a subsequent day, the latest day of sperm positivity was considered to be day 0 of gestation. Following the mating period, the male rats were returned to the wire-suspended cages. The female rats were caged individually in the polycarbonate cages. They were provided with nesting material in the form of the hardwood bedding.

At birth of the F_1 litter, the following observations were recorded:

- date of birth
- number of pups in litter
- number of stillborn pups
- number of live births
- sex
- birth weight
- any gross malformations

The dams and their litters were allowed to remain together in the polycarbonate shoe-box cages until the end of the lactation period. On postnatal day 4, the number of living pups in each litter was recorded, as well as sex and individual pup weights. Any physical or behavioral abnormalities were also recorded. Although

the authors' methods did not describe a culling procedure, Tables 35-39 of their report, the protocol, and the revised protocol (Appendix C) all indicate that litters with more than 8 pups were reduced to 8 pups, equally divided between males and females (if possible). The selection of pups was randomized by an undisclosed random numbers procedure.

The times for weaning of the different generations are not clearly described. The authors provide a range of dates for the birth of each generation, but a single date for weaning. Thus, the animals were either all weaned on that day, thereby resulting in different lengths of lactation periods for different litters; or the weaning date represents the day of weaning for the last litter born. MITRE has assumed the latter, and periods of lactation have been calculated as the number of days between the dates of the last litters' birth and weaning. The potential range according to the former possibility has also been calculated and is given parenthetically.

A further note of controversy surrounds the actual weaning date for the F_1 generation. According to protocol amendment 1 (Appendix C, page 84) weaning was to have occurred at day 21 followed by 1 week on control diet prior to initiation of dosing. This was not mentioned in the body of the report, and MITRE has assumed that the animals were not placed on control diets.

At weaning of the F_1 generation, which apparently occurred at 28 days (range: 28-42 days), pups were selected by an undescribed method to form the parental animals for the F_1 breeding period. Thirteen males and 26 females were used. The F_0 parent animals were sacrificed and apparently discarded without necropsy except for removal of the testes from the males, unless the animals were deemed "grossly abnormal". The date of sacrifice was not reported. Apparently, the remaining F_1 litter pups (non-parental animals) were sacrificed and discarded at the same time. The F_1 animals which were selected to be parents to the F_2 generation were immediately placed on the diet of their respective parents and dosed for a total of 120 days. At the end of that time, they were mated according to the procedures outlined above for the F_0 generation. The means, if any, for avoiding brother/sister matings were not reported, although the protocol stipulated that such matings were to be avoided. At birth of the F_{2a} generation, the same observations were recorded as described above for the F_1 generation; the litters again were culled on day 4. In this case, however, the reduced litters apparently were allowed to nurse with the lactating dam until 21 days (range: 21-35 days) at which time the litter was weaned. The litter was allowed to survive until one month after weaning, at which time the F_{2a} litter was sacrificed. Although it is implicit in the description of the

study that the weanlings were placed on the diets of their mothers, an explicit statement of that fact was not made either in the body of the report or in the protocol/revised protocol (Appendix C). Five male and five females from the F_2a litter were selected for gross necropsy and histopathological examination. At the same time (i.e., one month postweaning of the F_2a litter), the F_1 parents were rebred for the F_2b litters. The mating procedures were the same as described above. At birth the same observations were recorded as described above; litters were culled on day 4, the reduced litters were allowed to nurse from the dams until 20 days (range: 20-36 days), at which time weaning occurred. The F_2b parental animals were selected from the surviving pups and placed on the diet of the maternal animals. Ten male and 10 female rats per dose level were selected for histopathological examination if that number were available. The remaining pups of the F_2b litters were sacrificed, apparently without necropsy. All surviving F_1 parental animals of the control and each dose group were subjected to a complete gross necropsy and eventual histopathologic examination. The animals were sacrificed by being anesthetized and exsanguinated.

The F_2b animals which had been selected to become parents of the F_3 generation remained placed on the diets of the parents for 120 days after which time they were bred according to the procedures as described above; however, the method for avoiding brother/sister

matings was again not described. The offspring of that generation was designated the F_3a generation. The pups were allowed to nurse from their respective mothers for approximately 30 days (range: 30-40 days) at which time weaning occurred and the pups of the F_3a generation were immediately sacrificed[1]. Ten male and 10 female rats per dose level were selected for histopathological examination if that number were available. Following a resting period, the F_2b parental animals were re-mated (apparently in different pairs) to produce the F_3b generation. After birth of the F_3b generation, the pups were allowed to stay with their respective mothers until weaning, which allegedly occurred at 13 days postpartum (range: 13-27 days). According to the revised protocol in Appendix C the F_3b weanlings from dams of all groups were placed on the control diet for a period of approximately 3 1/2 months. The F_2b parental generation was allowed to survive for 30 days after the weaning of the F_3b generation, at which time they were sacrificed. Ten male and 10 female F_2b rats per dose level were selected for histopathological examination if that number were available. Similarly, 10 male and 10 female animals from each treatment group of the F_3b litters were selected for histopathological examination.

[1] The date of weaning was not provided, but the study milestones chart suggests minimum lactation period to be 30 days.

During the the necropsies of the animals for all three generations, the following tissues were dissected out and preserved in neutral buffered formalin for eventual histopathological examination:

- all gross lesions
- eyes and the contiguous harderian glands
- heart
- thyroid with parathyroids
- trachea
- esophagus
- stomach
- adrenal glands
- liver
- kidneys
- testes
- ovaries (including fallopian tubes)
- spleen
- skin
- sciatic nerve
- mammary gland
- bone (including marrow)
- skeletal muscles
- three coronal sections thru the head including nasal cavities, paranasal sinuses, tongue, oral cavity, naso-pharynx, and middle ear
- brain (at least 3 levels including forebrain, midbrain, and hindbrain)
- pituitary gland
- major salivary glands
- thymus
- lungs (including major lobes and mainstem bronchi)
- large and small intestine
- pancreas
- spinal cord (at least 2 levels)
- urinary bladder
- prostate gland
- corpus and cervix uteri
- lymph nodes
- vagina
- seminal vesicles
- epididymus

In addition to the observations mentioned above, the food and water consumption measurements were added to the protocol beginning on week 56 of the study (Appendix C). These observations were recorded for the F_1, F_2, and F_3 generations according to the schedules below.

The F_1 parental generation had daily food and water consumptions measured from the time of their second mating until they were

sacrificed. The F_2b parental animals were subjected to daily food and water consumption measurements during the 120 dosing period until the time of first breeding. Those measurements were omitted during the 8 weeks from the F_3a gestation period until the birth of the F_3b litters. The measurements were resumed during the F_3b lactation period and continued until the F_2 parents were sacrificed. The F_3b pups were subjected to food and water consumption measurements on a daily basis during the 75 day postweaning dosing period. Although the schedule for these observations was described in detail, the authors did not report the methodology for making these observations.

The statistical significance of differences between control and treated values for the analysis of fertility, viability, and lactation indices, were determined utilizing Chi-square procedures. Body weights, food and water consumption were analyzed utilizing Dunnett's "t" test for the comparison of several treated groups versus the control. Although the level of significance was not mentioned in the methods, it appears the authors used $p \leq 0.05$.

Results and Discussion:

Prior to discussing the findings of this study, several shortcomings in the experimental design, description of methods, and absence of some important data warrant comment.

Although the authors submitted an extensive section concerning the analysis of Tackle in the diet and concerning the purity of the chemical, the test material as received was only approximately 77% pure. This means that approximately 23% of the test material was composed of unidentified ingredients and/or impurities. Without some indication as to what the 23% of unidentified ingredients are, it is difficult to determine whether these may have affected the results of the study.

