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Absorption and Biotransformation of N-Ethyl FOSE
and Tissue Distribution and Elimination of Carbon-14
after Administration of N-Ethyl FOSE-¹⁴C in Feed

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Summary

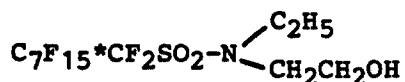
After a single oral dose of N-ethyl FOSE-¹⁴C (2-N-ethyl perfluorooctanesulfonamido ethanol) (mean dose, 10.13 mg/kg) in feed to fasted rats (groups of 3), at least 70% and probably more than 70% of the total carbon-14 is absorbed. Elimination of carbon-14 via urine and feces is very slow; from 0-32 days, only 63% of the dose is eliminated. Fecal elimination of carbon-14 is about 20-30 fold that of urine. The half-life of the disappearance from plasma of carbon-14 from day 1 to day 16 is 7.5 days. A metabolite of N-ethyl FOSE isolated from liver was identified by ¹⁹F-NMR analysis as the perfluorooctanesulfonate anion. In addition, another metabolite from liver has been tentatively identified as perfluorooctanesulfonamide.

Introduction

In addition to being a product sold by 3M, N-ethyl FOSE is a key intermediate in the production of other products. N-Ethyl FOSE is important in the study of FC-807 metabolism because the three esters of FC-807 are phosphate esters of N-ethyl FOSE and in addition FC-807 contains a trace (~0.8%) of N-ethyl FOSE. It would be expected that possible common metabolites occur from FC-807 and N-ethyl FOSE via different pathways or that after phosphate ester hydrolysis of FC-807 (systemic and/or gut) the biotransformation of N-ethyl FOSE and FC-807 follow similar pathways. Thus, the absorption, tissue distribution, and biotransformation of N-ethyl FOSE were investigated. The results are discussed in the context of other fluorochemical metabolism data from FC-807 and perfluorooctanesulfonate.

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 carbon-14 label is at the carbon α to the sulfur atom (see above structure). On the basis of the specific activity determination, chemical characterization, and radiochemical purity reported separately (1), the N-ethyl FOSE-¹⁴C was judged suitable for metabolism studies. The specific activity of the lot of N-ethyl FOSE-¹⁴C used in these studies (Riker Isotope Inventory Number 468) is 0.483 ± 0.020 μCi/mg. The radiochemical purity determination was repeated on the N-ethyl FOSE-¹⁴C dosing solution (see Appendix 1 - Table 1, and Appendix 1 - Figures 1-5) and the N-ethyl FOSE-¹⁴C was found to be at least 98% pure.

Animals

Male Charles River^a CD rats, eight weeks old, were conditioned to individual metal metabolism cages for 48 hours prior to dosing. The rats were fasted with free access to water for 24 hours prior to dosing. The body weights ranged from 220 to 329 g, mean 277 g. Rats were dosed in groups of 3; individual rats were selected so that the body weights of rats within each group of 3 were within ^b21 grams of each other. The rats were allowed free access to Purina^b Ground Chow and water after dosing.

Dosing

Preparation of Dose/Feed Mixture

An N-ethyl FOSE-¹⁴C solution was prepared by weighing N-ethyl FOSE-¹⁴C into a 1 liter Class A volumetric flask, adjusting to volume with absolute ethanol, and mixing by inverting. The N-ethyl FOSE-¹⁴C solution was transferred to a 4 liter beaker and Purina Ground Chow was added. The N-ethyl FOSE-¹⁴C/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 (see Appendix 2 and Appendix 2 - Table 1).

Administration of Dose

Each fasted rat was weighed immediately before being transferred to an individual metal metabolism cage. A feed cup was attached with wire to the inside of each cage to hold the single oral dose. The dose was a weighed amount of Purina Ground Chow containing 0.531 mg N-ethyl FOSE-¹⁴C/g. All of the rats in the groups sacrificed at 1, 2, and 4 days postdose consumed the dose administered in a two hour period. For rats sacrificed at 8, 16, and 32 days postdose, a longer period of time was allowed to consume the dose (12 hours) although most of the fasted rats ate the feed/dose immediately. Any dose/feed mixture not consumed was weighed and the weight subtracted from the total dose/feed mixture given to each rat. By inspection, it appeared that very little of the feed was spilled by the rats. The dose administered each rat was calculated from the weight of feed consumed. The mean dose was 10.13 mg/kg (see Appendix 3).

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

Sample Collection

Urine and feces were collected at 24 hour intervals for each rat sacrificed at 1, 2, 4, and 8 days postdose (groups of 3). Urine and feces were collected at intervals and respectively pooled for each rat sacrificed at 16 and 32 days postdose (groups of 3). At 1, 2, 4, 8, 16, and 32 days postdose, rats were anesthetized with diethyl ether; blood was drawn from the descending aorta and immediately transferred to a heparinized Vacutainer® tube. Plasma was prepared promptly by centrifugation. The rats were sacrificed by exsanguination and liver, spleen, kidneys, and lungs were collected as whole organs. Bone marrow was obtained from the femurs and tibias by splitting the bones and collecting the marrow on pieces of tared combustion pads^a. Samples of subcutaneous and abdominal fat, and muscle were collected. Digestive tract (esophagus, stomach, and intestines) and the remaining carcass were collected from rats sacrificed at 1 and 2 days postdose.

Radiometric Analyses

Sample Preparation for Radiometric Analyses

Feces and major organs 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 were weighed into combustion cones in duplicate by taring the cone and adding 1.0 g of the homogenate. Care was taken to mix the homogenate between samplings. Samples of bone marrow, fat and red blood cells were weighed into combustion cones. Care was taken to weigh these samples promptly to avoid loss of weight by drying. Homogenates and samples weighed directly were combusted with a Packard Model 306 Oxidizer. Recovery of carbon-14 from biological samples was determined by combusting suitable blank homogenates (feces, liver, kidney, muscle, and spleen) spiked with N-ethyl FOSE-¹⁴C solution; these reference samples were combusted at the beginning, middle, and end of the experimental sample set (see Appendix 4). Urine collections were sampled before freezing and were 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 1.0 ml aliquots or 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.

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

Analyses of Samples

All radiometric measurements were done using Packard Model 3380 and 3385 Tri-Carb Liquid Scintillation Spectrometers. 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 with the appropriate blanks and for counting efficiency, the carbon-14 content of each sample was calculated. For combusted samples, counting efficiency for each sample was determined by use of the AES (Automatic External Standard) 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 from the oxidizer. These recoveries were based on reference samples combusted with each sample set (see Appendix 4 - Tables 1-4).

Isolation and Identification of Metabolites

Extraction

Aliquots of the 9:1 homogenates of liver for rats sacrificed at 2 days after dosing were pooled. The aliquots (by weight) contained a total of 1,052 µg equivalents of N-ethyl FOSE-¹⁴C. The homogenate was extracted three times with diethyl ether with back extractions of the ether with water. The final volume of ether was 1,500 ml and the final volume of water was 1,200 ml. The ether was evaporated and the residue was redissolved in methanol (Fraction 1). The water layer was acidified with 250 ml of 10% HCl and stirred for an hour. The water layer was then extracted two times with diethyl ether. The ether was evaporated and the residue was redissolved in methanol (Fraction 2). The water was filtered and the filter cake was extracted two times with chloroform-methanol 1:1 (v/v). The chloroform-methanol was evaporated and the residue was redissolved in methanol (Fraction 3).

Column Chromatography

Three 2.0 cm in diameter columns were packed to 40 cm with silica gel^a in a chloroform slurry. The three fractions from the extraction of liver were chromatographed by placing the extract on the column and washing it into the bed with a small amount of chloroform. Each column was eluted with 500 ml of CHCl₃ (Eluate A), 500 ml of chloroform-methanol 1:1 (v/v) (Eluate B), and 500 ml of methanol (Eluate C). The nine fractions were evaporated to dryness and redissolved in methanol and then analyzed for carbon-14 content.

^a Unisil, activated silicic acid 100/120 mesh, Clarkson Chemical Company, Inc., Williamsport, Pennsylvania.

Thin-Layer Chromatography of Column Fractions

Each of the six column fractions that contained carbon-14 (not methanol elution) were assayed by thin-layer chromatography. Small aliquots of the material were streaked on pre-adsorbent silica gel plates^a and the plates were developed to 15 cm in the selected solvent system. The plates were then scraped in 0.5 cm segments into scintillation vials containing methanol and 7.5 ml of a modified scintillant^b was added. The contents of the scintillation vials were counted and the data were calculated and radioactivity per segment expressed as percent of total carbon-14 on the plate.

Results and Discussion

Extent and Route of Excretion and Tissue Distribution

The results indicate that at least 70% of an oral dose of N-ethyl FOSE-¹⁴C administered in feed to previously fasted rats is absorbed. The data from analyses of feces for individual rats are shown in Table 1, the data for urine analyses are shown in Table 2, and the data for total excretion (feces plus urine) are shown in Table 3. These data are expressed as cumulative percent of dose excreted. (The data are listed as µg equivalents N-ethyl FOSE-¹⁴C excreted per time period in Appendix 5 - Tables 1 and 2). As shown in Table 2, excretion of total carbon-14 via urine is not extensive; by 32 days postdose, <3.0% of the dose is eliminated via urine. The fecal elimination of carbon-14 is 20-30 fold more extensive than elimination via urine; however, the extent of carbon-14 elimination via feces is still only about 60% of the dose by 32 days postdose. The gastrointestinal transit time in fasted rats fed a nonabsorbed compound which does not affect the rate of excretion of feces is <30 hours (2). The fecal excretion rate of rats in this study is normal and does not seem to be affected by the N-ethyl FOSE-¹⁴C (see Appendix 7 and Appendix 7 - Table 1). The mean cumulative percent of dose excreted via feces by 48 hours postdose for the groups of 3 rats sacrificed at 2 days, 4 days, and 8 days postdose was 28.1, 14.4, and 15.2, respectively. Since carbon-14 levels found in feces at 48 hours or later after a single oral dose is most likely to represent material that has been absorbed and then excreted rather than unabsorbed compound, the cumulative percent of dose excreted via feces from time of dosing to 48 hours postdose (~14-28%) is the upper limit of the estimate for percent of dose not absorbed. Thus, at least 70 percent of the dose of N-ethyl FOSE-¹⁴C was absorbed.

The digestive tracts (esophagus, stomach, and intestines) were analyzed for carbon-14 content for individual rats in each of the groups of rats sacrificed at 24 and 48 hours postdose. The results (percent of dose) are shown in Table 4. (The data are expressed as µg N-ethyl FOSE equivalents/g of tissue in Appendix 6.) Feces data from Table 1 for these 6 rats are repeated in Table 4. The mean total carbon-14 content of feces plus digestive tract with contents is 38.3% of the dose at 24 hours postdose. However, the tissues and contents of the digestive tracts at 48 hours postdose contain a mean of 11.4% of the dose, and since the transit time of rats is faster than 48

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^a Analtech, 75 Blue Hen Drive, Newark, Delaware.
^b Modified TSS: 25.2 g PPO, 1.01 g Dimethyl POPOP, and 3.8 l toluene.

hours with normal rates of excretion (see Appendix 7 and Appendix 7 - Table 1), it is probable that the 11.4% of the dose observed at 48 hours is not unabsorbed N-ethyl FOSE-¹⁴C; the 11.4% of the dose likely comprises carbon-14 labeled material associated with the tissues of the digestive tract and carbon-14 labeled material in the feces as a result of absorption of N-ethyl FOSE-¹⁴C with subsequent elimination via bile. It follows that some of the carbon-14 in feces before 48 hours is likely absorbed N-ethyl FOSE-¹⁴C and then excreted N-ethyl FOSE derived material. Thus, the absorption of N-ethyl FOSE-¹⁴C is probably greater than 70%.

The results of analyses of liver, spleen, kidneys, lungs, and red blood cells for carbon-14 content by combustion and for carbon-14 in plasma by direct counting are shown in Table 5 for individual rats in all groups. (The data are expressed as percent of dose). Also included in Table 5 are results of analyses for carbon-14 content of digestive tract and carcass of rats sacrificed at 24 and 48 hours postdose. The data from analyses of liver, spleen, kidneys, lungs, red blood cells, plasma, bone marrow, digestive tract, carcass, subcutaneous fat, abdominal fat, and muscle are normalized to a 10 mg/kg dose and are shown in Table 6 as μg N-ethyl FOSE-¹⁴C equivalents/g of tissue. [The same data (not normalized) are listed in Appendix 8].

Mean total recovery of radioactivity from rats sacrificed at 24 hours and 48 hours [sum of amount in tissue (Table 5) and amount excreted (Table 3)] was 86%. Since a correction for recovery from the combustion analyses was made, these results seem low. It is probable that some of the N-ethyl FOSE-¹⁴C and/or its metabolites were lost during preparation of the samples (N-ethyl FOSE-¹⁴C is slightly volatile at room temperature). From Table 5, it is apparent that as with FC-807 (3), potassium perfluorooctanesulfonate (4), and ammonium perfluorooctanoate (5) a significant portion (mean, 9.5%) of the dose of carbon-14 is retained in the liver at 32 days after a single oral dose of the labeled compound. The mean log carbon-14 levels (normalized to a 10 mg/kg dose) in liver and plasma are plotted versus time in Figure 1 for groups of rats (3 rats/group). The ratio (liver/plasma) of carbon-14 levels are plotted versus time in Figure 2. From Figure 2, it is apparent that the liver/plasma ratio is not constant and the equilibrium is not established until 16 days postdose. Selective retention of a biotransformation product in the liver with more rapid clearance of N-ethyl FOSE-¹⁴C and/or other metabolites would be a possible explanation for this pattern. At 32 days after a single oral dose of N-ethyl FOSE-¹⁴C, the mean liver/plasma, spleen/plasma, and bone marrow/plasma carbon-14 level ratios were 11.8, 0.4, and 0.4, respectively. At 124 days after a single iv dose of FC-807-¹⁴C, the mean liver/plasma, spleen/plasma, and bone marrow/plasma carbon-14 level ratios were 22.1, 202.8, and 48.1, respectively. Thus, the pattern of distribution of carbon-14 after FC-807-¹⁴C administration is so different from the pattern after N-ethyl FOSE-¹⁴C administra-

tion that despite the two different routes of administration (oral versus iv) and the difference in duration of the experiments (time from dosing to sacrifice), it is probable that FC-807 biotransformation is more complex than a simple conversion to N-ethyl FOSE by hydrolysis of the phosphorus-oxygen bond with subsequent biotransformation of the released alcohol as N-ethyl FOSE.

The mean liver/plasma, spleen/plasma, and bone marrow/plasma carbon-14 level ratios calculated from data reported for potassium perfluorooctanesulfonate (6) are 9.3, 0.2, and 0.2, respectively, at 89 days after a single intravenous dose. Thus, in contrast to the tissue carbon-14 ratio data from FC-807, the perfluorooctanesulfonate-¹⁴C tissue/plasma ratio data are similar to the N-ethyl FOSE-¹⁴C tissue/plasma ratio data. Although perfluorooctanesulfonate is shown to be a metabolite of N-ethyl FOSE (see below, this report), these tissue/plasma ratio similarities should not be interpreted as evidence that the carbon-14 labeled material at later times (32 days) in liver is due only to perfluorooctanesulfonate.

The half-life of the disappearance from plasma of carbon-14 from day 1 to day 16 (Figure 1) is 7.5 days after N-ethyl FOSE-¹⁴C administration. However, after day 16 the disappearance is much slower since the mean carbon-14 levels on day 32 are about the same as the mean levels on day 16 (2.2 µg equivalents on day 16, 2.1 µg equivalents on day 32). The plasma half-life value of 7.5 days for carbon-14 after N-ethyl FOSE-¹⁴C oral administration is the same as that reported for carbon-14 after oral administration of potassium perfluorooctanesulfonate-¹⁴C to male rats (estimated from day 1 to day 6) (4). As with N-ethyl FOSE-¹⁴C, it is apparent that sometime after a few days (>6 days) the rate of disappearance of carbon-14 from plasma after administration of perfluorooctanesulfonate-¹⁴C decreases to a much lower rate.

