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Absorption of FC-143-^{14}C in Rats After a Single Oral Dose

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Summary

After a single oral dose of FC-143-^{14}C (mean dose, 11.0 mg/kg) in solution to groups of three male rats, at least 93% of the total carbon-14 is absorbed at 24 hours. The half-life for elimination of total carbon-14 from plasma is 4.8 days.

Introduction

$C_7F_{15}\overset{*}{C}O_2^-NH_4^+$

FC-143

* Denotes Position of Carbon-14

FC-143 is the ammonium salt of a perfluorinated carboxylic acid. A series of experiments has been planned to investigate possible means of increasing the rate of elimination of FC-143 from the body. FC-143 absorption, elimination from plasma, and excretion data were necessary to form a basis for efficient design of these experiments. This FC-143-^{14}C oral dosing experiment (FC-Experiment 5) which was designed to provide total carbon-14 absorption and plasma elimination data was paired with an FC-143-^{14}C iv dosing experiment (FC-Experiment 6) which was designed to provide data on the route and extent of total carbon-14 excretion.

Methods

Radiolabeled FC-143-^{14}C

The carbon-14 label is at the carbonyl carbon (see above structure). The specific activity of this lot of FC-143-^{14}C (Riker Isotope Inventory Number 459) is 0.5071 µCi/mg. The FC-143-^{14}C was found to be suitable for metabolism studies; details of the specific activity determination, chemical characterization, and radiochemical purity determination was reported separately. (4)

Animals

Male Charles River[a] CD rats, ten weeks old, were conditioned to individual metal metabolism cages for 24 hours prior to dosing. The body weights ranged from 304 to 398 g, mean 362 g. The rats were allowed free access to Purina[b] Ground Chow and water before and immediately after dosing.

Dosing

Each non-fasted rat was weighed immediately before being given a single oral dose of FC-143-^{14}C. The dose was 2.0 ml of a 0.9% NaCl solution containing 4.1412 mg FC-143-^{14}C/2.0 ml. The average dose was 11.4 mg/kg. The dose was administered with a 2.0 cc glass syringe (Trylon[c]) fitted with a stainless steel intubation tube. The dosing solution was prepared by weighing FC-143-^{14}C into a 100 ml Class A volumetric flask, adjusting to volume with 0.9% NaCl, and mixing by inverting by hand. The carbon-14 content of the dosing solution was determined by direct counting (see Appendix 1).

Sample Analysis for Carbon-14

Homogenizing of feces and tissues was done in Waring blenders by adding nine parts of water (w/w) to one part of biological material. The homogenates

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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, Missouri.

were weighed into combustion cones[a] in triplicate on a top-loading balance by taring the cone and adding 1.0 g 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 group 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 plasma spiked with dilutions of FC-143-^{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.

Results and Discussion

The results of the urine, feces, digestive tract plus contents, and carcass analyses are shown in Table 1. At 24 hours post dose, the digestive tract and contents contained 2.6% of the dose. The mean fecal excretion is 4.3% of the dose, at 24 hours and 5.0% at 48 hours. At 24 hours, the mean sum of total carbon-14 in feces and digestive tract plus contents is $\sim$ 7% of the dose.

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[a] Packard Instrument Company, Inc., 2200 Warrenville Rd., Downers Grove, Illinc

[b] New England Nuclear, Boston, Mass.

