(9-7-84)

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Na-Acifluorfen,(Tackle), Evaluation of a Rat Metabolism Study and a Contractor Review Thereof

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

FROM: Robert P. Zendzian PhD, Acting head
Review Section III
Toxicology Branch
HED (TS-769)

9/7/84

THROUGH: William Burnam, Chief
Toxicology Branch

WSB 9.4.84

Compound Na-Acifluorfen (Tackle) Tox Chem #

Registration #359-TNI Registrant Rhone-Poulenc Inc.

Accession #071321

Action Requested

The Registrant has submitted a study of the metabolism of Na-Acifluorfen in the rat which was reviewed and evaluated by Dr. B.H. Chin of MITRE Corp on contract with Toxicology Branch. Dr Chin concluded that certian parts of the study were not acceptable and should be repeated. The Registrant has objected to the evaluation and has presented reasons for the acceptability of the entire study. As the Branch expert on metabolism studies I have been asked to resolve this matter.

Conclusion

The study, as reported, satisfies the Agency requirements for a metabolism study of a pesticide. The compound is rapidly absorbed after oral dosing and rapidly excreted. The compound is excreted in urine and feces with fecal excretion being secondary to bilary secretion. The compound is excreted unchanged but the material secreted in the bile is converted into the amine by gut microflura. Sex related differences in rates of absorption, distrobution and excretion were observed

at the low doses with the female having more rapid absorption and excretion but less rapid distrobution. These parameters were similar in both sexes at the single high dose.

Background

The report in question is cited as;

Pharmakinetics and Metabolism of MC 10978 (Sodium 5-[2-Chloro-4-(Trifluoromethyl)Phenoxy]-2-Nitrobenzoate), L.K. Low, J.R. Meeks, C.R. Mackerer & M.A. Mehlman, Study No.63281(983-80F), Rhone-Poulec, Inc. Sept 9, 1982.

The study generally follows the Proposed Guidelines published in 1978. Radiolabeled (^{14}C) compound was utilized. Four dose groups were used, orally low dose, high dose and 14 times low dose and intravenously low dose. Urine, fecal and blood samples were collected periodically during the study and tissue samples at termination. Quantitative and qualitative analysis were conducted on appropriate samples by scientifically acceptable methodology.

Dr Chin, in his review, cited as a major deficiency;

"1. Overall recovery of radioactivity in urine, feces and tissues ranging form 70.9 to 92.5% of the dose is considered very poor. As much as 29.1% (100-70.7% = 29.1%) of the dose was not accounted for by the authors. This loss is probably due to poor technique."

And as minor deficiencies, that excreta was not collected for sufficient time, the exact amount of glucuronde conjugate was not conclusive, post-operative recovary and time of compound administration was not specified in the bile cannulation study and toxic signs did not occur with the high dose.

As a result of this review it was requested that certian dose regimens be repeated. Of particular concern was the single low oral dose in the male with total recovery of 74.2% and the single low intravenous does in the female with total recovery of 70.9%.

The Registrant has replied that the low recoveries are most probably a function of the experimental design in which the same rats were used for excretion studies and blood kinetics studies. Handling of these animals for blood collection could result in unavoidable loss of urine as rats tend to urinate when handled. In addition the low tissue residue and the short half-time indicate rapid excretion over the collection period used. The minor deficiences were considered to have little or no effect on the purpose of the metabolism study.

Data Available

The metabolism study on Na-Acifluorfen provides three independent measures of the quantitative removal of the compound from the body, the collection of excreta, the measurement of the residual material in the tissues and the half-time of disappearance from the blood. This information is presented in the following tables.

Table 1. Percent of total radioactive dose recovered in urine and feces.

Dose regimen	Percent of total radioactive dose recovered	
	Males	Females
Single low oral	71.1	91.5
Single high oral	88.5	82.4
Multiple low oral	87.2	80.4
Single low intravenous	82.5	70.8

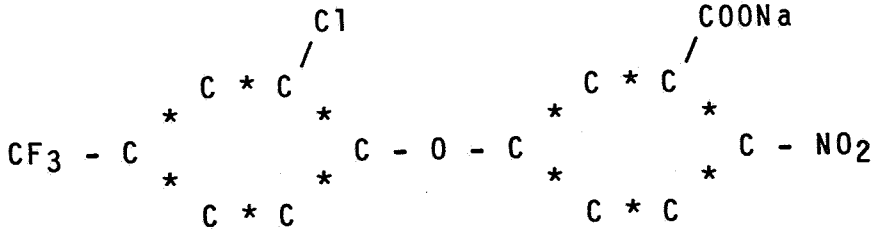
Table 2. Percent of total radioactive dose remaining in the total tissue at 96 hours.

