02/15/9)

PEER REVIEW FILES

007698

CHEMICAL NAME: Acifluorfen (Tackle/Blazer)
CASWELL NO.: 755D
CAS NO.: 62476-59-9
REVIEWER: Phang

CURRENT AGENCY DECISION

B2; 3.55 x 10-2
(HED)
B2/C (SAP)

TUMOR TYPE / SPECIES

Liver; B6C3F1 & CD-1 mice (M & F).
Stomach papillomas; B6C3F1 mice (M & F).

REVIEWER PEER REVIEW PACKAGE	PEER REVIEW MEETING DATE	PEER REVIEW DOCUMENTS	PEER REVIEW CLASSIFICATION
5. / /	5. / /	5. / /	5.
4. / /	4. / /	4. / /	4.
3. / /	3. / /	3. / /	3.
2. / /	2. 01/13/88	2. 03/17/88	2. B2; 3.55 x 10-2
1. 06/02/87	1. 06/10/87	1. 09/30/87	1. B2

SAP MEETING	SAP CLASSIFICATION
2. / /	2.
1. 12/15/87	1. B2/C

QUALITATIVE/QUANTITATIVE RISK ASSESSMENT DOCUMENT	GENETIC TOXICITY ASSESSMENT DOCUMENT
2. / /	1. / /
1. 07/25/88	

MISCELLANEOUS:

Publication: EPA Scientific Weight of the Evidence: Determination of the Oncogenicity of Pesticide Chemicals: Aciflurofen. Filed with SAP Report. Stamped 2/1/90; PR-007698; 185 p.; nha.

1 of 118

007698

Peer Review Documents
(Memo dates)

3/17/88

007698

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

FILE COPY

MAR 17 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Reevaluation of Classification of Carcinogenicity of Acifluorfen Following Science Advisory Panel (SAP) Review of Data.

FROM: John A. Quest, Ph.D. JAQuest 2/10/88
Mission Support Staff
Toxicology Branch/HED (TS-769)

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

The Peer Review Committee met on January 13, 1988, to examine the issues raised by the Science Advisory Panel (SAP) with respect to the classification of the carcinogenicity of Acifluorfen.

A. Individuals in Attendance

1. Peer Review Committee: (Signature indicates concurrence with the peer review unless otherwise stated.)

Theodore M. Farber — Theodore M. Farber
William Burnam — William L. Burnam
Robert Beliles — Robert Beliles
Richard Hill — Richard Hill
Esther Rinde — Esther Rinde
John Quest — John A. Quest
Richard Levy — Richard A. Levy
Marion Copley — Marion Copley
Kerry Dearfield — Kerry Dearfield
Judith Hauswirth — Judith W. Hauswirth

2. Scientific Reviewers: (Non-committee members responsible for presentation of data; signature indicates technical accuracy of panel report.)

Whang Phang — Whang Fang 2/10/88

Marcia van Gemert — M. van Gemert 2/10/88

C. J. Nelson — C J Nelson 2/11/88

3. Peer Review Members in Absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with overall conclusions of the Committee.)

Anne Barton — Anne Barton

Reto Engler — Reto Engler

Diane Beal

B. Initial Peer Review Meeting on Acifluorfen:

The Toxicology Branch Peer Review Committee met on June 10, 1987, to evaluate information relevant to the oncogenicity of Acifluorfen, the active ingredient of the herbicides Blazer and Tackle. The findings of the Committee from this meeting are summarized below:

1. Acifluorfen produced an elevated incidence of liver and stomach tumors in both sexes of $B6C3F_1$ mice.

- Liver adenomas, carcinomas, and adenomas/carcinomas combined were increased in males at dose levels both below (650 ppm) and above (2500 ppm) the MTD level, and in females at a dose level (2500 ppm) that approximated the MTD level.

- Stomach papillomas were increased in males at a dose level (2500 ppm) above the MTD and in females at a dose level (2500 ppm) that approximated the MTD.

2. Acifluorfen produced an elevated incidence of liver adenomas plus carcinomas combined in a second study in female CR-CD-1 mice at a dose level (270 ppm) that was below a MTD level. The observed incidences were: 7/80 or 8.7%, controls; 5/69 or 7.2%, 7.5 ppm; 4/80 or 5%, 45 ppm; and 15/66 or 22.7%, 270 ppm. It should be noted that the doses of Acifluorfen tested in this study in CR-CD-1 mice were much lower than those examined in $B6C3F_1$ mice where more pronounced oncogenic responses were observed.

3. No compound-related oncogenic effects were observed in a third study in F344 rats where the high dose level was in excess of an MTD.

4. Acifluorfen was positive for mutagenic activity in tests in insects and yeast, but negative in tests using mammalian cell in vivo and in culture.

5. Acifluorfen is structurally similar to six other diphenyl-ether type herbicides (Lactofen, Oxyfluorfen, Fomesafen, Nitrofen, Fusilade, and Haloxyfop-methyl). All six of these analogues have been tested for oncogenicity in mice ($B6C3F_1$ or CR-CD-1 strains) and have been shown to produce liver tumors. Some of them (Lactofen and Nitrofen) were also oncogenic in the rat.

6. Acifluorfen is a metabolite of Lactofen, a Category B_2 oncogen, in rodents.

The Peer Review Committee, applying the Agency's Guidelines for Carcinogen Risk Assessment, categorized Acifluorfen as a Category B_2 oncogen. The primary criteria employed for this classification were the production of an increased number of malignant, or combined malignant and benign tumors, of the liver in multiple strains of animals and in multiple experiments. Also noted were the facts that Acifluorfen produced uncommon stomach tumors in one of the mouse strains, that it showed some inherent mutagenic activity, and that it had strong structure -activity correlations to other oncogens.

C. Science Advisory Panel Meeting on Acifluorfen:

The above findings were presented to the EPA's Scientific Advisory Panel (SAP) on December 15, 1987. The SAP concluded that the weight of the evidence for Acifluorfen could be interpreted as supporting either a B_2 or C classification. They believed that the data supporting the B_2 classification was strongly dependent on the increased incidence of liver tumors (15/66 or 22.7% incidence of adenomas/carcinomas combined) seen at 270 ppm in female CR-CD-1 mice. They noted that this data was weak at best, and that it was called into further question during the SAP meeting, when it was indicated that a GLP audit reduced the number of liver tumors to a non-significant value. The latter point was raised during the SAP verbal comment period by Dr. Richard Kocslow of the Rohm and Haas Company. He indicated that his firm, which sold the registration of Acifluorfen (Blazer) to the BASF Corporation, had previously conducted a GLP audit on the CR-CD-1 mouse study. This audit was reported to reduce the liver adenomas/carcinoma incidence of 15/66 or 22.7% in females at 270 ppm to 14/69 or 20.2%, a value which was said to be no

longer statistically significant. The SAP indicated that if this contention was true, then a Category C classification would be more appropriate for Acifluorfen. (Note: A subsequent recalculation of the value of 14/69 liver tumors using the Fisher Exact test was reported to be significant from control at the $p < 0.05$ level by the Toxicology Branch Biostatistics Team.)

D. Follow-Up Peer Review Meeting on Acifluorfen:

Subsequent to the SAP meeting, the Toxicology Branch Peer Review Committee reconvened on on January 13, 1988, and reached the consensus conclusion that Acifluorfen should continued to be categorized as a B_2 oncogen (probable human carcinogen) for the following two reasons:

1. The Toxicology Branch has not received information from any registrant (Rohm and Haas, or BASF) of Acifluorfen regarding the reported GLP audit change in the incidence of liver tumors in high dose CR-CD-1 female mice. Our Registration Division (R. Mountfort) was reported by Dr. Phang to have made some inquiries of Rohm and Haas about the GLP audit, but the replies received were said to be incomplete. (The Committee did not have concise information available to them at this meeting but follow-up efforts will be made.) In the absence of GLP data from the Acifluorfen registrant(s), the Peer Review Committee was reluctant to alter the B_2 classification based on informally received comments.

2. Even if confirmatory GLP data ~~is~~ are received to negate the elevated tumor incidence in the CR-CD-1 mouse, the Committee believed that the toxicology information in the $B6C3F_1$ mouse nevertheless remains sufficiently robust to maintain it in the B_2 category. For example:

e) Benign and malignant liver tumors were significantly elevated (at "p" values of <0.01) in both sexes of $B6C3F_1$ mice, and were seen at more than one dose level (below and above the MTD) in males and at the high dose level (which approximated a MTD) in females. The observed increases frequently exceeded historical control rates.

b) In addition to liver tumors, uncommonly occurring stomach papillomas were also observed in both sexes of $B6C3F_1$ mice at the highest dose level tested in males (over the MTD) and females (at the approximated MTD). The observed incidences (10% in males and 13% in females) greatly exceeded the concurrent control rates (0% in males and

0% in females) and historical control rates (0.3% in males and 0.5% in females). The benign stomach tumors were noted to have the ability to progress to the malignant state.

c) Finally, as indicated previously, positive data from structure-activity correlations involving six other chemicals, positive mutagenicity tests, and metabolism data (Acifluorfen being a metabolite of Lactofen, a B_2 oncogen) also collectively support the oncogenicity findings in the $B6C3F_1$ mouse.

E. Conclusion of Peer Review Committee:

In summary, the Peer Review Committee considered the overall weight of the evidence on Acifluorfen in $B6C3F_1$ mice to be sufficient to categorize the chemical as a Category B_2 oncogen. The SAP indicated that the data from the CR-CD-1 female mouse appeared to be necessary to retain Acifluorfen in the B_2 category. However, the Peer Review Committee believed that even without this data the finding of a relatively high incidence of uncommonly occurring benign stomach papillomas (which have the ability to progress to the malignant type) plus the occurrence of benign and malignant liver tumors tumors in $B6C3F_1$ mice of both sexes, along with supporting structure-activity and metabolism data, was adequate to retain the compound in this oncogenicity category as indicated in our current guidelines.

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rew:2/9/88-sp

1/4/88

007698

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

JAN 4 1988

FILE COPY

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Reevaluation of Classification of Carcinogenicity of Pesticides Based on SAP Review of Previously Evaluated Chemicals

FROM: Reto Engler, Chief JA Squeet
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: Addressees

On December 15, 1987, the SAP reviewed the evidence on seven (7) chemicals which were previously evaluated by the Peer Review process. For these chemicals where the SAP disagreed with our previous evaluation we are now required to formulate and support our final evaluation by either agreeing with the SAP evaluation or upholding our previous evaluation. For each of the following chemicals the peer-review document and the SAP conclusion is attached.

Chemical	Classification Peer Review	SAP	Comments Issues
Aciflurofen	B_2	Possible B_2 C more appropriate	GLP of mouse study
Assure	C	E	Tumor response above MTD
Oxadixyl	C	C	1) Quantification of risk based on benign tumors 2) MTD of mouse study
Terbutryn	C	C	Quantification of risk

The SAP also disagreed on the original classification of Savey (B_2/C) and classified it as C. Although new information is still under review by the Toxicology Branch this chemical will also be discussed by the Committee at this meeting. In addition, 2,4-D will also be scheduled for discussion.

8

For Baytan and Methidathion, the SAP raised no issues requiring further evaluation.

A meeting to discuss the issues on Aciflurofen, Assure, Oxadixyl, Terbutryn, Savey and 2,4-D has been scheduled for Wednesday, January 13, 1988, from 9:00 to 3:30 (lunch 12:00 to 1:00) in Dr. Farber's office (CM-2, Rm. 821).

Attachments

ADDRESSEES

T. Farber
W. Burnam
J. Quest
E. Rinde
J. Hauswirth
W. Phang
M. Van Gemert
R. Levy
A. Barton
D. Barnes/R. Hill
K. Dearfield
R. Beliles
M. Copley

#23 1/4/88:sp

9/30/87

007698

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

FILE COPY

SEP 30 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Acifluorfen

FROM: John A. Quest, Ph.D. JAQ
Team Leader, Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Richard Mountford, Product Manager #23
Registration Division (TS-767)

The Toxicology Branch Peer Review Committee met on June 10, 1987 to review the toxicology data base on Acifluorfen. Attention was focused on the oncogenic potential of the chemical in mice.

A. Individuals in Attendance

1. Peer Review Committee: (Signature indicates concurrence with the peer review unless otherwise stated.)

Robert Beliles — Robert Beliles

William Burnam — William L Burnam

Reto Engler — Reto Engler

Theodore M. Farber — Theodore M. Farber

Judith Hauswirth — Judith W. Hauswirth

Richard Levy — Richard A. Levy

John A. Quest — John A. Quest

Esther Rinde — Esther Rinde

2. Scientific Reviewers: (Noncommittee members responsible for presentation of data; signature indicates technical accuracy of panel report.)

Whang Phang — Whang Phang 9/2/87

3. Peer Review Members in Absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with overall conclusions of the Committee.)

Anne Barton _____

Richard Hill _____

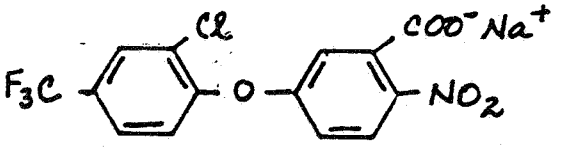
B. Material Reviewed

The material reviewed consisted of data summaries and one-liners prepared by the reviewer, and an updated qualitative risk assessment on Acifluorfen prepared by the Toxicology Branch Biostatistics Team. A copy of this information is attached to this report.

C. Background Information

Acifluorfen is the active ingredient of two herbicides, Tackle and Blazer, which are manufactured by Rhone-Poulenc, Inc. and Rohm & Haas, Inc., respectively. Both Tackle and Blazer are currently registered for use on food crops. Toxicological data are available on both products; however, the data on Tackle are more complete and acceptable. Therefore, discussions of the toxicology of Acifluorfen are predominantly based upon the data derived from the studies with Tackle, and where appropriate the data on Blazer is also utilized. It should be noted that Tackle contains about 20% to 24% of acifluorfen as the active ingredient, whereas Blazer contains approximately 40% of acifluorfen. Oncogenicity studies have been performed with Blazer/Tackle in mice and rats. The primary focus of the Peer-Review Committee was on the studies conducted in mice, where liver and stomach tumors were reported.

Structure:



(sodium 5-[2-chloro-4-(trifluoromethylethyl)phenoxy]-2-nitrobenzoate)

D. Evaluation of Oncogenicity Studies:

1. 18-Month Mouse Oncogenicity Study of Tackle:

Reference: Eighteen Month Mouse Oncogenicity Study of Tackle, Gulf South Research Institute (GSRI Project No. 413-984-41), November 3, 1982.

Tackle was administered in the diet to groups of 60 B6C3F1 mice/sex/dose level at levels of 0, 625, 1250 and 2500 ppm for 18 months. In each of the above experimental groups, 10 mice/sex were subjected to interim sacrifice at 12 months. The study, conducted by IRDC and completed in 1982, has been audited by EPA and classified as Core Minimum. Table 1 shows the increased incidence patterns of liver and stomach tumors in male and female mice.

TABLE 1 - Tumor Incidence in Tackle Treated B6C3F1 Mice†

	0 ppm	625 ppm	1250 ppm	2500 ppm
Male				
Liver:				
Adenomas	8/58(14)**	18/60(30)*	12/56(21)	25/59(42)**
Carcinomas	1/48(2)**	3/50(6)	4/46(9)	15/44(34)**
Ad/or Car	9/58(16)**	21/60(35)*	16/56(29)	40/59(68)**
Stomach papillomas	0/49(0)**	0/46(0)	0/43(0)	4/40(10)*
Female				
Liver:				
Adenomas	1/55(1)**	5/59(5)	4/57(4)	19/58(19)**
Carcinoma	0/45(0)**	1/47(2)	1/44(2)	5/46(11)*
Ad/or Car	1/55(2)**	6/59(10)	5/57(9)	24/58(41)**
Stomach papillomas	0/45(0)*	3/48(6)	3/44(7)	6/45(13)*

† : Tumor bearing animals/animal at risk (i.e., the animals, which died prior to the week of the first tumor occurrance for each tumor type, are removed from the number of at risk.

( )= Percent.

NOTE: Significance of trend analysis (Cochran-Armitage Trend Test) dencted at Controls: significance of pairwise comparison with control (Fisher's Exact Test) denoted at Dose level. * = $p < 0.05$ and ** = $p < 0.01$.

Tackle was associated with statistically significant positive trends for liver tumors (adenomas, carcinomas, and adenomas/carcinomas combined) and stomach tumors (papillomas) in both male and in female B6C3F1 mice. In addition, all of these tumor types were significantly increased at the highest dose level tested (2500 ppm) in male and female mice, and the liver tumors were also significantly increased at the lowest dose level tested (625 ppm) in male mice.

