1/26/84

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

003556

JAN 26 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE:

SUBJECT: Request For Review Of Data Submitted For TACKLE (359-TNI/3F2811) On Soybeans.
Caswell No. 818B

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

THROUGH: William Butler, Section Head William Butler 1-16-84
and
William Burnam, Branch Chief
Toxicology Branch/ HED (TS-769) WB

Pesticide Petition: 359-TNI/ 3F2811

Petitioner: Rhone-Poulenc

Action Requested: Review and indicate any additional studies which will be required prior to registration/establishment of permanent tolerances for soybeans.

Recommendations: The data submitted do not support establishing tolerances for TACKLE.

The following data have demonstrated serious problems:
1.) ONCOGENICITY: 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, 1982). A statisically 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 control males. The incidence of liver tumors was also statistically increased in the 2500 ppm females.

In addition, a dose related increase in rare stomach papillomas were observed in all treated female groups (625, 1250, and

1

2500 ppm) and in high dose males (2500 ppm). This type of tumor was not reported in the concurrent control animals of either sex.

An oncogenic risk assessment is currently underway.

2.) MUTAGENICITY: A mutagenic potential has been demonstrated in the following assays:

a.) Gene Mutation Tests: In Drosophilia melanogaster, a positive effect was observed in the dominant lethal assay (EG and G Mason Research Institute and Utah State University Foundation, dated April 13, 1981).

In the murine lymphoma assay (Mobil Environmental Health Sciences Laboratory, Report No. 512-80, dated November 12, 1980), TACKLE did not induce mutation. However, these data are not acceptable because mutation frequencies and positive controls were not available. Therefore, a data gap exists for mammalian gene mutation.

b.) Chromosome Aberations: In Drosophilia melanogaster a weak positive effect was demonstrated in the Y-chromosome loss assay (EG and G Mason Research Institute and Utah State University Foundation, dated April 13, 1981).

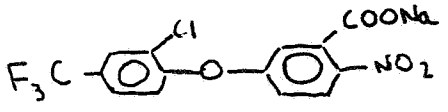
The metaphase analysis in rats (Mobil Environmental and Health Science Laboratory, Report No. 1041-80, dated March 13, 1981) indicated that TACKLE was toxic to bone marrow cells but did not demonstrate statistically significant clastogenic activity.

c.) Other Mechanisms Of Mutagenicity: An increase in recombination frequency was observed in the Saccharomyces cerevisiae strain D5 at 5.0 ul dose and frank toxicity was observed at 25.0 ul.

BACKGROUND: TACKLE is the 21.1% sodium salt of acifluorfen formulated as a water soluble concentrate that contains 2 lb a.i./ gallon. It is proposed for use on weed control in soybeans and will be applied by either ground or aerial equipment at a rate of 0.38 to 0.75 lb a.i./ acre.

TACKLE is applied between May and June and only one application is recommended.

CHEMICAL NAME: Sodium 5-(2-chloro-4-(trifluoromethyl phenoxy)-2-nitrobenzoate



003556

INERT INGREDIENT INFORMATION IS NOT INCLUDED

FORMULATION: TACKLE 2S

Commercial Components	% by Wt.	Purpose
TACKLE (Acifluorfen)	21 1 + [redacted]	Active Ingredient

8 POINT SUMMARY:

1.) Summary of Toxicology Data Attached In This Request:

STUDY	RESULTS
18-Month Feeding/Onco-mice	ONCOGENIC POTENTIAL at all doses tested: 625, 1250 and 2500 ppm in males and females
2-Year Feeding/Onco-rat	NOEL= 25 mg/kg
21-Day Dermal-rabbit	Systemic NOEL= 300 mg/kg/day Skin Irritation NOEL= not established
90-Day Feeding-mice	NOEL= 48 mg/kg
90-Day Feeding-rat	NOEL= 16 mg/kg
Teratology-rat	NOEL= 20 mg/kg
Teratology-rabbit	NOEL= 36 mg/kg
Acute Oral-rat	LD50 (male)= 2025 mg/kg LD50 (female)= 1370 mg/kg
Mutagenicity-Unscheduled DNA Synethesis (rat)	Negative
Mutagenicity-Bone Marrow (rat)	Negative

STUDY	RESULTS
Mutagenicity- Dominant Lethal (rat)	Negative
Mutagenicity- Forward Mutation	Unaceptable
Mutagenicity- Drosophilia	Negative for somatic reversion, biothorax test of Lewis, sex-lethals. Positive for dominant lethal, Y-chromosome loss.
Mutagenicity-Genetic Recombination (yeast)	Positive

2.) Toxicology Data Considered Lacking:
   a.) Reproduction: review in progress
   b.) 2-Year Feeding (dog): review in progress
   c.) Metabolism: Futher metabolism data are required because the overall recovery of radioactivity was not satisfactory.
   d.) Mutagenicity: Murine Lymphoma assay was not acceptable because positive control concentrations and mutation frequencies were not reported.

3.) Action Being Taken To Obtain Lacking Data:

None

4.) Summary Of Tolerances Granted:

None

5.) Summary Of How Total Tolerances Granted Affected The MPI:

Not applicable at this time.

6.) Acceptable Daily Intake Data:

Not application at this time.

7.) Pending Regulatory Action Against Registration:

None

f.) Other Considerations:

TACKLE is a biphenyl ether. Other similiarily structured chemicals have been reviewed.

Carolyn Gregorio, Toxicologist
Toxicology Branch/HED (TS-769)

Study Type: Acute Oral LD_{50} - Rats

Accession Number: 071306

MRID Number: Not assigned

Sponsor: Mobil Oil Corporation

Contracting Lab: Toxigenics, Inc. (Study No. 410-0249)

Date: October 13, 1980

Test Material: Tackle 2S
Purity: 20.2% (Liquid)

Protocol: In a range-finding study, "young adult" Sprague-Dawley Albino Rats (one animal/sex/dose) were given a single oral dose (by gavage) of 89, 178, 355, 708, 1413, 2818 or 5623 mg/kg of the "undiluted test article." The weight range for male rats was 260-311 grams and 188-217 grams for females. The animals were acclimated for at least 7 days before dosing.

Animals were fasted overnight prior to dosing. The animals were observed for gross signs of systemic toxicity and mortality "frequently throughout the day during the first day after dosing and twice daily for 7 days." The following results were recorded for the range-finding study:

Dose (mg/kg)	Time of Death	
	Males	Females
89	0/1	0/1
178	0/1	0/1
355	0/1	0/1
708	0/1	0/1
1413	0/1	1/1 at Day 1
2818	1/1 at Day 1	1/1 at Day 1
5623	1/1 at Day 1	1/1 at Day 1

In the main study, "young adult" Sprague-Dawley Albino rats (8 animals/sex/dose) were given a single oral dose (by gavage) of 200, 398, 794, 1000, 1259, 1413, 1585, 1778, 2239, 2818, 3548, or 5012 mg/kg of the "undiluted test article." The weight range for male rats was 208 to 270 grams and 177 to 236 grams for females. The animals were acclimated for at least 7 days before dosing.

Animals were fasted overnight prior to dosing. The animals were "frequently observed throughout the day (of dosing) for mortality and abnormal clinical signs" and then twice daily for 14 days.

Body weights were recorded on day of dosing and days 7 and 14 after dosing. All animals found dead or sacrificed were subjected to gross necropsy.

The Litchfield-Wilcoxon method of statistics was used.

RESULTS: All surviving animals gained weight until sacrificed. Clinical signs of toxicity that were observed were: lethargy, prostration, weakness, lacrimation, wheezing, crusty eyes, crusty nose, pilo erection, reddish exudate from urogenital area.

Necropsy revealed "multiple, red or tan, focal or diffuse lesions," in the lung, "multiple, red, diffuse lesions: in the stomach and "red, diffuse exudation" from the nasal area.

The mortality summary for the main study was as follows:

Dose (mg/kg)	Time of Death	
	Males	Females
200	0/8	0/8
398	0/8	1/8 at 1 day
794	None dosed	2/8 at 2 days
1000	None dosed	4/8 at 1,2 days
1259	None dosed	1/8 at 1 day
1413	2/8 at 1,2 days	None dosed
1585	None dosed	5/8 at 1,2 days
1778	5/8 at 1,2,11 days	None dosed
2239	3/8 at 1,2 days	None dosed
2818	4/8 at 1 days	None Dosed
3548	7/8 at 1,2 days	8/8 at 1,2 days
5012	8/8 at 1,2,3 days	8/8 at 1,2 days

CONCLUSION: The acute oral LD_{50} of Tackle 2S for male Sprague-Dawley rats is 2025 mg/kg (1556-2634).

The acute oral LD_{50} of Tackle 2S for female Sprague-Dawley rats is 1370 mg/kg (890-2107).

Toxicity Categor III

Classification: Guideline.

Study Type: Acute Dermal LD_{50} - Rabbits

Accession Number: 071306

MRID Number: Not Assigned

Sponsor: Mobil Oil Corporation

Contracting Lab: Toxigenics, Inc. (Study No. 410-0250)

Date: June 23, 1980

Test Substance: Tackle 2S
20.2% Purity (Liquid)

Protocol: Groups of New Zealand White Rabbits (5 animals/sex) were dermally dosed with 2.0 gm/kg of the test article. The body weight range of the animals was 2.3 to 2.8 kg.

All the animals were acclimated for 22 days before dosing. The backs of all the rabbits were shaved (approximately 10% of the body surface area). "Approximately 24 hours after clipping, the clipped area of each rabbit received longitudinal epidermal abrasions." The test material was applied and spread over the clipped/abraded site. The entire trunk of the animal was wrapped with an impervious binder consisting of Saran Wrap, adhesive tape and masking tape. After 24 hours, the binders were removed and the application site was cleaned by gently wiping the site with gauze sponges wetted with normal saline.

The animals were observed "frequently on the day of test article administration and twice daily thereafter" for 14 days. The animals were weighed, sacrificed and gross necropsied.

Results: One male rabbit was found dead on Day 9; "Death was attributed to naturally-occurring spontaneous disease." Gross necropsy report indicated "red discharge from cecum."

All surviving animals gained similiar weight through the 14-day observation period.

Gross necropsy of surviving rabbits revealed one female with "lesion, solitary, tan, focal in the liver."

Dermal reactions at the treatment site were reported as slight erythema until Day 2.

Conclusions: The acute dermal LD_{50} for Tackle 2S in New Zealand White Rabbits is greater than 2.0 gm/kg.

Toxicity Category: III

Classification: Minimum

Study Type: Primary Eye Irritation - Rabbits

Accession Number: 071306

MRID Number: Not Assigned

Sponsor: Mobil Oil Corporation

Contracting Lab: Toxigenics, Inc. (Study No. 410-0252)

Date: August 7, 1980

Test Substance: Tackle 2S
Purity: 20.3% (Liquid)

Protocol: Undiluted test material (0.1 ml) was instilled onto the everted lower lid of the right eye of each New Zealand white rabbit (2 males, 6 females). The upper and lower lids were held together for approximately one second. The left eye served as untreated control. The treated eyes of six rabbits were not washed. The remaining treated eyes of three rabbits were washed for 1 minute with lukewarm tap water approximately 30 seconds after instillation.

Ocular reactions were read with the aid of fluorescein at 24, 48 and 72 hours and 4 and 7 days after instillation of the test substance. Scoring was conducted according to Draize.

Results: Evaluation of mean eye irritation scores were as follows:

Non-Washed Eyes (6 animals)

	Pre-exposure	24 hrs.	48 hrs.	72 hrs.	Day 4	Day 7	Day 10*	Day 13*
Cornea	0	18	24	25	22	5[d]	5[d]	0[d]
Iris	0	5	5	6	6	0	0	0
Conjunctivae	0	12[a]	12[b]	8[c]	7[c]	1	1	0

* Only 3 animals examined.
a/ Blistering of conjunctivae in 3 animals.
b/ Blistering of conjunctivae in 4 animals.
c/ Blistering of conjunctivae in 1 animal.
d/ Pannus where opacity had been (2 animals).

Washed Eyes (6 animals)

	Pre-exposure	24 hrs.	48 hrs.	72 hrs.	Day 4	Day 7	Day 10*	Day 13*
Cornea	0	17	23	15	15	10	10[b/]	10[b/]
Iris	0	3	3	0	0	0	5	0
Conjunctivae	0	6	6[a/]	3	3	1	3	3

* Only 1 animal examined.
a/ Blistering of conjunctivae in 1 animal.
b/ Pannus where opacity had been.

Conclusion: The data indicate that Tackle 2S produces severe eye irritation (washed and non-washed).

Toxicity Category: I (severe irritation)

Classification: Minimum

Study Type: Primary Dermal Irritation - Rabbits

Accession Number: 071306

MRID Number: Not Assigned

Sponsor: Mobil Oil Corporation

Contracting Lab: Toxigenics, Inc. (Study No. 410-0285)

Date: September 8, 1980

Test Substance: Tackle 2S
20.2% purity (Liquid)

Protocol: Undiluted test material (0.5 ml, was applied under a one-inch square surgical gauze patch to two intact skin areas and two abraded skin areas on each of six female New Zealand White Rabbits.

At the end of 24-hour exposure period, the patches were removed and the skin wiped gently with normal saline (not washed). Skin reactions were scored immediately after removal of the patches (24-hour reading), again two days later (72-hour reading), and daily through eight days after application.

Scoring was conducted according to the Draize technique.

Results: Evaluation of mean skin reactions for erythema and edema for intact sites were as follows:

	24 hrs.	72 hrs.	Day 4	Day 5	Day 6	Day 7	Day 8
Erythema	2.5	2.0[a/]	2.0[a/]	1.6[a/]	1.0[a/]	0	0
Edema	0	0.2	0	0	0	0	0

[a/] Two animals demonstrated eschar formation.

Evaluation of mean skin reactions for erythema and edema for abraded sites were as follows:

	24 hrs.	72 hrs.	Day 4	Day 5	Day 6	Day 7	Day 8
Erythema	2.9	1.9[a/]	1.6[a/]	1.5[a/]	0.9[a/]*	0.8	0**
Edema	0	0.2	0.2	0	0	0	0

[a/] One animal demonstrated eschar formation.
* Five animals demonstrated desquamation.
** Three animals demonstrated desquamation.

Conclusions: The data indicate that Tackle 2S produces moderate to severe erythema irritant response (abraded and intact skin) at 24 hrs. Following dermal application, a well defined erythema persisted through Day 5. Skin was reported as normal at Day 7 for intact sites; three animals (abraded) continued to demonstrate the presence of desquamation at the application site through Day 8.

Toxicity Category: II (Severe irritation)

Classification: Core Minimum

003556

Study Type: Dermal Sensitization - Guinea Pigs

Accession Number: 071306

MRID Number: Not Assigned

Sponsor: Mobil Oil Corporation

Contracting Lab: Food and Drug Research Lab, Inc.
(Laboratory No. 6738)

Date: January 23, 1981

Test Substance: Tackle 2S
Purity: Not specified

Protocol: Groups of male Hartley-derived albino guinea pigs were shaved on the left flank area. The test material and positive control (DNCB in acetone) were topically applied 10-5 ml › 10 animals per group at Days 1, 8 and 15. Dermal irritation readings were taken 24 hours after each application.

Both groups were left untreated during the two-week period immediately following the above described treatment.

A single challenge application of 0.25 ml of the test substance on 0.5 ml of DNCB (positive control) was dermally applied to the respective groups. Skin reactions were assessed at 24, 48, and 72 hours after application and scored according to the Draize Method.

Results: The were no signs of systemic toxicity in the main study. However, one animal was found dead during the range finding study (0.5 ml). Gross autopsy indicated that "the death did not appear to be test material related."

Dermal reactions following the insult applications were:

Test Material: Barely perceptible erythema (mean score 0.1); no edema

Positive Control: Severe erythema (mean score 4.0); slight edema (mean score 0.3)

Dermal reactions following the challenge application were:

Test Material: No erythema; no edema

Positive Control: Slight erythema (mean score 1.0); no edema.

Conclusion: Due to the severe skin irritation potential demonstrated in tne primary dermal irritation study (Toxigenics, Inc., Study No. 410-0286; dated September 8, 1980) using rabbits, the skin sensitization study was conducted using the topical batch application technique. However the data, as reported, are insufficient to assess the dermal sensitization potential of the test substance (purity of test substance used was not reported; rationale for applying the test substance only three times (Days 1, 8 and 15) instead of the traditional 10 treatments).

Classification: Supplementary

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Study Type: 4-hour Acute Inhalation - Rats

Accession Number: 071306

MRID Number: Not Assigned

Sponsor: Mobil Oil Corporation

Contracting Lab: Toxigenics, Inc. (Report No. 420-0251)

Date: July 11, 1980

Test Substance: Tackle 2AS
Purity: 20.3% (Liquid)

Protocol: Sprague-Dawley Rats (5 animals/sex) were exposed for 4 hours to 6.91 mg/liter or the test substance. Untreated controls (5 animals/sex), were exposed to clean air only.

"The test atmosphere was generated by passing compressed outside air which was filtered, conditioned and dried through a 1/4 J SS Air Atomizing Nozzle Assembly equipped with a 1650 SS Fluid Cap and a 64 SS Air Cap. The test article was pumped in teflon tubing to the nozzle using an FMI Lab Pump." The resulting air-aerosol mixture was introduced into a stainless steel and glass inhalation chamber.

The chamber and room temperature was measured. The test atmosphere gravimetric concentration and the analytical aerosol concentrations were determined and particle size distribution was calculated.

Animals were observed during the exposure period for toxic signs and twice daily during the 14-day observation period. All animals were weighed prior to exposure and on Days 7 and 14 of the observation period. An animals were sacrificed and subjected to gross necropsy.

Results: The chamber and room temperature throughout the 4-hour exposure remained 70°C. The gravimetric concentration was calculated to be 2.6 mg/liter and the analyical aerosol concentration was calculated to be 6.91 mg/liter.

Particle size distribution indicated that 90-95% of the particles generated were less than or equal to 10 micrometers.

Clinical Observations: Squinting, nasal discharge, dypsnea, and lacrimation were observed during the exposure.

Nasal discharge, dypsnea, crusty nose and yellow/brown stained fur were seen shortly after the exposure.

Nasal discharge, crusty nose, yellow/brown stained fur, crusty mouth and poor coat quality were observed through Day 11 post exposure.

No abnormalities were observed in the untreated control animals.

Body Weights: Test and untreated control rats had similar weight gains during the observation period.

Gross Necropsy: Focal depressions of the lung were seen in one treated male. Lung lesions were seen in two untreated control males and one diaphragmatic hernia was observed in an untreated control female.

Conclusion: No mortality was observed during the conduct of this study. Therefore the acute inhalation LC_{50} for rats is greater than 6.9 mg/liter (4-hour exposure).

Toxicity Category: III

Classification: Minimum

MRID: Not assigned

Study Type: Teratogenicity in rabbits

Accession Number: ~~Not specified~~ 071519

MRID Number: Not assigned

Sponsor: Mobil Oil Company, Toxicology Division

Contracting Lab: Argus Research Laboratories, Inc., 2025 Ridge Road, Perkasie, PA. Argus Report # 113-003

Date: December 30, 1981

Test Material: Tackle ~~(TACU 06238001), purity 81.2%~~ 25, 22.4% Roaim

Protocol:

Tackle (TACU 06238001; purity 81.2%) was obtained from Mobil Oil Corporation, Toxicology Division, Princeton, NJ. The material was a light tan powder. The complete chemical composition of the test material was not supplied by Mobil Oil Corporation, although the aqueous stock solution (240 mg/ml) was stated to contain 22.4% active ingredient.

Ninety-six virgin New Zealand White female rabbits were received from Dutchland Laboratories, Inc., Denver, PA. The rabbits were 5 months of age upon receipt at Argus and were allowed to acclimate to laboratory conditions for approximately one month. The does were housed individually in stainless steel cages. The ambient room temperature was maintained between 64-72°F and the relative humidity was maintained between 35-80%. Filtered fresh air was changed 12-15 times per hour throughout the entire study period. The light cycle

was maintained at 12 hours of light to 12 hours of darkness with the light cycle beginning at 0600 hours. The rabbits were allowed filtered, local tap water ad libitum and 200 g of food per day (Ralston Purina Certified Rabbit Chow #5322) throughout the study.

The breeding males for this study were 4 proven sires from whom spermatozoa were collected for use in the artificial insemination technique. A different buck was the source for spermatozoa on each of 4 consecutive breeding days.

Following the acclimation period, 64 females which appeared to be in good health were randomly selected for artificial insemination. Sixteen does (4 per treatment group) were inseminated on each of 4 consecutive days by the following method. On the day of insemination, each doe was injected intravenously with 20 USP units/kg of human chorionic gonadotropin (PREGNYL, Organon, Inc.). Three hours later, she was inseminated with approximately 0.25 ml of semen which had been diluted with saline to a concentration of 6×10^6 spermatozoa/ml. The inseminated does were assigned to treatment groups on the day of insemination by means of a random numbers table with adjustments made for body weights.

The rabbits were observed for physical signs and general appearance on a daily basis throughout the acclimation period and during gestation until the beginning of treatment. The inseminated

does were weighed on day 0 and on a daily basis from days 6 through 29 of gestation and again on day 30, prior to time of sacrifice.

Tackle was administered via oral intubation to female rabbits on days 6-29 of gestation in dosages of 3, 12, or 36 mg/kg body weight. All dosages were maintained at a constant volume of 10 ml/kg. Batches of dosage solutions were prepared each week by diluting a stock solution of tackle with deionized water. The stock solution contained 240 mg active ingredient/ml. Each daily dosage of tackle was determined by the body weight of the subject doe on that day of gestation. Treatments occurred between 09:00 and 12:00 each day. Control does received 10 ml/kg of the vehicle only. During the period of treatment, the rabbits were observed several times each day for any signs of toxicity, abortion, and/or viability. The amount of food consumed each day was recorded. All pregnant or presumed pregnant rats which died prior to the time of sacrifice were necropsied to determine the cause of death.

On day 30 of gestation, the does were killed by carbon dioxide (CO_2) asphyxiation. The abdominal cavity of each doe was laparotomized and the uterus was exteriorized to examine its contents. The uterus was scored for presence, site and numbers of implantations, resorptions (both early and late), and living and dead fetuses. The ovaries were fixed in 10% neutral buffered formalin and

subsequently were examined with the aid of a dissecting microscope to determine the number of corpora lutea. Each individual fetus was identified with a tag, weighed, and examined for any gross external anomalies. All fetuses were examined for soft tissue anomalies of the thorax and abdomen by a variation of the Staples dissection technique. The brain was not examined. All fetuses were then eviscerated and prepared for skeletal evaluation by the Alizarin Red S technique.

The statistical analyses performed on the data included the following:

- maternal body weights were analyzed by Bartlett's Test of Homogeneity of Variance, Analysis of Variance, and Analysis of Covariance
- fetal body weights were analyzed utilizing Bartlett's Test of Homogeneity of Variance, and Analysis of Variance
- fetal anomalies were analyzed using Fisher's Exact Test, the Kruskal-Wallis Test, the Normal Approximation to the Binomial Distribution, and the Variance Test for Homogeneity of the Binomial Distribution
- data concerning ossification sites in the skeletal preparations were analyzed using Bartlett's Test of Homogeneity of Variance, and Analysis of Variance

Results and Discussion:

Prior to discussion of the results of this study, some comments concerning its method of execution are in order. This study was a well-designed, well-executed, and well-documented investigation. Only minor discrepancies were noted. In particular,

the animals were treated from day 6 of gestation through day 29 of gestation, rather than day 6 through day 18 which is the normal treatment period for a rabbit teratology study. Since the rabbits were treated beyond the stage of organogenesis, which ends on day 18, this should not impact adversely on the results of this study and is not considered to be a serious discrepancy. This deficiency is minor and does not weaken the conclusions which can be drawn from this study.

Relatively few clinical signs were observed in this study. They were distributed among all treatment and control groups. The most common signs were lacrimation, wheezing, and alopecia. A total of 7 rabbits delivered prior to scheduled sacrifice (1 control and 2 each of the treated groups). In addition, 4 rabbits died on test (3 controls and 1 from the 3 mg/kg group). All deaths were accidental: three animals died from intubation errors; the fourth animal was sacrificed after having sustained a back injury (presumably acquired during intubation).

The body weight and weight gain data for pregnant rabbits are presented in Table 1. There were no significant differences among the mean body weights of all groups of animals at the initiation of the study, at the initiation of dosing on day 6, or throughout the periods of treatment or gestation. Based upon the lack of adverse

TABLE 1

MATERNAL BODY WEIGHT AND BODY WEIGHT CHANGES FOR RABBITS INTUBATED WITH VARIOUS DOSES OF TACKLE DURING GESTATIONAL DAYS 6-29

Treatment	No. of Does Pregnant	Mean Body Weight (kg) at Gestational Day						Mean Change in Body Weight for Surviving, Pregnant Does Over Period			
								Treatment (day 6-29)		Gestation (day 0-30)	
		0	6	12	18	24	30 (sacrifice)	kg	%	kg	%
Control	13	4.08	4.20	4.26[a]	4.28[a]	4.18[b,c]	4.15[d]	0.07[d]	2	0.22[d]	6
Tackle (mg/kg)											
3	13	4.03	4.07	4.11	4.16[a]	4.12[a]	4.00[e]	0.02[e]	1	0.06[e]	2
12	12	3.94	4.03	4.05	4.10	4.18[b]	4.24[d]	0.12[d]	3	0.23[d]	6
36	11	4.00	4.10	4.17	4.20	4.04	4.09[d]	0.04[d]	1	0.17[d]	4

[a]N=12.
[b]N=11.
[c]Includes one rabbits which died on day 24.
[d]N=9.
[e]N=10.

effects on maternal body weights, body weight gains, and absence of clinical signs in the treated groups, the maternal systemic NOEL is estimated to be greater than 36 mg/kg body weight (highest dose tested).

Pertinent gestational data for control and tackle-treated litters are summarized in Table 2. The mean numbers of corpora lutea and implantations per litter were not adversely affected by treatment with tackle. Although the pregnancy rates (percent of inseminated does which were fertilized) of the mid- and high-dose tackle groups were lower than that of controls, the differences were not statistically significant. The implantation rates (percent of corpora lutea which were implanted) were not adversely affected by treatment with tackle. Similarly, the incidences of litters which exhibited some resorbed fetuses were similar among all groups of animals.

The embryotoxicity data for tackle are presented in Table 3. Tackle caused no changes in the incidence of resorptions or dead fetuses. Although the mean fetal weights of fetuses from the high-dose group were lighter than the weights of fetuses from other treatment groups, the difference was not statistically significant and was probably influenced by the larger mean litter size (8.3 pups/litter) compared to controls (5.6) or the low- and mid-dose

TABLE 2

GESTATIONAL DATA FOR RABBITS INTUBATED WITH
VARIOUS DOSES OF TACKLE DURING GESTATIONAL DAYS 6-29

Treatment	No. Fertilized/ No. Inseminated (%)	Cases of Maternal Mortality	No. Litters Evaluated	Corpora Lutea		Implantations		Implantation Rate (%)[a]	No. Litters	
				Total	Mean/ Litter	Total	Mean/ Litter		Partially Resorbed	All Resorbed
Control	13/16 (81.2)	3	9	84	9.3	61	6.8	73	4	0
Tackle (mg/kg)										
3	13/16 (81.2)	1	10	97	9.7	72	7.2	74	4	0
12	12/16 (75.0)	0	9	96	10.7	66	7.3	69	3	0
36	11/16 (68.8)	0	9	100	11.1	81	9.0	81	5	0

[a]Implantation Rate = (Total Implantations / Total Corpora Lutea) X 100.

tackle groups (6.8 and 6.7, respectively). Tackle treatment was associated with small changes in the mean fetal weights seen at term. These were apparent in both the mid- and high-dose groups. There was no change noted in the ratio of male to female fetuses.

Treatment with tackle was not associated with increased incidences of either malformations or anatomical variations. It should be noted that the authors of the original report considered all of the morphologic changes to be anatomical variations. MITRE has reclassified some of those observations as malformations based upon the magnitude of perturbation of embryologic processes which underlie those changes. Although the reclassification has resulted in 4 malformations, they were distributed equally among the treatment groups and only one of them (malformed diaphragm) is considered to be life-threatening. The malformations observed in tackle-treated rats consisted of one case each of a malformed diaphragm with atelectasis, an extra rib between ribs 5 and 6 and fused ribs at "L6-7" (the latter malformation may really be fusion of ribs 6 and 7). One control rabbit exhibited absence of the gall bladder.