Dose regimen	Percent of total radioactive dose recovered	
	Males	Females
Single low oral	3.05	0.99
Single high oral	1.92	2.49
Multiple low oral	3.08	0.63
Single low intravenous	1.87	0.13

Table 3. Elimination half-time from the blood

Dose regimen	Half-time (hours)	
	Males	Females
Single low oral	8.5	3.6
Single high oral	6.5	6.24
Multiple low oral	9.3	4.42
Single low intravenous	3.68	8.88

In addition one must consider the structure and physical /chemical properties of Na-Aciflourfen.



The compound is the sodium salt of a carboxilic acid having a pka of 3.8. It is freely soluble in water, has a low lipoid solubility and is ionized at plasma pH (7.4). Such a compound can be expected to be readily absorbed, through the stomach, and rapidly excreted by the kidneys

without the necessity of metabolic alteration. Tissue accumulation would not be expected.

Discussion

The 1978 Proposed Guidelines included a blood kinetic study to be performed on each of the dose groups. As a member and head of the Toxicology review group for metabolism studies I took part in discussions in which it was decided that the blood kinetics study would not be routinely required. The study would not contribute any additional useful information in relation to the safety of a pesticide for the effort required. It was immediately recognized that the excretion ballance studies and the blood kinetics studies could not be done on the same animals. Of particular concern was the passibility that handling the animals would lead to unavoidable loss of urine and feces as well as contaminating the handler with radio-labeled material. An independent blood kinetics study could require up to 45 additional animals. The Guidelines as subsiquently published do not include a requirement for blood kinetic studies.

Recovery in the urine and feces is the item of concern as shown in the relatively low values for the single low oral dose males and the single low intravenous dose females. However, urine and fecal collection concurrent with blood collection produces the single most serious potential for sample loss through reflex urination.

The tissue recovery data clearly shows that most of the radioactivity has left the experimental animals at the end of 96 hours. The percent of dose excreted ranges from 99.87% to 96.92%.

The worst case of the blood half-life (9.3 hrs) indicates that at the end of 96 hours 10 half-lives will have passed and less then 0.05% of the origional concentration of the compound will remain in the blood. Considering that the maximum volume of distrobution determined in the study was 106.2ml (multiple low oral dose, females) this indicates a potential whole body residue of 1.38ug. This is 0.01% of the initial dose. This theoretical reidue is one/tenth of the actual residue which is a reasonable approximation considering that at very low concentrations of a foreign substance the clearance is no longer a constant but will tend to decrease.

Thus, with all information available indicating rapid and essentially complete excretion of the test cmpound the most reasonable explanation of the two low excreta recoveries is loss of urine and/or feces during handling for blood samples.

The remaining concerns expressed by the Contract Reviewer can be most generously described as "trivial". The study as reported has answered the questions as to the rate and route of excretion and whether toxic metabolites are produced. Absolute adherance to peripheral portions of the experimental design will not improve on the answers to these critical questions.

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* CONFIDENTIAL BUSINESS INFORMATION *

Accession No. 071321

MRID: Not assigned

1. Chemical or Chemicals:

Acifluorfen-sodium

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

2. Type or Formulation:

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

3. Citation or Citations:

Mackerer, C.R., 1982. Pharmacokinetics and Metabolism of MC 10978 (Sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate), Mobil Environmental and Health Science Laboratory (Study No. 63281) Dated Sept. 30, 1982

4. Reviewed by:

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

Signature:
Date:

5. Approved by:

Signature:
Date:

6. Discipline/Topic or Test Type:

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

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

* CONFIDENTIAL BUSINESS INFORMATION *

7. Conclusions:

This DER reports the absorption, distribution, biotransformation, and excretion of acifluorfen-sodium (MC 10978) in female and male Fischer 344 rats (5 animals each) following four dosage regimens. The regimens include three oral dosages [single low (16-17 mg/kg), single high (116 mg/kg), and multiple low (10 -12 mg/kg daily for 14 days)] and an intravenous dosage (11-15 mg/kg).

Absorption

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

Distribution

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

Biotransformation

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

Excretion

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

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

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

8. Materials and Methods:

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

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

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

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

Preparation of Dosage Solutions

The four dosage regimens prepared were as follows:

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

Acifluorfen scientific reviews

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The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ 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

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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.