The increased incidences of liver adenomas/carcinomas combined in male (i.e., 68%) and female (i.e., 41%) B6C3F1 mice at the 2500 ppm dose level of Tackle exceeded historical control incidences of these tumor types in male (range: 16% - 58%) and female (range: 0% - 20%) B6C3F1 mice in studies conducted by the NTP (Handbook of Carcinogen Testing, H.A. Milman and E.K. Weisburger, eds., Noyes Publications, Park Ridge, N.J., pp 291, 1985). Similarly, the increased incidences of stomach papillomas produced in male (i.e. 10%) and female (i.e. 13%) B6C3F1 mice by the 2500 ppm dose level of Tackle exceeded the historical control incidences of this tumor type in male (0.3%) and female (0.5%) B6C3F1 mice in NTP studies (Haseman, et al., Toxicol. Pathol. 12: 126-135, 1984; no range data was available in this publication).

The highest dose tested in male mice (2500 ppm) exceeded a MTD level, based on findings of significantly increased mortality and reduced body weight gain (-18% to -23% throughout the study), as well as increases in liver weight and the presence of white foci and/or ulcers in the stomach of occasional animals. Doses of 625 and 1250 ppm did not exceed a MTD level in male mice; however, the Committee noted that a significant increase in liver tumors did occur at the 625 ppm dose level.

The highest dose tested in female mice (2500 ppm) was considered to approximate a MTD level, based on findings of a -14% reduction in body weight gain at week 13 of the chronic study, as well as increases in liver weight and the presence of white foci and/or ulcers in the stomach of occasional animals. Increased mortality was not observed in female mice.

2. 24-Month Mouse Oncogenicity Study of Blazer:

Reference: Two Year Feeding/Oncogenicity Study of Blazer in Mice. International Research and Development Corporation (Report No. 285-013a), March 6, 1979.

Blazer was administered in the diet to groups of 80 CR CD-1 mice/sex/dose level at levels of 0, 7.5, 45 and 270 ppm for 24 months. The highest dose level was initially administered to mice as a dose of 1.25 ppm on study weeks 1 to 16 before being increased to 270 ppm. In each of the above experimental groups, 10 mice/sex/group were subjected to interim sacrifice at 3 months and at 12 months. Table 2 shows the increased incidence pattern of liver tumors observed in female mice.

TABLE 2 - Tumors Incidence in Blazer-Treated CR CD-1 Mice

	0 ppm	7.5 ppm	45 ppm	270 ppm
Female				
Liver:				
Adenomas	5/80	2/69	4/80	11/66
Carcinomas	1/80	2/69	0/80	3/66
Ad and Car	1/80	1/69	0/80	1/66
Total (Ad or Car)	7/80	5/69	4/80	15/66*
Male				
Liver:				
Adenomas	9/79	3/69	14/80	12/70
Carcinoma	8/79	12/69	11/80	8/70
Ad and Car	2/79	3/69	3/80	7/70
Total (Ad or Car)	19/79	18/69	28/80	27/70

* = p= 0.05

Blazer produced a significant (p = 0.05) increase in the total number of liver tumors (primarily adenomas) in high dose (270 ppm) female mice. No significant increase in liver tumors occurred in male mice. No historical control data was available.

The highest dose tested in male and female mice (270 ppm) did not approximate a MTD level. The only toxicologic signs seen in this study included increased liver enzyme (alkaline phosphatase, SGPT) activities and liver weights in mid and high dose male mice, and increased kidney weights in high dose males.

3. 24-Montn Rat Oncogenicity Study of Tackle:

Reference: Two-Year Feeding/Oncogenicity Study in Kats. Gulf South Research Institute (Project No. 413-985-41), 1983.

Tackle was administered in the diet to groups of 73 F344 rats/sex/dose level at levels of 0, 25, 150, 500, 2500 and 5000 ppm for 2 years. The highest dose level was initially administered to rats as a dose of 10 ppm on study weeks 1 to 4 before being increased to 5000 ppm. In each of the above experimental groups, 8 rats/sex/group were subjected to interim sacrifice at 12 months. No oncogenic effects attributable to compound administration were noted in either male or female rats.

The highest dose tested in male and female rats (5000 ppm) exceeded a MTD level, based on findings of reduced weight gain, increased mortality, increased liver weights and liver enzyme changes (alkaline phosphatase, BUN, creatinine), renal changes (nephritis, pyelonephritis, glomerulonephritis), stomach ulcers and decreased testes size. Many of these changes were also seen at the 2500 ppm dose level as well (e.g., reduced weight gain in males and females, and liver, renal and stomach changes in females).

Another rat chronic feeding study with Blazer was also conducted by Rohm and Haas. The results indicated no increase in any tumor incidence in treated animals relative to the controls. However, the experimental design of the study used multiple shifts in dose levels at different durations of treatment throughtout the study. Therefore, it is difficult to establish at what dose levels or times that changes in biological parameters occur.

E. Additional Toxicology Information:

1. Mutagenicity:

Tackle produced positive results for gene mutations in D. melanogaster, for chromosome abberations in the sex-chromosome loss Assay in D. melanogaster, and for DNA damage in the mitotic recombination assay in yeast strain D5. The chemical was negative in other assays (e.g., gene

mutation in murine lymphoma cells; unscheduled DNA synthesis and DNA repair assays with mouse and rat hepatocytes; and metaphase analysis in rat bone marrow cells and dominant lethal assay in rats).

2. Reproduction and Teratology:

Tackle was not shown to be teratogenic in rats and rabbits at doses ranging from 36 to 90 mg/kg/day. In a 2-generation reproduction study in rats (0,25,500 and 2500 ppm), no adverse effects on adult reproductive performance occurred at doses up to 2500 ppm. However, reductions in body weight gain and viability occurred in high dose offspring, and increased incidences of renal pelvic dilation were seen in mid and high dose P1 and F1 females, in high dose F1 males, and in F2 offspring.

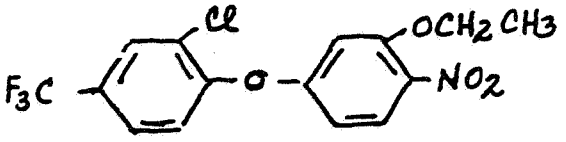
3. Metabolism:

Pharmacokinetic studies were performed in rats using radiolabelled acifluorfen. The compound is rapidly and almost completely absorbed from the GI tract within 96 hours after oral administration. No unusual organ or tissue accumulation of either the parent compound or metabolites occurred. About 70-97% of the administered radioactivity is excreted over a period of 96 hours; most of this (i.e., 46-82%) is found in the urine whereas smaller amounts (i.e., 5-41%) are found in the feces. Essentially all of the radioactivity excreted in the urine appears as unchanged acifluorfen, along with very small amounts of an amine metabolite and a glucuronide moiety. In contrast, the majority of the radioactivity excreted in the feces appears as the amine metabolite, along with smaller amounts of unchanged acifluorfen and acetamide.

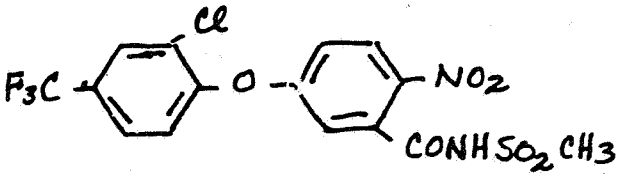
The Committee noted that no metabolism studies were performed in mice, the species in which tumors were found following the administration of both Tackle and Blazer.

4. Structure-Activity Relationships:

Acifluorfen is structurally related to the following four diphenyl ether type compounds, most of which are pesticidal in nature.



OXYFLUORFEN



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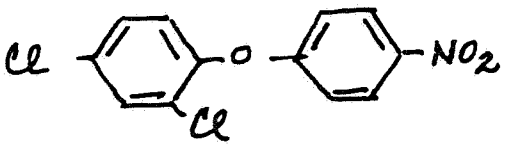
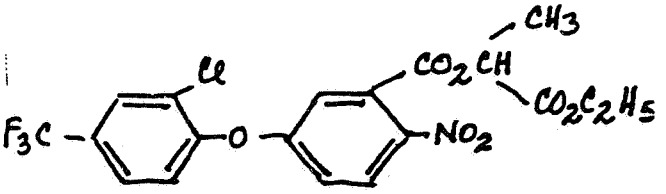


NITROFEN



LACTOFEN

The following information regarding oncogenicity testing of these chemicals was available to the Committee: (1) Oxyfluorfen produces hepatocellular carcinomas and liver hyperplastic nodules in male and female CR-CD-1 mice, respectively; (2) Fomesafen produces liver adenomas and carcinomas in both sexes of CR-CD-1 mice and has been classified as a Category C oncogen; (3) Nitrofen produces hepatocellular carcinomas in both sexes of B6C3F1 mice and pancreatic carcinomas in male rats; and (4) Lactofen procuces liver adenomas and carcinomas in both sexes of CR-CD-1 mice and liver neoplastic nodules in both sexes SD-CD rats, and has been classified as a Category B2 oncogen.

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Acifluorfen (i.e., Tackle/Blazer) to be of importance in a weight of the evidence determination of oncogenic potential.

1. Administration of Tackle was associated with an increased incidence of hepatocellular tumors (adenomas, carcinomas, and adenomas/carcinomas combined) and stomach papillomas in male and female B6C3F1 mice.

2. Liver and stomach tumors were seen in male B6C3F1 mice at a dose level (i.e., 2500 ppm) that exceeded a MTD level; the liver tumors also occurred at a dose level (i.e., 625 ppm) that was below a MTD level. In addition, liver and stomach tumors were seen in female B6C3F1 mice at a dose level (i.e., 2500 ppm) that approximated a MTD level.

3. The increased incidence of liver and stomach tumors produced by 2500 ppm Tackle in B6C3F1 mice of both sexes exceeded historical control range incidences for the same tumor types in recent studies conducted by NTP.

4. Administration of Blazer was also associated with an increased incidence of hepatocellular tumors (primarily adenomas) in a second strain of mice, namely female CR-CD-1 mice. The liver tumors occurred at the highest dose of Blazer (i.e., 270 ppm) tested, which was below a MTD level. No historical control data was available.

5. Tackle was not oncogenic when administered to F344 rats. The highest dose of Tackle (i.e., 5000 ppm) used in this test exceeded a MTD level.

6. Evidence for a mutagenic effect of Acifluorfen was provided by positive findings for gene mutations and chromosome abberations in D. melanogaster, and for DNA damage in yeast. Acifluorfen, however, was negative in other assays.

7. Tackle was not teratogenic in rats or rabbits. In a 2-generation reproduction study in rats, it caused reduced weight gain and viability, and renal pelvic dilation in offsprings.

8. No metabolism studies of Acifluorfen were performed in mice, the species where liver and stomach tumors were seen. Studies were conducted in rats, however, and the chemical was shown to be well absorbed, not to accumulate in specific body tissues, and to be excreted to the extent of 70% to 90% in urine (mainly as unchanged parent compound) and feces (mainly as the amine metabolite) over a period of 96 hours.

9. Acifluorfen is structurally related to four other diphenyl ether-type herbicides: oxyfluorfen, fomesafen, nitrofen and lactofen. All four chemicals have been tested for oncogenicity in mice (CR-CD-1 or B6C3F1 strains) and have been shown to produce liver tumors. In addition, liver neoplastic nodules were also evoked in rats in one case (lactofen) and pancreatic tumors in rats in another case (nitrofen).

G. Classification of Oncogenic Potential:

The Committee concluded that the data available for Acifluorfen (i.e., Tackle/Blazer) provided sufficient evidence for the oncogenicity of the chemical in mice. According to EPA Guidelines for Carcinogen Risk Assessment (CFR, September 24, 1986), the Committee classified Acifluorfen as a Category B_2 oncogen (probable human carcinogen). That is, the chemical produced an increased incidence of combined malignant and benign liver tumors in two different studies employing different strains (B6C3F1 and CR-CD-1) of mice. The compound also displayed positive mutagenic activity in several non-mammalian test systems, and is structurally similar to four other diphenyl ether herbicide compounds which cause increased incidences of liver tumors in B6C3F1 and CR-CD-1 mice.

#20 8/5/87 sp

11/23/83

007698

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

NOV 23 1983

MEMORANDUM

SUBJECT: Re-Review of The 24-Month Feeding/Oncogenicity Study in Mice Using Blazer. Caswell No. 755D

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

THRU: William Butler, Section Head
Toxicology Branch
Hazard Evaluation Division, (TS-767)
and
William Burnam, Branch Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

11-21-83

WDB 11-23-83

Registrant: Rohm-Haas

Background:

Blazer is structurally related to a series of chemicals which have been found to have either teratogenic, oncogenic and/or mutagenic potential (Tok, Goal, Flex, RH-0265, Hoelon, Fusiland and Tackle).

Tackle has the identical chemical structure as Blazer. Blazer which is registered by Rohm-Haas, has established permanent tolerances in or on the following (180.383):

-liver and kidney of cattle, goats, hogs, horses, sheep at 0.02 ppm

-meat, fat, meat byproducts of poultry at 0.02 ppm

-milk and eggs at 0.02 ppm

-soybeans at 0.1 ppm

RECOMMENDATION:

An oncogenic risk assessment is currently underway because an oncogenic potential was demonstrated in mice being fed Blazer for 24 months (International Research and Development Corp, Report No. 285-013a, dated March 6, 1979). As indicated in the appended review, a statistically significant (Chi-square) increase in liver tumors were observed in the high dose (270/1.25 ppm) females when compared to concurrent water controls. The following table summarizes the liver tumors:

Summary Of Liver Tumors In Mice Fed Blazer (24 months)

	Male Mice				Female Mice			
Dose (ppm)	a/ 0	7.5	45.0	270(1.25) b/	a/ 0	7.5	45.0	270(1.25) b/
# Livers examined		69	80	70	89	69	80	66
# Livers with carcinoma only	8	12	11	8	1	3	0	3
# Livers with adenoma only	9	3	14	12	5	1	4	11
# Livers with carcinoma and adenoma	2	3	3	7	1	1	0	1
Total # Livers with Tumors	19/79	18/69	28/80	27/70	7/80	5/69	4/80	15/66*

a/ Water control
b/ Animals fed 1.25 ppm until week 17 and then changed to 270 ppm until termination
* P = 0.05

NOTE: CHANGES IN TABLE [illegible] (CORROBORATED) WITH DHUMATT (11-21-83)

These above data are based on the individual animal data provided in the report and not the summary tables provided in the report, as there appear to be some counting discrepancies. Due to these discrepancies and the critical importance of this study to accurately establish the oncogenic potential of Blazer, a data audit has been formally requested. In the meantime the registrant should be requested to provide (a) either an applicator (mixer/loader/applicator) exposure study or estimates of applicator exposure for each application technique and use, and (b) either a dermal penetration study or estimate of dermal penetration with rationale.

