~~OCCA~~

File

Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single Intravenous Dose of FC-95-^{14}C

December 28, 1979

Conducted at: Riker Laboratories, Inc.
Subsidiary of 3M
St. Paul, Minnesota 55101

During: June to October, 1979

Conducted by: S. J. Gibson and J. D. Johnson

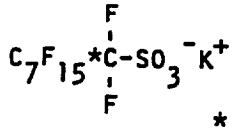
Report by: James D. Johnson 1/16/80
James D. Johnson, M.S. Date
Senior Biochemical Pharmacologist

Reviewed by: Robert E. Ober 1/16/80
Robert E. Ober, Ph.D. Date
Manager, Drug Metabolism

Summary

By 89 days after a single iv dose of FC-95-^{14}C (mean dose, 4.2 mg/kg) six male rats excreted a mean of 30.2% of the total carbon-14 via urine. Mean cumulative fecal excretion was 12.6%. At 89 days, mean tissue concentration of total carbon-14 expressed as µg FC-95-^{14}C equivalents/g were: liver, 20.6; plasma, 2.2; kidney, 1.1; lung, 1.1; spleen, 0.5; and bone marrow, 0.5. Lower concentrations ($\leq$ 0.5) were measured in adrenals, skin, testes, muscle, fat, and eye. No radioactivity (< 0.05) was detected in brain. The carbon-14 in liver and plasma represents 25 and 3 percent of the dose, respectively.

Introduction



* 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 intravenous experiment (FC-Experiment 3) which was designed to provide data on the route and extent of total carbon-14 excretion was paired with an FC-95-^{14}C oral dosing experiment which was designed to provide data on total carbon-14 absorption and elimination from plasma. (2) 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 (3) 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 synthesis, specific activity determination, and radiochemical purity determination have been reported. (1)

Animals

Six 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 262 to 303 g, mean 288 g. The rats were allowed free access to Purina[b] Ground Chow and water before and after dosing.

Dosing

Each rat was weighed, anesthetized with diethyl ether, and then given a single iv dose (via tail vein) 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 3.0 cc disposable plastic syringe (Monoject®[c]) fitted with a 26 gauge 1/2" needle. The

---

[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.

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).

Sample Collection

Urine and feces were collected at intervals (see Tables 1 and 2) for each of the six rats for 89 days. At 89 days post dose, the rats were anesthetized with diethyl ether; blood was drawn from the descending aorta and immediately transferred to a heparinized tube. Plasma was prepared promptly by centrifugation. The rats were sacrificed by exsanguination; and spleen, liver, brain, testes, adrenals, kidneys, lungs, and eyes were collected as whole organs. 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[a]. Samples of skin, thigh muscles, subcutaneous and abdominal fat, and the total remaining carcass were collected.

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 cone[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 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 were measured into combustion cones by weight. Whole eyes, adrenals, and samples of bone marrow, skin and fat were also weighed in combustion cones. Care was taken to weigh these samples promptly (1-2 minutes) after removal in order to avoid 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. Recovery of carbon-14 from biological samples was determined by combusting suitable blank homogenates (feces, liver, muscle, and spleen) and blank rat plasma spiked with dilutions of FC-95-^{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; triplicate 1.0 ml aliquots of each sample were pipetted directly into scintillation vials and 15 ml Aquasol® was added. 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.

---

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

Results and Discussion

The results of the fecal and urinary analyses are shown in Tables 1 and 2 and in Figures 1 and 2. Tables 1 and 2 list the total carbon-14 expressed as percent of dose. The data are expressed as cumulative percent of dose excreted versus time in Figures 1 and 2. As illustrated in Figures 1 and 2, excretion of total carbon-14 is slow. By 89 days after dosing, total excretion (urine + feces) is only 43 percent of the dose. Radioactive content in feces was too low to measure after 64 days; total carbon-14 excreted is thus the sum of fecal excretion (0 to 64 days) and urinary excretion (0 to 89 days). The mean ratio of cumulative excretion of total carbon-14 in urine (30.2%) to that in feces (12.6%) was 2.4 ± 0.2. This is in contrast to the ratio of cumulative excretion of total carbon-14 in urine to that in feces ($\leq$ 0.1) at 124 days post dose reported after a single iv dose of FC-807-^{14}C. (3) This indicates that FC-807-^{14}C is not likely extensively biotransformed to FC-95-^{14}C. Since 50-60 percent of the FC-95-^{14}C dose was not excreted by either feces or urine at 89 days post dose, selected tissues were analyzed for carbon-14 content to identify those tissues in which total carbon-14 might be retained.

