Biotransformation of ^{14}C-N-Ethyl FOSE in Rats After Administration in Feed for One Week

January 28, 1983

Conducted At: Riker Laboratories, Inc.
3M Center
St. Paul, Minnesota 55144

During: October 1980 to June 1981

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Summary

A major metabolite of ^{14}C-N-ethyl FOSE was isolated from rat feces and identified as 2-N-ethyl perfluorooctanesulfonamido acetic acid (FFSAA). The metabolite accounts for at least 50% of the carbon-14 excreted in feces between 24-48 hours after cessation of administration of a mixture of ^{14}C-N-ethyl FOSE/feed which rats were fed for 1 week. The mean total dose was 300 mg/kg/week.

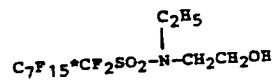
Although collection of feces for source material for metabolite isolation was the primary purpose of this study, the rats were fed normal chow for three weeks following the one week of ^{14}C-N-ethyl FOSE/feed administration and plasma and select tissues were collected at sacrifice and analyzed for carbon-14 content. The mean carbon-14 contents expressed as µg of N-ethyl FOSE equivalents/g of wet tissue in various tissues were: liver, 233; spleen, 20; and red blood cells, 30. Plasma contained 62 µg equivalents ^{14}C-N-ethyl FOSE/ml of plasma. The tissue concentrations at 21 days following the last dose after 1 week of feeding ^{14}C-N-ethyl FOSE are consistent with what would be expected after multiple dosing with a compound that is slowly eliminated.

Introduction

N-Ethyl FOSE is a product sold by 3M and is also a key intermediate in the synthesis of other products. In a previous study (1), it was shown that perfluorooctanesulfonate is a metabolite present in liver extracts from rats at 96 hours after administration in feed of a single dose of ^{14}C-N-ethyl FOSE. From the same liver extracts, a second metabolite was isolated and tentatively identified as perfluorooctanesulfonamide. Other unidentified metabolites were present in the liver extracts as was shown by thin-layer radiochromatograms. In the present experiment, three rats were administered ^{14}C-N-ethyl FOSE in feed for 1 week and feces were collected for two weeks after the dosing period. The feces collections from 24-48 hours after the last ^{14}C-N-ethyl FOSE dose were pooled and extracted. A metabolite was isolated and identified. The results are reported and discussed in the context of other fluorochemical metabolism studies.

Materials and Methods

Carbon-14 Labeled N-Ethyl FOSE



*Denotes position of carbon-14 label

N-Ethyl FOSE is 2-N-ethyl perfluorooctanesulfonamido ethanol.

The ^{14}C-N-ethyl FOSE used in this study was identical to the ^{14}C-N-ethyl FOSE used in the single dose ^{14}C-N-ethyl FOSE study reported earlier; the details of the radiochemical purity determination repeated for these two studies is reported (1). The specific activity of the lot of ^{14}C-N-ethyl FOSE used in these studies (Riker Isotope Inventory Number 468) is 0.483 $\pm$ 0.020 µCi/mg. On the basis of the specific activity determination, chemical characterization, and radiochemical purity reported separately (2), the ^{14}C-N-ethyl FOSE was judged suitable for metabolism studies.

Animals

Prior to dosing, three male Charles River CD rats[a] were conditioned overnight in individual stainless steel metabolism cages. Body weights were: Rat No. 1, 269 g; Rat No. 2, 210 g; and Rat No. 3, 219 g. The rats had free access to food[b] and water before and during the experiment.

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[a] Charles River Breeding Laboratories, Wilmington, Massachusetts.
[b] Purina Lab Chow, Ralston Purina Company, St. Louis, Missouri.

Dosing

Preparation of Dose/Feed Mixture

The ^{14}C-N-ethyl FOSE/feed mixture, prepared just prior to this study, was also used for the single dose study (1). The details of the preparation and the assay for the carbon-14 content are reported (1). A ^{14}C-N-ethyl FOSE solution was prepared by weighing 250 mg ^{14}C-N-ethyl FOSE into a 1 liter Class A volumetric flask, adjusting to volume with absolute ethanol, and mixing by inverting. The ^{14}C-N-ethyl FOSE solution was transferred to a 4 liter beaker and 500 g Purina Ground Chow was added. The ^{14}C-N-ethyl FOSE/feed mixture was stirred for one hour then transferred to a glass tray and the ethanol was evaporated. The carbon-14 content of the dose/feed mixture was determined by combustion (1).