Overall, the pattern of carbon-14 distribution in tissue and the half-life values for the first few days post oral dosage are similar for N-ethyl FOSE-¹⁴C and potassium perfluorooctanesulfonate-¹⁴C. In addition, for both compounds^a, the rates of disappearance of carbon-14 seem to decrease so that later plasma values for carbon-14 levels are somewhat higher than would be predicted from the earlier levels and the half-life values. Thus for these compounds in rats (and likely other species), predictions of plasma levels at later times based on half-life estimates of elimination from plasma are inaccurate and should not be attempted.

^a The change in rate of elimination of total carbon-14 from plasma is not unique to N-ethyl FOSE-¹⁴C and perfluorooctanesulfonate-¹⁴C; it is quite common for other compounds.

Isolation and Identification of Metabolites

The liver homogenate pool (9:1, water/tissue) from rats sacrificed at 48 hours after a single oral dose of N-ethyl FOSE-¹⁴C was extracted with ether (Fraction 1) then with acid-ether (Fraction 2); after filtration, the filter cake was extracted with chloroform-methanol 1:1 (Fraction 3). The percent of carbon-14 extracted from the liver homogenate for each fraction was: Fraction 1, 30.1%; Fraction 2, 25.1%; and Fraction 3, 11.8%. The water layer separated by filtration before the 1:1 chloroform-methanol extraction of the filter cake contained no detectable carbon-14 (0%). Thus, of the 1052 µg equivalents of N-ethyl FOSE-¹⁴C present in the feces pool, 705 µg equivalents (67%) was extracted.

The 3 fractions were chromatographed on separate silica gel columns and the columns were eluted successively with chloroform (Eluate A); 1:1 chloroform-methanol (Eluate B); and methanol (Eluate C). There was very little carbon-14 removed from any of the columns by methanol (Eluate C). For each of the liver homogenate extractions that were chromatographed on a silica gel column, there are two column eluates (A and B) containing carbon-14. The relative carbon-14 content of Eluates A and B for each fraction are shown in Table 7.

The six column fractions (Eluates A and B) were chromatographed by thin-layer chromatography. Radiochromatograms from thin-layer chromatography of these six fractions are shown in Figures 3-12. When chromatographed in the same solvent system (100 chloroform, 35 methanol, 5 ammonium hydroxide) (Figures 3-5), the three column eluates (B's) from the three extractions (ether, acid-ether, 1:1 chloroform-methanol) each have one major peak, and comparison of the radiochromatograms suggests that each contain the same metabolite. The extraction Fraction 2 (acid-ether) Eluate B was chromatographed in a second solvent system (100 butanol, 10 acetic acid, 10 water) (Figure 6) and in a third solvent system (100 chloroform, 100 methanol, and 2 acetic acid) (Figure 7). The three radiochromatograms of Fraction 2 Eluate B indicate that the radiochemical purity of the metabolite is ~95%.

The extraction Fraction 2 (acid-ether) Eluate B (chloroform-methanol) was labeled metabolite Fraction I and submitted to S. Pathre of the Central Analytical Laboratory for ¹⁹F-NMR analysis on the Varian XL-100 and XL-200 Spectrometers. The results are reported in Appendix 9; the metabolite was identified as the perfluorooctane-sulfonate anion.

From the percent of total carbon-14 extracted from the liver homogenate pool (67%) and the total carbon-14 recovered from the columns in 1:1 chloroform-methanol and the percent composition of these eluates by thin-layer chromatography (95%) it can be estimated that at least 22% of the carbon-14 in liver at 48 hours after a single dose of N-ethyl FOSE-¹⁴C is due to perfluorooctanesulfonate-¹⁴C. It is quite likely that due to loss during extraction and to loss during chromatography that the actual amount of perfluorooctanesulfonate is somewhat greater than 22%.

The chloroform column eluates from the three extraction fractions were chromatographed using thin-layer chromatography in the same solvent system (100 chloroform, 35 methanol, 5 ammonium hydroxide) (Figures 8-10). As with the chloroform-methanol eluates, comparison of the three column eluates suggests that most of the carbon-14 is the same metabolite. The extraction Fraction 1 (ether) Eluate A was chromatographed in a second solvent system (100 butanol, 10 acetic acid, 10 water) (Figure 11) and in a third solvent system (100 chloroform, 100 methanol, and 2 acetic acid) (Figure 12).

The extraction Fraction 1 (ether) Eluate A (chloroform) was labeled metabolite Fraction II and submitted to S. Pathre of the Central Analytical Laboratory for ¹⁴F-NMR analysis on the Varian XL-100 and XL-200 Spectrometers. The results are reported in Appendix 9. The metabolite was tentatively identified as perfluorooctanesulfonamide. Similar to the calculation for perfluorooctanesulfonate, it can be estimated that at least 32% of the carbon-14 present in liver at 48 hours after a single oral dose of N-ethyl FOSE-¹⁴C is metabolite Fraction II.

Further refinement of these extractions and separations would likely result in a higher estimate of the percentage of perfluorooctanesulfonate and the metabolite tentatively identified as perfluorooctanesulfonamide with respect to the total carbon-14 present. However, since these estimates establish that the two metabolites are present in substantial quantities, further refinement of the extraction and separation is not planned. The presence of other peaks in radiochromatograms (Figures 8-10) and the 33% of the carbon-14 in the liver homogenate that was not extractable by these conditions indicate that other unidentified metabolites are present.

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Table 1

Cumulative Excretion of Total Carbon-14
in Feces After an Oral Dose of N-Ethyl FOSE-¹⁴C
in Feed to Rats (Mean Dose, 10.13 mg/kg)

Collection Period (Days)	Rat Identification			Mean $\pm$ S.D.	
	A	B	C		
		<u>1 Day Group</u>			
0-1	29.98 ^a	12.41	22.55	21.65 $\pm$	8.82
		<u>2 Day Group</u>			
0-1	18.99	15.60	11.80	15.46 $\pm$	3.60
1-2	34.73	28.98	20.59	28.10 $\pm$	7.11
		<u>4 Day Group</u>			
0-1	7.74	5.27	9.53	7.51 $\pm$	2.14
1-2	14.04	12.32	16.89	14.42 $\pm$	2.31
2-3	20.03	17.78	21.74	19.85 $\pm$	1.99
3-4	23.56	22.48	26.08	24.04 $\pm$	1.85
		<u>8 Day Group</u>			
0-1	2.36	7.71	4.31	4.79 $\pm$	2.71
1-2	5.61	27.16	12.70	15.16 $\pm$	10.98
2-3	25.77	38.21	20.32	28.10 $\pm$	9.17
3-4	37.41	44.41	26.15	35.99 $\pm$	9.21
4-5	42.83	49.21	29.27	40.44 $\pm$	10.18
5-6	47.14	52.55	32.52	44.07 $\pm$	10.36
6-7	50.65	54.86	35.83	47.11 $\pm$	10.00
7-8	53.36	56.35	38.77	49.49 $\pm$	9.41
		<u>16 Day Group</u>			
0-16	66.27	60.39	62.79	63.15 $\pm$	2.96
		<u>32 Day Group</u>			
0-16	63.88	47.62	56.72	56.07 $\pm$	8.15
16-32	66.73	56.93	59.36	61.01 $\pm$	5.10

^a Data are expressed as percent of dose excreted during collection period.

Notebook Reference: NB-55673-37 and 38

C03492

Table 2

Cumulative Excretion of Total Carbon-14
in Urine After an Oral Dose of N-Ethyl FOSE-¹⁴C
in Feed to Rats (Mean Dose, 10.13 mg/kg)

Collection Period (Days)	Rat Identification			Mean $\pm$ S.D.
	A	B	C	
		<u>1 Day Group</u>		
0-1	0.13 ^a	0.12	0.11	0.12 $\pm$ 0.01
		<u>2 Day Group</u>		
0-1	0.14	0.09	0.11	0.11 $\pm$ 0.03
1-2	0.23	0.17	0.20	0.20 $\pm$ 0.03
		<u>4 Day Group</u>		
0-1	0.11	0.10	0.11	0.11 $\pm$ 0.01
1-2	0.25	0.24	0.22	0.24 $\pm$ 0.02
2-3	0.36	0.34	0.33	0.34 $\pm$ 0.02
3-4	0.50	0.44	0.43	0.46 $\pm$ 0.04
		<u>8 Day Group</u>		
0-1	0.10	0.06	0.09	0.08 $\pm$ 0.02
1-2	0.17	0.18	0.21	0.19 $\pm$ 0.02
2-3	0.30	0.28	0.34	0.31 $\pm$ 0.03
3-4	0.62	0.36	0.44	0.47 $\pm$ 0.13
4-5	0.88	0.61	0.53	0.67 $\pm$ 0.18
5-6	0.97	0.67	0.61	0.75 $\pm$ 0.19
6-7	1.06	0.72	0.71	0.83 $\pm$ 0.20
7-8	1.12	0.77	0.81	0.90 $\pm$ 0.19
		<u>16 Day Group</u>		
0-16	2.45	1.19	1.10	1.58 $\pm$ 0.75
		<u>32 Day Group</u>		
0-32	1.85	2.69	1.62	2.05 $\pm$ 0.56

^a Data are expressed as percent of dose excreted during collection period.

Notebook Reference: NB-55673-38

603493

Table 3

Cumulative Excretion of Total Carbon-14
in Urine and Feces After an Oral Dose of N-Ethyl FOSE-¹⁴C
in Feed to Rats (Mean Dose, 10.13 mg/kg)

Collection Period (Days)	Rat Identification			Mean	+ S.D.
	A	B	C		
		<u>1 Day Group</u>			
0-1	30.11 ^a	12.53	22.66	21.77	+ 8.82
		<u>2 Day Group</u>			
0-1	19.13	15.69	11.91	15.58	+ 3.61
1-2	34.96	29.15	20.79	28.30	+ 7.12
		<u>4 Day Group</u>			
0-1	7.85	5.37	9.64	7.62	+ 2.14
1-2	14.29	12.56	17.11	14.65	+ 2.30
2-3	20.39	18.12	22.07	20.19	+ 1.98
3-4	24.06	22.92	26.51	24.50	+ 1.83
		<u>8 Day Group</u>			
0-1	2.46	7.77	4.40	4.88	+ 2.69
1-2	5.78	27.34	12.91	15.34	+ 10.98
2-3	26.07	38.49	20.66	28.41	+ 9.14
3-4	38.03	44.77	26.59	36.46	+ 9.19
4-5	43.71	49.82	29.80	41.11	+ 10.26
5-6	48.11	53.22	33.13	44.82	+ 10.44
6-7	51.71	55.58	36.54	47.94	+ 10.06
7-8	54.48	57.12	39.58	50.39	+ 9.46
		<u>16 Day Group</u>			
0-16	68.72	61.58	63.89	64.73	+ 3.64
		<u>32 Day Group</u>			
0-32	68.58	59.62	60.98	63.06	+ 4.83

^a Data are expressed as percent of dose excreted during collection period.

Notebook Reference: NB-55673-39 and 40

003494

Table 4

Carbon-14 Content in Digestive Tract (plus contents)
and Feces After an Oral Dose of N-Ethyl FOSE-¹⁴C
in Feed to Rats (Mean Dose, 10.13 mg/kg)
at 24 and 48 Hours Postdose

Rat Identification	Time Post-Dose (Hours)	Digestive Tract (plus contents)	Feces
1A	24	12.84 ^a	29.98
1B	24	22.40	12.41
1C	24	14.60	22.55
Mean $\pm$ S.D.		16.61 $\pm$ 5.09	21.65 $\pm$ 8.82
2A	48	11.10	34.73
2B	48	13.03	28.98
2C	48	10.10	20.59
Mean $\pm$ S.D.		11.41 $\pm$ 1.49	28.10 $\pm$ 7.11

^a Data are expressed as percent of dose.

Notebook Reference: NB-51806-49-51

003495

Table 5
Carbon-14 Content in Tissues After an Oral Dose
of N-Ethyl FOSE-¹⁴C in Feed to Rats
(Mean Dose, 10.13 mg/kg)

Rat Identification	Liver ^a	Spleen ^a	Kidneys ^a	Lungs ^a	Red Blood Cells ^b	Plasma ^c	Digestive Tract ^a	Carcass ^a
1A	16.85	0.16	0.71	0.43	6.39	2.55	12.84	18.76
1B	17.98	0.13	1.12	0.60	6.80	3.10	22.40	23.48
1C	16.95	0.11	0.80	0.39	7.82	2.80	14.60	17.40
Mean	17.26	0.13	0.88	0.47	7.00	2.82	16.61	19.88
2A	17.72	0.14	0.66	0.35	5.89	2.06	11.10	12.30
2B	18.23	0.10	0.77	0.40	5.77	2.17	13.03	15.62
2C	24.17	0.16	1.00	0.59	3.62	3.32	10.10	19.29
Mean	20.04	0.13	0.81	0.45	5.09	2.52	11.41	15.74
4A	21.59	0.14	0.77	0.43	5.44	2.67	-- ^d	-- ^d
4B	18.47	0.12	0.98	0.43	5.85	2.96	--	--
4C	17.69	0.13	0.75	0.38	5.74	2.72	--	--
Mean	19.25	0.13	0.83	0.41	5.68	2.78	--	--
8A	15.44	0.04	0.32	0.16	2.78	1.15	--	--
8B	12.83	0.04	0.27	0.14	2.37	1.19	--	--
8C	18.30	0.09	0.54	0.36	3.84	2.05	--	--
Mean	15.52	0.06	0.38	0.22	3.00	1.46	--	--
16A	9.52	0.02	0.18	0.09	0.77	0.72	--	--
16B	12.10	0.02	0.16	0.09	0.99	0.85	--	--
16C	10.32	0.03	0.27	0.14	1.08	1.16	--	--
Mean	10.65	0.02	0.20	0.11	0.95	0.91	--	--
32A	7.84	0.02	0.18	0.07	0.28	0.78	--	--
32B	12.71	0.03	0.31	0.13	0.87	1.13	--	--
32C	8.04	0.01	0.16	0.07	0.30	0.64	--	--
Mean	9.53	0.02	0.22	0.09	0.48	0.85	--	--

^a Data are expressed as percent of dose in tissue.

^b Data are estimates of percent of dose present in red blood cells. Red blood cell volume = 26.3 ml/kg body weight (7).

^c Data are estimates of percent of dose present in plasma. Plasma volume = 31.3 ml/kg body weight (7).

^d Sample was not taken.