Carolyn Gregorio, Toxicologist CAG 10-6-83
Toxicology Branch/HED (TS-769

Tox 41:C Gregorio:LM:9/22/83:11745
Revised:DCR-32801:Tox 41:C Gregorio:bje:9/28/83:11745
REVISED:DCR-32804:CBI 5 TOX:C Gregorio:10/5/83:LM

Study/Lab/Study #/Date	Material	No.	LD_{50}, LC_{50}, PIS, NOEL, LEL	Cat. Category	Guid. Grade/ Doc. No.
Teratology- rabbit; Argus Research Labs; #113-003; 12/30/81	Tackle 2S (22.4% purity)	071319	Maternal NOEL > 36 mg/kg/day (HDT) Fetotoxic NOEL > 36 mg/kg/day (HDT) Teratogenic NOEL > 36 mg/kg/day (HDT) Levels tested by gavage to New Zealand White strain - 0, 3, 12, or 36 mg/kg/day.		Minimum 003556
Teratology- rat; Argus Research Labs; #113-004; 4/24/81	Tackle 2S (22.4% purity)	071319	Maternal NOEL = 90 mg/kg/day Maternal LEL = 180 mg/kg/day (HDT) (lower body weight) Fetotoxic NOEL = 20 mg/kg/day (LDT) Fetotoxic LEL = 90 mg/kg/day (reduced mean fetal weights) Teratogenic NOEL>180 mg/kg/day (HDT) Levels tested by gavage in CrL: COBS CD (SD) BR strain - 0, 20, 90, and 180 mg/kg/day.		Guideline 003556
90 Day Feeding- rat; Gulf South Research Institute; #413-971-40; 7/16/82	Tackle 2S (20.4-23.6% purity	071308 252826	NOEL = 320 ppm LEL = 1250 ppm (liver cell hypertrophy). Levels tested in young Fisher 344 strain - 0, 20, 80, 320, 1250, 2500, and 5000 ppm.		Supplementary 003556 Supplementary 003963
90 Day Feeding- mice; Gulf South Research Institute; #513-971-40; 7/16/82	Tackle 2S 20.4-23.2% purity)	071308 252826	NOEL = 320 ppm LEL = 1250 ppm (fatty intiltration of liver) Levels tested in young B6C3F1 strain 0, 20, 80, 320, 1250, 2500 and 5000 ppm		Supplementary 003556 Supplementary 003963
21 Day dermal - rabbit; Food and Drug Research Labs; #6718: 2/5/81	Tackle 2S (21.1% purity)	071311 252826	Systemic NOEL = 300 mg/kg/day Systemic LEL = 1000 mg/kg/day (19/20 animals died by day 8). Skin irritation NOEL < 100 mg/kg/day (edema and irritation) Levels tested in New Zealand white strain - 0, 100, 300 & 1000 mg/kg		Supplementary 003556 Minimum 003963

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
18-Month feeding/onco-mice; Gulf South Res. Inst.; GSRI #413-984-41; 11/3/82	Tackle 2S (24% Purity)	071312 071313 071314 250463 250464 252826	Liver tumors at 625, 1250 & 2500 ppm males and 2500 ppm (HDT) females Benign papilloma of stomach at 625, 1250, 2500 ppm (HDT) females and 2500 ppm (HDT) males. Oncogenic potential at all doses tested. Systemic effects (reduced body wt. for all treatment groups; increase WBC, RBC, lymphocytes & decrease MCV and segmented neutrophies in males) Levels tested in B6C3F1 strain = 0, 625, 1250 and 2500 ppm. [Recommended for data audit] Oncogenic NOEL < 625 ppm (LDT) Systemic NOEL < 625 ppm (LDT)		Supplementary 003409 Minimum 003963
2-Year feeding/onco-rat; Gulf South Research Inst.; #413-985-41; 3/30/83	Tackle 2S (19.1-25.6%) Purity	071315 071316 071317 250289 250290 250291 250292 252826	Oncogenic NOEL > 5000 ppm (HDT) Systemic NOEL = 500 ppm Systemic LEL = 2500 ppm (elevated bun and creatinine in males and females; nephritis and pyleneptritis of kidney in males. Levels tested = 0, 25, 150, 500, 2500 and 5000 ppm in the Fisher 344 strain. [Recommended for data audit]		Supplementary 003409 Supplementary 003963

24

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Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
2 Year feeding - dog; International Research and Development;450-039; 6/30/83	Tackle 2S 74.5 - 82.8% purity	251297 251298	NOEL = 20 ppm. LEL = 300 ppm (increased leukocyte count and platelet count in males; calcium, cholesterol and creatinine were decreased in females). Levels tested by diet in 3 month old beagles - 0, 20, 300 and 4500 ppm.		Supplementary 003723
Metabolism - DNA binding index - rat; Toxicology Lab, SRI International; #CSC-5573-4; 6/83	Aciflourofen lot #GF-266049-1	251293	purity of test material was not listed whole body half life = 21 hrs (acceptable)		Unacceptable 003723
Metabolism DNA binding index - mice; Toxicology Lab, SRI International; #CSC-5573-5; 6/83	Acitlurofen lot #GF-266049-1	251293	purity of test material was not listed whole body half life = 18 hrs (acceptable)		Unacceptable 003723
Metabolism - rat; Mobil Environ. Health Science Lab; #63281; 9/30/82	C^{13} or C^{14} labeled Acitluorfensodium Acid (99% purity)	071321 252826	Absorbtion: single low (16-17 mg/kg) single high (116 mg/kg), multiple low (10-12 mg/kg/day for 14 days). Doses demonstrated 70-97% excreted in males and females within 96 hrs. Distribution: very low tissue retention (0.13-3.08%) Metabolites: unchanged acitluorfen in blood (95-98%), urine (95%), and bile (93%). Amine metabolite in feces (60-80%). Excretion: Females: 60-82% in urine; 5-23% in feces within 96 hrs. Males: 46-58% in urine; 21-41% in feces within 96hrs.		Unacceptable 003556 Acceptable 003963
Mutagenic - unscheduled DNA synthesis -rat; Litton Bionetics; #1022-80; 4/29/81	Tackle 2S (purity unspecified)	071318	Negative in rat hepatocytes at doses 0.10-50.0 ug/ml.		Acceptable 003556

Study/Lab/Study #/Date	Material	No.	LD_{50}, LC_{50}, PIS, NOEL, LEL	Category	Doc. No.
Mutagenic - bone marrow-rat; Mobil Environmental and Health Science Labs; #1041-80; 3/13/81	Tackle 2S (purity unspecified)	071318	Negative in bone marrow cells in rats at doses 0.37, 1.11, or 1.87 g/kg of Tackle 2S.		Acceptable 003556
Mutagenic - dominant lethal- rat; Microbiological Assoc.; #11689-116; 9/23/81	Tackle 2S	071318	Negative at doses 80, 360, 800 mg/kg/day of Tackle 2S. Levels tested by gavage - 80, 360, and 800 mg/kg/day.		Acceptable 003556
Mutagenic- forward mutations (murine lymphoma cell line); Mobile Environmental Health Services Lab; #512-80; 11/12/80	Tackle 2S	071318 252826	Negative with/without activation.		Unacceptable 003556 Acceptable 003963
Mutagenic- drosophila; EG & G. Mason Research Institute and Utah State University; #009-275-533-9; 4/13/81	Tackle 2S	071318	Negative for somatic reversion of white-ivory, Bithorax test of Lewis, sex-lethals. Positive for Y-chromosome loss and dominant lethal mutations.		Acceptable 003556
Mutagenic- genetic recombination (yeast strain D_5); Litton Bionetics; #20988; 1/81	Tackle 2S 29.7% purity	071318	Positive at 5.0 and 10.0 ul/plate with/without activation.		Acceptable 003556
Mutagenic - DNA synthesis - mice; Toxicology Lab, SRI International; #LSU-83-16; 5/23/83	TACKLE lot # 25300D02201	251292	Tackle and an aqueous and a hexane extract of tackle were negative. Levels tested for tackle = 10,40 and 200 mg/kg. Levels tested for aqueous extract = 20, 150 and 750 mg/kg. Levels tested for hexane extract 50, 200, 700 and 1000 mg/kg by gavage. Purity of test material was not listed.		Unacceptable 003723

TOX CHEM NO. 0108

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic - DNA synthesis - rat; Toxicology Lab, SRI International; #LSU-83-16; 5/23/83	Tackle lot # 25300D02201	251292	Tackle and an aqueous and a hexane extract of tackle were negative. Levels tested for Tackle = 10,40 and 200 mg/kg/day by gavage. Purity of test material was not listed.		Unacceptable 003723
Acute oral LD_{50}-rat; Toxigenics, Inc.; #410-0249; 10/13/80	Tackle 2S (purity: 20.2%)	071306	LD_{50} (male): 2025 mg/kg LD_{50} (female): 1370 mg/kg	III	Guideline 003556
Acute dermal LD_{50}-rabbit; Toxigenics,Inc.; #410-0250; 7/23/80	Tackle 2S (purity: 20.2%)	071306	LD_{50} > 2.0 g/kg	III	Minimum 003556
Acute inhalation LC_{50}-rat; Toxigenics, Inc.; #420-0251; 7/11/80	Tackle 2AS (purity: 20.3%)	071306	LC_{50} > 6.9 mg/L/4 hrs.	III	Minimum 003556
Primary eye irritation-rabbit; Toxigenics,Inc.; #410-0252; 8/7/80	Tackle 2S (purity: 20.3%)	071306	Pannus of cornea in 1/3 of animals (washed) at day 13 observation. Pannus of cornea in 2/6 animals (non-washed) at day 10 observation.	I	Minimum 003556
Primary dermal irritation- rabbit; Toxigenics Inc.; #410-0286; 9/8/80	Tackle 2S (purity: 20.2%)	071306	Dermal irritation and edema persisted through day 5 for both abraded and non-abraded skin. Skin normal at day 7 - intact skin Desquamation at day 8 - abraded skin	II	Minimum 003556
Dermal sensitization-guinea pig; Food and Drug Res. Labs; #6738; 1/23/81	Tackle 2S (purity: not specified)	071306 252826	Not a sensitizer		Supplementary 003556 Supplementary 003963

27

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Dermal absorption-rat; Hazleton; 6188-101; 1/3/86	2AS (20.3%) Pure ^{14}C labeled	260951	Dermal absorption following 10.hrs exposure = 0.02% for doses of 2.1-3.3, 0.21-0.33 & 0.021-0.033mg/cm^2. Residue on skin = 1.30, 20.0 & 43.5 % of doses. Additional work to determine if remaining compound is absorbable is recommended.	N/A	Acceptable 004933
2-Generation reproduction - rat; Argus Research Lab; #218-002; 2/20/86	Tech Lot #305164001		Levels tested in Crl:COBS CD(SD)BR strain- 0, 25, 500 & 2500 ppm Parental NOEL = 25 ppm Parental LEL = 500 ppm(mortality & kidney lesions) Reproduction NOEL >2500 ppm(HDT) Offspring NOEL = 25 ppm Offspring LEL = 500 ppm(decreased viability & increased kidney lesions) At 2500 ppm - kidney lesions, reduced body weights in both parents and offspring		Minimum 005416
3-Generation reproduction - rat; Gulf South Res. Inst; 4/29/83	TACKLE 2S Lot #'s LCM 266821-3 LCM 266830-2 LCM 266830-4 RJH 276096 (purity approx. 77%)	071320 251294 251296	Levels tested by diet in Sprague Dawley strain - 0, 25, 500 and 2500 ppm		Invalid 003723

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - rabbit; Rohm & Haas Co; TRD 76P-11; 5/4/76	39.8% a.i. (2L) Aqueous Technical	095736	NOEL = 60 mg/kg (no toratological assessment possible at higher dose level tested due to embryotoxicity and maternal toxicity. Maternal Toxic NOEL = 60 mg/kg Maternal Toxic LEL = 180 mg/kg (death) Fetotoxic NOEL = 60 mg/kg Fetotoxic LEL = 180 mg/kg (death)		Minimum
3-Generation reproduction/teratology- rat; Hazleton Lab; #417-374; 9/2/77	39.4% a.i. (2L) Aqueous Technical	095736	NOEL = 540 ppm (HDT) Teratology NOEL = 540 ppm (HDT)		Minimum
2 Year feeding-dog; Hazleton Lab; #417-357; 9/11/78	39.8% a.i. (2L) Aqueous Technical	097705	NOEL= 50 ppm LEL = 300 ppm (blood coagulation effects) Results (High Dose = 1800 ppm) (2-year dog feeding) diffuse bilateral grayish discoloration of the tapetum and reduced vascularity of the retina and optic disc. Marked increase in quantity of brown granular pigment in renal tubule epithelium and occasional instances of residual parenchymal damage consisting of interstitial fibrosis. Slight hyperplasia of the mucosal epithelium with papillary infoldings.		001099 Guideline ???

62 29

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Lifetime oncogenic/feeding - mice; IRDC;#285-013a; 3/16/79	39.8% a.i. (2L) Aqueous Technical	098025	Oncogenic NOEL > 270 ppm (HDT) Systemic NOEL = 45 ppm Systemic LEL = 270 ppm (Statistically significant SGOT-SGPT were seen at 12-months in the 270 ppm group.		Minimum 001099
Lifetime oncogenic/feeding - rat; Dawson Research Lab; DCR-5800; 10/26/78	39.8% a.i. (2L) Aqueous Technical	097704	Oncogenic NOEL > 1080 ppm (HDT) Systemic NOEL = 90 ppm		Minimum 001099 Minimum 001251
24-Month feeding/oncomice; International Res. and Development Corp.; #285-013a; 3/6/79	Blazer (Purity 39.4-40.5%)	Not available on hard copy	Oncogenic potential demostrated in females at 270/1.25 ppm Dose (HDT). Hepatocellular arenomas and carcinomas. Levels tested = 1.25/270*, 7.5 and 45 ppm. *1.25 ppm change to 270 ppm on week 17 Data audit requested 9/9/83		Supplementary 003410
Acute oral LD50 - rat; Rohm & Haas Co; TRD 74P-30; 8/28/74	70% a.i. Solid Technical	095736	LD_{50} = 1.30 g/kg (lethargy) (male)	III	Minimum
Acute dermal LD50-rabbit ; Rohm & haas Co; TRD 74P-31; 8/28/74	70% a.i. Solid Technical	095736	LD_{50} = 3.54 g/kg (lethargy) (male)	III	Minimum
Primary eye irritation-rabbit; Rohm & Haas Co; TRD 74P-22; 5/1/74	70% a.i. Solid Technical	095736	Corneal opacity (not reversible in 7 days). Lesions to cornea, iris - not reversible.	I	Minimum
Primary dermal irritation - rabbit; Rohm & Haas Co; TRD 74P-19; 5/22/74	70% a.i. Solid Technical	095736	Slight irritation	III	Minimum

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute oral LD50 - rat; Rohm & Haas Co; TRD 76P-171; 11/12/76	40% a.i. (2L) Aqueous Technical	095736	LD_{50} = 1.54 g/kg	III	
Acute oral LD50- dog; Rohm & Haas Co; TRD 76P-34; 12/17/76	40% a.i. (2L) Aqueous Technical		LD_{50} = 186 mg/kg (emesis)	II	
Acute dermal LD50- rabbit;Rohm & Hass Co. TRD 74P	40% a.i. (2L) Aqueous Technical	095736	LD_{50} = 3.68 g/kg (male)	III	
Acute inhalation LC_{50}- rat; Rohm & Haas Co; TRD 77P; 12/19/76	40% a.i. (2L) Aqueous Technical	095736	LC_{50} = 8.90 mg/l	IV	
Primary dermal irritation - rabbit; Rohm & Haas Co; TRD 74P-19; 12/23/76	40% a.i. (2L) Aqueous Technical		Moderate		
Risk assessment					001017
Deposition and metabolism - mice; Rohm & Haas; #81R-0281; April 29, 1985	Acifluorfen Technical 39.9% a.i.	257956	Acifluorfen is mainly excreted in urine and feces within 2 to 3 days.		Minimum 004699
Chronic Bioassay - mice; Dr. S. Vesselinovitch; April 23, 1985	Report only	257956	Correct number of animals at risk for liver tumors not provided.		Supplementary 004699
Dermal absorption - rat; Rohm & Haas; #85R-063; April 26, 1985	^{14}C Acifluorfen Lot# 363.10	257956	Additional data required.		Unacceptable 004699

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute oral LD_{50} - rat; Rohm & Haas Co.; #80R-0200; 5/12/82	(1) Sodium salt of acifluorfen 20.1% (2) Sodium salt of acifluorfen 21.4%	249794	LD_{50} M 4.83 g/kg F 4.13 g/kg	III	Guideline 005472
Acute dermal LD_{50}-rabbit; Rohm & Haas Co.;#80R-0200; 5/11/82	(1) Sodium salt of acifluorfen 20.1% (2) Sodium salt of acifluorfen 21.4%	249794	LD_{50} > 5.0 g/kg	III	Guideline 005472
Primary dermal irr.-rabbit; Rohm & Haas Co.; #80R-0200; 5/11/82	(1) Sodium salt of acifluorfen 20.1% (2) Sodium salt of acifluorfen 21.4%	249794	24 hrs.: 6/6 severe erythema & edema (sc. 3 & 4) 72 hrs.: slight to severe erythema & edema (sc. 1 to 4) Day 7: 6/6 slight to severe erythema (sc. 1 to 3); 2/6 slight edema (sc. 1); Irr. score 7	II	Guideline 005472
Primary eye irr.-rabbit; Rohm & Haas Co.; #80R-0200; 5/11/82	(1) Sodium salt of acifluorfen 20.1% (2) Sodium salt of acifluorfen 21.4%	249794	24 hrs.: 9/9 corneal opacity, iris irr., conjun. irr. (sc. 2.5 to 20) Day 7: 6/6 & 2/3 corneal opacity (sc. 5 to 80); conjun. irr. (sc. 2 to 8) Day 21: 4/6 corneal opacity (sc. 10 & 46); 2/6 conjun. irr. (sc. 2 to 8)	I	Guideline 005472
Primary dermal irr.-rabbit; Rohm & Haas Co.; #80R-0200; 5/11/82	(1) Sodium salt of acifluorfen 20.1% (2) Sodium salt of acifluorfen 21.4%	249794	Irr. score zero	IV	Guideline 005472

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary dermal irr.-rabbit; Rohm & Haas Co.: # 81R-0186; 10/19/81	(1) Sodium salt of acifluorfen 20.1% (2) Sodium salt of acifluorfen 21.4%	249794	24 hrs.: moderate to severe erythema and edema (sc. 1 to 4) 72 hrs.: moderate to severe erythema (sc. 1 to 4); slight to well-defined edema (sc. 1 & 2); Irr. score: 4.9		Supplementary 005472

007698

SAP Executive Summary

12/15/87

007698

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

December 23, 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Transmittal of the Final FIFRA Scientific Advisory Panel Report on the December 15, 1987 Meeting

TO: Douglas D. Campt, Director
Office of Pesticide Programs (TS-766C)

The above mentioned meeting of the FIFRA Scientific Advisory Panel (SAP) was an open meeting held in Arlington, Virginia to review the following topics:

1. A Set of Scientific Issues Being Considered by the Agency in Connection with the Special Review of Tributyltin (TBT);

2. A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review of Aciflurofen as a Class B-2 Oncogen;

3. A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review of Terbutryn as a Class C Oncogen;

4. A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review of Triadimenol (Baytan) as a Class C Oncogen;

5. A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review of Methidathion as a Class C Oncogen;

6. A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review of Oxadixyl as a Class C Oncogen;

7. A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review of Savey as a Class B-2/C Oncogen;

8. A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review of Assure as a Class C Onccgen.

Please find attached the Panel's final report on the agenda items discussed at the meeting.

