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Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Male and Female Rats After a Single IV Dose of FC-143-^{14}C

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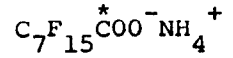
Summary

A very extensive sex difference in extent and rate of excretion of total carbon-14 was observed between male and female rats after a single iv dose (mean dose: female, 16.7 mg/kg; male 13.1 mg/kg) of FC-143-^{14}C. Female rats excreted essentially all of the dose via urine in 24 hours while at the same time period male rats excreted only 20 percent of the dose; male rats excreted 83% via urine and 5.4% via feces by 36 days post dose. No radioactivity was detected in tissues of female rats at 17 days post dose; male rats had 2.8% of the dose in liver and 1.1% in plasma at 36 days post dose with smaller levels (< 0.5% of the dose) in other organs. These sex differences are consistent with previous observations of differences in toxicity and tissue concentrations in a 90 day toxicity study.

Introduction



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. Information on FC-143 absorption, elimination from plasma, and excretion was necessary to form a basis for design of these experiments. A sex difference has been observed in the concentration of FC-143 in liver and plasma after repeated oral dosing of rats (1). A series of experiments has been planned to investigate these sex differences by comparing the route and extent of total carbon-14 elimination in male and female rats. These FC-143-^{14}C intravenous experiments for male (FC-Experiment 6) and female (FC-Experiment 7) rats, which were designed to provide data on the route and extent of total carbon-14 excretion, were paired with an FC-143-^{14}C oral dosing experiment (FC-Experiment 5), which was designed to provide data on total carbon-14 absorption and elimination from plasma (3). Urine and feces will be saved for possible study of metabolites.

Methods

Radiolabeled FC-143-^{14}C

The carbon-14 label is at the carbonyl carbon atom (see above structure). The specific activity of this lot of FC-143-^{14}C (Riker Isotope Inventory Number 459) is 0.51 μCi/mg. Details of specific activity, chemical characterization, and radiochemical purity have been reported separately (4); the FC-143-^{14}C was found to be suitable for metabolism studies.

Animals

FC-Experiment 6:

Six male Charles River[a] CD rats, ten weeks old and sexually mature, were conditioned to individual metal metabolism cages for 24 hours prior to dosing. The body weights ranged from 301 to 328 g (mean 317 g). The rats were allowed free access to Purina[b] ground chow and water before and immediately after dosing.

FC-Experiment 7:

Five female Charles River[a] CD rats, ten weeks old, and sexually mature, were conditioned to individual metal metabolism cages for 24 hours prior to dosing. The body weights ranged from 237 to 269 g (mean 248 g). The rats were allowed free access to Purina[b] ground chow and water before and immediately after dosing.

Dosing

Each rat was weighed, lightly anesthetized with diethyl ether, and then given a single iv dose (via tail vein) of FC-143-^{14}C. The dose was 2.0 ml of a 0.9% NaCl solution containing 4.14 mg FC-143-^{14}C/2.0 ml. The average dose for males (FC-Experiment 6) was 13.1 mg/kg. The average dose for females (FC-Experiment 7) was 16.7 mg/kg. The dose was delivered with a 3.0 cc disposable plastic syringe (Monoject®)[c] fitted with a 26 gauge 1/2" needle. 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 Collection

Urine and feces were collected at intervals (see Tables 1 through 4) for each of the female rats for 17 days and for each of the male rats for 36 days. At the time of sacrifice, rats were anesthetized with diethyl ether. Blood was drawn from the descending aorta and immediately transferred to a heparinized tube, and plasma was prepared promptly by centrifugation. The rats were then killed by exsanguination; and spleen, liver, brain, kidneys, and lung were collected. Bone marrow was obtained from the four major bones of the rear legs by splitting the bone and collecting the marrow on pieces of a tared combustion pad.[d] The total remaining carcass and samples of skin, thigh muscle, and subcutaneous and abdominal fat were collected.

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

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

[c] Sherwood Medical Industries, Inc., Deland, Florida 32720.

[d] Packard Instrument Co., Inc., 2200 Warrenville Road, Downers Grove, Illinois.

Sample Analysis for Carbon-14

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 (w/w) to one part of biological material. The homogenates were weighed into combustion cones in duplicate 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 were measured into combustion cones by weight. Samples of bone marrow, skin, and fat were weighed in combustion cones without homogenization. Care was taken to weigh these small samples within 1-2 minutes after removal in order to avoid relatively significant loss of weight by drying.

