Absorption of FC-95-^{14}C in Rats After a Single Oral Dose

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Summary

After a single oral dose of FC-95-^{14}C (mean dose, 4.2 mg/kg) in solution to groups of three male rats, at least 95% of the total carbon-14 is systemically absorbed at 24 hours. The half-life for elimination of total carbon-14 from plasma is 7.5 days.

Introduction

$$C_7F_{15}-\overset{*F}{\underset{F}{C}}-SO_3^- \; K^+$$

FC-95 *Denotes Position of Carbon-14

FC-95 is the potassium salt of a perfluorinated sulfonic acid. A series of experiments has been planned to investigate possible means of increasing the rate of elimination of FC-95 from the body. FC-95 absorption, elimination from plasma, and excretion data were necessary to form a basis for efficient design of these experiments. This FC-95-^{14}C oral dosing experiment (FC-Experiment 4) which was designed to provide total carbon-14 absorption and plasma elimination data was paired with an FC-95-^{14}C iv dosing experiment (FC-Experiment 3) which was designed to provide data on the route and extent of total carbon-14 excretion. In addition, these FC-95 absorption, plasma elimination, and excretion data will be used to interpret and guide other experiments on the possible biotransformation of FC-807 to FC-95.

Methods

Radiolabeled FC-95-^{14}C

The carbon-14 label is at the position α to the sulfur atom. (See above structure). The specific activity of this lot of FC-95-^{14}C (Riker Isotope Inventory Number 442) is 0.459 μCi/mg. The FC-95-^{14}C was found to be suitable for metabolism studies; details of the specific activity determination, chemical characterization, and radiochemical purity determination will be reported separately.

Animals

Male Charles River[a] CD rats, eight weeks old, were conditioned to individual metal metabolism cages for 24 hours prior to dosing. The body weights ranged from 243 to 315g, mean 285g. The rats were allowed free access to Purina[b] Ground Chow and water before and after dosing.

Dosing

Each non-fasted rat was weighed immediately before being given a single oral dose of FC-95-^{14}C. The dose was 2.0 ml of a 0.9% NaCl solution containing 1.21 mg FC-95-^{14}C/2.0 ml. The average dose was 4.2 mg/kg. The dose was delivered with a 2.0 cc glass syringe (Tryl[c]) fitted with a stainless steel intubation tube. The dosing solution was prepared by adding ∿ 200 mg of FC-95-^{14}C to 0.9% NaCl, shaking for one half hour at moderate speed in a mechanical shaker, and centrifuging. The supernatant was removed and used for dosing solution. The carbon-14 content of the dosing solution was determined by direct counting (see Appendix 1).

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[a] Charles River Breeding Laboratories, Wilmington, Mass.

[b] Purina Lab Chow, Ralston Purina Company, St. Louis, Missouri.

[c] Aloe Medical, Division of Brunswick, St. Louis 3, Missouri.

Sample Analysis for Carbon-14

Homogenizing of feces and tissue was done in Waring blenders by adding nine parts of water (w/w) to one part of biological material. The homogenates were weighed into combustion cones[a] in triplicate on a top-loading balance by taring the cone and adding 1.0g of the homogenate. Care was taken to mix the homogenate between samplings. Urine, red blood cells, and plasma samples (from the 24 and 48 hour post dose groups) were also measured into combustion cones by weight. The concentration of carbon-14 in plasma for the 3 rats in the 24 and 48 hour groups was determined by both combustion and direct counting. Plasma samples (including the 24 and 48 hour post dose groups) were counted directly by transferring 1.0 ml of plasma and 15 ml of Aquasol[®][b] to a scintillation vial and counting in a liquid scintillation spectrometer. Homogenate, plasma, urine and red blood cell samples from the 24 and 48 hour post dose group were combusted with a Packard Model 306 Oxidizer. Combustion recovery of carbon-14 from biological samples was determined by combusting blank fecal homogenates and blank red blood cells spiked with dilutions of FC-95-^{14}C dosing solution at the beginning, middle, and end of the analysis of the experimental sample set (see Appendix 2).

All radiometric measurements were done using a Packard Model 3385 Tri-Carb Liquid Scintillation Spectrometer. For plasma samples counted directly, the efficiency for each sample was determined by adding internal standard to each sample and recounting, correcting for background from appropriate blanks, and comparing the results to a sealed standard of known activity. After correcting for background with appropriate blanks and for efficiency, the carbon-14 content of each sample was calculated. For oxidized samples, counting efficiency for each sample was determined by use of the AES (Automatic External Standardization) ratio method. To calibrate the external standard, internal standard was added to selected samples from the group of samples (three with low AES ratios and three with high ratios) and these samples were recounted along with a sealed standard. Data were collected on punch tape and processed by the CDC 1700 Computer System in the 3M Central Research Data Processing Laboratory. Data reduction to dpm was accomplished with the Biological Automatic External Standardization computer program.