The other anatomical variations reported by the authors were considered by them to be of a minor nature. They included such changes as hematomata, accessory spleen, short tail,

TABLE 3

EMBRYOTOXICITY DATA FOR OFFSPRING OF RABBITS INTUBATED WITH VARIOUS DOSES OF TACKLE DURING GESTATIONAL DAYS 6-29

Treatment	Total Implantations	Total Resorptions	Dead Fetuses	Percent Dead or Resorbed	Live Fetuses		Mean Fetal Weight (g)	No. Malformed Fetuses (%)	No. Fetuses Exhibiting Anatomical Variation (%)	Male/Female Ratio of Live Fetuses (% Male)
					Total	Mean/ Litter				
Control	61	5	0	8	56	5.6	51.3	1[a] (2)	2 (4)	28/28 (50
Tackle (mg/kg)										
3	72	4	0	6	68	6.8	47.4	1[b] (1)	5 (7)	35/33 (52
12	66	6	0	9	60	6.7	53.3	1[c] (2)	1 (2)	33/27 (55
36	81	6	0	7	75	8.3	43.1	1[d] (1)	3 (4)	36/39 (48

[a] Absence of gall bladder.
[b] Rudimentary rib between ribs 5-6.
[c] Malformed diaphragm with atelectasis.
[d] Fused ribs (or fused vertebrae - records unclear).

and minor changes in ossification, such as fused or asymmetric sternebrae, or split xiphoid. The authors' assessment of these findings as minor changes is accurate.

Although the methods and procedures utilized by the personnel at Argus were exemplary, their analysis of the results is somewhat questionable. In particular, the conclusion portion of their report (Section II, 113-003:page 3) identified 36 mg/kg as the LEL for both maternal and fetotoxicity based upon the following statements:

> "Treatment with 36 mg/kg/day dosages of TACU 06238001 resulted in slight inhibition of maternal body weight gain, marked inhibition of maternal food consumption, possibly interfered with implantation and produced slight decreases in average fetal body weights.
>
> Dosages of 3 and 12 mg/kg/day of TACU 06238001 on days 6 through 29 of gestation did not result in agent-related toxicity in does or fetuses."

Those statements are not supported by the data submitted with the report for the following reasons:

1. "slight inhibition of maternal body weight gain" (Table 1)

Although does in the high-dose tackle-treated group gained less weight thar controls during treatment (0.04 kg vs. 0.07 kg) and during the entire gestational period (0.17kg vs. 0.22 kg) the differences were not statistically significant according to analysis of covariance (performed by the authors, covariate unspecified) nor

by analysis of variance (calculated by MITRE). In addition, the greatest reduction in weight gain relative to controls was observed in the low-dose group (0.02 kg during treatment; 0.06 kg during entire gestational period). Statistically significant levels were not attained by the low-dose group.

2. "marked inhibition of maternal food consumption" (Argus Table 6, 113-003 page 35)

The authors reported average food consumption for rabbits at 6 intervals during gestation from the day prior to mating until day 0, and from gestational days 5-6, 11-12, 17-18, 23-24, and 29-30. The average food consumption for the high-dose group was higher than controls for all intervals except days 23-24. Finding a single food consumption measurement to be lower than controls does not support the authors' conclusion. The data were analyzed by analysis of covariance; however, the authors did not identify the covariate.

Inspection of Argus Table 7 (Individual Food Consumption, pages 36-39) suggests that two high-dose females may have had hairballs which could account for their lack of eating during the last week of gestation. Were animals checked for the presence of hairballs in the stomach at sacrifice? Did the authors' conclusion refer to the trend towards lower food consumption rather than comparison to controls? These points should be clarified.

3. "possibly interfered with implantation"

The authors' conclusion was based upon the decreased pregnancy rate in the high-dose group (68.75%) compared to controls (81.25%), and upon the finding of involuting corpora lutea and congested uteri in 4 non-pregnant high-dose rabbits compared to 1 control. It should be noted that the incidences of pregnant vs. non-pregnant animals in the control treated group are not statistically different ($p > 0.3$ Fisher's Exact Test). In addition, the implantation rate, which is a measure of ova which were implanted, was higher in the high-dose group (81%) than in controls (73%) (c.f. Table 2).

The finding of involuting corpora lutea and congested uterine mucosa is worthy of note; however, its meaning is not clear. The authors did not perform any staining techniques of the uteri to identify possible occult implantations, which may have resorbed early in gestation, but rather relied strictly upon gross observation. Thus, neither the presence nor absence of implantations was established in the rabbits.

4. "slight decrease in average fetal body weights"

Although the mean fetal body weight of pups in the high-dose group (43.1 g) was lighter than controls (56.3 g), the difference was not significant when subjected to analysis of variance.

Furthermore, the large litter size of does which received the high-dose of tackle (8.3 pups/litter) compared to controls (5.6 pups/litter) is a better explanation of the reduction in mean pup weight. In addition, it should be noted that the aggregate litter weights (sum of the weight of all live pups in a litter) was larger in the high-dose tackle group (358 g) than in the control group (287 g).

5. Other signs of toxicity were not observed in the lower tackle dosages

This statement is misleading because signs of toxicity were not observed in any treatment group. The authors did utilize the slight decrease in maternal body weight gain among high dose animals to support maternal toxicity in the high-dose group. In contrast to the authors' conclusions, the treatment group which exhibited the greatest decrease in body weight gains over both the treatment period (0.02 kg) and gestation (0.06 kg), was the low- dose tackle group. By the authors' definition, this should have qualified as a deleterious effect in the low dose group. Due to the fact that none of the decreased weight gains attained statistical significance, these effects are not considered supportive of maternal toxicity.

In spite of the comments concerning the authors' analysis of data, tackle does not appear to be teratogenic to New Zealand White

rabbits. Under the conditions of this study, no evidence of fetotoxicity was observed in any dose group, as evidenced by decreases in mean fetal weights, increases in anatomical variations or alterations in sex ratios of surviving pups. Thus, the NOEL for fetotoxicity is considered to be at least 36 mg/kg (highest dose tested).

Conclusions:

In this teratology study, tackle (TACU 06238000; purity 81.2%; 18.8% unidentified ingredients/impurities) was diluted with deionized water and administered by gastric intubation to groups of 16 inseminated New Zealand White rabbits at dosages of 3, 12, or 36 mg active ingredient/kg on gestational days 6-29. Control does received deionized water only. Few clinical signs were observed in any treatment group throughout gestation. No statistically significant differences were noted in maternal body weights, body weight gains, numbers of corpora lutea, implantations, resorptions, pregnancy rate, or implantation rate among all groups.

Based on these data, the maternal systemic NOEL for tackle in pregnant rabbits is greater than 36 mg/kg (highest dose tested).

No tackle-related adverse effects were seen in the pups in terms of reduction of mean fetal weights, increased incidence of

malformations or increased anatomical variations. The malformations observed in tackle-treated pups included one case of malformed diaphragm (12 mg/kg) and two minor skeletal malformations (one case of extra rudimentary ribs and one case of fused ribs). From these data, the NOEL for fetotoxicity caused by tackle in rabbits is estimated to be greater than 36 mg. Tackle was not teratogenic to rabbits under the conditions of this study.

CORE Classification:

~~Conditional~~ Core Minimum.

* CONFIDENTIAL BUSINESS INFORMATION *

MRID: Not assigned

Study Type: Teratogenicity in rats

Accession Number: ~~Not specified~~ 071319

MRID Number: Not assigned

Sponsor: Mobil Oil Company, Toxicology Division

Contracting Lab: Argus Research Laboratories, Inc., 2025 Ridge Road, Perkasie, PA. Argus Report # 113-004

Date: April 24, 1981

Test Material: Tackle ~~(TACU 06238001), purity unspecified~~ 25 purity: 22.4%

Protocol:

Tackle (TACU 06238001), ~~purity unspecified~~, was obtained from Mobil Oil Corporation, Toxicology Division, Princeton, NJ. The material was a light tan powder. The complete chemical composition of the test material was not supplied by Mobil Oil Corporation.

One-hundred and fifty Crl:COBS CD (SD) BR virgin female rats were received from Canadian Breeding Farms Laboratories, Ltd., a subsidiary of Charles River Breeding Laboratories. The rats were 72 days of age upon receipt at Argus and were allowed to acclimate to laboratory conditions for approximately 2 weeks. The dams were housed individually in suspended wire bottom cages, except during periods of mating. The ambient room temperature was maintained between 70-78°F and the relative humidity was maintained between 35-65%. Filtered fresh air was changed 12-15 times per hour throughout the entire study period. The light cycle was

maintained at 12 hours of light to 12 hours of darkness with the light cycle beginning at 0600 hours. Filtered, local tap water and food (Ralston Purina Certified Rodent Chow #5002) were available ad libitum throughout the study.

The breeding males for this study were proven sires which were eight months old and had been received from the Charles River Breeding Laboratories, Portage, Michigan. Following the acclimation period, females which appeared to be in good health were placed in cages with males in a one-to-one ratio for a maximum of four days. Female rats which exhibited vaginal plugs in situ or vaginal plugs on the cage liners beneath their cages were considered to be pregnant; that date was designated day 0 of gestation. A total of 100 female rats (25 per group) were assigned to treatment groups through use of a random numbers table with adjustments made for body weights. Each female rat was identified with a unique, self-piercing ear tag. The rats were observed for physical signs and general appearance on a daily basis throughout the acclimation period and during gestation until the beginning of treatment. The female rats were weighed on day 0 and on a daily basis from days 6 through 19 of gestation and again on day 20, prior to time of sacrifice.

Tackle was administered via oral intubation to female rats on days 6-19 of gestation in dosages of 20, 90, or 180 mg/kg body

weight. All dosages were maintained at a volume of 10 ml/kg. Batches of dosage solutions were prepared each week by diluting a stock solution of tackle with deionized water. The stock solution contained 240 mg active ingredient/ml. Each daily dosage of tackle was determined by the body weight of the subject dam on that day of gestation. Treatments occurred between 10:15 and 12:15 each day. Control rats received 10 ml/kg/day of the vehicle only. During the period of treatment, the rats were observed several times each day for viability and any signs of toxicity and/or abortion. All pregnant or presumed pregnant rats which died prior to the time of sacrifice were necropsied to determine the cause of death.

On day 20 of gestation, the dams were killed by carbon dioxide (CO_2) asphyxiation. The abdominal cavity of each dam was laparotomized and the uterus was exteriorized to examine its contents. The uterus was scored for presence, site, and numbers of implantations, resorptions (both early and late), and living and dead fetuses. The ovaries were fixed in 10% neutral buffered formalin and subsequently were examined with the aid of a dissecting microscope to determine the number of corpora lutea. Each individual fetus was identified with a tag, weighed, and examined for identification of sex and for determination for any gross external anomalies. Approximately one-third of the fetuses were examined for soft tissue

anomalies by means of a free hand razor blade sectioning technique. The remaining 2/3 of the fetuses were prepared for skeletal evaluation by the Alizarin Red S technique.

The statistical analyses performed on the data included the following:

- maternal body weights were analyzed by Bartlett's Test of Homogeneity of Variance, Analysis of Variance, Analysis of Covariance, and the Test of Equality of Means, and Dunnett's Test
- fetal body weights and litter data were analyzed utilizing Bartlett's Test of Homogeneity of Variance, Analysis of Variance, Dunnett's Test, and Dunn's Test of Multiple Comparisons
- fetal anomalies were analyzed using Bartlett's Test of Homogeneity of Variance, the Kruskal-Wallis Test, Dunn's Method for Multiple Comparisons, the Normal Approximation to the Binomial Distribution, and the Variance Test for Homogeneity of the Binomial Distribution
- data concerning ossification sites in the skeletal preparations were analyzed using Bartlett's Test of Homogeneity of Variance, Analysis of Variance, Dunnett's Test, the Kruskal-Wallis Test, and Dunn's Method for Multiple Comparisons

Results and Discussion:

Prior to discussion of the results of this study, some comments concerning its method of execution are in order. This study was a well-designed, well-executed, and well-documented investigation. Only minor discrepancies were noted. In particular, the following data were not reported: maternal food consumption data and weights

of the gravid uteri. In addition, the animals were treated from day 6 of gestation through day 19 of gestation, rather than day 6 through day 15 which is the normal treatment period for a teratology study. Since the animals were treated beyond the stage of organogenesis, which ends on day 15, the treatment schedule should not impact adversely on the results of this study and is not considered to be a serious discrepancy. These deficiencies are minor and do not, in any way, weaken the conclusions which can be drawn from this study.

Clinical signs were observed primarily in the high-dose tackle treated group of females. The predominant clinical signs included excessive salivation (19 cases), chromorhinorrhea (14 cases), urine-stained fur of the abdomen (7 cases), rales (5 cases), decreased motor activity and chromodacryorrhea (4 cases each). In addition, three females from the high-dose group died on test. Two animals died on day 10 and one on day 17. At necropsy, one of the day 10 rats was noted to have hemorrhagic lungs, the other exhibited multiple erosions of the stomach. No mention was made of the necropsy findings for the day 17 rat.

Relatively few clinical signs were seen in the other groups, although alopecia was observed in both the vehicle control and mid-dose tackle group (two animals per group). Mid-dose tackle treated rats also exhibited excessive salivation (8 cases) and piloerection (3 cases).

The body weight and weight gain data for pregnant rats are presented in Table 1. There were no significant differences among the mean body weights of all groups of animals at the initiation of the study nor at the initiation of dosing on day 6. By gestational day 13, the animals in the high-dose group were lighter than the other groups of animals, including the controls, and they remained lighter throughout the remainder of gestation. Indeed, the mean change in body weights over the treatment period, i.e., days 6-19, and over the entire gestational period, i.e., days 0-20, were significantly reduced in the high-dose group when compared to all other groups of animals ($p \leq 0.01$ by ANOVA and Dunnett's Test, calculated by the authors). Since the authors did not report the food intake of the animals throughout the course of the study, it is not possible to determine whether the loss in body weight in the high-dose group of animals was due to decreased intake of food or decreased feed efficiency. Nevertheless, based upon the decreased body weight changes and decreased mean body weights during the latter part of gestation in these animals, and the increase in clinical signs and mortality observed in the high-dose group, it can be estimated that the maternal systemic LEL for tackle in rats is 180 mg/kg body weight. The NOEL is estimated to be 90 mg/kg body weight.

Pertinent gestational data for control and tackle-treated litters are summarized in Table 2. The mean numbers of corpora lutea

TABLE 1

MATERNAL BODY WEIGHT AND BODY WEIGHT CHANGES FOR CHARLES RIVER RATS INTUBATED WITH VARIOUS DOSES OF TACKLE DURING GESTATIONAL DAYS 6-19

Treatment	No. of Dams	Mean Body Weight (g) at Gestational Day					Mean Change in Body Weight Over Period			
							Treatment (day 6-19)		Gestation (day 0-20)	
		0	6	13	19	20 (sacrifice)	g	%	g	%
Control	22	240.4	267.7	295.7	355.0	369.6	87.3	33	129.2	54
Tackle (mg/kg)										
20	21	240.2	267.2	293.9	355.5	373.2	88.3	33	133.0	55
90	19	239.8	268.4	288.7	349.7	364.5	81.3	30	124.7	[redacted]
180	21[a]	240.0	269.0	277.6[b,c]	325.4[b,c]	335.4[b,c]	56.5[b,c]	21	96.1[b,c]	40

[a]Excludes the weight of one pregnant rat which resorbed her entire litter.
[b]N=19; 2 pregnant rats died on gestational day 10.
[c]Significantly lighter than control, $p \leq 0.01$; ANOVA and Dunnett's test (Authors' calculations).

TABLE 2

GESTATIONAL DATA FOR CHARLES RIVER RATS INTUBATED WITH VARIOUS DOSES OF TACKLE DURING GESTATIONAL DAYS 6-19

Treatment	No. Fertilized/ No. Inseminated (%)	Cases of Maternal Mortality	No. Litters Evaluated	Corpora Lutea		Implantations		Implantation Rate (%)[a]	No. Litters	
				Total	Mean/ Litter	Total	Mean/ Litter		Partially Resorbed	All Resort
Control	22/25 (88)	0	22	323	14.7	288	13.1	89	16	0
Tackle (mg/kg)										
20	21/25 (84)	0	21	309	14.7	286	13.6	93	11	0
90	19/25 (76)	0	19	293	15.4	260	13.7	89	13	0
180	22/25 (88)	3	20	292	14.6	272	13.6	93	15	1

[a]Implantation Rate = (Total Implantations / Total Corpora Lutea) X 100.

and implantations per litter were similar among all groups. The pregnancy rates (percent of inseminated dams which were fertilized) and the implantation rates (percent of corpora lutea which implanted) were also similar among all groups of animals. Although the incidences of litters which exhibited some resorbed fetuses were similar among all groups of animals, it should be pointed out that the only completely resorbed litter occurred in the high-dose group. Taken together with the three cases of maternal mortality, this is a further indication of maternal toxicity in the high-dose group.

The embryotoxicity data for tackle are presented in Table 3. Tackle caused an increase in resorptions in the high-dose group of animals. There were also tackle related changes in the mean fetal weights seen at term. These were apparent in both the mid- and high-dose groups. There was no change noted in the ratio of male to female fetuses.

Although the authors reported an apparent dose-related increase in the number of fetuses exhibiting anatomical variation, it should be pointed out that no malformations were reported in any treatment group. The incidence of fetuses which exhibited anatomical variations attained statistical significance in the mid- and high-dose groups. The anatomical variations reported by the authors were of a minor nature. The variations

TABLE 3

EMBRYOTOXICITY DATA FOR OFFSPRING OF CHARLES RIVER RATS INTUBATED WITH VARIOUS DOSES OF TACKLE DURING GESTATIONAL DAYS 6-19

Treatment	Total Implantations	Total Resorptions	Dead Fetuses	Percent Dead or Resorbed	Live Fetuses		Mean Fetal Weight (g)	No. Fetuses Exhibiting Anatomical Variation (%)	Male/Female Ratio of Live Fetuse (% Male)
					Total	Mean/ Litter			
Control	288	20	0	7	268	12.2	3.72	9 (3)	137/131 (51
Tackle (mg/kg)									
20	286	19	0	7	267	12.7	3.77	12 (4)	145/122 (54
90	260	27	0	10	233	12.3	3.39[b]	17[c] (7)	112/121 (48
180	272	44[a]	0	16	228	11.4	3.03[b]	23[d] (10)	107/121 (47

[a]Significantly greater than control at $p \leq 0.0001$ by Fisher's Exact Test (Calculated by MITRE).
[b]Significantly lighter than controls at $p \leq 0.01$ by ANOVA, Dunnett's test (Authors' calculations).
[c]Significantly greater than controls at $p \leq 0.05$ by Kruskal-Wallis test and Dunn's method of multiple comparisons (Authors calculations).
[d]Significantly greater than controls at $p \leq 0.01$ by Kruskal-Wallis test and Dunn's method of multiple comparisons (Authors calculations).

included such changes as slightly dilated lateral ventricles of the brain, hemorrhage in the eyeball, slight dilation of the renal pelvis, hemorrhage in either the peritoneal cavity or subcutaneous spaces, and minor changes in ossification, such as incomplete ossification of supra-occipital sternebra or thoracic centra. The authors' assessment of these findings as minor changes is accurate.

Under the conditions of this study, tackle was not teratogenic to Charles River Rats. Fetotoxicity was observed in both the high-dose and in the mid-dose groups, as evidenced by decreased mean fetal weights and, to some extent, by the increase in anatomical variations seen among the fetuses. Thus, the LEL for fetotoxicity is considered to be 90 mg/kg; the NOEL is 20 mg/kg.

Conclusions:

In this teratology study, tackle (TACU 06238000; purity unspecified) was diluted with deionized water and administered by gastric intubation at groups of 25 mated female Charles River Crl:COBS CD (SD) BR rats at dosages of 20, 90, or 180 mg active ingredient/kg on gestational days 6-19. Control dams received deionized water only. High-dose treated dams exhibited numerous clinical signs throughout gestation. Three dams from the high-dose group died on test and one dam completely resorbed her litter. At terminal sacrifice, high-dose treated rats were significantly lighter

than animals in the other groups and exhibited significantly smaller mean body weight gains over the period of treatment and gestation. Based on these data, the maternal systemic LEL for tackle in pregnant rats is 180 mg/kg; the NOEL is 90 mg/kg.

The only adverse effects seen in the pups included a significant increase in resorptions in the high-dose group and reduced mean fetal weights compared to controls for both the mid- and high-dose groups. Although no malformations were recorded in any treatment group, the incidence of fetuses exhibiting minor anatomical variations was increased significantly for the mid- and high-dose groups. From these data, the LEL for fetotoxicity caused by tackle in rats is estimated to be 90 mg/kg; the NOEL is 20 mg/kg. Tackle was not teratogenic to rats under the conditions of this study.

CORE Classification:

Conditional Core Minimum. The purity of tackle used in the study was not specified, nor were other ingredients or contaminants identified. That information should be requested from the registrant. Upon satisfactory analysis of the requested information, this study could be elevated to Core Guideline.

003556

* CONFIDENTIAL BUSINESS INFORMATION *

Accession No. 071321

MRID: Not assigned

1. Chemical or Chemicals:

Acifluorfen-sodium

Sodium-5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate

2. Type or Formulation:

^{13}C or ^{14}C labelled acifluorfen-sodium

3. Citation or Citations:

Mackerer, C.R., 1982. Pharmacokinetics and Metabolism of MC 10978 (Sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate), Mobil Environmental and Health Science Laboratory (Study No. 6425) Dated Sept. 30, 1982

4. Reviewed by:

B.H. Chin
MTS
The MITRE Corporation
1820 Dolley Madison Blvd.
McLean, Virginia 22102
(703) 827-2974

Signature: _____
Date:

5. Approved by:

Signature: _____
Date:

6. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC METABOLISM.

This study relates to the Proposed Guidelines data requirement 163.85-1.

* CONFIDENTIAL BUSINESS INFORMATION *

7. Conclusions:

This DER reports the absorption, distribution, biotransformation, and excretion of acifluorfen-sodium (MC 10978) in female and male Fischer 344 rats (5 animals each) following four dosage regimens. The regimens include three oral dosages [single low (16-17 mg/kg), single high (110 mg/kg) and multiple low (10 -12 mg/kg daily for 14 days)] and an intravenous dosage (11-15 mg/kg).

Absorption

The single low, single high, and multiple low oral dose studies indicate that acifluorfen-sodium is rapidly and almost completely absorbed into the systemic circulation (70-97% bioavailability and excreted) in both female and male rats within 96 hours after dosing.

Distribution

Very little radioactivity was detectable in tissues of rats 96 hours after administration of each of the four dosage regimens as shown in Table 4.

Biotransformation

The major radioactive component present in blood (95-98%), urine (95%), and bile (93%) was unchanged acifluorfen. The major component in feces was the amine metabolite which accounted for 60-80% of the radioactivity.

Excretion

The radioactivity recovered in the urine and in the feces in female animals, 96 hours after administration, was 60-82 and 5-23% of

the dose, respectively. In contrast, the radioactivity recovered in the urine and feces in male rats was 46-58% and 21-41% of the dose, respectively.

CORE CLASSIFICATION: Not Applicable. The guidelines for classification of metabolism studies are not available. Further metabolism studies are required because the overall recovery of radioactivity accounts for 70.9 to 92.5% of the dose which is not considered satisfactory.

8. Materials and Methods:

The following description of the materials and methods used for this study was abstracted and paraphrased from the original report.

The structure, IUPAC names, and acronyms of acifluorfen-sodium and its related derivatives are shown in Figure 1.

^{13}C- and ^{14}C-Labelled Acifluorfen

Acifluorfen free acids (MC 10109) labelled with ^{13}C or ^{14}C were synthesized by Pathfinder Laboratories, Inc., St. Louis, Missouri. The ^{13}C- or ^{14}C-labelled-acifluorfen acids had a radiochemical purity of 99%. Acifluorfen-sodium (unlabelled, ^{13}C-, or ^{14}C-labelled) was prepared from the corresponding acifluorfen acid by addition of sodium hydroxide. The ^{13}C-acifluorfen used was either CF_3- or NO_2-ring labelled material while the ^{14}C-acifluorfen used was approximately equal amounts of CF_3- and NO_2-ring labelled materials.

Preparation of Dosage Solutions

The four dosage regimens prepared were as follows:

- single low oral dose of 16-17 mg/kg

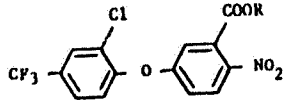
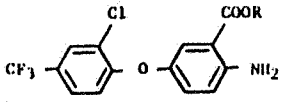
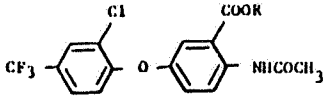
Structure		IUPAC Name	Acronym	Designation
	R=Na	Sodium 5-[2-chloro-4(trifluoromethyl)phenoxy]-2-nitrobenzoate	Acifluorfen-sodium	MC 10978
	R=H	5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid	Acifluorfen acid	MC 10109
	$R=CH_3$	Methyl 5-[2-chloro-4-(trifluorometyl)phenoxy]-2-nitrobenzoate	Acifluorfen methyl ester	MC 10108
	R=H	5-[2-Chloro-4-(trifluoromethyl)-2-amino-benzoic acid	Amine acid	MC 14620
	$R=CH_3$	Methyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-aminobenzoate	Amine methyl ester	MC 14621
	R=H	N-Acetyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-aminobenzoic acid	Acetamide acid	--
	$R=CH_3$	N-Acetyl methyl 5-[2-chloro-4-(trifluoromethyl)-pnenoxy]-2-aminobenzoate	Acetamide methyl ester	--

FIGURE 1

STRUCTURE OF ACIFLUORFEN-SODIUM (MC 10978) AND RELATED DERIVATIVES

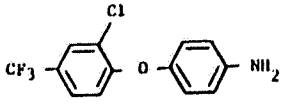
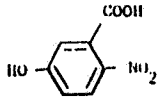
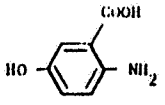
Structure	IUPAC Name	Acronym	Designation
	4-[2-Chloro-4-(trifluoromethyl)phenoxy]aniline	Descarboxy amine	MC 16412
	2-Nitro-5-hydroxybenzoic acid	--	AGRT 1106
	2-Amino-5-hydroxybenzoic acid	--	AGRT 1136

FIGURE 1

CONCLUDED

- single high oral dose of 116 mg/kg
- multiple low oral dose of 10-12 mg/kg daily for 14 days
- single low intravenous dose of 11-15 mg/kg

The single doses consisted of approximately a 1:1 mixture of unlabelled and ^{13}C-labelled acifluorfen-sodium plus 18-28 microcuries of ^{14}C-labelled acifluorfen-sodium. The multiple low oral dose involved administration of unlabelled acifluorfen-sodium daily for 13 days followed by a radioactive low dose on the 14th day. The mean dose of acifluorfen-sodium administered per dosage group for female and male rats is given in Table 1. All oral doses of acifluorfen-sodium were administered by gastric intubation. Saline was used as the injection vehicle. Intravenous doses of acifluorfen-sodium were administered via cardiac puncture. Throughout this report, the term "intravenous" will be used for the cardiac puncture method.

Animals

Young adult male and female Fischer 344 rats were fasted for 16 hours prior to dosing.

Collection of Blood, Urine, Feces, and Tissues

Blood, urine, feces, and tissues were collected in the following manner. Blood samples (ca. 100-200 microliters) from the tail vein of each rat were collected in heparinized Vacutainer tubes. Blood

TABLE 1

DOSE AND ROUTE OF ADMINISTRATION OF ACIFLUORFEN-SODIUM
IN FEMALE AND MALE FISCHER 344 RATS FOR THE FOUR REGIMENS STUDIED

Dose Level	Route	Sex	Dose (mg/kg)	Total Radioactivity Received (μCi)
Single Low	Oral	M	16.5	19.37
Single High	Oral	M	115.8	17.93
Multiple Low	Oral	M	11.5	20.88
Single Low	Intravenous	M	14.6	28.37
Single Low	Oral	F	17.0	20.33
Single High	Oral	F	116.5	18.59
Multiple Low	Oral	F	10.3	17.83
Single Low	Intravenous	F	11.3	20.24

was taken at 0.25, 0.5, 1, 2, 4, 8, 16, and 24 hours after oral dosing. In the case of intravenous administration, two additional blood samples were taken at 5 and 10 minutes after dosing. Urine was collected after 12, 24, 48, 72, and 96 hours following dosing (the method of urine collection and storage was not specified). Feces were collected at 24, 48, 72, and 96 hours. After 96 hours, animals were killed by cervical dislocation or carbon dioxide asphyxiation. For tissue distribution studies, the following tissues were collected and weighed: brain, kidney, bone (femur), liver, muscle, fat (retroperitoneal), gonads (testes or ovaries), large intestines, small intestines, stomach, bladder, spleen, heart, and residual carcass.