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

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

Animals

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

Collection of Blood, Urine, Feces, and Tissues

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

TABLE 1

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

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

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

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

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

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

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

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

Bile Cannulation Studies

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

Qualitative Analysis of Acifluorfen and Metabolites

A. Thin Layer Chromatography

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

- Ethyl acetate/isopropyl alcohol/water, 65:24:11 (v/v)
- Dichloromethane/acetic acid, 96:4 (v/v)
- Toluene/diethyl ether/acetic acid. 90:60:2 (v/v)

B. High Performance Liquid Chromatography

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

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

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

C. Gas Chromatographic and Mass Spectrometric Analysis

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

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

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

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

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

A. Urine

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

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

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

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

B. Feces

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

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

C. Blood, Bile, Kidney and Liver

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

Pharmacokinetic Calculations and Analysis

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

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

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

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

Statistical Analysis

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

9. Results and Discussion:

A. Absorption

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

1. Single Low Oral Dose

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

2. Single High Oral Dose

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

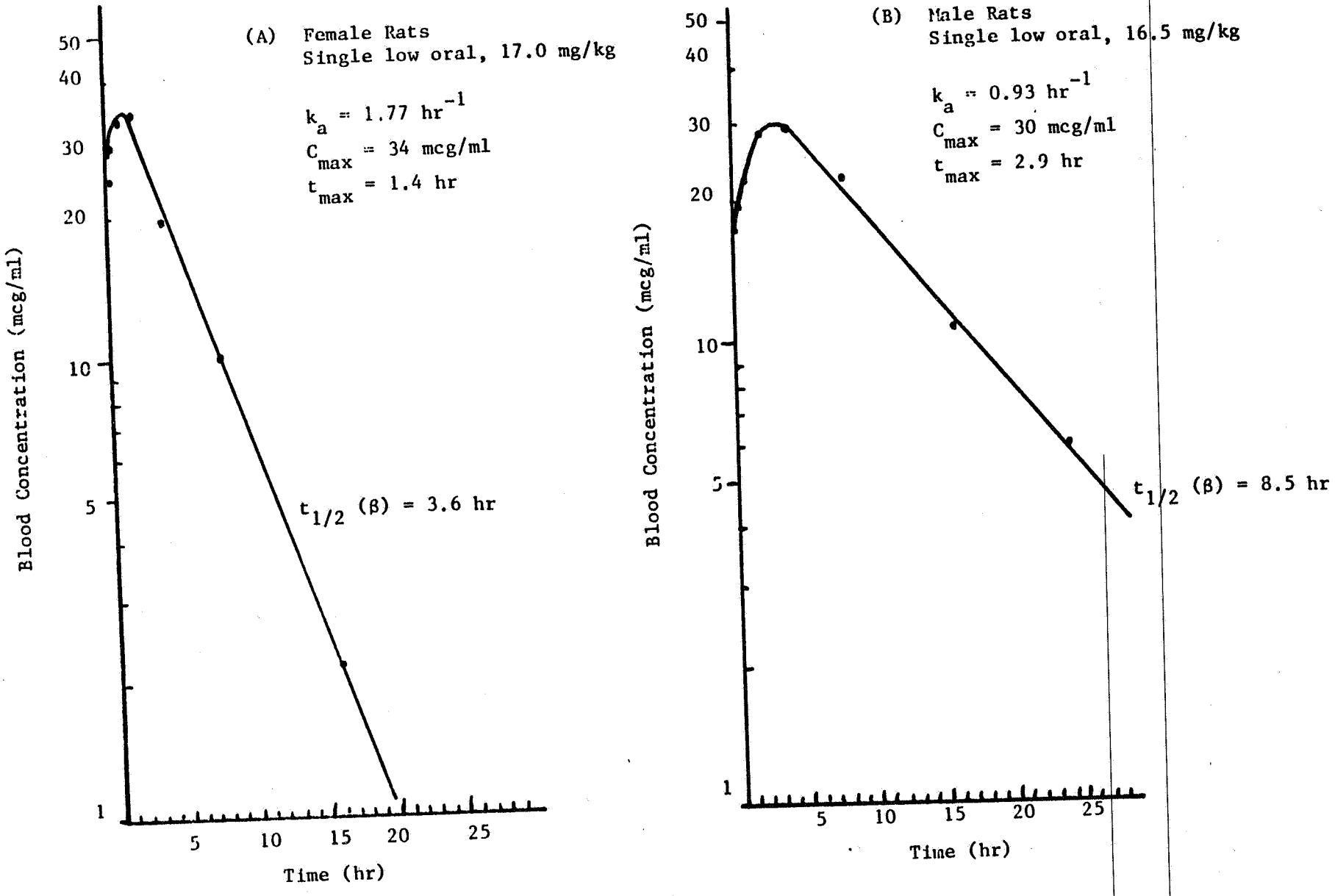

FIGURE 2

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

TABLE 2

ABSORPTION PHARMACOKINETIC PARAMETERS FOR
VARIOUS ACIFLUORFEN-SODIUM DOSAGE REGIMENS

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

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

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

3. Multiple Low Oral Dose

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

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

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

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

B. Distribution

1. Distribution Pharmacokinetics