Administration of Dose

A feed cup was attached with wire to the inside of each cage. A 130 g portion of Purina Ground Chow containing 0.53 mg ^{14}C-N-ethyl FOSE/g of chow was weighed into a separate wide-mouth, brown glass jar for each rat and portions of the mixture were frequently transferred to the corresponding feed cups. Care was taken not to place so much of the mixture in the cups that significant spilling by the rats could occur. The jars containing the ^{14}C-N-ethyl FOSE/feed stock mixture were kept tightly sealed at room temperature. It took one week for the rats to eat 130 g of the ^{14}C-N-ethyl FOSE/feed mixture. The total dose of ^{14}C-N-ethyl FOSE administered was for Rat No. 1, 256 mg/kg; Rat No. 2, 329 mg/kg; and Rat No. 3, 315 mg/kg. After the ^{14}C-N-ethyl FOSE/feed mixture was consumed (168 hours), the rats were given free access to normal chow.

Sample Collection

During the dosing period, urine and feces were collected and stored frozen (not analyzed at this time). Urine and feces were collected at 24 hour intervals for 1 week following cessation of administration of the ^{14}C-N-ethyl FOSE/feed mixture; thereafter, urine and feces were collected as pools for individual rats until sacrifice. At 21 days after the last ingestion of ^{14}C-N-ethyl FOSE, the rats were anesthetized with diethyl ether; blood was drawn from the descending aorta and immediately transferred to a heparinized tube. Plasma was prepared promptly by centrifugation. Aliquots were taken for analysis and the samples were then frozen. The rats were sacrificed by exsanguination and liver, spleen, brain, kidneys, lungs, and testes were collected as whole organs. The femurs and tibias were collected for bone marrow sampling. Samples of subcutaneous and abdominal fat, skeletal muscle, and digestive tract (esophagus, stomach, and intestines), and the remaining carcass were collected for each rat.

Radiometric Analyses

Sample Preparation

Feces, liver, and spleen were prepared for carbon-14 analysis by homogenizing and aliquoting a sample of the homogenate into combustion cones[a]. Homogenizing was done in Waring blenders by adding nine parts of water to one part of biological material. The homogenates (1.0 g) were weighed into combustion cones in duplicate. Care was taken to mix the homogenate between samplings. Samples of red blood cells were weighed into combustion cones. All other samples collected (see sample collection) were not analyzed; these samples remain available for further analysis or for metabolite isolation/identification studies.

Homogenates and samples weighed directly were combusted with a Packard Model 306 oxidizer. Recovery of carbon-14 was determined by combusting suitable blank homogenates (feces and liver) spiked with ^{14}C-N-ethyl FOSE solution; these reference samples were combusted at the beginning, middle, and end of the analytical sample set. Urine collections were sampled and counted directly; duplicate 1.0 ml aliquots of each sample were pipetted directly into scintillation vials and 15 ml Aquasol® was added. Plasma was sampled before freezing and counted directly; duplicate 0.5 ml aliquots plus 0.5 ml water were pipetted directly into scintillation vials and 15 ml Aquasol® added. All samples were cold and dark adapted before counting.

Analyses of Samples

All radiometric measurements were done using a Packard Model 3380 Tri-Carb Liquid Scintillation Spectrometer. For plasma and urine samples counted directly, the counting efficiency for each sample was determined by adding a known amount of internal standard to each sample and recounting. After each sample was corrected for background and for counting efficiency, the carbon-14 content was calculated. For combusted samples, counting efficiency for each sample was determined by use of the AES (Automatic External Standardization) ratio method. To calibrate the external standard, a known amount of internal standard was added to selected samples in the group (three with low AES ratios and three with high ratios) and these samples were recounted. For combusted samples, a correction was made for the recovery (mean, 95%) from the oxidizer. The percent recovery was based on reference samples of ^{14}C-N-ethyl FOSE combusted with each sample set.

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[a] Packard Instrument Company, Inc., 2200 Warrenville Road, Downers Grove, Illinois.