For tissue distribution studies for shorter sacrifice times, one male and one female rat from each of the dosage regimens were sacrificed at the following times and tissues described above were collected: 0.25, or 0.5, 4, and 16 hours.

Determination of Radioactivity of Acifluorfen and Related Derivatives in Various Biological Matrices

The radioactivity in various biological matrices was determined using a Beckman Model LS 9000 liquid scintillation system interfaced to a Texas Instruments Silent 700 data readout terminal.

Serial blood samples (25 microliters) were incubated with 150 microliters of a 2:1 solution (v/v) of isopropyl alcohol and Solulyte

digesting agent at 50°C for 30 minutes in 7 or 20 ml scintillation vials. The resulting mixture was then bleached with 100 microliters of 30% hydrogen peroxide and allowed to stand for 30 minutes. One-hundred microliters of 1.0 M acetic acid and 5 or 15 ml of Dynagel liquid scintillation cocktail were added, the mixture was vortexed well, and the radioactivity in the sample was determined. Determinations of radioactivity in urine and bile samples were carried out in duplicates. The radioactivity in fecal and tissue samples were determined using a Harvey Biological Oxidizer.

Bile Cannulation Studies

Rats were anesthetized with ether and a midline incision was made through the abdominal wall. The bile duct was located and a polyethylene tubing (PE-50) inserted and ligated. The cannula was directed posteriorly and fastened to the back of the animal by sutures. After intravenous administration of radiolabelled acifluorfen-sodium via cardiac puncture, the rat was placed in a restraining tube and the bile was collected from 0-4, 4-8, 8-12, and 12-24 hours. All animals received water only intraperitoneally several times during the course of the 24-hour collection to prevent dehydration.

Qualitative Analysis of Acifluorfen and Metabolites

A. Thin Layer Chromatography

Thin layer chromatography (TLC) was utilized for the characterization, isolation, and purification of metabolites of acifluorfen-sodium. Silica gel plates containing a fluorescent indicator were used in preliminary identification of urinary and fecal metabolites. Visualization of TLC spots was accomplished with a short and long wavelength hand-held UV lamp or Chromato-Vue lamp cabinet (Ultra-Violet Products, San Gabriel, Calif.) and by iodine vapor. Bratton-Marshall reagent was sprayed on developed TLC plates to visualize aromatic primary amines [Bratton and Marshall, J. Biol. Chem. 128:537 (1939)]. The identity of the metabolite was obtained by comparing the chromatographic properties (R_f value) of the radioactive metabolite with those of synthetic standards. Solvent systems utilized in TLC analysis include the following:

- Ethyl acetate/isopropyl alcohol/water, 65:24:11 (v/v)
- Dichloromethane/acetic acid, 96:4 (v/v)
- Toluene/diethyl ether/acetic acid. 90:50:2 (v/v)

B. High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) was used for qualitative and quantitative analysis of the metabolites of acifluorfen-sodium. The HPLC system consisted of a Beckman Model 330

liquid chromatograph with a Hewlett Packard 3390A or Beckman CORlA integrating recorder.

Ion-pair reverse phase HPLC was employed to separate acifluorfen and its derivatives from urine, feces, blood, bile, and tissues. Tetrabutyl ammonium phosphate (TBA) was chosen as the countercation to form the ion-pair complex with acifluorfen. The HPLC analysis was performed isocratically employing a mobile phase consisting of methanol and 0.01M TBA/0.04 M ammonium phosphate (pH 7.8) solution.

C. Gas Chromatographic and Mass Spectrometric Analysis

Gas chromatographic (GC) analyses were performed on a Hewlett Packard 5880A equipped with a flame ionization and nitrogen-phosphorus detector. All gas chromatography-mass spectrometric (GC-MS) analyses were performed on a Hewlett Packard 5895 interfaced to a 7920 data system. Extracted biological samples and the standard references were methylated with diazomethane before analysis by GC or GC-MS.

The ion cluster or doublet ion technique (Baille, Pharmacol. Review 33:81-132, 1981) was employed in MS analyses to detect metabolites. Since the molecular weight difference between $^{13}C_6$- and ^{12}C-acifluorfen is 6 atom mass units (amu), doublet ion clusters having a difference of 6 amu were sought as markers in the mass spectral scans for the metabolites derived from acifluorfen-sodium.

In GC-MS analysis, specific mass/charge ratios (m/e) were used for the selected ion monitoring of the GC effluent to determine whether any of the following acifluorfen metabolites or derivatives were present in various biological fluids or tissues:

- m/e 345 Acifluorfen amine methyl ester (MC 14620)
- m/e 375 Acifluorfen methyl ester (MC 10108)
- m/e 387 Acifluorfen acetamide methyl ester [N-acetyl methyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-aminobenzoate]
- m/e 405 Possible aromatically hydroxylated (phenolic) acifluorfen metabolites (as their methyl ether and methyl ester derivatives)
- m/e 432 Glycine conjugate of acifluorfen acid (methyl ester derivative)
- m/e 287 Descarboxy amine (MC 15412)

Quantitation of Acifluorfen and Its Metabolites In Urine, Feces, Blood, Bile, and Tissues

A. Urine

Urine samples were pooled (0-48 hour) and a 1.0 ml aliquot of urine was mixed with 1 ml of 0.05 M TBA in a Teflon-lined screw-capped Pyrex (7 ml) or Corex (thick-walled, 25 ml) test tube. The pH of the mixture was adjusted with 0.05 M NaOH to pH 10-12 and was extracted with 5 ml of diethyl ether/ethyl acetate, 1:1 (v/v). The organic layer was transferred to tubes (with tapered tips) and the aqueous layer extracted again with 5 ml of diethyl ether/ethyl acetate. The combined organic layers were evaporated under a stream

of nitrogen on a Meyer N-Evap analytical evaporator (Organomation Associates, Northborough, MA).

For quantitation of urinary metabolites, 0.5 minute fractions of HPLC effluent were collected and the radioactivity determined. Radioactivity histograms were constructed from the collected fractions and the radioactivity under each major peak was summed and divided by the total collected radioactivity. This number multiplied by 100 was used to determine the relative percentage of each individual urinary metabolite.

Enzymatic hydrolysis of urine samples was carried out in the following manner. Urine (1 ml) was adjusted to pH 7 and incubated with 1000 units of β-glucuronidase (from E. coli) at 37°C for 20-24 hours on a metabolic shaker. At the end of the incubation period, the urine mixture was extracted using the ion-pair method described above.

B. Feces

Quantitation of acifluorfen and its metabolites in fecal samples was determined by a procedure analogous to that used for quantitation in urine with the following minor modifications. After removal of particulates by filtration, twenty microliter aliquots were analyzed by HPLC and the separated fractions of the effluent collected. Quantitative determination of the individual metabolites followed the same method described previously for urine.

Enzymatic hydrolysis of fecal pellets after ion-pair extraction was carried out in the following manner. The aqueous fraction remaining after ion-pair extraction was adjusted with 1 M HCl to neutrality. Glucuronidase (2000 units per gm of fecal homogenate) was added and the mixture incubated at 37°C for 20-24 hours. After the incubation period, the fecal mixture was extracted in the usual manner with TBA and ethyl acetate.

C. Blood, Bile, Kidney and Liver

Extracts of the blood, bile, kidney, and liver were analyzed by the same experimental procedures used to quantitate acifluorfen and its metabolites in urine. Enzymatic hydrolysis of bile samples was carried out in the following manner. The aqueous fraction remaining after ion-pair extraction was adjusted to pH 7 and incubated with 1000 units of β-glucuronidase at 37°C for 24 hours. At the end of the incubation period, any liberated radioactivity was removed by ion-pair extraction and determined.

Pharmacokinetic Calculations and Analysis

Pharmacokinetic parameters were calculated according to methods described by the following individuals:

- Gibaldi and Perrier ("Pharmacokinetics", Marcel Dekker 1975),
- Wagner ("Biopharmaceutics and Relevant Pharmacokinetics" and "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications 1971 and 1975, respectively),

- Rowland and Tozer ("Clinical Pharmacokinetics: Concept and Applications," Lea and Febiger 1980),
- Shargel and Yu ("Applied Biopharmaceutics and Pharmacokinetics," Appleton-Century-Crofts 1980).

For each individual animal, the blood concentration (based on radioactivity) of acifluorfen-sodium was plotted against time on semilogarithmic graph paper. Peak blood levels (C_{max}) and the times to attain peak blood concentration (t_{max}) were estimated by visual inspection of the curves. The rate constant for absorption (k_a) was obtained by estimating t_{max} from the blood concentration-time curve and calculating k (elimination rate constant) by linear regression. The latter two parameters, t_{max} and k, were substituted into $t_{max} = [\ln (k_a-k)]/(k_a-k)$ and ka was subsequently determined. The absorption half-life [$t_{1/2}$ (absorption)] was obtained from the equation $t_{1/2} = 0.693/k_a$. For most orally administered regimens, the terminal excretion half-life [$t_{1/2}$ (β)] was determined by linear regression.

Statistical Analysis

The statistical significance of the difference in the mean values of various pharmacokinetic parameters among the dosage regimens and among the two sexes was determined using Student's t test.

9. Results and Discussion:

A. Absorption

A number of absorption pharmacokinetic parameters have been summarized (Table 2) for both female and male rats after oral administration of various doses of acifluorfen-sodium.

1. Single Low Oral Dose

A single low dose (16-17 mg/kg) of acifluorfen-sodium was rapidly and efficiently absorbed into systemic circulation by botn female (97%) and male (80%) rats. From blood concentration-time curves (see Figure 2), C_{max} (34 mcg/ml) of acifluorfen was found to occur at 1.5 hours (t_{max}) in female rats. C_{max} (30 mcg/ml) in male rats occurred at 3 hours (t_{max}). Based on these t_{max} values as well as k_a and $t_{1/2}$ (absorption) values, acifluorfen-sodium is more rapidly and extensively absorbed in female than in male rats.

2. Single High Oral Dose

The time required for absorption of acifluorfen-sodium was slower in a single high dose (116 mg/kg) group in comparison to a single low dose group in both male and female animals (Table 2). For example, the $t_{1/2}$ (absorption) in male rats was twice as long (1.84 hr for the high dose versus 0.74 hr for the low dose). Similarly, in female rats the $t_{1/2}$ (absorption) was also 6 times longer (2.38 hr for the high dose versus 0.39 hr for the low dose). C_{max} (210 mcg/ml) of acifluorfen

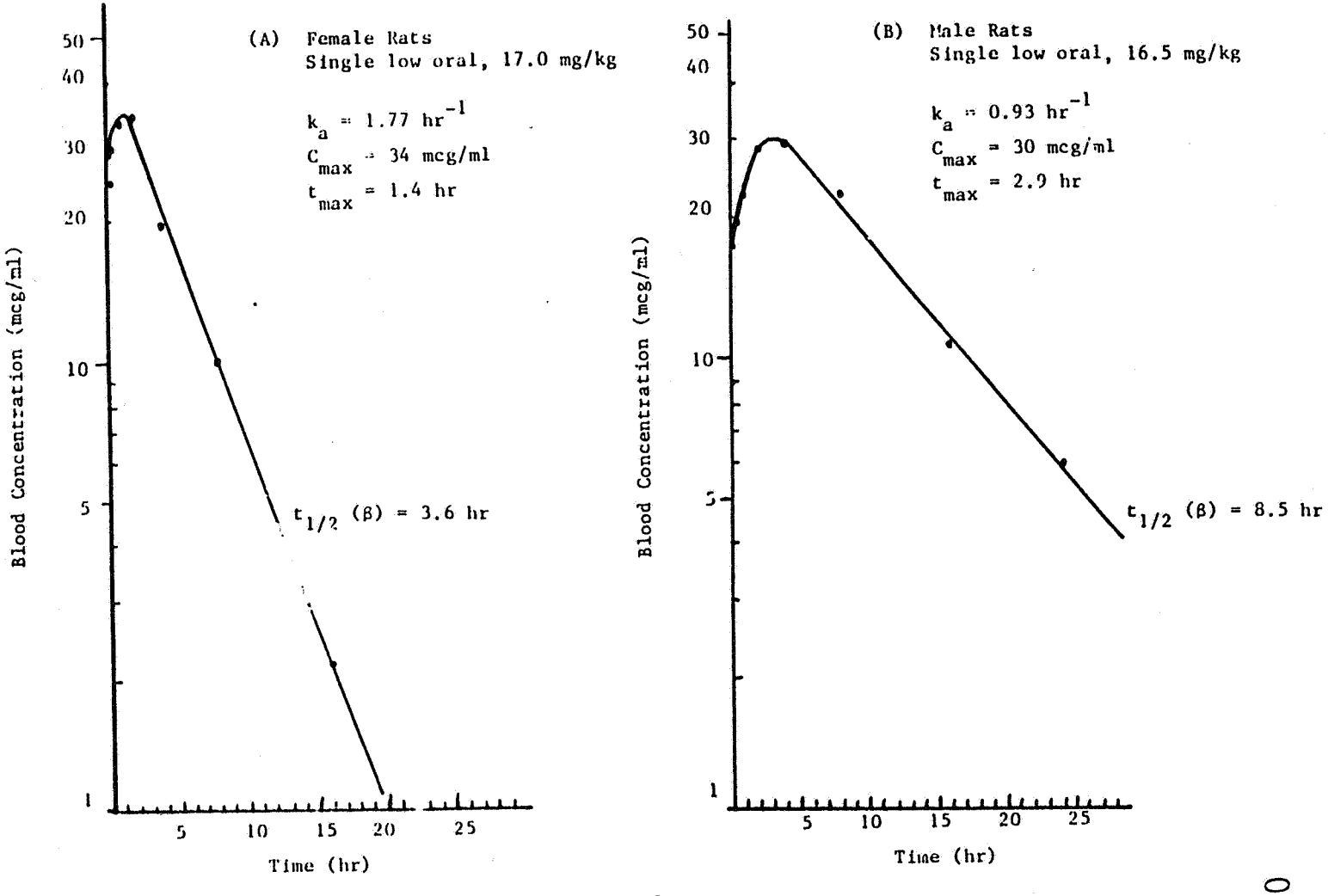


FIGURE 2

MEAN BLOOD CONCENTRATION-TIME CURVE AFTER ORAL ADMINISTRATION
OF A SINGLE LOW ORAL DOSE OF ACIFLUORFEN-SODIUM IN FEMALE AND MALE RATS

TABLE 2

ABSORPTION PHARMACOKINETIC PARAMETERS FOR
VARIOUS ACIFLUORFEN-SODIUM DOSAGE REGIMENS

Dose Regimen	Sex	Dose (mg/kg)	n	k_a (hr^{-1})	$t_{1/2}$ (absorption) (hr)	t_{max} (hr)	C_{max}[a] (mcg/ml)	AUC[b] (% dose-hr)	Bioavailability or % Absorption (%)
Single Low	M	16.5	5	0.93	0.74	2.9	30	151.0	80[c]
	F	17.0	5	1.77	0.39	1.4	34	71.5	97[c]
Single High	M	115.8	5	0.38	1.84	4.6	210	--	76[d]
	F	116.5	6	0.29	2.38	5.4	270	--	70-90[d]
Multiple Low	M	11.5	5	2.02	0.34	1.7	27	--	92-95[c,d]
	F	10.3	5	8.5	0.08	0.5	15	--	81[d]

[a] C_{max}, in most instances, was obtained from visual inspection of log blood concentration-time curves (see Figure 2).
[b] AUC = Area under the blood concentration-time curves, AUC for single low i.v. for males and females were 188.2 and 74.1, respectively.
[c] Bioavailability obtained by comparing AUC following oral and intravenous administration.
[d] Bioavailability was estimated by comparing the total amount of acifluorfen excreted unchanged in the urine (0-96 hr) following oral and intravenous administration.

was reached in 4.6 hours in male rats while C_{max} (270 mcg/ml) was reached at 5.4 hours in female rats. The acifluorfen-sodium absorbed into systemic circulation by female and male rats was 70-90% and 76% of the dose, respectively (Table 2).

3. Multiple Low Oral Dose

In the multiple low oral dose studies, acifluorfen-sodium was rapidly absorbed by the female rats since C_{max} (15 mcg/ml) was achieved within 0.5 hr. In males, absorption also occurred quite rapidly since C_{max} (27 mcg/ml) was observed at 1.7 hrs. (Table 2). The amount of the parent compound absorbed into systemic circulation was 81% for females and 92-95% for males.

In male rats, since the elimination half-life of acifluorfen-sodium (low dose) was about 8.5 hrs (Figure 2), a 14-day multiple dosage regimen would result in some blood accumulation (27 mcg/ml) of acifluorfen in male animals after 24 hours (i.e., before administration of the next dose). On the other hand, blood accumulation (15 mcg/ml) of acifluorfen-sodium after multiple low doses is not expected to occur to any significant extent in female rats since female animals eliminate acifluorfen-sodium quite rapidly (elimination half-life of 3.6 hours).

Absorption (k_a) is significantly slower after a high oral dose (0.29 - 0.38 hr^{-1}) than after a low oral dose (0.93 - 1.77 hr^{-1}). In

addition, the t_{max} for a high oral dose is much longer (4.6 - 5.4 hr) than that for a single or multiple low dose (Table 2). Thus, increasing the oral dose tenfold significantly diminishes the rate of absorption of acifluorfen-sodium in both female and male rats.

B. Distribution

1. Distribution Pharmacokinetics

Pharmacokinetic analysis after intravenous administration revealed a significant difference in the distribution phase (α-phase) of acifluorfen-sodium in male and female rats (Table 3). The α-phase of acifluorfen-sodium in females (α = 0.58 hr) occurred about five times slower than in males (α = 2.8 hr). While acifluorfen-sodium is more slowly distributed in females than in males, it is more rapidly eliminated from female rats than from male rats. The elimination half-life [$t_{1/2}$ (β)] of the parent compound was 3.7 hours in females versus 8.8 hours in males (Table 3).

The apparent volume of distribution (Vd) of acifluorfen-sodium, was approximately 66 and 82 ml in female and male rats, respectively (Table 3). Since the Vd is about 5-6 times the blood volume (ca. 12-15 ml), it appears that tissue binding occurs to some extent and acifluorfen-sodium is not extensively bound to plasma or protein.

2. Distribution of Radioactivity in Tissues

The distribution of total radioactivity in the individual tissues at 0.25 or 0.5, 4, 16, and 96 hours from the four dosage

TABLE 3

PHARMACOKINETIC PARAMETERS OF ACIFLUORFEN-SODIUM (MC 10978) AFTER INTRAVENOUS ADMINISTRATION IN MALE AND FEMALE FISCHER 344 RATS

Parameter[a]	Male	Female
Body Weight (gm)	225.5	169.8
Dose (mg)	2.51	1.92
A (mcg/ml)	10.59	31.92
B (mcg/ml)	27.9	14.18
$\alpha(hr^{-1})$	2.78	0.576
$\beta(hr^{-1})$	0.078	0.188
$t_{1/2}\ \alpha$ (hr)	0.25	1.20
$t_{1/2}\ \beta$ (hr)	8.88	3.68
K_{10} (hr^{-1})	0.107	0.352
K_{12} (hr^{-1})	0.714	0.105
K_{21} (hr^{-1})	2.035	0.307
AUC (% Dose-hr)	188.2	74.0
Cl_B (ml/hr)	6.73	12.76
V_d (ml)	81.5	65.9
Cl_{bile} (ml/hr)[b]	1.83	3.48

[a] Pharmacokinetic parameters were calculated from mean blood concentration values for groups of female (n=4) and male (n=5) Fischer rats. Blood data were fitted to an open two-compartment model either by the NONLIN computer program or by the method of feathering.

[b] Cl_{bile}, biliary clearance, was calculated by dividing the amount of unchanged acifluorfen in the bile in 24 hr by the corresponding AUC (0-24 hr). Two rats of each sex were used in the bile cannulation experiment.

regimens in male and female animals are compared. In orally dosed animals at 0.25 or 0.5 hours, the majority of the radioactivity was found in the stomach. At 4 hours, significant levels of radioactivity were present in the gastrointestinal tract (i.e., stomach, small and large intestines), liver, and kidney. The data in Table 4 are the percentages of the total radioactive dose found in various tissues 96 hours after administration of each of the four dosage regimens. At progressively longer sacrifice times, the radioactivity within the alimentary tract appears to be located further along the intestinal tract. In general, the radioactivity present in liver, kidney, and gastrointestinal tract diminishes with time such that, 96 hours after administration, very little radioactivity is detectable in these three tissues. At 96 hours, the percent of radioactivity remaining in body tissues in female rats and in male rats was low (e.g., 0.99% of total radioactivity in females versus 3.05% of total radioactivity in males after single low oral dose).

TABLE 4

DISTRIBUTION OF RADIOACTIVITY IN TISSUES OF FISCHER 344 RATS AT 96 HOURS FOR VARIOUS DOSAGE REGIMENS OF ACIFLUORFEN-SODIUM[a]

	Percent of Total Radioactive Dose[b]							
	Low Oral		High Oral		Multple Oral		Intravenous	
Tissue	Male	Female	Male	Female	Male	Female	Male	Female
Brain	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	0.01	0.00
Kidney	0.12	0.02	0.02	0.02	0.11	0.01	0.07	0.01
Bone	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Liver	0.31	0.08	0.14	1.08	0.29	0.26	0.18	0.03
Muscle	0.02	0.03	<0.01	<0.01	0.03	0.01	0.00	0.00
Fat	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.00	0.00
Gonads	0.03	<0.01	0.01	0.01	0.04	<0.01	0.01	<0.01
Large Intestine	.1.63	0.70	1.26	1.02	1.40	0.21	1.30	0.06
Small Intestine	0.80	0.10	0.46	0.33	0.96	0.06	0.28	0.03
Stomach	0.09	0.03	0.01	0.01	0.13	0.08	<0.01	0.00
Bladder	<0.01	<0.01	<0.01	<0.01	0.06	<0.01	<0.01	0.00
Spleen	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.00
Heart	0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.00
Residual Carcass	0.04	0.01	0.02	0.02	0.04	<0.01	0.02	<0.01
Total	3.05	0.99	1.92	2.49	3.08	0.63	1.87	0.13

[a]The data on earlier sacrifice times (0.25 or 0.5, 4 and 16 hours) are not included in this table.
[b]In males, values are the mean of 5 rats per each dosage regimen. In females, values are the mean of n=5 rats for single low and multple low, n=6 for single high oral and n=4 for intravenous administration.

C. Biotransformation (Isolation and Identification of Acifluorfen and Its Metabolites in Various Matrices)

1. Urine

Urine samples were pooled from all male or female rats administered low or high oral doses of acifluorfen-sodium. When these samples were extracted with acid and base, most of the total urinary radioactivity was found in the acidic fraction. Based on TLC, HPLC, and GC-MS analyses, unchanged acifluorfen (95% of the total urine radioactivity) and the amine derivative (1-3% of the urinary radioactivity), [4-[2-chloro-4-(trifluoro-methyl)phenoxy-2-aminobenzoic acid] (MC 14621), were identified in urine. The mass spectrum of the methyl ester of amine metabolite shows the characteristic doublet ion clusters (6 mass units difference) at m/e's 345 and 351 (M^+) and at m/e's 313 and 319 ($M+1-OCH_3$) indicating that the observed amine metabolite originates from the ^{12}C - ^{13}C (1:1 ratio) acifluorfen-sodium. The amount of glucuronide conjugate is less than 5% in urine since greater than 95% of the radioactivity in urine can be recovered by ion-pair extraction which removes the non-conjugated portion.

2. Feces

TLC, HPLC, and GC-MS analyses revealed that the major fecal component was the amine metabolite (approximately 60-80% of the total fecal radioactivity). The parent compound and the acetamide

metabolite, N-acetyl 5[2-chloro-4-(trifluormethyl)phenoxy]-2-aminobenzoic acid, were minor components found in the feces.

Two unknown fecal metabolites (each less than 5% of total fecal radioactivity) were observed. Based on the HPLC retention times of available standards, the following compounds were ruled out as possible structures for the two unidentified metabolites: MC 10074 (descarboxy acifluorfen), MC 15412 (descarboxy amine), MC 10879 (desnitro acifluorfen) and AGRT 1136. The glucuronide conjugate comprises at most 3% of the total radiolabelled materials in feces based on the β-glucuronidase hydrolysis.

3. Blood, Bile, Kidney and Liver

HPLC analysis of blood samples obtained from rats after administration (0-6 hours) of various dose regimens of acifluorfen-sodium showed that the major component (95-98%) present in blood was the unchanged parent compound. The amine metabolite was also found in some blood samples (less than 10-15% of the total blood radioactivity) at longer times after dosing (16 or 24 hours).

The principal component identified in bile was the unchanged parent compound (more than 93% of total bile radioactivity). Small amounts (less than 2-3% of total bile radioactivity) were also detected as amine metabolite.

Table 5 shows the amount of acifluorfen and its major amine metabolite found in renal and hepatic tissues of animals 4-hours

TABLE 5

CONCENTRATION OF ACIFLUORFEN AND ITS AMINE METABOLITE IN LIVER AND KIDNEY TISSUES OBTAINED FROM MALE AND FEMALE RATS (SACRIFICED AT 4 HOURS) FOR VARIOUS DOSAGE REGIMENS

Dosage Regimen and Route	Sex	Percent of Total Radioactive Dose					
		Liver			Kidney		
		Acifluorfen	Amine Metabolite	Total Residue	Acifluorfen	Amine Metabolite	Total Residue
Single low oral	M	0.64	4.70	11.31	1.30	0.09	1.93
Single high oral	M	3.83	0.44	7.34	0.80	0.07	1.08
Multiple low oral	M	0.38	8.15	12.13	0.79	0.39	1.49
Single intravenous	M	0.80	5.69	14.26	0.89	0.13	1.65
Single low oral	F	4.34	0.66	8.53	1.03	0.06	2.27
Single high oral	F	3.13	1.69	7.31	1.23	0.08	1.59
Multiple low oral	F	2.08	0.87	4.54	0.69	0.06	1.06
Single intravenous	F	1.46	2.69	7.52	0.99	0.09	1.77

after the administration of four different dosage regimens. In the kidneys, the amount of acifluorfen and its amine metabolite ranged from 0.7 to 1.3% and 0.06 to 0.4% of the dose, respectively. In the liver, acifluorfen and its amine metabolite ranged from 0.4 to 4.3% and 0.4 to 8.2% of the dose, respectively.

4. Metabolic Pathways of Acifluorfen-Sodium

Table 6 summarizes the quantitative results obtained from analyses of male and female rat urine and fecal samples for the four dosage regimens using the HPLC radiometric assay. A scheme summarizing the metabolic pathway of acifluorfen-sodium in rats is shown in Figure 3. In general, acifluorfen-sodium is primarily excreted unchanged in the urine. The parent compound does not appear to undergo conjugation to any significant extent with glucuronic acid. The major fecal metabolite was the amine derivative arising from the nitroreduction of the parent compound. The findings that a significant portion (28-29% of the total dose in 24 hours) is excreted in the bile and that the amine metabolite is the major component in feces suggest that gut microflora play an important role in the reductive metabolism of acifluorfen in rats. The minor fecal metabolite was the acetamide derivative arising from N-acetylation of the amine metabolite.