Sample Collection

Groups of three rats were sacrificed by exsanguination at 1, 2, 6, 12, 24, 48, 96, and 144 hours post dose. Rats were anesthetized with diethyl ether and blood was drawn from the descending aorta of each rat and immediately transferred to a heparinized tube. Plasma was prepared promptly by centrifugation. In addition to plasma and red blood cells, total urine, total feces, spleen, digestive tract plus contents (esophagus, stomach, small intestine, large intestine, and colon), and remainder of carcass were saved from each of the three rats in the 24 and 48 hours post dose groups for carbon-14 analysis.

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

b New England Nuclear, Boston, Mass.

Results and Discussion

The results of the urine, feces, digestive tract plus contents, and carcass analyses are shown in Table 1. The digestive tract and contents contained on the average, 3.45% of the dose. The mean fecal excretion is 1.55% of the dose at 24 hours and 3.24% at 48 hours. At 24 hours, the mean sum of total carbon-14 in feces and digestive tract plus contents is 5% of the dose. Some of this 5% likely represents systemically absorbed carbon-14 present either in the digestive tract tissues or in the digestive tract contents as a result of excretion. The data from the 48 hour post dose group of rats are consistent with the 24 hour post dose data. Thus, at least 95% of the FC-95-^{14}C dose was absorbed from solution after administration to non-fasted rats. The major portion of the radioactivity recovered was found in the carcass. The carcass data are not as reliable as the other tissue data since large volume homogenates were necessary and homogeneity of sample aliquots was difficult to assure. There is some excretion of total carbon-14 in urine (1-2%/day); this is in contrast to the much slower rate of urinary excretion of total carbon-14 after an iv dose of FC-807-^{14}C (0.067%/day) (1). The spleens from the 24 hour and 48 hour post dose rats were analyzed for total carbon-14 content, and the percent of the dose in the whole organ was ∿ 0.2%. This is much lower than the 6% of the dose in spleen observed at 124 days post iv dose for FC-807-^{14}C (1).

The concentrations of total carbon-14 in red blood cells and plasma were compared (Table 2). The mean ratio of red blood cell to plasma concentration at 24 and 48 hours is 0.25 and 0.39, respectively. Thus, at 24 and 48 hours after a single oral dose of FC-95-^{14}C, there is no selective retention of carbon-14 in red blood cells.

The results from analysis of plasma samples from groups of three rats at 1, 2, 6, 12, 24, 48, 96, and 144 hours after a single oral dose of FC-95-^{14}C are shown in Table 3. The log of mean concentration versus time for these data is plotted in Figure 1. The least squares line through the individual points from 24 to 144 hours for these data fits the equation:

$C_p = 15.65e^{-0.00387t}$; C_p is plasma concentration. The half-life of elimination from plasma is 179 hours (7.5 days). Thus, elimination from plasma of total carbon-14 after a single oral dose of FC-95-^{14}C is slow.

References

1. Johnson, JD: Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single IV Dose of FC-807-^{14}C (Report), September 29, 1979.

2. Johnson, JD: Absorption of FC-807-^{14}C in Rats after a Single Oral Dose (Report), July 10, 1979.

List of Tables and Figures

Table 1

Percent Recovery of Total Carbon-14 after a Single Oral Dose of FC-95-^{14}C to Rats (Mean Dose, 4.2 mg/kg) at 24 and 48 Hours Post Dose

Rat Number	Time Post Dose (Hours)	Percent of Dose			
		Carcass	Digestive Tract plus Contents	Feces	Urine
1	24	77.34[a]	3.27	1.68	1.67
2	24	77.06	3.45	1.72	1.94
3	24	82.62	4.02	1.24	1.09
Mean		79.01	3.58	1.55	1.57
4	48	103.61	3.55	3.25	1.91
5	48	86.21	3.17	3.10	2.78
6	48	92.63	3.24	3.37	2.88
Mean		94.15	3.32	3.24	2.52

[a] Data were adjusted for combustion recovery (see Appendix 2).