Homogenates, tissue samples weighed directly, and weighed samples of plasma, red blood cells, and urine were combusted with a Packard Model 306 Oxidizer. (Urine was combusted for comparison to the direct counting method, see below.) Recovery of carbon-14 from biological samples was determined by combusting suitable blank homogenates (feces and liver) spiked with dilutions of FC-143-^{14}C dosing solution at the beginning, middle, and end of the experimental sample set (see Appendix 2). 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 (see Appendix 3). All samples were cooled to refrigerator temperature in the dark before counting. All radiometric analyses were done using a Packard Model 3385 Tri-Carb Liquid Scintillation Spectrometer. 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.

Results and Discussion

The results of analyses of urine and feces are shown in Tables 1 and 2 (female rats, FC-Experiment 7) and in Tables 3 and 4 (male rats, FC-Experiment 6). The data are expressed as percent of dose excreted versus time in Figure 1. As shown in Table 1 and Figure 1, female rats excrete nearly all of the administered carbon-14 via urine within 24 hours after a single iv dose. In contrast, male rats, as shown in Table 3 and Figure 1, excrete only 83% of the administered dose via urine by 36 days. This unusual sex difference

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

in rate and extent of excretion is in agreement with sex differences suggested by discoloration of liver in a 90 day subacute toxicity study (2) and in concentration of unchanged FC-143 observed in liver after a 90 day study with administration of FC-143 in feed (1). The urine samples are stored frozen for possible study of metabolites as related to sex difference in excretion.

The total carbon-14 excretion via feces is shown in Table 2 for female rats (FC-Experiment 7) and in Table 4 and Figure 1 for male rats (FC-Experiment 6). On the average, only 1-2% of the dose was excreted by female rats in feces by three days post dose. Since the major portion of this small amount of total carbon-14 was collected during the time when >90% of the dose was being excreted via urine, it is likely that some of the total carbon-14 found in feces is a result of contamination by urine (especially rat number 5, 0-0.5 days, Table 2). Male rats (FC-Experiment 6) excreted $\approx$ 5.4 percent of the dose via feces by 36 days postdose. In male rats, the ratio of total urine carbon-14 excretion to total fecal carbon-14 excretion is 15.4: 1.

The results of the tissue analyses of male rats (FC-Experiment 6) are shown in Table 5. Mean tissue concentrations above 1 µg FC-143-^{14}C equivalents/g were as follows: liver, 8.0; plasma, 3.2; and kidney, 2.3. Other tissues such as lung, red blood cells, skin, spleen, bone marrow, fat, muscle, and brain had concentrations ranging from 0.1 to 0.8 µg/g. There was a difference in mean concentration observed in subcutaneous fat (0.28 µg/g) as compared to abdominal fat ($\leq$ 0.12 ug/g). The same tissues were analyzed for female rats (FC-Experiment 7) at 17 days post FC-143-^{14}C dose. No carbon-14 was detectable in any tissue ($\leq$ 0.05 ug/g); thus, the female rat appears to effectively eliminate all FC-143 and/or metabolites from the body by 17 days. The percent of dose present in whole organs in male rats (FC-Experiment 6) at 36 days post FC-143-^{14}C dose is listed in Table 6. Only liver (2.8%) and plasma (1.1%) appear to contain a significant portion of the dose.

References

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3. Johnson JD: Absorption of FC-143-^{14}C in Rats After a Single Oral Dose (Report) December 4, 1979.

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6. 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 28, 1979.

7. Altman PL, Dittmer DS: Blood and Other Body Fluids. Bethesda, Maryland, Federation of American Societies for Experimental Biology, 1979, p 5.