Some of this 7% likely represents systemically absorbed carbon-14 present either in the digestive tract tissues or in the digestive tract or feces as a result of elimination. The data from the 48 hour post dose group of rats are consistent with the 24 hour post dose data. Thus, at least 93% of the FC-143-^{14}C dose was absorbed from solution after administration to non-fasted rats. The major portion of the radioactivity recorded was found in the carcass. The carcass data are not as accurate as the other tissue data since large volume homogenates were necessary and homogeneity of sample aliquots from carcass homogenates was difficult to assure. The quantity of total carbon-14 excreted via urine was on the average 9.2% of the dose at 24 hours and 14.8 of the dose at 48 hours. This is a much higher rate of excretion than that observed for FC-95-^{14}C at 24 hours post dose (1.6%) (1) or for FC-807-^{14}C at 24 hours (0.02%) (3). The spleens from the 24 and 48 hour post dose group of rats were analyzed for total carbon-14 content, and the percent of the dose in the whole organ was $\sim$ 0.1%. This is much lower than the 6% of the dose in spleen observed at 124 days post iv dose for FC-807-^{14}C. (2) It is similar to the $\sim$ 0.2% of the dose found in spleen at 24 and 48 hours post oral dosing with FC-95-^{14}C.

The concentrations of total carbon-14 in red blood cells and plasma were compared (Table 2). The mean ratios of red blood cell to plasma concentration at 24 and 48 hours are 0.25 and 0.22, respectively. Thus, at 24 and 48 hours after a single oral dose of FC-143-^{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-143-^{14}C are shown in Table 3. The log of mean concentration versus time is plotted in Figure 1. The least squares line through the individual points from 24 to 144 hours for these data fits the equation: $Cp = 64.64e^{-0.006005t}$; Cp is plasma concentration. The half-life of elimination from plasma is 115 hours(4.8 days). The half-life of elimination of total carbon-14 after oral dosing with FC-95-^{14}C is 7.5 days (1). Thus, elimination from plasma of total carbon-14 after a single oral dose of FC-143-^{14}C is slow but more rapid than the elimination of total carbon-14 after oral dosing with FC-95-^{14}C.

References

(1) Johnson JD: Absorption of FC-95-^{14}C in Rats After a Single Oral Dose (Report), October 26, 1979.

(2) 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.

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

(4) Behr FE, Johnson JD: Synthesis and Characterization of FC-143-^{14}C (Report), December 28, 1979.

List of Tables and Figures

Table 1

Percent Recovery[a] of Total Carbon-14 After a Single Oral Dose of FC-143-^{14}C to Rats (Mean Dose, 11.4 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	56.04	2.58	4.49	13.98
2	24	60.90	2.62	3.43	7.36
3	24	62.58	2.53	4.92	6.29
Mean		59.84	2.58	4.28	9.21
4	48	54.38	1.68	5.32	15.54
5	48	51.60	1.49	4.37	17.20
6	48	55.04	1.80	5.32	11.69
Mean		53.67	1.66	5.00	14.81

[a] Mean total recovery is 75.9 and 75.8 for the 24 and 48 hour groups, respectively; this is likely a reflection of the inherent inaccuracy of the carcass data (see text).

Table 2

Comparison of Total Carbon-14 Content[a] of Red Blood Cells and Plasma After a Single Oral Dose of FC-143-^{14}C to Rats (Mean Dose, 11.0 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	9.16	46.34	0.20
2	24	14.80	60.68	0.24
3	24	14.45	48.54	0.30
Mean				0.25
4	48	10.20	50.66	0.20
5	48	12.69	42.76	0.30
6	48	7.50	43.72	0.17
Mean				0.22

[a] Data are expressed as µg equivalents of FC-143-^{14}C/g.

Table 3

Concentration of Carbon-14[a] in Rat Plasma
After a Single Oral Dose of FC-143-^{14}C (Mean Dose, 11.4 mg/kg)

Hours Post Dose	Rat[a] A	B	C	Average ± SD
1	27.75[b]	30.04	24.12	27.30 ± 2.98
2	68.41	48.40	41.58	52.80 ± 13.94
6	64.67	58.29	64.62	62.53 ± 3.67
12	63.86	59.39	77.10	66.78 ± 9.21
24	49.89	64.53	53.66	56.03 ± 7.61
48	54.63	45.74	----[c]	50.19 ± 6.29
96	34.91	42.42	29.73	35.69 ± 6.38
144	29.87	23.79	29.51	27.72 ± 3.41

