CASWELL FILE

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

004699

SEP 27 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Blazer 2S, 2L; EPA Registration Nos. 707-149, 707-150; Acifluorfen

Caswell No.: 755D
Accession No.: 257956

FROM: William Dykstra, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: Richard Mountfort
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THRU: Robert P. Zendzian, Ph.D. 9/18/85
Acting Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769) 9/27/85

Requested Action:

Review of (1) dermal penetration study in rats with Blazer 2L, (2) acifluorfen disposition and metabolism study in mice and rats, and (3) report of histopathological findings on examination of livers and lungs of mice from the Blazer mouse bioassay by Dr. Stan D. Vesselinovitch.

Conclusion:

1. The dermal penetration study is unacceptable. The results presented are internally inconsistant and inconsistant with the pattern expected for this type of study. Additional data are required to further evaluate the study and determine if it can be utilized. See attached DER for details of the information needed.

2 The disposition and metabolism study is acceptable.

3. The report of the histopathological assessment of the liver slides in the mouse bioassay is not acceptable. The report states that male mice sacrificed at 12 months (week 53) were not included in calculating the number of animals at risk. Three male mice in group VII sacrificed at 53 weeks had heptocellular adenomas. They were animals 14268, 14271 and 14273. These male mice survived until week 53 and exceeded the 50-week period used to calculate animals at risk.

   This issue needs to be resolved by the registrant in order to complete the evaluation of the report.

Background:

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

Tackle has the identical chemical structure as Blazer. Blazer which is registered by Rohm and Haas has permanent tolerances established in 40 CFR 180.383. No permanent tolerances have been established for Tackle.

In the rereview of the 2-year mouse feeding study in the memo of November 23, 1983, by Toxicology Branch, it was determined that Blazer was oncogenic in female mice of the high-dose group. A statistically (chi-square) significant increase in liver tumors was observed.

As a consequence of these results, a 3(c)(2)(B) letter dated February 11, 1985, was sent to the registrant requiring an applicator (mixer/loader/applicator) exposure study.

In response to this letter, the registrant (Rohm and Haas) submitted (April 30, 1985) a dermal penetration study, a reevaluation of the mouse liver histopathology report by Dr. Stan D. Vesselinovitch and a disposition and metabolism study in mice and rats.

Based on the positive oncogenic results in the mouse feeding study with Blazer and Tackle, a risk estimate was developed by Toxicology Branch.

Review:

1. ^{14}C-Acifluorfen Dermal Absorption Study in Male Rats (Rohm and Haas report no. 85R-063; April 26, 1985).

The review of this study is contained in the attached DER by Zendzian (9/11/850

Classification: Unacceptable

2. Acifluorfen Deposition and Metabolism Study in Mice and Rats (Rohm and Haas No. 81R-0281; April 29, 1985.

Test Material:

1. Acifluorfen technical 39.9 percent ai; TD 78-052; Lot No. 76-8077; aqueous solution.

2. ^{14}C-Acifluorfen; 40.0 percent ai; Lot No. 308.074; specific activity: 3.32 mCi/g; radiopurity of 98.8 percent;

The dosages chosen for this metabolism study were equivalent to the low-dose and high-dose in the mouse bioassay (1.5 and 54 mg/kg/day; 7.5 and 270 ppm) and the high-dose in the rat bioassay (54 mg/kg/day; 1080 ppm).

The rodents used in the study were male and female young adult CD-1 mice and Sprague-Dawley female rats.

