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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

AUG 3 1983

MEMORANDUM

SUBJECT: PP#3F2811. Acifluorfen in Soybeans. Evaluation of analytical method and residue data.

FROM: Alfred Smith, Chemist
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

THRU: Charles L. Trichilo, Chief
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Hazard Evaluation Division (TS-769)

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

and

Toxicology Branch
Hazard Evaluation Division (TS-769)

The Agrochemical Division of Rhone-Poulenc, Inc. proposes a tolerance for combined residues of the herbicide acifluorfen, sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate, and its metabolites (the corresponding acid, methyl ester, and amino analogues) in or on soybeans at 0.1 ppm.

Permanent tolerances have been established for combined residues of the sodium salt of acifluorfen and its metabolites (as above) in or on the following commodities (§180.383):

Liver and kidney of cattle, goats, hogs, horses, and sheep at 0.02 ppm; meat, fat, and meat byproducts of poultry at 0.02 ppm; milk and eggs at 0.02 ppm; soybeans at 0.1 ppm.

These tolerances were established as a result of PP#9F2158 submitted by the Rohm & Haas Company. Residue Chemistry Branch, HED, has been requested to review the subject petition from Rhone-Poulenc, Inc., also proposing a tolerance on soybeans

(see the Registration Division memo entitled "Review Guidance for Tackle" by James W. Akerman dated 2/15/83). The RD memo outlines the procedures to follow in our review of this data package. We are requested to review the data submitted to determine if the studies will support registration/tolerance and to review only the data submitted by Rhone-Poulenc. The conclusions and recommendations of this review reflect only the data submitted in this petition by Rhone-Poulenc.

Conclusions

[redacted]

2. The nature of the residue is adequately understood. The significant components in animal and plant residues are the parent compound acifluorfen or its sodium salt, and its metabolite amino-acifluorfen. (Traces, <5%, of the methyl ester and the amino ester metabolites also occur. Therefore, the proposed tolerance is appropriate as expressed.)

3. Adequate analytical methods are available for residue determinations. A successful method trial was conducted previously for residues of acifluorfen in soybeans, meat and milk in connection with an earlier petition and has been submitted for inclusion in PAM II. The methods submitted in this petition have not been validated for enforcement purposes.

4. The data submitted in this petition show that no real residues are likely to occur in soybeans or its byproducts (meal, hulls, oil, and soapstock). The proposed tolerance level represents the combined sensitivities of the residue components analyzed for.

5. Because the data in this petition show the absence of detectable residues in soybeans, we conclude that no residues are likely to result in eggs, milk, meat, fat, and meat byproducts of livestock from the proposed use [§180.6(a)(3)].

6. There are no Codex, Canadian, or Mexican tolerances for acifluorfen on soybeans. Therefore, there is no problem of compatability of tolerances.

Recommendations

TOX and EAB considerations permitting, we could recommend for the proposal in this petition. The favorable recommendation is contingent upon identification of [redacted] in Conclusion 1.

Detailed Considerations

Acifluorfen Manufacturing Process

[redacted]

MANUFACTURING PROCESS AND INERT INGREDIENT INFORMATION IS NOT INCLUDED

Proposed Use

Tackle® herbicide, an aqueous formulation containing the sodium salt of acifluorfen (21% act., 2 lbs act/gal.), is a postemergence herbicide proposed for weed control in soybeans.

Tackle is to be applied when the soybeans reach the 1-2 trifoliate leaf stage at rates of 0.50-0.75 lb act/A. Only one application per growing season and a maximum of 0.75 lb act/A are permitted. No application is to occur within 100 days of harvest (100-day PHI).

Treated plants are not to be used for feed or forage.

For the control of certain weeds (e.g., Velvetleaf) and suppression of escaped Giant Foxtail seedlings, Tackle [redacted]

(*) Tentative identification

Acifluorfen scientific reviews

Page _____ is not included in this copy.

Pages 4 through 5 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

X Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

___ FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

[redacted]

Nature of the Residue

Plants

Several studies were performed with the radiolabelled sodium salt of C^{14}-acifluorfen and soybeans. The acifluorfen was uniformly labelled in either the nitrophenyl or the trifluoromethyl ring.