Notebook References: NB-56531-8 and 9, NB-51806-49, and NB-53102-49

003496

Table 6

Carbon-14 Content in Tissues After an Oral Dose of
N-Ethyl FOSE-¹⁴C in Feed to Rats (Mean Dose, 10.13 mg/kg)

Rat Identification	Liver ^a	Spleen ^a	Kidneys ^a	Lungs ^a	Red Blood Cells ^a	Plasma ^a	Bone Marrow ^a	Digestive Tract ^a	Carcass ^a	Subcut. Fat ^a	Abdom. Fat ^a	Muscle ^a
1A	32.4	6.72	8.87	8.07	21.80	7.31	12.31	11.77	2.27	6.40	8.04	0.40
1B	48.7	6.88	14.39	11.68	27.24	10.42	14.73	31.41	3.17	7.79	9.51	0.42
1C	30.4	6.06	8.54	7.78	25.69	7.74	11.02	10.78	2.03	4.92	6.15	0.34
Mean	37.2	6.55	10.60	9.18	24.91	8.49	12.69	17.99	2.49	6.37	7.90	0.39
2A	30.4	5.76	6.70	6.21	18.83	5.53	10.42	8.27	1.43	3.96	3.26	0.22
2B	41.8	4.68	8.00	6.57	20.10	6.36	10.59	12.03	1.93	4.18	3.99	0.37
2C	41.4	7.26	10.44	8.76	12.25	9.46	11.35	8.08	2.39	3.95	4.28	0.52
Mean	37.9	5.90	8.38	7.18	17.06	7.12	10.79	9.46	1.92	4.03	3.84	0.37
4A	43.1	6.55	8.56	8.06	18.28	7.53	7.97	-- ^b	-- ^b	2.15	2.08	1.58
4B	43.8	7.41	10.98	8.83	20.86	8.84	9.80	--	--	2.99	1.75	1.36
4C	39.2	6.65	9.54	7.59	19.34	7.69	8.67	--	--	1.82	1.74	1.32
Mean	42.0	6.87	9.69	8.16	19.49	8.02	8.81	--	--	2.32	1.86	1.42
8A	26.1	2.09	3.25	2.79	8.19	2.85	3.27	--	--	0.53	0.37	0.33
8B	24.0	1.75	3.35	2.55	7.46	3.15	2.83	--	--	0.98	0.22	0.24
8C	33.6	3.87	6.16	6.34	12.27	5.50	5.48	--	--	0.69	0.47	0.95
Mean	27.9	2.57	4.25	3.89	9.31	3.83	3.86	--	--	0.73	0.35	0.51
16A	20.4	0.81	1.88	1.43	2.18	1.70	0.96	--	--	0.03	0.04	0.16
16B	27.9	0.84	1.99	1.76	2.95	2.13	0.98	--	--	0.13	0.01	0.16
16C	26.3	1.08	2.98	2.29	3.16	2.84	1.48	--	--	0.35	0.03	0.20
Mean	24.9	0.91	2.28	1.83	2.76	2.22	1.14	--	--	0.17	0.03	0.17
32A	19.7	0.74	1.81	1.61	0.81	1.89	0.67	--	--	0.08	0.00	0.13
32B	32.8	1.33	3.47	2.38	2.58	2.79	1.46	--	--	0.28	0.00	0.28
32C	20.4	0.62	1.75	1.32	0.86	1.54	0.61	--	--	0.06	0.00	0.09
Mean	24.3	0.90	2.34	1.77	1.42	2.07	0.91	--	--	0.14	0.00	0.17

^a Data is normalized to a 10 mg/kg dose and expressed as µg N-ethyl FOSE-¹⁴C equivalents/g.
^b Sample was not taken.

Notebook References: NB-56531-5 and 6, NB-53102-43 and 44

603497

Table 7

Relative Carbon-14 Content of Eluates A and B
for Extraction Fractions 1, 2 and 3

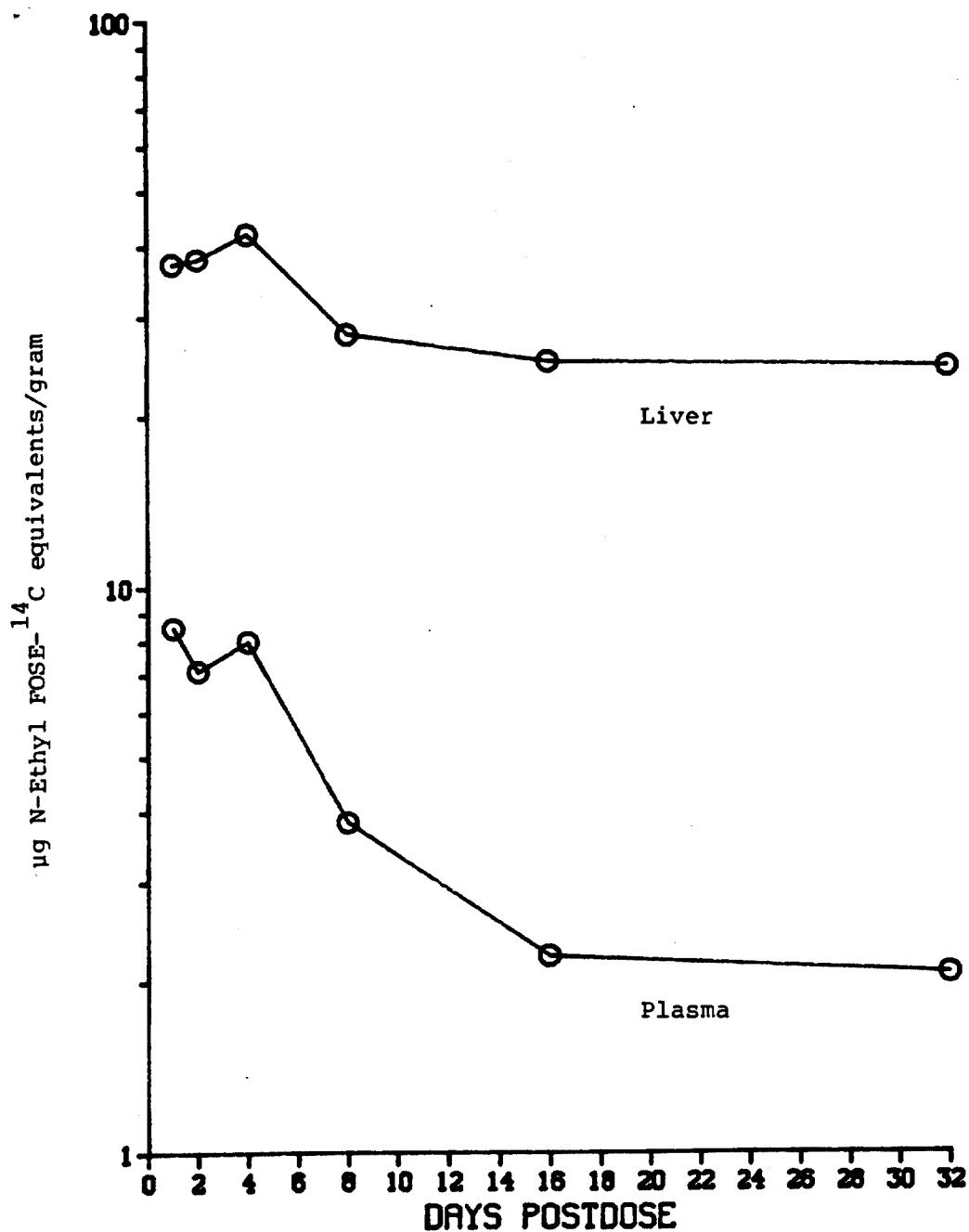
	Fraction 1 (ether)	Fraction 2 (acid-ether)	Fraction 3 (1:1 chloroform-methanol)
Eluate A (chloroform)	93%	23%	61%
Eluate B (1:1 chloroform- methanol)	19%	61%	22%
Total % recovered	112%	84%	83%

Notebook Reference: NB-51579-34

003498

Figure 1

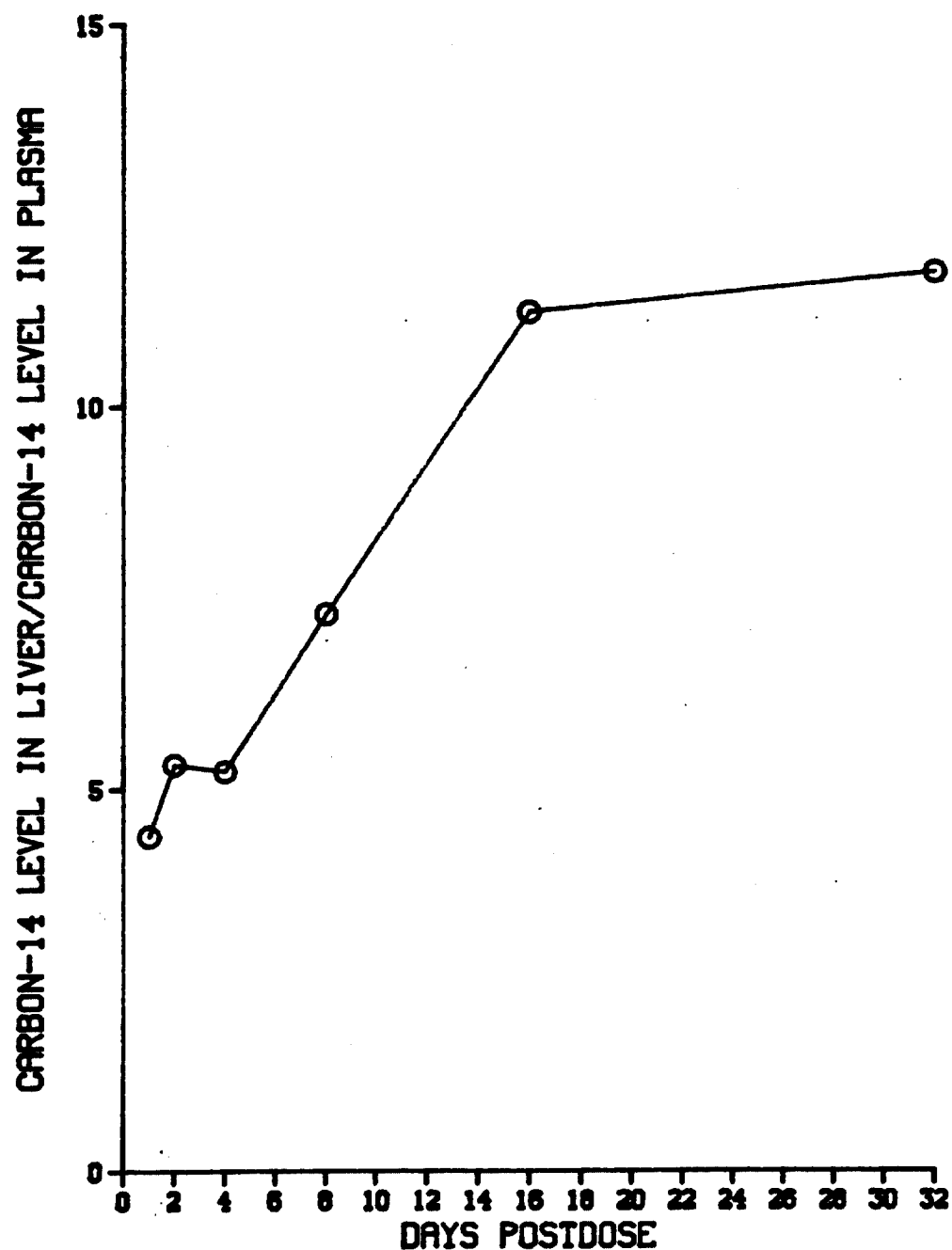
Mean Log Carbon-14 Levels (Normalized to a 10 mg/kg Dose) in Liver and Plasma of Rats (Groups of 3) at 1, 2, 4, 8, 16, and 32 Days Post Oral Dose of N-Ethyl FOSE-¹⁴C in Feed



C03499

Figure 2

Ratio of Carbon-14 Level in Liver/Carbon-14
Level in Plasma of Rats (Groups of 3) at 1,
2, 4, 8, 16, and 32 Days Post Oral Dose of
N-Ethyl FOSE-¹⁴C in Feed



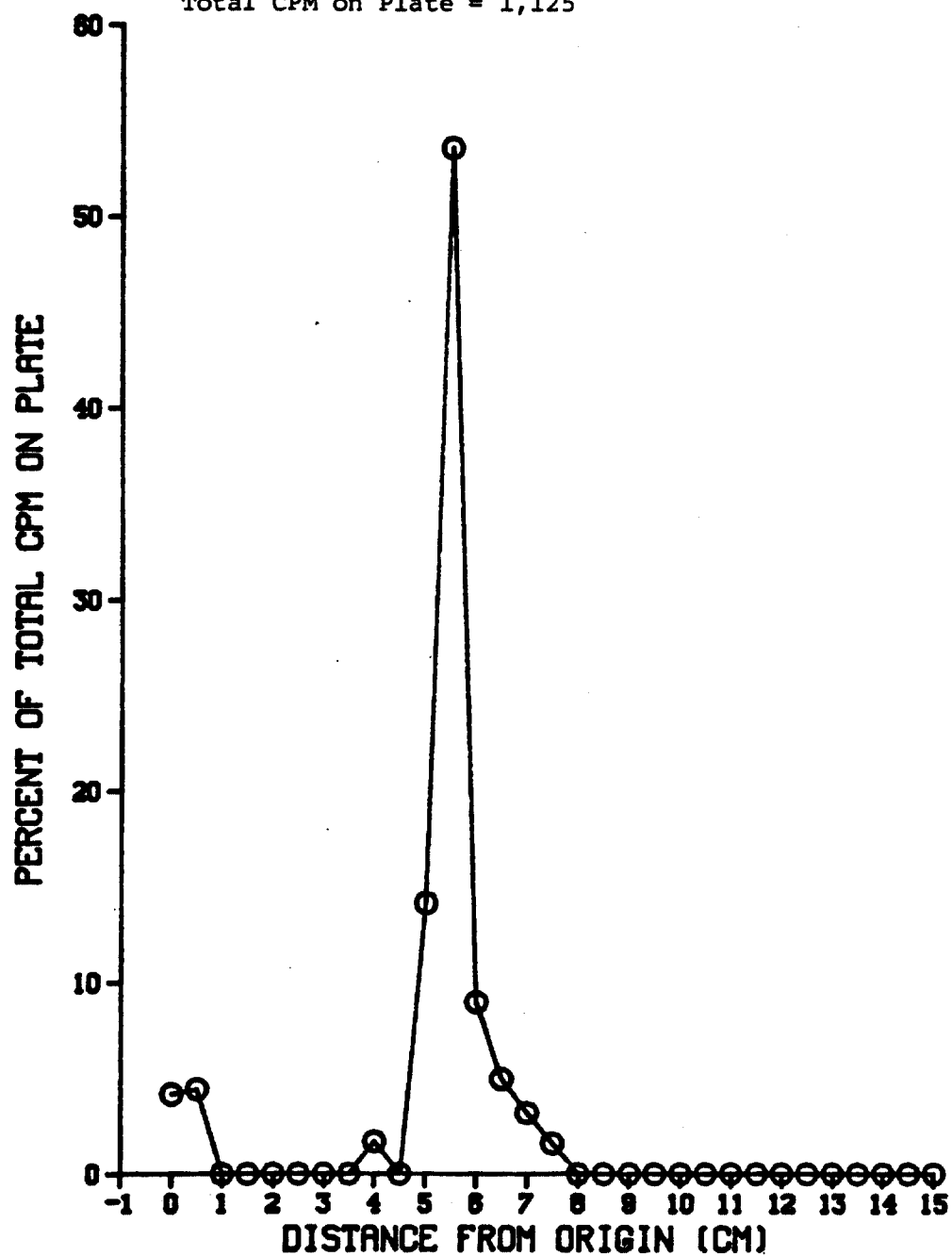
003500

Figure 3

Thin-Layer Radiochromatogram of Extraction Fraction 1
(ether) Eluate B (1:1 chloroform-methanol)