A second problem with the study involves the manner in which the protocol was designed. The experimental design appears to have evolved as the study was in progress. The evolution is verified by the protocol, revised protocol, and protocol amendments found in Appendix C of the original report. The original protocol submitted by Mobil described a two-generation reproduction study with each generation being composed of one litter. Due to apparent difficulties in the ability of the rats to reproduce, it was determined that the second generation should be expanded to two litters. At a later date, Rohne-Poulenc expanded the study from two generations to three generations; the third generation was to be composed of two litters. Food and water consumptions were added to the investigation after 13 months on test. According to the amended protocol, the rats were weaned at different times during different generations: F_1 at approximately 30 days; F_2 at approximately

21 days; F_3 at approximately ~~21~~ 13 days. Thus, the experimental design failed to treat all 3 generations in the same manner. As will be documented later, the dates mentioned in the protocol were not adhered to. In addition, protocol amendment 1 (Appendix C) specifies that all groups of F_1 weanlings were to receive control diets for 1 week prior to initiation of dosing. This amendment implies that similar procedures would be followed for the F_2 generation. No statement to that effect is made in the methods section of the report or in the study milestones chart. Thus, it is not clear whether or not the weanlings were placed on control diets.

Another shortcoming of the study is that those rats which were examined for possible effects on the testes were only those sires from the F_0 or very first parental generation. In most reproduction studies, the effects of a particular compound may be exacerbated in the later generations, that is to say in the F_2 and F_3 male animals, rather than in the first generation of animals that are treated.

In addition to the shortcomings relating to the experimental design, this study also exhibits shortcomings in presentation of the data. The methods of the study, as well as the results are poorly described. Pertinent information is scattered throughout the report in a random manner. This not only makes it difficult to understand exactly what happened in the study but also makes analysis of the

data difficult. In particular, the rather voluminous appendices of the report contain only group mean data and therefore are not amenable to independent analysis by MITRE. Data from some litters were apparently counted twice (e.g., litter AF219 of F_2a). Several pages of data were omitted from the original report and had to be requested. In addition, some pages of the report were transposed and misnumbered (e.g., pgs 116 and 117 of Vol. 1).

The mating procedures and methods of recording data relating to the matings were not of acceptable quality to determine readily whether any effects exerted by Tackle on the reproductive process were centered on male or female reproductive tracts. The authors did not summarize records concerning which males sired a particular litter, or which sires were found to exhibit spermatogenic problems.

Figure 2 presents a list of definitions for the terms and indices to be discussed in this report.

The reproductive and gestational data for all three generations of rats are summarized in Table 1. The mean numbers of live born pups per litter fell within the range to be expected (8-12) for most albino rat strains. The numbers presented in the original document do not agree with those calculated by MITRE. The reason for this discrepancy appears to be that in the first two generations, the F_1 and F_2a/F_2b litters, the authors included the stillbirths among the

$$\text{Birth Index} = \frac{\text{Number of Pregnancies}}{\text{Number of Females Co-habited with Males}} \times 100$$

$$\text{Fertility Index} = \frac{\text{Number of Females Conceiving}}{\text{Number of Females Co-habited with Males}} \times 100$$

$$\text{Gestation Index} = \frac{\text{Number of Liveborn Litters}}{\text{Number of Pregnancies}} \times 100$$

$$\text{Incidence of Infertile Breeding} = \frac{\text{Number of Barren Dams Giving Evidence of Copulation (copulatory plug or sperm positivity)}}{\text{Number of Dams Bearing Litters} + \text{Number of Barren Dams Giving Evidence of Copulation but No Pregnancy}}$$

$$\text{Lactation Index} = \frac{\text{Number of Pups Alive at Weaning}}{\text{Number of Pups Alive on Post-natal Day 4}} \times 100$$

$$\text{Viability Index} = \frac{\text{Number of Pups Alive on Post-natal Day 4}}{\text{Number of Liveborn Pups}} \times 100$$

FIGURE 2

DEFINITION OF TERMS AND INDICES

TABLE 1

REPRODUCTION AND GESTATIONAL DATA FOR 3 GENERATIONS OF RATS RECEIVING VARIOUS DOSAGES OF DIETARY TACKLE

Generation by Litter	Dosage (ppm)	Females Cohabited With Males	Number of Litters With Liveborn Young	Number of Stillbirths	Live Born Pups		Male/Female (% Male)	Mean Pup Birth Weight (g)
					Total	Mean/ Litter		
F_1	0	26	17	4	208 (214)[a]	12.2 (12.5)[b]	101/107 (49)	6.4
	25	26	21	2	224 (226)	10.7 (10.8)	116/108 (52)	7.0
	500	26	20	1	245 (234)	12.3 (12.4)	131/114 (53)	6.4
	2500	26	26	1	334 (335)	12.8	156/178 (47)	6.0
F_{2a}	0	22	2	5	12 (17)	6.0 (8.5)	5/7 (42)	7.3
	25	25	7[e]	4	56 (68)[f,g]	8.0 (9.5)	30/26 (47)	6.4
	500	24	11	0	96	8.7	47/49 (49)	6.9
	2500	25	14	1	116 (117)	8.3	57/59 (49)	6.6
F_{2b}	0	22	10	3	96 (99)	9.6 (9.9)	53/43 (55)	6.9
	25	24	16	5	154 (159)	9.6 (9.9)	77/77 (50)	6.7
	500	24	17[h]	8	169 (177)	9.9 (10.4)	86/83 (51)	6.7
	2500	25	15	3	153 (156)	10.2 (10.4)	73/80 (48)	5.9[c]
F_{3a}	0	21	17	4	207	12.2 (12.4)	95/112 (46)	6.6
	25	25	23	3	276	12.0 (12.1)	137/139 (50)	6.5
	500	25	20	4	232	11.6 (11.8)	108/124 (47)	6.7
	2500	25	20	7	220	11.0 (10.3)[c]	94/126 (43)	6.0[d]
F_{3b}	0	21	21	3	234 (233)	11.1 (11.7)	111/123 (47)	6.6
	25	25	23	6	273 (276)	11.9 (12.2)	141/132 (52)	6.2
	500	24	21	5	257	12.2 (12.5)	129/128 (50)	6.6
	2500	25	20	9	206 (211)	10.3 (10.9)	97/109 (47)	5.7[d]

[a]The number calculated by MITRE is the sum of live male plus live female fetuses; numbers in parentheses are the numbers presented in the original report.
[b]The number calculated by MITRE is the total live fetuses divided by the number of liveborn litters; numbers in parentheses are the numbers presented in the original report.
[c]Significantly different from control value by Dunnett's "t" test, $p \leq 0.05$ (Authors' statistics).
[d]Significantly different from control value by Dunnett's "t" test, $p \leq 0.01$ (Authors' statistics).
[e]Includes one dam (AF223) which cannibalized her litter - the number of pups was not reported.
[f]Does not include cannibalized pups of litter AF223.
[g]Litter AF219 was reported twice in Table 25, p. 90 of Vol. 1.
[h]Includes one stillborn litter.

number of liveborn litters. In at least one instance (litter AF219 of F_2a) data were counted twice in some cases (e.g., Table 25, p. 90 of Vol. 1) but only once in other cases (e.g., Table 36, p. 119 of Vol. 1). Mistakes were made in the data for the F_3a and F_3b litters as well. These, however, are not so readily understandable. Since the number of male plus female pups should sum to the total of liveborn pups, MITRE used that criterion to determine the total number of liveborn pups. Thus, MITRE calculated the total number of births by examining the appendix materials and summing the number of males and females for each litter. Due to their errors in computation of this parameter, the authors erroneously reported that the mean number of pups/litter in the F_3a high-dose group was significantly smaller than the controls; however, when MITRE recalculated the statistics based on the corrected numbers of pups per litter, this difference was not significant.