Stephen L. Johnson
Executive Secretary
FIFRA Scientific Advisory
Panel (TS-769)

Attachments

cc: Panel Members
John A. Moore
James Lamb
Al Heir
Susan H. Wayland
Anne Barton
Rick Tinsworth
Janet Anderson
John Quest
Judy Hauswrith
George Ghali
Marion Copley
Reto Engler
Wang Phang

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Aciflurofen as a Class B-2 Oncogen

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The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed review of a set of scientific issues being considered by the Environmental Protection Agency's peer review classification of Aciflurofen as a Class B-2 oncogen. The review was conducted in an open meeting held in Arlington, Virginia, on December 15, 1987. All Panel members, except Drs. Edward Bresnick and Thomas W. Clarkson were present for the review.

Public notice of the meeting was published in the Federal Register on November 30, 1987.

Oral statements were received from staff of the Environmental Protection Agency and from Dr. Philip Leber, PPG Industries; and Dr. Ronald Baron, Rhone-Poulenc Ag Company.

In consideration of all matters brought out during the meeting and careful review of all documents presented by the Agency, the Panel unanimously submits the following report.

REPORT OF PANEL RECOMMENDATIONS

Aciflurofen

The Agency requested the Panel to focus its attention upon a scientific issue relating to the Peer Review of Aciflurofen. There follows the issue and the Panel's response to the issue:

Issue:

The Agency specifically requests any comments that the Panel may wish to make regarding our assessment of the weight-of-the-evidence and subsequent classification according to the Agency's Guidelines for Carcinogen Risk Assessment.

Panel Response:

The Panel believes the weight of evidence for acifluorfen can be interpreted as supporting either a B_2 or C classification. Data supporting a B_2 classification is strongly dependent on liver tumors in the CD-1 mouse study. These data are weak at best and were called into further question during the meeting when the Panel was told that a Good Laboratory Practice audit reduced the number of liver tumors to a non-significant value. If this is so, the Panel believes a category C classification is more appropriate.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

Stephen L. Johnson
Executive Secretary
FIFRA Scientific Advisory Panel

Date: 12-23-87

007698

EPA SCIENTIFIC WEIGHT OF THE EVIDENCE DETERMINATION OF THE ONCOGENICITY OF PESTICIDE CHEMICALS: ACIFLUORFEN

John A. Quest, Whang Phang, Marcia van Gemert, Karen Hamernik, Richard A. Levy, Theodore M. Farber and Reto Engler

Toxicology Branch
Hazard Evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington, D.C.

Abbreviated Title: EPA SCIENTIFIC EVALUATION OF ACIFLUORFEN

Address Correspondence to: John A. Quest, Ph.D.,
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U.S. Environmental Protection Agency
401 M Street, S.W.
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ABSTRACT

The Toxicology Branch of the Office of Pesticide Programs evaluates the oncogenic properties of pesticides by a consensus peer review process in which the weight of the evidence is assessed according to EPA's guidelines for cancer risk assessment. In many cases, pesticides are also evaluated by an external group of accomplished scientists who comprise the Agency's Scientific Advisory Panel. The herbicide Acifluorfen was evaluated by these processes and was classified as a Category B_2 oncogen based upon evidence of an increased incidence of malignant, or combined benign and malignant, tumors in multiple experiments involving two different strains of mice. The compound produced benign and malignant liver tumors in male and female $B6C3F_1$ mice and in male and female CD-1 mice. Stomach papillomas were also observed in male and female $B6C3F_1$ mice. Acifluorfen was mutagenic in bacteria and yeast, but not in mammalian cell systems. In addition, Acifluorfen is structurally related to eight other diphenyl-ether pesticides, all of which evoke liver tumors in mice or rats. The data was found to be sufficient to quantify human risk to Acifluorfen.

INTRODUCTION

The assessment of potential adverse health effects of pesticides is a complex task that often requires an interdisciplinary evaluation of data. This is especially true if the identified hazard concerns oncogenic effects, reproductive or mutagenic events, or other irreversible or unusual effects such as those involving the neuromuscular and immune systems. If hazards such as these are identified in the pesticide data review process, they can and frequently do result in significant regulatory activity. As such, it is imperative that the scientific conclusions derived for pesticide toxicity are valid and of consistently high quality.

In an effort to provide a comprehensive analysis of toxicology data on pesticides, the Toxicology Branch of the Hazard Evaluation Division, Office of Pesticide Programs, established a Peer Review Committee in October 1984. This committee consists of senior staff members of EPA's Office of Pesticide Programs, Office of Toxic Substances, and Carcinogen Assessment Group who have expertise in one or more areas of toxicology plus experience in performing weight-of-biological-evidence analyses of data for pesticide chemicals.[1] For the purpose of relating the experimental biological data observed in animals to oncogenicity effects in human, the EPA has developed guidelines for Carcinogen Risk Assessment (EPA, 1986). This classification system is a modification of the approach developed by the International Agency for Research on Cancer (IARC, 1984) for classifying weight of the evidence

information obtained from animal studies. In brief, the categories in the EPA classification system include: Group A, Carcinogenic to Humans; Group B, Probably Carcinogenic to Humans; Group C, Possibly Carcinogenic to Humans; Group D, Not Classifiable as to Human Carcinogenicity; and Group E, Evidence of Non-Carcinogenicity.

The conclusions of the Toxicology Branch Peer Review Committee are used by the Office of Pesticide Programs for a variety of reasons including further comprehensive risk assessment, the registration of new chemicals, the allowance of additional uses for older pesticides, registration standards and special reviews. For the latter function, the Office of Pesticide Programs required to present relevant data to the EPA's Scientific Advisory Panel[2] to assess decisions made by the Agency concerning the risks of a pesticide chemical. However, the Agency also frequently solicits the advice of the Science Advisory Panel regarding pesticide risks on a case-by-case basis early on in a chemical's risk assessment process.

Since its inception, the Toxicology Branch Peer Review Committee has evaluated oncogenicity and other toxicological issues for approximately 60 pesticides. From these deliberations the OPP has learned that weight-of-biological-evidence determinations for pesticides are highly complex and debatable processes that involve numerous complicated and complex scientific issues. These include controversial tumor issues (e.g., male

mouse liver tumors), the interpretation of evolving mechanisms for tumor formation (e.g., peroxisome proliferation and renal alpha 2 u-globulin hyaline droplet formation), maximum tolerated dose considerations, and genetic toxicology, structure-activity relationship, and statistical problems. To illustrate this complexity and at the same time to broaden public awareness of the pesticide scientific review process and its limitations, we are initiating a series of articles in which scientific evaluations of pesticides will be described for individual chemicals. This paper represents the first in the series and describes the weight-of-the-biological-evidence analysis for the herbicide, Acifluorfen.

DATA BASE

Background Information:

Acifluorfen (sodium 5-[2-chloro-4-(trifluoromethylethyl) phenoxy]-2-nitrobenzoate), a diphenylether compound (see Figure 1), is the active ingredient of two herbicides, Tackle® and Blazer®, which are manufactured by Rhone-Poulenc, Inc. and Rohm & Haas, Inc., respectively. Tackle® contains about 20% to 24% Acifluorfen, whereas Blazer® contains approximately 40% Acifluorfen. Both Tackle® and Blazer® are currently registered for use on food crops. Toxicological data are available on both products; however, data on Tackle® are more extensive. Therefore, discussions of the toxicology of Acifluorfen presented below are predominantly based upon data derived from studies with Tackle®, and, where appropriate, data on Blazer® is also presented.

A weight-of-biological-evidence analysis was made of the information contained in the toxicology data base on Acifluorfen (Tackle®/Blazer®) by the Toxicology Branch Peer Review Committee on June 10, 1987 (EPA, 1987). The herbicide was evaluated for potential oncogenic and genotoxic activity, and its relationship to other chemicals of similar structure was also assessed. Reproductive and teratogenicity studies, and pharmacokinetic information, were also considered.

Oncogenicity Studies:

Acifluorfen (Tackle®/Blazer®) was tested for tumorigenic activity in an 18 month study in B6C3F$_1$ mice (Tackle®), a 24 month study in CR-CD-1 mice (Blazer®), and a 24 month study in F344 rats (Tackle®).

B6C3F$_1$ Mouse Study:

Tackle® was fed to groups of 60 male and 60 female B6C3F$_1$ mice at levels of 0, 626, 1250 and 2500 ppm for 18 months (EPA, 1987). The data presented in Table 1 indicates that Tackle® was associated with statistically significant positive trends for liver tumors (adenomas, carcinomas, and adenomas/carcinomas combined) and stomach tumors (papillomas) in both males and females. The liver and stomach tumor were also significantly increased at the highest dose level tested (2500 ppm) in both sexes. In addition, the liver tumors were significantly increased at the lowest dose level tested (625 ppm) in males.

The increased incidences of liver adenomas/carcinomas combined in male (i.e., 68%) and female (i.e., 41%) B6C3F$_1$ mice at the 2500 ppm dose level of Tackle® exceeded historical control incidences of these tumor types in male (range: 16% - 58%) and female (range: 0% - 20%) B6C3F1 mice (Goodman, et.al., 1985). Similarly, the increased

incidences of stomach papillomas produced in male (i.e. 10%) and female (i.e. 13%) $B6C3F_1$ mice by 2500 ppm Tackle® exceeded the historical control incidences of this tumor type in male (0.3%) and female (0.5%) B6C3F1 mice in National Toxicology Program (NTP) studies (Haseman, et al., 1984).

The highest dose tested in male mice (2500 ppm) appeared to be excessively toxic and above that considered to be adequate for evaluating oncogenic activity. This dose was associated with significantly increased mortality and reduced body weight gain (-18% to -23% throughout the study), as well as slight increases in liver weight and the presence of white foci and/or ulcers in the stomach of occasional animals. Doses of 625 and 1250 ppm, however, were not overly toxic in male mice. The only changes of interest seen of these doses doses were reduction in body weight gain of 8% to 10% throughout the study. The highest dose tested in female mice (2500 ppm) was also not considered to be overly toxic, based on findings of a -14% reduction in body weight gain at week 13 of the chronic study, as well as slight increases in liver weight and the presence of white foci and/or ulcers in the stomach of occasional animals. Increased mortality was not observed in female mice.

CR-CD 1 Mouse Study:

Blazer® was administered in the diet to groups of 80 CR CD-1 mice/sex/dose level at levels of 0, 7.5, 45 and 270 ppm for 24 months (EPA, 1987). The highest dose level was initially administered to mice as a dose of 1.25 ppm on study weeks 1 to 16 before being increased to 270 ppm. Table 2 indicates that Blazer® produced statistically significant trends for liver tumors (adenomas in females and adenomas/carcinomas combined in both sexes). These categories of tumors were also significantly elevated at the highest dose level tested (270 ppm) in female and/or male mice. No historical control data were available.

The highest dose tested in male and female mice (270 ppm) was not sufficient to fully evaluate the oncogenic potential of Acifluorfen. Toxicologic signs seen in the study were slightly increased liver enzyme (alkaline phosphatase, SGPT) activities and liver weights in mid and high dose female mice, and increased kidney weights in high dose males. These changes were seen only at 24 months.

F344 Rat Study:

Tackle® was administered in the diet to groups of 73 F344 rats/sex/dose level at levels of 0, 25, 150, 500, 2500 and 5000 ppm for 2 years (EPA, 1987). The highest dose level was initially administered to rats as a dose of 10 ppm on study weeks 1 to 4 before being increased to 5000 ppm. No oncogenic effects attributable to compound administration were noted in either male or female rats.

The highest dose tested in male and female rats (5000 ppm) produced excessive toxicity and was above that considered to be adequate for evaluating oncogenic activity. This was based on findings of reduced weight gain, increased mortality, increased liver weights and liver enzyme changes (alkaline phosphatase, BUN, creatinine), renal changes (nephritis, pyelonephritis, glomerulonephritis), stomach ulcers and decreased testes size. Many of these changes were also seen at the 2500 ppm dose level as well (e.g., reductions in weight gain of -10% to -26% throughout the study in males and females, as well as liver, renal and stomach changes in females).

Mutagenicity Studies:

Genotoxic effects of Acifluorfen were evaluated in vitro and in vivo in a variety of test systems (EPA, 1987). The data presented in Table 3 indicates that the herbicide had mutagenic activity in insects and yeast but not in mammalian cells in culture and in vivo. That is, the only positive mutagenicity findings with Aciflurofen were noted for DNA damage (mitotic recombination) in yeast strain D5, and chromosome abberations (dominant lethal mutations and loss of Y Chromosome) in D. melanogaster.

Structure Activity Correlations:

As shown in Figure 1, Acifluorfen is structurally related to 8 other diphenyl-ether type herbicides: Bifenox, Fomesafen, Fusilade, Haloxyfop-methyl, Hoelon, Lactofen, Nitrofen, and

Oxyfluorfen (EPA, 1987). All of these chemicals have been tested for oncogenicity in mice ($B6C3F_1$, CR-CD 1, HOE-NMRKF or ICI strains) and have been shown to produce liver tumors (Table 4). In addition, liver adenomas were also evoked in Sprague-Dawley rats with Lactofen, and pancreatic tumors in Osborne Mendell rats with Nitrofen (Table 4). Metabolic studies have shown Acifluorfen to be a metabolite of Lactofen.

Reproduction and Teratology Studies:

Tackle® was not teratogenic in rats and rabbits at doses ranging from 36 to 90 mg/kg/day (EPA, 1987). In a 2-generation reproduction study in rats (0,25,500 and 2500 ppm), no adverse effects on adult reproductive performance occurred at doses up to 2500 ppm (EPA, 1987). However, reductions in body weight gain and viability occurred in high dose offspring, and increased incidences of renal pelvic dilation were seen in mid and high dose P1 and F1 females, in high dose F1 males, and in F2 offspring.

Pharmacokinetic Information:

Pharmacokinetic studies were performed in rats using radiolabelled Acifluorfen (EPA, 1987). The compound is rapidly and almost completely absorbed from the GI tract within 96 hours after oral administration. No unusual organ or tissue accumulation of either the parent compound or metabolites occurred. About 70-97% of the administered radioactivity was excreted over a period of 96 hours; most of this (i.e.,

46-82%) was found in the urine whereas smaller amounts (i.e., 5-41%) were found in the feces. Essentially all of the radioactivity excreted in the urine appears as unchanged Acifluorfen, along with very small amounts of an amine metabolite and a glucuronide moiety. In contrast, the majority of the radioactivity excreted in the feces appears as the amine metabolite, along with smaller amounts of unchanged Acifluorfen and acetamide.

No metabolism studies were performed in mice, the species in which tumors were found following the administration of both Tackle® and Blazer®.

WEIGHT-OF-BIOLOGICAL-EVIDENCE DETERMINATION OF ONCOGENICITY OF ACIFLUORFEN

The information contained in the toxicology data base for Acifluorfen was evaluated by the Toxicology Branch Peer Review Committee for the purpose of classifying the oncogenic potential of the compound. Subsequent to this process, all information was presented to the Agency's Scientific Advisory Panel for evaluation. The findings of the latter group were then reconsidered at a reconvened meeting of the Toxicology Branch Peer Review Committee.

Toxicology Branch Peer Review Committee Weight-of-Evidence Determination of oncogenic pontential

According to EPA's Guidelines for Carcinogen Risk Assessment (EPA, 1986), data from animal studies considered to be sufficient evidence of oncogenicity and supportive of a Group B2 (probable human)carcinogen classification are the occurrence of an increased incidence of malignant tumors or combined malignant and benign tumors in multiple species of animals, or in multiple experiments. The toxicology data available for Acifluorfen (Tackle®/Blazer®) was considered by the Toxicology Branch Peer Review Committee to meet these criteria:

1. Tackle® produced an increased incidence of hepatocellular tumors (adenomas, carcinomas, and adenomas/carcinomas combined) and stomach papillomas in male and female B6C3F1 mice. In male B6C3F1 mice, the liver and

stomach tumors were seen at dose levels that were excessively toxic (2500 ppm); liver tumors also occurred at a dose level that was not overly toxic (625 ppm). In female B6C3F1 mice, liver and stomach tumors were seen at a dose level (2500 ppm) that was adequate for assessing oncogenic potential. In both sexes of $B6C3F_1$ mice, the increased incidences of liver and stomach tumors produced by 2500 ppm Tackle® exceeded historical control range incidences for the same tumor types in recent studies conducted by the NTP. On the basis of the above information, the data in $B6C3F_1$ mice was considered to constitute clear evidence of carcinogenicity in males and some evidence in females, using the NTP's description of the levels of evidence of carcinogenicity (NTP, 1986).