Metabolite Isolation and Identification

In this section, a lengthy procedure is described. A short description of this lengthy procedure follows: Feces homogenates were pooled and extracted with ether. The extract was chromatographed on a silicic acid column and a fraction was rechromatographed on a second column. A major metabolite was located by thin-layer chromatography (TLC) and isolated using preparative TLC. Comparison of R_f's in various systems with standard reference compounds suggested the structure to be $C_8F_{17}SO_2NCH_2CH_3$ with CH_2COOH on the nitrogen.

The data from gas chromatography/mass spectroscopy (GC/MS) confirm the identification.

The fecal homogenate pool from feces collected 24-48 hours after the last administration of ^{14}C-N-ethyl FOSE was placed into twelve 45 ml centrifuge tubes (25 ml homogenate/tube). To each tube was added 15 ml diethyl ether and the mixtures were shaken for 15 minutes on a mechanical shaker. The tubes were centrifuged and the ether was removed. (Care was taken not to remove any of the interphase material). The extraction was repeated five times. The ether was pooled and reduced with a rotating evaporator so that what remained was mostly the water that had been carried over with the ether. This water layer was extracted with chloroform (1:1, v:v). The layers were separated. Radiometrically, the chloroform contained 5.9 mg of N-ethyl FOSE equivalents. The extraction removed ~70% of the carbon-14 from the fecal homogenate. The chloroform layer was chromatographed by column chromatography (Unisil[a] was packed in chloroform into a 2.4 cm x 40 cm glass column). The chloroform extract of the aqueous residue from the ether extract was placed on the column. Five hundred ml of chloroform was passed through the column and collected as one fraction. Following the 500 ml of chloroform, 500 ml of methanol was passed through the column. Radiometrically, the chloroform fraction contained 4.6 mg equivalents of ^{14}C-N-ethyl FOSE and the methanol contained 1.3 mg. Thus, the chloroform column fraction contained ~55% of the material in the fecal homogenate.

The procedure for thin-layer chromatography (TLC) analysis of carbon-14 labeled compounds has been described previously (1). The chloroform column fraction was analyzed by TLC and the radiochromatogram is shown in Figure 1. A component at R_f 0.43 comprised ~90% of the carbon-14 in this column fraction or ~50% of the carbon-14 in the feces at 24-48 hours after the last ^{14}C-N-ethyl FOSE ingestion.

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[a] Unisil, activated silicic acid 100/120 mesh, Clarkson Chemical Company, Inc., Williamsport, PA.

The chloroform column fraction was evaporated with a rotating evaporator to a few ml and chromatographed by column chromatography. Unisil® was packed in benzene-chloroform (1:1, v:v) in a 2.4 cm x 40 cm glass column and the chloroform column fraction was placed on this second column. Fifty ml fractions were collected as 500 ml of benzene-chloroform (1:1, v:v), 500 ml of benzene-chloroform (1:2, v:v), and then 500 ml of chloroform were passed through the column. Aliquots (100 µl) of the 50 ml fractions were analyzed radiometrically, and most of the carbon-14 appeared to be in three 50 ml fractions collected from the 1:2 benzene-chloroform elutions (Fractions 17, 18, and 19). By inspection of the column during the chromatography and of the various fractions, it was apparent that much of the fecal pigments were separated from the carbon-14 by this column chromatography procedure. The pooled fractions 17, 18, and 19 contained 3.9 mg of ^{14}C-N-ethyl FOSE equivalents.

The pooled fractions 17, 18, and 19 from the second column were evaporated to a small volume and streaked on six 20 cm x 20 cm silica gel plates[a]. The plates were developed to 15 cm above the pre-adsorbent band in 100 ml of a mixture of 100 ml chloroform, 35 ml methanol, and 5 ml concentrated ammonium hydroxide. The carbon-14 was located by scraping 0.5 cm segments laterally from a center 5 cm x 20 cm section of the 20 cm x 20 cm plate. (Only a narrow strip ~1 cm was scraped.) The carbon-14 was located at R_f 0.33. A 1.0 cm wide zone centered at R_f 0.33 was scraped from the six plates and the silica gel was eluted with methanol.