Inspection of Table 1 also discloses that Tackle exerted no apparent effect on the male/female sex ratio. The authors reported that the mean birth weights of pups were significantly reduced for the high-dose animals of the F_2b, F_3a, and F_3b generations. These data were not able to be verified by MITRE because the raw data were not available; however, the statistical significance as noted there do appear reasonable. It should be noted that although the treated

pups were lighter than the F_2a control litter, which were the heaviest in the entire study, this weight difference did not attain statistical significance. The reason for this is the small number of surviving control pups (12).

In the original report, the authors calculated a "fertility index" which is defined as the percent of matings resulting in pregnancy. Accurate determination of the fertility index requires an examination of the uterus shortly after birth; such examinations are carried out at autopsy. Thus, fertility indices could not have been accurately calculated for the F_2a or F_3a litters. Furthermore, due to revisions in the protocol, the F_0, F_1b, and F_2b parents survived well beyond the weaning of the second litter in their generation. The uteri of those dams would no longer have displayed evidence of implantation or resorption sites. For these reasons, MITRE calculated a birth index which is defined as the percent of females cohabited with males which gave birth to a litter. The results of those calculations are presented in Table 2. Interestingly, the birth index as presented in Table 2 and calculated by MITRE, corresponds to the fertility index presented in the original report.

Inspection of Table 2 reveals several places at which the birth index of treated groups were significantly different from those of

TABLE 2

BIRTH INDEX* FOR RATS RECEIVING VARIOUS DOSAGES
OF TACKLE OVER 3 GENERATIONS

Treatment by Litter / Generation	Control						25 ppm						500 ppm						2500 ppm					
	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ	%
F_1	17/26	65	—	—	17/26	65	21/26	81	—	—	21/26	81	20/26	77	—	—	20/26	77	26/26[e]	100	—	—	26/26[e]	100
F_2	2/22	9	10/22	45	12/44	27	7/25[c]	28	16/24	67	23/49[c]	47	11/24[d]	46	17/24	71	28/48[d]	58	14/25[e]	56	15/25	60	29/50[d]	58
F_3	17/21	81	21/21	100	38/42	90	23/25	92	23/25	92	46/50	92	20/25	80	21/24	88	41/49	84	20/25	80	20/25	80	40/50	80
Overall					67/112	60					90/125[c]	72					89/123[c]	72					95/126[c]	75

*Birth Index = $\frac{\text{Number of females bearing a litter}}{\text{Number of females mated cohabited with a male}} \times 100$

Σ = a + b.

[c]Data for all groups are heterogeneous by χ^2 ($p \leq 0.05$); this group is statistically different from controls at $p \leq 0.05$ by Fisher's Exact test (calculated by MITRE).
[d]Data for all groups are heterogeneous by χ^2 ($p \leq 0.05$); this group is statistically different from controls at $p \leq 0.01$ by Fisher's Exact test (calculated by MITRE).
[e]Data for all groups are heterogeneous by χ^2 ($p \leq 0.05$); this group is statistically different from controls at $p \leq 0.01$ by Fisher's Exact test (calculated by MITRE).

controls. In particular, the high-dose group of the F_1 generation, all Tackle groups of the F_2 generation, and the total birth index for all treated groups were significantly different from controls. It should be noted, however, that in all cases, the birth index was significantly elevated compared to controls. This is also true for the overall birth index (birth index calculated for all 3 generations). This finding is somewhat problematical and deserves some discussion. In particular, it should be noted that in the F_2a generation, the controls delivered only 2 litters out of a total of 22 mated dams. This is far lower than would be expected. The birth indices for all animals in all groups over both the a and b litters of the F_2 generation are lower than would be expected. Indeed the highest birth index is that of the F_2b 500 ppm group (71%) which approximates the expected 70-80% value. A revision to the protocol noted in Appendix C of the original report states that during the F_2 generation, the sponsor suggested that handling of the animals may have been related to some of the low incidence of births in this generation. Consequently, the animals were to be handled less frequently. Thus, for the F_2b generation litter and both F_3 litters, the females were checked for pregnancy only by inspection of the vagina for a copulatory plug. Since the performance of a vaginal lavages in rats has not been associated with interruption of

pregnancy or adverse effects on reproduction, this leads one to suspect that animal husbandry, in general, may have been a problem. This potential problem will be discussed at a later point in this data evaluation.

The gestation index is presented in Table 3. The gestation index is defined as the percent of pregnancies resulting in live litters. The numbers used to calculate this index are normally obtained by an inspection of the uterus as they are for the fertility indices. Since autopsies were not performed at a time close enough to birth of the pups, it is difficult to understand how this index was calculated by the authors. The authors do, however, report gestation indices in Tables 22 and 23 of their report (pages 80-81 of Vol. 1). Since it was impossible for MITRE to verify the gestation indices, The authors' figures are presented without further comment.

Due to MITRE's inability to verify the gestation indices, MITRE has calculated the incidence of infertile breedings. This incidence is presented in Table 4. The incidence of infertile breedings is defined as the number of barren dams which had evidence of copulation (either sperm positivity by vaginal lavage or presence of a copulatory plug) divided by the total number of dams which bore litters plus the number of barren animals which had evidence of copulation but no pregnancy. Inspection of the table reveals a high

TABLE 3

GESTATION INDEX FOR RATS RECEIVING VARIOUS DIETARY DOSAGES OF TACKLE OVER 3 GENERATIONS

Treatment by Litter / Generation	Control		25 ppm		500 ppm		2500 ppm	
	a (%)	b (%)	a (%)	b (%)	a (%)	b (%)	a (%)	b (%)
F_1	100	—	100	—	100	—	100	—
F_2	100	91	78	100	100	94	100	100
F_3	100	100	100	100	100	100	100	100

*Gestation Index = $\frac{\text{Number of Liveborn Litters}}{\text{Number of Pregnant Rats}}$ x 100

MITRE is unable to confirm the accuracy of the numbers used to calculate the Gestation Index and, therefore, cannot corroborate the veracity of this index.

TABLE 4

INCIDENCE OF INFERTILE BREEDINGS* FOR RATS RECEIVING VARIOUS DIETARY DOSAGES OF TAKCLE OVER 3 GENERATIONS

Treatment by Litter / Generation	Control						25 ppm						500						2500 ppm					
	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ	%
F_1	5/22	23	--	--	5/22	23	1/22	5	--	--	1/22	5	2/22	9	—	—	2/22	9	0/26	0	--	--	0/26	0
F_2	15/17	88	4/15	27	19/32	59	4/13	31	2/18	11	6/31	19	3/14	21	0/18	0	3/32	9	2/16	13	2/17	12	4/33	12
F_3	2/19	11	0/21	0	2/40	5	1/24	4	1/24	4	2/48	4	3/23	13	1/22	5	4/45	9	3/23	13	1/21	5	4/44	9
Overall					26/94	28					9/101	9					9/99	9					8/103	8

* Barren dams that exhibited copulatory plug or sperm positivity
Gravid dams + Barren dams that exhibited copulatory plugs or sperm positivity.

Σ = a + b

incidence of infertile breeding among the F_1 and F_2 generations. This is particularly evident in the control groups, especially the F_2a litter which demonstrated an 88% infertile breeding rate. By contrast, the treated groups of the F_2a litter demonstrated infertile breedings in only 13-31% of cases. The high number of unsuccessful breedings among the control animals in the first two generations, as well as the small number of fetuses which were produced by those litters, make it difficult to draw valid conclusions concerning most of the reproductive indices which will be discussed in future portions of this document. The reasons for such problems in reproduction in control animals are difficult to ascertain from inspection of the report. It should be noted, however, that poor reproductive outcome among rodents has been associated with stress which could be related to temperature, methods of animal handling, poor nutrition, and other generic problems.