In a greenhouse study the trifoliate leaves of young soybean plants were treated with C^{14}-acifluorfen sodium salt, sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate (also known as MC 10978), at a rate of 0.6 kg/ha (0.53 lb/A). The plants were sampled over a 4-week period and analyzed.

The radiolabelled residues were confined to the treatment site and did not translocate to other plant parts. Acifluorfen was metabolized, and at least 8 components were noted (i.e., the parent and 7 more polar C^{14}-labelled components). At maturity no activity was found in the grain. (Any activity present would be less than the method's sensitivity of 0.05 ppm). The petitioner believes the 7 components are conjugates.

Three major components were noted in the residue by thin-layer chromatography: the parent compound which had 72% of the activity at 3 days, 58% at 7 days, and 35% at 14 days; an unidentified component containing 13% at 3 days, 10% at 7 days, and 8% at 14 days; and a second unidentified component at the origin which increased with time. This material had 11% at 3 days, 26% at 7 days, and 46% of the activity at 14 days. The other 5 unidentified components increased slowly with time over this period. However, only one component was greater than 2.5%, and this component was 5.6% at 14 days.

The conversion of the parent could readily be noted in an examination of the amount of methanol-extractable C^{14}-activity present during the study period. At 3 days after treatment, about 96% of the activity in the plants was methanol extractable, and 69% of the activity was the parent compound. At 14 days 66% of the plant activity was methanol extractable, and 23% of the activity was the parent compound. At 28 days 65% of the plant activity was methanol extractable, and no analysis for the parent was performed. As a result about 77% of the parent compound was metabolized and/or degraded to other forms in two weeks.

In order to determine if the radioactivity was translocated from the application site, 3 sections of the plant were examined: top section; treated section; and bottom section. Each section was analyzed for C^{14}-activity at intervals of 3-28 days after treatment. No activity was noted in the top or bottom section at any time. All activity was present in the treated section.

No radioactivity (<0.05 ppm, method sensitivity) was noted in mature soybeans obtained from plants treated with C^{14}-labelling in either the nitro ring or the trifluoromethyl ring.

Samples were initially solvent-extracted, and the radioactivity was determined by liquid scintillation techniques (LSC). The radioactivity was also extracted by sample combustion with oxygen. The extracted activity was determined by LSC. The residue components were separated and characterized using radioautographic techniques and thin-layer chromatography.

In another study, young field grown soybean plants were sprayed once with C^{14}-sodium acifluorfen at 0.56 kg/ha (0.5 lb/A). Plant samples were collected at intervals of 0-12 weeks. (Weeks 0-8 included only forage; week 12 included straw, pod, and grain samples.) The samples were examined for total radioactivity, acetonitrile-water extractable activity, and unextractable activity. The results were expressed as equivalent residues of the sodium salt of acifluorfen.

The residue levels of total activity were highest in the forage at week-0 (23-41 ppm) and declined thereafter. At week 1 residue levels were 4-12 ppm. Residues were 0.57-1.66 ppm at 2 weeks; 0.093 ppm at 4 weeks; and 0.022 ppm at 8 weeks. At 8 weeks the radioactivity had decreased to less than 0.1% of its initial value. While all levels decreased with time (i.e., total activity; extractable activity; unextracted activity), the percentage of unextracted activity in the forage increased with time from 33% at 0-week to 81% at 4 weeks (based on percent of total activity).

The study shows that sodium acifluorfen is metabolized and/or degraded when applied to young field grown soybean plants. This behavior is similar to that noted in the greenhouse study. Moreover, the residue levels dissipate during the growing period. At 8 weeks the forage levels have decreased to total activity of 0.022 ppm. Undoubtedly, much of the decrease is due to growth dilution.

The straw had total activity of 0.049 ppm. The pods and the grain had less than 0.021 ppm. The levels for the straw, pods, and grain reflect unextracted activity which suggests reincorporation of the radiolabelled atoms into naturally-occurring components of the soybean plant.

A second field study was performed in New Jersey to complement the field study from Maryland. Young soybean plants were spray-treated with radiolabelled C^{14}-sodium acifluorfen at a rate equivalent to 0.5 lb/A. The plants were sampled by cutting the stem just above the soil level at intervals of 0-28 days.