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

Total CPM on Plate = 1,125



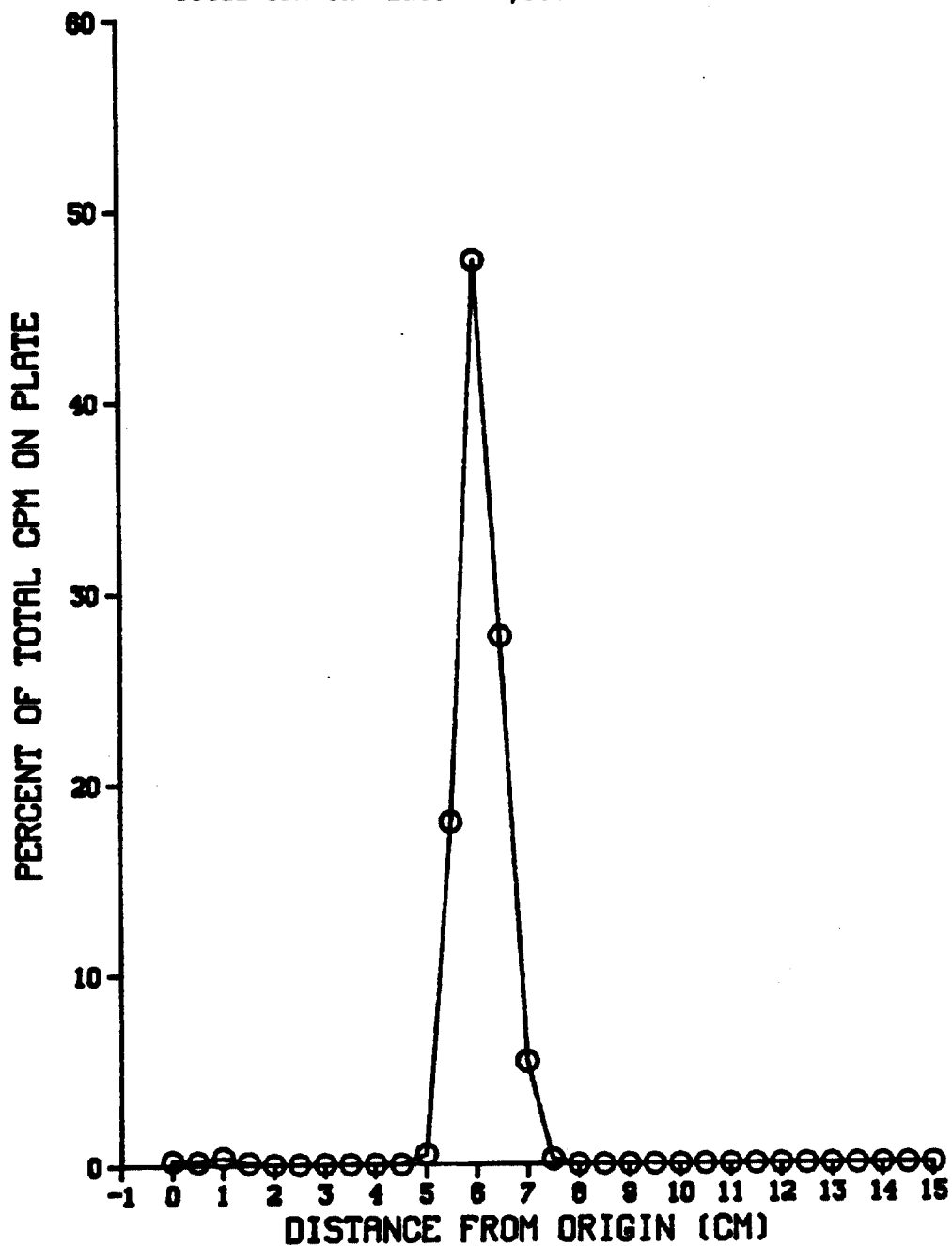
003501

Figure 4

Thin-Layer Radiochromatogram of Extraction Fraction 2
(acid ether) Eluate B (1:1 chloroform-methanol)

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

Total CPM on Plate = 4,363

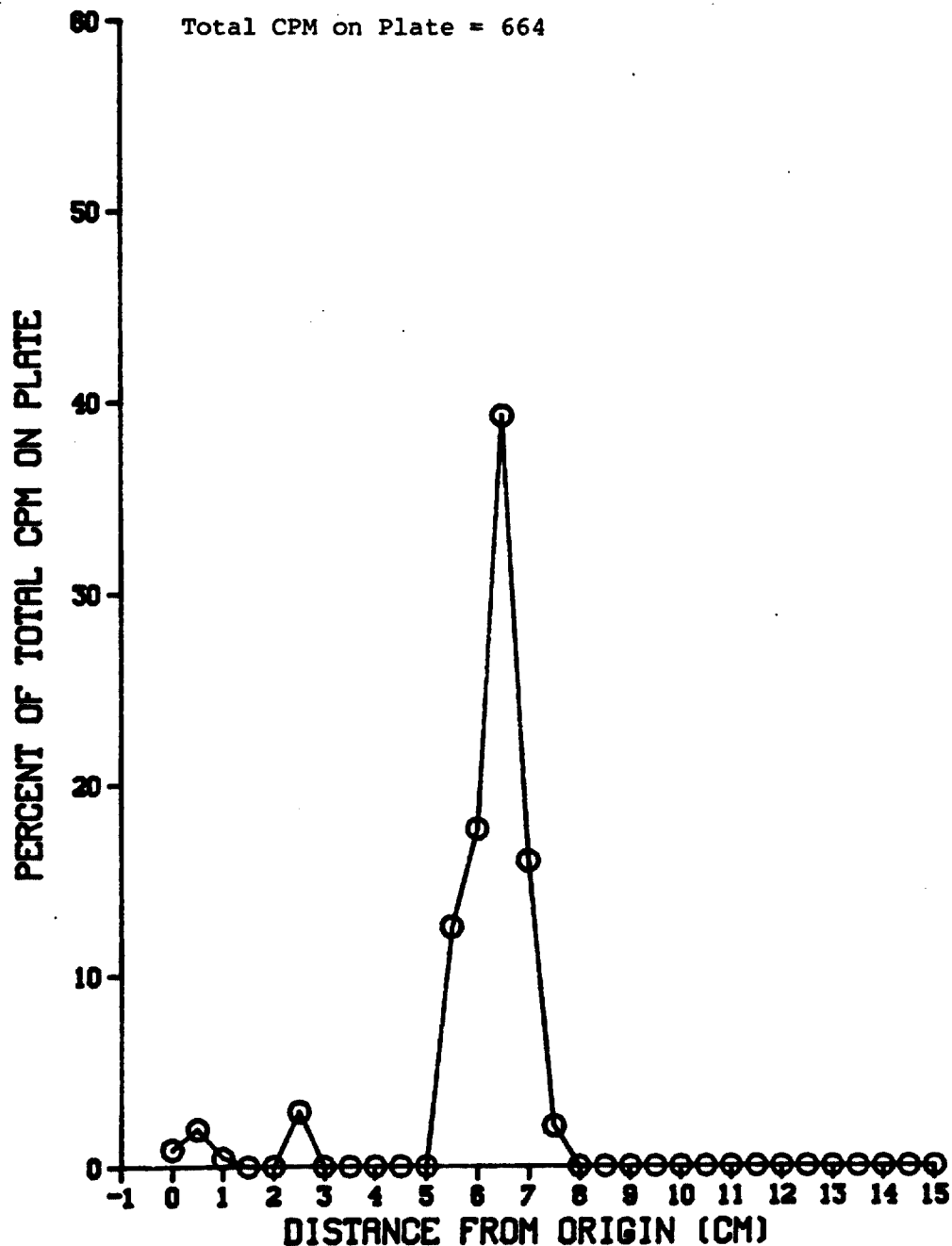


003502

Figure 5

Thin-Layer Radiochromatogram of Extraction
Fraction 3 (1:1 chloroform-methanol) Eluate B
(1:1 chloroform-methanol)

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



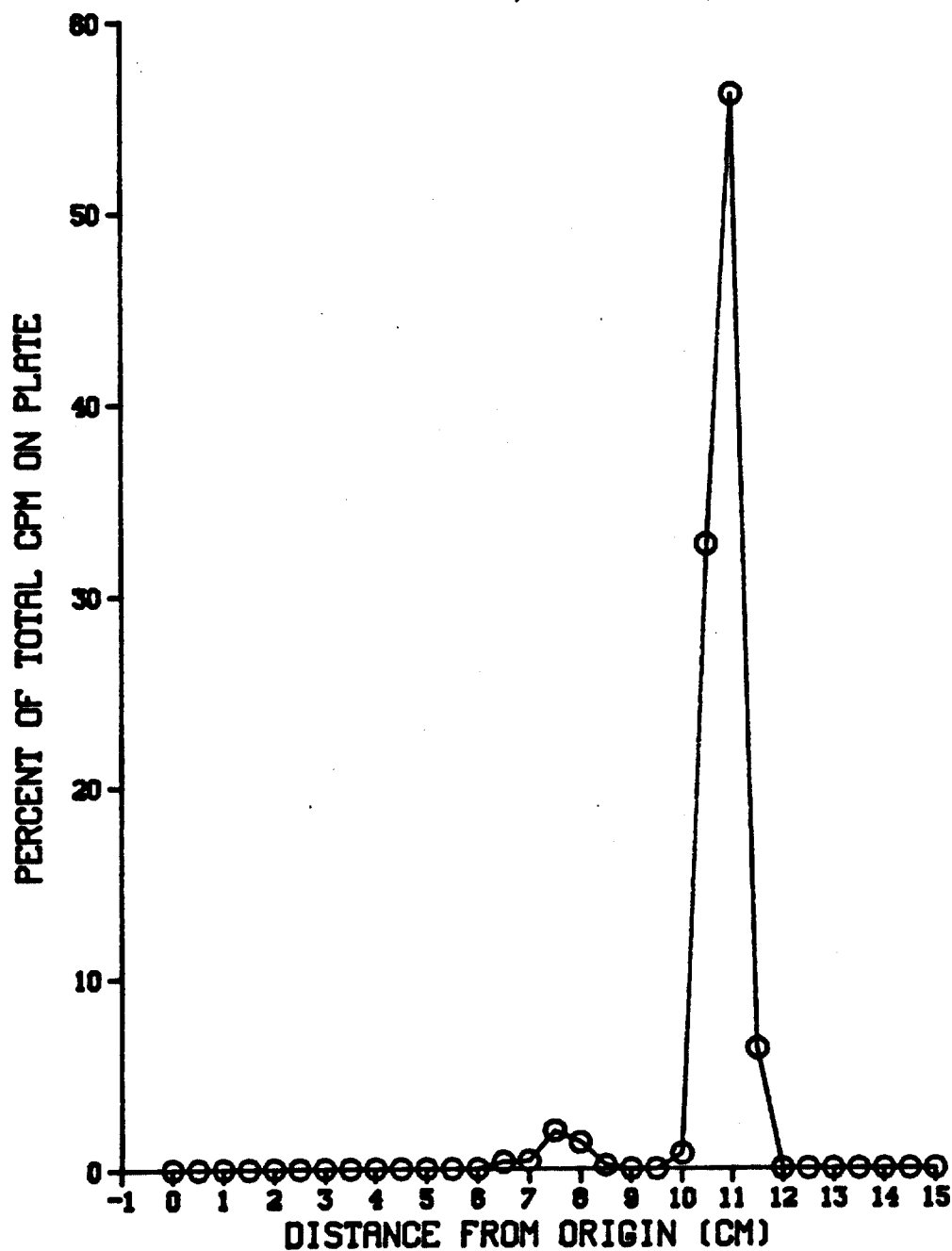
003503

Figure 6

Thin-Layer Radiochromatogram of Extraction Fraction 2
(acid-ether) Eluate B (1:1 chloroform-methanol)

Pre-adsorbent SGF Uniplate: 100 butanol
10 water
10 acetic acid

Total CPM on Plate = 1,650



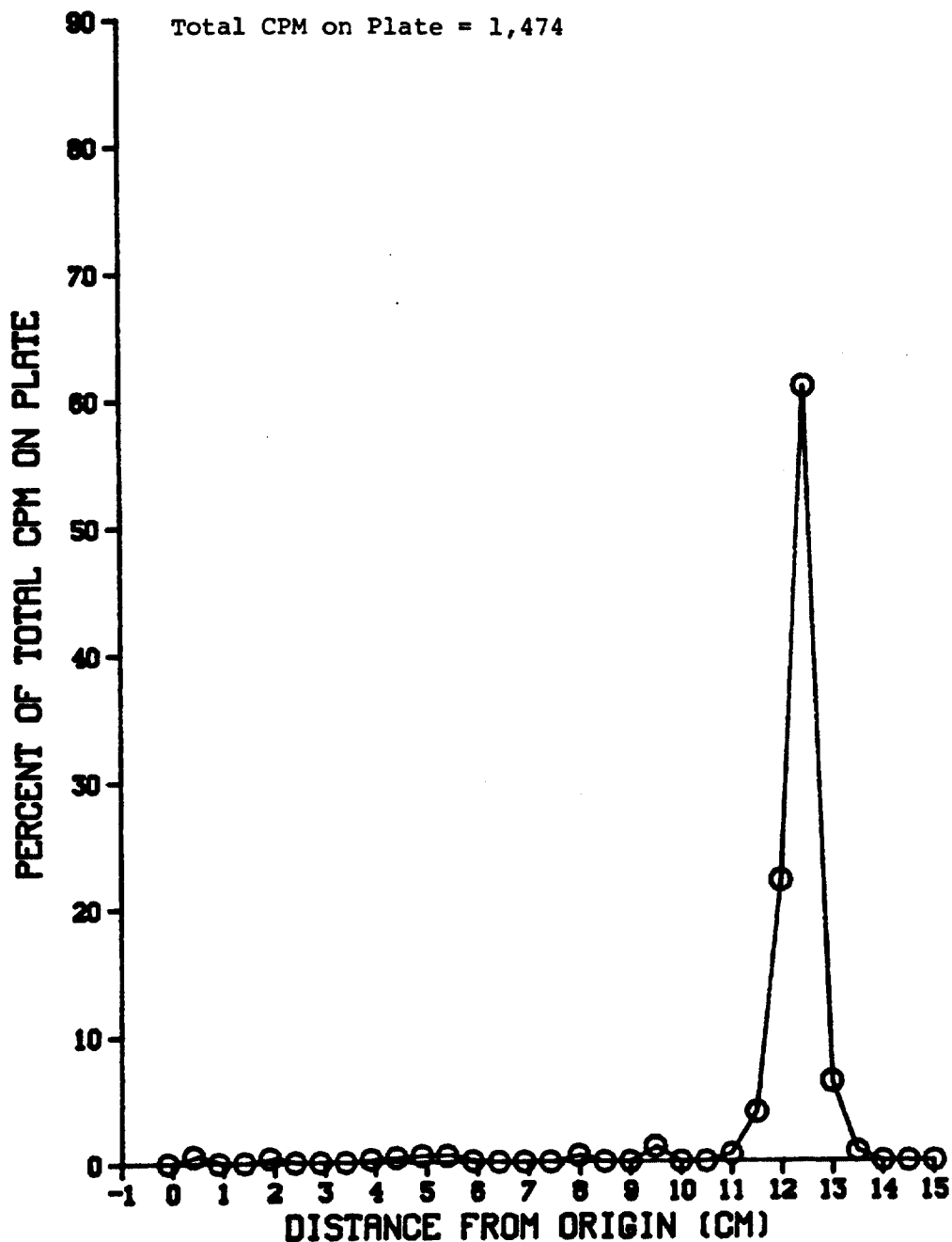
003504

Figure 7

Thin-Layer Radiochromatogram of Extraction
Fraction 2 (acid-ether) Eluate B (1:1
chloroform-methanol)