The remaining reproductive indices concern observations made on surviving pups. Prior to discussing those indices, the statement made by the registrants in the Summary and Conclusion section relating to fetal malformations (p. 27, Vol. 1) that administration of Tackle was not associated with the "presence of physical abnormalities...at any dose level" must be corrected. In fact, at least 2 pups exhibiting major malformations were born of mothers treated with Tackle. One pup in the high-dose F_3a litter

displayed a short tail; a second pup in the low-dose F3b litter displayed a malformed right hindleg and no tail. The latter pup was mentioned in the body of the report (page 20); the former pup was not mentioned.

Table 5 presents the viability indices for all three generations of rats. The viability index is defined as the percentage of liveborn pups which survive until day 4 post-birth. Inspection of the table reveals that survival of the pups until day 4 for all treatment groups and control groups over all three generations was excellent. Indeed, the only point at which a treated group differed significantly from the control group was in the F3a generation, wherein the high-dose Tackle treated group was significantly smaller than the control group. It should be noted, however, that the Tackle treated rats exhibited a 91% survival rate, whereas, the control groups exhibited a 98% survival to day 4. The overall viability indices for all three treatment groups and the control groups across all three generations were not significantly different from one another; they ranged from 94% overall survival in the high-dose group to 98% survival in the low- and mid-dose Tackle treated groups.

Table 6 presents the lactation indices for three generations of Tackle-treated rats. The lactation index is defined as the percentage of pups alive on day 4 which survive until weaning. This index is calculated for survival of pups after the litters have been culled to not more than eight pups per litter. Inspection of

TABLE 5

VIABILITY INDEX FOR RATS RECEIVING VARIOUS DOSAGES OF TACKLE OVER 3 GENERATIONS

Treatment by Litter / Generation	Control						25 ppm						500 ppm						2500 ppm					
	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ
F_1	202/208	97	—	—	202/208	97	220/224	98	—	—	220/224	98	241/245	98	—	—	241/245	98	316/334	95	—	—	316/334
F_2	12/12	100	89/92	93	101/108	94	56/56	100	131/136[c]	96	187/192	97	90/96	94	165/169	98	255/265	96	114/116	98	139/153	91	253/269
F_3	203/207	98	222/234	95	425/441	96	276/276	100	262/273	96	538/549	98	231/232	100	253/257	98	484/489	99	201/220[d]	91	202/206	98	403/426
Overall					728/757	96					945/965	98					980/999	98					972/1029

[a]Viability Index = $\frac{\text{Number of pups alive on day 4}}{\text{Number of live born pups}} \times 100$

Σ = a + b.

[c]18 pups for 2 litters were sacrificed after their mothers were found dead on days 1 and 2. These pups are not included in the 136.

[d]Data for all groups are heterogeneous by X_2 ($p \leq 0.05$); this group is statistically different from controls at $p \leq 0.01$ by Fisher's Exact Test (calculated by MITRE).

TABLE 6

LACTATION INDEX FOR RATS RECEIVING VARIOUS DOSAGES OF TACKLE OVER 3 GENERATIONS

Treatment by Litter / Generation	Control						25 ppm						500 ppm						2500 ppm					
	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ	%
F_1	130/130	100	---	---	130/130	100	148/148	100	---	---	148/148	100	155/156	100	---	---	155/156	99	199/202	99	---	---	199/202	99
F_2	11/11	100	75/78	96	86/89	97	65/65	100	115/115	100	180/180	100	64[c]/64	100	135/142	95	199/206	97	107/110	97	126/131	96	233/241	97
F_3	135/135	100	142/144	99	277/279	99	183/183	100	180/181	99	363/364	100	160/160	100	167/168	99	327/328	100	156/157	99	149/150	99	305/307	99
Overall					493/498	99					691/692	100					681/690	99					737/750	98

[a]Lactation Index = $\frac{\text{Number of pups alive at weaning}}{\text{Number of living pups after culling on day 4}} \times 100$

Σ = a + b.

[c]4 pups reportedly died in this group, however, they cannot be included because record keeping errors required the elimination of that litter from the study.

the table reveals that survivability was excellent across all treatment and control groups and across all three generations. In fact, the smallest lactation index was demonstrated by the F_2b, 500 ppm, Tackle-treated group which demonstrated a 95% lactation index. The overall lactation indices for all three generations for Tackle treatment and control treatment range from 98% in the high-dose Tackle treated group to 100% survival in the 25 ppm Tackle treated group.

Analysis of the preceeding reproductive data allows one to conclude that pup survival is not adversely affected by the dosages of Tackle utilized in this study; however, the difficulties which were noted for reproductive performance among control animals, greatly weakened the utility of such conclusions.

The authors did note behavioral changes among the rats during the period of lactation. In particular, female rats tended to cannibalize the litters more than would be expected. According to the authors' tabulation of maternal observations, cannibalization occurred among all groups, although it was most prominent among Tackle treated groups. However, when the observations in the pup records are compared to those in the maternal records, several discrepancies concerning the incidence of cannibalization become apparent (Table 7). In addition to differences among the identities

TABLE 7

LITTERS REPORTED TO HAVE CASES OF CANNIBALIZATION OR MISSING PUPS ACCORDING TO PUP OBSERVATIONS OR OBSERVATIONS OF MATERNAL BEHAVIOR FOR 3 GENERATIONS OF RATS RECEIVING VARIOUS DOSAGES OF DIETARY TACKLE

Treatment/ Record Type / Generation	Control		25 ppm		500 ppm		2500 ppm	
	Maternal	Pup	Maternal	Pup	Maternal	Pup	Maternal	Pup
F_1	XF165 C[a] XF172 C	-- --	AF162 C AF165 C AF183 C AF184 C	AF162 C AF165 C AF183C AF184 C	BF164 C BF178 C BF184 C	BF164 C BF178C BF184 C	CF162 C CF167 C CF169 C CF175 C CF180 C CF184 C	CF162 C CF167 C CF169 C CF175 C -- CF184 C
(Total)	(2)	(0)	(4)	(4)	(3)	(3)	(6)	(5)
F_{2a}	--	--	AF223 C	AF223 C	BF203 C BF207 C	BF203 C BF207 C	CF210 C CF215 C CF217 C CF224 C	CF210 C CF215 C CF217 C CF224 C
(Total)	(0)	(0)	(1)	(1)	(2)	(2)	(4)	(4)
F_{2b}	XF201 C XF202 C XF211 C	XF201 C XF202 C XF211 C	AF214 C AF223 C	AF214 C AF223 C	BF205 C BF211 C --	-- BF211 C BF215 MC[b]	CF208 C CF223 C	CF208 C CF223 C
(Total)	(3)	(3)	(2)	(2)	(2)	(2)	(2)	(2)
F_{3a}	--	XF249 C[c]	--	--	--	--	CF238 C CF242 C --	CF238 C -- CF244 ME[g]
(Total)	(0)	(1)	(0)	(0)	(0)	(0)	(2)	(2)
F_{3b}	-- --	XF229 C[c] XF246 MC[d]	--	AF227 MC[e]	--	CF249 MC[f]	--	CF238 MC[h]
(Total)	(0)	(2)	(0)	(1)	(0)	(1)	(0)	(1)

[a]Suffixes: C = cannibalization; MC = missing presumed cannibalized; ME = missing presumed escaped.
[b]1 Male missing on day 7.
[c]A tail chewed off one pup.
[d]Entire litter (12 pups) missing on day 2.
[e]One malformed pup missing on day 3.
[f]One pup missing on day 14.
[g]One female missing on day 21 - presumably escaped.
[h]One female missing on day 14.