The methanol from the elution was evaporated to a small volume and applied to a 20 cm x 20 cm silica gel plate that had been pre-washed in methanol and air dried. The plate was developed to 15 cm in 100 ml of solvent prepared by mixing 100 ml chloroform, 100 ml methanol, and 2 ml concentrated acetic acid. The carbon-14 was located radiometrically (a narrow band from the origin to the solvent front was scraped from the center of the plate laterally in 0.5 cm segments and counted). The R_f of the carbon-14 labeled material was 0.77. A 1.0 cm zone centered at R_f 0.77 was scraped and the silica gel was eluted with acetone and then chloroform-methanol (1:1, v:v). The eluates were combined, evaporated to a small volume, and streaked on a silica gel plate and developed to 15 cm in the same chloroform-methanol-acetic acid solvent system as before. The carbon-14 was located by scraping a segment and counting. The carbon-14 was again located at R_f 0.77 . A 1.0 cm zone centered at R_f 0.77 was scraped and the silica gel was eluted with acetone. The acetone eluate contained 1.0 mg of N-ethyl FOSE equivalents.

Figure 2 is a radiochromatogram of the carbon-14 labeled material eluted from the second plate with acetone; the TLC plate was developed in 100 ml of a mixture prepared from 100 ml chloroform, 100 ml methanol, and 2 ml concentrated acetic acid.

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[a] Analtech, 75 Blue Hen Drive, Newark, Delaware.

Reference standards were prepared in methanol and spotted along with aliquots of the purified (radiochemically) metabolite on TLC plates. The structures of the reference standards were:

1) $C_8F_{17}SO_2NH_2$

2) $C_8F_{17}SO_2NHC_2H_5$

3) $C_8F_{17}SO_2NCH_2CO_2H$ (with C_2H_5 on N)

4) $C_8F_{17}SO_2NHCH_2CO_2H$

The plates were developed in 100 ml of a mixture prepared from 100 ml chloroform, 35 methanol, and 5 ml concentrated ammonium hydroxide (System A) or in 100 ml of a mixture prepared from 100 ml chloroform, 100 ml methanol, and 2 ml concentrated acetic acid (System B). The spots on the plate were located with acidified palladium chloride or bromphenol blue spray reagents. The R_f of the metabolite was confirmed radiometrically by scraping and counting. The R_f was 0.28 in System A and 0.77 in System B.

The TLC of the metabolite and the reference standards showed that the R_f of standard 3 ($C_8F_{17}SO_2NCH_2CO_2H$ with C_2H_5 on N) was the same in both systems and different than the R_f of standards 1, 2, and 4. The ^{14}C-metabolite was submitted to 3M Central Research (J. N. Schroepfer) for GC/MS analysis (see Appendix 1).

GC/MS data indicate identical retention times and an exact match of EI spectrum with standard 3, and the CI spectrum of the metabolite matches exactly with the CI spectrum of standard 3. In addition, it is possible to assign the major fragments observed consistent with the structure of standard 3.

Overall, the identical thin-layer chromatography R_f's, identical gc retention times, identical EI and CI spectrum, and the interpretation of structural data from mass spectra show that the metabolite is $C_8F_{17}SO_2NCH_2CO_2H$ (with C_2H_5 on N).

The methanol fraction from the first silica gel column was evaporated and spotted on a silica gel plate and developed to 15 cm in 100 ml of a mixture of 100 ml chloroform, 100 ml methanol, and 2 ml acetic acid. The radiochromatogram is shown in Figure 3.

Results and Discussion

A major metabolite of ^{14}C-N-ethyl FOSE was isolated from feces and identified as 2-N-ethyl perfluorooctanesulfonamido acetic acid (PFSAA). The metabolite accounts for at least 50% of the carbon-14 excreted in a feces pool at 24-48 hours after the last dose (see Appendix 2) in a regimen in which rats were fed ^{14}C-N-ethyl FOSE for 1 week. Since the extraction is from feces collected (24-48 hours after the last dose) following a week of dosing, the contents reflect what would be expected at various times after a single dose. As reported previously (1) by one week following a single dose of ^{14}C-N-ethyl FOSE, ~50% of the carbon-14 is eliminated via feces. These data suggest that a large portion of the carbon-14 eliminated via feces is PFSAA. In addition, three other metabolites were extracted from feces. Together, these other unidentified metabolites account for ~15% of the carbon-14 in this pool.

In a previous experiment, perfluorooctanesulfonate was identified in liver extracts from rats at 48 hours after a single oral dose of ^{14}C-N-ethyl FOSE (1). Perfluorooctanesufonate accounted for at least 22% of the carbon-14 in the liver pool (4.4% of dose). From the same extracts, a second metabolite which accounted for at least 32% of the carbon-14 in the liver pool was tentatively identified as perfluorooctanesulfonamide (6.4% of dose) (1).