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

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

2. Distribution of Radioactivity in Tissues

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

TABLE 3

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

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

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

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

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

TABLE 4

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

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

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

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

1. Urine

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

2. Feces

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

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

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

3. Blood, Bile, Kidney and Liver

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

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

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

TABLE 5

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

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

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

4. Metabolic Pathways of Acifluorfen-Sodium

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

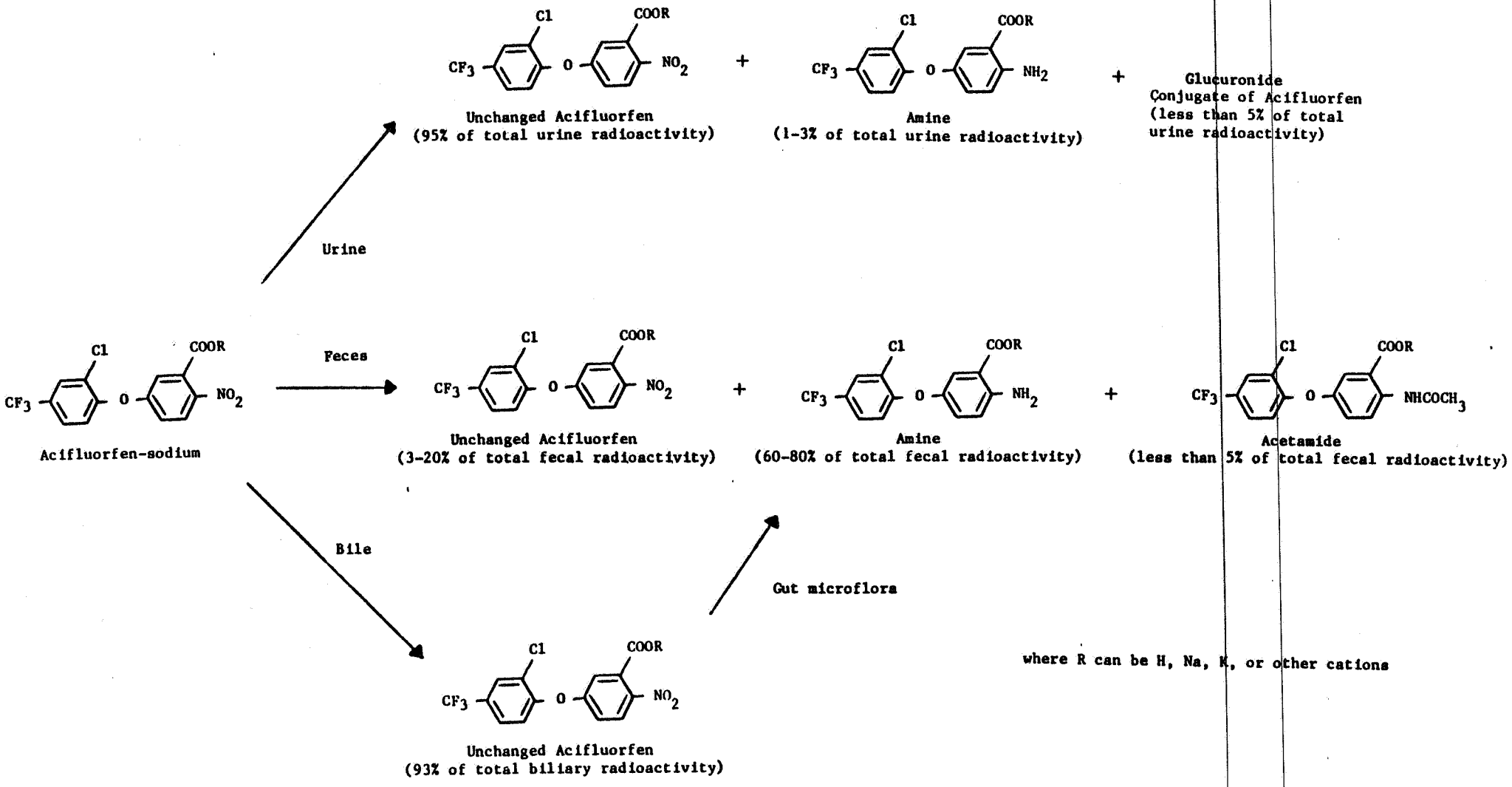

FIGURE 3

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

B

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TABLE 6

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

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

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

D. Excretion

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

1. Biliary Excretion Studies

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

TABLE 7

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

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

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

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

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

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

TABLE 8

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

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

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

half-life was 6.49 hr for high orally dosed male rats, it was not statistically different from the half-life of 8.34 hr for intravenously dosed animals (Table 9). The total body clearance for acifluorfen-sodium after administration of a high oral and an intravenous dose was not statistically different [5.81 ml/hr (high oral) versus 6.73 ml/hr (iv)] (Table 9).

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

A. Major Deficiency

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

B. Minor Deficiencies

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

TABLE 9

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

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

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

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

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

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

10. Technical Review Time: 72 hours.