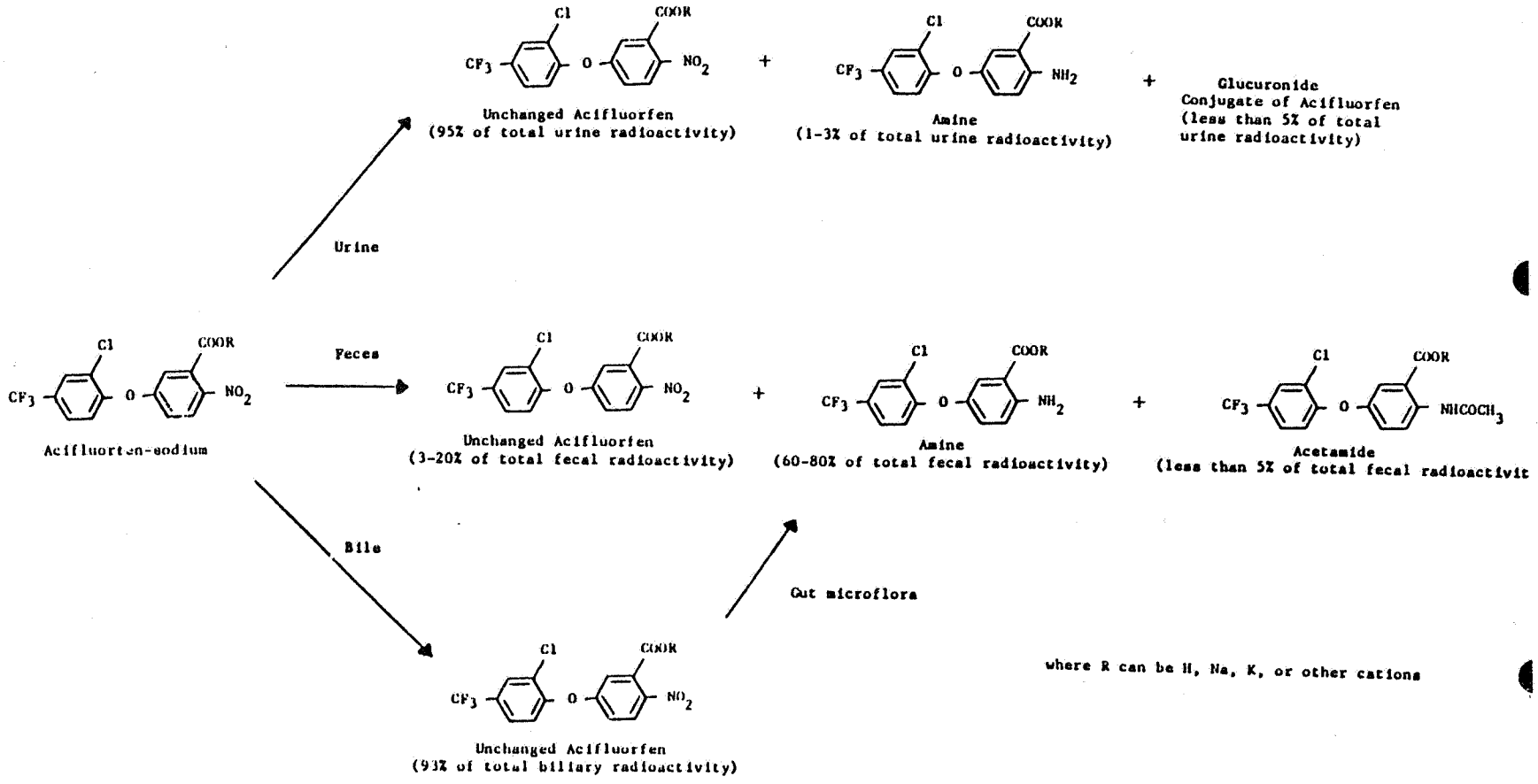


FIGURE 3

THE METABOLISM AND DISPOSITION OF ACILFUORFEN-SODIUM IN FISCHER 344 RATS

TABLE 6

URINARY AND FECAL EXCRETION OF ACIFLUORFEN AND ITS METABOLITES IN MALE AND FEMALE FISCHER RATS AFTER VARIOUS DOSAGE REGIMENS

Dosage Regimen[a]	Route	Sex	Percent of Total Urinary Radioactivity[b]		Percent of Total Fecal Radioactivity[b]				
			Acifluorfen	Amine	Acifluorfen	Amine	Acetamide	#1 Unknown	#2 Unknown
Single low	oral	M	98.3	0.1	5.7	59.0	3.5	4.6	3.7
Single high	oral	M	97.4	0.9	12.5	64.0	1.6	4.5	3.0
Multiple low	oral	M	94.7	0.9	12.3[c]	69.9[c]	4.3[c,d]	2.6	3.7[c]
Single low	intra-venous	M	93.3	0.6	3.8	82.7	0.9	2.2	2.9
Single low	oral	F	98.3	0.8	13.6	58.5	5.0[d]	5.1	3.1
Single high	oral	F	97.3	1.6	6.1	71.2	3.5	2.8	2.4
Multiple low	oral	F	97.5	1.3	18.4	62.7	1.0	3.4	5.4
Single low	intra-venous	F	97.2	1.0	12.2	66.3	3.3	3.9	4.2

[a]See Table 3 for dose (mg/kg) for each regimen.
[b]Values represent the mean of 4-5 animals in most instances.
[c]Only two animals were used in this dosage regimen because not enough fecal material was available for extraction and quantitation.
[d]Acetamide was detectable only in the feces of one animal in this dosage regimen group.

D. Excretion

Urinary and fecal recoveries of radioactivity after administration of each of the four dosage regimens in both female and male rats are listed in Table 7. In female rats, most of the radioactivity is recovered in the urine (60-82%) for all four dose regimens within 96 hours after the administration. The remaining 5-23% is recovered in the feces. In contrast, male rats excreted 46-58% of the dose in the urine and approximately 21-41% in the feces during the 96 hours after the administration.

1. Biliary Excretion Studies

Bile was collected for 24 hours after intravenous administration of a low dose of acifluorfen-sodium. Within this 24-hour period, females and males excreted 28% and 29% of the radioactive dose, respectively. In females, entereohepatic circulation of acifluorfen must occur to a major extent in order to account for the relatively small amounts of radioactivity (5% of the dose) were recovered in the feces of non-cannulated intravenously dosed rats. In comparsion, very little entereohepatic circulation appears to take place in males since large amounts of radioactivity (35% of the dose) were recovered in the feces of non-cannulated intravenously dosed male rats. The cause of this sex difference in the enterohepatic circulation of acifluorfen was not explored by the authors.

TABLE 7

EXCRETION OF RADIOACTIVITY IN URINE, FECES, AND TISSUES IN MALE AND FEMALE RATS AT VARIOUS DOSAGE REGIMENS

Dose Level	Route	Sex	Dose (mg/kg)	% of Dose Recovered[a]			
				Urine	Feces	Tissues	Total
Single low	oral	M	16.8	50.3 ± 3.5[b]	20.8 ± 8.3[b]	3.1	74.2
Single high	oral	M	115.8	47.8 ± 6.3	40.7 ± 13.2	1.9	90.4
Multiple low	oral	M	11.3	58.0 ± 7.4	29.2 ± 4.7	3.1	90.3
Single low	intravenous	M	13.8	46.5 ± 6.6	36.0 ± 11.6	1.9	84.4
Single low	oral	F	17.9	82.3 ± 21.2	9.2 ± 7.3	1.0	92.5
Single high	oral	F	116.8	59.8 ± 3.1	22.6 ± 4.6	2.5	84.9
Multiple low	oral	F	10.7	68.8 ± 22.0	11.6 ± 5.7	0.6	81.0
Single low	intravenous	F	8.3	65.6 ± 19.5	5.2 ± 0.6	0.1	70.9

[a]Recoveries were based on the total radioactivity administered; urine and feces were collected over a 96 hour interval.
[b]Values represent the mean (±S.D.).

2. Elimination Half-Lives, Total Body Clearance, and Volume of Distribution

The data on elimination half-life [$t_{1/2}$ (β)], total body clearance (Cl_B) and volume of distribution (Vd) for female and male rats for the four regimens are tabulated in Table 8. The elimination half-life and total body clearance values for female and male rats were statistically different for every dosage regimen except for the high oral dose. For the intravenous, single low oral and multiple low oral dosage regimens, the elimination half-life was 8.34, 8.25, and 9.22 hr, respectively, for male rats and 3.67, 3.43, and 4.03 hr, respectively, for female rats. It appears that acifluorfen-sodium is eliminated from female rats approximately twice as fast as from male rats based on these half-lives. Also, the radioactivity is eliminated twice as fast in female animals compared to male animals based on the total body clearance values (12.76 to 18.31 ml/hr for female rats versus 5.27 to 7.37 ml/hr for male rats, Table 8).

In the single high oral dosage regimen, female rats eliminated acifluorfen-sodium at a much slower rate in comparison to the other three dosage regimens as indicated by the elimination half-life of 5.93 hr and a total body clearance of 4.14 ml/hr (Table 8). In contrast to female rats, male rats did not display any dose-dependent differences in elimination of acifluorfen-sodium after oral administration of a high dose. Although the elimination

TABLE 8

COMPARISON OF PHARMACOKINETIC PARAMETERS AMONG THE VARIOUS ROUTES OF ADMINISTRATION OF ACIFLUORFEN-SODIUM AND BETWEEN MALE AND FEMALE RATS

Dosage Regimen	Sex (p-value)	Elimination $t_{1/2}$ (β) (hr)	AUC[b] (% Dose-hr)	Total Body Clearance (ml/hr)	Volume of Distribution (ml)
Single intravenous	M F p-value	8.34 3.67 <0.01*	188.2 74.1 <0.01*	6.73 12.76 <0.01*	81.5 65.9 <0.5[ns]
Single low oral	M F p-value	8.25 3.43 <0.01*	151.0 71.5 <0.01*	5.27 13.79 <0.01*	61.2 67.9 >0.5[ns]
Single high oral	M F p-value	6.49 5.93 >0.05[ns]	139.4 195.3 <0.1[ns]	5.81 4.14 <0.1[ns]	53.3 33.3 <0.01*
Multiple low oral	M F p-value	9.22 4.03 <0.001*	178.1 44.5 <0.001*	7.37 18.31 <0.001*	97.4 106.2 >0.5[ns]

[a]Statistically significant if p-value <0.01, denoted by * (asterisk). Not statistically significant if p-value >0.05, denoted by ns. The authors included p-values of >0.5, <0.5, and <0.1.
[b]AUC = Area under the blood concentration-time curve.

half-life was 6.49 hr for high orally dosed male rats, it was not statistically different from the half-life of 8.34 hr for intravenously dosed animals (Table 9). The total body clearance for acifluorfen-sodium after administration of a high oral and an intravenous dose was not statistically different [5.91 ml/hr (high oral) versus 6.73 ml/hr (iv)] (Table 9).

The following major and minor deficiencies were noted in this report.

A. Major Deficiency

1. Overall recovery of radioactivity in urine, feces, and tissues ranging from 70.9 to 92.5% of the dose is considered very poor. As much as 29.1% (100-70.9% = 29.1%) of the dose was not accounted for by the authors. This loss is probably due to poor technique. The possibility of loss as volatile materials (such as CO_2) in exhaled air is not likely due to the structural stability of acifluorfen.

B. Minor Deficiencies

1. The excretion study should have been conducted for 7 days or until 95% of the administered dose was excreted (whichever occurs first). This study was only conducted for 4 days or until only 70.9 to 92.5% of the administered dose was excreted.

TABLE 9

COMPARISON OF PHARMACOKINETIC PARAMETERS BY THE THREE ORAL ROUTES OF ADMINISTRATION WITH THE INTRAVENOUS ROUTE IN MALE AND FEMALE RATS

Dosage Regimen	Sex	Elimination $t_{1/2}$ (β) (hr)	AUC (% Dose-hr)	Total Body Clearance (ml/hr)	Volume of Distribution (ml)
Single intravenous	M	8.34	188.2	6.73	81.5
Single low oral	M	8.25	151.0	5.27	61.2
Single high oral	M	6.49	139.4	5.81	53.3
Multiple low oral	M	9.22	178.1	7.37	97.4
Single intravenous	F	3.67	74.1	12.76	65.9
Single low oral	F	3.42	71.5	13.79	67.9
Single high oral	F	5.93*	195.3**	4.14***	33.3***
Multiple low oral	F	4.03	44.5**	18.31*	106.2*

Student t test (two-sided) used for statistical analysis (oral routes compared to intravenous route for test of significance: Significant at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

2. The exact amounts of glucuronide conjugate of acifluorfen-sodium present in urine or feces samples are not conclusive since the efficiency of the enzymatic hydrolysis system employed for this study was not stated by the authors.

3. In the bile cannulation study, the authors did not specify (a) post-operative recovery period and (b) time of administration of test substance.

4. The high dose level should be chosen such that toxic signs occur. However, data on the observation of animals after the administration of the high dose level were not available in this report. Therefore, it is not certain if the high dose level was selected properly.

10. Technical Review Time: 72 hours.

TACKLE

Study Type: 90-Day Feeding - Rats.

Accession Number: ~~071308~~ 071308

MRID Number: None assigned.

Sponsor: Rhone-Poulenc, Inc., Monmouth Junction, NJ.

Contracting Laboratory: Gulf South Research Institute, New Iberia, LA.
GSRI Project No. 413-971-40; Rhone-Poulenc No. 372-80.
DATE: JUNE, 1982
Test Material: Tackle 2S (Acifluorfen Sodium; MC 10109) Purity: 20.4-23.6 percent.

Concentration in Feed: Analysis of samples of prepared diets obtained eleven times during the study indicated that the actual dose levels were close to the desired levels.

Stability: Assays indicated that the aqueous solution of the sodium salt of Tackle (Tackle 2S) is stable for 4 months as 50°C, pH 7, 8 and 9, and at 37°C, pH 8.

Stability in Feed: Not available.

PROTOCOL

Procedure: Five hundred and twenty-nine 6-week old Fisher 344 rats obtained from the Charles River Breeding Labs were acclimated for two weeks prior to commencement of the study. From these animals, 30 males (weighing approximately 130 grams) and 30 females (weighing approximately 100 grams) were randomly selected for each test group. Tackle 2S was given in the diet at concentrations of 0, 20, 80, 320, 1,250, 2,500, and 5,000 ppm for 90 days.

The diets were prepared by mixing aqueous Tackle 2S solution with acetone and blending with basic rodent diet. "The test diets were prepared weekly, approximately one week in advance to allow formulation analyses prior to feeding the animals." However, the latter "3 mixes were approximately two weeks apart." Diets and city tapwater were available ad libitum. The basic diet for the study was NIH-07 open formula which was prepared and analyzed by Zeigler Brothers.

The rats were individually housed in hanging wire cages in a temperature- (74°F ± 2°F), humidity- (40-70 percent), and light- (12-hour light, 12-hour dark) controlled room. "No other species or test material were under concurrent investigation in this animal room."

General Observations: Animals were examined twice daily for morbundity and mortality; once weekly for signs of toxicity.

Body Weights and Food Consumption: Individual body weights and food consumption were recorded weekly.

Hematology: At interim sacrifice (30 days) and final sacrifice (90 days) blood was taken from 10 animals/sex/dose. Hematological determinations were: hematocrit, hemoglobin, erythrocyte count, mean corpuscular volume, total and differential leukocytes counts, platelet count, and reticulocyte count.

Clinical Chemistry: At interim sacrifice (30 days) and final sacrifice serum or plasma was taken from 10 animals/sex/dose. Clinical chemistry determinations were: serum lactate dehydrogenase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and alkaline phosphatase activities, calcium, albumin, creatinine phosphokinase, creatimine, uric acid, chloride, potassium, glucose, blood urea nitrogen, direct and indirect bilirubin, total bilirubin, total cholesterol, globulin, triglyceride, total protein, sodium, and inorganic phosphorous.

Urinalysis: At interim sacrifice (30 days) and final sacrifice (90 days) urine was examined from 10 animals/sex/dose. The parameters measured in the urinalysis were: specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, nitrite, hemoglobin, and cells and formed elements by microscopic examination of sediment.

Organ Weights: Liver, kidneys, heart, testes, and brain were weighed from animals sacrificed at interim and termination.

Gross pathology: Animals sacrificed (30 and 90 days), or sacrificed moribund, or dying during treatment were examined for gross pathologic changes.

Histopathology: Histopathology was performed by Mobil Oil Corporation pathologists (Choudari Kommineni, D.V.M., Ph.D. and Peter H. Craig, V.M.D.) on the following tissues/organs for the control and 5,000 ppm dose (HDT) groups:

Eyes with Harderian gland	Testes*	Salivary glands
Heart*	Ovaries	Thymus
Thyroid with parathyroid	Spleen	Lungs with bronchi
Trachea	Skin	Coronal sections of head
Esophagus	Sciatic nerve	Brain
Stomach	Mammary gland	Intestines (large and small)
Adrenal Glands	Gross lesions*	Pancreas
Liver*	Bone	Skeletal muscle
Kidneys*	Spinal cord	Urinary bladder
	Lymph node	Corpus and cervix uteri

Those tissues indicated by asterisk (*) were examined for animals in the 20-2,500 ppm groups. In addition, tissues with lesions and all tissues from the high-dose animals were to be examined.

Statistics: Quantitative data (e.g., body weight, food consumption, hematologic parameters) were analyzed using analysis of variance and Duncan's Multiple Range Test. Differences were considered significant when p was less than 0.05.

General Observations: No deaths were reported during the study.

The authors reported "the most frequent clinical sign observed during the testing period was hair loss on the dorsal areas of the rats...similarly distributed between control and treated groups...summarized in Appendix D." However, in Appendix D of the submitted report (page 98-102) "loss of hair" was never mentioned. The most frequent observations were "bad eye" and "red eye lids."

Body Weights and Food Consumption: Mean body weight data for male rats showed a statistically significant decrease in weight for the 2,500 and 5,000 ppm groups when compared to their appropriate controls. Statistically significant decreases in mean body weights were observed in the 5,000 ppm females when compared to control females. These data are presented in the following table.

TABLE 1. Mean Body Weights (Grams)

Group (ppm)	Weeks 0 M	0 F	1-4 M	1-4 F	5-10 M	5-10 F	11-13 M	11-13 F
0	133.0	101.4	203.6	136.7	298.6	172.3	331.3	182.3
2,500	129.7	101.7	194.6*	132.1	275.7*	166.3	314.3*	184.3
5,000	132.2	98.7	137.0*	114.5*	195.0*	151.2*	235.8*	165.1*

* $p<0.05$ by Duncan's multiple range test performed by the authors of study.

Mean food consumption for the 5,000-ppm males and females was decreased throughout most of the study. Food consumption for these animals was 5-14 grams/day less than control animals.

Hematology: The following significant changes were observed in the hematological parameters (see Table 2). The other parameters showed no time or dose-related alterations.

TABLE 2. Selected Mean Hematology Values

Sex/ Group (ppm)	Erythrocyte[a] 30 Days	Erythrocyte[a] 90 Days	Hemoglobin[b] 30 Days	Hemoglobin[b] 90 Days	Hematocrit[c] 30 Days	Hematocrit[c] 90 Days
Males						
0	8.56	8.69	16.5	16.5	44.9	44.0
320	8.47	8.19	16.7	16.4	45.9	41.1
1,250	8.47	7.74[d]	16.4	16.0	45.3	39.3[d]
2,500	8.43	7.87[d]	16.3	15.4[d]	45.0	40.1[d]
5,000	7.85[d]	7.71[d]	15.2[d]	14.4[d]	41.6[d]	38.4[d]
Females						
0	8.21	7.95	16.7	15.9	43.1	42.5
320	8.16	7.74	16.5	16.3	43.9	41.5
1,250	8.07	7.50	16.3	15.9	45.0	40.0
2,500	8.17	7.34[d]	16.1	15.4	43.9	39.2[d]
5,000	7.62[d]	7.56	15.1[d]	15.3[d]	41.2	39.9

[a] Units are $10^6/mm^3$.

[b] Units are g/dl.

[c] Units are calculated percent.

[d] $p<0.05$ as compared to controls by Duncan's multiple range test performed by the authors.

Clinical Chemistry: Treatment related changes were observed in the serum electrolytes levels of calcium and phosphorous primarily in males at the 5,000 ppm dose level as summarized in the following table:

TABLE 3. Mean Calcium and Phosphorus Levels (mg/dl)

Group (ppm)	Calcium				Phosphorus			
	30 Days		90 Days		30 Days		90 Days	
	M	F	M	F	M	F	M	F
0	10.91	9.96	11.08	10.27	8.49	6.88	6.06	6.12
2,500	10.64	10.35	10.67	9.17	8.74	8.06*	7.77*	6.25
5,000	10.15*	9.47	10.01*	10.14	8.37	7.22	7.45*	6.63

*$p<0.05$ as compared to controls using Duncan's multiple range test.

Liver function enzymes, serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (AP) displayed increased activity primarily in males at the 2,500 and 5,000 ppm dose levels as presented in the following table:

TABLE 4. Mean SGPT and AP Levels (IU/l)

Group (ppm)	SGPT				AP			
	30 Days		90 Days		30 Days		90 Days	
	M	F	M	F	M	F	M	F
0	38.10	32.63	64.60[b]	44.90	244.40	189.26	128.00	133.30
2,500	47.30[a]	51.60[a]	39.50	38.40	221.00[a]	187.80	132.44	103.30
5,000	71.40[a]	33.44	101.30	39.70	314.70[a]	178.67	168.67[a]	110.90[a]

[a] $p<0.05$ compared to controls using Duncan's new multiple range test.

[b] "Statistical analyses not performed due to heterogenity o. variances."

Some indications of reduced renal function were evident in males and females at the 2,500 ppm and 5,000 ppm doses. Blood urea nitrogen and creatinine levels were significantly increased as seen in the following table:

TABLE 5. Mean Blood Urea Nitrogen and Creatinine Levels (mg/dl)

Group (ppm)	Blood Urea Nitrogen				Creatinine			
	30 Days		90 Days		30 Days		90 Days	
	M	F	M	F	M	F	M	F
0	16.70	14.63	16.70	15.60	0.52	0.43	0.57	0.51
2,500	20.00*	18.10*	19.00*	14.00	0.52	0.53*	0.57	0.56
5,000	21.50*	18.67*	18.30	15.60	0.56	0.54*	0.61	0.59

[a] $p<0.05$ compared to controls using Duncan's multiple range test.

Statistically significant decreases in total serum protein, albumin, and globulin levels were observed primarily in males at the 5,000 ppm doses (Table 6).

All other clinical chemistry parameters listed in the Protocol Section were similar to control values, or, if significant differences were noted, were not considered biologically relevant.

Urinalysis: Two of the urinalysis parameters showed possible treatment-related alterations. Slight increases in the excretion of urobilinogen were noted in the high-dose male rats at 30 and 90 days, in the high-dose female rats at 90 days, and in the rats of both sexes in the second highest dose group at 90 days. Nitrite levels in urine of the two high dose female groups were slightly higher than controls at 90 days. In addition, protein excretion was slightly lower in both sexes of the 5,000-ppm group at both time points. No differences between control and dosed groups were noted in the appearance of the urine, microscopic elements, glucose, ketone, bilirubin, and hemoglobin levels. Due to the qualitative nature of the urinalysis data, no statistical comparisons were performed.

Organ Weights: Statistically significant increases in absolute and relative (to body weights) liver and kidney weights were observed at 1,250, 2,500 and 5,000 ppm doses as presented in the Table 7.

Gross Pathology: Interim Sacrifice: Discoloration was observed in the liver (9/10 males, 3/10 females) and the kidneys (9/10 males, 6/10 females) of the 5000 ppm dose animals.

Terminal Sacrifice: Discoloration was observed in the liver (19/20 males, 2/20 females) and the kidneys (19/20 males, 11/20 females) of the 5000 ppm dose animals.

TABLE 6. Mean Total Serum Protein, Albumin, and Globulin Levels (g/dl)

Group (ppm)	DAY	Total Protein				Albumin				Globulin			
		30		90		30		90		30		90	
		M	F	M	F	M	F	M	F	M	F	M	F
0		5.99	5.71	6.46	6.04	3.56	3.48	3.68	3.50	2.43	2.23	2.43	2.34
2,500		6.21	5.64	6.36	5.53	3.84	3.56	3.89*	3.31	2.37	2.08	2.37	2.24
5,000		5.26	5.18*	5.58*	5.68	3.44*	3.40	3.44*	3.58	1.82*	1.78	1.82*	2.10

*$p<0.05$ as compared to controls.

TABLE 7. Mean Absolute and Relative Liver and Kidney Weights

Sex and Group ppm	Liver				Kidney			
	Absolute (g)		Relative (g/100 g Body Weight)		Absolute (g)		Relative (g/100 g) Body Weight)	
	30 Day	90 Day	30 Day	90 Day	30 Day	90 Day	30 Day	90 Day
Male								
0	7.25	9.38	3.053	2.804	1.74	2.14	0.734	0.639
20	6.47*	9.74	2.921	2.932	1.69	2.20	0.762	0.660
80	6.68*	10.04	3.006	2.962	1.65	2.20	0.743	0.648
320	7.94	9.84	3.168	2.841	1.76	2.27*	0.746	0.654
1,250	8.68*	11.38*	3.576*	3.427*	1.97*	2.38*	0.811*	0.716*
2,500	9.51*	12.89*	4.274*	4.070*	1.77	2.38*	0.796	0.750*
5,000	8.40*	9.78	5.307*	3.971*	1.39	1.97*	0.879*	0.805*
Female								
0	4.24	4.85	2.767	2.633	1.12	1.24	0.724	0.672
20	3.96	4.81	2.860	2.639	1.08	1.21	0.776	0.662
80	4.22	4.75	2.909	2.634	1.09	1.26	0.752	0.702
320	4.19	4.99	2.987*	2.561	1.08	1.25	0.769	0.645
1,250	4.32	5.31	2.840	2.825	1.15	1.21	0.755	0.642
2,500	4.70*	5.29	3.214*	2.874	1.21	1.23	0.827*	0.695
5,000	5.22*	5.45*	4.154*	3.277*	1.03	1.18	0.820*	0.708*

*$p<0.05$ as compared to control using Duncan's multiple range test.

Histopathology: Histopathology of individual animal tissues was not included in this report. Summary tables presented in the text demonstrated treatment-related effects in liver of both sexes at 1,250, 2,500, and 5,000 ppm doses. Table 8 presents these data. Other tissues had no apparent treatment-related pathology.

TABLE 8. Summary of Histopathology Data

Interval/Organ/Lesion	Males/Group (ppm)							Females/Group (ppm)						
	0	20	80	320	1,250	2,500	5,000	0	20	80	320	1,250	2,500	5,000
Interim Sacrifice														
Liver														
Hypertrophy of liver cells	0/10	0/10	0/10	0/10	0/10	6/10	10/10	0/10	0/10	0/10	0/10	0/10	1/9	10/10
Mitotic Activity	0/10	0/10	0/10	0/10	0/10	0/10	4/10	0/10	0/10	0/10	0/10	0/10	0/9	8/10
Individual cell death	0/10	0/10	0/10	0/10	0/10	4/10	7/10	0/10	0/10	0/10	0/10	0/10	0/9	8/10
Proliferation of oval or bile duct cells	0/10	0/10	0/10	1/10	2/20	1/10	0/10	0/10	0/10	0/10	0/10	0/10	5/9	0/10
Final Sacrifice														
Liver														
Hypertrophy of liver cells	0/20	0/20	0/19	0/20	13/20	17/20	20/20	0/20	0/20	0/20	0/20	4/18	9/20	8/20
Mitotic Activity	--[a]	--	--	--	--	--	--	0/20	0/20	0/20	0/20	0/18	3/20	0/20
Individual cell death	6/20	0/20	0/19	0/20	0/20	5/20	20/20	0/20	0/20	0/20	0/20	2/18	1/20	0/20
Proliferation of oval or bile duct cells	0/20	0/20	1/19	2/20	0/20	2/20	17/20	6/20	1/20	1/20	7/20	3/18	2/10	2/20

[a]This lesion was not listed for the males at final sacrifice.

CONCLUSIONS

Changes in body weights, hematology (erythrocyte count and hemoglobin concentration), clinical chemistry (serum electrolytes, calcium and phosphorous; SGPT and alkaline phosphatase activities; BUN, creatinine, total serum protein, albumin, and globulin concentrations), urine (nitrate and urobilinogen content), liver and kidney weights (absolute and relative), and histopathologic changes in the liver (increased cellular hypertrophy, cell death, mitotic activity, and proliferation of oval or bile duct cells) were noted at the 2,500 and 5,000 ppm (HDT) dose levels.

Changes in hematology (erythrocytes count and hematocrit), liver and kidney weights (absolute and relative), and histopathological changes in the liver (cell hypertrophy) were observed in one or both sexes at the 1,250 ppm dose. Based on these parameters, the following NOEL and LEL were identified: NOEL—320 ppm; LEL—1,250 ppm.

Classification: Supplementary. (The registrant is requested to submit the following: a) Individual animal histopathology on the animals of the 20–2,500 ppm groups; b) An explanation of why in the author's report the least frequent clinical observation was "loss of hair" while the submitted daily record of clinical signs did not mention this event [pg. 98–102 of report].)

TACKLE

Study Type: 90-Day Feeding - Mice.

Accession Number: 071308.

MRID Number: Not assigned.

