11/23/83

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

003409

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

NOV 23 1983

MEMORANDUM

SUBJECT: Review of Toxicology Data (2-Year Feeding/Onco In Rats and 18-Month Feeding/Onco In Mice) for TACKLE (Acifluorfen sodium) Caswell No. 818B

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

THRU: William Butler, Section Head
Hazard Evaluation Division (TS-769)
and
William Burnam, Branch Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

William M. Butler 11-21-83

WB 11-22-83

Pesticide Petition: 359-TNI/3F2811

Petitioner: Rhone-Poulenc

Action Requested: Request for review of the 2-year feeding/onco in rats and 18-month feeding/onco in mice data submitted for TACKLE (acifluorfen sodium) and its metabolites (the corresponding acid, methyl ester and amino analogues).

Due to the request to expedite this information for regulatory action, the additional toxicology data received in this submission will follow shortly.

Recommendations: An oncogenic potential has been demonstrated in mice being fed TACKLE for 18 months, as indicated in the attached review (Gulf South Research Institute, Report No. 413-984-41, dated November 3, 1982). A statistically significant (Chi-square) increase in liver tumors was observed in the 625 ppm (lowest dose tested) and the 2500 ppm (highest dose test) treated males when compared to concurrent control males. The incidence of liver tumors in the

a statistically significant increase in liver tumors was observed in the 2500 ppm (highest dose tested) females.

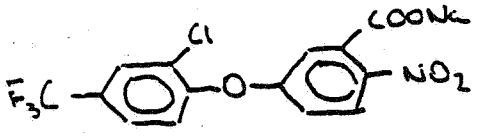
In addition, a dose related increase of rare stomach papillomas were observed in all treated female groups and in high dose males. This type of tumor was not reported in the concurrent control animals of either sex.

An oncogenic risk assessment is currently underway.

Background: Tackle is the 21.1% sodium salt of acifluorfen formulated as a water soluble concentrate that contains 2 lb ai/gal. It is used for weed control in soybeans and is applied either by ground or aerial equipment at a rate of 0.38 to 0.75 lb ai/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.



Sodium Acifluorfen

Formulation: TACKLE 2S

Commercial Components	% by Wt.	Purpose
Tackle (Acifluorfen)	21.1 ±	Active Ingredient
[redacted]		

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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 = 500 ppm (25 mg/kg)

2. Toxicology Data Considered Lacking:

None

3. Action Being Taken to Obtain Lacking Data:

None

4. Summary of Tolerances Granted:

None

5. Summary of How Total Tolerances Granted Affect the MPI:

Not applicable at this time.

6. Acceptable Daily Intake Data:

Not applicable at this time.

7. Pending Regulatory Action Against Registration:

None

8. Other Considerations:

Tackle is structurally related to a series of chemicals which have been found to have either teratogenic, oncogenic and/or mutagenic potential [Fomosofen (Flex), RH-0265, Acifluorfen (Blazer), Oxyflurofen (Goal), Nitrofen (TOK), Hoelon, Fluzifop-Butyl (Fusilade)]. Tackle has the identical chemical structure as Blazer.

CAG 11-10-83

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

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

	Males						Females					
Dose (ppm)	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 003409

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	5
Kidney												
- Discolored	0	3	2	2	1	21	0	1	0	1	0	
- Distended Pelvis/Calculi	0	1	0	0	0	2	0	0	0	0	12	
- Granular	0	5	1	3	2	1	0	0	0	0	4	
Liver												
- Discolored	5	0	1	2	0	10	1	3	0	1	0	
- Granular	5	2	3	1	3	0	3	4	2	2	1	
- Diaphragmatic Hernia	3	0	2	0	0	0	2	3	1	1	0	
- Mass(es)	2	1	2	4	2	3	0	0	2	0	1	
Stomach												
- Ulcer(s)	0	2	0	0	1	22	4	1	1	0	5	
- Foci	1	0	1	0	0	0	1	0	0	0	0	
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	0.841 (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	-						

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

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

003409

TACKLE
(Acifluorfen)

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

John R. Strange, Ph.D.
Department Director
Dynamac Corporation

Signature John R. Strange
Date 19 August 1983

Richard L. Hebert, M.S.
Staff Scientist
Dynamac Corporation

Signature R L Hebert
Date August 19, 1983

Carolyn Gregorio, Ph.D.
EPA Scientist

Signature _____
Date _____

003409

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.

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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)

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."

RESULTS

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

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.

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	625	1,250	2,500	Females 0	625	1,250	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	5	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								
- Benign papilloma	48	46	43	40	45	48	45	47
	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

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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 Burnam to Toukey, dated September 9, 1983.

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

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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.