List of Tables and Figures

Table 1

Excretion of Total Carbon-14 in Urine[a] in Female Rats (FC-Experiment 7) After a Single Intravenous Dose of FC-143-^{14}C (Mean Dose, 16.7 mg/kg)

Collection Period (days)	Rat Number 1	2	3	4	5	Mean ± S.D.
0-0.5	94.92	94.17	95.76	101.65	85.32	94.36 ± 5.86
0.5-1	8.16	9.85	3.46	6.59	7.66	7.14 ± 2.37
1-2	0.86	1.05	0.50	0.99	0.79	0.84 ± 0.22
2-3	0.11	0.21	0.17	0.15	0.24	0.18 ± 0.05
3-4	0.04	0.11	0.12	0.07	0.10	0.09 ± 0.03
4-5	0.03	0.10	0.04	0.08	0.07	0.06 ± 0.03
5-6	0.02	0.05	0.04	0.08	0.04	0.05 ± 0.02
6-7	0.01	0.04	0.05	0.02	0.04	0.03 ± 0.02
7-9	b	0.06	0.04	0.07	0.09	0.05
9-10	b	0.02	0.01	0.02	0.08	0.03
10-12	b	0.03	0.03	0.02	0.54	0.16
12-14	b	0.04	0.02	0.01	0.37	0.09
14-16	b	b	0.05	b	0.09	0.04
16-17	b	0.014	0.02	b	0.05	0.02
TOTAL	104.0	105.7	100.3	109.8	95.5	103.1 ± 5.4

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

b Radioactive content too low to measure (≤ 20 dpm/ml).

Table 2

Excretion of Total Carbon-14 in Feces[a] in Female Rats (FC-Experiment 7) After a Single Intravenous Dose of FC-143-^{14}C (Mean Dose, 16.7 mg/kg)

Collection Period (days)	Rat Number					
	1	2	3	4	5	Mean ± S.D.
0-0.5	0.59	1.11	0.75	0.06	3.09	1.12 ± 1.16
0.5-1	0.35	0.51	0.20	0.44	0.06	0.31 ± 0.18
1-2	[b]	0.05	[b]	0.12	0.08	0.05
2-3	[b]	[b]	[b]	[b]	0.05	0.02
TOTAL	0.94	1.67	0.95	0.62	3.28	1.5 ± 1.1

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

[b] Radioactive content too low to measure (≤ 20 dpm/ml).

Table 3

Excretion of Total Carbon-14 in Urine [a] in Male Rats (FC-Experiment 6) After a Single Intravenous Dose of FC-143-^{14}C (Mean Dose, 13.1 mg/kg)

Collection Period (days)	Rat Number						
	1	2	3	4	5	6	Mean ± S.D.
0.05	13.45	21.87	8.30	10.42	12.01	7.98	12.34 ± 5.12
0.5-1	8.16	12.42	5.27	6.18	7.27	5.95	7.54 ± 2.60
1-2	11.77	13.43	8.44	9.06	11.95	8.24	10.48 ± 2.18
2-3	8.52	6.19	5.24	5.59	7.28	5.72	6.42 ± 1.25
3-4	5.23	3.82	3.88	3.95	5.61	4.71	4.53 ± 0.77
4-5	5.47	3.32	3.39	4.18	3.58	4.25	4.03 ± 0.81
5-6	5.12	2.63	3.12	3.53	4.01	3.06	3.58 ± 0.89
6-7	4.50	2.60	2.79	3.42	3.68	3.33	3.39 ± 0.68
7-9	6.30	3.29	5.14	6.28	5.28	5.42	5.29 ± 1.10
9-10	2.66	2.64	2.93	2.29	2.34	2.67	2.59 ± 0.24
10-12	3.51	3.17	4.35	4.02	3.60	3.53	3.70 ± 0.42
12-14	2.83	3.11	4.15	3.03	2.79	1.88	2.97 ± 0.73
14-16	2.32	2.43	3.66	3.43	2.65	3.42	2.99 ± 0.58
16-18	1.79	1.94	3.21	2.70	2.06	3.89	2.60 ± 0.83
18-22	2.10	1.87	4.76	3.74	2.95	5.52	3.49 ± 1.46
22-26	1.28	1.54	3.69	4.23	2.32	4.34	2.90 ± 1.36
26-30	1.01	0.93	3.06	2.66	1.39	3.01	2.01 ± 1.01
30-34	0.69	0.77	2.37	2.26	1.17	2.04	1.55 ± 0.76
34-36	0.52	0.20	0.77	0.72	0.50	0.86	0.60 ± 0.24
TOTAL	87.2	88.2	78.5	81.7	82.4	79.8	83.0 ± 3.9

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

Table 4

Excretion of Total Carbon-14 in Feces[a] in Male Rats (FC-Experiment 6) After a Single Intravenous Dose of FC-143-^{14}C (Mean Dose, 13.1 mg/kg)