The samples were ground up and homogenized with dry ice, stored in a freezer, and the dry ice was permitted to dissipate. Samples of plant tissue were combusted to radiolabelled C^{14}-carbon dioxide before extraction. Aliquots of the macerate remaining after extraction and the filtrate were also combusted to carbon dioxide. The resulting radioactivity was measured by liquid scintillation counting techniques (LSC).

Identification and quantitation of the solvent-extracted residues were performed with thin-layer chromatography, gas chromatography, and mass spectrometry.

The results of the study are similar to the first two studies (greenhouse and field studies). The total radioactivity decreases with time. The maximum level occurred after one day (131 ppm) and decreased to 0.2 ppm at 28 days after treatment. Some metabolism and/or degradation occurred as evidenced by the increasing amount of C^{14}-labelled insoluble material (from 3.7% at 0-day to 42% at 14 days) and the decreasing amount of radiolabelled material extracted by chloroform (from 88% at 0-day to 35% at 14 days after treatment). A third fraction of radioactivity was noted. This fraction had 0.5% of the total activity at 0-day and increased to 40% at 14 days.

The parent compound decreased from 88% of the radioactivity at 0-day to 0.3% at 14 days and no detectable residues at 28 days (<0.01 ppm, method detection limit). A probable metabolite LS-82-5281 (MC-14621), the amino analog of the parent, was noted. This metabolite resulted from conversion of the nitro group to the amino group. The metabolite appeared at day-1 with 2.3% of the total radioactivity, and had decreased to 0.9% at 14 days after treatment. Two additional fractions containing radioactivity were identified with TLC and each was characterized as composites of several fractions. One fraction had maximum activity of 8% at 2 days and had decreased

to 3% at 14 days. The second fraction consisted of radioactivity at the origin and had 0.9% of the total activity at 0-day. The level had increased to a maximum of 18% at 7 days. No further characterization of the plant residues was performed.

Standard compounds of potential metabolites were compared with the components of the plant residues (see chart for identity of compounds used). Only the amino analog, MC-14621, could be identified as a part of the residue.

An examination of the TLC plates showed four major radiolabelled components (arbitrary designation based on intensity of spots). However, greater than 10 radiolabelled components were present.

The studies indicate that the parent compound is appreciably metabolized and/or degraded, and the radioactivity is reincorporated in the naturally-occurring plant components.

The significant components of soybean plant residues are the parent compound acifluorfen and its amino metabolite.

Since no translocation of acifluorfen and its metabolites occurs from leaf treatment and treatment takes place before the soybeans begin to form, then no residues of acifluorfen or its metabolites are expected in soybean from direct foliar contact.

Animals

Rats

Rats were administered single doses of radiolabelled C^{14}-acifluorfen sodium (ring label) at levels of 10-116 mg/kg. The radioactivity was essentially eliminated within 96 hours following dosing.

Aclifluorfen is ingested, absorbed, metabolized, and largely excreted by rats in the urine and feces (>96%). Some deposition of activity occurs in tissues. The liver and kidney had <0.1-0.3% of the administered dose, and the intestines had 1-3%.

The metabolism of acifluorfen involves reduction of the nitro group to the amino group to form 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-aminobenzoic acid. This was the major metabolite and accounted for 1-3% of the urine activity and 60-80% of the activity in the feces. The unchanged acifluorfen

represented 95% of the urine activity, 3-20% of the fecal activity, and 93% of the bile activity. The amino group is further metabolized to the acetamide, N-acetyl-5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-aminobenzoic acid. This metabolite represents less than 5% of the feces activity, or less than 2% of the administered dose. The feces also contained two unidentified components and each comprised <5% of the fecal activity, or less than 2% of the total dose.

No ring hydroxylation, glucuronide or glycine conjugation, or decarboxylated components were noted in the residues.