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

Total CPM on Plate = 1,474

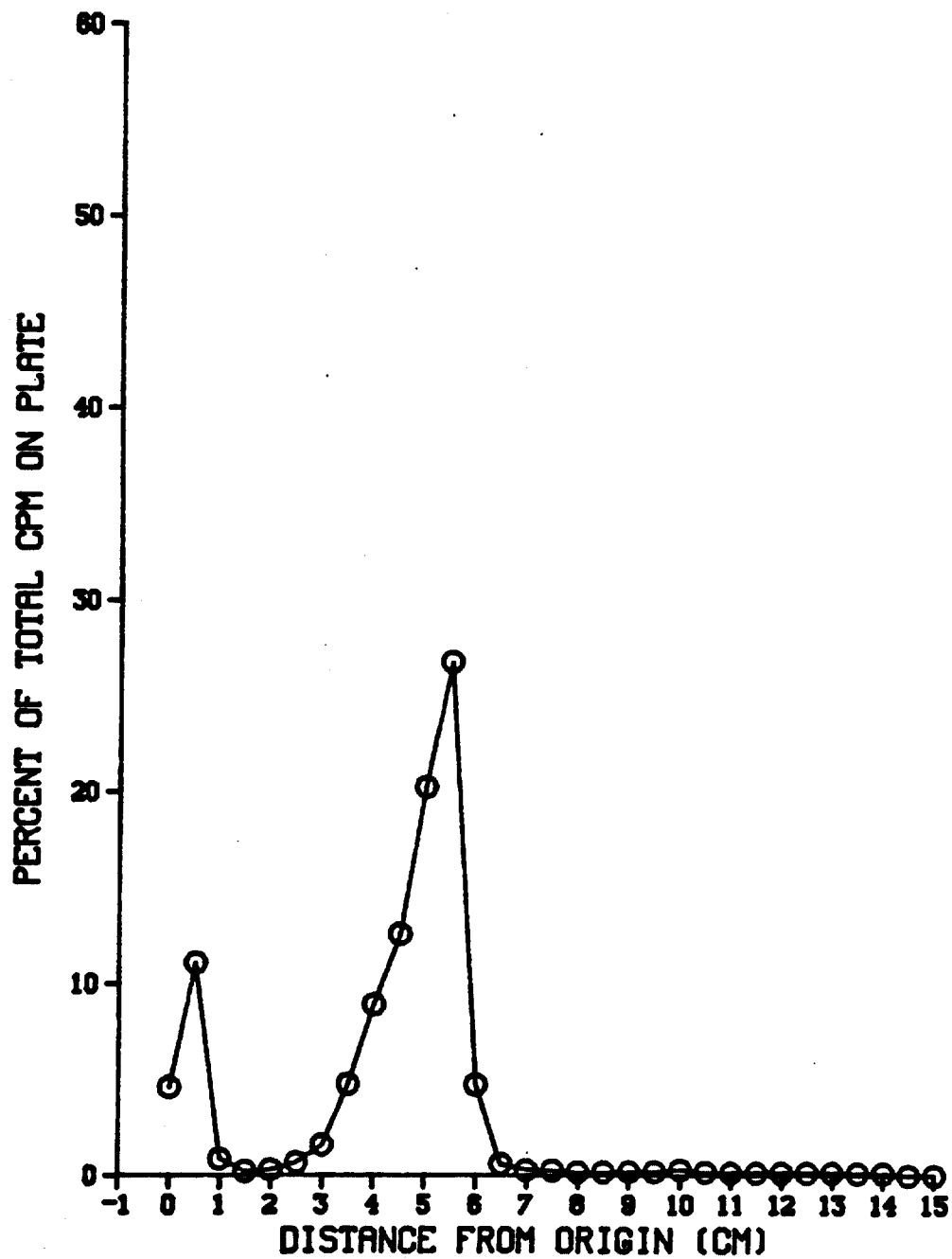


003505

Thin-Layer Radiochromatogram of Extraction
Fraction 1 (ether) Eluate A (chloroform)

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

Total CPM on Plate = 4,983



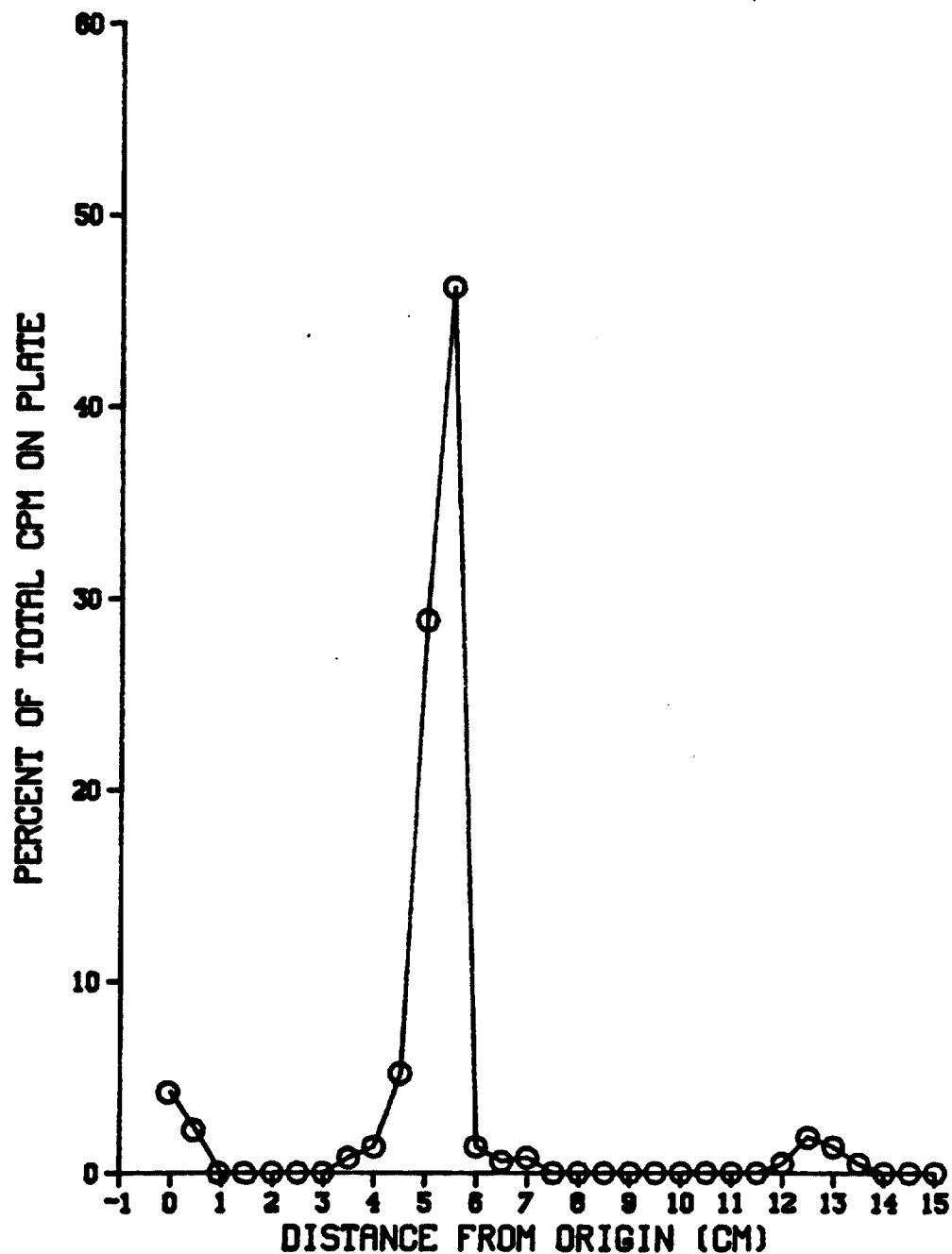
003506

Figure 9

Thin-Layer Radiochromatogram of Extraction
Fraction 2 (acid-ether) Eluate A (chloroform)

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

Total CPM on Plate = 1,020



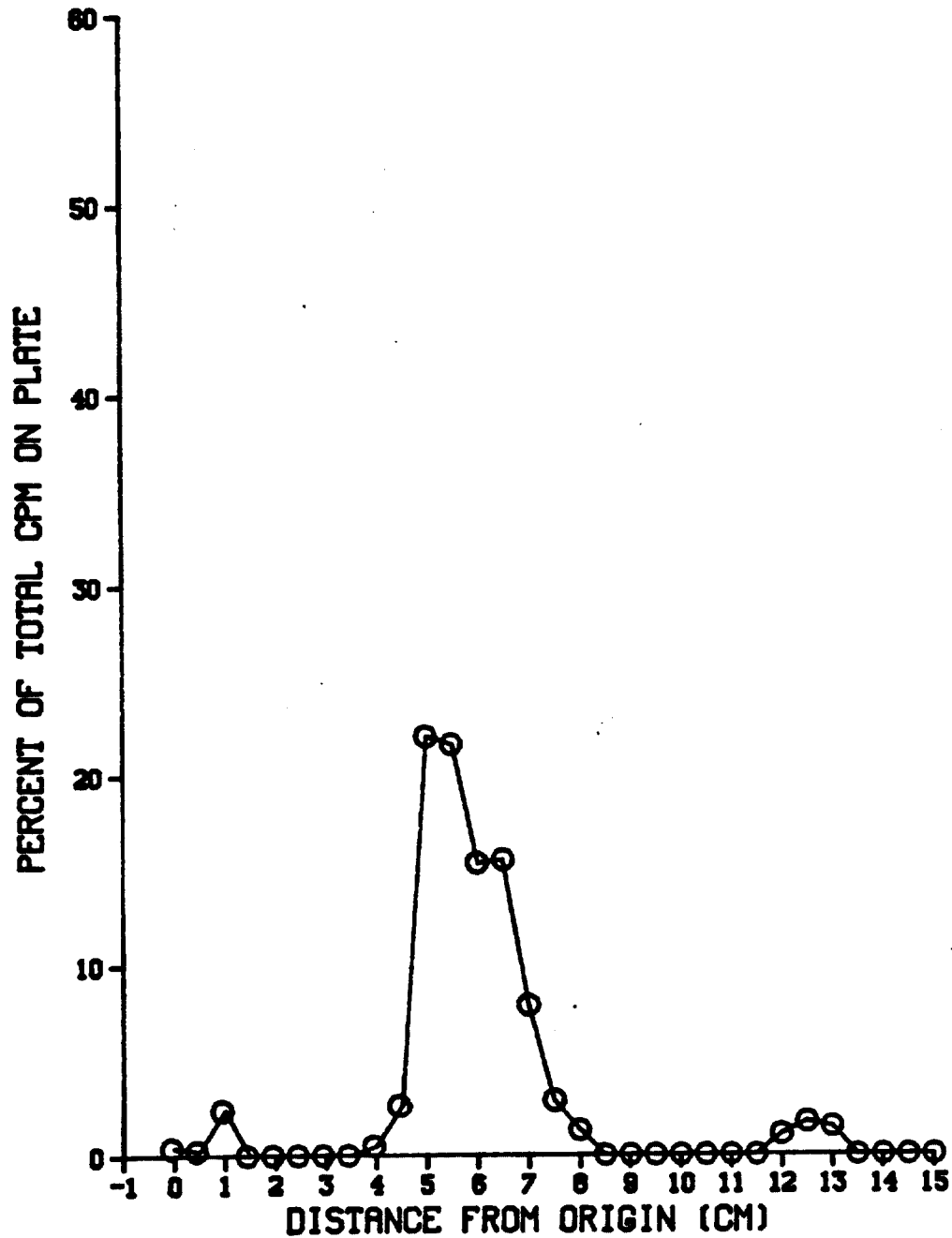
003507

Figure 10

Thin-Layer Radiochromatogram of Extraction Fraction 3
(1:1 chloroform-methanol) Eluate A (chloroform)

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

Total CPM on Plate = 1,216



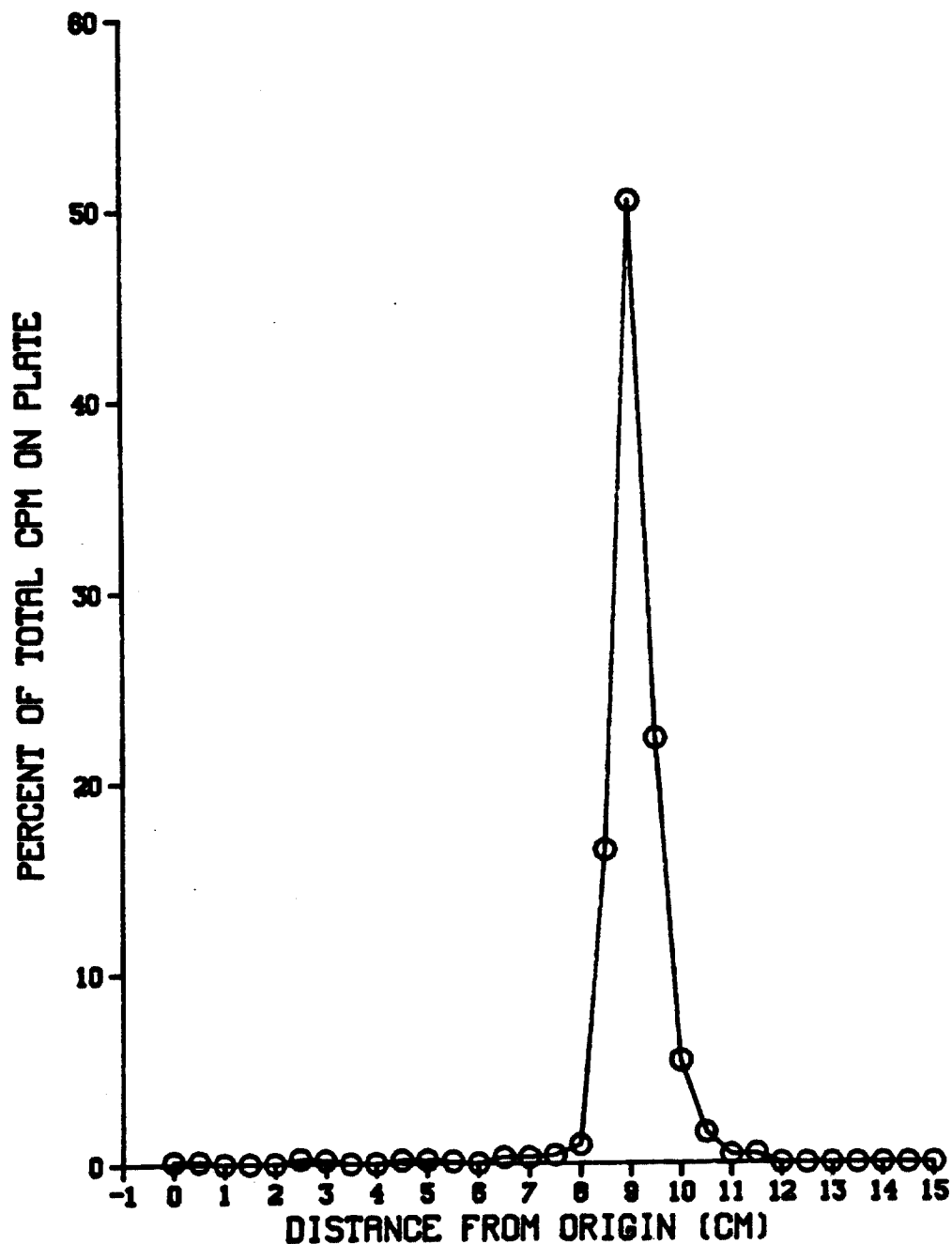
003508

Figure 11

Thin-Layer Radiochromatogram of Extraction
Fraction 1 (ether) Eluate A (chloroform)