2. Blazer® also produced an increased incidence of hepatocellular tumors (adenomas in females, and adenomas/carcinomas combined in both males and females) in a second strain of mice, namely female CR-CD-1 mice. The liver tumors occurred at the highest dose of Blazer® (i.e., 270 ppm) tested, which was not an overly toxic level. As such the study was compromised by inadequate experimental design, i.e., improper dose selection. No historical control data was available. Given these limitations, the data in CR-CD 1 mice was considered to constitute equivocal evidence of carcinogenicity in males and some evidence in females (NTP, 1986).

3. Tackle® was not oncogenic when administered to F344 rats. The highest dose of Tackle® (i.e., 5000 ppm) used in this test was excessively toxic. The data in F344 rats was considered to constitute no evidence for a carcinogenic effect in either males or females (NTP, 1986).

The oncogenicity findings with Acifluorfen were supported by results from short term genotoxicity assays and by structure-activity relationships. Evidence for a mutagenic effect of Acifluorfen was provided by positive findings for gene mutations and chromosome abberations in D. melanogaster, and for DNA damage in yeast. Acifluorfen, however, was negative in other assays. In terms of structural relationships to known oncogens, Acifluorfen was similar to numerous other diphenyl -ether pesticides which, like Acifluorfen, have been shown to produce liver tumors in mice. Of interest is the fact that some of these herbicides (e.g., Lactofen) have been identified as being inducers of hepatic peroxisomes (Butler, et.al., TAP 93:72, 1988). Peroxisome proliferation has been implicated as a possible mechanism for tumor induction in animals (Rao and Reddy, 1987). However, scientific information available at the present time is insufficient for the purpose of evaluating the role of the mechanism with regard to human risk. Species differences exist in the sensitivity to chemicals inducing peroxisome proliferation, but the degree to which human peroxisomes are involved remains unclear.

EPA Scientific Advisory Panel Evaluation Of Data:

Information on Acifluorfen was presented to the EPA's Scientific Advisory Panel (SAP) on December 15, 1987. The Scientific Advisory Panel concluded that the weight of the evidence for Acifluorfen could be interpreted as supporting either a B_2 or C classification. They believed that the data supporting the B_2 classification was strongly dependent on the increased incidence of liver tumors seen in CD-1 mice (Table 2). This data was called into question during the meeting by information that an unconfirmed GLP audit reduced the number of mouse liver tumors to non-statistically significant values. The Scientific Advisory Panel indicated that if this was true, then a Category C classification would be more appropriate for Acifluorfen. To resolve this issue the Toxicology Branch Peer Review Committee reevaluated the statistics using the revised tumor numbers and found that both trend and pairwise values remained significant at the $p < 0.05$ level.

Conclusion and Regulatory Dispositon:

In summary, the Toxicology Branch Peer Review Committee reached a final consensus conclusion to describe Acifluorfen as a Category B2 (possible human) carcinogen. The decision was based on the findings that the compound produced an increased incidence of combined malignant and benign liver tumors in two different studies employing different strains (B6C3F1 and CR-CD-1) of mice. The liver tumors occurred

in both sexes of mice. In addition, an elevated incidence of uncommonly occurring stomach papillomas also occurred in both sexes of $B6C3F_1$ mice. The compound displayed positive mutagenic activity in several non-mammalian test systems, and is structurally similar to several other diphenyl ether herbicide compounds that produced liver tumors in $B6C3F_1$ and CR-CD-1 mice.

The upper bound on unit risk, Q_1^* $(mg/kg/day)^{-1}$, of Tackle® was based on male $B6C3F_2$ mice combined liver tumors (adenoma/carcinoma) since they are the most sensitive in terms of pairwise comparison and trend, and comprise the larger contribution to total risk of carcinomas.

Using the Weibull 82 increased multistage time-to-death with tumor model (since there is a survival disparity for male mice) a unit risk, Q_1^*, is estimated in human equivalent (surface area conversion) as 3.55×10^{-2} $(mg/kg/day)^{-1}$ $[B_2]$. It is noted that Q_1^* is an estiamte of upper (95%) bound on risk and the true value of risk is unknown and may be as low as zero.

Several tolerances for Acifluorfen on raw agricultural commodities such as soybeans, peanuts and rice were established between 1980 and 1982 (40 CFR 180.383). The oncogenic property of Acifluorfen at those times was not known. With the more recent findings that Acifluorfen produces hepatocellular and stomach tumors in mice, the risks from dietary exposure and to applicators for the registered uses were calculated

(EPA, 1984). This risks ranged from 10^{-6} to 10^{-5} for both dietary exposure and for applicators. Because of these relatively low risks, Acifluorfen has not been referred for additional risk assessment, i.e., special review, by the Agency.

TABLE 1

Neoplastic Response of the Liver and Stomach In B6C3F$_1$ Mice Given Dietary Doses of Tackle® for 18 Months

Tumor Site and Type	Sex	Dose Level 0 ppm	625 ppm	1250 ppm	2500 ppm
	Male				
Liver:					
Adenomas		8/58(14)**	18/60(30)*	12/56(21)	25/59(42)**
Carcinomas		1/48(2)**	3/50(6)	4/45(9)	15/44(34)**
Combined		9/58(16)**	21/60(35)*	16/56(29)	40/59(68)**
Stomach					
papillomas		0/49(0)**	0/46(0)	0/43(0)	4/40(10)*
	Female				
Liver:					
Adenomas		1/55(1)**	5/59(5)	4/57(4)	19/58(19)**
Carcinoma		0/45(0)**	1/47(2)	1/44(2)	5/46(11)*
Combined		1/55(2)**	6/59(10)	5/57(9)	24/58(41)**
Stomach					
papillomas		0/45(0)*	3/48(6)	3/44(7)	6/45(13)*

† : Tumor bearing animals/animal at risk (i.e., the animals which died prior to the week of the first tumor occurrance for each tumor type are removed from the number of at risk).

( )= Percent.

NOTE: Significance of trend analysis (Cochran-Armitage Trend Test) denoted at Controls: significance of pairwise comparison with control (Fisher's Exact Test) denoted at Dose level. * = $p < 0.05$ and ** = $p < 0.01$.

TABLE 2

Neoplastic Response of the Liver in CR CD-1 Mice
Given Dietary Doses of Blazer® for 24 Months

Liver Tumor Type	Sex	Dose Level 0 ppm	7.5 ppm	45 ppm	270 ppm
	Female				
Adenomas		5/80(6)**	2/69(3)	4/80(5)	11/66(17)*
Carcinomas		2/80(2)	3/69(4)	0/80(0)	4/66(6)
Combined		7/80(8)**	5/69(7)	4/80(5)	15/66(23)*
	Male				
Adenomas		9/79(11)	3/69(4)	14/80(18)	12/70(17)
Carcinoma		10/79(13)	15/69(22)	14/80(18)	15/70(21)
Combined		19/79(24)*	18/69(26)	28/80(35)	27/70(38)*

( ) = percent

Note: Significance of trend analysis (Cochran-Armitage Trend test) denoted at Controls: significance of pairwise comparison with control (Fisher's Exact test) denoted at Dose level.

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

TABLE 3

Mutagenicity Studies With Acifluorfen

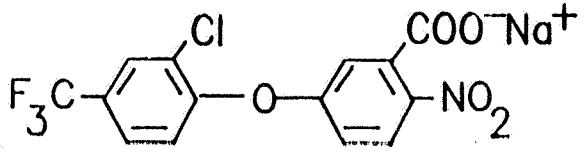
Category and Type of Test	Result
DNA Damage	
Mitotic Recombination assay with yeast strain D5	+
Unscheduled DNA Synthesis in rat and mouse hepatocytes	−
DNA repair assays in rat and mouse hepatocytes	−
Chromosomal Abberation	
Sex Chromosone loss in *D. melanogaster*	+
Dominant lethal mutation in *D. melanogaster*	+
Metaphase analysis in rat bone marrow cells	−
Dominant lethal assay with rats	−
Gene Mutation	
Mouse lymphoma cells (forward mutation)	−

TABLE 4

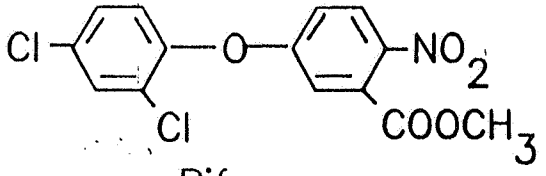
Pesticides Structurally Related to Acifluorfen and Oncogenic Effects in Various Strains of Rodents

	Oncogenicity Findings					
Chemical	Mouse Strain	Sex	Results	Rat Strain	Sex	Results
Acifluorfen	$B6C3F_1$	M/F	+(Liver)	F344	M/F	-
	CR-CD1	F	+(Liver)			
Bifenox	$B6C3F_1$	M	+(Liver)	-		
Fomesafen	CR-CD1	M/F	+(Liver)	Wistar	M/F	-
Fusilade	ICI Strain	M	+(Liver)	Sprague-Dawley	M/F	-
Haloxyfopmethyl	$B6C3F_1$	M/F	+(Liver)	F344	M/F	-
Hoelon	HOE-NMRKF	M	+(Liver)	Wistar	M/F	-
Lactofen	CR-CD1	M/F	+(Liver)	Sprague-Dawley	M/F	+(Liver)
Nitrofen	$B6C3F_1$	M/F	+(Liver)	Osbone Mendel	M	+(Pancreas)
Oxyfluorfen	CR-CD1	M/F	+(Liver)	Long Evans	M/F	-

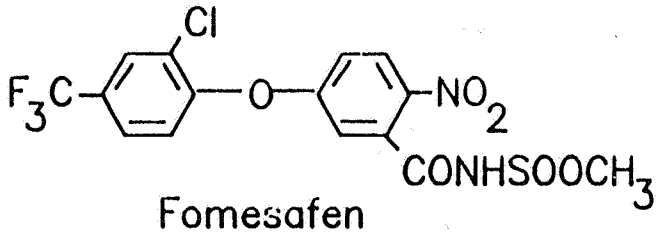
FIGURE 1: Chemical Structures of Acifluorfen and Related Diphenyl Ether Type Pesticides



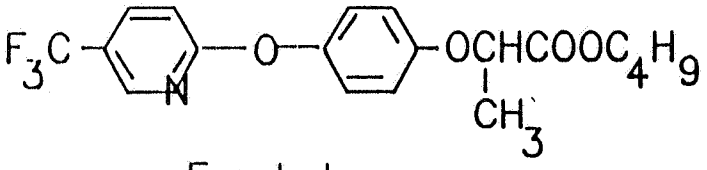
Acifluorfen



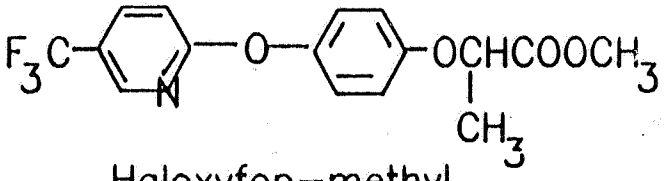
Bifenox



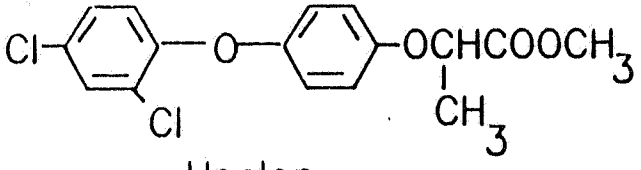
Fomesafen



Fuselade



Haloxyfop–methyl



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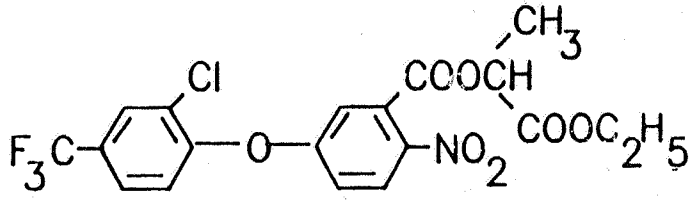
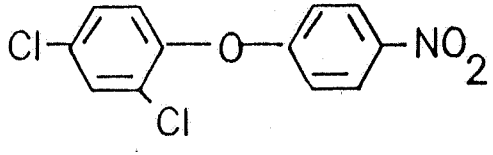
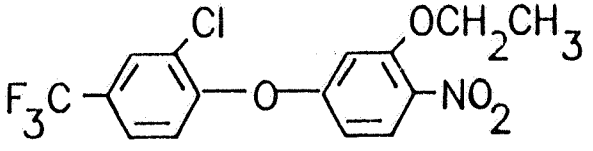


Lactofen



Nitrofen



Oxyfluorfen

FOOTNOTES:

1. Toxicology Branch Peer Review Committee members: Donald Barnes, Anne Barton, Diane Beal, Robert Beliles, William Burnam, Marion Copley, Kerry Dearfield, Reto Engler, Theodore M. Farber, Judith W. Hauswirth, Richard Hill, Stephen L. Johnson, Richard Levy, John A. Quest, Esther Rinde, and Lynnard Slaughter

2. EPA's Scientific Advisory Panel members: Robert Anthony, Edward Bresnick, Thomas W. Clarkson, Joseph W. Grisham (chairperson), Stephen L. Johnson (Executive Secretary), Mont R. Juchaw, James A. Swenberg, and James M. Tiedje.

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INFORMATION CONCERNING ACIFLUROFEN

FOR THE

SCIENTIFIC ADVISORY PANEL

December 15, 1987

55

TABLE OF CONTENTS

I. Scientific Issues Considered by the Agency in Connection with the Classification of Aciflurofen as an Oncogen

A. Introduction

B. Assessment of Oncogenicity

C. Issues for the SAP

II. Background Documents

I. Scientific Issues Considered by the Agency in Connection with the Classification of Aciflurofen as an Oncogen

A. Introduction

Acifluorfen (sodium 5-[2-chloro-4-(trifluoromethylethyl) phenoxy]-2-nitrobenzoate) Acifluorfen is the active ingredient of two herbicides, Tackle and Blazer, which are manufactured by Rhone-Poulenc, Inc. and Rohm & Haas, Inc., respectively. Both Tackle and Blazer are currently registered for use on food crops. Toxicological data are available on both products; however, the data on Tackle are more complete and acceptable. Therefore, discussions of the toxicology of Acifluorfen are predominantly based upon the data derived from the studies with Tackle, and where appropriate the data on Blazer is also utilized. It should be noted that Tackle contains about 20% to 24% of acifluorfen as the active ingredient, whereas Blazer contains approximately 40% of acifluorfen. Oncogenicity studies have been performed with Blazer/Tackle in mice and rats. The primary focus of the Agency is on two separate studies that have been conducted in different strains of mice; liver tumors were reported in both of these tests and stomach tumors in one of them. The basic issue for consideration by members of the Scientific Advisory Panel is whether the data available of Acifluorfen supports the Toxicology Branch Peer Review Committee (PRC) classification of this chemical as a Category B_2 oncogen.

B. Assessment of Oncogenicity

The PRC considered the following data in their consideration of the oncogenicity of Acifluorfen (Attachment A).

In an 18-month study in B6C3F1 mice (dietary doses of 0, 625, 1250 and 2500 ppm), Acifluorfen (i.e., Tackle) produced elevated incidences of liver adenomas/carcinomas and stomach papillomas in both sexes of animals (Table 1).

57

TABLE 1 - Tumor Incidence in Tackle Treated B6C3F1 Mice†

	0 ppm	625 ppm	1250 ppm	2500 ppm
Male				
Liver:				
Adenomas	8/58(14)**	18/60(30)*	12/56(21)	25/59(42)**
Carcinomas	1/48(2)**	3/50(6)	4/46(9)	15/44(34)**
Ad/or Car	9/58(16)**	21/60(35)*	16/56(29)	40/59(68)**
Stomach papillomas	0/49(0)**	0/46(0)	0/43(0)	4/40(10)*
Female				
Liver:				
Adenomas	1/55(1)**	5/59(5)	4/57(4)	19/58(19)**
Carcinoma	0/45(0)**	1/47(2)	1/44(2)	5/46(11)*
Ad/or Car	1/55(2)**	6/59(10)	5/57(9)	24/58(41)**
Stomach papillomas	0/45(0)*	3/48(6)	3/44(7)	6/45(13)*

† : Tumor bearing animals/animal at risk (i.e., the animals, which died prior to the week of the first tumor occurrance for each tumor type, are removed from the number of at risk.