5

of affected litters in the F_1 and F_2 generations, it appears that cannibalizations occurred in the F_3 generation, as well as F_1 and F_2, since several young pups were reported to be "missing" within the first 2 weeks. This included one case of an entire litter of 12 pups which disappeared on day 2. Since the authors did not report finding any pups outside their cages, and since cannibalizations were prevalent among the first 2 generations, MITRE has assumed that pups which were missing within the first 2 weeks were cannibalized. Pups reported missing after 14 days were assumed to have escaped.

Table 8 presents the incidence of litters which were cannibalized either in whole or in part by the maternal dams. The incidences of cannibalization were obtained from Table 7 by counting the numbers of different litters which had cannibalizations recorded in either maternal or pup records (i.e., duplicate litters were excluded). Inspection of Table 8 reveals that although most of the cannibalizations were restricted to the F_1 and F_2 generations, cannibalizations did occur in all generations at approximately equal rates among all groups. Although cannibalization is a behavioral change, it is difficult to relate that change directly to an effect of Tackle treatment. Indeed, cannibalization can be related to stress on the animals due to such disparate causes as elevated, ambient temperatures, starvation, or poor animal handling techniques. Consequently, this may be considered part of the

TABLE 8

INCIDENCE OF CANNIBALIZATION OR PRESUMED CANNIBALIZATION*
AMONG RATS RECEIVING VARIOUS DOSAGES
OF DIETARY TACKLE OVER 3 GENERATIONS

Treatment by Litter / Generation	Control						25 ppm						500 ppm						2500 ppm Tackle					
	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ	%
F_1	2/17	12	--	--	2/17	12	4/21	19	--	--	4/21	19	3/20	15	--	--	3/20	15	6/26	23	--	--	6/26	23
F_2	0/2	0	3/10	30	3/12	25	1/7	14	2/16	13	3/23	13	2/11	18	3/17	18	5/28	18	4/14	29	2/15	13	6/29	21
F_3	1/17	6	2/21	10	3/38	8	0/23	0	1/23	4	1/46	2	0/20	0	1/21	5	1/41	2	2/20	10	1/20	5	3/40	8
Overall					8/67	12					8/90	9					9/89	10					15/93	16

*$\frac{\text{Number of litters exhibiting one or more pups which were cannibalized or presumed cannibalized}}{\text{Total Number of Litters}}$

$\Sigma = a + b$.

spectrum of potentially stress-related changes which was first demonstrated by the infertile breedings. It should also be noted that although the incidence of cannibalization was higher among Tackle treated groups, the total incidence of successful litters was much higher among the Tackle treated animals than among the control animals at the same time.

Since it is possible that the cannibalization, as well as the incidence of infertile breedings, may have been caused by a common mechanism, such as stress on the animals, Table 9 presents the combined incidences of infertile breedings and cannibalizations over three generations. In this table, the numerator is the combined incidence of infertile breedings plus cannibalizations and the demoninator is the number of actual pregnancies plus the number of sperm positive dams which did not produce litters. Inspection of this table reveals that the F_1 and F_2 generations exhibit the majority of these observations. It is clear, however, that the control rats demonstrated a much greater incidence of this type of reproductive failure than did any of the Tackle treated groups. In fact, the overall percentages across all three generations show the controls to have a 36% incidence of this type of reproductive failure as opposed to incidences of 17, 18, and 22% in the low-, mid-, and high-dose Tackle treated groups, respectively. Although

TABLE 9

COMBINED INCIDENCES OF INFERTILE BREEDINGS AND CANNIBALIZATIONS AMONG RATS RECEIVING VARIOUS DOSAGES OF DIETARY TACKLE OVER 3 GENERATIONS

Treatment by Litter / Generation	Control						25 ppm						500 ppm						2500 ppm					
	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ	%	a	%	b	%	Σ	%
F_1	7/22	32	--	--	7/22	32	5/22	23	--	--	5/22	23	5/22	23	--	--	5/22	23	6/26	23	--	--	6/26	23
F_2	15/17	88	7/15	47	22/32	69	9/13	38	4/18	22	9/31	29	5/14	36	3/18	17	8/32	22	6/16	38	4/17	24	10/33	30
F_3	3/19	16	2/21	10	5/40	13	1/24	4	2/24	8	3/48	6	3/23	13	2/22	9	5/45	9	5/23	22	2/21	10	7/44	14
Overall					34/94	36					17/101	17					18/99	18					23/103	22

* Numbers of Infertile Breedings + Cannabilizations / Total of Gravid Dams + Barren Dams Exhibiting Copulatory Plugs or Sperm Positivity

Σ = a + b.

* CONFIDENTIAL BUSINESS INFORMATION *

cannibalization has been associated with general types of stress among rodents, there may be other plausible reasons for this behavior. Among the possibilities, the following is another potential scenario. The authors reported the presence of subdermal hemorrhages among pups at birth. Some investigators have suggested that cannibalization tends to be more prevalent among animals whose litters have subdermal hemorrhages, although such statements have not been investigated experimentally. Since the authors of the original report did not tabulate their clinical findings, or their observations at birth in a summary form, MITRE attempted to correlate the observations of cannibalization with observations of pups which had subdermal hemorrhages. The incidences of subdermal hemorrhage and cannibalization were not concordant in any case throughout all 3 generations Thus, it does not appear that the subdermal hemorrhage may have been the stimulus which caused the animals to cannibalize their young.

Taken together, the poor reproductive performance of the control animals during the F_1 and F_2 generations and the high incidence of cannibalizations suggest potential stress-related problems and greatly weaken any conclusions concerning adverse effects of Tackle treatment on reproduction in rats.

The body weights of male and female rats during the first 10 weeks post-dosing are presented in Tables 10 and 11, respectively.

TABLE 10

BODY WEIGHTS OF 4 GENERATIONS OF MALE RATS RECEIVING VARIOUS DOSAGES OF DIETARY TACKLE FOR 10 WEEKS AFTER THE INITIATION OF DOSING

Generation by Litter	Dosage (ppm)	Body Weight (g)					
		Week Post-Dosing					
		0	2	4	6	8	10
F_0	0	168	268	342	396	425	458
	25	164	281	342	399	425	461
	500	162	275	340	392	420	458
	2500	164	263	324	373	399	428
F_1	0	114	217	312	370	420	450
	25	133+	225	317	373	419	448
	500	130	236	330	377	425	448
	2500	105	204	291	340	383	413
F_{2b}	0	132	240	308	371	398	429
	25	132	231	301	352	378	408
	500	142	246	315	371	398	437
	2500	108=	191=	263=	321=	343=	372=
F_{3b}	0	51	130	240	306	370	415
	25**	51	139+	251	318	382	423
	500**	50	129	242	310	378	423
	2500**	42=	111=	219=	284=	352	399

+Significantly heavier than controls, $p \leq 0.05$ (Dunnett's "t" test) (Authors' statistics).
=Significantly lighter than controls, $p \leq 0.01$ (Dunnett's "t" test) (Authors' statistics).
**All animals received control feed, however, they were exposed to Tackle via milk during lactation.