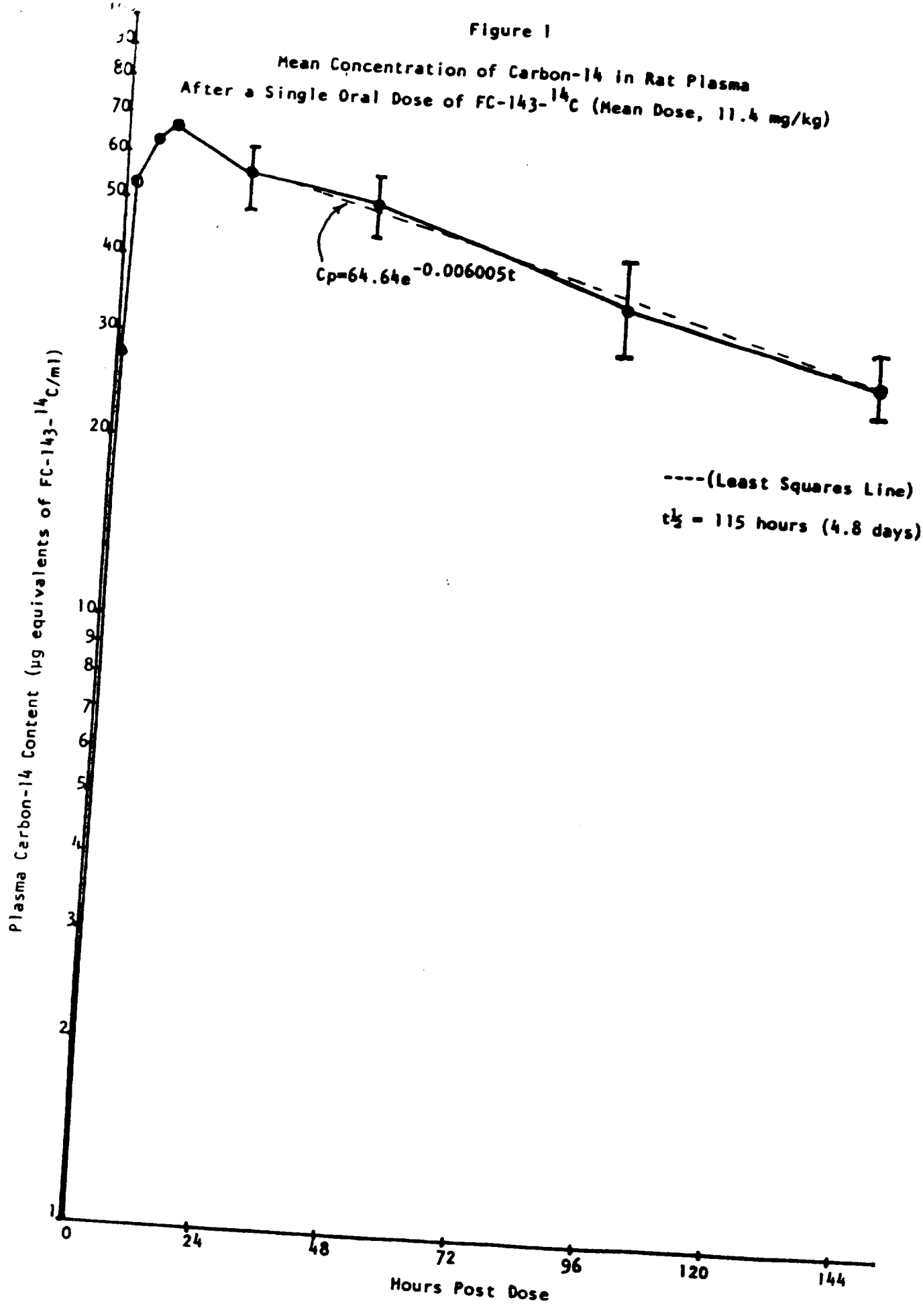
[a] Data were obtained by direct counting; data are expressed as µg equivalents of FC-143-^{14}C/ml of plasma.