( )= Percent.

NOTE: Significance of trend analysis (Cochran-Armitage Trend Test) denoted at Controls: significance of pairwise comparison with control (Fisher's Exact Test) denoted at Dose level. * = $p < 0.05$ and ** = $p < 0.01$.

Liver tumors were also produced by Acifluorfen (i.e., Blazer) in a 24 month feeding study (doses of 0, 7.5, 45 and 270 ppm) in female (but not in male) CR CD-1 mice (Table 2).

63

TABLE 2 - Tumors Incidence in Blazer-Treated CR CD-1 Mice

	0 ppm	7.5 ppm	45 ppm	270 ppm
Female				
Liver:				
Adenomas	5/80	2/69	4/80	11/66
Carcinomas	1/80	2/69	0/80	3/66
Ad and Car	1/80	1/69	0/80	1/66
Total (Ad or Car)	7/80	5/69	4/80	15/66*
Male				
Liver:				
Adenomas	9/79	3/69	14/80	12/70
Carcinoma	8/79	12/69	11/80	8/70
Ad and Car	2/79	3/69	3/80	7/70
Total (Ad or Car)	19/79	18/69	28/80	27/70

* = p= 0.05

Acifluorfen (i.e. Tackle) was also tested for oncogenicity in F344 rats at dietary dose levels of 0, 25, 150, 500, 2500 and 5000 ppm for 2 years and was found not to be oncogenic.

In terms of non- tumor toxicology findings, Acifluorfen (Tackle) produced positive results for gene mutations in D. melanogaster, for chromosome abberations in the sexchromosome loss Assay in D. melanogaster, and for DNA damage in the mitotic recombination assay in yeast strain D5. However, the chemical was negative in other assays (e.g., gene mutation in murine lymphoma cells; unscheduled DNA synthesis and DNA repair assays with mouse and rat hepatocytes; and metaphase analysis in rat bone marrow cells and dominant lethal assay in rats). The compound was not teratogenic in rats or rabbits. In a 2-generation reproduction study in rats, it caused reduced weight gain and viability, and renal pelvic dilation in offsprings. No metabolism studies were performed in mice, the species in which tumors were found following the administration of Acifluorfen.

Acifluorfen is structurally related to four other diphenyl-ether-type herbicides: oxyfluorfen, fomesafen, nitrofen and lactofen. All four chemicals have been tested for oncogenicity in mice (CR-CD-1 or B6C3F1 strains) and have been shown to produce liver tumors. In addition, liver neoplastic nodules were also evoked in rats in one case (lactofen) and pancreatic tumors in rats in another case (nitrofen).

69

Based upon the above information, Acifluorfen was classified as a Category B_2 oncogen ((probable human carcinogen) using EPA's Guidelines for Carcinogen Risk Assessment. That is, the chemical produced an increased incidence of combined malignant and benign liver tumors in two different studies employing different strains (B6C3F1 and CR-CD-1) of mice. It also displayed positive mutagenic activity in several non-mammalian test systems, and is structurally similar to four other dipnenyl ether herbicide compounds which cause increased incidences of liver tumors in B6C3F1 and CR-CD-1 mice.

C. Issues for the SAP

The Agency specifically requests any comment that the Panel may wish to make regarding our assessment of the weight of evidence and subsequent determination of oncogenicity according to the Agency's Guidelines for Carcinogen Risk Assessment.

II. Background Documents

A. Memorandum, 9/30/87, Peer Review of Acifluorfen

Qualitative/Quantitative Risk Assessment

7/25/88

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

Memorandum

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Tackle (acifluorfen,sodium salt)
Quantitative Risk Assessment- Mouse Study

caswell no. 755D

From: Bernice Fisher, Biostatistican
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

B. Fisher 7/25/88

To: Whang Phang, Ph.D.
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Thru: for Richard Levy, M.P.H., Leader-Biostatistics Team
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

C J Nelson
7/25/88

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Summary

The oncogenetic unit risk, $Q_1^*(mg/kg/day)^{-1}$ of tackle, based upon liver tumors(adenomas and/or carcinomas) in male mice, in terms of human equivalents, is $3.55 \times 10^{-2}[B_2]$.

72

Background

In June, 1987 the Peer Review Committee reviewed the tackle and blazer feeding/oncogenicity studies in $B_6C_3F_1$ and CD-1 mice respectively. Tackle and blazer, both contained the same active ingredient of sodium salt of aciflorfen. The tackle study was conducted by Gulf South Reasearch Institute(GSRI Project No. 413-984-41) for Rhone-Poulenc, November 3, 1982 and the blazer study, by International Research and Development Corp.(Report No. 285-013a) for Rohm and Haas, March 6, 1979.

The Peer Review Committee recommended that a risk assessment be prepared from the tackle data only, since blazer was classified as supplementary because the highest(270 ppm) dose in it was substantially less than the lowest(625 ppm) dose used in tackle and that a MTD was not approached in blazer as indicated by the tackle study in terms of survival rates, body weights, food consumption and the dose levels used in the study. In addition the dosing regimen in the blazer study was changed at 17 weeks in the highest dose group (R. Levy-Aciflurfen (Tackle); Updated Risk Assessment, June 10,1987). The highest dose of 270 ppm. was changed from 1.25 at week 17.

The qualitative risk assessment indicated that female mice did not have any significant differential survival with dose increments of tackle. Male mice did show a significant survival disparity in the pairwise comparison of control with both the mid(1250 ppm) and high(2500 ppm) dose groups; and a significant increasing mortality trend with dose increments of tackle.

Liver tumors(adenomas and/or carcinomas) were significantly increased in both sexes with dose increments of tackle. The component parts of liver tumors (i.e. adenomas, carcinomas), also exhibited significant increasing trends with dose increments of tackle in both sexes. These results were based upon the Peto Prevalence trend test in male mice and the Cochran-Armitage trend test in the females. In addition, pair-wise comparisons of control and the highest (2500 ppm) dose, in both sexes, also resulted in significant differences. These results were based upon Peto's Prevalence test in male mice and the Fisher Exact test in females.

In male mice, the liver carcinoma tumor rate increases were more pronounced than in females; and the liver adenoma tumor rate increases were more severe in females. See Tables 1. and 2. for details.

Dose-Responce Analysis

Since for male mice there is a survival disparity, a time-to-death with tumor (linearized) multistage model (Weibull83) is used to estimate the unit risk for liver tumors.

Since the female mice have no survival disparity the linearized multistage model (Global86) is used to estimate the unit risk for liver tumors.

In following the recommendations of EPA Cancer Guidelines to indicate the contribution of the benign tumors to total risk- The quantitative models are first fit to the malignant tumors and then fit to the combined tumors. The difference in the unit risk between the combined and the malignant is the result of considering the benign tumor to progress to malignant.

The unit risk, Q_1^* $(mg/kg/day)^{-1}$ for mice, was converted to human equivalents by the application of the interspecies surface area adjustment+, as recommended by the EPA Cancer Guidelines (F.R. 51: 33993-34014) and under the assumption that the adult weight of the mouse is .025 kg. and of humans is 60 kg. .

Since the male mice were the more sensitive sex and species in terms of tumor incidence pairwise comparisons with control and dose related trends, and because the estimated level of total unit risk was largely due to the malignant tumors, their associated unit risk on combined tumors was selected.

It is to be noted that Q_1^* $(mg/kg/day)^{-1}$ is an estimate of the upper (95%) bound on risk and that (as stated in the EPA Risk Assessment Guidelines) "the true value of the risk is unknown, and may be as low as zero."

The resulting estimates of $Q_1^*(mg/kg/day)^{-1}$ are as follows:

	Liver Tumors		Liver Carcinomas	
	Mouse	Human Equivalent	Mouse	Human Equivalent
Males (Weibull83)	2.65×10^{-3}	3.55×10^{-2}	8.37×10^{-4}	1.12×10^{-2}
Females (Global86)	1.05×10^{-3}	1.40×10^{-2}	4.25×10^{-4}	5.69×10^{-3}

+ $\left[\frac{\text{human wt.-grams}}{\text{animal wt.-grams}}\right]^{1/3}$

Table 1. Tackle - Mouse Study, Liver Tumor Rates+, Males with Peto's Prevalence test Results

	Dose (ppm)			
Liver tumors	0	625	1250	2500
Adenomas only	8/58 (14)**	18/60 (30)*	12/56 (25)	25/59 (42)a**
Carcinomas	1/48 (2)**	3/50 (6)b	4/45 (9)	15/45 (33)**
Both	9/58 (16)**	21/60 (35)*	16/56 (29)*	40/59 (68)**

a-appearance of first adenoma-week 25
b-appearance of first carcinoma-week 62

+ Number of tumor-bearing animals/number of animals examined(excluding those that died before the appearance of the first tumor).

( ) percent

Note: Significance of Trend Analysis denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

*p < .05, **p < .01 .

Table 2. Tackle - Mouse Study, Liver Tumor Rates+, Females with Cochran-Armitage Trend test and Fisher Exact test Results

	Dose (ppm)			
Liver Tumors	0	625	1250	2500
Adenomas only	1/55 (2)**	5/59 (8)	4/57 (7)	19/58 (33)**
Carcinomas	0/45 (0)**	1/47 (2)	1/44 (2)	5/46 (11)a*
Both	1/55 (2)**	6/59 (10)	5/57 (9)	24/58 (41)b**

a- appearance of the first adenoma-week 53
b- appearance of the first carcinoma-week 79

+ Number of tumor-bearing animals/number of animal examined(excluding those that died before the appearance of the first tumor).

( ) percent

Note: Significance of Trend Analysis denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

*p < .05, **p < .01 .

References

Farrell, R. (1983) - Notes on Weibull82, Vector Research, Inc. VRI - EPA - 8 WN83-1.

Howe, R. B., Crump, K.S. and Van Landingham, C. (1986) A Computer Program to Extrapolate Quantal Animal Toxicity Data to Low Doses (unpublished report), 25 pgs.

Reviewer's Peer Review Package for 1st Meeting

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

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JUN 2 - 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer-Review of Acifluorfen

FROM: Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

Attached for your review please find a package on Acifluorfen prepared by Dr. Whang Phang. A more detailed discussion of the negative rat study will be provided at the meeting, as well as an expansion of the SAR to include other Biphenyl ethers.

A meeting to discuss the weight-of-the-evidence on Acifluorfen has been scheduled for Wednesday, June 10, 1987, at 9:30 AM in Dr. Farber's office (Rm. 821 CM-2).

Attachment

ADDRESSEES

T. Farber
W. Burnam
E. Rinde
J. Hauswirth
J. Quest
L. Kasza
R. Levy
M. Van Gemert
W. Phang
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#19 6/2/87 sp

79

007698

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Toxicology Summary for Peer Review of Acifluorfen

TO: Reto Engler, Ph.D.
Chief, Mission Support Staff
Toxicology Branch/HED (769c)

FROM: Whang Phang, Ph.D. 5/14/87
Pharmacologist
Toxicology Branch/HED (769c)

THROUGH: Marcia van Gemert, Ph.D. 5/15/87
Head, Section III
Toxicology Branch/HED (769c)

In the attached peer review package are toxicology summary of acifluorfen and the DER's of oncogenicity study with mice.

80

TOXICOLOGY SUMMARY FOR PEER REVIEW OF ACIFLUORFEN

Table of Contents

	Pages
1. Summary of Issues	2
2. Introduction	3
3. Summary of Non-oncogenic Toxic Effects	3
4. Summary of Oncogenic Studies in Mice	3
5. Structure Activity Relationship	5
6. Mutagenicity	6
7. Metabolism	7
8. Discussion and Summary	7
9. References	8

Appendices

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

Attachment B: 24-Month Feeding/Oncogenicity Study of Blazer in Mice

81

TOXICOLOGY SUMMARY FOR ACIFLUORFEN
prepared for
The Toxicology Branch Peer Review Committee on Oncogenicity
by
Whang Phang, Ph.D.

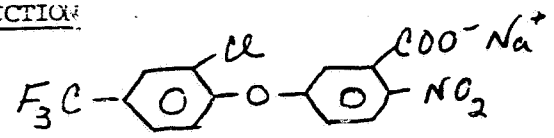
Summary of Issues

The Peer Review Committee is requested to consider, comment on the following findings/issues, and to provide a tentative weight of the evidence classification for acifluorfen with respect to its oncogenic potential.

1). Findings of liver adenomas/or carcinomas in two strains of male and female mice.

2). Findings of increased incidence of stomach papillomas in one strain of male and female mice.

I. INTRODUCTION



Acifluorfen

Acifluorfen (sodium 5-[2-chloro-4-(trifluoromethylethyl)phenoxy]-2-nitrobenzoate) is an active ingredient in two herbicides, Tackle and Blazer which are manufactured by Rhone-Poulenc, Inc. and Rohm & Haas, Inc., respectively. Both Tackle and Blazer are currently registered to be used on food crops. Toxicological data are available on both products; however, the data on Tackle are more complete and acceptable. Therefore, the following discussions of the toxicology of acifluorfen will be predominantly based upon the data derived from the studies with Tackle, and where appropriate the data of Blazer will also be discussed to provide additional evidence in assessing the oncogenicity of acifluorfen.

It should noted that Tackle used in majority of the studies contain approximately 20% to 24% of acifluorofen; Blazer, 40% of acifluorofen.

II. SUMMARY OF NON-ONCOGENIC TOXIC EFFECTS

Tackle possesses a low order of acute oral toxicity (Category III). The acute oral LD_{50} of Tackle for male rats is 2.0 gm/kg; female rats, 1.4 gm/kg. Tackle is a moderate dermal irritant (Category II) and a strong eye irritant (Category I). However, it is not a dermal sensitizer.

Tackle had not been demonstrated to be a teratogen in both rats and rabbits. In a 2-generation rat reproduction study, no adverse effects on adult reproductive performance were observed in Tackle treated rats. Decreases in body weight and viability of offspring were observed in high dose pups. Increased incidence of renal pelvic dilations was observed in mid and high dose P_1 and F_1 females and high dose F_1 males. Dose-related renal pelvic dilatation was also found in F_2 offspring.

Chronic rat feeding study showed increased incidence of kidney damage in mid (2,500 ppm) and high dose (5,000 ppm) animals relative to the controls. The kidney damage include nephritis, pyelonephritis, and glomerulonephritis. There was an increased incidence of stomach ulcer in both high dose males and females compared to the controls (Males: control, 0/73; high dose, 22/73. Females: control, 4/73; hgih dose, 16/73).

III. SUMMARY ONCOGENICITY STUDIES IN MICE

A. Tackle: 18-Month Mouse Oncogenicity Study (Rhone-Poulenc)
Project No. 413-394-4 (Gulf South Research Institute)
This study had been audited by EPA (Jan 14, 1984) and subsequently classified as a minimun study.

Doses Tested: 0; 625; 1,250; and 2,500 ppm in the diet (nominal concentrations).

Strain: B6C3F1 mice (Charles River Breeding Labs); 60 mice/sex/dose with a 12-month interim sacrifice of 10/sex/dose.

Testing Laboratory: Gulf South Research Institute (Nov 3, 1982)

The report of this study was evaluated by Dynamac Corporation and approved by Toxicology Branch.

Groups of mice were treated for 18 months with a diet containing the designated nominal concentrations. In male mice, there was a dose-related increase in mortality (Table I). Mean body weights of both treated males and females were decreased relative to the controls throughout the major duration of the experiment whereas the food consumption of the treated mice was not affected.

TABLE I

Mortality of Tackle Treated Animals

Dose	Male	Female
0	1	5*
625	3	2
1250	7†	5*
2500	10	3

* One animal was listed as accidental death
† The cause of the death of four animals was reported to be due to cage flooding.

There were changes in hematologic parameters in high dose males at final sacrifice; the changes included decreases in mean corpuscular volume and segmented neutrophils and increases in red blood cells and lymphocytes. Mean liver weights of both high dose males and females were significantly increased at both interim and final sacrifices. Mid dose males and females also showed significantly increased liver weight at final sacrifice. There was dose-related decrease in efficiency of food utilization in both treated males and females relative to the controls despite consuming near normal quantity of food.

The results of gross pathology indicate an increased incidence of liver masses in treated males which died prior to the final sacrifice. At the final sacrifice, increased incidence of liver masses was observed in both treated males and females in comparison to the controls (Table II). In all treated females and high dose males, there was an increased incidence of white foci on the non-glandular portion of the stomach. Stomach ulceration was found in one male and one female of the high dose groups (Table II).