TABLE 11

BODY WEIGHTS OF 4 GENERATIONS OF FEMALE RATS RECEIVING VARIOUS DOSAGES OF DIETARY TACKLE FOR 10 WEEKS AFTER INITIATION OF DOSING

Generation by Litter	Dosage (ppm)	Body Weight (g)					
		Week Post-Dosing					
		0	2	4	6	8	10
F_0	0	135	195	226	246	267	278
	25	133	190	220	243	259	272
	500	132	191	223	245	260	274
	2500	137	194	220	243	251^{-}	267
F_1	0	104	173	216	234	252	256
	25	116^{+}	180	213	230	253	259
	500	108	174	212	235	263	266
	2500	98	162	189$^{=}$	220^{-}	238	249
F_2b	0	112	167	200	228	238	252
	25	124^{+}	178	210	235	249	262
	500	114	172	206	232	248	262
	2500	97$^{=}$	148$^{=}$	187	211^{-}	224	238
F_3b	0	50	121	174	208	231	249
	25**	50	124	179	208	233	247
	500**	49	118	173	205	235	256
	2500**	36$^{=}$	98$^{=}$	154$^{=}$	186$^{=}$	213$^{=}$	233

$^{+}$Significantly heavier than controls, $p \leq 0.05$ (Dunnett's "t" test) (Authors' statistics).
$^{-}$Significantly lighter than controls, $p \leq 0.05$ (Dunnett's "t" test) (Authors' statistics).
$^{=}$Significantly lighter than controls, $p \leq 0.01$ (Dunnett's "t" test) (Authors' statistics).
**All animals received control feed, however, they were exposed to Tackle via milk during lactation.

Although all adult rats were weighed during the first 10 weeks of dosing, the animals of different generations were not the same age at the onset of dosing and therefore it is difficult to compare body weights. For instance, at week 0 of dosing, the F_0 rats were 38 days old; the F_1 rats were 28 days old; F_2b rats were 20 days old; and F_3b rats were 13 days old. In addition, it must be recalled that all groups of F_3b weanlings were placed on control diets. The apparent differences in ages of the rats at the initiation of dosing probably account for the differences in initial body weights of rats among the 4 generations presented in Tables 9 and 10. Thus, valid comparison can be made only within a given generation.

Among male rats, the high-dose Tackle treated group of the F_2b generation was significantly lighter than controls throughout the initial 10 weeks post-dosing. It should be recalled that as demonstrated in Table 1, the high-dose treated groups for the F_2b and both F_3 litters were significantly lighter than controls at birth. A large difference was also apparent between the control and treated pup weights of the F_2a generation, however, that difference was not significant due to the extremely small number (i.e., 12) of pups in the control group. The F_3b pups of the high-dose treated group were significantly lighter than controls during the first six weeks post-dosing. The observations in the F_3b pups are weakened by the

fact that the pups were placed on the control diet at the time of weaning and consequently, the only exposure to Tackle was received during gestation and lactation. These data do suggest, however, that the effects of Tackle were reversible given the pups were able to approach control weights after exposure to Tackle was terminated.

Among female rats, significant differences from control weights were seen primarily in the high-dose group in the F_2b and F_3b generations. The F_2b generation demonstrated significantly lighter weights during approximately the first six weeks post-dosing; but by 10 weeks, they had attained weights which were within control limits. In the F_3b generation, significant differences were seen throughout the first eight weeks post-dosing but once again these conclusions are weakened by the fact that the animals were on control feed during the post-dosing period.

Based upon the reductions in weights among male and female rats, seen both at birth (Table 1) and during the post-dosing period (Tables 10 and 11), it can be concluded that the 2500 ppm dosage of Tackle was the low effect level. The no effect level is the 500 ppm dosage.

Although a major portion of the final report submitted by Gulf South Reseach Institute, was comprised of tabulated mean food consumption and mean water consumption data, these will not be

discussed in the present data evaluation record. The data submitted in the original report comprised 36 pages of mean data, however, the numbers of animals comprising each treatment group were not disclosed. This makes independent statistical evaluation of those data impossible. In addition, due to the great number of weaknesses in the experimental design of this study, these data add little to our understanding of either the effects of Tackle on reproduction or the mechanism by which those effects may be mediated. For these reasons, the food and water consumption data are considered superfluous.

Evaluation of spermatogenesis was performed on all males in the F_0 parental generation. As discussed previously, the weakness in conducting such an evaluation on the F_0 generation is that any potential effects seen here should be magnified had they been evaluated in the males of the F_3 generation. It should be noted that according to the amended protocol (Appendix C, p. 78), spermatogenic analyses were supposed to have been carried out for all breeding males of all generations. Nevertheless, a report concerning histologic examination of the testes from all males in all treatment and control groups was submitted for the F_0 sires only. Examination of these testes showed no treatment-related changes in any of the Tackle treated groups. Indeed, the only adverse testicular effects

occurred in 2/13 of the controls. Those two cases included one case of severe testicular atrophy with oligospermia in a male which was reported to have copulated with 2 dams resulting in no litter; and one case of chronic inflammation of the epididymus in a male which successfuly sired 2 litters.

The major histopathological findings over three generations are summarized in Table 12. The material in that table was extracted from three histopathologic reports which were appended to the original document. The findings in general were of a minor nature. The organs which were most frequently affected were the kidney and the liver. By far, the most common of the lesions, were hydronephrosis or nephropathy in the kidney and a condition described as eosinophilic hepatocytes in the liver.

Hydronephrosis occurred in control as well as treated groups. Inspection of Table 12 reveals that hydronephrosis, as a histological lesion, appeared more frequently in young animals than in older animals, that is to say, hydronephrosis was found among the 8-week old F_1 animals and was prevalent among the 8-week old F_2a and 4 to 5-week old F_2b animals as well as the 3 to 4-week old F_3a and 14-week old F_3b animals. However, in older animals, such as the 42 and 43-week old animals, that lesion was not reported as often. It should be noted that hydronephrosis when described as a gross lesion during teratological evaluation, frequently disappears as the animal

TABLE 12

INCIDENCES OF HISTOPATHOLOGICAL LESIONS AMONG MALE AND FEMALE RATS RECEIVING DIETARY TACKLE OVER 3 GENERATIONS

Generation by Litter	Age (Weeks)	Dosage (ppm)	Kidney				Liver	
			Hydronephrosis		Nephropathy		Eosinophilia	
			Male	Female	Male	Female	Male	Female
F_1	8	0	2/5	2/5	0/5	0/5	4/5	2/5
		25	0/5	0/5	0/5	0/5	2/5	1/5
		500	1/5	0/5	0/5	0/5	4/5	0/5
		2500	2/5	0/5	0/5	0/5	4/5	4/5
F_1	44	0	0/13	0/26	0/13	2/26	0/13	5/26
		25	0/13	0/26	0/13	2/26	0/13	4/26
		500	0/13	2/26	0/13	3/26	7/13	9/26
		2500	0/13	4/25	0/13	21/25	4/13	13/25
F_2[a]	8	0	0/5	1/6	0/5	0/6	0/5	1/6
		25	1/10	3/9	0/10	0/9	0/10	0/9
		500	1/9	0/11	0/9	0/11	1/9	0/11
		2500	4/9	4/11	0/9	0/11	0/9	0/11
F_2[b]	4-5	0	5/7	2/4	0/7	0/4	0/7	0/4
		25	2/10	5/10	0/10	0/10	0/10	0/10
		500	3/10	4/10	0/10	1/10	0/10	0/10
		2500	3/10	4/10	0/10	1/10	0/10	0/10
F_2[b]	42	0	0/14	0/21	1/14	2/21	0/14	0/21
		25	0/15	0/26	7/15	1/26	1/15	1/26
		500	0/15	1/25	5/15	1/25	1/15	2/25
		2500	0/15	6/25	8/15	12/25	1/15	5/25
F_3[a]	3-4	0	1/10	6/10	0/10	0/10	1/10	0/10
		25	5/10	8/10	0/10	0/10	2/10	0/10
		500	9/11	5/9	0/11	0/9	1/11	1/9
		2500	6/10	5/10	0/10	0/10	3/10	2/10
F_3[b]	14	0	7/11	2/9	0/11	0/9	1/11	0/9
		25	4/10	3/10	0/10	0/10	0/10	0/10
		500	4/10	4/10	1/10	0/10	0/10	0/10
		2500	7/10	9/10	1/10	1/10	1/10	0/10

ages. These histopathologic findings may be a reflection of the same phenomenon.