Sponsor: Rhone-Poulenc, Inc., Monmouth Junction, NJ.

Contracting Laboratory: Gulf Southern Research Institute, GSRI Project No. 513-971-40, Rhone-Poulenc No. 374-80.

Date: July 16, 1982, to sponsor.

Test Material: Tackle 2S (Acifluorfen Sodium; MC10109).

Purity: 20.4-23.2 percent.

Concentration in Feed: The compound concentrations were determined in the eleven samples taken from each dose level and the mean concentrations were close to the desired levels throughout the samplings. Assay results for control feeds were not reported.

Stability: Assays indicated that the aqueous solution of the sodium salt of Tackle (Tackle 2S) is stable for 4 months at 50°C, pH 7, 8, and 9, and at 37°C, pH 8.

Stability in Feed: Not available in report.

PROTOCOL

Procedure: Four hundred and ninety-one 6 week old B6C3F1 mice from the Charles River Breeding Labs were acclimated for 2 weeks prior to commencement of the study. The animals, 30 males (weighing approximately 22 grams) and females (weighing approximately 18 grams), were randomly selected for each test group. Tackle 2S was given in the diet at concentrations of 0, 20, 80, 320, 1,250, 2,500 and 5,000 ppm for 90 days.

The diets were prepared by mixing an aqueous Tackle 2S solution with acetone and blending with basic rodent diet. The test diets were prepared weekly, approximately one week in advance to allow formulation analyses prior to feeding the animals. However, the "latter 3 mixes were approximately 2 weeks apart." Diets and city tapwater were available ad libitum. The basal diet for the study was NIH-07 open formula which was prepared and analyzed by Zeigler Brothers.

The mice were housed 5 (per sex) in polycarbonate cage in a temperature-(74°F), humidity-(40-70 percent) and light-(12-hour light, 12 hour dark) controlled room. "No other species or test material were under concurrent investigation in this animal room."

General Observations: Animals were examined twice daily for moribundity and mortality; once weekly for signs of toxicity.

Body weight and food consumption: Body weights and food consumption were recorded weekly.

Hematology: At 30 days and termination of treatment, blood was taken from 10 animals/sex/dose. Hematological determinations included: hematocrit, hemoglobin, erythocyte count, mean corpuscular volume, total and differential leukocyte counts, platelet counts and reticulocyte counts.

Clinical Chemistry: At 30 days and termination of treatment, samples of serum or plasma were taken from 10 animals/sex/dosage. Derminations included: serum glutamic pyruvic transaminase, serum alkaline phosphatase, glucose, blood urea nitrogen.

Urinalysis: At 30 days and termination of treatment urine was examined from 10 animals/sex/dose. Urinalysis included: specific gravity, pH, protein, glucose, ketone, bilirubin, urobilinogen, nitrite, hemoglobin, and microscopic examination of sediment.

Organ Weights: The weights of the liver, kidney, heart, testes, and brain were recorded at 30 days and termination.

Pathology: Animals sacrificed after 30 days (10 animals/sex/group) or 90 days of treatment, or sacrificed moribund, or dying during the treatment were examined for gross pathology.

Tissue samples were taken for residue analysis from: liver, kidneys, skeletal muscle, testes, mesenteric adipose tissue, heart, and one-half of the brain.

Histopathology: The histopathologic evaluation was performed by the sponsor (Mobil Oil Corp.) on the following tissues/organs for the control and high-dose groups; tissues indicated by an asterisk were examined for animals in the 20-2,500 ppm groups. In addition, tissues with lesions and all tissues from the high-dose animals were to be examined.

Eyes with Harderian gland	Ovaries	Thymus
Heart*	Spleen	Lungs with bronchi
Thyroid with parathyroid	Skin	Coronal sections of head
Trachea	Sciatic nerve	Brain
Esophagus	Mammary gland	Intestines (Large and small)
Stomach	Gross lesions*	Pancreas
Adrenal glands	Bone	Skeletal muscle
Liver*	Spinal cord	Urinary bladder
Kidneys*	Lymph Node	Corpus and cervix uteri
Testes*	Salivary glands	

Statistics: Quantitative data (e.g., body weight, food consumption, hematologic parameters) were analyzed using analysis of variance and Duncan's Multiple Range Test. Differences were considered significant when p was less than 0.05.

RESULTS

General Observations: No signs of toxicity were observed. The reporting of a "skinny" or "emaciated" appearance was scattered throughout the study with no apparent dose-related trend. An item of interest was the footnote in the observations, "Water mechanical problem resolved." No further elaboration was provided.

Mortality: This reviewer concludes that there were no compound-related mortalities at any dose levels. The distribution of mortality was as follows:

TABLE I. Distribution of Mortality

Group (ppm)	Male	Female
0	0	1(wk 3)
20	1(wk 4)	0
80	4(wk 4)[a] 2(wk 12)	0
320	0	0
1,250	0	1(wk 3)
5,000	2(wk 1) 1(wk 4)[b]	0

[a] A notation in the report for 2 animals that were found dead was "cage flooding by equipment malfunction; death possibly from drowning". Also, 1 animal death was described as a "moribund sacrifice of surviving animal from cage which was flooded."

[b] The notation in the report for 1 animal was "Mouse missing for several days from cage; sacrified when found in dirty corridor."

Body Weights and Food Consumption: Individual body weights were not included in this report. Mean body weight data showed a statistically significant ($p<0.05$ by Duncan's new multiple range test reported by the authors of the study) decrease in weight for the 2,500 and 5,000 ppm male and female dose groups when compared to control animals throughout the study. These data are presented in Table 2.

TABLE 2. Mean Body Weights of Control, 2,500 and 5,000 ppm Groups (grams)

Group (ppm)	Weeks 0 M	0 F	1-4 M	1-4 F	5-10 M	5-10 F	11-13 M	11-13 F	Weight Gain (percent change) M	Weight Gain (percent change) F
0	22.1	18.8	23.3	19.6	27.9	23.3	29.2	25.6	+39	+39
2,500	23.5	18.6	20.0	16.6	22.0	18.8	24.3	20.5	-2	+7
5,000	22.8	18.0	17.7	16.1	19.8	17.1	21.6	19.0	-10	-3

Mean food consumption was similar for all groups throughout the study.

Hematology: No consistent dose or time-related effects were noted in the parameters observed with the exception of a depression in total white blood cell number in both males and females in the 5,000 ppm group. This depression was observed at 30 and 90 days, but was not tested for statistical significance ("Statistical analyses not performed due to heterogeneity of variances"). In addition, the calculated mean corpuscular volume (MCV) was significantly depressed ($p<0.05$ using Duncan's Multiple Range Test performed by the authors of the study) for males and females in the 2,500- and 5,000-ppm groups at 90 days. Reticulocyte counts were statistically increased at the 2,500-ppm females at 30 and 90 days and 5,000-ppm females at 90 days.

Clinical Chemistry: Serum glutamic pyruvic transaminase (SGPT) and serum alkaline phosphatase activity were increased in males and females at the 2,500- and 5,000-ppm doses at 30 and 90 days as compared to control values (Table 3). "Statistical analyses not performed due to heterogeneity of variances." An increase in alkaline phosphatase activity was also observed for both sexes and at both time points for animals of the 1,250-ppm group (Table 3).

Serum glucose was statistically decreased in males and females in 1,250-, 2,500-, and 5,000-ppm groups at 30 and 90 days (Table 3).

Urinalysis: No consistent dose- or time-related effects were observed in the parameters with exception of decreased excretion of protein in males and females at the 320-, 1,250-, 2,500- and 5,000-ppm doses at 30 and 90 days. In addition, urobilinogen levels were increased in males and females in the 5,000-ppm group at 90 days only.

Organ Weights: Statistically significant increases in absolute or relative (to body weight) liver weights were observed in both sexes at 1,250, 2,500, and 5,000 ppm as presented in the Table 4.

TABLE 3. Mean Clinical Chemistry Data[a]

Group (ppm)	SGPT				Alkaline Phosphatase				Glucose			
	30 Days		90 Days		30 Days		90 Days		30 Days		90 Days	
	M	F	M	F	M	F	M	F	M	F	M	F
0	20.98	15.43	21.32	15.98	57.77	80.59	37.38	56.53	257.03	326.80	234.97	271.64
	(7.08)	(2.78)	(2.75)	(6.30)	(18.21)	(15.35)	(4.21)	(16.71)	--	(44.29)	--	--
1,250	31.41	24.76	20.75	12.54	118.56	115.63	118.18	115.35	205.88	260.41	167.28[b]	227.40
	(39.19)	(12.89)	(10.71)	(5.32)	(22.46)	(7.94)	(20.01)	(13.77)	--	(70.14)	--	--
2,500	360.72	171.81	471.98	198.60	229.09	261.66	290.85	144.53	143.28[b]	122.67	118.58[b]	192.65[b]
	(217.40)	(111.66)	(235.07)	(108.57)	(31.37)	(53.76)	(96.16)	(144.06)	--	(32.75)	--	--
5,000	373.47	282.34	383.57	444.20	348.06	310.25	269.37	322.60	136.07[b]	125.86	193.40	163.42[b]
	(125.05)	(84.11)	(129.68)	(153.81)	(46.00)	(37.11)	(55.84)	(48.07)	--	(25.24)	--	--

[a] Units for SGPT and alkaline phosphatase are international units/l; units for glucose are mg/dl; standard deviations are in parentheses. Except for the data for glucose (males at 30 and 90 days, females at 90 days), these data were not statistically analyzed due to "heterogeneity of variances."

[b] $p<0.05$ as compared to controls.

TABLE 4. Absolute and Relative Liver Weights for Males and Females

Sex/ Group (ppm)	Absolute Weight (g) 30 Days	Absolute Weight (g) 90 Days	Relative Weight (g/100 g body weight) 30 Days	Relative Weight (g/100 g body weight) 90 Days
Male				
Control	1.37	1.19	5.06	4.16[a]
20	1.30	1.45[b]	5.53	4.55
80	1.23	1.60[b]	5.20	5.16
320	1.15[b]	1.29	5.23	4.65
1,250	1.44	1.71[b]	6.41[b]	6.24
2,500	1.28	1.84[b]	6.65[b]	8.19
5,000	1.56[b]	1.73[b]	8.30[b]	8.48
Female				
Control	1.05	1.10	4.71[a]	4.61[a]
20	0.78[b]	1.17	3.76	4.61
80	0.93[b]	1.14	4.18	4.65
320	0.93[b]	1.06	4.64	5.04
1,250	1.27[b]	1.66[b]	6.36	7.20
2,500	1.31[b]	1.82[b]	8.33	9.30
5,000	1.34[b]	1.59[b]	8.30	8.77

[a]These data were not statistically analyzed due to "heterogeneity of variances."

[b]$p<0.05$ as compared to controls.

Gross Pathology: Table 5 presents pertinent necropsy findings.

Histopathology: Histopathologic observations on individual animals were not presented in the final report. The summary data indicated treatment-related effects in liver and bone marrow tissue in animals of both sexes at the 1,250-, 2,500-, and 5000-ppm dose levels. A summary of these observations is presented in Table 6.

TABLE 5. Necropsy Findings in Mice Administered Tackle 2S over 90 Days.

Group (ppm)	Males							Females						
	0	20	80	320	1,250	2,500	5,000	0	20	80	320	1,250	2,500	5,000
30 Day Interim														
No. animals sacrified	10	10	10	10	10	10	10	10	10	9	10	10	10	10
Liver- foci or general discoloration	0	0	0	0	0	1	0	0	0	0	0	0	0	1
90 Day Sacrifice														
No. animals sacrified	20	19	12	21	19	20	17	19	20	21	20	18	19	19
Liver- foci or general discoloration	0	0	0	0	2	1	0	0	0	0	0	0	1	1
Stomach- white firm structure	0	0	0	0	2	1	0	0	0	0	0	0	0	0
Unscheduled deaths														
No. animals examined	0	1	8	0	0	0	3	1	0	0	0	2	0	0
Liver- foci or general discoloration	0	0	0	0	0	1	0	0	0	0	0	0	0	1

TABLE 6. Summary of Histopathological Observations in Mice Administered 1250-, 2500-, and 5000-ppm Tackle 2S for 90 Days

Interval/ Tissue/Observation	Incidence[a] Control Male	Control Female	1,250-ppm Male	1,250-ppm Female	2,500-ppm Male	2,500-ppm Female	5,000-ppm Male	5,000-ppm Female
30-Day Interim Sacrifice								
Liver								
Hypertrophy	0/10	0/10	0/10	0/10	5/10	1/10	10/10	3/10
Increased Mitotic Activity	0/10	0/10	0/10	0/10	4/10	4/10	2/10	5/10
Individual Cell Death	0/10	0/10	0/10	0/10	8/10	6/10	10/10	10/10
Oval Cell Proliferation	0/10	0/10	0/10	0/10	4/10	5/10	4/10	8/10
Sternum								
Fatty Infiltration of Marrow	0/10	0/9	—[b]	—[b]	—[b]	—[b]	8/10	9/10
90-Day Final Sacrifice								
Liver								
Hypertrophy	0/20	0/18	0/20	0/18	15/20	10/19	17/17	19/19
Increased Mitotic Activity	0/20	0/18	1/20	0/18	5/20	7/19	17/17	19/19
Individual Cell Death	0/20	0/18	1/20	0/18	16/20	18/19	17/17	19/19
Oval Cell Proliferation	0/20	0/18	0/20	0/18	9/20	12/19	17/17	19/19
Fatty Infiltration	5/20	8/18	17/20	17/18	20/20	16/19	0/17	3/19
Focal Necrosis	1/20	0/18	5/20	2/18	0/20	0/19	0/17	0/19
Sternum								
Fatty Infiltration of Marrow	0/19	0/19	—[b]	—[b]	—[b]	—[b]	16/16	18/18

[a] Number of animals in which observation was made/number of animals examined. These data were taken directly from the final report and could not be substantiated because individual animal histopathology was not reported.

[b] This tissue was not examined in these animals.

CONCLUSIONS

Conclusions: Changes in mean body weights, hematology (total white blood cell numbers, MCV, reticulocyte counts), clinical chemistry (SGPT, SAP, serum glucose), liver weights (absolute and relative), and histopathological changes in the liver (hypertrophy, increased mitotic activity, individual cell death, focal necrosis) were noted in males and females at the 2,500 and 5,000 ppm dose at 30 and 90 days.

Fatty infiltration of the liver was observed in males and females at the 1,250, 2,500 and 5,000 ppm doses at 30 days and at the 1,250 and 2,500 ppm doses at 90 days. The authors stated that "these data suggest that ingestion of high levels of the test article may influence liver metabolism of lipids."

The results reported in this study indicated the following effect and no-effect levels based on histopathologic changes (fatty infiltration) of the liver: NOEL: 320 ppm, LEL: 1,250 ppm.

Classification: Supplementary. (Histopathologic examination was not conducted on bone marrow tissue of lower dose groups although a treatment-related effect was noted in the high-dose animals. In addition, stomach lesions that were noted in a few animals of intermediate dose groups were not examined histopathologically. No explanation was provided for the high mortality in the 80-ppm group (8 from a total of 60 study animals; over half the total mortality was from this group). The registrant is requested to clarify the following: (a) submit individual animal histopathology and (b) explain "water mechanical problems" and "cage flooding by equipment malfunction.")

TACKLE

Study Type: 21-Day Dermal - Rabbits.

Accession Number: 071311.

MRID Number: Not assigned.

Sponsor: Rhone-Poulenc Inc., Monmouth, NJ.

Contracting Laboratory: Food and Drug Research Laboratories, Inc.

Testing Facility: Food and Drug Research Laboratories, Inc. (Lab No. 6718).

Date: February 5, 1981.

Material: Tackle 2S
Purity 21.1 percent

PROTOCOL

Procedure: New Zealand white rabbits, age unspecified, supplied by H.A.R.E., Hewitt, New Jersey, weighing approximately 2.0-4.0 kg, were housed individually in hanging wire mesh cages in a temperature-, humidity-, and light-controlled environment. The animals were given commerical rabbit feed (Charles River Rabbit Chow) and tapwater ad libitum. The animals were acclimated for 10 days prior to the study initiation and were randomly assigned to the study groups.

The back of each animal was clipped free of hair prior to the initiation of the study and weekly thereafter to keep it relatively free of hair. From each test group (10 animals/sex/dose), 5 males and 5 females were further prepared by abrading the test site (sites were abraded weekly thereafter) using a clean hypodermic needle to penetrate the stratum corneum but not cause bleeding.

The test material was mixed in aqueous NaOH and adjusted to a pH of 7.5-7.6 to obtain a solution that was to be applied so as to achieve dose levels of 0, 100, 300, and 1000 mg/kg/day. The control group received

the NaOH solution only. The dose volume for each dose level was as follows:

Table 1. Dose Volume (ml/2.6 kg of Body Weight)

Group (mg/kg/day)	Application Number		
	1-3	4	5-15
0	10	10	5[a]
100	1	1	1
300	3	3	3[b]
1000	10	5[a]	5[c]

[a] Due to overt toxicity in the 1000 mg/kg/day group, the control and high dose volumes were reduced starting on days 4 and 5 of the study, respectively.

[b] Analysis of the dose solutions found these applications equivalent to 342 mg/kg/day.

[c] Analysis of the dose solutions found these applications equivalent to 570 mg/kg/day.

The animals were exposed to test material for a 6-hour period, 5 consecutive days, for 3 weeks. The treated skin was covered with two layers of gauze and an occlusive binder. The wrappings were removed at the end of each daily exposure and the treated areas were wiped with clean gauze.

Clinical Observations: The animals were observed daily for signs of toxicity and twice daily for mortality.

Body Weights and Food Consumption: Individual body weights and food consumption were recorded twice weekly.

Dermal Irritation: The animals were observed for dermal irritation daily, and the observations were quantified using the Draize (1975) method of dermal irritation scoring.

Blood and urine samples were taken from 5 animals randomly selected from each sex and group before treatment and at termination of the study. Animals were fasted 14-16 hours prior to blood drawing. Urine samples were collected during 14-16-hour fasting period.

Hematology: Hematological determinations included total and differential leukocyte counts, erythrocyte count hematocrit, hemoglobin, and platelet count.

Clinical Chemistry: Serum or plasma was assayed for alkaline phosphatase (AP), urea nitrogen, glutamic pyruvate transaminase (GPT), glutamic oxaloacetic transaminase, calcium, potassium, lactic dehydrogenase, glucose, bilirubin (total and direct), total cholesterol, albumin, globulin and total protein.

Urinalysis: Urinalysis included appearance, specific gravity, blood, protein, pH, bilirubin, urobilinogen, ketones, glucose, microscopic examination of sediment (e.g., crystals, bacteria, cells).

Pathology: Tissues and organs of surviving and moribund animals were examined for gross and histopathological changes.

Organ Weights: Brain, heart, kidneys, liver, adrenals, pituitary, thyroid and parathyroid, ovaries, and testes were taken from all animals and weighed.

Tissues Preserved and Examined: Representative sections of the following tissues were examined: untreated skin, treated skin, kidneys, liver, and "grossly abnormal tissue."

Statistics: Analysis of body weight, food consumption, clinical chemistry, and organ weight data were performed using a one-way analysis of variance. Differences between groups were identified using the least significant difference test. Dermal irritation and urinalysis data were analyzed using the Mantel-Haenszel chi-square test. Pathology incidence data were analyzed using a chi-square test with Yates correction for 2x2 contingency tables. Results were considered significant when $p<0.05$.

RESULTS

Mortality: Nineteen of twenty high dose (1000 mg/kg/day) animals died or were sacrificed moribund during the study. The distribution of the deaths observed throughout the study are summarized in the following table:

Table 2. Summary of Mortality

Group[a] (mg/kg/day)	Days on Study							Total Mortality
	3	4	5	6-7	8	9-14	15-21	
0	-	-	-	-	-	-	1M, 1F	1M, 1F
100	-	-	-	-	1M, 0F	-	-	1M, 0F
300	-	-	-	-	-	1M, 0F	1M, 0F	2M, 0F
1000	3M, 0F	1M, 4F	4M, 4F	1M, 1F	0M, 1F	-	-	9M, 10F

[a]Control Group: 11 males, 9 females; other groups: 10/animals/sex.

Daily Observations: Mortality seen in the high dose group were preceded by the following signs of toxicity: respiratory difficulty which was seen in 4 males and 3 females, nasal discharge (3 males and 3 females), excessive salivation (6 males and 3 females), ataxia (2 males and 2 females), and tremors (2 males). Animals in the other groups, including control, displayed intermittent "diarrhea, bloated appearance and decreased activity," but with no consistency or pattern.

Dermal Irritation: As stated by the authors, "At all dose levels, repetitive daily application of the test article caused severe dermal irritation with eschar formation." It was not specified if the irritations observed were of equal severity at the abraded and unabraded skin sites. Average daily Draize scores were as follows:

Table 3. Mean Skin Irritation Scores - Males

Group (mg/kg/day)	Erythema/Eschar				Edema			
Day	0	100	300	1000	0	100	300	1000
1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1
2	0.0	0.5	0.6	0.7	0.0	0.4	0.2	0.2
3	0.0	0.8	1.0	1.0	0.0	0.5	0.6	0.7
4	0.0	1.3	1.6	1.4	0.0	0.6	0.9	1.1
5	0.0	2.2	2.3	2.8	0.0	1.4	1.6	2.3
6-7	0.0	3.1	3.9	-	0.0	2.5	3.0	-
8	0.0	3.7	3.7	-	0.0	2.5	2.8	-
9-14	0.0	3.8	3.9	-	0.0	2.8	3.1	-
15-21	0.1	3.1	3.9	-	0.0	2.4	3.0	-

Table 4. Mean Skin Irritation Scores - Females

Group (mg/kg/day)	Erythema/Eschar				Edema			
Day	0	100	300	1000	0	100	300	1000
1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0
2	0.4	0.3	0.5	0.6	0.0	0.3	0.0	0.0
3	0.3	0.8	1.0	0.8	0.0	0.3	0.4	0.5
4	0.3	0.8	1.0	1.8	0.0	0.7	1.0	1.1
5	0.7	1.8	2.7	4.0	0.3	1.2	2.3	2.0
6-7	0.6	3.2	3.9	-	0.4	2.3	2.9	-
8	0.2	3.4	4.0	-	0.2	2.4	3.0	-
9-14	0.2	3.3	4.0	-	0.2	2.3	3.1	-
15-21	0.4	2.9	4.0	-	0.3	2.3	3.0	-

Body Weights and Food Consumption: Mean body weights and food consumption were similar to control for the low and mid-dose animals throughout the study.

Body weights were 9 percent and 13 percent lower than controls for high dose males and females, respectively, at day 4 of the study. Further

105

comparison could not be made due to the deaths of high dose animals prior to day 7 of the study. Food consumption was 50 percent lower for these animals when compared to controls.

Hematology: Although "no significant differences were observed between any groups in any of the parameters analyzed" comparisons could not be made for the high dose animals due to the high mortality rate prior to day 7.

Clinical Chemistry: Scattered changes occurred, but were not of biological significance. Comparisons could not be made for the high dose animals due to the high mortality rate prior to day 7.

Urinalysis: Although "no significant differences were observed between groups in any parameter during the study" Comparisons could not be made for the high dose animals due to the high mortality rate prior to day 7.

Gross Pathology: Gross pathologic examination of the high-dose animals that died or were sacrificed in extremis during the study did not identify a treatment related pattern of pathologic changes.

In the animals that were sacrificed at the end of the study, the most frequent findings were white streaks or spots in the liver and accumulation of mucus or mucus-like material in the intestinal tract. The white streaks or spots on the liver, which occurred in 2-8 of the animals per sex in all groups, were attributed to generalized infection or active or healed lesions of a coccidiosis infection that was confirmed histologically. The mucoid accumulations, found in 1-3 animals per sex per group, were said to be due to subacute inflammation or to parasitic organisms.

Distinct differences between the control and dosed animals were noted in the appearance of the treated areas of skin. Animals receiving the test material had higher incidences of hemorrhage, discoloration, crust formation, and other changes (Table 5). No differentiation was made between the abraded and nonabraded sites in the treated animals.

Organ Weights: Organ weight data were determined at necropsy for the control, low-, and mid-dose animals; data were not presented on the high-dose animals that died during the study. With one exception, no statistical differences were found between the control and treated animals in the absolute organ weights or the organ weights relative to body or brain weight. The female animals of the mid-dose group had adrenal gland weights relative to body weight that were 35 percent greater than the control ratio, a statistically significant difference. The mean weight of the adrenal and the adrenal-to-brain-weight ratio were not statistically different for this group. It was also noted that these animals had slightly lower terminal body weights than the control and low-dose animals which may have contributed to the apparent increase in relative adrenal weight.

1

Histopathology:

Skin: Histopathologic examinations were conducted on sections of treated and untreated skin, liver, kidneys, and any grossly abnormal tissues from all study animals. Only sections of treated skin were observed to have lesions that differed in severity and incidence between the control and treated groups. Animals in all of the groups administered the test article had focal or diffuse eschar formation with acute inflammation and necrosis of the epidermis and outer dermis. In addition, the study's pathologist stated that "Scattered epidermal ulcers (active, healing, or healed) with overlying crusts or eschars and underlying acute inflammation, which at times extended to the subcutaneous muscle, were also frequently seen in these animals." High-dose group animals tended to have a higher incidence of eschar formation than the other groups, but less of an incidence of epidermal thickening (hyperplasia). In the low- and mid-dose animals, acute or chronic inflammation, fibrosis of the superficial dermis, and regeneration of the injured skin were prevalent. None of the incidences of these changes appeared to be related to dose (Table 5). In the control group, a low incidence of focal acute or chronic inflammation, crust formation, and epidural thickening occurred, most likely a result of shaving and vehicle administration. No differentiation was made between abraded and unabraded skin sites.

Liver: In all groups, 1-6 animals per sex were found to have single or multiple cellular necrosis and inflammations in liver tissue, and 4 animals had lesions of the liver resulting form coccidiosis infection.

Kidney: In kidney tissue, 2-3 animals per sex per group in all groups showed focal areas of nonsuppurative chronic interstitial nephritis with tubular dilation and interstitial fibrosis.

Intestine: Several animals (9 out of a total of 80) had inflammation, necrosis, and/or coccidiosis of the intestine, all of which were considered characteristic of mucoid enteropathy. These lesions were felt to have resulted from disease processes common to laboratory rabbits and not a result of treatment.

Histopathologic examination of the high-dose animals that died during the first week of the study did not reveal any common pattern of pathology. These deaths were attributed to an acute toxic effect of the test material. Histopathologic examination was conducted on adrenal tissue of 5 males and 2 females from various groups; mid-dose females were not examined for a possible lesion related to the observed increase in adrenal weight relative to body weight. One male animal of the mid-dose group was found to have necrosis of the adrenal gland.

Table 5. Summary of the Incidence of Gross Pathologic and Histopathologic Changes in Skin

Observation	Group (mg/kg/day) and Animals/Sex/Group							
	0		100		300		1000	
	M	F	M	F	M	F	M	F
	11	9	10	10	10	10	10	10
Gross								
Diffuse or focal discoloration; hemorrhage	3	0	1	4	5	2	10	10
Sores	1	3	5	3	4	9	1	0
Crust formation	0	0	6	7	7	8	1	0
Focal scars, dryness, scales, or other[a]	1	1	12	10	13	10	9	4
Histopathologic								
Eschar formation, necrosis, ulceration	0	0	5	9	7	9	9	10
Epidermal thickening (acanthosis), hyperkeratosis	3	4	7	9	9	10	1	0
Inflammation, acute	4	7	9	10	8	10	10	10
Inflammation, chronic; fibrosis	2	4	7	8	10	9	6	5

[a]More than one observation per animal is possible and therefore the total is greater than the number of animals examined.

CONCLUSIONS

003556

Conclusions: Daily application of 1000 mg/kg/day resulted in 19/20 animals dying by day 8 of the study and thereby the termination of this high-dose group. The necropsy and histopathologic examination of the organs of these high dose animals did not reveal any pattern of pathology.

No systemic effects were reported for the hematology, clinical chemistry, and urinalysis parameters examined in this study for any of the groups that survived the study (evaluations could not be made for the high dose group [1000 mg/kg/day] due to the high mortality rate by day 8 of the study). Pathology was unremarkable for these groups as well.

Dermal irritation was observed at all treatment groups (100, 300, and 1000 mg/kg/day) beginning on day 2 and continuing throughout the study.