The results of the tissue analyses are shown in Table 3. Mean tissue carbon-14 concentrations above one µg FC-95-^{14}C equivalents/g were as follows: liver, 20.6; plasma, 2.2; kidney, 1.1; and lung, 1.1. Other tissues such as muscle, skin, bone marrow, and spleen had concentrations ranging from 0.2 to 0.6 µg/g. There was a difference in carbon-14 content of subcutaneous fat (0.2 µg/g) and abdominal fat ($\leq$ 0.08µg/g). Very little total carbon-14 was found in whole eye (0.16 µg/g) and no detectable total carbon-14 was found in brain. There is a striking difference in the pattern of total carbon-14 retention when liver, bone marrow, and spleen after iv dosing with FC-95-^{14}C is compared to the pattern of retention found for these tissues after dosing with FC-807-^{14}C. (3) The mean carbon-14 content for FC-807-^{14}C dosed rats normalized to liver content are: liver, 1.0; spleen, 8.9; and bone marrow, 2.4. For FC-95-^{14}C dosed rats, the mean ratios are liver, 1.0; spleen, 0.02; and bone marrow, 0.02. Since the spleen/liver ratio following FC-95-^{14}C is 8.9 and bone marrow/liver is 2.4, this data supports the conclusion that FC-807 is not extensively or rapidly biotransformed to FC-95.

The total carbon-14 contents expressed as percent of dose for selected whole organs and plasma and red blood cells are listed in Table 4. Only liver and plasma contain a substantial percentage of the dose at 89 days post dose. The low levels of radioactivity found for kidney, lung, testes, and spleen, are (in view of the relatively high carbon-14 content of plasma and red blood cells) due in part to the blood still contained in these organs when homogenized and analyzed.

References

(1) Johnson JD, Behr FE: Synthesis and Characterization of FC-95-^{14}C (Report), November 2, 1979.

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

(3) 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.

(4) Altman PL, Dittmer DS: Blood and Other Body Fluids. Bethesda, Maryland, Federation of American Societies for Experimental Biology, 1971, p. 5.

List of Tables and Figures

Table 1

Excretion of Total Carbon-14 in Urine in Rats after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg)

Collection Period (days)	Rat Number 1	2	3	4	5	6	Mean
0-0.5	1.09[a]	0.69	1.03	0.77	1.03	0.86	0.91
0.5-1	0.70	0.90	0.72	0.60	0.77	0.94	0.77
1-2	1.56	1.11	1.47	0.78	1.08	1.27	1.21
2-3	1.31	1.05	1.14	0.68	0.91	1.11	1.03
3-4	1.16	0.87	1.06	0.68	0.93	0.85	0.93
4-5	1.09	0.80	0.81	0.60	0.80	0.90	0.83
5-6	0.86	0.72	0.67	0.49	0.76	0.77	0.71
6-7	1.07	0.71	0.75	0.52	0.74	0.75	0.76
7-8	0.90	0.69	0.85	0.54	0.76	0.74	0.75
8-9	0.86	0.57	0.70	0.60	0.68	0.67	0.68
9-10	0.82	0.66	0.79	0.59	0.48	0.74	0.68
10-11	0.60	0.36	0.65	0.64	0.63	0.64	0.59
11-12	0.62	0.52	0.54	0.69	0.55	0.57	0.58
12-13	0.56	0.53	0.63	0.60	0.60	0.59	0.59
13-14	0.55	0.53	0.51	0.58	0.57	0.55	0.55
14-15	0.65	0.51	0.46	0.57	0.55	0.51	0.54
15-16	0.54	0.48	0.56	0.45	0.58	0.46	0.51
16-17	0.57	0.43	0.40	0.45	0.48	0.55	0.48
17-18	0.53	0.40	0.34	0.35	0.49	0.44	0.43
18-19	0.41	0.39	0.28	0.42	0.38	0.46	0.39
19-21	0.93	0.81	0.79	0.77	0.84	0.92	0.84
21-23	0.91	0.75	0.69	0.77	0.66	0.88	0.78
23-25	0.63	0.59	0.59	0.66	0.70	0.77	0.66
25-27	0.76	0.67	0.64	0.66	0.68	0.64	0.68
27-29	0.73	0.66	0.73	0.68	0.66	0.64	0.68
29-32	1.04	0.88	0.69	0.89	0.82	0.86	0.86
32-36	1.06	1.05	1.16	1.21	1.14	0.66	1.05
36-40	0.97	1.08	0.97	1.01	1.01	0.91	0.99
40-43	0.59	0.58	0.88	0.82	0.79	0.83	0.75
43-47	0.59	0.78	1.00	0.94	0.96	1.25	0.92
47-50	0.70	0.52	0.66	0.70	0.69	0.79	0.68
50-54	0.52	0.77	0.82	0.83	0.75	0.98	0.78
54-57	0.63	0.56	0.62	0.63	0.58	0.61	0.61
57-61	0.56	0.82	0.74	0.89	0.81	0.94	0.79
61-69	1.38	1.33	1.41	1.64	1.66	1.59	1.50
69-78	1.51	1.77	1.49	1.60	1.64	1.81	1.64
78-89	1.59	2.03	1.97	2.06	2.45	2.39	2.08
TOTAL	31.6	28.6	30.2	28.4	30.6	31.8	30.2