Identification of perfluorooctanesulfonate in liver as a metabolite at 4.4% of the dose after a single dose and identification of PFSAA as a metabolite comprising 50% of the carbon-14 in feces during the second twenty-four hour period after the last dose following a week of feeding ^{14}C-N-ethyl FOSE establishes that both compounds are major metabolites of N-ethyl FOSE. However, these data do not lead to conclusions as to the relative proportion of the biotransformation to either compound.

Since it can be assumed that the time course of elimination of PFSAA and perfluorooctanesulfonate is vastly different [based on the amount of PFSAA in feces and on previous metabolism studies on perfluorooctanesulfonate which is eliminated slowly via feces (<5% of dose/week) (3)] and since the possibility that PFSAA is further biotransformed to perfluorooctanesulfonate exists, it would require a kinetic study to ascertain the rate and extent of biotransformation of N-ethyl FOSE to these compounds.

For the same reasons given for PFSAA, the relative extent of the biotransformation of N-ethyl FOSE to the three metabolites tentatively identified as perfluorooctanesulfonamide (1) and the metabolites in the methanol column fraction (Figure 3) (which, since they have not been identified, may include perfluorooctanesulfonate and perfluorooctanesulfonamide) is not established with these data.

The mean carbon-14 content expressed as µg of N-ethyl FOSE equivalents/g of wet tissue in various tissues were: liver, 233; spleen, 20; and red blood cells, 30. Plasma contained 62 µg equivalents ^{14}C-N-ethyl FOSE/ml of plasma. (Individual tissue and plasma concentrations are listed in Appendix 3.) The tissue concentrations at 21 days following the last dose after 1 week of feeding ^{14}C-N-ethyl FOSE are consistent with what would be expected from multiple dosing with a slowly eliminated compound.

References

1. Gibson SJ, Johnson JD: Absorption and Biotransformation of N-Ethyl FOSE and Tissue Distribution and Elimination of Carbon-14 After Administration of N-Ethyl FOSE-^{14}C in Feed to Rats (Report) January 19, 1983.

2. Johnson, JD and Behr, FE: Synthesis and Characterization of N-Ethyl FOSE-^{14}C (Report) December 11, 1980.

3. Johnson JD: Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats After a Single Intravenous Dose of FC-95-^{14}C (Report) December 28, 1979.