The following LELs and NOELs were identified in this study:

Systemic NOEL = 300 mg/kg/day.
Systemic LEL = 1000 mg/kg/day (19/20 animals died by day 8).
Skin Irritation NOEL: Not established.
Skin Irritation LEL: 100 mg/kg/day (LDT).

Classification: Supplementary. (Loss of 19/20 of the high-dose animals by day 8 resulted in the study effectively having only two dose groups and prevented meaningful evaluation of possible treatment related effects. In addition, histopathologic examinations were limited to the liver and kidneys in all animals and to organs with gross lesions; consequently, toxic effects on tissue and organ structure in animals of the low- and mid-dose groups may have been overlooked).

112

003556

DATA EVALUATION RECORD
(TACKLE - TECHNICAL)

MUTAGENICITY

DATA EVALUATION RECORD 003556

(1) CHEMICAL: Tackle.

(2) FORMULATION: Technical.

(3) CITATION: Myhr, BC and McKeon M. Evaluation of 06238001 in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay. (Unpublished report prepared by Litton Bionetics, Inc. March, 1981.) Accession No. 071318. Litton Bionetics Report No. 1022-80; April 29, 1981

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(5) APPROVED BY:

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(6) STUDY TYPE: Mutagenicity.

(7) CONCLUSION:

An unscheduled DNA synthesis assay was conducted in rat hepatocytes using doses of 0.10-50.00 ug/ml of Tackle to determine this chemicals' ability to cause DNA damage and subsequent repair. The results of the assay indicated that a dose of 50 ug/ml was toxic to the cells while the remaining doses were essentially nontoxic. At these doses, the extent of DNA synthesis (as measured by thymidine

incorporation) was not appreciably greater than the negative control level of synthesis. A positive control substance (2-aminoacetyl-fluorene) produced significantly greater DNA synthesis. Thus, these data indicated that Tackle was inactive in the unscheduled DNA synthesis assay.

(8) CORE CLASSIFICATION/EVALUATION: Acceptable.

(9) MATERIALS AND METHODS:

1. The test material was identified as 06238001, Lot LCM 266830-7, a light tan powder. An aqueous solution of this material was prepared according to procedures provided by the sponsor. The powdered test material was dissolved in a sodium hydroxide solution, adjusted to a pH of 8.0, and diluted with water to a final concentration of 240 mg/ml of the sodium salt of 0628001.

2. The indicator cells used in the assay were hepatocytes from adult male Fischer 344 rats from Charles River Breeding Laboratories. The cells were obtained by in situ perfusion of the liver with a collangenase solution, followed by mechanical dispersion of excised liver tissue and concentration of the free cells by centrafugation. Cultures were then established with approximately 5 x 10^5 cells in Williams' Medium E supplemented with serum and antibiotics.

3. A cytotoxicity test was conducted by exposure of cell cultures for 1 hour to arange of test material concentrations. A dose of 500 ug/ml was found to be completely lethal after 2-4 hours. A subsequent assay with doses of 1.95 - 250 ug/ml was considered not acceptable due to toxicity at the high dose and a high level of thymidine incorporation in the negative control cultures. A second assay, as described below, was conducted using a dose range of 0.10 - 50.00 ug/ml of the test material, a negative control (medium alone), and a positive control (0.10 ug/ml of 2-acetyl-aminofluorene).

4. The assay utilized cells that were collected at 80% viability. Each dose level, including positive and negative controls, was applied to seven cultures, four of which did not receive labelled thymidine and were used to measure toxicity. Exposure was for 18 hours, followed by washing and incubation of three cultures at each dose level with 1 uCi/ml of labelled thymidine for 3 hours. After washing, samples of the cells on a cover slip were treated with a photographic emulsion. After an 8-day development time, the extent of DNA synthesis was measured by counting the nuclear grains and subtracting out an average count from three nucleus-sized areas. Net counts were made on 50 randomly selected cells from each culture.

5. Three evaluation criteria were used to identify a positive treatment effect: 1) an increase in mean nuclear grain count of at least 6 grains/nucleus over the negative control count, and/or 2) an increase in the percent of nuclei with 6 or more grains to a level of 10% above the examined population, and/or 3) the percent of nuclei with 20 or more grains is equal to or more than 2% of the examined population.

(10) REPORTED RESULTS: The results of the assay were that 50 ug/ml was highly toxic (2.1% survival); little toxicity was evident in the lower doses (Table 1). Although the treated cells tended to have more grains per nucleus, these values did not exceed the control value by 6, nor did the percentage of nuclei with more than 6 grains exceed the control percentage by 10%. In addition, no dose-related trend was evident. The positive control cultures provided clearly positive results (Table 1). Finally, a count of the cells for excessive labeling (500 cells per culture were screened) found approximately 0.4% were heavily labeled, and therefore, was not considered an impediment.

Table 1. Summary of Data from the Rat Hepatocyte Unscheduled DNA Synthesis Assay

Compound	Dose (ug/ml)	Grains/ Nucleus[a]	Nuclei with ≥6 Grains (%)	Nuclei with ≥20 Grains (%)	Survival at 21-24 hours[b]
None	--	0.40	1.3	0.0	100.0
2-AAF[c]	0.10	24.40	93.3	56.0	80.4
06238001	50.00		Grains Not Countable		2.1
	25.00	0.86	2.7	0.0	95.8
	10.0	0.55	0.7	0.0	102.4
	5.00	0.49	0.7	0.0	106.5
	2.50	0.61	2.7	0.0	95.9
	1.00[d]	0.81	4.3	0.0	107.5
	0.50[d]	0.91	1.7	0.0	103.1
	0.25[d]	1.20	4.7	0.0	102.7
	0.01[d]	0.94	2.3	0.0	Not determined

[a]Average of net nuclear grain counts in 150 cells (triplicate samples).

[b]Number of viable cells per unit area relative to negative control x 100%.

[c]2-aminoacetylfluorene.

[d]Counts performed in two samples; one was counted twice.

003556

DATA EVALUATION RECORD
(TACKLE 2S - TECHNICAL)

MUTAGENICITY

118

DATA EVALUATION RECORD

(1) CHEMICAL: Tackle 2S.

003556

(2) FORMULATION: Technical.

(3) CITATION: Skinner, MJ and Schreiner, CA. Metaphase Analysis of Rat Bone Marrow Cells Treated In Vivo with Tackle 2S. (Unpublished report prepared by Mobil Environmental and Health Science Laboratory, Report No. 10311-82 March 13, 1981.) Accession No. 071318.

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(6) STUDY TYPE: Mutagenicity.

(7) CONCLUSION:

In this study, the ability of Tackle to induce clastogenic changes in the bone marrow cells of rats was studied. Groups consisting of 6 male Sprague-Dawley rats each were given either 10 ml/kg of Methocel (the vehicle), 0.37, 1.11, or 1.87 gm/kg of Tackle 2S by gavage daily for five consecutive days. An additional group received 60.0 mg/kg of cyclophosphamide 24 hours prior to sacrifice and served as a

positive control. Signs of toxicity were observed in animals of the mid- and high-dose groups, and two high dose animals died. Analysis of the blood of one animal from each of the groups given Tackle 2S showed that the test material was absorbed and the absorption was dose-related. Examination of approximately 50 bone marrow cells from 5 rats of each group showed a statistically significant increase in the number of chromosomal aberrations in the cells of animals given cyclophosphamide, but not in the animals dosed with Tackle 2S or the vehicle. These data indicated that the test material did not induce physical damage to the chromosomes of bone marrow cells. In addition, it was observed that one animal from each of the groups dosed with Tackle, two given cyclophosphamide, but no animals given the vehicle had insufficient cells to examine, suggesting that Tackle 2S may have had a toxic effect on these cells. Otherwise, it appears that Tackle 2S lacked statistically significant clastogenic activity under the conditions of the experiment.

(8) CORE CLASSIFICATION/EVALUATION: Acceptable.

(9) MATERIALS AND METHODS:

1. The test material was identified as Tackle 2S, Lot LCM 266830-1A, TACU Sample No. 10318001.

2. Thirty male Sprague-Dawley rats (201 ± 12 g each) were randomly assigned to 5 groups. The rats were caged individually, given food and water ad libitum, and housed in an environmentally controlled room.

3. The animals were dosed with the vehicle and the test material daily for 5 consecutive days. The positive control substance was given 24 hours prior to sacrifice. The doses were as follows:

Group	Dose
I	1.37 gm/kg Tackle 2S
II	1.11 gm/kg Tackle 2S
II	0.37 mg/kg Tackle 2S
IV	60.0 mg/kg Cyclophosphamide
V	10 mg/kg Methocel (vehicle)

The animals were observed for signs of toxicity 1, 2, and 6 hours after dosing. One animal from each group except IV was bled by cardiac puncture 2 hours after the last dose. These samples were analyzed for Tackle content (the free acid) by high pressure liquid chromatography. After the final dose, the animals were sacrificed, a femur was removed from each, and bone marrow cells were processed for metaphase analysis. Fifty cells from each animal, when possible, were examined for clastogenic effects.

4. Chi-Square analysis was used to compare test values to negative control values.

(10) REPORTED RESULTS:

Toxic signs were observed in the mid- and high-dose groups and included hypoactivity, nasal discharge, labored breathing, and increased or persistant urination. Two high-dose animals died and were replaced by randomly selected animals. Analysis of the blood samples found the following levels of Tackle:

Dose	Tackle (ug/ml)
1.87 gm/kg	287
1.11 gm/kg	87.8
0.37 gm/kg	64.4
10 ml/kg Methocel	0.0

One animal from each of the groups treated with Tackle and two animals dosed with cyclophosphamide had too few bone marrow cells for analysis. Cells were taken for analysis from the animals selected for the determination of levels of Tackle in blood. No increase in the number of cells with aberrant chromosomes was observed in the rats treated with Tackle 2S; a significant increase in aberrations was seen the cells of animals dosed with cyclophosphamide (Table 1).

Table 1. Aberrant Bone Marrow Cells from Rats Dosed with Tackle 2S

003556

Group	Number of Aberrations[a]						Total Per Cells Observed
	CSB	CTB	EX	FR	PV	Other	
I (1.87 gm/kg Tackle 2S)	-	1	-	-	-	-	1/266
II (1.11 gm/kg Tackle 2S)	-	-	-	-	-	-	0/250
III (0.37 gm/kg Tackle 2S)	-	-	-	-	-	-	0/257
IV (60.0 mg/kg cyclophosphamide)	8	127	77	15	21	2[b]	146/172[c]
V (10 ml/kg Methocel)	-	-	-	-	-	-	0/250

[a]CSB = chromosomal break, CTB = chromatid break, EX = exchange, FR = fragment, PV = pulverized.

[b]A translocation.

[c]Significantly different from control, p 0.001.

003556

DATA EVALUATION RECORD
(TACKLE 2S - TECHNICAL)

CHROMOSOMAL ABERRATION

DATA EVALUATION RECORD

003556

(1) CHEMICAL: Tackle 2S.

(2) FORMULATION: Technical.

(3) CITATION: Putwam, DL and Schechtman, LM. Activity of T 1689 in the Dominant Lethal Assay in Rodents. (Unpublished report prepared by Microbiological Associates, September 23, 1981.) Accession No. 071318. Microbiological Associates Report No. T1689-116)

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(6) STUDY TYPE: Chromosomal aberration.

(7) CONCLUSIONS:

In this dominant lethal mutagenicity assay, groups of 10 Sprague-Dawley rats received by gavage 80, 360, or 800 mg/kg/day of Tackle 2S (236 mg/ml a.i.) for 5 consecutive days and allowed to mate over a 7-week period. A negative control group that received water and a positive control group dosed with triethylenelamine were also tested. Statistically significant effects were found at most time

points in all but two of the nine indices that were measured in the group dosed with the positive control material. No dose-related differences from control were found in any of the indices calculated for the groups treated with Tackle, except for a higher preimplantation loss relative to the control. However, this was a cluster effect coming from one to three pregnant females where the group size was 12-18. Since this was only marginally significant, it is the opinion of this reviewer that the clustering effect biased the data to the extent that a positive response should be considered uncertain. The authors clearly covered this concern in their concluding statement.

(8) CORE CLASSIFICATION/EVALUATION: Acceptable.

(9) MATERIALS AND METHODS:

1. The test material was identified as Tackle 2S, a dark yellow liquid, code 10318001, Lot #266830-1A, with a concentration of 236 $\pm$ 7 mg/ml of active ingredient. The positive control substance was triethylenelamide (TEM), Lot #6-246, and was dissolved in distilled water.

2. Rats of the Sprague-Dawley strain were used after a 10-14 day quarantine period. They were maintained in environmentally controlled rooms and provided food and water ad libitum. Males were caged individually; females were caged 2-4 per cage after mating. Identification was by ear tags. The weight of the male rats was given as 290-400 g each. The male rats were randomly assigned to 5 groups of 10 animals each.

3. The groups of male rats were dosed with the test material by gavage with 80, 360, or 800 mg/kg/day for 5 consecutive days. The negative control group received 5 ml/kg/day of distilled water for 5 days; the positive control group was given a single intraperitoneal injection of 0.05 mg/kg of TEM on the fourth day of dosing. Three days after the last dose, each male was mated with two females over a 5 days period and allowed to rest for 2 days. This procedures was repeated until each male had been mated with 2 males per week for a total of 7 weeks.

4. Fourteen-fifteen days from the mid-point of the mating period, the females were sacrificed by CO_2 asphyxiation and the number of corpora lutea for each ovary and the number of live and dead implants for each uterine horn were determined.

5. The results were analyzed statistically using Chi-Square analysis (2x2 contigency table) and one and two-sided t-tests. Statistically significant effects were further analyzed using analysis of regression, analysis of linear trend, and analysis of variance.

(10) REPORTED RESULTS:

Nine indices were calculated: the fertility index (the number of pregnancies divided by the number of mated animals), implantations per pregnant animal, corpora lutea per pregnant animal, preimplantation losses per pregnant female (estimated by the difference between the number of corpora lutea and total implantations), average number of dead implants per pregnant female, proportion of pregnant females with one or more dead implants on weeks 2 and 4, proportion with two or more dead implants on weeks 1 and 4, average number of dead implants per total implants, and the average number of live implants per pregnant female. Treatment with the positive control substance (TEM) produced statistically significant effects in all but two. TEM reduced the number of implants on weeks 1-4 and 7, increased preimplantation losses (weeks 1-7), increased the number of dead implants (weeks 1-6), and reduced the number of live implants (weeks 1-7). Exposure to the test material resulted in several statistically significant differences from control in four of the indices. The number implants was lower than control for mid-dose group (360 mg/kg/day) at week 7 (p 0.05). Preimplantation losses were higher at several points for the low and mid-dose groups (Table 1). Treatment with Tackle also increased the average number of dead implants per total implants at the 800 mg/kg/day level at week 5 (p 0.05), and 360 mg/kg/day reduced the average number of live implants at week 7 (p 0.05). However, inspection of these statistically significant differences did not find any dose-related changes, and thus indicated that the changes were not the result of exposure to the test material. Also, the increases in preimplantation losses found for the dosed groups were not statistically significant when compared to historical controls and were not paralleled by concomitant reductions in the number of total implantations.

Table 1. Preimplantation Losses Per Pregnant Female

Week	Negative Control	Tackle (mg/kg/day) 80	360	800	Positive Control
1	0.18	1.76*	0.27	1.54	4.50**
2	1.20	2.33	1.46	1.29	8.95**
3	0.38	1.00	2.00*	0.32	9.56**
4	0.18	0.53	1.17*	0.47	3.86**
5	0.00	0.73**	0.40*	0.13	2.40*
6	0.00	1.43*	0.33	0.67	0.33*
7	0.19	0.79	1.19*	0.20	1.20*

*Significant greater than control, p=0.05.
**Significant greater than control, p=0.01.

003556

DATA EVALUATION RECORD
(TACKLE 2AS - TECHNICAL)

MUTAGENICITY

DATA EVALUATION RECORD

003556

(1) CHEMICAL: Tackle 2AS.

(2) FORMULATION: Technical.

(3) CITATION: Mackerer, CR, Schreiner, CA, and Mehlman, MA. A Murine Lymphoma (Heterozygous for Thymidine Kinase) Mutagenicity Assay for the Determination of Potential Mutagenicity of Tackle. (Unpublished report prepared by Mobil Environmental Health Sciences Laboratory, Report No. 512-5 November 12, 1980.) Accession No. 071318.

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(5) APPROVED BY:

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(6) STUDY TYPE: Mutagenicity.

(7) CONCLUSION:

In this study, Tackle 2AS, a solution containing 227.8 mg/ml of the sodium salt, was tested for the abilility to induce forward mutations in murine lymphoma cells with and without metabolic activation. The activating system was the S-9 homogenate fraction of Arochlor

1254-induced rat liver. The volumes tested were 0.11-1.7 ul/ml (25 - 387.3 ug/ml) in the nonactivated system and 0.08-0.56 ul/ml (18 - 127.6 ug/ml) in the activated system. A preliminary toxicity study, for which the data were not presented, identified these doses to be between the LD_{10} and LD_{90} for the cells. The results of the assay indicated that Tackle 2AS did not induce forward mutations either with or without activation.

Although volumes were reported for the positive controls, EMS and DMBA, the solution concentrations were not reported. Also, the induced mutagenic frequency was not given and the absolute numbers of TK-/- was not reported for the positive controls. Hence, this reviewer cannot determined if the assay was capable of giving positive responses under the conditions of the experiment.

(8) CORE CLASSIFICATION/EVALUATION: Unacceptable. (Data would be acceptable if the positive control concentrations were given and if the mutation frequencies were reported.)

(9) MATERIALS AND METHODS

1. The test material was identified as Tackle 2AS Herbicide, Lot LCM-254889-380, TACU Sample No. 64038001. An addendum stated that the Tackle 2AS solution contained the equivalent of 227.8 mg/ml of the active ingredient (the sodium salt). Positive control substances were ethylmethane sulfonate (EMS) for the non-S-9-activated system and 7,12-dimethylbenzanthracene (DMBA) for the activated system.

2. The cell line was Murine Lymphoma Cell Line (L51784-3.7.2c) which is characterized at the thymidine kinase locus (TK+/-). The cells were derived from frozen aliquots and grown on a complete medium. Homozygotes resulting from a forward mutation (TR-/-) were selected for in a trifluorothymidine-hypoxanthine methotrexate-glycine supplemented medium.

3. Tackle 2AS was tested at 0.11, 0.19, 0.32, 0.56, 0.97, and 1.7 ul/ml of cell suspension [range from 25 to 387.3 ug/ml) in the nonactivated system and at 0.08, 0.11, 0.19, 0.32, and 0.56 ul/ml [18 to 127.6 ug/ml] in the activated system. The S-9 fraction was supplied by Litton Bionetics and was prepared from the livers of Sprague-Dawley rats treated with Arochlor 1254. The basic procedure was as follows. First, 6×10^5 murine lymphoma cells/ml were exposed to the test material on positive control substances and S-9 fraction, if any. After 180 minutes of exposure, the cells were washed, incubated for 48 hours, and adjusted to a concentration of 3×10^6 cells/ml. Aliquots of this cell suspension were cloned by addition to Viable Count and Restrictive Medium flasks and incubation for 15 minutes. A 33 ml quantity from each flask was placed in a culture dish and incubated for 10 days at 37 C. Resultant colonies were counted on a Biotran III automatic counter.

(10) REPORTED RESULTS

The results of the assays without S9 and with activation are presented in Tables 1 and 2, respectively. These data indicated that the doses used were not cytotoxic and did not induce mutations at the thymidine kinase locus. In addition, the report stated that preliminary toxicity studies at doses of 10 to 0.0001 ul/ml [2,278 ug/ml to .023 ug/ml] determined the LD_{90} to be greater than 0.56 ul/ml [127.6 ug/ml] and the LD_{10} to be 0.08 ul/ml [18 ug/ml] for the metabolically activated system. For the nonactivated system these values were 1.7 [387.3] and 0.11 ul/ml [25 ug/ml], respectively. No data for the preliminary toxicity study were presented.

Table 1. Total Cell Growth and Induced Mutation Frequency - Nonactivated System

Compound	Dose (ul)	Total Growth[a]	Induced Mutation Frequency
Tackle 2AS	1.7	0	2.0
	0.97	1	0.0
	0.56	35	0.0
	0.32	79	0.0
	0.19	106	0.0
	0.11	106	0.0
EMS	0.5	39.7	--[b]
	0.1	20.6	--

[a]Total growth = % growth in suspension x % cloning growth/100.

[b]--Indicates information not provided.

Table 2. Total Cell Growth and Induced Mutation Frequency - Activated System

Compound	Dose (ul)	Total Growth[a]	Induced Mutation Frequency
Tackle 2AS	0.56	74.6	0.0
	0.32	113.9	0.1
	0.19	102.5	0.1
	0.11	75.3	0.1
	0.08	97.6	0.0
DMBA	5.0	39.0	—[b]
	7.5	24.4	--

[a]Total growth = % growth in suspension x % cloning growth/100.

[b]—Indicates information not provided.

003556

DATA EVALUATION RECORD
(TACKLE 2S - TECHNICAL)

MUTAGENICITY

DATA EVALUATION RECORD

003556

(1) CHEMICAL: Tackle 2S.

(2) FORMULATION: Technical.

(3) CITATION: Bowan, JT, Bowman, SA and Bergener DR. Drosphilia Mutagenicity Assays of Mobil Chemical Company Compund MC 10109 (MRI #533). (Unpublished report prepared by EG&G Mason Research Institute and Utah State University Foundation, April 13, 1981.) Accession No. 071318. EG&G Mason Report No. 009-275-533-9

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(6) STUDY TYPE: Mutagenicity.

(7) CONCLUSION:

The following five mutagenicity tests were carried out using Drosophilia melanogaster: somatic reversion of white-ivory, Y chromosome loss, dominant lethal mutations, bithorax test of Lewis, and sex-lethals. In each assay, male flies were exposed to a Kimwipe paper towel saturated with a 15 mg/ml Tackle 2S solultion for 2 hours

(somatic reversion of white-ivory assay) or 24 hours (remaining fo. - assays). Exposure to Tackle 2S resulted in an increase of exceptions over control frequencies in the Y chromosome loss and dominant lethal assays, but not in the other three tests. These results indicate a disruptive effect of Tackle 2S on mitosis and meiosis. Therefore, it is the opinion of this reviewer that a genetic effect is induced by the assay chemical, Tackle 2S.

(8) CORE CLASSIFICATION/EVALUATION: Acceptable

(9) MATERIALS AND METHODS:

1. The test material, Tackle 2S, was described as a tan powder. It had a Mobil Chemical identification number of MC-10109 and a Lot No. of LCM-266830-7.

2. The test species was Drosophila melanogaster. The stock used for each assay were:

   A. Somatic reversion of white-ivory

   $\underline{w}^{i}/\underline{w}^{i}$

   $\underline{v}$ Qn(1)$\underline{w}^{i}$ $\underline{ec}$/Y

   B. Y chromosome loss

   $\underline{w}/\underline{w}$

   $\underline{w}/\underline{w}$+Y

   C. Dominant lethal mutations

   Canton-S

   D. Bithorax test of Lewis

   $\underline{Ubx}$/Ins(3+3R)P

   $\underline{bx}^{34e}/\underline{bx}^{34e}$

   E. Sex-linked lethals

   Basc (x-chromosome balancer)
   Canton-S

3. The test procedures followed for each of the assays are described below. For each assay, the male flies were exposed to Kimwipes

paper that had been saturated with a solution of 15 mg/ml Tackle. The duration of the exposure were for assay A, 2 hours, and for assays B through E, 24 hours.

A. Somatic reversion of white-ivory

Cross: $\underline{w^i}/\underline{w^i}$ X $\underline{v}$ Qn(1)$\underline{w^i}$ $\underline{ec}/\underline{Y}$

Males with X chromosomes carrying five copies of the white-ivory ($\underline{w^i}$) mutant and marked with the recessive mutants yellow body ($\underline{y}$) and echinus eyes ($\underline{ec}$) are carried in stock mated to attached-X females with normal red eyes, yellow body, and forked bristles ($\underline{f}$). In this stock, the X chromosomes are inherited by the males from their fathers, and by the female from their mothers. The cross is placed on baker's yeast for egg-laying periods of up to 15 hours and then removed. Three days later larvae are collected by washing the yeast through a 60-mesh screen. These larvae are treated with the test substance, placed on standard culture medium to complete their development, and then scored. Mutagenic activity is indicated by a significant increase in the frequency of red mosaic spots in the eyes of the females.

B. Y chromosome loss

Cross: $\underline{w}/\underline{w}$ X $\underline{w}/\underline{w^+}Y$ (treated)

Induction of loss of the Y chromosome is assayed by mating treated males which carry a duplication of the gene for normal (red) eyes on the Y chromosome and a mutant allele white ($\underline{w}$) on the X chromosome to white-eyed ($\underline{w}/\underline{w}$) females. Since each male offspring of this cross normally receives his Y chromosome from his father, each male offspring is expected to carry the gene for red eyes. The occurrence of a white-eyed male therefore signals the loss of the Y chromosome from the spermatozoon, i.e., an X-O male is produced.

C. Dominant lethal mutations

Any genetic change which blocks development prior to hatching from the egg may be termed a dominant lethal mutation. Treated males of the Canton-S strain are mated to Canton-S females in nylon net cages on Welch's grape juice solidified with 2% agar. After an egg laying period of not more than 12

hours the plates are stored at 25° C for approximately 30 hours. The agar plates are then scored to determine the fraction from which larvae have not hatched.

D. Bithorax test of Lewis

Cross: UBx/Ins(3L+3R)P X $\underline{bx}^{34e}/\underline{bx}^{34e}$ (treated)

The occurrence of rearrangements with a break-point between the centromere of chromosome 3 and the locus of bithorax (bx) can be determined by scoring the offspring of treated bithorax-34e males and Ultrabithorax (Ubx) females. These $\underline{Ubx}/\underline{bx}^{34e}$ offspring, which have their halteres enlarged to form a second set of wings, develop a band of hairy tissue, the metanotum, between the thorax and the abdomen if a chromosomal aberration with a break between bx and the centromere has been induced.

E. Sex-linked lethals

Parental Cross: Basc/Basc X +/Y (treated

F_1 Cross: Basc/+ X Basc/Y

Expected F_2: Basc/Basc extreme bar, apricot eyed female

Basc/+ wide bar, red eyed female

Basc/Y extreme bar, apricot eyed male

+/Y non-bar, red eyed male

Females homozygous for the X-chromosome balancer Basc are mated to Canton-S (wild type) males which have been treated with the test substance. All males from this parental cross carry the Basc X chromosome. All daughters carry one Basc chromosome and a treated X from their fathers. In the F_1, individual daughters are mated to their brothes. If the treatment applied to the parental males induce a lethal mutation in a sperm, the F_1 females which inherited the chromosome will produce no +/Y sons. Each single-female culture, therefore, is scored for the presence of red-eyes males. Any such culture which contains at least 20 flies at least 8 of which are males but none of which are red-eyed males is scored as a lethal. If these conditions are not met the culture is retested.

6. Poisson data analysis was carried out on results from each of the five assays according to the method described by Steven (1942).

(10) REPORTED RESULTS:

The results for each assay were as follows:

A. Somatic reversion of white-ivory

	Qn(1)w^i, Y ec/w^i Females		
Group	Number Scored	Mosaics	Frequency
Control	1096	5	0.005
Treated	2164	10	0.005*

*f_e does not exceed $2f_c$ with a 99% probability.

B. Y chromosome loss

Group	Males		
	Total	X/O	Freqency of Loss
Control	1213	4	0.003
Treated	2172	18	0.083*

*f_e exceed f_c with a 95% probability.

C. Dominant lethal mutations

Group	Eggs		
	Number Scored	Inflated	Freqency of Lethals
Control	3554	64	0.018
Treated	2153	309	0.014*

*f_e exceeds $6f_c$ with a 99% probability.

D. Bithorax Test of Lewis

Group	Ubx/bx^{34e}		
	Number Tested	Transvections	Frequency
Control	2135	1	0.0005
Treated	2031	1	0.0005*

*f_e does not exceeds f_c with a 90% probability.

E. Sex-linked lethals

Group	Single Female F_2 Cultures		
	Number Scored	Lethals	Freq. of Lethals
Control	6760	7	0.001
Treated	2261	2	0.0009*

*f_e does not exceeds $3f_c$ with a 95% probability.

(11) REFERENCES:

Stevens, WL. 1942. Accuracy of mutation rates. J. Genetics 43:301-307.

003556

DATA EVALUATION RECORD
(TACKLE - TECHNICAL)

MUTAGENICITY

DATA EVALUATION RECORD

003556

(1) CHEMICAL: Tackle.