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

Table 2

Excretion of Total Carbon-14 in Feces in Rats after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg)

Collection Period (days)	Rat Number						Mean
	1	2	3	4	5	6	
0-0.5	0.043[a]	-	0.069	0.004	0.178	-	0.049
0.5-1	0.711	0.780	0.919	0.988	0.777	0.877	0.842
1-2	0.955	0.770	0.865	0.465	0.682	1.032	0.795
2-3	0.761	0.572	0.726	0.466	0.652	0.719	0.649
3-4	0.736	0.604	0.599	0.667	0.639	0.689	0.656
4-5	0.764	0.630	0.606	0.395	0.538	0.529	0.577
5-6	0.636	0.595	0.352	0.397	0.502	0.578	0.510
6-7	0.560	0.464	0.911	0.434	0.522	0.635	0.588
7-8	0.539	0.423	0.832	0.306	0.343	0.451	0.482
8-9	0.441	0.336	0.597	0.337	0.411	0.401	0.421
9-10	0.478	0.400	0.342	0.299	0.371	0.433	0.387
10-11	0.524	0.407	0.327	0.295	0.294	0.372	0.370
11-12	0.311	0.278	0.240	0.304	0.269	0.374	0.296
12-13	0.319	0.279	0.301	0.363	0.300	0.296	0.310
13-14	0.351	0.255	0.280	0.299	0.222	0.278	0.281
14-15	0.328	0.233	0.265	0.309	0.247	0.275	0.276
15-16	0.291	0.276	0.276	0.252	0.252	0.283	0.272
16-17	0.246	0.174	0.174	0.176	0.190	0.164	0.187
17-18	0.228	0.130	0.171	0.156	0.117	0.178	0.163
18-19	0.124	0.105	0.170	0.111	0.110	0.153	0.129
19-21	0.378	0.274	0.295	0.294	0.325	0.301	0.311
21-23	0.357	0.258	0.280	0.303	0.276	0.340	0.302
23-25	0.088	0.349	0.294	0.255	0.280	0.308	0.262
25-27	0.288	0.176	0.138	0.204	0.218	0.226	0.208
27-29	0.266	0.140	0.197	0.181	0.149	0.280	0.202
29-32	0.270	0.114	0.220	0.260	0.239	0.237	0.223
32-36	0.570	0.366	0.490	0.646	0.587	0.499	0.526
36-50	1.397	1.074	1.562	1.768	1.352	2.028	1.530
50-64	1.086	0.663	0.957	1.125	0.452	0.712	0.833
TOTAL	14.0	11.1	13.5	12.1	11.5	13.6	12.6

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

Table 3

Tissue Distribution of Total Carbon-14 in Rats at 89 Days after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg)