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

[c] Sample was very hemolized; data for this sample (obtained by direct counting) was not used.

Figure 1

Mean Concentration of Carbon-14 in Rat Plasma
After a Single Oral Dose of FC-143-^{14}C (Mean Dose, 11.4 mg/kg)



Appendix I

Determination of Carbon-14 Content of Dosing Solution

Just prior to dosing of rats, the FC-143-^{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-143-^{14}C already determined[c], the µCi/2.0 ml was calculated. The data are shown in Appendix Table I. The dose administered each rat was 2.0 ml of solution containing 2.100 µCi of FC-143-^{14}C which is 4.1412 mg of FC-143-^{14}C. This is a mean dose of 11.4 mg/kg for the whole group of rats and 11.0 mg/kg for the 24 and 48 hour groups of rats.

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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 = 0.5071 µCi/mg. (Specific activity determination to be reported separately.)

Appendix

Table I

Carbon-14 Content of Dosing Solution

10 µl Aliquot µCi/ 1ml	50 µl Aliquot µCi/ 1ml
1.0393	1.0804
1.0449	1.0365
1.0598	1.0328
1.0427	1.0655
1.0709	1.0447
1.0473	1.0348

Overall average = 2.100 µCi/2.0 ml dose

$\frac{2.100\ \mu Ci}{0.5071 \mu Ci/mg}$ = 4.1412 mg FC-143-^{14}C/2.0 ml

Appendix 2

Recovery of Total Carbon-14 From Blank Biological Samples Spiked with FC-143-^{14}C

Good recovery of total carbon-14 from combusted FC-807-^{14}C and FC-95-^{14}C has been reported (2, 3). In these labeled molecules, the carbon-14 label is α to the sulfur atom and is fluorinated. For FC-143-^{14}C, the carbonyl carbon is labeled, and the carbon-14 atom should be readily released by combustion. Thus, in view of the good recovery of total carbon-14 observed for FC-807-^{14}C and FC-95-^{14}C, it was anticipated that good recovery of total carbon-14 from combusted FC-143-^{14}C would be realized.

For the set of samples four replicates of 10 µl, 50 µl, and 100 µl of diluted FC-143-^{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 and 100 µl were aliquoted directly into combustion cones which already contained blank biological material (fecal homogenates or plasma). 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-143-^{14}C had been added directly. One each of the 10 µl, 50 µl, and 100 µl FC-143-^{14}C spiked pellets were 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 are shown in Appendix Table 2. The mean recovery is 97.3%. Thus, combustion is a suitable means of preparing samples containing FC-143-^{14}C for counting in a liquid scintillation counter.

Appendix

Table 2

Recovery of Total Carbon-14 From Fecal Homogenate of Plasma Samples Spiked with FC-143-^{14}C

Direct Addition

10 µl[a]	50 µl	100 µl
1244[b]	6171	12284
1159	6088	12233
1127	5982	12132
1172	6155	12158
$\overline{1176} \pm 49$	$\overline{6099} \pm 86$	$\overline{12202} \pm 70$

Spiked and Oxidized

10 µl	50 µl	100 µl
1149	5969	11689
1195	5964	11816
1197	5572	11798
1164	5734	11793
$\overline{1176} \pm 24$	$\overline{5809} \pm 193$	$\overline{11774} \pm 58$

$\frac{1176}{1176} \times 100 = 100\%$ $\quad \frac{5809}{6099} \times 100 = 95.3$ $\quad \frac{11774}{12201} \times 100 = 96.5$

Overall Average = 97.3

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[a] Amount of FC-143-^{14}C spiking solution added.

[b] Data are expressed as dpm.

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