Table 2

Comparison of Total Carbon-14 Content of Red Blood Cells and Plasma after a Single Oral Dose of FC-95-^{14}C to Rats (Mean Dose, 4.2 mg/kg) at 24 and 48 Hours Post Dose

Rat Number	Time Post Dose (Hours)	Red Blood Cells	Plasma	Ratio Red Blood Cells/Plasma
1	24	3.63[a,b]	16.34[b]	0.22
2	24	4.15	14.62	0.28
3	24	3.18	13.40	0.24
Mean				0.25
4	48	2.80	14.27	0.20
5	48	7.85	14.25	0.55
6	48	4.94	11.76	0.42
Mean				0.39

[a] Data are expressed as µg equivalents of FC-95-^{14}C/g.

[b] The data were obtained by combustion and were adjusted for recovery (see Appendix 2).

Table 3

Concentration of Carbon-14 in Rat Plasma
after a Single Oral Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg)
at 1, 2, 6, 12, 24, 48, 96, and 144 Hours Post Dose

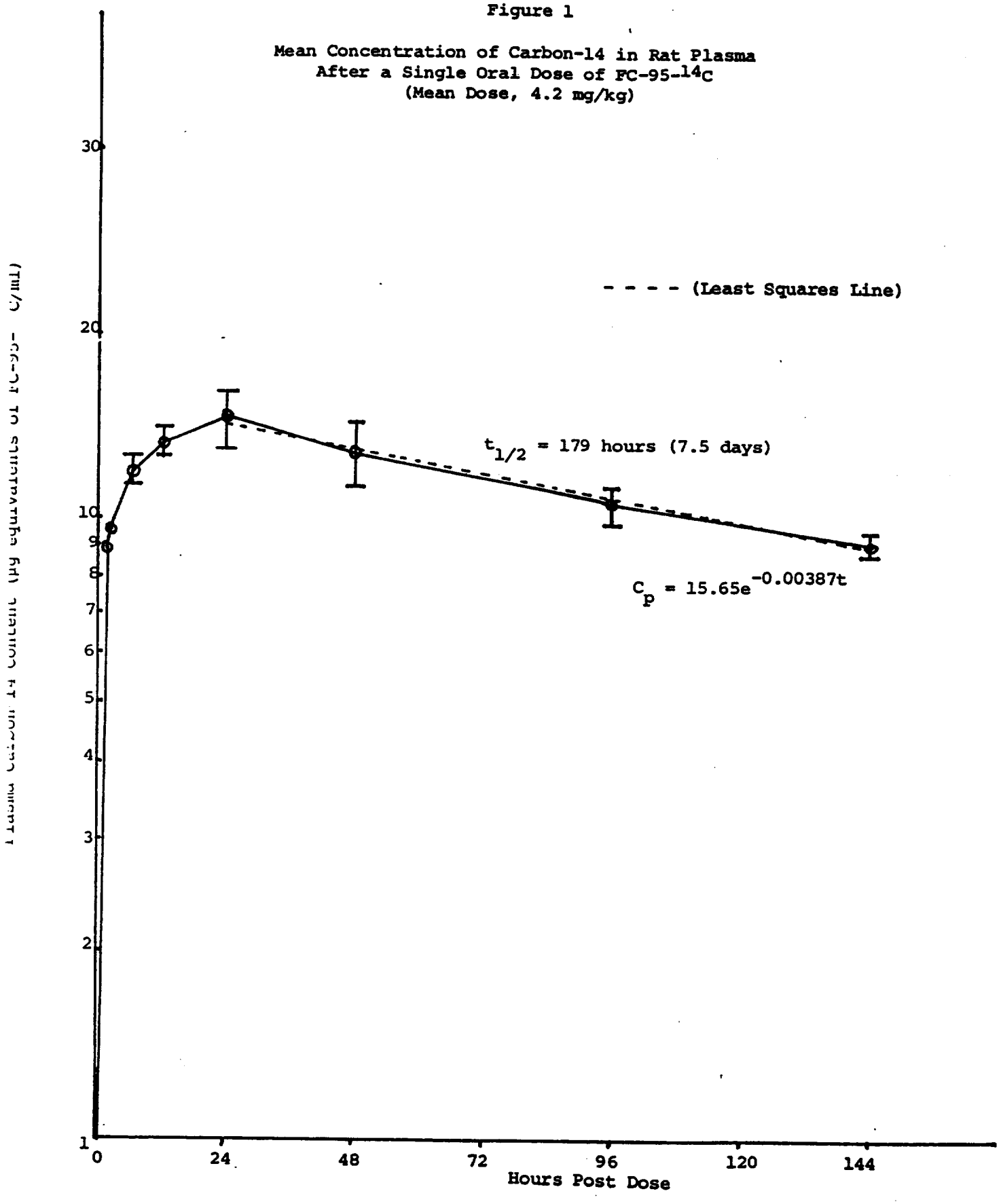
Hours Post Dose	Rat[a]			Average ± SD
	A	B	C	
1	11.35[b]	7.47	7.62	8.81 ± 2.20
2	9.84	12.88	6.12	9.61 ± 3.39
6	11.74	11.59	12.60	11.98 ± 0.55
12	13.20	14.14	12.61	13.32 ± 0.77
24	16.35	14.43	13.02	14.60 ± 1.60
48	13.64	13.98	11.06	12.89 ± 1.60
96	11.05	9.67	11.00	10.57 ± 0.78
144	8.71	9.53	9.09	9.11 ± 0.41

