DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date October 29th, 2006

From Toxicology Group 2: DFCN
William Roth, Ph.D., D.A.B.T. (HFS-275)

Subject FCN 628: Special Review of Biopersistence Screening Study (DuPont No. 4650)

To Yan Gu, Ph.D. (HFS-275)
Primary Toxicology Reviewer, FCN 628

FCN 628

Clariant Corporation
4000 Monroe Road
Charlotte, NC 28205

We have reviewed the subject biopersistence screening study in rats at the request of the primary toxicology reviewer and Toxicology management. Major conclusions from our review of this and related studies are as follow:

1. The half life of residues from H-24542 (PFOE-acrylate) in male rats is at least 21 days, and may be as long as 100 days after steady-state has been achieved.

2. The residual material is qualitatively similar in partitioning properties to those observed for the telomer alcohol H-24538 (PFOE) in earlier DuPont studies, but appears to have a higher fat:blood partitioning ratio (11.6 versus the PFOE fat:plasma ratio of 7.0). The half-life of PFOE-acrylate residues however, are much longer, as noted above. It is therefore unlikely that these residues represent hydrolysis products from PFOE-acrylate.

3. It is not possible to develop an accurate mass balance from the limited tissue and blood samples described in this 90-biopersistence study. This study contained no measurements of fluoride excretion, and no measurements for the carcass remaining after removal of blood, liver and fat samples. The partitioning into fat is large enough relative to liver and blood to allow the assumption that fat, liver and blood should account for most of the absorbed test substance. We believe that the absorption is less than 3 % of the dose, but cannot reduce the estimated absorption much below this number in the absence of data on the excretion of fluoride during the experimental period.

[redacted]
William L. Roth, Ph.D.

Clariant (DuPont) Study No. 4650

Title: [redacted] Biopersistence Screening 10 Dose Oral Gavage Study in Rats (DuPont Study No. 4650)

Testing Laboratory: Du Pont Haskell Laboratory for Health and Environmental Sciences ; Elkton Road, PO Box 50, Newark, Delaware 19714

Study Author: Carol Finlay, B.A.
Date: December 18, 2002 (Study completed on 11/20/2001)
GLP/QA: Good laboratory practice and quality assurance statements are not included; however, a signed certification is included in the study.

Location of Study: FCN 628, pages 0000942 – 0001059

Test Substance:

[redacted]
12 %, others 6 %). 95% Active, 63% fluorine (F) content in active.
Molecular Weight: 518 (C8-2 ester), 618 (C10-2 ester), 718 (C12-2 ester)

Positive Controls: Potassium perfluoroalkyl sulfonate (H-24019, PFOS, CAS #2795-39-3, 3871-99-6, 29420-49-3, 60272-25-1); [redacted] (H-24538, PFOE, CAS# 678-39-7) in deionized water

Negative Control: 0.5% aqueous methylcellulose (100 ml/100g)

Dose Preparation:

The test substance was heated in at 50°C water bath until clear, followed by stirring for 10 minutes. The test substance was then aliquoted to 10 containers which were used for dosing on each day. Dosing was prepared by mixing the liquefied test substance in 0.5% aqueous methylcellulose. Rats were dosed with a volume of 1 ml/100g. Mixtures were stirred continually during the dosing period.

Protocol:

The test animals were Crl:CD®(SD)IGS BR rats, males, five per group (see below), from Charles River Laboratories, Inc., Raleigh North Carolina. Animals were housed (one/cage) in stainless steel, wire-mesh cages. Tap water was supplied ad libitum. Certified chow (Rodent LabDiet® 5002, PMI Nutrition International, Inc.) was used as feed.

Animals (5/group) were dosed consecutively up to 10 days with either the test substance, positive controls or negative control:

Table 1
Dosage Forms

Substance	Vehicle	Dosage (mg/kg)	Number of Animals
Negative Control	0.5% Aqueous methylcellulose	0	30
Positive Controls			
H-24019 (PFOS)	0.5% Aqueous methylcellulose	10	30
H-24538 (PFOE)	Deionized water	20	30
Test Substance			
H-24542	0.5%Aqueous methylcellulose	1000	30

Body weights were measured on each day of dosing and weekly thereafter.