The following groups of animals were treated as reported and are shown below:

Group	Species	Sex	N	Acifluorfen in Diet (ppm)	Pulse Dose ^{14}C-Acifluorfen (mg/kg)	Time after initiation of Dietary Treatment 14d	28d	29d	32d
A	Mouse	F	1	7.5	1.5	D	D	K	
A	Mouse	F	1	7.5	1.5	D	D		K*
B	Mouse	F	1	270	54	D	D	K	
B	Mouse	F	1	270	54	D	D		K*
C	Mouse	M	1	270	54	D	D	K	
C	Mouse	M	1	270	54	D	D		K*
D	Rat	F	1	1080	54	D	D	K	
D	Rat	F	1	1080	54	D	D		K*

N: Number of animals.
D: A single oral (pulse) dose of ^{14}C-acifluorfen was administered.
K: Animals were killed and blood and liver were collected and analyzed for ^{14}C-label. Livers were analyzed for ^{14}C-metabolites.
K*: Animals were killed and blood, liver, skin, and remaining carcass were collected and analyzed for ^{14}C-label.
Urine and feces were collected twice daily for two days after each ^{14}C-pulse dose and daily thereafter for ^{14}C-analysis. A majority of the samples were analyzed for ^{14}C-metabolites.

Each group (A, B, C, and D) of animals were administered acifluorfen technical in the diet for 14 days prior to the ^{14}C-pulse doses. The pulse doses were given orally by gavage on days 14 and 28 of the study.

A majority of urine and fecal samples from all groups were analyzed for ^{14}C-metabolites. Livers collected from animals 24 hours after the second pulse dose were also analyzed for ^{14}C-metabolites.

A two-way analysis of variance was performed on individual animal data and Duncan's or Scheffe's multiple-range test was used to determine significant differences among tests. $P < 0.05$ was used as the level of statistical significance.

Results:

All animals gained weight during the study.

The mg/kg/day of acifluorfen consumed in the study corresponded to the administered pulse dose for all groups.

Practically the entire pulse dose of ^{14}C-acifluorfen was excreted in the urine and feces (107 to 144 percent of dose).

Urinary excretion ranged from 37 to 82 percent of the dose.

Fecal excretion ranged from 34.6 to 65.1 percent. A large amount (90 to 111% of dose) was excreted within two to three days.

Liver concentrations of ^{14}C-material were 92 to 163 percent of the corresponding ^{14}C-blood concentrations.

The residual dose in the skin and carcass at 92-hour postdose ranged from nondetectable (group A carcass) to 1.88 percent (group C carcass).

Blood and liver ^{14}C-concentrations were 3.1 to 11.1 fold greater in female mice (group B) than female rats (group D) at 24 and 92 hours postdose. Blood and liver ^{14}C concentrations were 1.9 to 5.2 fold greater in male mice (group C) than female mice (group B).

RH-5781, the free acid of acifluorfen, was the major metabolite produced representing 32.9 to 90.7 percent of the total dose.

RH-4514, the reduced amino metabolite of RH-5781, and origin material were also produced mainly in the feces. RH-4514 and origin material comprised 11.1 to 37.8 percent and 5.6 to 34.6 percent of the total dose, respectively.

Origin material appeared to consist largely of acid hydrolyzable conjugates of RH-5781 and RH-4514.

Female rats had lower body burdens of ^{14}C-material than female mice based on percent of ^{14}C-acifluorfen.

Conclusion:

Acifluorfen is mainly excreted in the urine and feces within 2 to 3 days. Metabolites consist mainly of free acid (RH-5781), reduced amino form (RH-4514) or as origin material which consists of acid hydrolyzable conjugates of RH-5781 and RH-4514.

Classification: Minimum

3. Report on Liver Nodular Lesions Observed in the Chronic Bioassay Study in Mice Exposed to RH-6201 (Blazer) (Dr. Stan D. Vesselinovitch, April 23, 1985).

Slides of liver tissues for mice in the mouse bioassay were examined without prior knowledge of the treatment. The examined groups were numbered 2, 6, 7, and 8 for each of the sexes. At the end of the evaluation of the slides, the code was broken, and group 2 was identified as the nontreated control animals. Group 5 were the mice exposed to the high-dose, while groups 7 and 8 were exposed to the low and mid dose of Blazer, respectively.