Qualitative and quantitative characterizations were carried out using the analytical techniques of thin-layer chromatography, high performance liquid chromatography, capillary gas chromatography, and gas chromatography-mass spectrometry combined. Hydrolysis studies with the enzyme beta-glucuronidase were performed on the residues to determine the absence or presence of conjugated and/or bound components. Additionally, derivatization with diazomethane (causes methylation of carboxyl and phenolic-hydroxyl groups) was used in the analytical separation and characterization schemes. (See chart of rat metabolism for chemical structures.)

Goats

Two lactating goats were fed the sodium salt of radiolabelled C^{14}-acifluorfen (MC 10978) for seven days at levels equivalent to 11 ppm and 128 ppm in the daily diet. (The compound was uniformly labelled in the nitrophenyl ring.) Samples of milk, blood, urine, and feces were collected during the treatment period and examined for radioactivity. The animals were slaughtered 24 hours after the final dosing, and tissue samples were taken for radioactivity analyses.

Total radioactivity (bound and extractable) was determined by sample combustion. Radioactivity (solvent extracted or combustion) was quantitated by liquid scintillation counting techniques. The components of the radiolabelled residues were identified through radiometric techniques using thin-layer chromatography and gas chromatography.

Acifluorfen is ingested, metabolized, and excreted by goats with some retention of residues in milk and tissues. The urine and feces contained 61-79% of the administered radioactivity. The tissues (fat, heart, kidney, liver, muscle) had 0.7-1.7% of the dosage, and the milk had 0.04-0.07% of the dosage. Approximately 88% of the administered radioactivity was recovered from both animals.

Acidic digestion of aqueous extracts of urine and feces samples were performed in order to hydrolyze bound and/or conjugated components. The results showed that less than 10% of the total radioactivity was present as bound and/or conjugated residues. The data also indicate that some of the bound activity reflects C^{14}-activity that has been reincorporated in naturally-occurring components.

Reference standards of possible metabolites of acifluorfen were used to aid in the identification of the components of the residues (see chart of reference standards). The residue components and their maximum levels, as percent of total radioactivity, are as follows: the amino metabolite (MC 14621, 56% in urine, 24% in kidney); the decarboxylated amino metabolite (MC 15412, 1.6%); the decarboxylated component with an hydroxy group in place of the nitro group (MC 15437, 3.8% in urine); the acid form (parent) of acifluorfen (MC 10109, 3.5% in blood, 2.4% in kidneys); the methyl ester of the amino metabolite (MC 14620, 0.4% in feces); the hydroxy metabolite (MC 15598, 2.7% in feces).

Unknown and unidentified components were present in tissues and excreta. The maximum levels noted were: 12% in kidneys; 12% in liver following hydrolysis; (the amino metabolite was also noted following acid hydrolysis of liver tissue). The unknown components were characterized as groups of 3 or more components. As a result, none of the unidentified components consists of greater than 4% of the residue.

The appearance of components following hydrolysis indicates that conjugation and/or binding of residue components occur.