Histopathology data indicate that there were increases in the incidence of hepatocellular adenomas and carcinomas in both treated males and females at all dose levels relative to the controls. The increased tumor incidence was statistically significant in both high dose males and females (Table III). The Statistics Team of the Toxicology Branch found the incidence of liver tumor in treated males and females to show a dose-related trend with Cochran-Armitage Trend Test.

There was also a statistically significant increase in incidence of stomach papilloma in high dose males and females relative to the controls (Males: control, 0/49; high dose, 4/40. Females: control, 0/45; high dose, 6/45).

In summary, all Tackle treated male and female mice developed liver tumor, and both males and females which received high dose Tackle developed papilloma of the stomach.

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

Time/Nature of death	Organ/Finding	Males 0 ppm	Males 625 ppm	Males 1,250 ppm	Males 2,500 ppm	Females 0 ppm	Females 625 ppm	Females 1,250 ppm	Females 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.

DATA TAKEN From the DER

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TABLE III

Tumor Incidence in Tackle Treated Mice †

		0 ppm	625 ppm	1250 ppm	2500 ppm
	Male				
Liver	Adenomas	8/58 (14)**	18/60 (30)*	12/56 (21)	25/59 (42)**
	Carcinomas	1/48 (2)**	3/50 (6)	4/46 (9)	15/44 (34)**
	Ad/or Car	9/58 (16)**	21/60 (35)*	16/56 (29)	40/59 (68)
Stomach	papillomas	0/49 (0)**	0/46 (0)	0/43 (0)	4/40 (10)*
	Female				
Liver	Adenomas	1/55 (1)**	5/59 (5)	4/57 (4)	19/58 (19)**
	Carcinomas	0/45 (0)**	1/47 (2)	1/44 (2)	5/46 (11)*
	Ad/or Car	1/55 (2)**	6/59 (10)	5/57 (9)	24/58 (41)**
Stomach	papillomas	0/45 (0)*	3/48 (6)	3/44 (7)	6/45 (13)*

† : tumor bearing animals/animal at risk (i.e. the animals, which died prior to the week of the first tumor occurrance for each tumor type, are removed from the number of at risk).

( ) : percent

Note: Significance of trend analysis (Cochran-Armitage Trend Test) denoted at Controls; significance of pairwise comparison with control (Fisher's Exact Test) denoted at Dose level.

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

Statistical analyses shown in this table were carried by the Statistics Team of toxicology Branch.

Data taken from the individual animal data which were compiled by Carolyn Gregorio.

B. Blazer: 24-Month Feeding/Oncogenicity Study in Mice
(Blazer containing approximately 40% of acifluorfen)
A data audit was requested, but no report about the audit was found. This study is still classified as Supplementary.

Doses Tested: 0, 0, 1.25(changed to 270 ppm at week 17), 7.5, and 45 ppm of Blazer in diet. Two control diets were prepared; one was with distilled water and the other with acetone.

Strain: Charles River CD-1 mice; 80 mice/sex/dose; interim sacrifices were carried out at 3- and 12-month periods (10 mice/sex/dose).

Test Laboratory: The study was conducted by International Research and Development Corporation (Report No. 285-013a). March 6, 1979.

The report of this study had originally evaluated by Dynamac Corp. and later re-evaluated by another reviewer of Toxicology Branch. All the changes made by that reviewer and the original DER of this study are in Attachment B.

Groups of mice (80/sex/dose) were fed a diet containing Blazer at the designated nominal concentrations for 24 months. Interim sacrifices were carried out at 3 and 24 months. The survival rates of treated and control male and female rats were comparable. Body weight, food consumption, and hematologic parameters were similar for treated and control animals.

Clinical chemistry data showed increases in the values of alkaline phosphatase and serum glutamic pyruvic transaminase for 45 and 270 ppm males at 24 months. There were also increases in liver weight in the mid and high dose males. Kidney weight of the high dose males was also increased relative to the controls.

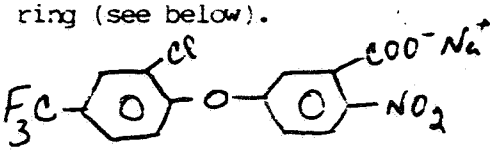
During gross examination, increased incidence of liver masses were found in mid and high dose males and high dose females relative to either the distilled water or acetone controls (Males: water control, 10/78; mid dose, 19/80; high dose, 20/70. Females: water control, 2/79; high dose 13/66).

With histologic examination, increased incidence of liver adenomas and carcinomas was observed in both high dose male and female mice relative to the controls (Table IV). The increased liver tumor incidence in high dose females was statistically significant relative to the controls and confirmed the results seen in the study with Tackle in B6C3F1 mice. It should be noted that the highest dose used in this study (270 ppm) was substantially less than the lowest dose used in the study with Tackle (625 ppm), even though the percentage of acifluorfen in Blazer is twice that in Tackle.

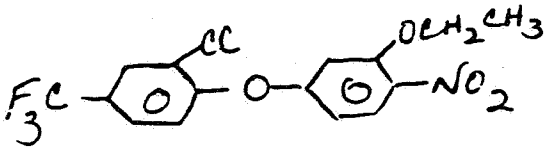
The survival rate, body weight, food consumption, and the dosages of Tackle used in the other study indicate that the highest dose of Blazer used in this study had not approached MTD.

IV. STRUCTURE ACTIVITY RELATIONSHIP:

Structurally, acifluorfen (active ingredient of Tackle and Blazer) closely resembles oxyfluorfen; they differ only on the side chain on the nitro benzene ring (see below).



Acifluorfen



Oxyfluorfen

TABLE IV

Summary of Liver Tumors in Mice Fed Blazer (24 Months)

	MALES			a/	FEMALES			a/
DSE (PPM)	0	7.5	45.0	270(1.25)	0	7.5	45.0	270(1.25)
Month Sacrifice								
Livers Examined	10	0	10	0	10	0	10	0
Carcinoma (only)	0	-	0	-	0	-	0	-
Adenoma (only)	0	-	0	-	0	-	0	-
Carcinoma & Adenoma	0	-	0	-	0	-	0	-
OTAL - 3 MONTHS	0/10	0	0/10	0	0/10	0	0/10	0
Month Sacrifice								
Livers Examined	12	10	11	10	10	9	7	7
Carcinoma (only)	0	3	0	0	0	0	0	0
Adenoma (only)	0	0	0	1	0	0	0	0
Carcinoma & Adenoma	0	0	0	0	0	0	0	0
OTAL - 12 MONTHS	0/12	3/10	0/11	1/10	0/10	0/9	0/7	0/7
ULY DEATHS 0-12 MONTHS								
Livers Examined	3	7	5	3	9	7	10	6
Carcinoma (only)	0	0	0	0	0	0	0	0
Adenoma (only)	0	0	0	0	0	0	0	0
Carcinoma & Adenoma	0	0	0	0	0	0	0	0
OTAL - EARLY DEATHS (3-12 MONTHS)	0/3	0/7	0/5	0/3	0/9	0/7	0/10	0/6
ULY DEATHS 12-24 MONTHS								
Livers Examined	26	26	31	26	23	31	31	37
Carcinoma (only)	5	4	7	4	1	0	0	1
Adenoma (only)	2	0	7	3	1	0	1	4
Carcinoma & Adenoma	0	1	0	2	0	0	0	0
OTAL - EARLY DEATHS (12-24 MONTHS)	7/26	5/26	14/31	9/26	2/23	0/31	1/31	5/37
MINAL SACRIFICE- 4 MONTHS								
Livers Examined	28	26	23	31	28	22	22	16
Carcinoma (only)	3	5	4	4	0	2	0	2
Adenoma (only)	7	2	7	8	4	2	3	7
Carcinoma & Adenoma	2	3	3	5	1	1	0	1
OTAL - TERMINAL SACRIFICE	12/28	10/26	14/23	17/31	5/28	5/22	3/22	10/16
TOTALS								
Livers Examined	79	69	80	70	80	69	80	66
Carcinoma (only)	8	12	11	8	1	2	0	3
Adenoma (only)	9	3	14	12	5	1	4	11
Carcinoma & Adenoma	2	3	3	7	1	1	0	1
TOTAL	19/79	18/69	28/80	27/70	7/80	5/69	4/80	15/66*

a/ Animals fed 1.25 ppm until week 17 and then changed to 270 ppm until term.

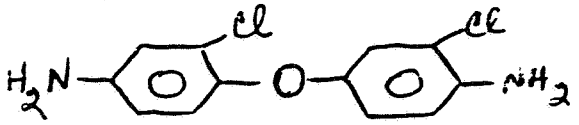
*P = 0.05.

NOTE: CHANGES ON TABLE CORROBORATED WITH DRUMMAC (11-21-83)

DATA TAKEN FROM FINAL REP

A mouse-oncogenicity study with oxyfluorfen at concentrations of 2, 20, and 200 ppm was carried out in Charles River CD-1 mice for 20 months. After one year, the concentration of 200 ppm was increased to 800 ppm. The high dose females showed increased incidence of hyperplastic nodules in the liver (control, 0/41; high dose, 4/44). High dose males had increased incidence of hepatocellular carcinomas (control, 2/39; high dosé, 7/44). The concentrations of oxyfluorfen used in this study was markedly less than those in the Tackle study, and the highest dose used in this study also had not approached the MTD.

Another compound, which is structurally related to acifluorofen, is 3,3'-dichloro-4,4'-diaminodiphenyloxide (DCDP). Also DCDP is related to the amine metabolite of acifluorfen. This amine metabolite was found in the urine and feces of acifluorfen treated rats (see figure below and Figure I).



3,3'-dichloro-4,4'-diaminophenyloxide (DCDP)

When DCDP was administered subcutaneously in a total dose of 10.5 mg/kg to rats for 190 days, carcinomas of the ear ducts were found in 37/40 rats. A scattering of internal tumors not centered in any particular location was found (Arcos and Argus, 1974).

Data of the structurally related compounds lend some support to the oncogenic effects of acifluorfen.

V. MUTAGENICITY

The mutagenicity studies indicate that Tackle is not mutagenic in mammalian cell systems, but it causes gene mutation and chromosomal aberration in Drosophila melanogaster and DNA damage in yeast.

A. Gene Mutation
- Murine lymphoma cells (forward mutation) (negative)
- Drosophila melanogaster (positive)

B. DNA Damage
- Unscheduled DNA synthesis with mouse and rat hepatocytes (negative)
- DNA repair assays with rat and mouse hepatocytes (negative)
- mitotic recombination assay with yeast strain D_5 (positive)

C. Chromosomal Aberration
- Metaphase analysis in rat bone marrow cells (negative)
- Domirant lethal assay with rats (negative)
- Sex-chromosome loss assay in Drosophila melanogaster (positive)

VI. METABOLISM

The results of rat metabolism studies indicate that acifluorfen was absorbed rapidly and almost completely from the gastro-intestinal tract 96 hrs after oral dosing. Very little acifluorfen or its metabolite was found in tissues of either male or female rats at 96 hrs after dosing. A possible metabolic pathway is presented in Figure I.

In urine, much of the radioactivity was found to be unchanged acifluorfen and a small amount of amine metabolite of the test compound. In comparison, in feces the amine metabolite of acifluorfen was the major metabolite.

VII. DISCUSSION AND SUMMARY

Acifluorfen was found to cause liver tumors in two strains of mice (B6C3F1 and charles River CD-1 mice), and it did not produced any tumor in Tackle treated rats. Although historical control B6C3F1 mice had a high spontaneous rate of liver adenomas/or carcinomas (males, 4.2 to 31.1%; females, 3.1 to 7.9%) (Haseman, 1984), the incidence of liver adenomas/or carcinomas found in high dose (2,500 ppm) Tackle treated B6C3F1 mice (males, 68%; females, 41%) was substantially greater than that seen in the historical controls. The occurrence of liver tumor in Tackle treated B6C3F1 mice was dose related.

The male and female B6C3F1 mice which received high dose Tackle also developed stomach papillomas (male, 10%; female, 13%). No incidence of stomach papillomas was found in the concurrent controls, and the incidence of stomach papillomas in historical control B6C3F1 mice was small (male, < 0.1%; female, 0.1%).

The structure activity relationship analysis of two other compounds, oxyfluorfen and DCDP, both of which caused tumor development, provides additional support to the oncogenic effects of acifluorfen.

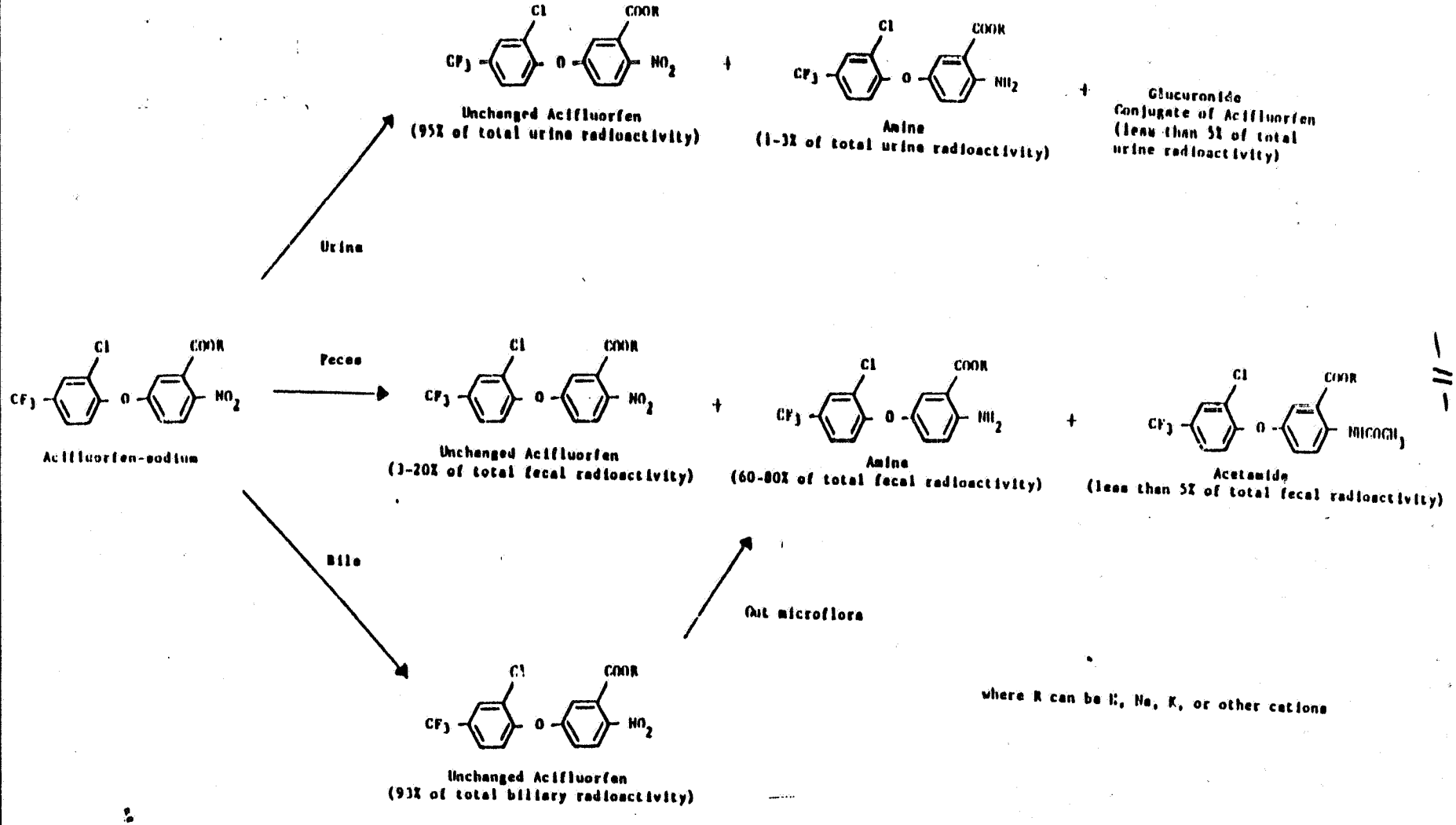


FIGURE I

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

DATA TAKEN FROM DER OF THE METABOLISM STUDY (DOC. NO. 003963)

16 - 91

867400

REFERENCES:

1. Arcos, J.C. and Argus, M.F. (1974). Chemical Induction of Cancer: Structural Bases and Biological Mechanisms. Vol. IIB. Academic Press, New York. pp. 17-19.

2. Haseman, J.K., Huff, J., and Boorman, G.A. (1984). Use of Historical Control Data in Carcinogenicity Studies in Rodents. Toxicologic Pathology 12(2): 126-135.

ATTACHMENT A

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	190±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
- Interim Sacrifice											
# animals examined	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
- Final Sacrifice											
# animals examined	46	25	58	51	58	-	42	48	55	58	51
# animals with cataract	31	NR	31	30	34	-	35	12	35	35	26

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.