Pre-adsorbent SGF Uniplate: 100 butanol
10 water
10 acetic acid

Total CPM on Plate = 2,879



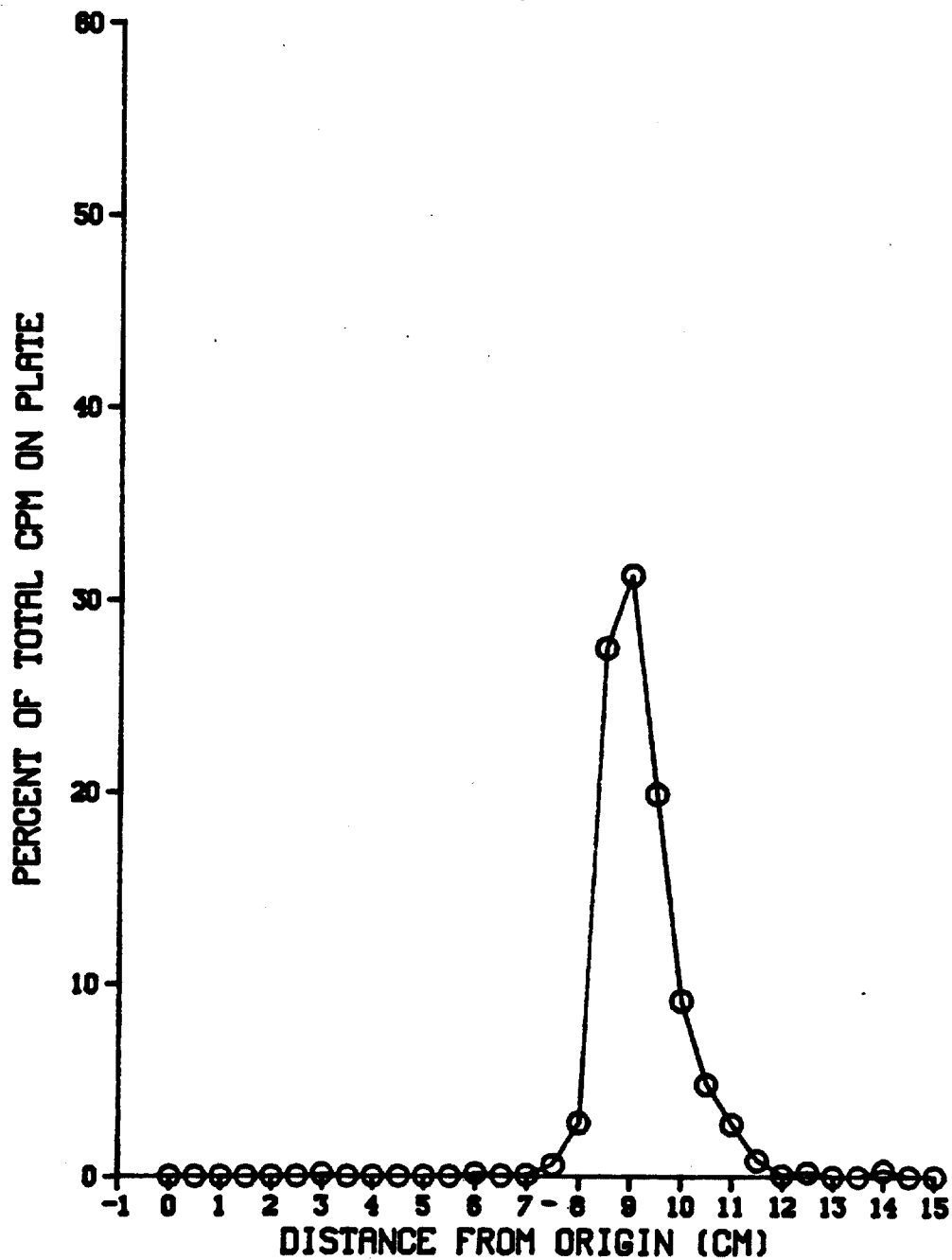
003509

Figure 12

Thin-Layer Radiochromatogram of Extraction
Fraction 1 (ether) Eluate A (chloroform)

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

Total CPM on Plate = 2,882



003510

Appendix 1 - Table 1

Thin-Layer Chromatography Systems for N-Ethyl FOSE-¹⁴C

Plate No.	Solvent System ^a	R _f ^b of N-Ethyl FOSE- ¹⁴ C
1	100 chloroform 100 acetone	0.70
2	100 chloroform 100 methanol 2 acetic acid ^c	0.90
3	150 chloroform 50 methanol 5 ammonium hydroxide ^c	0.90
4	100 chloroform 35 methanol 5 ammonium hydroxide ^c	1.00
5	100 butanol 10 water 10 acetic acid ^c	0.77

^a Solvents were prepared volume:volume; a 100 ml aliquot of solvent mixture was added to the chromatography tank.

^b R_f is of major (> 98%) peak on the thin-layer chromatography plate.

^c Acetic acid and ammonium hydroxide were concentrated.