	Rat Number						
	1	2	3	4	5	6	Mean
Tissue[a]	µg/g[b]	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Liver	17.17	21.46	21.22	22.25	18.99	22.27	20.56
Plasma	2.37	2.17	2.37	2.27	2.05	2.05	2.21
Kidney	1.05	0.89	1.10	1.20	1.10	1.22	1.09
Lung	0.98	0.90	1.03	1.31	1.10	1.03	1.06
Spleen	0.48	0.50	0.51	0.57	0.48	0.49	0.51
Bone Marrow	0.41	0.43	0.39	0.79	0.35	0.38	0.46
Red Blood Cells	0.43	0.59	0.58	0.37	0.37	0.34	0.45
Adrenals	0.44	0.42	0.49	0.32	0.35	0.41	0.41
Testes	0.33	0.33	0.35	0.41	0.35	0.37	0.36
Skin	0.38	0.38	0.35	0.32	0.34	0.32	0.35
Muscle	0.41	0.31	0.31	0.29	0.26	0.18	0.29
Subcutaneous Fat	0.15	0.28	0.19	0.27	0.13	0.15	0.20
Eye	0.18	0.15	0.16	0.18	0.13	0.16	0.16

[a] Brain and abdominal fat were also assayed but level of carbon-14 was too low to quantitate ($\leq$ 0.08 µg/g) in abdominal fat, and too low to detect in brain (< 0.05 µg/g).

[b] Total carbon-14 concentration is expressed as µg equivalents of FC-95-^{14}C/g.

Table 4

Percent of Dose Present in Tissues
at 89 Days after a Single Intravenous
Dose of FC-95-^{14}C (Mean Dose, 4.2 mg/kg)

Tissue	Rat Number						Mean
	1	2	3	4	5	6	
	%	%	%	%	%	%	%
Liver[a]	20.16	27.11	25.39	29.06	24.96	24.58	25.21
Plasma[b]	2.81	2.82	2.84	3.00	2.75	2.64	2.81
Red Blood Cells[c]	0.42	0.64	0.58	0.41	0.41	0.37	0.47
Kidney[a]	0.27	0.22	0.24	0.30	0.27	0.30	0.27
Lung[a]	0.15	0.11	0.12	0.17	0.13	0.11	0.13
Testes[a]	0.10	0.09	0.11	0.12	0.10	0.10	0.10
Spleen[a]	0.02	0.02	0.02	0.03	0.02	0.02	0.02

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

[b] Data is estimate of percent of dose present in plasma.
Plasma volume = 31.3 ml/kg body weight (4).

[c] Data is estimate of percent of dose present in red blood cells.
Red blood cells = 26.3 ml/kg body weight (4).

Figure 1

Cumulative Excretion of Total Carbon-14 in Urine
after a Single IV Dose of FC-95-^{14}C
(Mean Dose, 4.2 mg/kg)
Mean of 6 Rats