List of Figures

Figure 1

Thin-Layer Radiochromatogram of
Chloroform Column Fraction

Pre-adsorbent SGF Uniplate: 100 chloroform
35 methanol
5 ammonium hydroxide

Total CPM on Plate = 19,221

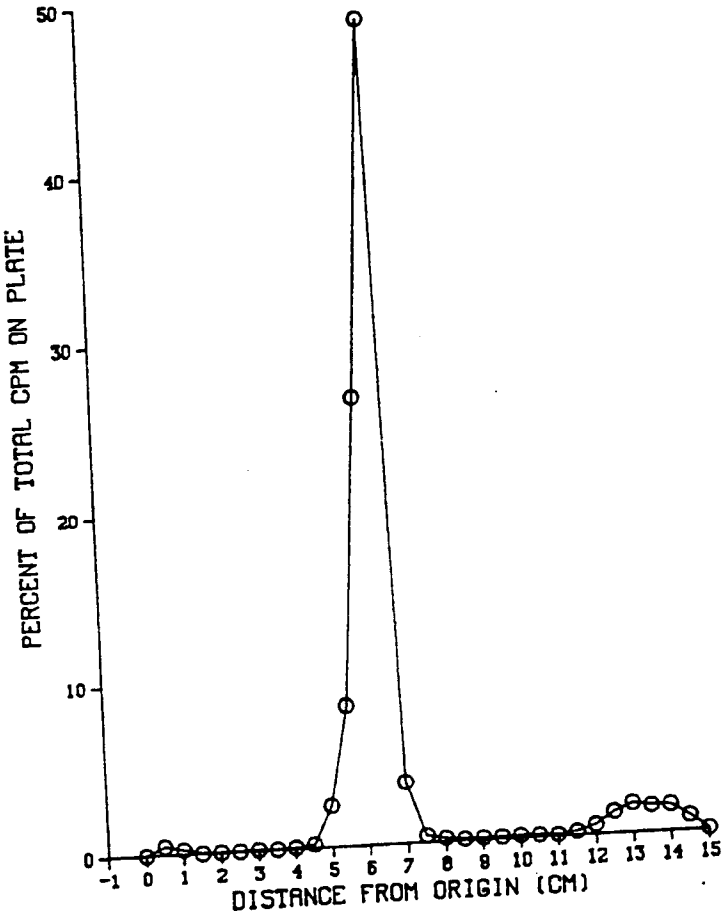


Figure 2

Thin-Layer Radiochromatogram of
Column Fractions 17, 18, and 19

Pre-adsorbent SGF Uniplate: 100 chloroform
100 methanol
2 acetic acid

Total CPM on Plate = 18,428

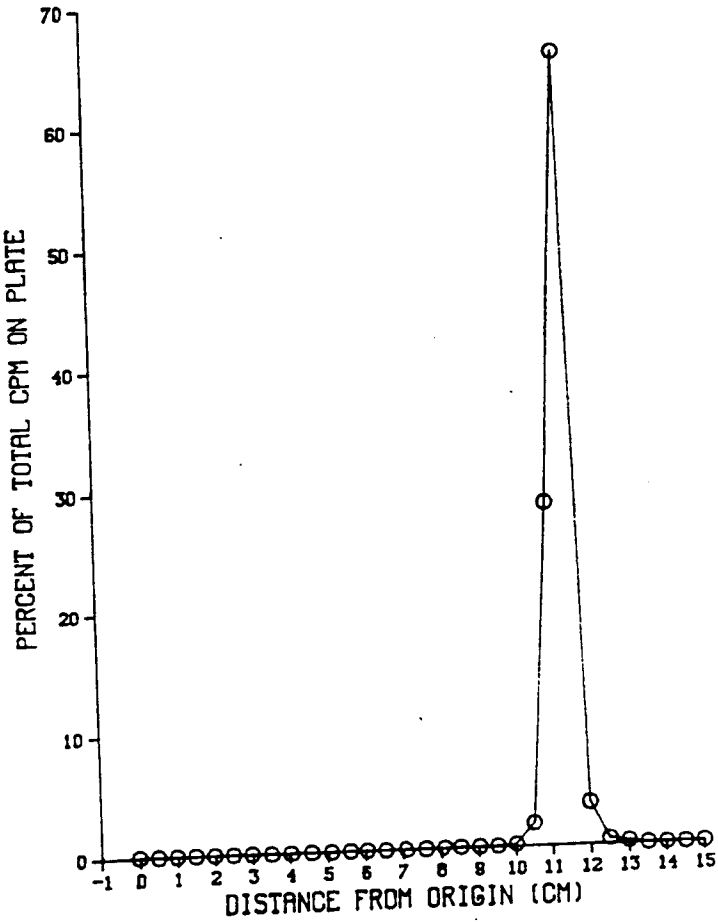
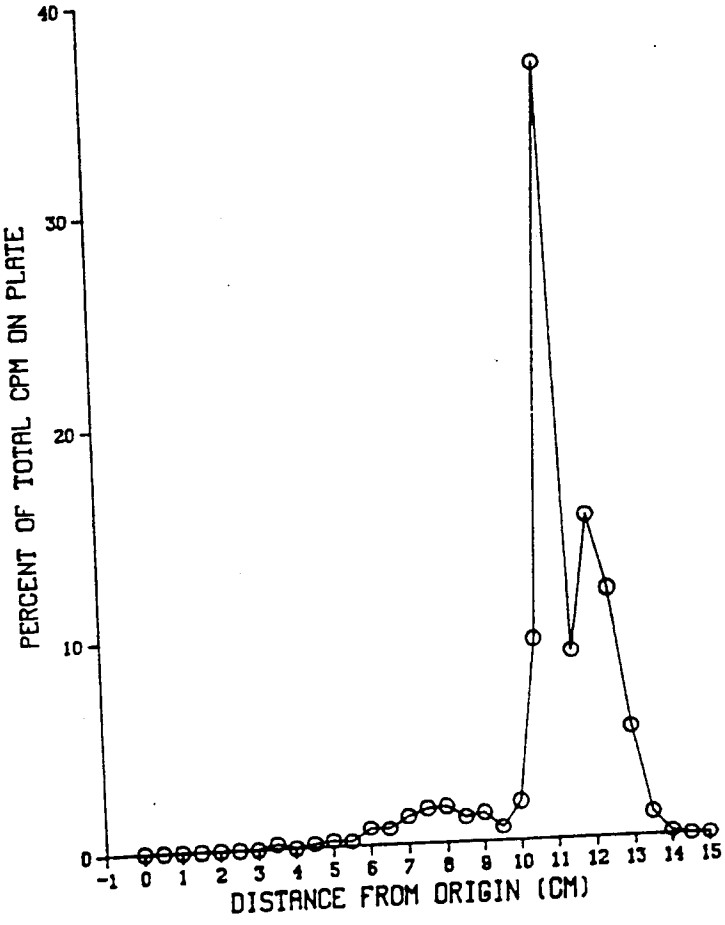