97

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

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*	51.5*
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	500
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 3. 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 3: SUMMARY OF NON-NEOPLASTIC LESIONS

	ppm	Males						Females					
		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.

	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

00769

ATTACHMENT B

Study Title: 24-Month Feeding/Onco - Mice

Accession Number: Not Reported

MRID Number: Not Reported

Sponsor: Rohm and Haas Company

Contracting Lab: International Research and Development Corporation (Report No. 285-013a)

Date: March 6, 1979

Material: Blazer (Sodium Salt of Acifluoren; RH-6201)
Purity: 39.4-40.5%

Compound Stability: No data were presented in the submitted report with regard to compound stability.

Concentration of Compound in Feed: In an appended report (Report No. TR-34H-77-14) "food samples used in the first year of chronic toxicity studies" were reported. Food analysis was taken on the following dates: 6-9-76, 7-28-76 and 12-1-76. These dates, however, do not correspond to one year, but approximately 6 months (study was initiated on June 9, 1976 and terminated May 18 and 19, 1978). The compound concentration varied acceptably for the 7.5 and 45 ppm levels. The high dose of 270 ppm (changed from 1.25 ppm at week 17) was analyzed only once. Analysis of control diets indicated an average 0.02 ppm of test compound.

In another appended report (Report No. 34H-78-34), samples were taken "from feed used in week 104 of the study." However, the table found in the report provides analyses for "week 1, 4, 8, 26 and 104," (the high dose was only analyzed on week 26 and 104.) Based on the provided analyses, the compound concentration in the feed varied acceptably. Analysis of the control diets indicated an average 0.05 $\pm$ 0.10 ppm of test compound.

Stability of Compound in Feed: No data were presented in the submitted report.

Protocol: Eight hundred Charles River CD-1 mice obtained from Charles River Breeding Labs (Wilmington, Massachusetts) were "arbitrarily placed into temporary groups, as they were removed from shipping crates, and assigned temporary numbers." The animals, 80 males (weighing 24-39 grams) and 80 females (weighing 20-33 grams) were assigned to each dose group. Blazer was given in the diet at concentrations of 0, 1.25 [changed to 270 ppm at week 17], 7.5 and 45 ppm.

The diets were prepared in the following manner: "RH-6201 was dissolved in a small quantity of distilled water to facilitate mixing with the basal diet (ground Purina Laboratory Chow). Two control groups of 80 mice/sex/group were fed basal diet mixed with similar quantities of either acetone or distilled water." It should be noted that the report states (section labeled: II. Compound, page 9) that the compound is a "brown, slightly viscous liquid." "The appropriate amount of RH-6201 was mixed with 7 ml distilled water and premixed with 500 grams basal diet using a Hobart food mixer." Then the premix was added and mixed with additional basal diet "to yield 8 kg of prepared test diet. Control diets were prepared by the addition of 7 ml of ACS grade acetone or distilled water to basal diet to provide an identical quantity of premix as for treated groups." Water and test diets were available ad libitum. For purposes of this review, the water control was used for all comparisons since it was the substance mixed in the diets.

"The mice were housed individually in hanging wire-mesh cages and were maintained in a temperature, humidity and light controlled room."

General Observations: All mice wre observed daily for overt signs of toxicity, morbundity, and mortality.

Body Weights and Food Consumption: Individual body weights and "sex-group" food consumption were recorded weekly for the first 13 weeks and monthly thereafter.

Hematology: Blood samples were obtained from 10 animals/sex/dose at 3, 12, 18 and 24 months. Hematological studies included: hemoglobin, hematocrit, total erythrocyte count, total and differential leucocyte counts, clotting times, prothrombin time, and cell morphology were done at 3, 12 and 18 months and termination. "At 18 months, formation of slides for differential count revealed a large number of platelets. These were recorded as increased (inc) where applicable."

Biochemistry: Blood samples were obtained from 10 animals/sex/dose at 3, 12, 18 and 24 months. Biochemical studies included: fasting glucose, blood urea nitrogen (BUN), total protein, albumin, glutamic pyruvic transamine activity (SGPT), alkaline phosphatase activity, globulin, and the albumin:globulin ratio. Serum oxaloacetic transaminase activity (SGOT) was inadvertently determined at 12 months of study only.

Pathology:

Gross Pathology: Complete necropsy was conducted on terminally sacrificed animals (supervised by Dr. Curt Barthel, consultant veterinary pathologist). "Fixed tissues of terminally sacrificed mice and mice that died or were moribund from 12

months to termination were sent to Histolabs, Inc. for routine histological processing, then sent to Dr. Bartel for microscopic examination."

"Fixed tissues of mice sacrificed at 12 months and mice which died or were sacrificed in extremis during the first 12 months of the study were delivered to American Histolabs, Inc. (Silver Spring, Maryland) for histological processing, then returned to IRDC for microscopic examination."

Organ Weights: The weights of the following organs were recorded: spleen, liver, kidneys, brain, heart, thyroid, adrenals, testes/ovary.

Histopathology:

a. 3 Month Interim Sacrifice: The following tissues were examined from 10 animals/sex/dose "in both control groups and the 45 ppm dosage level" (asterisk denotes those tissues examined from the 1.25 [changed to 270 ppm at week 17] and 7.5 ppm doses):

aorta	liver	spleen*
adrenals	lungs	stomach
bone marrow*	mandibular lymph glands	thyroid
brain	mammary gland	urinary bladder
eye	pancreas	uterus/prostate
gallbladder	salivary gland	
gonads	skin	
heart	spinal cord	
small intestine	pituitary	gross lesions
large intestine	skeletal muscle	tissue masses
kidneys	with nerve	and tumors

b. 12 Month Interim Sacrifice: The following tissues were examined from 10 animals/sex dose "in the control groups, the 270 (1.25) and 45 ppm dosage levels" (asterisk denotes those tissues examined from the 7.5 ppm dose):

Spleen and bone marrow were microscopically examined from mice at the 7.5 and 1.25 ppm dosage levels. In addition, special stains for iron were performed on tissues of selected mice at the 45.0 ppm dosage level.

At the 12 month interim sacrifice, the following tissues from each mouse in the control groups, the 270 (1.25) and 45.0 ppm dosage levels were embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically:

adrenals*	kidneys	spleen*
bone marrow*	liver*	thyroid/
brain	lung	parathyroid*
gonads*	mandibular lymph glands*	
heart with coronary vessels	pancreas*	any other
	pituitary*	gross lesions*

c. Terminal Sacrifice: The Protocol Section of the report does not state which tissues were to be examined in which dose groups.

Statistics: "All statistical analyses compared treatment groups with each of the control groups by sex. Body weights at 13, 26, 39, 52, 66, 78, 91 and 100, and hematological and biochemical parameters (with the exception of SGOT) at 3, 12 and/or 18 months and termination were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances, and the appropriate t-test (for equal and unequal variances) as described by Steel and Torrie. Dunnett's multiple comparison tables were used to judge significance of differences.

Results:

General Behavior: The authors reported, "The following observations were noted for both control and treated mice: an accumulation of yellow material in the anogenital region; pale color to skin and/or eyes; ocular changes including corneal opacity and eccentric pupils; and moribundity usually preceded by tremors, labored breathing and an apparent reduction in body heat. A slightly higher frequency of these observations, particularly the yellow material in the anogenital region and paling of eyes and/or skin, was noted for the treated mice predominantely males, although the incidence of these signs was greatest for mice in the 45- and 270-ppm dosage levels. The small numerical differences between these two groups and mice treated at the lowest dosage level precludes a definitive conclusion as to a dosage-related effect. The incidence of these signs in the treated males versus the control males is indicative of probable compound effect. Incidental findings included: tonic convulsions, circling, swelling of extremities or body, and red, extended penis or red material at the vaginal openings."

Mortality: Similar mortality rates were observed for all groups. Survival was as follows:

Treatment Level	No. Surviving/No. Initiated* Male	Females
Control (acetone)	21/55	27/60
Control (distilled water)	27/58	32/60
7.5 ppm	26/60	23/59
45.0 ppm	23/59	28/60
270 (1.25) ppm	31/60	21/60

*Interim sacrifices not included.

Body Weights: Mean body weights were similar for all groups throughout the study.

Food Consumption: Although the mean food consumption appears to be similar for all groups throughout the study, explanation of the Table 4 is needed. It is not clear what "grams/kilograms/day" and "mcg/kg/day" represent.

Hematology: Mean hematological values were similar for all groups throughout the study.

Biochemistry: Mean biochemical values were similar for all groups throughout the study with the exception of alkaline phosphatase (AP) and serum glutamic pyruvic transaminase (SGPT) activity.

The following table demonstrates the apparent dose related elevation of alkaline phosphatase and SGPT in males at the 45 and 270 (1.25 ppm) doses. However, due to the variability of the standard deviation for these parameters, statistical analysis was not performed. NOTE: A series of statistical tests will be performed during the conduct of the oncogenic risk assessment.

Mean Alkaline Phosphatase and SGPT Values

	Males					Females				
Dose (ppm)	Control (Acetone)	Control (Water)	270(1.25)*	7.5	45.0	Control (Acetone	Control (Water)	270 (1.25)*	7.5	45.0
Alkaline Phosphatase										
- 12 Month	47±26.4	82±76.1	95±42.7	61±26.6	69±42.8	100±49.6	83±27.4	113±63.4	111±46.7	105±30.5
- 24 Month	132±125.9	85±44.8	347±355.4	77±39.9	169±194.8	122±48.6	179±105.3	122±81.5	140±95.8	111±46.4
SGPT										
- 12 Month	71±33.5	95±35.4	146±54.7	105±37.8	107±52	72±21.2	74±31.9	174±51.0	88±24.6	142±65.9
- 24 Month	43±20.7	37±17.1	169±165.5	62±27	61±35.5	60±35.2	31±14.4	42±20.6	40±18.7	52±20.4

*Dose increased from 1.25 ppm to 270 ppm at week 17.

Organ Weights:

a. 3-Month Sacrifice: Organ-to-bodyweight ratio for spleen, liver, kidneys, testes/ovaries, heart, thyroid and adrenals were similar for all groups.

b. 12-Month Sacrifice: A dose-related increase in absolute and relative (% body weight) weights of the liver and kidneys were observed in treated males.

Mean Absolute (G) and Relative (% Body Weight) for Liver - Males

Dose (PPM)	Control (Acetone)	Control (Water)	270(1.25)*	7.5	45.0
Bodyweight(G)	36	38	37	37	38
- Liver					
Absolute(G)	1.53	1.54	1.76	1.64	1.72
Relative(%)	4.24	4.11	4.73	4.44	4.55

*Dose increased from 1.25 ppm to 270 ppm at week 17.

c. Terminal Sacrifice: A dose-related increase in absolute and relative (% body weight) weights were observed in the liver and kidneys in treated males.

Mean Absolute (G) and Relative (% Body Weight) for Liver, Kidneys - Males

Dose (PPM)	Control (Acetone)	Control (Water)	270(1.25)*	7.5 ppm	45.0
Bodyweight(G)	32	32	31	31	32
- Liver					
Absolute(G)	1.98	1.85	2.40[a,b]	2.03	2.08
Relative(%)	6.23	5.86	7.65[a,b]	6.41	6.58
- Kidneys					
Absolute(G)	0.842	0.843	0.937	0.832	0.837
Relative(%)	2.66	2.66	3.00[a,c]	2.68	6.58

*Dose increased from 1.25 ppm to 270 ppm at week 17.
a/ = Significantly different from acetone control, p <0.05.
b/ = Significantly different from water control, p <0.01.
c/ = Significantly different from water control, p <0.05.

Gross Pathology: Gross masses/nodules of the liver were reported as follows:

Summary of Liver Masses/Nodules Seen Grossly

	Control (Acetone)	Control (Water)	270/1.25* ppm	7.5 ppm	45.0 ppm
Males					
- 0-12 mo. Sacrifice	0	0	1	4	0
- Early Deaths	6	4	6	6	10
- Final Sacrifice	5	6	13	6	9
# Livers Examined	61	78	70	69	80
Total	11/61	10/78	20/70	16/69	19/80
Females					
- 0-12 mo. Sacrifice	0	0	0	1	0
- Early Deaths	2	0	5	3	2
- Final Sacrifice	4	2	8	4	0
# Lives Examined	71	79	66	69	80
Total	6/71	2/79	13/66	8/69	2/80

*Dose increased from 1.25 ppm to 270 ppm at week 17.

Histopathology: A statistically significant (Chi-square) increase in liver tumors were observed in the high dose (270/1.25 ppm) females when compared to concurrent water controls. The following table summarizes the liver tumor incidence:

Summary of Liver Tumors in Mice Fed Blazer (24 Months)

DOSE (PPM)	MALES 0	7.5	45.0	270(1.25) a/	FEMALES 0	7.5	45.0	270(1.25) a/
3 Month Sacrifice								
# Livers Examined	10	0	10	0	10	0	10	0
- Carcinoma (only)	0	-	0	-	0	-	0	-
- Adenoma (only)	0	-	0	-	0	-	0	-
- Carcinoma & Adenoma	0	-	0	-	0	-	0	-
TOTAL - 3 MONTHS	0/10	0	0/10	0	0/10	0	0/10	0
12 Month Sacrifice								
# Livers Examined	12	10	11	10	10	9	7	7
- Carcinoma (only)	0	3	0	0	0	0	0	0
- Adenoma (only)	0	0	0	1	0	0	0	0
- Carcinoma & Adenoma	0	0	0	0	0	0	0	0
TOTAL - 12 MONTHS	0/12	3/10	0/11	1/10	0/10	0/9	0/7	0/7
EARLY DEATHS 0-12 MONTHS								
# Livers Examined	3	7	5	3	9	7	10	6
- Carcinoma (only)	0	0	0	0	0	0	0	0
- Adenoma (only)	0	0	0	0	0	0	0	0
- Carcinoma & Adenoma	0	0	0	0	0	0	0	0
TOTAL - EARLY DEATHS (3-12 MONTHS)	0/3	0/7	0/5	0/3	0/9	0/7	0/10	0/6
EARLY DEATHS 12-24 MONTHS								
# Livers Examined	26	26	31	26	23	31	31	37
- Carcinoma (only)	5	4	7	4	1	0	0	1
- Adenoma (only)	2	0	7	3	1	0	1	4
- Carcinoma & Adenoma	0	1	0	2	0	0	0	0
TOTAL - EARLY DEATHS (12-24 MONTHS)	7/26	5/26	14/31	9/26	2/23	0/31	1/31	5/37
TERMINAL SACRIFICE- 24 MONTHS								
# Livers Examined	28	26	23	31	28	22	22	16
- Carcinoma (only)	3	5	4	4	0	2	0	2
- Adenoma (only)	7	2	7	8	4	2	3	7
- Carcinoma & Adenoma	2	3	3	5	1	1	0	1
TOTAL - TERMINAL SACRIFICE	12/28	10/26	14/23	17/31	5/28	5/22	3/22	10/16
TOTALS								
# Livers Examined	79	69	80	70	80	69	80	66
- Carcinoma (only)	8	12	11	8	1	2	0	3
- Adenoma (only)	9	3	14	12	5	2	4	11
- Carcinoma & Adenoma	2	3	3	7	1	1	0	1
TOTAL	19/79	18/69	28/80	27/70	7/80	5/69	4/80	15/66*

a/ Animals fed 1.25 ppm until week 17 and then changed to 270 ppm until term.

*P = 0.05.

NOTE: CHANGES ON TABLE CORROBORATED WITH DYNAMAC (11-21-83)

Note: Statistical calculations were done with the "water" control since "for each treated group, the appropriate amount of RH-6201 [Blazer] was mixed with 7 ml distilled water...."

In the submitted report, the summary table provided for "selected histopathologic findings in the liver from 3 months through termination" does not agree with the individual animal data submitted in the report, as seen in the following table:

Total Numer Livers Examined

Dose (PPM)	Acetone	Water	7.5	45.0	270 (1.25)
Males					
- Registrant Count	70	69	70	70	70
- Reviewer Count	61	78	69	80	70
Females					
- Registrant Count	69	70	69	70	69
- Reviewer Count	71	79	69	80	66

This type of noted discrepancy does impede the assessment of the oncogenic potential of this compound. In order to clarify this issue, a data audit has been formally requested (memo W. L. Burnam to J. G. Touhey, Dated 9-9-83).

Conclusion: Based on the presented data, an oncogenic potential was demonstrated in mice being fed Blazer for 24 months. A statistically significant (Chi-square) increase in total liver tumors was observed in the high dose (270/1.25 ppm) females when compared to concurrent water controls. Further resolution of counting discrepancies (total number livers examined) is necessary. A data audit has been formally requested. In addition, an oncogenic risk assessment is currently underway.

Classification: Supplementary (resolution of histopatholgy questions; explanation of why the 270 ppm diet was analyzed only once; provide stability of the compound in feed data. Explanation of Table 4 (Food Consumption).