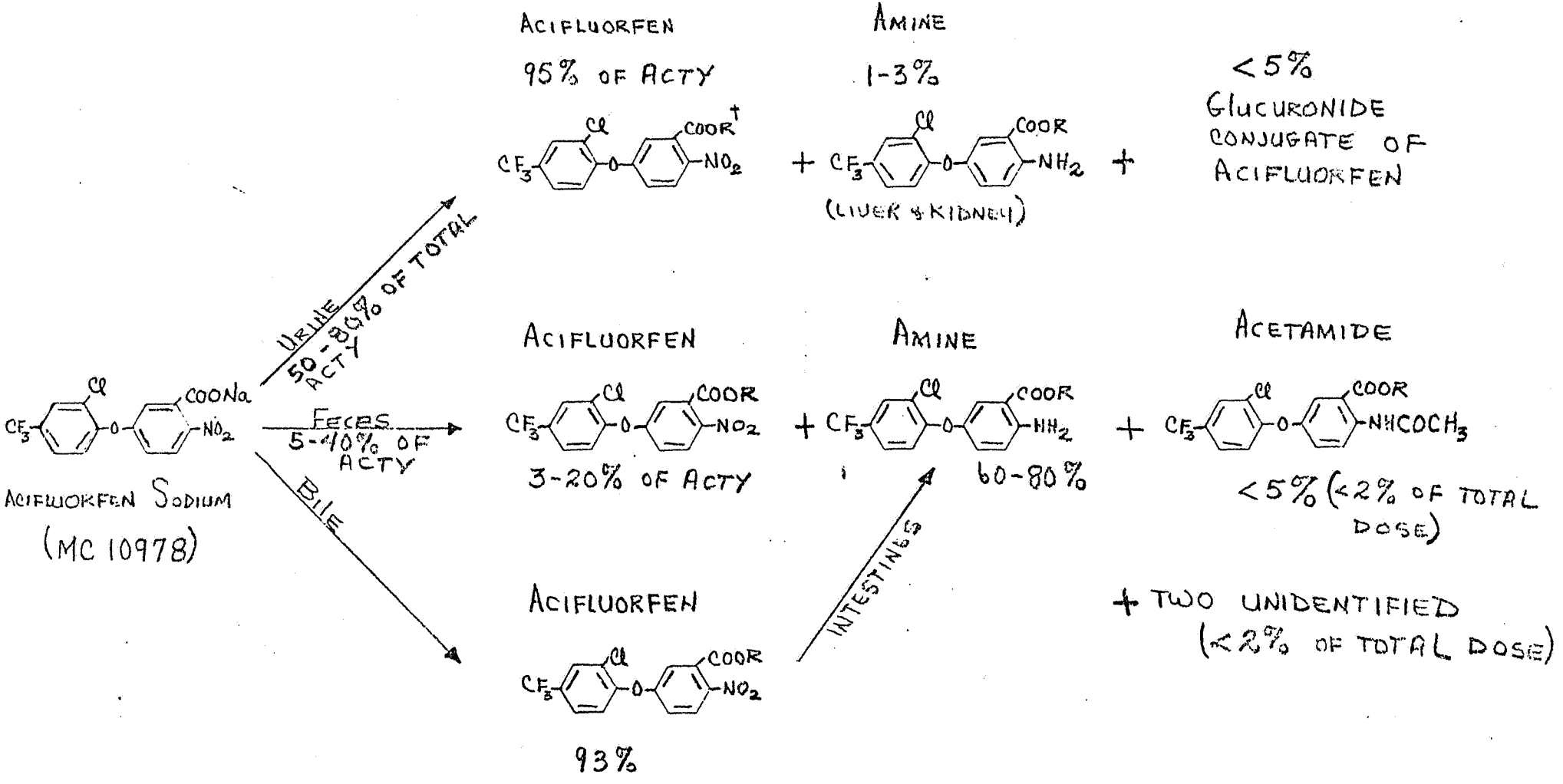
No identification of milk residue components were attempted due to the low residue levels.

The metabolic behavior of acifluorfen in goats and rats is similar. The significant residue components are the parent compound acifluorfen and its amino metabolite.

Analytical Method

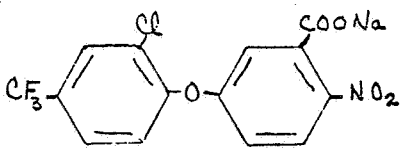
The method (No. 160) determines five residue components. Four of the five components are converted to the fifth component which is then determined by gas chromatography. The results may then be expressed in terms of the parent compound acifluorfen.

RAT METABOLISM OF ACIFLUORFEN SODIUM

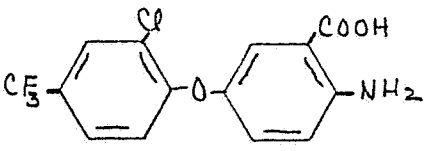


†(R CAN BE H, Na, K, OR OTHER CATIONS)

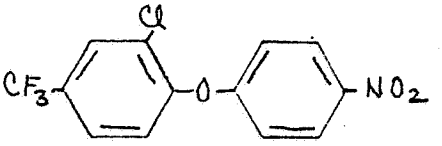
P



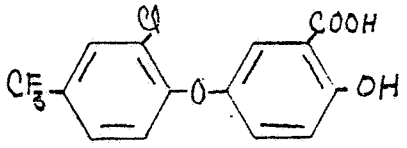
ACIFLUORFEN-SODIUM
LS-80-1213 (MC-10978)