Collection Period (days)	Rat Number						
	1	2	3	4	5	6	Mean
0-0.5	0.37	0.86	0.24	0.33	0.42	0.29	0.42 ± 0.23
0.5-1	0.28	0.43	0.36	0.28	0.36	0.34	0.34 ± 0.06
1-2	0.72	0.91	0.69	0.41	0.64	0.66	0.67 ± 0.16
2-3	0.38	0.53	0.48	0.29	0.54	0.36	0.43 ± 0.10
3-4	0.25	0.22	0.31	0.24	0.35	0.51	0.31 ± 0.11
4-5	0.28	0.19	0.42	0.19	0.25	0.40	0.29 ± 0.10
5-6	0.28	0.18	0.34	0.26	0.22	1.02	0.38 ± 0.32
6-7	0.29	0.15	0.25	0.18	.20	0.19	0.21 ± 0.05
7-9	0.34	0.42	0.69	0.37	0.41	0.38	0.44 ± 0.13
9-10	0.17	0.25	0.18	0.16	0.11	0.16	0.17 ± 0.05
10-12	0.17	0.38	0.43	0.29	0.23	0.29	0.30 ± 0.10
12-14	0.30	0.16	0.28	0.35	0.23	0.13	0.24 ± 0.08
14-16	0.17	0.15	0.32	0.19	0.15	0.15	0.19 ± 0.07
16-18	0.19	0.11	0.25	0.18	0.20	0.27	0.20 ± 0.06
18-22	[b]	0.24	0.55	0.27	0.16	0.51	0.29
22-26	[b]	0.08	0.35	0.22	0.23	0.29	0.20
26-30	[b]	[b]	0.23	0.20	[b]	0.23	0.11
30-34	[b]	[b]	0.23	0.21	[b]	0.20	0.11
34-36	[b]	[b]	0.07	0.09	[b]	0.08	0.04
TOTAL	4.2	5.3	6.7	4.7	4.7	6.5	5.4 ± 1.0

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

[b] Radioactive content too low to measure (≤ 20 dpm/100 mg).

Table 5

Tissue Distribution[a] of Total Carbon-14 in Male Rats (FC-Experiment 6) After a Single Intravenous Dose of FC-143-^{14}C (Mean Dose 13.1 mg/kg)

Tissue	Rat Number 1	2	3	4	5	6	Mean ± S.D.
Liver	2.99	5.76	14.04	11.06	5.80	8.16	7.97 ± 4.02
Plasma	1.07	1.44	4.75	4.57	2.56	4.76	3.19 ± 1.72
Kidney	1.87	0.80	3.96	3.13	1.62	2.58	2.33 ± 1.13
Lung	0.19	0.30	1.28	1.27	0.52	1.17	0.79 ± 0.51
Red Blood Cells	0.27	0.21	1.37	1.06	0.86	0.85	0.77 ± 0.45
Skin	0.09	0.13	0.69	0.76	0.28	0.46	0.40 ± 0.28
Spleen	0.11	0.14	0.48	0.43	0.27	0.56	0.33 ± 0.19
Bone Marrow	0.07	0.13	0.69	0.50	0.28	[b]	0.33 ± 0.26
Subcutaneous Fat	0.06	0.17	0.49	0.36	0.13	0.46	0.28 ± 0.18
Muscle	0.11	0.11	0.32	0.23	0.15	0.28	0.20 ± 0.09
Brain	0.06	0.09	0.11	0.13	0.10	0.18	0.11 ± 0.04
Abdominal Fat	[c]	[c]	0.26	0.17	[c]	0.16	≤ 0.12

[a] Total carbon-14 concentration is expressed as µg equivalents of FC-143-^{14}C/g.

[b] Malfunction of oxidizer; sample was lost.

[c] Total carbon-14 concentration too low to quantitate (≤0.05 µg/g).