Sampling:

Sampling times, dosing and tissues collected are shown below in Table 2.
At sampling times prior to euthanasia blood (1-2 mL) was collected from the orbital sinus into tubes containing EDTA. Animals were sacrificed two hours after the last dose. Euthanasia was via carbon dioxide and exsanguination prior to collection liver and fat samples. Samples were preserved using refrigeration or freezing and sent to Haskell Laboratory (blood) or Jackson Laboratory, Deepwater, NJ (liver and fat) for F analysis.

Fluorine Analysis:

Total F content was measured by the Wickbold torch combustion method followed by analysis with a fluoride ion selective electrode. Samples were decomposed or volatilized in the presence of wet oxygen and swept through an oxy-hydrogen flame in a closed quartz apparatus. Combustion products were collected in an aqueous absorbing solution and analyzed using a F ion selective electrode.

The limit of detection (LOD) of 0.2 ppm was subtracted from each sample and the limit of quantification (LOQ) was determined to be 0.5 ppm. Any values below the LOQ were excluded from the treatment. Data were converted to μM equivalents (report Appendix D).

Because the test material and positive controls have different potencies the study authors "normalized" the dose to an arbitrary 0.1 mmoles/kg. This meant, effectively, multiplying measured ppm F for the positive control substances (PCS) by the factor

(Dose (mg/kg) H-24542)(M.W. PCS)/ (Dose PCS)(M.W. H-24542) [= 25.25 for H-24538]

Table 2
Sampling Schedule

Group	Dosing Days	Tissues Collected	Sampling Times
I	1- 5	Blood only	Day 1, @ 2 hr
I	1-5	Blood, Liver, Fat	Day 5, at sacrifice
III	1-10	Blood, Liver, Fat	Day 10
V	1-10	Blood, Liver, Fat	Day 13
VII	1-10	Blood, Liver, Fat	Day 24
IX	1-10	Blood, Liver, Fat	Day 52
XI	1-10	Blood, Liver, Fat	Day 94

Results:

Body Weights Body weights shown in Review Table 3 are averages of individual animals weights recorded in Study report Tables 1 and 2, Appendix A. All animals (control and test substance treated) grew at rates which exceed rates for historical controls recorded by FDA in similar rat strains and studies. Over the entire recovery period, animals in the test substance group had a body weight gain 12% higher than negative controls. Overall body weight gain was increased as compared to the positive controls. During recovery, test substance and H-24538 had comparable body weight gain, whereas body weight gain was 11% higher for test substance of positive control H-24019.

Tissue Weights Average liver weights for rats treated with the test substance are shown along with corresponding weights for vehicle controls in Review Table 3. Body weight, liver and estimated (this reviewer) values for blood volume and total body fat composition are shown in Report Figure 1. Estimates for fat and blood volumes are required for kinetic and mass balance analyses because the samples do not represent the contents of the entire compartment and because these compartments increase in size during the 90 + day course of the experiment, diluting the total residual fluoride mass into these increasing volumes.

Mass Balance The mass balance can be bounded between extreme values, using the known properties of positive control (PFOE) from previous studies, but no better values can be obtained in the absence of data for excretion and remaining carcass. Tissue distribution analyses described below allow us to state that the mass of H-24542 in fat at 10 days is approximately 2 x that in liver, and 15 x that in blood. The mass in other tissues is unknown, and in the absence of excretion data, the mass balance and fractional absorption can only be bounded within a maximum limit.