The results of the histopathological evaluation are presented below as reported:

> The incidence of focal and modular liver lesions observed in mice was reported for the number of animals at risk. The number of animals alive at the time the first focal or nodular liver lesion has been observed. In males, animal 14328 of group VII had an adenoma at week 50. In females, animal number 14409 of group VII had a carcinoma at week 74.
>
> The report, however, states that male mice sacrificed at 12 months (week 53) were not included in calculating the number of animals at risk. Three male mice in group VII sacrificed at 53 weeks had hepatocellular adenomas. They were animals 14268, 14271, and 14273.

This discrepancy needs to be further addressed by the registrant.

Additional evaluation of the report cannot be completed until this issue is explained.

Classification: Supplementary

Data Evaluation Report

004699

Compound Aciflourfen (Blazer)

Citation

Dermal Absorption Study in Male Rats, R.B. Steigerwalt & S.L. Longacre, Rohm and Haas Co, Protocol No. 85P-058, Report No. 85R-063, April 26, 1985

Reviewed by 9/11/85
Robert P. Zendzian PhD
Pharmacologist

Core Classification Unacceptable

Conclusion

The results presented are internally inconsistant and inconsistant with the pattern expected for this type of study. Additional data are required to further evaluate the study and determine if it can be utilized.

Materials

^{14}C-Acifluorfen: Sodium 5-(2-chloro-4-trifluoromethyl-phyenoxy)2-nitrobenzoate (^{14}C-Blazer Herbicide) uniformly labeled in the nitrophenyl ring.
Lot No. 363.10
TD No. 85-57
Specific activity 10.05mCi/gm
Radiopurity 95% by TLC

Solvent system for Blazer 2L, TD No. 85-56

Male Sprague Dawley Rats, 4 to 6 weeks of age, from Charles River-Kingston.

Methods

Twenty rats were assigned to each of four groups and dosed dermally with A. 7.56, B. 75.6, C. 756 or D. 7560 ug/rat of radiolabled aciflourfen. Animals were placed into individual metabolism cages and urine and feces collected for the total duration of exposure. Four rats per dose were sacrificed at 0.5, 1, 2, 4 and 10 hours after dosing. Residual urine, blood and skin at the application site were collected for analysis. The skin was not washed. The carcass was stored frozen.

The solution for dosing group D was prepared in 2L solvent system and the solutions for the remaining groups prepared by serial dilution of the solution in distilled water. The solution for group D was equilivant to the Blazer 2L end use product and for group C equilivant to the use dilution.

The back of each rat was clipped 24 hours prior to dosing. Immediately prior to dosing a contoured glass ring (3.6 cm diameter, 10.2 cm^2) was glued to the clipped area with cyanoacrylate adhesive. Measured amounts of aciflourfen was applied with a pipette. The glass ring was then covered with a porous top. At termination the glass ring and porous top were washed for analysis.

Results

Essentially all excreted aciflourfen was found in the urine. Table 1 from the report provides the mean percent of dose excreted.

Table 1. Mean ^{14}C-urinary excretion in male rats after dermal application of ^{14}C-aciflourfen. Percent of dose excreted in urine and urine funnel wash.

Dose ug/rat	Exposure time (hr) 0.5	1.0	2.0	4.0	10.0
7.56	0.0000 (0.0000)	0.0957 (0.1424)	0.0000 (0.0000)	0.0380 (0.0266)	1.8514 (3.5884)
75.6	0.0292 (0.0357)	1.0277 (2.0554)	6.2407 (12.4186)	0.1883 (0.3570)	0.4026 (0.4416)
756.0	0.0099 (0.0130)	0.1752 (0.3400)	1.1765 (2.2778)	0.3020 (0.5147)	1.0100 (1.5500)
7560.0	0.0014 (0.0023)	0.0017 (0.0019)	0.0009 (0.0017)	0.0096 (0.0017)	0.0162 (0.0145)

The percent of dose found in the blood at sacrifice is taken from Tables 7, 8, 9 and 10 of the report and presented in Table A

Table A. Mean percent of dose in blood of male rats after dermal application of ^{14}C-aciflourfen.