Table 6

Percent of Dose Present in Male Rat Tissues (FC-Experiment 6) at 36 Days After a Single Intravenous Dose of FC-143-^{14}C (Mean Dose, 13.1 mg/kg)

Tissue	Rat Number 1	2	3	4	5	6	Mean ± S.D.
Liver[a]	1.16	1.91	4.69	3.45	2.18	3.09	2.75 ± 1.26
Plasma[b]	0.39	0.50	1.68	1.51	0.90	1.75	1.12 ± 0.60
Red Blood Cells[c]	0.08	0.06	0.41	0.29	0.25	0.26	0.23 ± 0.13
Kidney[a]	0.13	0.05	0.24	0.20	0.11	0.18	0.15 ± 0.07
Lung[a]	0.01	0.02	0.05	0.05	0.02	0.06	0.04 ± 0.21
Spleen[a]	0.002	0.003	0.008	0.007	0.004	0.008	0.005 ± 0.003
Brain[a]	0.003	0.004	0.005	0.004	0.004	0.008	0.005 ± 0.002

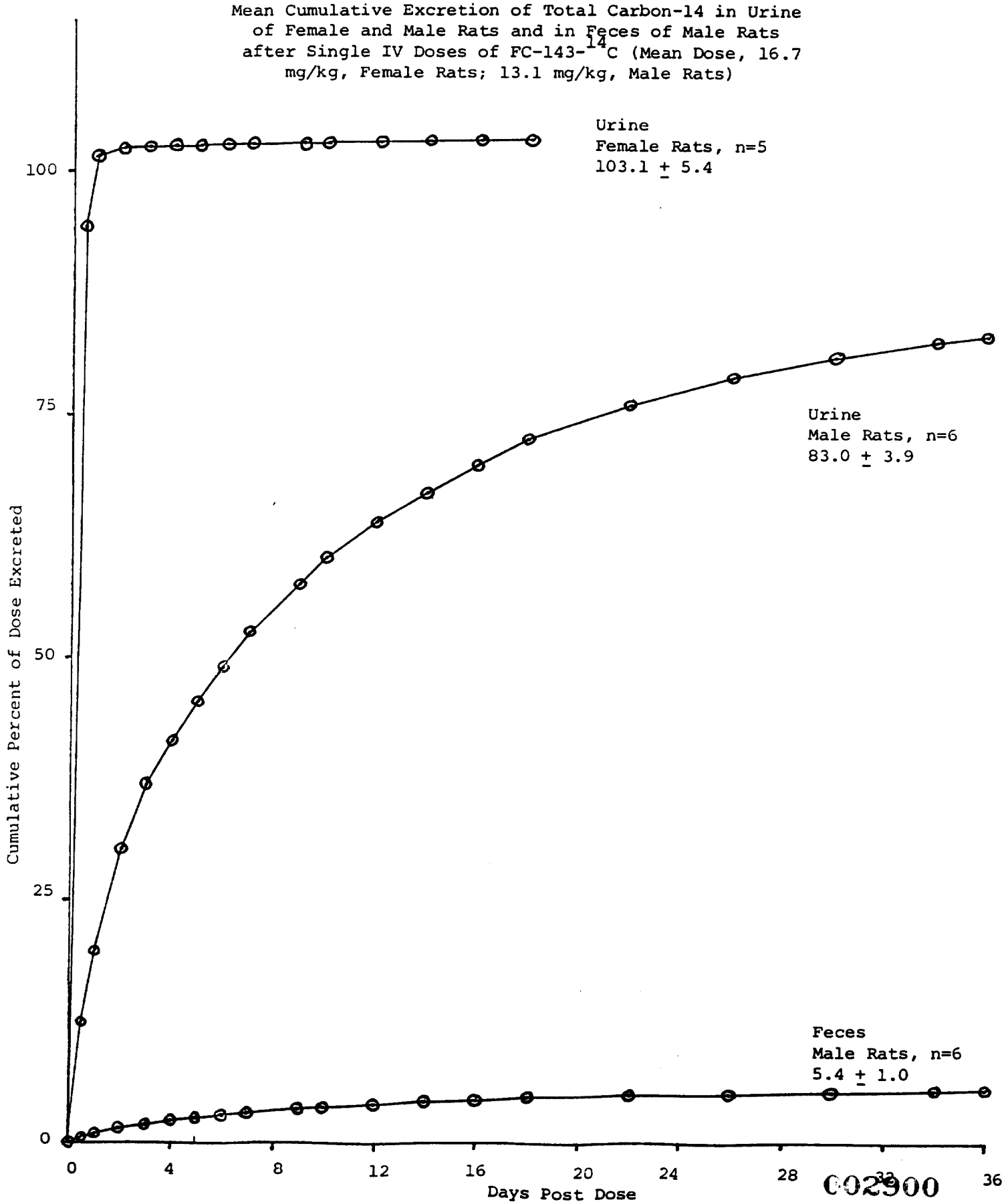
[a] Data expressed as percent of dose present in whole organ.