(2) FORMULATION: Technical.

(3) CITATION: Jagannath, DR. Mutagenicity Evaluation of 06238001 Lot LCM 266830-7 in the Mitotic Recombination Assay with the Yeast Strain D_5. (Unpublished report prepared by Litton Bionetics, Inc. January, 1981.) Accession No. 071318. Litton Bionetics Report No. 20988

(4) REVIEWED BY:

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Date: 22 June 1983

(5) APPROVED BY:

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(6) STUDY TYPE: Mutagenicity.

(7) CONCLUSION:

Tackle was tested for its ability to induce genetic recombination in the Saccharomyces cerevisiae strain D_5 in the presence and absence of a metabolic activating system prepared from Arochlor-1254-induced

142

rat livers. Positive results (an increase in recombination frequency) were obtained in an initial test at a 5.0 ul dose without the activating system and at a 10.0 ul dose with activation; toxicity was observed at 25.0 ul. A repeat assay found a dose-related increase in recombination frequency without activation and a positive result (not dose-related) with activation. These results indicated that Tackle was capable of inducing recombination in this indicator strain without metabolic activation.

There are two minor criticisms which do not undermine the validity of te report, but should be answered. First, it would be useful to either have the molecular weight or specific gravity of the test material. Second, the positive controls are obviously working but their volumes do not tell the reviewer the actual concentration.

(8) CORE CLASSIFICATION/EVALUATION: Acceptable. (Well conducted and evaluated study.)

(9) MATERIALS AND METHODS:

1. The test material was identified is Mobil Chemical No. 06238001 Lot LCM-266830-7, a tan powder. A 29.7% solution of the test material was prepared by adding it to a sodium hydroxide solution and adjusting the pH to 8.0.

2. The indicator strain that was used was Saccharomyces cerevisiae strain D_5 which is diploid and heteroallelic at ade_2-40 and ade_2-119 loci. The S-9 metabolic activation fraction was obtained from male rat livers induced by Arochlor 1254 and was prepared by Bionetics Laboratory Products, Inc.

3. Initially, 5 concentrations of the test material were tested (0.1 - 50.0 ul/plate) with and without activation. In addition, a negative control (distilled water) and positive control substances (ethylmethanesulfonate for the nonactivated system and dimethylnitrosamine for the activated system) were tested concurrently. Positive results in the initial assay prompted additional testing at 2.5, 5.0, and 7.5 ul/plate of the test material in the nonactivated system and 7.5, 10.0, and 25.0 ul/plate in the activated system. [It may be possible to assume that 297 mg/ml is the concentration, but this fact has not been established.] The basic procedure was as follows. A mixture of the appropriate dose of the test substance, 0.1-0.2 ml of the indicator strain, and buffer was incubated for 3 hours (S-9 fraction was substituted for the buffer in the activated system). Aliquots were then diluted and plated onto complete yeast medium and the plates incubated at 30 C for 4 days. After 1-3 days of refrigeration to heighten color differences, the plates were scored for pigmented colonies and sectors.

4. The evaluation criterion was the appearence of red-pink cells that result from the expression of a nonreciprocal recombination of two recessive allevels. A positive effect was said to have occurred if the frequency of the recombinations in the treated cells was 2 times or more than the spontaneous frequency and if the increase in frequency was proportional to dose and could be replicated.

(10) REPORTED RESULTS:

The initial test obtained a positive response at the 5.0 ul dose level without activation and a marginally positive response at 10.0 ul with activation (Table 1). The repeat test found a dose-related response in the nonactivated system and a positive, but not clearly dose-related, response (due to toxicity at the highest dose) in the activated system (Table 2). Thus, these results indicated that Tackle was capable of inducing recombination in both nonactivated and activated systems. It should be noted that the quantitative response was greater in the nonactivated than activated systems, thus suggesting that the S-9 fraction was responsible for converting the test material to a less active metabolite.

Table 1. Initial Test of Tackle in the Recombinant Assay with S. cerevisiae

Compound	Dose (ul)	Survivors (%)	Recombinants Per 10^5 Survivors			Percent Cross Overs
			Reciprocal	Nonreciprocal	Total	
Nonactivated						
Water	50.0	100.0	1.0	2.0	3.0	0.078
EMS[a]	10.0	90.3	56.0	213.0	269.0	7.775
Tackle	0.1	104.4	2.0	3.0	5.0	0.125
	1.0	121.4	0.0	1.0	1.0	0.022
	5.0	103.7	1.0	6.0	7.0	0.176
	10.0	65.5	0.0	1.0	1.0	0.039
	50.0	3.7	0.0	0.0	0.0	0.000
Activated						
Water	50.0	100.0	1.0	4.0	5.0	0.129
DMN[b]	100.0	0.3	0.1	1.1	1.1	9.913
Tackle	0.1	108.7	3.0	4.0	7.0	0.165
	1.0	109.0	1.0	0.0	1.0	0.024
	5.0	94.1	0.0	1.0	1.0	0.027
	10.0	88.2	2.0	7.0	9.0	0.262
	50.0	0.0	0.0	0.0	0.0	0.000

[a]EMS = Ethylmethanesulfonate.

[b]DMN = Dimethylnitrosamine.

Table 2. Repeat Test of Tackle in the Recombinant Assay with S. cerevisiae

Compound	Dose (ul)	Survivors (%)	Recombinants Per 10^5 Survivors			Percent Cross Overs
			Reciprocal	Nonreciprocal	Total	
Nonactivated						
Water	50.0	100.0	0.0	2.0	2.0	0.109
EMS[a]	10.0	100.0	50.0	119.0	169.0	9.235
Tackle	2.5	109.8	0.0	3.0	3.0	0.149
	5.0	96.2	2.0	2.0	4.0	0.227
	7.5	90.2	0.0	5.0	5.0	0.303
Activated						
Water	50.0	100.0	0.01	0.09	0.10	0.011
DMN[b]	100.0	4.6	0.03	1.14	1.17	2.868
Tackle	7.5	56.6	0.00	0.16	0.16	0.032
	10.0	48.0	0.00	0.19	0.19	0.045
	25.0	2.9	0.03	0.13	0.16	0.635

[a]EMS = Ethylmethanesulfonate.

[b]DMN = Dimethylnitrosamine.

003556

TACKLE
(Acifluorfen)

Evaluation of Potential Oncogenic and Toxicological Effects
of Long-Term Dietary Administration of Tackle to B6C3F1 Mice

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Dynamac Corporation

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Date 14 August 1983

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Staff Scientist
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Signature R L Hebert
Date August 19, 1983

Carolyn Gregorio, Ph.D.
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003556

TACKLE
(Acifluorfen)

Study Type: 18-month mouse oncogenicity study.

Accession Number: 071312, 071313, 071314, 250463, 250464

MRID Number: Not assigned.

Sponsor: Rhone-Poulenc.

Contracting Laboratory: Gulf South Research Institute (GSRI Project No. 413-984-41).

Date: November 3, 1982.

Test Material: Tackle "2S" (Acifluorfen, sodium salt; MC 10978).

Tackle Solution Stability: Tackle "2S" is a solution (24 percent; 2 lb/gal) of the sodium salt of the technical acid (MC 10109). In this study, the technical acid was taken from Lot Nos. LCM 266821-3, LCM 266830-2, and LCM 266830-4 from Mobil Oil (purity of these lots not stated), and Lot No. RJH276096 from Rhone-Poulenc (77 percent purity). The purity of solutions made from these lots was not described. Tackle 2S purity data given in Appendix A of the report was taken from two different lot numbers (LCM-254889 and LCM-254892). These data indicated that at zero-time, the Tackle concentration ranged from 20.4 percent to 23.2 percent. After 6 months, "no significant potency changes were observed at any of the three storage temperatures [room temperature, 37°, and 50°C], at the specified pH 8 [two lots], or at the outside pH range of 7 and 9."

Stability of Tackle Acid: The purity of Lot No. RJH276096 was 77 percent. Assays done by Rhone-Poulenc showed that Tackle acid was stable for two months when stored at 50°C.

PROTOCOL

Animals: Six hundred B6C3F1 mice obtained from the Charles River Breeding Labs were acclimated for 2 weeks prior to commencement of the study. The animals, 60 males and 60 females (weighing approximately 22 and 18 grams, respectively) were randomly selected for each dose group. Tackle 2S was given in the diet at concentrations of 0, 625, 1,250, and 2,500 ppm.

143

The mice were housed 5 per polycarbonate cage in a temperature, ("generally held at 74°F"), humidity ("generally maintained between 40-70 percent") and light (12-hour light, 12-hour dark) controlled room. "No other species or test material were under concurrent investigation in this animal room."

Diet Preparation: The diets were prepared by mixing aqueous "2S" solution with acetone. "The control feed was mixed with acetone. The material was dried and mixed in a steel Hobart mixer." Diets and city tapwater were available ad libitum. "The tapwater was analyzed annually by the city for contaminants." The basal diet for the study was NIH 07 open formula mash which was prepared and analyzed by Ziegler Brothers, Gardeners, Pennsylvania.

"Mice to be fed 625 ppm were given feed containing 1,250 ppm of the test compound and animals to receive 1,250 ppm were given 625 ppm during the dates of 7/14/81 - 7/30/81 resulting in incorrect dosing of the animals of these two levels for this time period."

Concentration of Tackle 2S in Feed: Samples from the 625-, 1,250-, and 2,500-ppm dose groups were mixed and analyzed immediately or up to 9 days after mixture. Control diets were not analyzed.

The compound concentrations in the feed varied acceptably within the 10 percent tolerance limits with the exception of the following times:

a) February 15, 1982 (pg. 226) - illegible

b) March 20, 1982 (pg. 228) - the 625 ppm dose level exceeded the ± 10 percent tolerance limit, and notation indicated "remix" will be done. No "remix" sheet was included in the report.

Stability of Tackle 2S in Feed: Not available in report.

General Observations: All mice were observed twice daily for overt signs of toxicity, moribundity, and mortality. Detailed clinical observations were recorded weekly.

Body Weights and Food Consumption: Individual body weights were recorded weekly for the first 13 weeks and twice weekly thereafter. Food consumption was recorded weekly for the first 13 weeks and "twice monthly for 7 days thereafter for each cage."

Ophthalmology: All mice were examined at 0, 12, and 18 months for eye abnormalities "as detected by direct and indirect ophthalmoscopy."

Hematology: At 12 and 18 months, blood samples were taken from 10 animals/sex/dose. Blood was taken by cardiac puncture after fasting overnight. The following determinations were made: hematocrit, erythrocyte count, hemoglobin, total and differential leukocyte counts, reticulocyte counts ("if indications of anemia were noted"), and platelet counts.

Residue Analysis: "At approximately 4 months, urine and feces were collected from 4 animals/sex/dose (2 animals/pooled sample). Samples of urine and feces were frozen and shipped to the sponsor (Mobil). From each animal at the 12 and 18 month sacrifices, samples of the following tissues were collected: liver, skeletal muscle, heart, mesenteric adipose tissue, kidney and one testis/male. The collected tissues were placed in labeled vials, frozen in dry ice/alcohol and stored at -20°F."

Pathology: All surviving animals and interim sacrificed animals (10 animals/sex/dose at 12 months) were "anesthetized, exsanguinated and necropsied." Mice found moribund or dead were also subjected to complete necropsy.

Organ Weights: The weights of the following organs were recorded: liver, kidneys, heart, testes, brain including entire brain stem, spleen, lungs, and adrenals.

Histopathology: The following tissues were preserved in 10 percent buffered neutral formalin:

- Eyes and Harderian glands
- Heart
- Thyroid with parathyroid
- Trachea
- Esophagus
- Stomach
- Adrenal glands
- Liver (multiple sections if tumor)
- Kidneys (multiple sections if tumor)
- Spleen (multiple sections if tumor)
- Lungs - all lobes and mainstem bronchi (multiple sections if tumor)
- Testes
- Ovaries
- Skin
- Sciatic nerve
- Mammary gland
- Bone with marrow - tibio-femoral joint, vertebra, or sternum
- Skeletal muscle
- All gross lesions
- Brain - at least three levels from forebrain, midbrain, and hindbrain
- Pituitary
- Salivary glands
- Thymus
- Small and large intestines
- Pancreas
- Urinary bladder
- Prostate
- Corpus and cervix uteri
- Gall bladder
- Lymph nodes - mesenteric, non-mesenteric, and any abnormal nodes
- Spinal cord - at least three levels (10 animals/sex/group at termination)
- 3 Coronal sections of head - nasal cavity, paranasal sinuses, tongue, oral cavity, nasopharynx, and middle ear (10 animals/sex/group at termination)

150

003556

Tissues were inventoried at GSRI and shipped to WIL Labs for processing and analysis.

Statistics: "Quantitative data such as body weights were analyzed by Dunnet's "t" comparison of control versus treated groups. Group mortality was examined by the Chi-square test for significance."

GENERAL OBSERVATIONS

Reporting of "weight loss marked," sores and "generalized hair loss" was scattered through week 22 and then constant for the rest of the study in all dose groups.

An item of interest was the reporting of "lacked water, mechanical problems" at weeks 1, 2, 11, 17, 19, 31, 33, 38, 39, 41, 57, 67, 73, and 74.

"Beginning in week 52 and continuing with increasing frequency to termination, abdominal masses were observed." The distribution of "masses appeared to be dose-related, with the mid- and high-dose males and high-dose females showing a considerably greater number of observations than the control." The respective number of animals with abdominal or inguinal masses for control, low-, mid-, and high-dose groups were 8, 10, 11, and 16 for males, and 1, 2, 2, and 6 for females.

MORTALITY

The distribution of mortality among test groups was as follows:

Dose (ppm)	Males	Females
0	1	5[a]
625	3	2
1250	7[b]	5[a]
2500	10	3

[a]One animal in each of these groups was listed as an accidental death.

[b]Four animals "died due to cage flooding".

Two animals (control females XF-79 and XF-83) were listed as natural deaths in Appendix H of this report and as accidental deaths in Appendix F of this report.

The time to death as a function of dose was examined. All natural deaths among control animals occurred during weeks 17-23, whereas 16/25 deaths in treatment groups occurred after 52 weeks (Table 1).

TABLE 1. Time Distribution of Natural Deaths

Sex	Study Time Interval (weeks)	No. of Deaths in Groups			
		Control	625 ppm	1,250 ppm	2,500 ppm
Males	0–26	1[a]	0	1	1
	27–52	0	0	1	3
	53–79	0	3	1	6
Females	0–26	2[a]	0	0	1
	27–52	0	0	2	0
	53–79	0	2	2	2

[a]These deaths occurred during weeks 17–23. The deaths of two female control animals at weeks 18 and 21 are not included here because they were listed as both natural and accidental deaths.

BODY WEIGHTS AND FOOD CONSUMPTION

Beginning at week 2 for mid- and high-dose males, and week 6 for low-dose males, body weights were significantly reduced ($p<0.01$) relative to controls. The effect was dose related, as shown in Table 2. Similar results were obtained with females, except that reduced body weights of low- and mid-dose females were not significant until week 13.

TABLE 2. Mean Body Weights Relative to Controls (percent)[a]

Sex	Dose (ppm)	Week				
		4	13	29	53	79
Males	625	-2	-8	-7	-7	-10
	1,250	-3	-10	-13	-14	-13
	2,500	-18	-23	-22	-29	-25
Females	625	+1	-6	-6	-12	-11
	1,250	-1	-5	-11	-21	-22
	2,500	-12	-14	-19	-32	-34

[a] Body weights measured weekly through week 13, and twice weekly thereafter.

Mean group weekly food consumption values for females were generally similar, with no pattern of significant differences observed. Mean food consumption data for males are shown in Table 3.

TABLE 3. Mean Food Consumption in Males

Dose (ppm)	Weekly Measurements		Twice Weekly Measurements		
	Weeks 1–2	Weeks 3–13	Weeks 15–25	Weeks 27–41	Weeks 43–79
625	–[a]	Increase[b]	–	–	–
1,250	–	Increase[b]	–	–	–
2,500	Decrease	Increase[b]	Increase[b]	–	Increase[b]

[a] Comparable to controls.

[b] All or most of the measurements during interval were statistically significant ($p<0.05$).

Eighteen month overall mean values for daily food consumption, diet sample analyses, and mean test material intake (mg/kg/day) for each group were also provided (Table 4).

TABLE 4. Mean Test Material Concentrations in Diet, Mean Daily Food Consumption Values, and Test Material Intake

Parameter	Sex	Test Group			
		Control	625 ppm	1,250 ppm	2,500 ppm
Test material in diet (ppm)	Males	0	627	1,249	2,477
	Females	0	627	1,249	2,477
Daily food consumption(g)	Males	6.5	6.6	6.9	7.9*
	Females	6.6	6.5	6.8	6.9
Test material intake (mg/kg/day)	Males	0	119	259	655
	Females	0	143	313	711

*Statistically significant at $p<0.01$.

NOTE: These data were provided in the report.

During the first two weeks of the study, high-dose males and females lost weight despite consuming near normal quantities of food. The calculated efficiency of food utilization (EFU) values for selected times during the first 25 weeks of the study are shown in Table 5. There was a dose-related effect on EFU for both males and females.

TABLE 5. Efficiency of Food Utilization
(g body weight gain/kg food consumed)

	Week					
Sex/Dose (ppm)	1	2	4	8	13	25
Males						
0	21.98	15.69	8.11	3.31	1.46	0.60
625	42.86	14.72	6.64	2.11	1.00	0.45
1,250	11.90	9.27	6.43	2.12	0.93	0.37
2,500	-2.60	-21.63	-0.44	1.15	0.41	0.23
Females						
0	9.37	7.61	5.78	2.12	1.04	0.49
625	18.87	6.83	5.60	1.93	0.85	0.43
1,250	-7.79	3.51	4.48	1.96	0.83	0.34
2,500	-25.06	-11.34	1.12	1.22	0.54	0.22

OPHTHALMOLOGY

The ophthalmic examinations before study initiation and at 12 months were performed by David Moore, D.V.M. Terminal sacrifice eye examinations were conducted by William E. Field, D.V.M. "Ophthalmic exams were performed on the 001-T chronic mice prior to randomization...Any mice with eye abnormalities were eliminated from the randomization selection pool." Ophthalmic abnormalities reported at 12 and 18 months are summarized in Table 6.

TABLE 6. Results of Ophthalmic Examinations[a]

Abnormality	Males				Females			
	0 ppm	625 ppm	1,250 ppm	2,500 ppm	0 ppm	625 ppm	1,250 ppm	2,500 ppm
12-Month Sacrifice								
Cataracts	1	0	0	0	0	0	0	0
Corneal ulcers	0	2	0	0	0	1	2	1
Total abnormalities	1	2	0	0	0	1	2	1
Terminal Sacrifice								
Cataracts	6	8	7	5	3	1	0	3
Corneal ulcers	0	0	0	0	0	0	0	1
Keratitis	1	2	1	0	0	1	2	5
Total abnormalities	7	10	8	5	3	2	2	9

[a] Total number of animals examined per group could not be determined from the data presented as presented in the report.

HEMATOLOGY

No individual data were provided in this report.

Males: Mean corpuscular volume (MCV) was decreased in all treated male groups at interim and final sacrifice when compared to control males (Table 7). Treated males also had higher RBC counts than controls at final sacrifice (Table 7). Segmented neutrophil counts were reduced and lymphocyte counts were increased in high-dose males at interim sacrifice, and in all treated male groups at final sacrifice (Table 7).

Females: Segmented neutrophil counts were reduced and lymphocyte counts were increased in all treated female groups at interim sacrifice and in the 1,250- and 2,500 ppm-dose females at final sacrifice (Table 7).

TABLE 7. Hematologic Parameters Affected in Mice Fed Tackle for 18 Months

Parameter	Interim sacrifice (12 mos.)				Final sacrifice (18 mos.)			
	0 ppm	625 ppm	1,250 ppm	2,500 ppm	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Males								
MCV (μ^3)	51.1	47.3	47.8	46.4	52.7	49.9	49.7	48.1*
RBC ($10^6/mm^3$)	8.8	8.6	9.3	9.1	8.3	8.4	9.1	10.0*
Segmented neutrophils[a]	35.0	49.7*	40.5	30.5	43.2	40.6	35.8	27.8*
Lymphocytes[a]	48.8	35.2*	44.2	57.8	52.8	54.5	59.6	67.4*
Females								
Segmented neutrophils[a]	46.6	43.5	30.0*	38.1	38.8	43.4	34.0	32.6
Lymphocytes[a]	42.1	45.5	55.7*	50.8	57.6	53.9	63.6	64.6

[a]Percent.

*Statistically significant ($p<0.05$).

RESIDUE ANALYSIS

003556

No data on residue analyses were available in this report.

ORGAN WEIGHTS

Mean liver weights and liver-to-body weight ratios of treated males and females were greater than controls at interim and final sacrifice (Table 8).

TABLE 8. Mean Liver Weights - Interim and Final Sacrifice

	Males		Females	
Dose (ppm)	Liver weight (g)	Liver/body weight (g/100g)	Liver weight (g)	Liver/body weight (g/100g)
Interim sacrifice - 12 months				
0	1.554	4.037	1.305	3.721
625	1.952*	5.102	1.275	4.261
1,250	1.874	5.852	1.469	5.478
2,500	2.199**	7.533	1.778**	7.857
Final sacrifice - 18 months				
0	2.030	4.633	1.699	3.999
625	2.412	6.013	1.585	4.229
1,250	2.947**	7.529	1.909*	5.760
2,500	3.753**	11.374	2.320**	8.435

*Statistically significant ($p<0.05$).
**Statistically significant ($p<0.01$).

NOTE: Liver/body weight not statistically analyzed.

Organ weights of animals found dead or sacrificed moribund were highly variable. For all groups, weights of livers were generally much higher than normal. Factors related to the cause of death and the period of time from death to necropsy may have been responsible. Therefore, an evaluation of these data would not be useful.

158

GROSS PATHOLOGY

Interim Sacrifice: Liver masses were observed in one control male, one low-dose male, one mid-dose male, four high-dose males, and one-high dose female (Table 9). In addition, focal or general discoloration of livers was observed in one mid-dose male, two high-dose males, one low-dose female, and one mid-dose female.

Early Deaths: Liver masses were observed more frequently in treated males and treated females, when compared to controls (see Table 9). Appendix I presented results for only 10/25 early deaths among treated animals, 6/6 early deaths among control animals. The following animals are listed in Appendix H (Unscheduled Deaths) but are not listed in Appendix I (Gross Necropsies, Interim Kill and Early Deaths): GF-75, GM-10, GF-119, HM-2, FM-8, HM-15, HM-20, HM-4, HF-105, FM-53, HM-19, FM-43, HM-48, HF-106, GM-26, GM-27, GM-29, GM-30, FF-66. As a result, Appendix I does not present an accurate comparison of necropsy findings for the animals that died early in the study.

Final sacrifice: Liver masses were observed in an apparent dose-related increase in treated males when compared to controls. In addition, high dose females showed a 37 percent increase in incidence of liver masses when compared to controls (Table 9). White foci (1 mm) on the nonglandular portion of the stomach were seen in 3 high-dose males, 1 control female, 2 low-dose females, 3 mid-dose females, and 7 high-dose females (Table 8). In addition, one high-dose male and one high-dose female each had an ulcer of the stomach.

Histopathology: "The tissue processing was supervised and microscopic examinations were performed by Fred W. Sigler, D.V.M., Veterinary Pathologist at WIL Research Laboratories."

An increased incidenced of liver tumors was observed in all treated groups when compared to control animals. (See Table 10).

In addition, a dose-related increase in the incidence of benign stomach papillomas was observed in treated females at final sacrifice (Table 11). Stomach papillomas were also observed in high dose males at the final sacrifice (Table 11).

TABLE 9. Summary of Remarkable Necropsy Findings in Mice Fed Tackle in the Diet for 18 Months

Time/Nature of death	Organ/Finding	Males				Females			
		0 ppm	625 ppm	1,250 ppm	2,500 ppm	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Interim sacrifice-12 months	Number examined	10	10	10	10	10	10	10	10
	Liver								
	-masses	1	1	1	4	0	0	0	1
Early deaths	Number examined	1	3	7	10	5	2	5	2[a]
	Liver								
	-masses	0	2	2	5	0	1	1	1
Final sacrifice-18 months	Number examined	48[a]	47	43	40	44[a]	48	45	47
	Liver								
	-masses	7	13	17	28	0	4	3	18
	Stomach								
	-round white elevated foci on non-glandular portion	0	0	0	3	1	2	3	7
	-ulceration	0	0	0	1	0	0	0	1

[a]Not including animals autolyzed or not completely examined.

TABLE 10. Summary of Liver Tumors in Mice Fed Tackle for 18 Months

Dose (ppm)	Males 0	Males 625	Males 1,250	Males 2,500	Females 0	Females 625	Females 1,250	Females 2,500
12-Month Sacrifice								
Interim sacrifice-12 months								
No. of livers examined	10	10	10	10	10	10	10	10
- Carcinoma (only)	0	0	0	0	0	0	0	0
- Adenoma (only)	1	2	1	5	0	0	0	1
- Carcinoma and adenoma	0	0	0	0	0	0	0	0
Total at 12 months	1/10	2/10	1/10	5/10	0/10	0/10	0/10	1/10
Early Deaths								
No. livers examined	1	3	7[a]	10	4	1	5[a]	2
- Carcinoma (only)	0	1	0	2	0	1	0	0
- Adenoma (only)	0	1	0	3	0	0	0	2
-Carcinoma and adenoma	0	0	1	1	0	0	0	0
Total - early deaths	0/1	2/3	1/7	6/10	0/4	1/1	0/5	2/2
18-Month Sacrifice								
Final sacrifice-18 months								
No. livers examined	48	47	42[b]	40[b]	45[a]	47	45	47[c]
- Carcinoma (only)	1	2	2	6	0	1	1	4
- Adenoma (only)	7	15	11	18	1	4	3	16
- Carcinoma and adenoma	0	0	1	b	0	0	0	1
Total - final sacrifice	8/48	17/47	14/42	29/40	1/45	5/47	4/45	21/47
TOTALS								
Total no. livers examined	59	60	59	60	59	58	60	59
Total carcinoma (only)	1	3	2	7	0	2	1	4
Total adenoma (only)	8	18	12	26	1	4	3	19
Total carcinoma and adenoma	0	0	2	7	0	0	0	1
Total liver tumors over 18 months	9/59	21/60*	16/59[d]	40/60**	1/59	6/58	4/60	24/59**

*p<0.0025 (Chi-square).

**p<0.001 (Chi-square).

[a]"Round...firm mass" on liver of one animal was observed at necropsy, but was not histologically reported (see Table 12 for details).

[b]"Round...firm mass(es)" on the liver three animals were observed at necropsy, but were not histologically reported (see Table 12 for details).

[c]"Round...firm mass(es)" on the liver of four animals were observed at necropsy, but were not histologically reported (see Table 12 for details).

[d]"Round...frim mass" on liver of four animals were observed at necropsy, but were not histologically observed after sectioning.

Dose (ppm)	Males				Females			
	0	625	1,250	2,500	0	625	1,250	2,500
Interim sacrifice-12 months								
No. stomach examined	10	10	10	10	10	10	10	10
- Benign papilloma	0	0	0	0	0	0	0	0
Early deaths								
No. stomach examined	0	2	7	8	3	1	2	1
- Benign papilloma	0	0	0	0	0	0	0	0
Final sacrifice-18 months	48	46	43	40	45	48	45	47
- Benign papilloma	0	0	0	4	0	3	4	6
TOTALS								
Total no. stomachs examined	58	58	60	58	58	59	57	58
Total benign papillomas	0	0	0	4	0	3	4	6

CONCLUSIONS

003556

ONCOGENICITY

As indicated in the histopathology section of this review, a significant ($p<0.025$; Chi-square) increase in liver tumors was observed in the 625-ppm [LDT] and 2500-ppm (HDT) treated males (Table 10). The incidence of liver tumors among mid-dose males (1250 ppm) was not significant; however four males in this group were described at necropsy as having a "round...firm mass on the liver which were either not fully described or included in the corresponding histopathology data. Further explanation of the diagnoses of these animals is necessary to fully assess the oncogenic response in this mid-dose male group.

Correspondingly, a significant ($p<0.001$; Chi-square) increase in liver tumors was observed in the 2,500-ppm (HDT) females (Table 9).

A dose-related increase of a rare benign stomach pallimas was observed in all treated female groups, (Table 11). In addition, this stomach tumor was observed in high-dose males (Table 11). Although these incidences do not achieve significance, they are biologically significant and there are contribute to the assessment of the oncogenic potential of this compound.