Dose ug/rat	Exposure time (hr) 0.5	1.0	2.0	4.0	10.0
7.56	1.5855 (0.4907)	3.9943 (2.2234)	5.8034 (8.2188)	4.8438 (2.8720)	2.5915 (1.6569)
75.6	0.5452 (0.6166	0.1876 (0.2227)	1.5597 (2.0430)	0.3811 (0.2209)	1.1115 (1.2541)
756.0	1.6924 (3.1359)	0.3947 (0.3789)	0.4879 (0.5179	0.5173 (0.5036)	2.3116 (3.2954)
7560.0	0.0171 (0.0115)	0.0246 (0.0203)	0.0572 (0.0963)	0.0215 (0.0212)	0.0401 (0.0273)

The mean percent of the dose remaining at the application site (± S.D.) is presented in Table 5 from the report.

Table 5. Mean ^{14}C recovery from the application sites of male rats after dermal application of ^{14}C-aciflourfen.

Dose ug/rat	Exposure time (hr) 0.5	1.0	2.0	4.0	10.0
7.56	97.6026 (10.5975)	104.5033 (12.9170)	79.9341 (20.6743)	92.7496 (6.7741)	96.8499 (7.7438)
75.6	92.2620 (15.6453)	99.6483 (7.9774)	91.9575 (11.5843)	104.9388* (5.9458)	106.1085 (7.6143)
756.0	71.3664* (15.6111)	79.5426* (18.7650)	83.9152 (3.7998)	73.0290 * (10.6372)	75.5621* (12.6841)
7560.0	89.5196 (17.5215)	80.0433* (0.8861)	81.3738 (2.7422)	82.5675 (5.7384)	104.0876 (13.9320)

*Statistically different from other treatment groups at that exposure period.

Discussion

The data generated in this study are inconsistant with what one usually expects in the study of the dermal absorption of a foreign compound. The quantity absorbed at each dose is small, as is the percentage of dose absorbed. This is to be expected by the physical/chemical properties of the compound. Aciflourfen is the water soluble sodium salt and is ionized at neutral pH. However the relative absorption in relation to time and dose in this study do not follow the pattern expected in this type of study.

For any single dose of a compound which is absorbed dermally the percent absorbed can be expected to increase with time. Conversely for a compound that is absorbed dermally the percent absorbed per unit time can be expected to decrease with increasing dose. These relationships may not hold at extremes of dose or duration of exposure but are generally true throughout the middle ranges. Experience has shown that the percent absorpion at half or one hour exposures for varying doses often does not follow this rule.

Both the urinary excretion data and the blood content date differ, in a nonuniform manner, from the expected pattern. The skin recovery data is of no assistance in verifying or disputing these results. Skin recovery is generally a rather crude measure which does not indicate differences in the order of a few percent or less. In this study the skin recovery

data for the 756ug dose adds to the confusion reflecting an absorption in the order of 20-30 percent while the urine and blood data for this dose indicate absorption in the order of 0.1-2.3 percent.

A further complication is present in the comparative data from blood and urine. Absorbed aciflourfen passes through the skin, enters the blood and is excreted in the urine. Because of its' physical/chemical properties one can expect urinary excretion to be rather rapid. However, the data do not reflect such expectations. For example the data for a dose of 75.6ug show 3.9 and 5.8 percent of the dose in the blood at 1 and 2 hours respectively but the excretion data show only 1.8 percent excreted at the end of 10 hours. There are additional but less obvious inconsistancies in the data from other doses. In general the blood data appear to be higher than expected in an order(s) of magnitude relationship to the excretion data.

In order to clearify these inconsistancies the following data is requested.

1. The complete individual animal and sample values for radioactivity recovary and a stepwise persentation showing how they were converted into the data format as presented in the report.

2. Data on the excretion of aciflourfen, particularly kinetic data. Blood half-life data and/or urinary excretion half-life data would be of particular value in resolveing the apparent inconsistancies between the blood and urine data presented in the report.