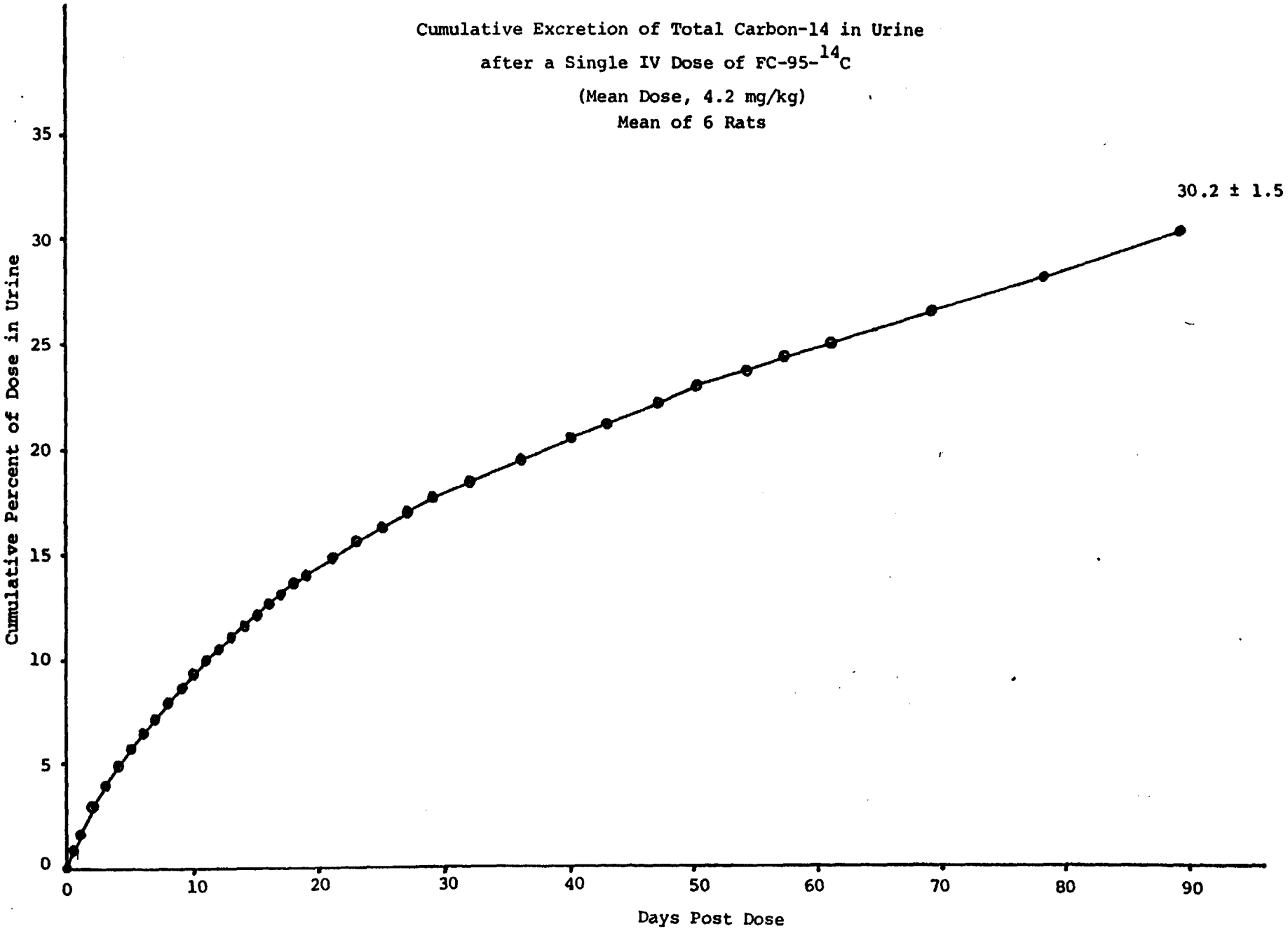
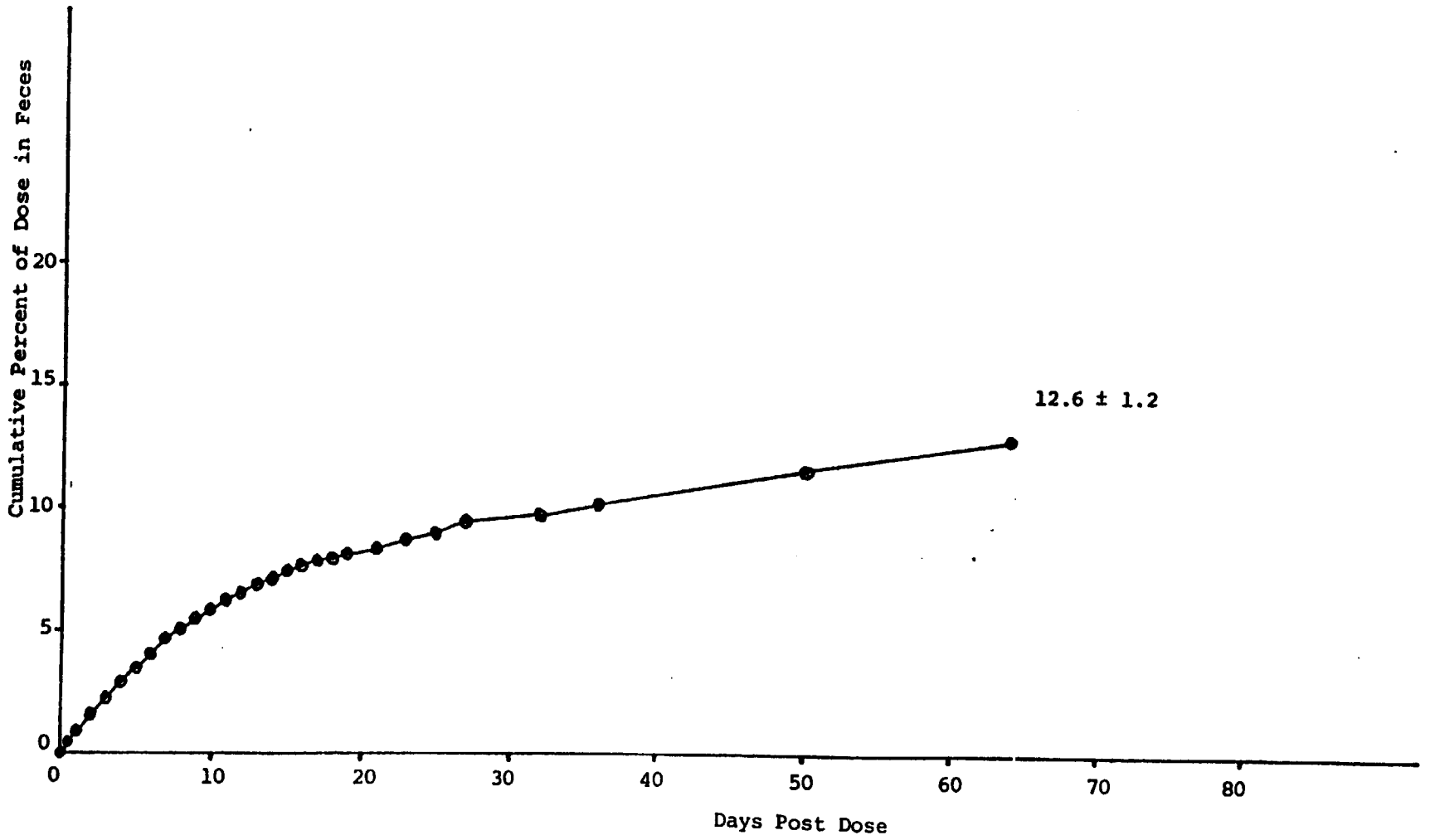