The other two common lesions were nephropathy and eosinophilia of hepatocytes. Nephropathy includes pyelitis, pyelonephritis, and other renal lesions of a chronic nature. Although they were frequently observed in the treated animals of the F_1 and F_2b litters, they also occurred at a lower incidence in the control animals. Eosinophilia of hepatocytes occurred sporadically among all treatment groups. It was not observed in the F_2b litter and it was virtually nonexistent in the F_2a and F_3b litters. As in the case of the kidney lesions, eosinophilia was also noted among control animals. In general, the lesions noted in this study commonly occur among laboratory rats. Treatment with Tackle was associated with a higher incidence of only those lesions which also occurred among controls and are considered to be spontaneous. Thus, the effects of Tackle were probably not due to a direct action of Tackle on the animals, rather they should be interpreted as exacerbating a pre-existing tendency for spontaneous lesions to occur. MITRE concurs with the conclusions of the histopathology reports. It should also be noted that the histopathologists stated that these lesions are generally associated with animals which are placed under stress. Thus, these lesions may also be a reflection of the stress-related phenomena which can cause the reproductive problems seen earlier in the study.

Conclusions:

In this three generation reproduction study, Tackle (acifluorfen; purity approximately 77%; approximately 23% unidentified ingredients/impurities) was administered to rats in the feed at dosages of 25, 500, and 2500 ppm. Control animals received feed only.

Reproductive performance of the control rats was not within acceptable limits during the first two generations of this study. This led to a lack of sufficient control animals and the absence of appropriate baseline data with which to judge the effects of Tackle. As a result, the reproductive indices generated by this report are of dubious value.

Among the clinical and behavioral signs noted in this study were a high number of infertile breedings among the control animals and a high incidence of cannibalization among both control and Tackle treated animals. These may reflect problems of a generic nature, such as stress, which may have had an adverse impact on this study.

The authors also experienced difficulty in record keeping as evidenced by the numerous errors in the tabulation of data as put forth in the present document. Thus, it is not possible, based on the submitted data, to determine whether Tackle in fact adversely affected reproduction in rats.

The postnatal growth of pups was apparently affected by the high-dose level of Tackle and, thus, the high-dose level (2500 ppm) may be considered the low effect level for growth in weanling animals; 500 ppm is the no effect level.

CORE CLASSIFICATION: Invalid. The purity of Tackle used in this study was not technical grade and the other ingredients or contaminants were not identified. This information should be requested from the registrant. The problems concerning the reproductive performance of control animals should be addressed by the authors; in the absence of adequate control data, it is not possible to evaluate the effects of Tackle treatment on reproduction. The methods used in the study need to be clearly stated in a single place; the present report contains mutually exculsive or contradictory statements in different sections (i.e., Methods vs. Protocol vs. Amended Protocol). Verification of the numbers in the report should be requested since many tables contain contradictory values.

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NATIONAL SECURITY INFORMATION EO 12065

DRAFT

EPA: 68-01-6561
TASK: 21
January 9, 1984

DATA EVALUATION RECORD

TACKLE

Mutagenicity

CITATION: Mirsalis JC. 1983. An investigation of the potential of Tackle and aqueous and hexane extracts of Tackle to induce unscheduled DNA synthesis in the in vivo and in vitro hepatocyte DNA repair assay in rats and mice. [An unpublished study report prepared by SRI International, submitted by Rhone-Poulenc, Inc., Project No. LSU-83-16, dated May 23, 1983.]

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - unscheduled DNA synthesis.

CITATION: Mirsalis JC. 1983. An investigation of the potential of Tackle and aqueous and hexane extracts of Tackle to induce unscheduled DNA synthesis in the in vivo and in vitro hepatocyte DNA repair assay in rats and mice. [An unpublished study report prepared by SRI International, submitted by Rhone-Poulenc, Inc., Project No. LSU-83-16, dated May 23, 1983.]

ACCESSION NUMBER: 251292.

MRID NUMBER: Not provided.

LABORATORY: Toxicology Laboratory, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025.

TEST MATERIAL: Three materials were tested; all three were provided by the sponsor. Tackle (Lot No. 25300D02201) was provided as a 20 percent aqueous solution; its purity was not specified. An aqueous extract of Tackle was also tested; purity and method of preparation were not specified. A hexane extract of Tackle, a solid, was administered in corn oil; purity and method of extract preparation were not specified. In addition, dimethylnitrosamine (DMN) was used as a positive control article. It was obtained from Aldrich Chemical Co. and had a purity greater than 99.9 percent.

PROTOCOL:

1. Experimental Design: Male Charles River F-344 rats (220-300 g) and B6C3F1 mice (18-30 g) were administered the test material by gavage and were sacrificed either 2 or 12 hours later. Each group of 3 animals received 10, 40, or 200 mg/kg of Tackle (both rats and mice); 50, 200, 700, or 1000 mg/kg of the hexane extract of Tackle (mice only); or 20, 150, or 700 mg/kg of the aqueous extract of Tackle (mice only). The report state that "all doses were calculated as weight of solid material." Groups of 3 animals were dosed concurrently in each assay with either water or 10 mg/kg DMN.

2. Cultures: Primary liver cell cultures were prepared from the livers of the animals after sacrifice according to the procedure of Mirsalis et al.[1] The cell cultures were inoculated into cultures dishes

[1] Mirsalis JC, Tyson CK, and Butterworth BE. 1982. Environ. Mutagen. 4:553-562.

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containing coverslips in Williams' Medium E supplemented with glutamine, gentamicin, and fetal bovine serum. After 1.5–2.0 hours, the cells were washed with medium and incubated in medium containing 10 µCi/ml ^{3}H-thymidine (specific activity, 40–60 Ci/mole) for 4 hours, followed by a 14–16 hour incubation in 0.25 mM unlabelled thymidine. The cells were washed and fixed, and the coverslip were mounted, dipped in photographic emulsion, and exposed for 12–14 days at -20°C. After development, the cells were stained with 1 percent methy-green Pyronin Y and read.

3. Measurement of Unscheduled DNA synthesis (UDS): The number of photographic grains was determined for 50 morphologically unaltered cells on a minimum of 3 slides for each animal for a minimum total of 450 cells/dose. Grain counting was accomplished automatically by an ARTEK Model 880 or colony counter that was interfaced through an ARTEK TV camera to a Zeiss Universal Microscope. The highest count from 2 nuclear-sized areas in the most heavily labeled cytoplasm adjacent to the nucleus was substracted from the nuclear count to give the net count. The percentage of cells in repair was calculated as those cells with net counts of at least 5. The data collected were considered acceptable if control values were within historical ranges and if the total net grain count for each animal was within 2 standard deviations of the other animals at that dose.

RESULTS:

None of the doses of Tackle produced net grain counts significantly greater than the control values, and no dose-related trends were evident (Table 1). In each assay, positive results were obtained with doses of 10 mg/kg of DMN.

DISCUSSION:

This study was adequately conducted and reported. The positive results seen with DMN indicated that the test system was responsive to mutagens that require metabolic activation. The negative results obtained with Tackle indicate that this compound does not induce unscheduled DNA synthesis and therefore is not likely to be metabolized to a genotoxic compound capable of interacting with hepatocytes in vivo. However, chemical characterization of the Tackle solution, and of the aqueous and hexane Tackle extracts were not provided. In addition, the nuclear-sized areas of cytoplasm which were used to estimate background counts should have been those with a representative number of counts rather than those which were the most heavily labelled. This would have facilitated reporting the findings as positive mean net grain counts for the 3 test materials and the water control groups.