In summary, oncogenic potential was demonstrated at all dose levels for males and females.

NONONCOGENIC EFFECTS

Reduction in body weight gain was observed in all groups treated male groups beginning at week 4 of the study, and in high-dose females beginning at week 1 of the study. Hematology findings showed that all treated male and female groups had increased lymphocyte counts and decreased segmented neutrophil counts. In addition, treated males had decreased mean corpuscular volumes and increased WBC and RBC counts.

In summary, toxic effects were observed in males and females at the lowest dose tested, 625 ppm.

Note: This study has been recommended for a data audit (memo from Burnham to Toukey, dated September 9, 1983.

163

CLASSIFICATION: Reserved.
Resolution of the following items is requested:

1) Thirteen animals (animal numbers reported in Table 12 of this review) were reported at necropsy to have a "tissue mass" on the liver, but were either not described or not included in the corresponding microscopic pathology data. Further explanation of these "tissue mass[es]" is needed.

2) The following animals are listed in Appendix H (Unscheduled Deaths) but are not listed in Appendix I (Gross Necropsies, Interim Kill and Early Deaths): GF-75, GM-10, GF-119, HM-2, FM-8, HM-15, HM-20, HM-4, HF-105, FM-53, HM-19, FM-43, HM-48, HF-106, GM-26, GM-27, GM-29, GM-30, FF-66. An explanation is needed.

3) The following animals were listed in Appendix H (Unscheduled Deaths) and listed in Appendix J (Gross Necropsies Final Kill): XM-24, FM-8, FM-43, FM-53, GM-10, GM-26, GM-27, GM-28, GM-29, GM-30, GM-45, HM-2, HM-4, HM-15, HM-16, HM-19, HM-20, HM-28, HM-34, HM-35, HM-48, XF-66, XF-79, XF-83, XF-84, XF-95, FF-66, FF-100, GF-74, GF-75, GF-95, GF-116, HF-103, HF-105, HF-106. An explanation is needed.

4) On March 30, 1982, the feed with the 625-ppm dose level was analyzed. The analysis exceeded the ±10 percent limit (figure recorded was -13.4 percent) and the notation read "remix." No remix sheet was included in the report. An explanation is needed.

5) The stability of TACKLE 2S in the feed was not included in the report. An analysis should be provided.

6) Control diets were not analyzed for possible TACKLE 2S contamination. An explanation is needed.

7) Clinical observation sheets reported that animals occasionally "lacked water, mechanical problems." Further explanation of the occurrence (how long, how often, etc.,) is needed.

8) The Moribundity and Mortality Section of the report states for mid-dose males "four animals found dead in cage flooding." Further explanation of the occurrence (how long, were all animals enjoying this experience, or only the dose group in which deaths occurred, etc.) is needed.

9) High-dose males and females lost weight during the first two weeks of the study, yet consumed food in quantities similar or slightly less than controls. The clinical observation records did not indicate that animals exhibited diarrhea or spilled their food. An explanation is needed.

TABLE 12. Liver Masses Observed at Necropsy but not Described in Histopathology Data

Dose Group	Animals No.[a]	Necropsy Observation of Liver	Microscopic Finding for Liver
Final Sacrifice			
Males–1,250 ppm	GM–3	Round, tan, firm tissue mass (5 mm) on caudate lobe.	No significant changes observed.
	GM–34	Round, tan tissue mass (2 mm) embedded in left lobe.	- -[b]
	GM–42	Round, tan, firm tissue mass (0.2 mm) on caudate lobe.	Steatosis.
Males–2,500 ppm	HM–23	Round, tan tissue mass (0.5 mm) on left lobe.	Steatosis.
	HM–29	Elevated, firm, tan, round tissue mass (4 mm), and three gray foci (1–2 mm) on left lobe.	Massive necrosis and hemorrhage, and acidophilic cells.
	HM–30	Two brown irregular tissue masses (8 mm) involving right and median lobes.	Massive necrosis and hemorrhage, and acidophilic cells.
Females–625 ppm	FF–101	Tan, soft, tissue mass (1.5 x 1 x 0.5 cm) on right lobe.	- -[b]
Females–2,500 ppm	HF–62	Two tan, soft tissue masses (6 mm and 2 mm); one elevated from median lobe, the other embedded in left lobe.	Massive necrosis and hemorrhage, and multilocular cysts.
	HF–92	Round, tan elevated tissue mass (0.5 cm) on right lobe.	No significant changes observed.
	HF–99	Round, tan soft tissue mass (4 mm) elevated from caudate lobe.	Caudate lobe with mass was not present for trimming.
	HF–104	Round, firm, red tissue mass (0.3 mm) on right lobe.	No significant changes observed.
Early Deaths			
Males–1,250 ppm	GM–30	Round, firm, greenish tissue mass (7 mm) on caudate lobe.	Massive necrosis and hemorrhage.
Females–1,250 ppm	GF–74	Round, dark brown tissue mass (0.7 x 0.5 x 0.4 cm) on left lobe.	No significant changes observed.

[a] F, G, H = low, mid, and high dose, respectively.

[a] There were no findings described, nor was it specified if liver was not examined microscopically.

TACKLE
(Acifluorofen)

STUDY TYPE: Two-year feeding/oncogenicity - rats.

CITATION: Barnett J.W., Jenkins, L.J., Parent, R.A. 1983. A combined oncogenicity/chronic feeding study of Tackle in Fischer 344 rats. An unpublished report.

ACCESSION NUMBER: 071315 thru 071317; 250289 thru 250792

MRID NUMBER: Not assigned.

SPONSOR: Rhone-Poulenc, Inc.

CONTRACTING LABORATORY: Gulf South Research Institute (GSRI Project No. 413-985-41).

DATE: March 30, 1983.

TEST MATERIAL: Tackle "2S" (Acifluorofen, sodium). Purity 19.1 to 25.6 percent.

TECHNICAL TACKLE STABILITY: Tackle Technical Acid (Lot No. RJH-276096) was stable for 2 months stored at 50°. Assayed purity ranged from 76.8 to 77.9 percent.

TACKLE "2S" STABILTY: Tackle "2S" is a 2 lb/gallon solution of the sodium salt of Technical Tackle Acid. Tackle 2S purity data (Lot No. LCM 254889, Lot No. LCM 254892) ranged from 19.1-25.6%. Tackle 2S was stable for 6 months at pH 7, 8 and 9 and storage temperatures of 37°C, 50°C and room temperature.

PROTOCOL:

Fisher 344 rats (554 males and 352 females) were received from Charles River Breeding Laboratories, Wilmington, MA and acclimated to laboratory conditions for two weeks. Before assigning the animals to a dose group, each received an ophthalmologic examination; only those animals free of eye lesions were included in the study. The animals were randomized into 5 dose groups and one control group of 73 animals of each sex. The animals were approximately 47 days old and mean body weights ranged from 133.8 to 139.0 g for males and 110.1 to 113.7 g for females. The final test material concentrations in the diet were 0, 25, 150, 500, 2,500, and

5,000 ppm. The 5,000 ppm group received 10 ppm for the first 4 weeks of the study.

Diets were prepared by dissolving the powdered acid in NaOH, adjusting to pH 8.0, and diluting the solution to contain 240 g/L of Tackle as the sodium salt. This solution was mixed with acetone and added to feed to obtain the required concentrations. The diets were then dried and mixed in a Hobart mixer. Diets were prepared twice weekly and analyzed in advance of feeding to ensure that the actual concentrations of Tackle were within 10 percent of the nominal concentration. The average concentrations for each dietary level throughout the study are shown below:

Nominal Concentration (ppm)	Analytical[a] Concentration (ppm)
25	24.9 ± 1.1
150	149.0 ± 6.4
500	496.0 ± 21.7
2,500	2,488.3 ± 109.3
5,000	4,981.0 ± 215.53

[a] Values are means and standard deviations of all prepared diets at each dose level.

NOTE: This table reproduced from Registrant's Submission.

No information was present on the stability of test compound in feed.

Animals were housed 5 per cage in polycarbonate cages suspended on stainless steel racks. Food and water were available ad libitum. The animal rooms were maintained at 74°F, had 12 changes of air per hour, and a 12 hour dark/light cycle.

Observations: All animals were checked twice daily for mortality and moribundity. Detailed examinations and palpations were conducted monthly thereafter. Food consumption (over a 3-4 day interval) was measured weekly for 14 weeks and twice monthly thereafter.

Eye examinations were conducted on all rats prior to dosing and at 12 and 24 months using a direct ophthalmoscope and transillumination.

Hematology, clinical chemistry, and urinalysis determinations were performed on 10 animals/sex/group at 3, 6, 12, and 24 months. Animals were fasted for 24 hours and blood taken from the orbital plexus at all sampling times except at 24 months when cardiac puncture was used.

Hematology parameters included hematocrit, hemoglobin, erythrocyte count, total and differential leukocyte counts, prothrombin and clotting times. Blood chemistry parameters included: calcium, sodium, potassium, serum lactic dehydrogenase, serum glutamic pyruvic transaminase, creatine phosphokinase, serum glutamic oxaloacetic transaminase, glucose, blood urea nitrogen, direct and total bilirubin, total cholesterol, triglyceride, serum alkaline phosphatase, albumin, globulin, total protein, chloride, uric acid, blood creatinine and gamma-glutamyl transpeptidase. Blood analysis at 3, 6, and 12 months was by SMA-18 automated analyzer, and at 18 and 24 months by a Centrifichem automated system.

Urinalysis parameters measured were specific gravity, pH, protein, glucose, ketones, bilirubin and urobilinogen and the presence of formed elements.

Complete necropsy was performed on all animals that died or were sacrificed. At 12 months, 8 animals/sex/group were sacrificed. All surviving animals were sacrificed at 24 months. At necropsy, the following organ weights were recorded: liver, kidneys, heart, testes, brain and brain stem, spleen, lungs, and adrenals.

Tissues were fixed in neutral formalin, trimmed, processed, and stained with hematoxylin-eosin. The slides were examined and diagnosed by Fred W. Sigler, D.V.M. at WIL Research Laboratories, Cincinnati, Ohio. The following tissues were examined:

Adrenal glands	Aorta	Bone	Bone marrow
Brain	Colon	Duodenum	Esophagus
Eyes/optic nerve	Ileum	Jejunum	Kidney
Lymph node, Ma.	Lymph node, ME.	Lung/bronchi	Mammary gland
Nerve, sciatic	Pacreas	Pituitary	Prostate
Salivary glands	Skeletal muscle	Skin	Spinal cord
Spleen	Stomach	Thyroid gland	Trachea
Urinary bladder	Harderian gland	Cecum	Heart
Thymus	Testes	Liver	

Statistics: Quantitative data were analyzed by Dunnett's t-test for multiple comparisons and significant differences were identified at the 95 and 99 percent confidence level. Mortality was analyzed by Chi-square analysis. Histopathologic changes were analyzed during the Kolmorgorov-Smirnov one tailed test.

RESULTS:

Clinical Observations: Eye abnormalities ("lacrimation both eyes," "eye abnormal," "eye closed," "smaller eye," "eye opacity") were noted frequently in all groups throughout the study. In addition, the 5,000 ppm males and females became progressively emaciated in the second year of the study.

Body Weights and Food Consumption: Mean body weight was significantly decreased in males and females at the 2,500 and 5,000 ppm doses throughout the study when compared to controls (Table 1). In addition, 500 ppm females displayed a significant decrease in mean body weight from week 0 through 17 of the study, and intermittently through week 40.

TABLE 1. Mean Body Weights of Rats (Grams) at Selected Intervals

Dose (ppm)	Sex	Weeks 0	17	40	80	104
0	M	136±14	336±17	400±23	437±25	412±44
2,500	M	139±12	306±28	377±28	408±24	378±31
5,000	M	139±12	276±16	323±16	283±51	a
0	F	114±6	192±8	226±10	280±19	300±29
500	F	110±8	187±7	216±11	275±28	295±27
2,500	F	112±7	180±8	206±10	234±24	250±31
5,000	F	111±7	171±10	198±10	202±19	191±39

[a] All animals on this group died before week 104.

Mean food consumptioon data showed no consistent trends.

Mortality: All the males in the 5000 ppm group died before term, in fact 60% of these animals died by week-84 of the study. High-dose females, also, demonstrated poor survivability as 45% of these animals had died by week-92 of the study. The following table shows mortality of all groups at termination of the study:

TABLE 2. Mortality Data[a]

Dose (ppm)	Males		Females	
	No. Died	Percent Died[a]	No. Died	Percent Died[a]
0	17/65	26.2	12/65	18.3
25	15/65	23.1	18/65	27.7
150	5/65	12.3	9/65	13.8
500	11/65	16.9	28/65	30.8
2500	7/65	10.8	15/65	23.1
5000	65/65	100.0	61/65	78.5

[a] Interim sacrifice animals not included in calculation.

Ophthalmology:

The incidence of animals with cataracts was similar for all groups:

TABLE 3. OPHTHALMIC EXAMINATION - CATARACTS

Dose (ppm)	Males						Females					
	0	25	150	500	2500	5000	0	25	150	500	2500	5000
- Interim Sacrifice												
# animals examined	73	73	73	73	73	73	73	73	73	73	73	73
# animals with cataract	0	3	1	0	0	0	1	7	1	1	6	1
- Final Sacrifice												
# animals examined	46	25	58	51	58	-	42	48	55	58	51	14
# animals with cataract	31	NR	31	30	34	-	35	12	35	35	26	34

NR = none reported

The predominance of eye lesions noted in the interim sacrifice animals were in those animals used for blood samples via the orbital plexus.

It should be noted that incidence of cataracts reported at the final sacrifice did not correspond to the histopathology of the final sacrifice, retinal degeneration was the predominant eye lesion reported histologically.

Hematology: Red cell count, hematocrit and hemoglobin values were significantly lower in 5,000 ppm males at 6, 12, and 18 months (there were no values at 24 months due to the death of all high dose males) when compared to controls. Treated female groups did not demonstrate any significant hematologic changes.

Clinical Chemistry:

Males: Blood glucose, triglycerine, serum globulin, total serum proteins were lower in the 2500 and 5000 ppm dose groups when compared to control animals (Table 4). BUN levels, creatinine and alkaline phosphatase were elevated in the 5000 pm group when compared to control animals (Table 4).

No consistent response was reported for cholesterol, uric acid, serum electrolytes (sodium, chloride, potassium and calcium), creatinine phosphokinase (CPK), lactic dehydrogenase (LDH), serum glutamic-oxaloacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT).

Females: Blood glucose, triglycerides, serum globulin and total serum proteins were lower in the 2500 and 5000 ppm females when compared to control females (Table 5). BUN levels and creatinine levels were evaluated in the 5000 ppm females when compared to control females. Alkaline phosphatase was elevated in the 2500 and 5000 ppm females when compared to control females (Table 5).

No consistent response was reported for cholesterol, uric acid, serum electrolytes (sodium, chloride, potassium and calcium), creatinine phosphokinase (CPK), lactic dehydrogenase (LDH), serum glutamic-oxaloacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT).

TABLE 4. SELECTED CLINICAL CHEMISTRY VALUES - MALES

Group (ppm)	Month 3	6	12	18	24
Glucose (mg/dl)					
Control	74.3	51.1	81.1	92.1	134.5
2,500	66.1	44.7	42.7*	80.9	133.7
5,000	56.7	31.9	45.1*	53.5*	--
Triglyceride (mg/dl)					
Control	27.9	63.2	140.3	94.2	94.1
2,500	14.2	53.8	87.3*	60.3	62.4
5,000	7.5	34.4	57.3*	101.1	--
Globulin (mg/dl)					
Control	3.2	2.9	3.3	3.5	3.0
2,500	2.7	2.6	2.8*	3.0*	3.1
5,000	2.4*	2.1*	2.5*	2.6*	--
Total Protein (g/dl)					
Control	7.2	7.0	7.1	7.2	7.6
2,500	7.0	6.8	6.8	6.7	7.6
5,000	6.4**	6.3**	6.3**	5.6**	--
BUN (mg/dl)					
Control	13.8	14.4	11.6	13.4	19.7
2,500	15.3	15.8	14.5*	15.4	18.1
5,000	17.7*	16.7	16.2*	35.2*	--
Creatinine (mg/dl)					
Control	0.6	0.9	0.8	0.6	0.61
2,500	0.7	0.9	0.8	0.7	0.64
5,000	0.8	1.1*	0.9	0.9*	--
Alkaline Phosphatase (IU/L)					
Control	140.2	101.8	131.9	61.0	47.3
2,500	153.5	122.2	149.9	66.7	50.3
5,000	208.9	164.1*	206.3*	146.4*	--

* P <0.05
** P <0.01
-- All animals died before 24 months.

TABLE 5. SELECTED CLINICAL CHEMISTRY VALUES - FEMALES 003556

Group (ppm)	Month 3	6	12	18	24
Glucose (mg/dl)					
Control	65.5	51.3	55.5	85.6	141.0
2,500	64.8	45.1	38.6	84.3	130.1
5,000	28.7*	28.4*	36.7	61.1	126.3
Triglycerine (mg/dl)					
Control	5.8	37.0	54.5	55.6	120.6
2,500	13.3	28.5	41.3	43.1	54.1*
5,000	6.7	27.9	31.0	54.8	69.2*
Globulin (mg/dl)					
Control	3.4	3.0	3.4	3.6	2.5
2,500	3.1	2.7*	3.8*	3.2*	3.6
5,000	2.3	2.3*	2.4*	2.9*	2.3
Total Protein (g/dl)					
Control	7.5	7.4	7.9	7.5	7.6
2,500	6.7	7.0	7.1	7.0	8.4
5,000	6.6	6.4**	6.3**	6.4**	6.3**
BUN (mg/dl)					
Control	16.7	15.2	12.9	14.0	15.0
2,500	13.6	14.4	13.9	13.4	23.9
5,000	14.8	16.0	13.8	21.8*	27.4*
Alkaline Phosphatase (IU/L)					
Control	101.8	70.2	71.8	39.3	34.2
2,500	125.8	79.5	98.3	42.5	61.5*
5,000	137.1	100.7*	135.2**	59.4*	61.5*
Creatinine (mg/dl)					
Control	0.7	1.0	0.7	0.6	0.59
2,500	0.7	0.9	0.8	0.6	0.63
5,000	0.8	1.1	0.8	0.8**	0.71

* $p < 0.05$
** $p < 0.01$

Urinalysis: No consistent changes were seen between control and treated animals.

Gross Necropsy:

Males: An increased incidence of kidney and liver discoloration, stomach ulcers and reduced testes size were recorded for the 5000 ppm males when compared to control males (TABLE 6).

Females: An increased incidence of kidney lesions and stomach ulceration was observed in the 2500 and 5000 ppm females when compared to control females (TABLE 6).

Organ Weights:

Males: Mean liver weights and mean relative liver weights (as percent of body weights) were significantly higher for the 2500 and 5000 ppm males when compared to control males at the 12 month sacrifice, but not at terminal sacrifice (TABLE 7).

Mean heart weights were lower for the 2500 ppm males when compared to respective controls at the 12 month sacrifice and terminal sacrifice. The 5000 ppm males had lower mean heart weights at the 12 month sacrifice (TABLE 7).

Mean spleen weights and mean relative spleen weights (as percent of body weight) were lower for the 500, 2500 and 5000 ppm males at the 12 month sacrifice and lower for the 500 and 2500 males at the terminal sacrifice. (TABLE 7).

Mean kidney weights were lower for the 5000 ppm males at the 12 month sacrifice (TABLE 7).

Females: Mean liver weights and mean relative liver weights (as percent body weight) were significantly higher for 2500 and 5000 ppm females at the 12 month and final sacrifice (TABLE 8).

Mean heart weights and mean relative heart weights were significantly lower for the 2500 and 5000 ppm females at the 12 and 24 month sacrifice (TABLE 8).

Mean relative spleen weights were significantly higher for the 2500 and 5000 ppm females at the 12 month and final sacrifice. Mean spleen weights were higher at the final sacrifice at 5000 ppm.

Mean relative kidney weights were higher for the 2500 and 5000 ppm females at the 12 month and terminal sacrifice (TABLE 8).

Histopathology:

Neoplastic Lesions: No increase in either benign or malignant tumors in any organ were reported in treated animals when compared to control animals. However, a very low tumor incidence was recorded which is quite curious.

Non-Neoplastic Lesions: A summary of the non-neoplastic lesions is in Table 9.

It should be noted that in the histological assessment of this study, the liver (acidiophilic cells) and kidney lesions (chronic nephritis) were unusually low in the control and low dose groups. These above mentioned lesions are commonly seen in aging rats and it is curious as to the low reporting.

Conclusion:

TACKLE 2S did not demonstrate oncogenic potential under the conditions of this study. An apparent low response for tumors and non-neoplastic lesions has somewhat compromised the outcome of this study. This study has been recommended for a data audit to clearly substantiate the reported findings. In addition the registrant is requested to submit historical control data for this species within GSRI for the past 5 years.

TACKLE 2S does cause kidney damage (demonstrated by changes in clinical chemistry values and histology evaluation of nephritis, pyelonephritis and glomerulonephritis) at the 2500 and 5000 ppm groups. TACKLE 2S has a strong effect on the stomach mucosa as demonstrated by the high incidence of stomach ulcers at the 5000 ppm dose. And, testicular atrophy was noted in the 5000 ppm males.

Onco NOEL = Greater than 5000 ppm (HDT)
Onco LEL = Greater than 5000 ppm (HDT)

Systemic NOEL = 500 ppm
Systemic LEL = 2500 ppm

Classification: Supplementary. A very low neoplastic and non-neoplastic lesion response was reported in this study. As a result, these data have been recommended for a data audit. Pending the outcome of the data audit a complete classification will be done.

NOTE: Tables 1, 3, 5, 7 and 8 were selective items reproduced from the registrant's submission.

TABLE 6. REMARKABLE GROSS NECROPSY FINDINGS

	Males[a]						Females[a]					
	0	25	150	500	2500	5000	0	25	150	500	2500	5000
Kidney												
- Discolored	0	3	2	2	1	21	0	1	0	1	0	7
- Distended Pelvis/Calculi	0	1	0	0	0	2	0	0	0	0	12	5
- Granular	0	5	1	3	2	1	0	0	0	0	4	5
Liver												
- Discolored	5	0	1	2	0	10	1	3	0	1	0	2
- Granular	5	2	3	1	3	0	3	4	2	2	1	2
- Diaphragmatic Hernia	3	0	2	0	0	0	2	3	1	1	0	1
- Mass(es)	2	1	2	4	2	3	0	0	2	0	1	1
Stomach												
- Ulcer(s)	0	2	0	0	1	22	4	1	1	0	5	16
- Foci	1	0	1	0	0	0	1	0	0	0	0	3
Testes												
- Small	7	7	6	5	5	19	-	-	-	-	-	-

[a] Number of animals examined = 73

TABLE 7. MEAN ABSOLUTE AND RELATIVE WEIGHTS OF SELECTED ORGANS - MALES (GRAMS)

Dose (ppm)	0	500	2500	5000
Liver				
-12 Month	12.377 (2.895)	12.010 (2.796)	14.020** (3.504**)	15.266** (4.769**)
-24 Month	11.299 (2.769)	11.081 (2.642)	10.356 (2.738)	NR
Heart				
-12 Month	1.073 (0.251)	1.035 (0.242)	0.982 (0.246)	0.863 (0.270)
-24 Month	1.088 (0.267)	1.102 (0.263)	1.013* (0.270)	NR
Spleen				
-12 Month	0.775 (0.181)	0.692 (0.162)	0.694 (0.158*)	0.650** (0.203)
-24 Month	1.464 (0.355)	1.224 (0.292)	1.131 (0.299)	NR
Kidney				
-12 Month	2.472 (0.579)	2.421 (0.565)	2.586 (0.645)	2.349 (0.735)
-24 Month	2.651 (0.650)	2.638 (0.630)	2.670 (0.710*)	NR

* P <0.05
** P <0.01
NR = Not recorded. All males died before terminal sacrifice
Values in parentheses are relative weights as percent of body weight

TABLE 8. MEAN ABSOLUTE AND RELATIVE WEIGHTS OF SELECTED ORGANS - FEMALES (GRAMS)

Dose (ppm)	0	2500	5000
Liver			
-12 Month	6.891 (2.883)	7.014 (3.266**)	8.457** (4.274**)
-24 Month	7.875 (2.628)	8.109 (3.306**)	9.262** (5.123**)
Heart			
-12 Month	0.667 (0.279)	0.595** (0.277)	0.537** (0.272)
-24 Month	0.836 (0.281)	0.759** (0.311*)	0.657** (0.357**)
Spleen			
-12 Month	0.446 (0.187)	0.492 (0.229*)	0.445 (0.225*)
-24 Month	0.641 (0.267)	0.784 (0.324)	1.046 (0.541)
Kidney			
-12 Month	1.513 (0.635)	1.471 (0.685)	1.486 (0.749**)
-24 Month	1.890 (0.633)	1.817 (0.744)	1.891 (1.027)

* P <0.05
** P <0.01
Values in parentheses are relative weights as percent of body weights.

TABLE 9. SUMMARY OF NON-NEOPLASTIC LESIONS

	Males						Females					
ppm	0	25	150	500	2,500	5,000	0	25	150	500	2,500	5,000
12 MONTH SACRIFICE												
No. of animals examined	8	8	8	8	8	8	8	8	8	8	8	8
Eyes, retinal degeneration	1	1	1	0	1	4	1	0	0	0	1	3
Heart, myocardial degeneration and fibrosis	6	2	3	1	1	1	0	1	1	0	0	0
Kidney, glomerulonephrosis	4	0	2	0	0	2	1	0	0	0	1	1
nephritis/pyelonephritis	0	0	0	0	0	1	0	0	0	0	1	1
Liver, acidophilic cells	0	0	0	0	0	8*	0	0	0	0	0	7*
FINAL SACRIFICE												
Kidney,												
- Number examined	45	50	56	54	56	0	53	48	56	45	49	14
- nephritis/pyelonephritis	0	0	0	0	1	-	0	0	0	0	31*	11*
- chronic pyelonephritis with papillary necrosis	0	0	0	0	1	-	0	0	0	0	11	2
- glomerulonephrosis	42	42	47	46	28	-	14	13	19	16	0	1
Liver,												
- Number examined	45	50	57	54	57	0	53	48	56	45	50	14
- acidophilic cells	0	0	0	0	0	-	0	0	0	0	11*	12*
Stomach												
- Number examined	44	49	57	54	56	0	53	48	56	45	49	14
- ulcers	0	0	0	0	1	-	4	1	0	0	0	3
Heart												
- Number examined	45	50	56	54	57	0	49	48	56	45	49	14
- myocardial degeneration and fibrosis	13	16	28*	31*	25	-	4	3	5	7	2	0
Testes												
- Number examined	45	50	56	54	57	0						
- Atrophy	10	5	3	5	9	-						

TABLE 9. SUMMARY OF NON-NEOPLASTIC LESIONS (Continued)

	Males						Females					
ppm	0	25	150	500	2,500	5,000	0	25	150	500	2,500	5,000
EARLY DEATHS												
Kidney,												
- Number examined	20	13	8	11	8	61	11	17	6	20	15	51
- nephritis/pyelonephritis	0	1	0	0	0	9	1	5	1	5	0	3
- chronic pyelonephritis with papillary necrosis	0	1	0	0	0	48*	0	0	0	0	5	38*
- glomerulonephrosis	12	5	4	5	1	9	1	5	0	5	0	3
Liver,												
- Number examined	20	15	8	11	8	65	12	17	9	20	15	50
- acidophilic cells	0	0	0	0	0	52*	0	0	0	0	3	41*
Stomach												
- Number examined	18	13	8	10	6	65	12	16	16	19	14	51
- ulcers	1	0	0	1	1	32*	1	1	1	0	2	23*
Heart												
- Number examined	20	13	8	11	8	64	11	16	7	20	15	51
- myocardial degeneration and fibrosis	4	2	1	2	1	2	1	3	1	1	0	0
Testes												
- Number examined	20	13	8	11	7	61						
- Atrophy	3	3	2	4	1	31*						

* Significantly different from controls at P = 0.05 with Kolmorgorov-Smirnov one tailed test.

TOX:GREGORIO:TOX-35:DCR-11741:09/07/83
REVISED-9/14/83:DCR-11744:TOX-35:efs
REVISED-10/5/83:DCR-32803:CBI-4-TOX:efs
REVISED-10/11/83:DCR-32980:pad