Figure 2

Cumulative Excretion of Total Carbon-14 in Feces
after a Single IV Dose of FC-95-^{14}C
(Mean Dose, 4.2 mg/kg)
Mean of 6 Rats



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, 1 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 is 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.

---

[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 (1).

[c] Specific activity = 0.459 µCi/mg. Specific activity determination has been reported (1).

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 mg FC-95-^{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. (3) 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 and 100 µl were aliquoted directly into combustion cones which already contained blank biological material (fecal, liver, muscle, or spleen 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-^{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) are shown in Appendix Table 2. 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. Recovery of total carbon-14 from spiked tissue homogenates (not shown) was also 98%. Thus, good recovery of total carbon-14 from FC-95-^{14}C spiked biological material is shown.

Appendix

Table 2

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% (mean ± S.D.)

[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 3

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

The urine carbon-14 excretion data reported in this report are based on direct counting in Aquasol®. However, there is a concern that an insoluble metabolite or complex might exist in the urine so that not all of the radioactivity in the urine is counted. To investigate this possibility, concentration data from combustion were compared to concentration data obtained from direct counting of the same urine samples. The data are shown in Appendix Table 3. The mean ratio of the results from the comparison is 0.97. Thus, direct counting in Aquasol® is a valid method to assay total carbon-14 in the 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	0.98	0.95	0.97
2	1.04	1.01	0.97
3	0.76	0.75	0.99
4	0.66	0.62	0.94
5	0.86	0.72	0.84
6	0.77	0.84	1.09
		Mean	0.97

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

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

Distribution List

M.T. Case	218-2
S.F. Chang	218-2
G.J. Conard	218-2
H.E. Freier	201-1S
F.D. Griffith	220-2E
L.I. Harrison	218-2
T.L. Kerley	218-1
J.D. LaZerte	236-1
L.J. Magill	223-6S
R.A. Nelson	218-1
R.E. Ober	218-2
J.A. Pendergrass	220-2E
R.A. Prokop	236-3B
A.L. Rosenthal	230-3
F.A. Ubel	220-2E
P. Venkateswarlu	236-3A
DMG Central File	218-2
Tech Communications	201-2CN
Central Files	218-1