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

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

Figure 1

Mean Cumulative Excretion of Total Carbon-14 in Urine of Female and Male Rats and in Feces of Male Rats after Single IV Doses of FC-143-^{14}C (Mean Dose, 16.7 mg/kg, Female Rats; 13.1 mg/kg, Male Rats)



Appendix 1

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 1. The dose administered each rat was 2.0 ml of solution containing 2.10 µCi of FC-143-^{14}C which is 4.14 mg of FC-143-^{14}C. This is a mean dose of 16.7 mg/kg for the female rats and 13.1 mg/kg for the male rats.

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

[b] Aquasol® was found to be a suitable solvent-scintillant for FC-143-^{14}C during specific activity determination (4).

[c] Specific activity = 0.507 µCi/mg (4).

Appendix

Table 1

Carbon-14 Content of Dosing Solution

10 µl Aliquot µCi/1.0 ml	50 µl Aliquot µCi/1.0 ml
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 = 1.050 uCi/ml

$$\frac{1.050\ \mu Ci/ml}{0.507\ \mu Ci/mg} = 2.07\ mg\ FC\text{-}143\text{-}^{14}C/ml$$

$$= 4.14\ mg/2.0\ ml\ dose$$

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 (5,6). In these labeled molecules, the carbon-14 atom is α to the sulfer atom and is fluorinated. For FC-143-^{14}C, the carbonyl carbon atom is labeled. 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.

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 or 100 µl were aliquoted directly into combustion cones which already contained blank biological material (fecal or liver 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-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. The fourth set was also combusted at the end. 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-143-^{14}C) are shown in Appendix Table 2. The mean recovery is 97.4 ± 1.6%. Each entry in the table is the mean of at least four determinations; this mean recovery is based on 46 determinations. Recovery of total carbon-14 from spiked liver homogenates (shown in the bottom half of Appendix Table 2) was 97.3 ± 1.8% (based on nine determinations). Thus, good recovery of total carbon-14 from FC-143-^{14}C spiked biological material is shown. No corrections for percent recovery were made.

Appendix

Table 2

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

Recovery of Total Carbon-14 From Blank Fecal Homogenate Samples Spiked With FC-143-^{14}C		
10 µl[a]	50 µl	100 µl
97.6[b], [c]	97.8	98.3
100.3	95.3	96.8
98.5	95.1	96.5
98.8	96.1	97.2

Overall Average = 97.4 ± 1.6

Recovery of Total Carbon-14 From Blank Liver Homogenate Samples Spiked With FC-143-^{14}C		
10 µl[a]	50 µl	100 µl
99.3[b], [d]	96.3	96.2

Overall Average = 97.3 ± 1.8

[a] Amount of 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 at least 4 combustion recovery determinations.

[d] Each entry is the mean of 3 combustion recovery determinations.

Appendix 3

Comparison of Carbon-14 Analysis of Urine Samples by Oxidation Versus Direct Counting in Aquasol®

The urine carbon-14 excretion data reported and discussed in this report are based on direct counting in Aquasol®. However, there is a concern that an insoluble metabolite or complex might exist in urine so that not all the radioactivity is counted. To investigate this possibility, concentration data obtained from combustion were compared to concentration data obtained from direct counting of the same urine samples. The data are shown in Table 3. The mean ratio of the results from the comparison (oxidized/direct counting) is 0.88. Thus, direct counting in Aquasol® is a valid method to assay total carbon-14 in these urine samples.

Appendix

Table 3

Comparison of Carbon-14 Analysis of Urine Samples by Oxidation Versus Direct Counting in Aquasol®

Sample Number	Direct Counting (µg/ml)[a]	Oxidized (µg/g)[b]	Ratio Oxidized/Direct Counting
1	38.82[c]	34.64	0.89
2	18.57	16.77	0.90
3	15.68	14.01	0.89
4	26.02	22.94	0.88
5	29.93	26.03	0.87
6	28.98	24.69	0.85
		Mean	0.88

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

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

[c] Each entry is the mean of 3 determinations.