Figure 3

Thin-Layer Radiochromatogram of
Methanol Column Fraction

Pre-adsorbent SGF Uniplate: 100 chloroform
100 methanol
2 acetic acid

Total CPM on Plate = 1,742



List of Appendices

Form 8747-11-A

Appendix I

TECHNICAL REPORT SUMMARY

Date: March 13, 1981

TO: TECHNICAL COMMUNICATIONS CENTER – 201-2CN

(Important – If report is printed on both sides of paper, send two copies to TCC.)

Division: CENTRAL RESEARCH LABORATORIES, Analytical and Properties Research Laboratory
Dept. Number: 0502

Project: Service to Riker - Isolation of Trace Fluorochemicals
Project Number: A000007

Report Title: Mass Spectral Analysis of N-Ethyl AR No. 7454 - FOSE-^{14}C Labeled - March 13, 1981
Report Number: 454

To: J. D. Johnson - 218-2-02

Author(s): J. N. Schroepfer
Employee Number(s): 076647

Notebook Reference:
No. of Pages Including Coversheet: 3

SECURITY ▶ ☐ Open (Company Confidential) ☒ Closed (Special Authorization)

3M CHEMICAL REGISTRY ▶ New Chemicals Reported ☐ Yes ☒ No

KEYWORDS: (Select terms from 3M Thesaurus. Suggest other applicable terms.)

CRLAP
Analytical Report
Chemical Analysis
[illegible]/MS
[illegible]/MS
CI/MS
Negative MS

CURRENT OBJECTIVE:

Request No. C58236

Project No. 91505026

Requestor - J. D. Johnson

REPORT ABSTRACT: (200-250 words) This abstract information is distributed by the Technical Communications Center to alert 3M'ers to Company R&D. It is Company confidential material.

Based on the agreement of the results from the two samples using GC, EI MS, and CI MS, the structure of the major component in the sample metabolite is probably identical to the structure of the major component in the standard-N-ethyl FOSE.

Negative CI MS of the methylated standard can help detect this compound but cannot confirm the structure by itself.

Information Liaison Initials: [illegible]
3/20/81

Form 2843-C rev.

CENTRAL ANALYTICAL LABORATORY

Report No. 7454

Date March 13, 1981

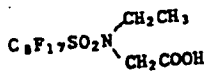
Subject: Mass Spectral Analysis of N-Ethyl FOSE-^{14}C Labeled

Requestor: L. D. Johnson Dept. Name Riker Proj. No. 91505026

Request No. C58236 Dated March 3, 1981

Report:

The requestor submitted, for GC/MS analysis, a sample metabolite of N-Ethyl FOSE-^{14}C labeled and a standard of N-Ethyl FOSE having the following structure:



The sample metabolite was an extract from rat feces which the requestor separated on various TLC systems and divided equally into three vials each containing 100,000 cpm of ^{14}C, or about 100µg.

Experimental

Diazomethane (CH_2N_2) reacted with the sample metabolite and standard; each sample had an air stream blown over it to evaporate excess CH_2N_2. Electron Impact (EI) and Chemical Ionization (CI) mass spectra resulted from the sample metabolite and standard introduced into the mass spectrometer (MS) via a gas chromatograph (GC). Table I lists the sample analyzed, the method of introduction, the mode of MS operation and the mass spectrum number. Table II lists the conditions used. Negative CI mass spectra resulted from the standard methylated and unmethylated introduced directly into the MS.