Notebook Reference: NB-51806-41-42

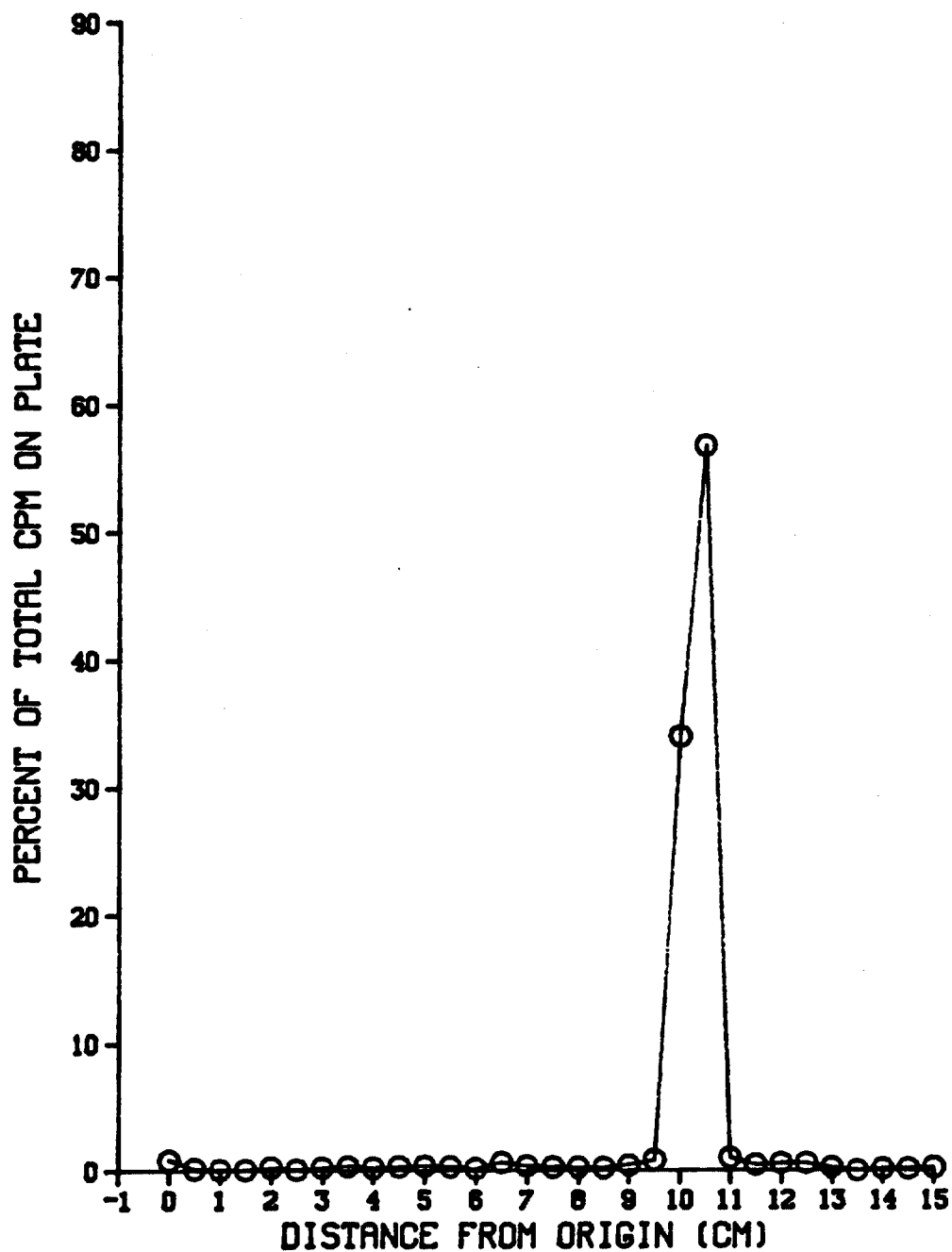
003511

Appendix 1 - Figure 1

Thin-Layer Radiochromatogram of N-Ethyl
FOSE-¹⁴C Dosing Solution, Plate No. 1

Pre-adsorbent SGF Uniplate: 100 chloroform
100 acetone

Total CPM on Plate = 5,393



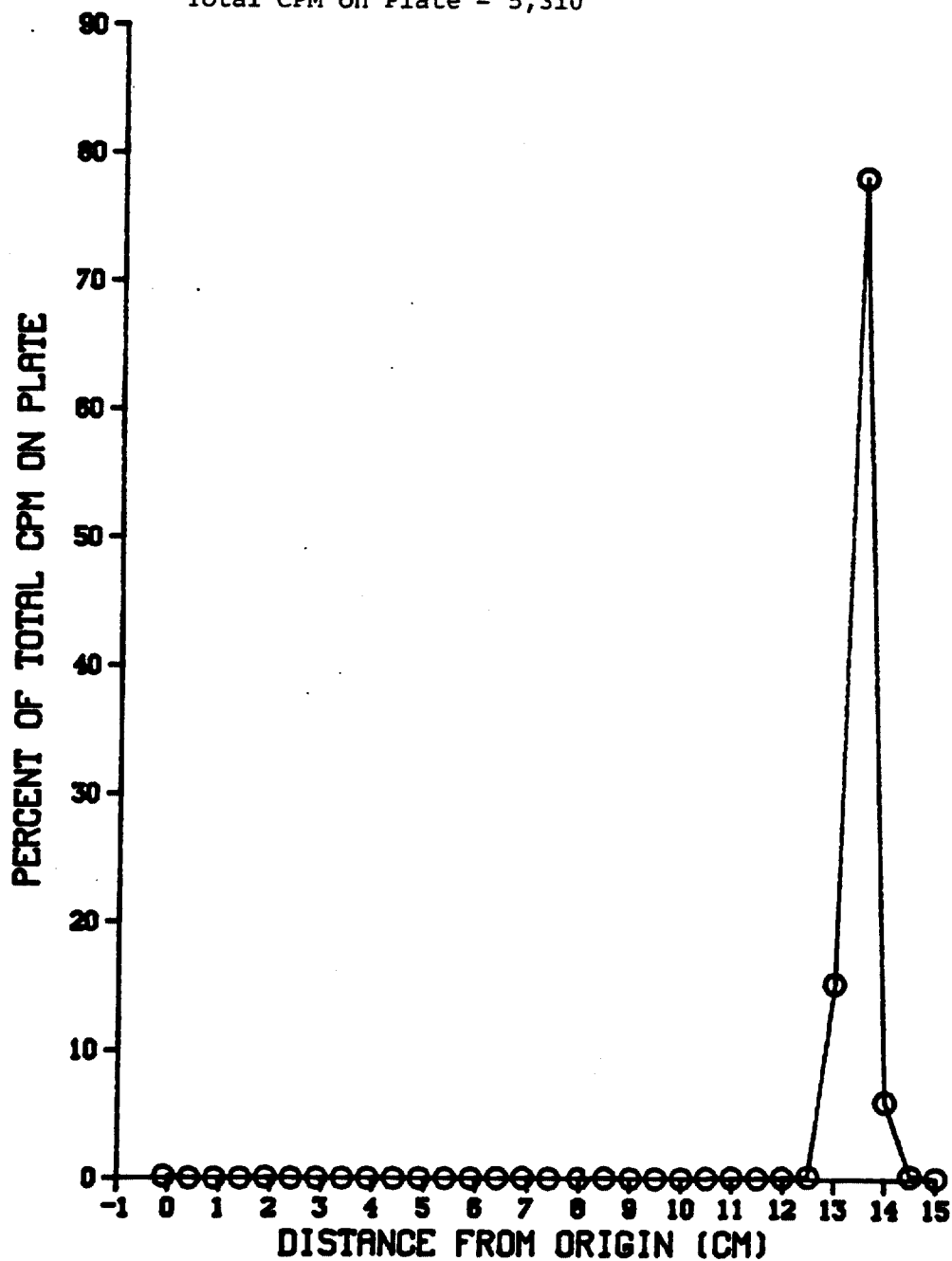
003512

Appendix 1 - Figure 2

Thin-Layer Radiochromatogram of N-Ethyl
FOSE-¹⁴C Dosing Solution, Plate No. 2

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

Total CPM on Plate = 5,310



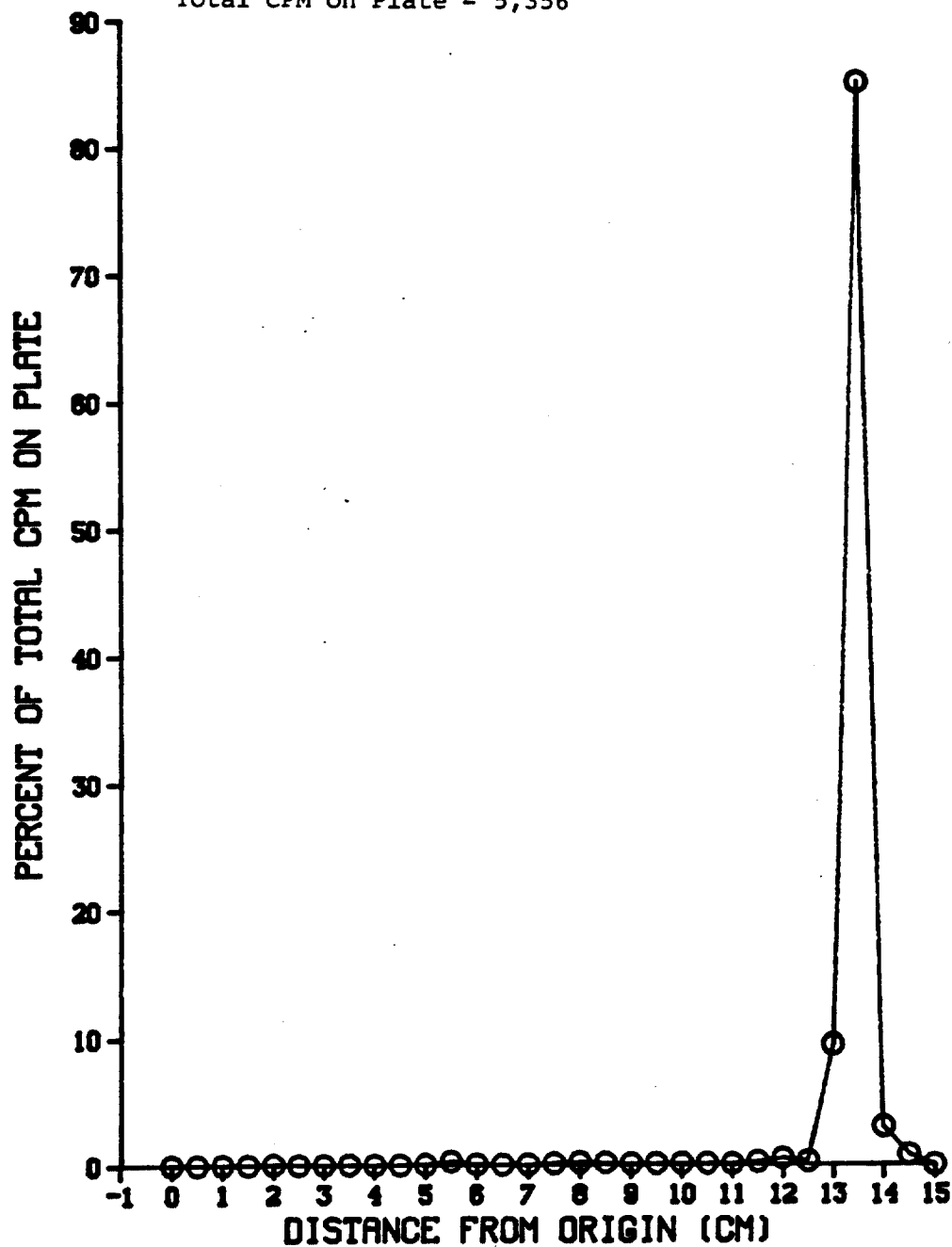
G03513

Appendix 1 - Figure 3

Thin-Layer Radiochromatogram of N-Ethyl
FOSE-¹⁴C Dosing Solution, Plate No. 3

Pre-adsorbent SGF Uniplate: 150 chloroform
50 methanol
5 ammonium hydroxide

Total CPM on Plate = 5,356



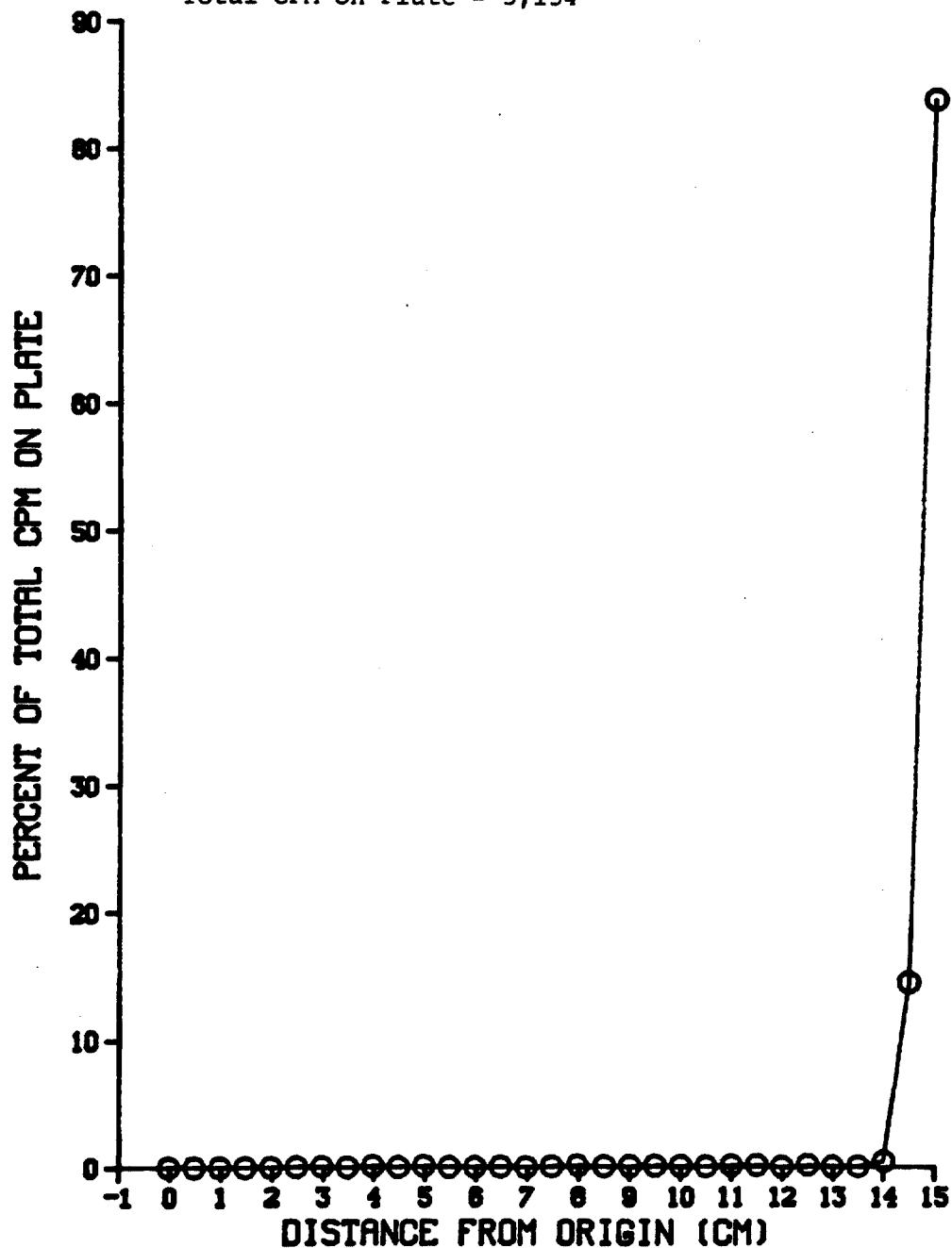
003514

Appendix 1 - Figure 4

Thin-Layer Radiochromatogram of N-Ethyl
FOSE-¹⁴C Dosing Solution, Plate No. 4

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

Total CPM on Plate = 5,154



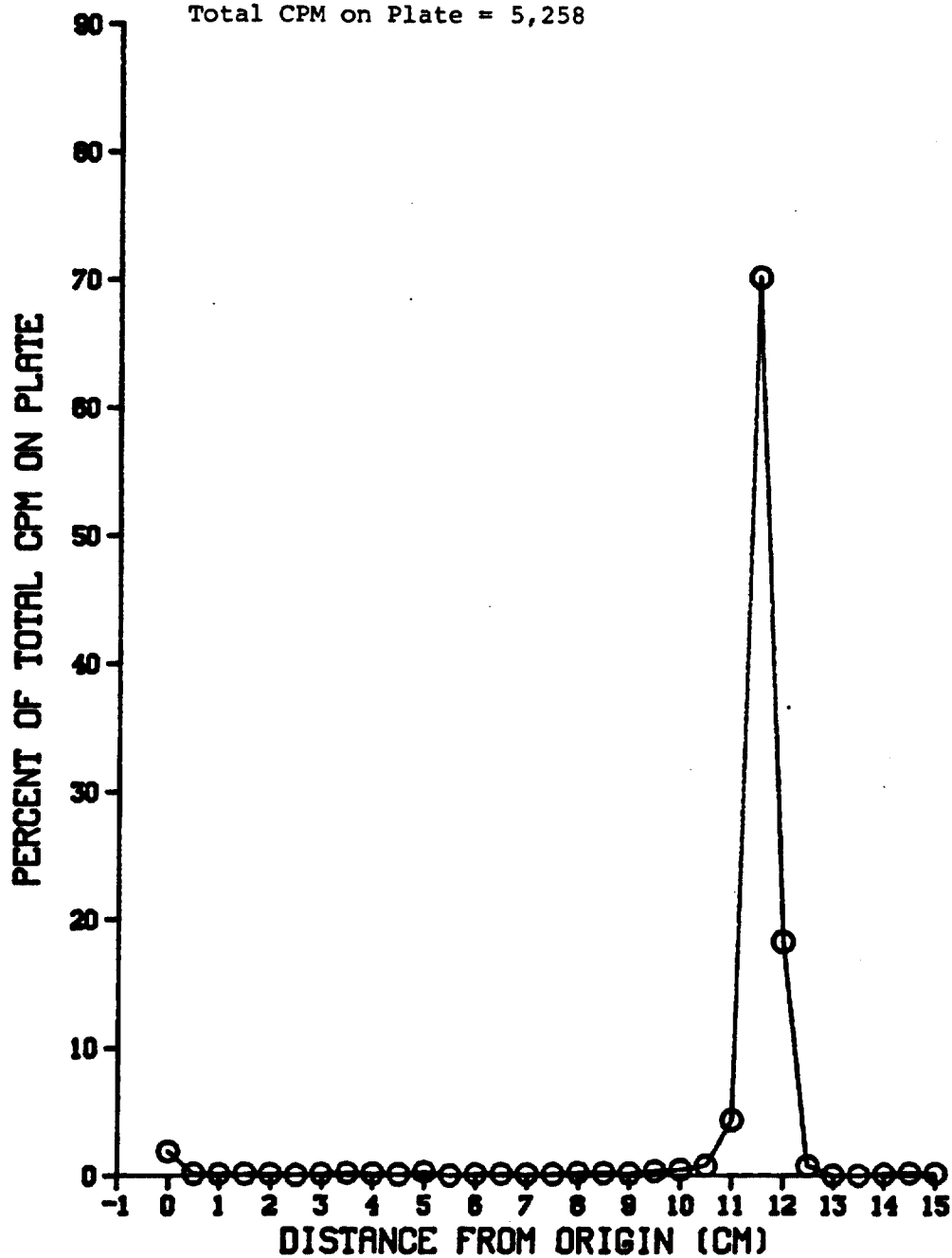
003515

Appendix 1 - Figure 5

Thin-Layer Radiochromatogram of N-Ethyl
FOSE-¹⁴C Dosing Solution, Plate No. 5

Pre-adsorbent SGF Uniplate: 100 butanol
10 water
10 acetic acid

Total CPM on Plate = 5,258



003516

Appendix 2

Determination of Carbon-14 Content of N-Ethyl FOSE-¹⁴C Dose/Feed Mixture

Five aliquots of the dose/feed mixture were weighed into tared combustion cones and pads^a on a five-place analytical balance. The carbon-14 content of the dose/feed aliquots was determined by combustion with a Packard Model 306 Oxidizer. Recovery of carbon-14 was determined to be 84.1% (see Appendix 4 and Appendix 4 - Table 1). This recovery was uniformly low throughout the combustion sample set, thus the data were corrected using this recovery factor.

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

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Appendix 2 - Table 1

Carbon-14 Content of N-Ethyl FOSE-¹⁴C
Dose/Feed Mixture

$\mu\text{g N-Ethyl FOSE-}^{14}\text{C equivalents/g}$	
	523.69
	535.61
	517.61
	517.37
	<u>559.24</u>
Overall $\bar{x}$ =	530.7 $\pm$ 17.58

Notebook Reference: NB-51806-45

003518

Appendix 3

Rat Weights and Amount of N-Ethyl FOSE-¹⁴C Dose/Feed Mixture Administered to Each Rat

Rat Identification	Weight (g) at Time of Dose	Amount (g) of Dose/ Feed Mixture Consumed	Dose in mg/kg
1A	236	4.48	10.07
1B	221	4.24	10.18
1C	242	3.61	7.92
2A	231	4.48	10.29
2B	220	4.26	10.28
2C	237	4.49	10.05
4A	315	6.11	10.30
4B	329	6.37	10.28
4C	327	6.34	10.29
8A	270	5.23	10.28
8B	289	5.61	10.30
8C	288	5.58	10.28
16A	280	5.42	10.27
16B	289	5.61	10.30
16C	276	5.35	10.29
32A	317	6.15	10.30
32B	309	5.99	10.29
32C	301	5.84	10.30

$$\bar{x} \pm \text{S.D.} = 10.13 \pm 0.56$$

Notebook Reference: NB-56531-16b-s and NB-51806-46

003519

Appendix 4

Determination of Recovery of Total Carbon-14 From Blank Biological Samples Spiked With N-Ethyl FOSE-¹⁴C

For each of the four sets of samples combusted, five replicates of 10 μ l, 50 μ l, and 100 μ l of diluted N-ethyl FOSE-¹⁴C dosing solution were aliquoted with calibrated micropipettors directly into scintillation vials. At the same time using the same solution and pipets, either five or six replicates of 10 μ l, 50 μ l, and 100 μ l were aliquoted directly into combustion cones containing 1 g blank biological material (fecal, liver, spleen, or muscle homogenates). The combustion cones were dried and then pelletized with 5 cm ashless filter paper. Blank filter paper pellets were combusted and the solvents collected in the vials to which the FC-95-¹⁴C had been added directly. One of each of the 10 μ l, 50 μ l, and 100 μ l N-ethyl FOSE-¹⁴C spiked pellets were routinely combusted at the beginning, middle, and end of each set of samples. After correction for background and counting efficiency, percent recovery was calculated by comparing mean results from direct addition and combustion. The recovery data for four sets of samples that were analyzed on different days for total carbon-14 (N-ethyl FOSE-¹⁴C) are shown in Appendix 4 - Tables 1-4. The mean recoveries for the four sample sets are 84.1%, 92.7%, 93.3%, and 95.0%. The recoveries were uniformly low throughout the combustion sample sets, so the data were corrected using the appropriate recovery factors.

C03520

Appendix 4 - Table 1

Recovery of Total Carbon-14 From Blank
Biological Samples Spiked With N-Ethyl FOSE-¹⁴C,
Combustion Set No. 1

Combusted Spiked Biological Samples							
	<u>Feces</u>	<u>Feces</u>	<u>Feces</u>	<u>Liver</u>	<u>Liver</u>	<u>Liver</u>	<u>$\bar{x}$ of Liver and Feces</u>
10 μ l ^a	1563 ^b	1648	1682	1750	1748	1713	1684
50 μ l	8328	8329	7768	8219	7605	7475	7954
100 μ l	16381	14905	15581	15724	15693	14498	15464
Direct Addition Samples							<u>$\bar{x}$</u>
10 μ l	1917	1907	1912	1946	1962		1929
50 μ l	9547	9558	9609	9605	9819		9628
100 μ l	18943	18415	18654	19154	18741		18781
<u>10 μl</u>		<u>50 μl</u>		<u>100 μl</u>			
$\frac{1684}{1929} \times 100 = 87.3\%$		$\frac{7954}{9629} \times 100 = 82.6\%$		$\frac{15464}{18781} \times 100 = 82.3\%$			

Overall $\bar{x} \pm$ S.D. recovery used for correction of oxidized
samples = $84.1 \pm 2.8\%$.

^a Amount of N-ethyl FOSE-¹⁴C spiking solution added.
^b Data are expressed as dpm.

Notebook Reference: NB-51806-44

003522

Appendix 4 - Table 2

Recovery of Total Carbon-14 From Blank
Biological Samples Spiked With N-Ethyl FOSE-¹⁴C,
Combustion Set No. 2

Combusted Spiked Biological Samples								
	<u>Spleen</u>	<u>Spleen</u>	<u>Muscle</u>	<u>Muscle</u>	<u>Liver</u>	<u>$\bar{x}$ of Spleen</u>	<u>$\bar{x}$ of Muscle</u>	<u>$\bar{x}$ of Spleen, Muscle, and Liver</u>
10 μ l ^a	1650 ^b	1892	1701	1622	1985	1771	1661	1806
50 μ l	9392	7837	9397	9387	8775	8615	9392	8927
100 μ l	18645	18613	16306	-- ^c	19135	18629	16306	18023
Direct Addition Samples								<u>$\bar{x}$</u>
10 μ l	1933	1938	1713	1925	1919			1886
50 μ l	9877	9829	9883	9861	9931			9876
100 μ l	19743	19735	19546	19523	19338			19577
	<u>10 μl</u>		<u>50 μl</u>		<u>100 μl</u>			
	$\frac{1806}{1886} \times 100 = 95.76\%$		$\frac{8927}{9876} \times 100 = 90.36\%$		$\frac{18023}{19577} \times 100 = 92.06\%$			

Overall $\bar{x} \pm$ S.D. recovery used for correction of oxidized samples = $92.7 \pm 2.8\%$.

- ^a Amount of N-ethyl FOSE-¹⁴C spiking solution added.
^b Data are expressed as dpm.
^c Spiking error; sample was not used.

Notebook Reference: NB-53102-46

003522

Appendix 4 - Table 3

Recovery of Total Carbon-14 From Blank
Biological Samples Spiked With N-Ethyl FOSE-¹⁴C,
Combustion Set No. 3

Combusted Spiked Biological Samples								
	<u>Spleen</u>	<u>Spleen</u>	<u>Feces</u>	<u>Feces</u>	<u>Feces</u>	<u>$\bar{x}$ of Spleen</u>	<u>$\bar{x}$ of Feces</u>	<u>$\bar{x}$ of Spleen and Feces</u>
10 μ l ^a	1978 ^b	2071	2084	2052	1979	2025	2038	2032
50 μ l	9530	-- ^c	10347	10061	10049	9530	10152	9841
100 μ l	18332	18070	18944	19641	19778	18201	19454	18828
Direct Addition Samples								<u>$\bar{x}$</u>
10 μ l	2050	2055	2085	2087	2105			2076
50 μ l	10624	10664	10609	10525	10466			10578
100 μ l	21668	20617	21263	20810	21253			21122
<u>10 μl</u>			<u>50 μl</u>			<u>100 μl</u>		
$\frac{2032}{2076} \times 100 = 97.9\%$			$\frac{9841}{10578} \times 100 = 93.0\%$			$\frac{18828}{21122} \times 100 = 89.1\%$		

Overall $\bar{x} \pm$ S.D. recovery used for correction of oxidized samples = $93.3 \pm 4.4\%$.