[a] Three rats were dosed and sacrificed for each time interval.

[b] Data were obtained by direct counting; data are expressed as µg equivalents of FC-95-^{14}C/ml of plasma.

Figure 1

Mean Concentration of Carbon-14 in Rat Plasma
After a Single Oral Dose of FC-95-^{14}C
(Mean Dose, 4.2 mg/kg)



Appendix 1

Determination of Carbon-14 Content of Dosing Solution

Just prior to dosing of rats, the FC-95-^{14}C dosing solution was sampled with calibrated micropipettors[a] directly into counting vials; six 10 µl and six 50 µl aliquots were pipetted. One ml of water and 15 ml of Aquasol® were added[b], corrections were made for background and counting efficiency and, using the specific activity of FC-95-^{14}C already determined[c], the µCi/2.0 ml was calculated. The data are shown in Appendix Table 1. The dose administered each rat was 2.0 ml of solution containing 0.553 µCi of FC-95-^{14}C which is 1.21 mg of FC-95-^{14}C. This is a mean dose of 4.2 mg/kg.

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[a] L/I Micropipettor, Lab Industries, Berkeley, California.

[b] Aquasol® was found to be a suitable solvent-scintillent for FC-95-^{14}C during specific activity determination (to be reported separately).

[c] Specific activity = 0.459 µCi/mg. (Specific activity determination to be reported separately).

Appendix

Table 1

Carbon-14 Content of Dosing Solution

10 µl Aliquot µCi/2.0 ml	50 µl Aliquot µCi/2.0 ml
0.5602	0.5624
0.5554	0.5532
0.5614	0.5290
0.5628	0.5390
0.5612	0.5382
0.5554	0.5534

Overall average = 0.5526

$$\frac{0.553}{0.4586\ \mu Ci/mg} = 1.21\ \mu Ci\ FC\text{-}95\text{-}^{14}C/2.0\ ml$$

Appendix 2

Recovery of Total Carbon-14 from Blank Biological Samples Spiked with FC-95-^{14}C

The good recovery of total carbon-14 from combusted FC-807-^{14}C in biological samples has been reported (2). Since the carbon label is α to the sulfur atom in both FC-95-^{14}C and the components of FC-807-^{14}C, it is reasonable to expect that good recovery of total carbon-14 from FC-95-^{14}C and/or its metabolites in biological samples can be attained. For each set of samples combusted, four replicates of 10 µl, 50 µl, and 100 µl of diluted FC-95-^{14}C dosing solution were aliquoted with calibrated micropipettors directly into scintillation vials. At the same time using the same solution and pipettes, either three or four replicates of 10 µl, 50 µl or 100 µl were aliquoted directly into combustion cones which already contained blank biological material (fecal homogenates, plasma, or red blood cells). 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-^{14}C had been added directly. One each of the 10 µl, 50 µl, and 100 µl FC-95-^{14}C spiked pellets were routinely combusted at the beginning, middle, and end of each set of biological samples. After correction for background and counting efficiency, percent recovery was calculated by comparing mean results from direct addition and combustion. The mean recovery data for three sets of fecal samples that were analyzed on different days for total carbon-14 (FC-95-^{14}C) for FC-Experiments 3(to be reported later) are shown in Appendix Table 2A. The mean recovery is 98.0 ± 1.5%. Since each entry in the table is the mean of four determinations, this mean recovery is based on 36 determinations. Thus, good recovery of total carbon-14 from FC-95-^{14}C spiked biological material is shown. The mean recovery for the set of samples in the report (FC-Experiment 4) which is based on triplicate 10 µl, 50 µl, and 100 µl FC-95-^{14}C spiked blank fecal homogenates and red blood cells was 93.6%. (See Appendix Table 2B). This slightly lower recovery was due to a minor malfunction of the Packard Oxidizer and was fairly uniform throughout the set of samples. Thus, the total carbon-14

data obtained from combustion in this report were adjusted for recovery.