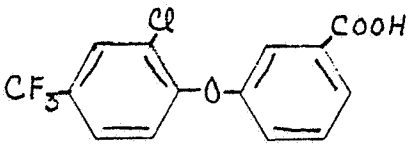
AMINO ANALOG (MC-14621)
LS-82-5281



LS-82-5280
(MC-10074)



LS-82-5277
(MC-15598)



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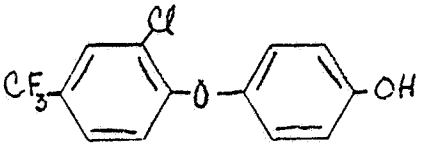
(MC-10879)



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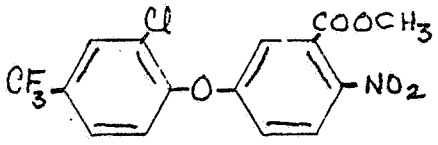
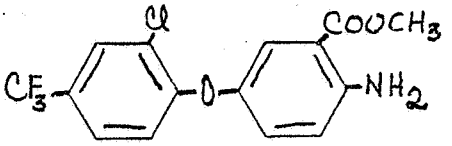


LS-81-5875
(MC-10108)



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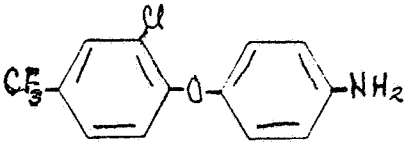
(MC-14620)



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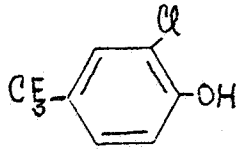
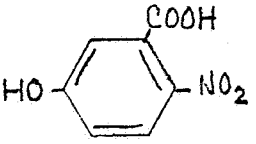
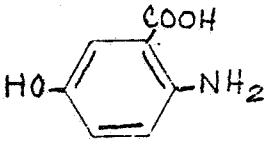


LS-82-5284



LS-82-5285



LS-82-5286

B

POTENTIAL METABOLITES OF ACIFLUORFEN

The five residue components are as follows (see chart for structures).

Acifluorfen sodium, MC-10978 (LS-80-1213)

Acifluorfen, MC 10109 (LC-82-5276)

Acifluorfen-methyl, MC-10108 (LS-81-5875)

Amino analogue, MC-14621 (LS-82-5281)

Amino ester analogue, MC 14620 (LS-82-5282)

All components are converted to the acifluorfen ester which is then determined.

For residue analysis, an homogenized sample is extracted with a 1% acetic acid in acetonitrile solution, and an aliquot is then concentrated and treated with peroxytrifluoroacetic acid (this reagent oxidizes the amino group to the nitro group). The mixture is acidified with hydrochloric acid and extracted with dichloromethane which is evaporated to dryness.

The residue is taken up in ethyl ether, treated with diazomethane and evaporated to dryness. (This process converts the acid forms to the methyl ester form.)

The residue is taken up with hexane and cleaned up on a florisil column. The residue is eluted with a solution of ethyl acetate in hexane which is evaporated.

The residue is taken up with hexane and determined by gas chromatography using an electron capture detector (ECGC). A limit of detection of 0.02 ppm is reported for each component.

Untreated (control) soybean samples had no detectable acifluorfen-equivalent residues (<0.02 ppm). Control samples of beans, straw, and forage were fortified with each component at levels of 0.01-0.2 ppm. Overall recoveries were 32-125%.

Control soybean grain samples were fortified with the four components which contained C^{14}-radiolabelling at levels of 0.3 ppm. The fortified samples were then analyzed by liquid scintillation techniques (LSC) and gas chromagraphy (GC). Recoveries were 77-100% (LSC) and 56-100% (GC). The results support a conclusion that residues are adquately determined by gas chromatography.

The analytical method does not, however, distinguish between residues of the various components. The method is therefore not specific. In order to enhance the method's specificity, a second method is submitted.

The method (No. 161) determines the components listed above as separate entities. The limit of detction for each component is reported to be 0.02 ppm.