- ^a Amount of N-ethyl FOSE-¹⁴C spiking solution added.
^b Data are expressed as dpm.
^c Spiking error; sample was not used.

Notebook Reference: NB-53102-53

003523

Appendix 4 - Table 4

Recovery of Total Carbon-14 From Blank
Biological Samples Spiked With N-Ethyl FOSE-¹⁴C,
Combustion Set No. 4

Combusted Spiked Biological Samples

	<u>Kidney</u>	<u>Kidney</u>	<u>Kidney</u>	<u>Feces</u>	<u>Feces</u>	<u>$\bar{x}$ of Kidney and Feces</u>
10 μ l ^a	2420 ^b	2386	2210	2341	2310	2333
50 μ l	11892	11876	11844	11584	10782	11596
100 μ l	22569	22444	22622	22867	21969	22494

Direct Addition Samples

						<u>$\bar{x}$</u>
10 μ l	2412	2394	2384	2413	2425	2406
50 μ l	11299	12222	12232	12214	12127	12019
100 μ l	24816	24405	24289	24873	24753	24627

<u>10 μl</u>	<u>50 μl</u>	<u>100 μl</u>
$\frac{2333}{2406} \times 100 = 96.97\%$	$\frac{11596}{12019} \times 100 = 96.48\%$	$\frac{22494}{24627} \times 100 = 91.34\%$

Overall $\bar{x} \pm$ S.D. recovery used for correction of oxidized
samples = $95.0 \pm 3.1\%$.

^a Amount of N-ethyl FOSE-¹⁴C spiking solution added.
^b Data are expressed as dpm.

Notebook Reference: NB-56531-10

003524

Appendix 5 - Table 1

Total Carbon-14 in Feces After an Oral Dose of
N-Ethyl FOSE-¹⁴C in Feed to Rats
(Mean Dose, 10.13 mg/kg)

Collection Period (Days)	Rat Identification			Mean <u>+</u> S.D.
	A	B	C	
<u>1 Day Group</u>				
0-1	712.8 ^a	279.3	432.1	474.7 <u>+</u> 219.9
<u>2 Day Group</u>				
0-1	451.5	352.8	281.2	361.8 <u>+</u> 85.5
1-2	374.4	302.6	209.5	295.5 <u>+</u> 82.7
<u>4 Day Group</u>				
0-1	251.0	178.0	320.6	249.9 <u>+</u> 71.3
1-2	204.2	238.5	247.8	230.2 <u>+</u> 23.0
2-3	194.4	184.7	163.2	180.8 <u>+</u> 16.0
3-4	114.4	159.0	146.0	139.8 <u>+</u> 22.9
<u>8 Day Group</u>				
0-1	65.6	229.6	127.7	141.0 <u>+</u> 82.8
1-2	90.3	579.0	248.3	305.9 <u>+</u> 249.4
2-3	559.5	329.0	225.7	371.4 <u>+</u> 170.9
3-4	323.1	184.6	172.7	226.8 <u>+</u> 83.6
4-5	150.4	142.8	92.3	128.5 <u>+</u> 31.6
5-6	119.6	99.4	96.2	105.1 <u>+</u> 12.7
6-7	97.5	68.7	98.0	88.1 <u>+</u> 16.8
7-8	75.3	44.2	86.9	68.8 <u>+</u> 22.1
<u>16 Day Group</u>				
0-16	1906.0	1797.8	1782.6	1828.8 <u>+</u> 67.3
<u>32 Day Group</u>				
0-16	2085.0	1513.8	1757.6	1785.5 <u>+</u> 286.6
16-32	93.0	295.8	81.7	156.8 <u>+</u> 120.5

^a Data are expressed as μ g N-ethyl FOSE-¹⁴C equivalents/sample collection period.

Notebook References: NB-51806-50, NB-55673-30-33

Appendix 5 - Table 2

Total Carbon-14 in Urine After an Oral Dose of
N-Ethyl FOSE-¹⁴C in Feed to Rats
(Mean Dose, 10.13 mg/kg)

Collection Period (Days)	Rat Identification			Mean $\pm$ S.D.	
	A	B	C		
		<u>1 Day Group</u>			
0-1	3.15 ^a	2.73	2.11	2.66 $\pm$	0.52
		<u>2 Day Group</u>			
0-1	3.30	2.11	2.65	2.69 $\pm$	0.60
1-2	2.16	1.71	2.18	2.02 $\pm$	0.27
		<u>4 Day Group</u>			
0-1	3.72	3.31	3.76	3.60 $\pm$	0.25
1-2	4.67	4.62	3.62	4.30 $\pm$	0.59
2-3	3.72	3.31	3.76	3.60 $\pm$	0.25
3-4	4.71	3.25	3.22	3.73 $\pm$	0.85
		<u>8 Day Group</u>			
0-1	2.86	2.05	3.12	2.68 $\pm$	0.56
1-2	2.34	3.95	4.08	3.46 $\pm$	0.97
2-3	4.20	3.31	4.86	4.12 $\pm$	0.78
3-4	10.42	2.60	3.53	5.52 $\pm$	4.27
4-5	8.46	8.34	3.12	6.64 $\pm$	3.05
5-6	2.45	1.85	2.47	2.26 $\pm$	0.35
6-7	2.63	1.51	2.92	2.35 $\pm$	0.74
7-8	1.78	1.37	2.81	1.99 $\pm$	0.74
		<u>16 Day Group</u>			
0-16	70.34	35.46	31.27	45.69 $\pm$	21.45
		<u>32 Day Group</u>			
0-32	60.36	85.44	50.07	65.29 $\pm$	18.19

^a Data are expressed as μ g N-ethyl FOSE-¹⁴C equivalents/sample collection period.

Notebook Reference: NB-51806-52-55

003526

Appendix 6

Carbon-14 Content in Digestive Tract (plus contents)
and Feces After an Oral Dose of N-Ethyl FOSE-¹⁴C
in Feed to Rats (Mean Dose, 10.13 mg/kg)

Rat Identification	Time Post-Dose (Hours)	Digestive Tract (plus contents)	Feces
1A	24	11.85 ^a	64.04
1B	24	31.98	105.79
1C	24	8.54	57.38
Mean $\pm$ S.D.		17.46 $\pm$ 12.69	75.74 $\pm$ 25.24
2A	48	8.51	91.79
2B	48	12.37	115.74
2C	48	8.12	44.15
Mean $\pm$ S.D.		9.67 $\pm$ 2.35	83.89 $\pm$ 36.44

^a Data are expressed as μ g N-ethyl FOSE-¹⁴C equivalents/g.

Notebook Reference: NB-51806-49-50

C03527

Appendix 7

Comparative Data Showing Normal Fecal Excretion for Rats

Appendix 7 - Table 1 shows comparative data showing normal fecal excretion for rats. Grams of feces excreted by rats at 24 hour intervals for a 7 day postdose period are given for rats in this study and for rats used as control groups in previous studies (FC-Experiments 8 and 9). These data show that the fecal excretion rates of rats in this study are comparable to the excretion rates of rats used as control groups in 2 other studies.

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Appendix 7 - Table 1

Comparative Data Showing Normal Fecal Excretion for Rats

Collection Period (Days)	$\bar{x} \pm \text{S.D. of 3 rats from the present study}$	$\bar{x} \pm \text{S.D. of 5 control rats from FC-Exp. 8}$	$\bar{x} \pm \text{S.D. of 5 control rats from FC-Exp. 9}$
0-1	$8.0 \pm 2.68^{a,b}$	10.29 ± 3.68	7.04 ± 4.38
1-2	9.47 ± 2.90^b	7.79 ± 1.58	9.00 ± 2.21
2-3	10.79 ± 1.14^c	10.27 ± 2.04	8.30 ± 1.20
3-4	11.61 ± 0.91^c	10.33 ± 2.22	9.74 ± 1.25
4-5	8.92 ± 2.47^c	8.51 ± 2.63	9.15 ± 0.86
5-6	9.78 ± 2.17^c	9.11 ± 1.79	9.57 ± 1.54
6-7	10.69 ± 1.27^c	8.65 ± 1.93	8.62 ± 1.07

^a Data are expressed as grams of feces excreted per collection period.
^b This data is from the 2 Day Group of this study.
^c This data is from the 8 Day Group of this study.

Notebook References: NB-56531-16u-16v and 16dd-16hh, and
 NB-53102-29t-29bb and 42q

003529

Appendix 8

Carbon-14 Content in Tissues After an Oral Dose of N-Ethyl POSE-¹⁴C in Feed to Rats (Mean Dose, 10.13 mg/kg)

Rat Identification	Liver ^a	Spleen ^a	Kidneys ^a	Lungs ^a	Red Blood Cells ^a	Plasma ^a	Bone Marrow ^a	Digestive Tract ^a	Carcass ^a	Subcut. Fat ^a	Abdom. Fat ^a	Muscle ^a
1A	32.55	6.77	8.93	8.13	21.95	7.36	12.40	11.85	2.29	6.44	8.10	1.79
1B	49.58	7.00	14.65	11.89	27.73	10.61	15.00	31.98	3.23	7.93	9.68	2.21
1C	24.05	4.80	6.76	6.16	20.35	6.13	8.73	8.54	1.61	3.90	4.87	0.97
Mean	35.39	6.19	10.11	8.73	23.34	8.03	12.04	17.46	2.38	6.09	7.55	1.66
2A	31.25	5.93	6.89	6.39	19.38	5.69	10.72	8.51	1.47	4.07	3.35	1.13
2B	42.95	4.81	8.22	6.75	20.66	6.54	10.89	12.37	1.98	4.30	4.10	1.65
2C	41.58	7.30	10.49	8.80	12.31	9.51	11.41	8.12	2.40	3.97	4.30	1.88
Mean	38.59	6.01	8.53	7.31	17.45	7.25	11.01	9.67	1.95	4.11	3.92	1.55
4A	44.42	6.75	8.82	8.30	18.83	7.76	8.21	-- b	-- b	2.21	2.14	1.63
4B	44.95	7.62	11.29	9.08	21.44	9.10	10.07	--	--	3.07	1.80	1.40
4C	40.34	6.84	9.82	7.81	19.90	7.92	8.92	--	--	1.87	1.79	1.36
Mean	43.24	7.07	9.98	8.40	20.06	8.26	9.07	--	--	2.38	1.91	1.46
8A	26.81	2.15	3.34	2.87	8.42	2.94	3.36	--	--	0.54	0.38	0.34
8B	24.65	1.80	3.45	2.63	7.68	3.24	2.91	--	--	1.01	0.23	0.25
8C	34.52	3.98	6.33	6.52	12.61	5.66	5.63	--	--	0.71	0.48	0.98
Mean	28.66	2.64	4.37	4.01	9.57	3.95	3.97	--	--	0.75	0.36	0.52
16A	20.97	0.83	1.93	1.47	2.24	1.75	0.99	--	--	0.03	0.04	0.16
16B	28.67	0.87	2.05	1.81	3.04	2.19	1.01	--	--	0.13	0.01	0.16
16C	27.09	1.11	3.07	2.36	3.25	2.92	1.52	--	--	0.36	0.03	0.21
Mean	25.58	0.94	2.35	1.88	2.84	2.29	1.17	--	--	0.17	0.03	0.18
32A	20.25	0.76	1.86	1.66	0.83	1.95	0.69	--	--	0.08	0.00	0.13
32B	33.78	1.37	3.57	2.45	2.65	2.87	1.50	--	--	0.28	0.00	0.29
32C	20.95	0.64	1.80	1.36	0.89	1.59	0.63	--	--	0.06	0.00	0.09
Mean	24.99	0.92	2.41	1.82	1.46	2.14	0.94	--	--	0.14	0.00	0.17

^a Data are expressed as µg N-ethyl POSE-¹⁴C equivalents/g.
^b Sample was not taken.

Notebook References: NB-51806-47-49, NB-53102-43-44, 47-52, and 54, NB-56531-11-12

603530



Form 6747-11-A

Appendix 9

TECHNICAL REPORT SUMMARY

Date
June 9, 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 Perfluorooctane Sulfonic Acid - A Rat-Liver		Report Number 474
AR No. 7474 - Metabolite of FM-3422 - June 9, 1981		
Author(s) S. J. Gibson, J. D. Johnson - 218-2-02		Employee Number(s) 233150
Notebook Reference S. V. Pathre		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

CURRENT OBJECTIVE:

Request No. C57427
Project No. 91505026
Requestor - S. J. Gibson, 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.

A rat-liver metabolite is identified as perfluorooctane sulfonic acid by ^{19}F -NMR.

003531

Information Liaison
Initials: _____

3M CONFIDENTIAL

CENTRAL ANALYTICAL LABORATORY

Report No.7474.....

Date.....June 9, 1981.....

Subject: Perfluorooctane Sulfonic Acid - A Rat-Liver Metabolite of FM-3422
S. J. Gibson

Requestor: ...J. D. Johannp...

Dept. NameRiker.....

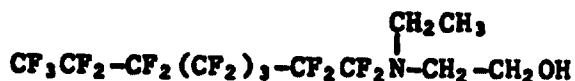
Proj. No. 91505026..

Request No. ...C57427.....

Dated ..December 10, 1980...

Report:

Two metabolites isolated from the liver of a rat administered ^{14}C -labeled FM-3422 were submitted for spectroscopic analysis. These two metabolites were labeled as I- CH_2Cl -MeOH eluted and II- CH_3 eluted.



FM-3422

Experimental

Both samples were reconstituted in CD_3OD . The ^{19}F -NMR spectra on these samples were obtained on the Varian XL-100 and XL-200 NMR spectrometers.

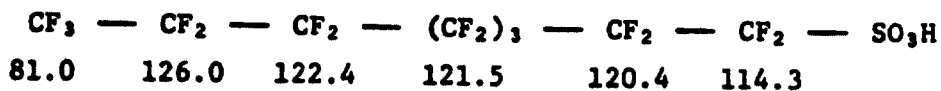
Metabolite I ^{19}F NMR 13875NMetabolite II ^{19}F NMR 30190XResults

The chemical shifts (ppm upfield from CFCl_3) of the major peaks in the fluorine spectra are given below. The peak frequencies are normalized to $\delta(\text{CF}_3) = 81.0$ ppm.

I	81.0	114.3	120.4	121.5	122.4	126.0
II	81.0	112.9	120.2	121.8	122.8	126.2

Discussion

Both spectra were typical of perfluorooctane sulfonyl derivatives. The metabolite I was identical to perfluorooctane sulfonic acid as determined by comparing the reference ^{19}F NMR (11252X) of the latter with that of I.

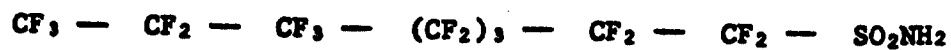


(I)

The spectrum of the metabolite II was very similar to that of I except the chemical shift of the fluoromethylene alpha to the sulfonyl group. It is observed at 112.9 ppm in II, 1.4 ppm upfield from that in I. The 112.9 ppm peak,

C03532

although not unambiguously, can be assigned to the alpha fluoromethylene of the sulfonamide ($-\text{SO}_2\text{NH}_2$) group:



112.9

(II)

Conclusion

The liver metabolite labeled I- CHCl_3 -MeOH is identified as perfluorooctane sulfonic acid and that labeled II- CHCl_3 is suggested as perfluorooctane sulfanamide.



S. V. Pathre

SVP/rs

003533