Discussion

For this discussion, when I mention the sample metabolite and the standard, I will refer to their methylated forms unless specified otherwise. The major component in the GC trace of the sample metabolite and standard showed identical retention time (8.6 minutes). The EI mass spectrum of this component in the sample metabolite compared exactly to the EI mass spectrum of the major component in the standard. They each contain the same base peak--116 m/z--probably due to ($-N(CH_2CH_3)CH_2COOCH_3$). The largest abundant high-mass fragment is the same in each sample -540 m/z which is due to $C_8F_{17}SO_2N(CH_2CH_3)-CH_2-$. Both mass spectra show a very small fragment at 600 m/z—a parent ion of 599 m/z + 1, which is probably due to a self CI phenomenon observed in other samples. The CI mass spectra of the two samples compare exactly. Both sample spectra show primarily three mass spectral fragments: 116, 540, and 600 m/z. The negative CI mass spectrum of the unmethylated standard shows a 586 for the parent ion + 1.

The negative CI mass spectrum of the methylated standard shows a barely detectable fragment at 600 m/z, a fragment at 527 m/z (loss of 72 m/z from 599 m/z), a fragment at 483 m/z (probably due to $C_8F_{17}SO_2^-$), and the base peak at 181 m/z (probably due to loss of 419 m/z C_8F_{17}).

Conclusion

Based on the agreement of the results from the two samples using GC, EI MS, and CI MS, the structure of the major component in the sample metabolite is probably identical to the structure of the major component in the standard-N-ethyl FOSE.

TABLE I

Sample	Method of Introduction	Mode of Operation	Mass Spectrum Number
Sample metabolite of N-Ethyl FOSE ^{14}C methylated	GC	EI	AA5624
	GC	CI	AA5625
Standard of N-Ethyl FOSE methylated	GC	EI	AA5622
	GC	CI	AA5623
	DP	-CI	AA5639
Standard of N-Ethyl FOSE	DP	-CI	AA5638

TABLE II

Varian 2740 Gas Chromatograph

Column: 2 meter, glass, 5% OV101 on 80/100 mesh Chrom G-HP

Oven Program: 100°C to 330°C at 10°C/min.

Injector temperature: 270°C

Detector temperature: 260°C (Varian FID)

Carrier gas: helium at 25 mls/min.

duPont 21-491B Mass Spectrometer

Source: Chemical Ionization

CI gas: Isobutane

Mass Range: 15-617 m/z

Separator: Single stage jet

Multiplier: Bendix Channeltron® (set to 4200v for Negative CI)

J. N. Schroepfer

J. N. Schroepfer

JNS/rs

Appendix 2

Excretion of Total Carbon-14 in Feces After an Oral Dose of ^{14}C-N-Ethyl FOSE Fed to Rats for 1 Week[a]

Time Period of Collection (Hrs)	Rat Number 1	2	3	$\bar{x} \pm$ SD
0-24	5.74[b]	4.68	5.28	5.23 + 0.53
24-48	2.10	3.12	3.23	2.82 ± 0.63
48-72	1.52	1.92	1.87	1.77 ± 0.22
72-96	1.14	1.12	1.33	1.19 ± 0.12
96-120	0.66	0.65	0.66	0.66 ± 0.00
120-144	0.49	0.52	0.65	0.55 ± 0.09
144-168	0.48	0.43	0.44	0.45 ± 0.03
168-504	2.35	2.23	2.39	2.32 ± 0.09

[a] Total doses fed rats were: Rat No. 1, 256 mg/kg; Rat No. 2, 329 mg/kg; and Rat No. 3, 315 mg/kg.

[b] Data are expressed as mg of N-ethyl FOSE equivalents excreted during time period.

Appendix 3

Carbon-14 Content at 21 Days in Tissues After an Oral Dose of ^{14}C-N-Ethyl FOSE Fed to Rats for 1 Week[a]

Rat Number	Liver	Spleen	Plasma	Red Blood Cells
1	220.8[b]	21.6	61.9	28.3
2	211.3	17.5	55.5	24.8
3	266.6	21.8	68.9	37.4
Mean ± SD	232.9 ± 29.6	20.3 ± 2.4	62.1 ± 6.70	30.2 ± 6.5

[a] Total doses fed rats were: Rat No. 1, 256 mg/kg; Rat No. 2, 329 mg/kg; and Rat No. 3, 315 mg/kg.

[b] Data are expressed as µg of N-ethyl FOSE equivalents/g of wet tissue or ml of fluid.