There was no apparent differences in recovery between spiked blank red blood cells and spiked fecal homogenates.

Appendix

Table 2A

Recovery of Total Carbon-14 from Blank Fecal Homogenate Samples Spiked with FC-95-^{14}C

10 µl[a]	50 µl	100 µl
97.4[b,c]	99.5	97.7
100.6	97.0	98.1
97.9	95.4	98.1
98.6	97.3	98.0

Overall average = 98.0 ± 1.5%

[a] Amount of FC-95-^{14}C reference solution added.

[b] Data are expressed as % recovery $\left(\frac{\text{dpm from combustion}}{\text{dpm from direct addition}} \times 100\right)$

[c] Each entry is the mean of 4 combustion recovery determinations.

Appendix

Table 2B

Recovery of Total Carbon-14 from Fecal Homogenate and Red Blood Cell Samples Spiked with FC-95-^{14}C

DIRECT ADDITION

10 µl[a]	50 µl	100 µl
756[b]	3868	7403
756	3688	7404
727	3719	---[c]
723	3671	7394
741 ± 18	3737 ± 90	7400 ± 6

SPIKED AND OXIDIZED

	10 µl		50 µl		100 µl	
	Fecal Homogenates	Red Blood Cells	Fecal Homogenates	Red Blood Cells	Fecal Homogenates	Red Blood Cells
	619	671[d]	3581	3583	7408	7093
	613	---[d]	3775	3583	7104	7098
	677	711	3484	3347	7105	7081
$\bar{X}$	636	691	3613	3504	7106	7091
	$\frac{636}{741} \times 100 = 85.8\%$		$\frac{3613}{3737} \times 100 = 96.7\%$		$\frac{7106}{7400} \times 100 = 96.0\%$	
	$\frac{691}{741} \times 100 = 93.3\%$		$\frac{3504}{3737} \times 100 = 93.8\%$		$\frac{7091}{7400} \times 100 = 95.8\%$	

Overall average = 93.6%

[a] Amount of FC-95-^{14}C spiking solution added.

[b] Data are expressed as dpm.

[c] Sample was not used; cracked vial.

[d] Spiking error; sample was not used.

Appendix 3

Comparison of Oxidation with Direct Counting of Plasma Samples from Rats at 24 and 48 Hours after a Single Oral Dose of FC-95-^{14}C

Concentration of Carbon-14 in plasma for the three rats in both the 24 and 48 hour post dose groups was determined by both oxidation and direct counting. Samples were counted directly by transferring 1.0 ml of plasma and 15 ml of Aquasol® to scintillation vials and counting in the liquid scintillation counter. Corrections for efficiency and background were made for each sample. For determination by oxidation, plasma samples were measured by weight into combustion cones and carbon-14 was determined by combusting and counting. The data from the plasma oxidation - direct counting comparison are shown in Appendix Table 3. The mean ratio of oxidized to direct counting is 0.97± 0.02. Thus, the plasma level data obtained via either of these two methods will be essentially the same. The direct counting method, being much more convenient, was chosen to analyze the plasma, and the data quoted for the plasma concentration versus time (Figure 1) is from direct counting. Though a direct measurement of absolute recovery from plasma was not done, these results indicate that recovery of total carbon-14 from plasma spiked with FC-95-^{14}C would be similar to that found for recovery from fecal homogenates (98%, Appendix 2).

Appendix

Table 3

Comparison of Oxidation with Direct Counting of Plasma Samples from Rats at 24 and 48 Hours after a Single Oral Dose of FC-95-^{14}C

Rat	Direct Counting (µg/ml)[a]	Oxidation (µg/g)[a]	Ratio Oxidation/ Direct Counting
1 (24 hours)	16.35	15.30	0.94
2 (24 hours)	14.43	13.99	0.97
3 (24 hours)	13.02	12.55	0.96
1 (48 hours)	13.64	13.36	0.98
2 (48 hours)	13.98	13.33	0.95
3 (48 hours)	11.06	11.01	1.00
			X ± SD 0.97 ± 0.02

[a] Data are expressed as µg equivalents of FC-95-^{14}C.