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TABLE 1. Results of Assay of Unscheduled DNA Synthesis with Tackle

Treatment	Dose (mg/kg)*	Mean Net Grain Count	Cells in Repair (percent)
Assay 1 - Rats			
Control (water)	—	-8.1	3
DMN	10	64.7	98
Tackle	10-200	-5.7 to -14.4	2-4
Assay 2 - Mice			
Control (water)	—	-8.1	1
DMN	10	21.9	80
Tackle	10-200	-5.3 to -10.0	1-4
Assay 3 - Mice			
Control (water)	—	-8.3	1
DMN	10	24.7	85
Aqueous extract of Tackle	10-700	-6.1 to -9.5	0-4
Assay 4 - Mice			
Control (water	—	-8.1	1
DMN	10	25.4	89
Hexane extract of Tackle	50-1000**	-3.5 to -9.7	0-7

* "Calculated as weight of solid material."
** Cytotoxicity was seen at 1000 mg/kg.

CONCLUSIONS:

The results of this assay indicated that under the conditions of the study Tackle and its aqueous and hexane extracts, when administered to rats and mice by gavage, did not induce unscheduled DNA repair.

CLASSIFICATION: Unacceptable in its present form; however, if information on the chemical characterization of the three test materials is provided, so as to permit verification of the dose and nature of these materials, the study classification will be reconsidered.

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

DRAFT

EPA: 68-01-6561
TASK: 21
January 10, 1984

DATA EVALUATION RECORD

TACKLE

Metabolism

CITATION: Mitoma C, and Green CE. 1983. Whole-body half-life and DNA-binding index of ^{14}C-aciflurorен in rats and mice. [An unpublished study (Project No. CSC-5573-4 and -5) prepared by SRI International for Rhone-Poulenc, Inc.] [illegible] 1983.

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DATA EVALUATION RECORD

STUDY TYPE: Metabolism.

CITATION: Mitoma C, and Green CE. 1983. Whole-body half-life and DNA-binding index of ^{14}C-acifluroren in rats and mice. [An unpublished study (Project No. CSC-5573-4 and -5) prepared by SRI International for Rhone-Poulenc, Inc.]

ACCESSION NUMBER: 251293.

MRID NUMBER: None provided.

LABORATORY: Toxicology laboratory, SRI International, 333 Ravenswood Avenue, Menro Park, CA 94025.

TEST MATERIAL: The test material was identified as CF_3 - [URL-^{14}C] aciflurofen in toluene, a total of 1 mCi (specific activity 13.74 mCi/mmole). The lot number was given as GF-266049-1. No purity was given. The test material was provided by the Agrochemical Division, Rhone-Poulenc, Inc.

PROTOCOL:

1. Dose Solutions: An aqueous solution of the sodium salt of the labeled acifluorfen was prepared by shaking a measured quantity of the toluene solution with 0.1 N sodium hydroxide and by evaporating the toluene. For the half-life study, the aqueous solution was diluted to 2.62 µCi/ml; for the binding study, the solution was diluted to 90.9 µCi/ml. The purity of the solutions was checked using high performance liquid chromatography.

2. Animals: Male Fischer 344 rats (175 to 225 g) and male B6C3F1 mice (18 to 25 g) were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA, and quarantined for 10 days prior to use.

3. Half-Life Study: Thirty rats (231 g average body weight) and 30 mice (23 g average) were orally dosed with 0.6 ml (1.57 µCi) and 0.2 ml (0.52 µCi) of the prepared labeled aciflurofen solution, respectively, after a 16-hour fast. Three animals of each species were sacrificed 1, 2, 4, 8, 13, 32, 61, 128, 253, and 512 hours after dosing. After the cleansing of the gastrointestinal tract with water and draining of the bladder, each animal was homogenized with chloroform. The homogenates were filtered and re-extracted, and aliquots were mixed

with scintillation reagent for assay of radioactive content. The efficiency of the extraction process was determined by the addition of labeled test material directly to homogenates of untreated animals.

4. DNA Binding Study: Four rats (272 g average) and 4 mice (26 g average) were orally dosed with 0.6 ml and 0.2 ml of the prepared labeled acifluorfen solution, respectively. The rats were sacrificed 50.8 hours after dosing, and the mice, 44.5 hours after dosing. The livers of these animals were removed and the DNA extracted after tissue homogenation, protein removal, and clean-up with diethyl ether. The resulting aqueous extract was applied to a hydroxylapatite column and the DNA trapped and eluted. For each sample, at least 80 percent to 95 percent of the eluent was used to determine radioactivity; the DNA content of the remainder was determined spectrophotometrically. Livers from untreated animals were also processed for the determination of background counts.

   Data from each sample were compared to background and tested using Student's t-test ($p<0.001$). Significant values were used to calculate the covalent binding index (CBI) which was defined as:

$$CBI = \frac{\mu\text{mole chemical bound per mole nucleotides}}{\text{mmole chemical administered per kg animal.}}$$

RESULTS:

1. Half-Life Study: The extraction efficiencies were 37 and 55 percent for the rat and mouse, respectively. In both species, the maximum amount of acifluoren was absorbed at 8 hours, no radioactivity was detected after 128 hours. Using a curvilinear regression analysis of the corrected means data, half-life values of 21.40 and 18.26 hours were obtained for the rat and mouse, respectively.

2. DNA Binding Study: The average recovery of DNA from the hydroxylapatite columns with the mouse liver samples was 79.0 percent ± 56.2; the large standard deviation was attributed mainly to an "inexplicably low" recovery from one sample. The limit of detection for acifluoren bound to mouse liver DNA was equivalent to CBI = 1.18. Three of the 4 mouse samples had amounts of radioactivity higher than background. The CBI calculated for these 3 samples was 6.38 ± 3.3 and 4.79 ± 4.18 for all 4 samples.

   Average recovery of DNA from the columns with rat liver was 38.8 percent ± 11.2. None of the samples had levels of radioactivity higher than the background count. The limit of detection was equivalent to CBI = 8.70.

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DISCUSSION:

The half-life study was adequately conducted and reported. However, there are several points in the conduct of the DNA-binding study that require resolution. As pointed out by Lutz (Mutat. Res. 65:289-356, 1979), it is important to assure that the binding of the test chemical to DNA is maximum at the time of sacrifice and DNA extraction. This can best be determined by understanding the metabolism of the compound. Although in this study the interval between dosing and sacrifice was long enough to assure complete absorption of the test material (over 45 hours), it was also sufficient to allow possible DNA repair and excision mechanisms to reduce the amount of bound radioactive compound. The study would have been improved if multiple sacrifices at various time intervals had been performed. In addition, the reported recovery of DNA for the assay in rats was low with a mean of 38.3 ± 11.2 percent. This problem also occurred in the mouse assay for which the authors noted that recovery was very low (18.8 percent). Some explanation of these low recoveries is needed, since this is important in interpreting the data. Overall, the DNA-binding assay would have been more meaningful if clearly positive results had been obtained, since negative or borderline negative results are questionable due to the possibility that the labeled portion of the test material may have been eliminated by metabolism and an unlabeled metabolite bound to the DNA. Knowledge of the test material's metabolism is essential for evaluating and interpreting the reported findings.

CONCLUSIONS:

Whole-body half-life determinations conducted in rats and mice with labeled acifluoren indicate that the maximum amount of absorption was at 8 hours and that the half-life values were 21 and 18 hours for rats and mice, respectively. The DNA-binding study did not provide clear evidence that acifluoren forms, or does not form, in vivo an adduct with hepatic DNA of mice or rats.

CLASSIFICATION: Half-life study is acceptable; DNA-binding study is not acceptable in its present form; however, if the relevant metabolic information is made available and the DNA recovery levels are explained, the study classification will be reconsidered.

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