An homogenized sample is extracted with acetic acid in acetonitrile. An aliquot is made alkaline and extracted with hexane (the aqueous phase is saved). The extract is filtered and evaporated to dryness (this residue contains acifluorfen-methyl, MC 10108, and acifluorfen-methyl amino analog, MC 14620). The residue is cleaned up on a florisil column, and the two components are eluted with ethyl acetate in hexane. The eluate is evaporated to dryness.

The residue is treated with heptafluorobutyric anhydride (HFBA) which forms the amide derivative with MC 14620 (the MC 10108 remains unchanged). The two components are determined as separate entities by gas chromatography.

The aqueous phase from the initial extract is acidified with sulfuric acid and extracted with ethyl acetate. The aqueous phase is made more acidic and again extracted with ethyl acetate. All ethyl acetate extracts are combined, evaporated to dryness, and taken up with hexane.

An aliquot of the hexane solution is taken for the determination of acifluorfen (MC 10109) and evaporated to dryness (the remaining portion is saved). The residue is treated with diazomethane (which forms the methyl ester of acifluorfen, MC 10108), cleaned up on a florisil column, and determined by gas chromatography.

The remaining hexane phase saved from the preceding paragraph (which contains the amino analog, MC 14621) is evaporated to dryness, treated with the diazomethane reagent which converts the component to the methyl amino analog, MC 14620. The solution is evaporated to dryness and treated with HFBA (forms the butyric amide). The derivative is cleaned up on a florisil column, eluted with hexane, and determined by gas chromatography.

Control grain samples were fortified with MC 10108, MC 10109, MC 14620, and MC 14621 at levels of 0.02-0.1 ppm. Recoveries were 70-138%.

The proposed enforcement method (No. 160) and the confirmatory method (No. 161) are adequate for the determination of residues of acifluorfen and its metabolites.

Method trials for acifluorfen on soybeans, meat and milk were successfully completed in connection with an earlier petition.

Residue Data

Field studies were performed in Nebraska, Alabama, Georgia, Missouri, Arkansas, Illinois, North Carolina, Minnesota, South Dakota, and Indiana. The crops were treated at rates of 0.25-1.0 lb act/A (up to 1.3X maximum proposed rate). 88 samples of soybeans were collected at intervals of 97-171 days after treatment (PHI proposed:100 days) and examined.

The beans were analyzed for: acifluorfen-sodium, MC-10978; acifluorfen, MC-10109; acifluorfen-methyl ester, MC-10108; amino acifluorfen, MC-14621; methyl ester of amino acifluorfen, MC-14620. (Bean samples were fortified with the various components at levels of 0.02-0.05 ppm. Average recoveries were 69-97%.) No residues of any component were detected (<0.02 ppm) due to any application rate.

Byproducts

The absence of detectable residues (>0.02 ppm) at the proposed rates and the showing of low levels of total activity in soybeans in the metabolism study indicates that residues, if any, in soybean processing fractions (meal, oil, hulls, soapstock) would be less than those in the grain. Therefore, a food additive tolerance is not necessary.

Forage

No data are submitted for soybean forage. However, the labelling states that treated plants are not to be used for feed or forage. As a result, residue data are not needed.

The plant metabolism studies indicate that no real residues are likely to occur in soybeans from the proposed use. The proposed tolerance level of 0.1 ppm represents the combined sensitivity levels for the five components sought in the residue (the sensitivity for each component is 0.02 ppm).

Meat, Milk, and Eggs

Soybeans, its forage and hay, and its byproducts (meal, hull, soapstock) may be used as livestock feeds. The labelling bans the use of plants for food or feed. This removes soybean forage and hay as feeds.

The residue data and metabolism studies indicate no real residues are expected in soybeans. As a result, no residues will occur in the byproducts (meal, hulls, soapstock).

Since the feed items contain no residues, then no residues will occur in eggs, milk, meat, fat, and meat byproducts of livestock [§180.6(a)(3)].

cc: R.F.
Circu
Alfred Smith
TOX
EEB
EAB
Petition No. 3F2811
FDA, Robert Thompson
RDI:Section Head:RJH:Date-7/1/83:RDS:Date-7/19/83:DCR-11720
TS-769:RCB-28:AS:pad:RM:810:CM#2:7/20/83
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