Table 3
Average Body and Liver Weights

Test Days	Body Wt	Liver Wt.	Liver: Body Ratio
VEHICLE (NEGATIVE CONTROL)			
5	267.4	12.153	0.045
10	297.6	14.428	0.045
13	323.3	17.241	0.042
24	406.1	24.460	0.042
69	515.9	20.419	0.034
94	592.1		
H-24542 (TEST SUBSTANCE)			
5	256.9	12.347	0.048
10	306.3	16.018	0.052
13	321.5	16.581	0.052
24	403.4	17.339	0.043
52	487.9	18.268	0.037
94	623.2	25.473	0.041

Tissue Distribution and Disappearance Accurate fluoride measurements were not available for H-24542 in blood for Day 94 (4 of 5 non detectable), and no fluoride measurements were reported for liver or fat on day 52. This lack of data limits our analysis of both tissue:blood partitioning and elimination rates. Reported "normalized" concentrations in blood were:

Table 4
Blood Fluorine Concentration Normalized to Dose

Test Day	H-24019 uM F Equivalents	H-24538 uM F Equivalents	H-24542 uM F Equivalents
1	86.15(7.0)	210.61 (39.0)	0.25(0.0)
5	656.62(25.5)	1282.83 (54.2)	1.31(0.4)
10	971.54(77.4)	1808.08 (159.8)	1.69(0.2)
13	863.08(72.8)	869.70 (243.9)	1.00(0.3)
24	521.85(39.0)	398.48 (136.0)	0.34(0.1)
52	311.15 (29.0)	41.92 (29.0)	0.10(0.1)
94	178.46(16.2)	32.83 (7.1)	***

*** 4 out of 5 samples reported as non-detectable (ND)

The "normalized" concentrations are not the same as actual concentrations (see below).

Table 5
Liver F^- Concentration Normalized to 0.1 mmol/kg Dose

Test Days	Positive Controls H-24019 uM Equivalents	Positive Controls H-2453 8 uM Equivalents	Test Substance H-24542 uM Equivalents
5	2269.23 (153.5)a	2147.98 (159.7)	12.18 (2.7)
10	3963.38 (444.2)	3309.09 (562.6)	14.25 (2.0)
94	827.69 (68.4)	35.86 (18.6)	0.62 (0.3)

The distribution of the test substance and positive controls in blood, liver and fat were reported in study Tables 5 - 9, with "normalization" to a micromolar (uM) fluoride basis. Since dosing ended after 10 days of the 94 day experiment, determination of elimination rates by this reviewer required correction for growth and consequent dilution of the systemic mass of chemical by increased tissue weights (Review Figure 1). Blood volume was estimated by this reviewer using an allometric formula based on body weight (Brown et al., 1997):

$$V_{blood}(BW,Age) = 0.0935(BW)^{0.9}$$

Blood concentrations of H-24542 were recomputed by this reviewer from net ppm F^-, and were then multiplied by estimated blood volumes to give the mass of H-24542 in blood, plotted in Review Figure 3. Disappearance rates were estimated by linear regression analysis of individual values for total H-24542 in blood at 10 days and 52 days. Later time points were not available. Note that the half-life computed from concentrations (15.4 days) is little different from that computed from total mass in blood (17 days). Blood volume is almost a constant fraction of body weight so this result is to be expected.

Liver concentrations of H-24542 were recomputed by this reviewer from net ppm F^-, and were then multiplied by individual liver weights to give H-24542 mass in liver, plotted in Review Figure 4. Disappearance rates were estimated by linear regression analysis of individual values for total H-24542 in liver at 10 days and 94 days. Time points earlier than 94 days were not available. Again, the half-life computed from concentration (18 days) is little different from that computed from total mass in liver (21 days). Although liver enlargement was noted in treated animals, this change occurred early in the experiment and was a relatively small change in proportion to body weight.
A liver:blood partition coefficient $R_{liver:blood} = 9.6$ was estimated from 10 day concentrations.

Concentrations of H-24542 in fat, recomputed from net ppm F^- were multiplied by body fat composition estimated from a body weight and age-dependent formula (Roth, 2006):

$$V_{fat}(BW,Age) = 0.04 * BW + 0.15(1 - e^{-0.0055 * Age})$$

The change in body fat composition during the experimental period was predicted to increase from about 7 % at 50 days of age (start of experiment) to about 12 % at the end of the experiment (50 + 90 = 140 days of age). Regression of total H-24542 in fat indicate that fluoride mass in fat disappeared at a much lower rate ($t_{1/2} = 98$ days) than was apparent from

concentrations ($t_{1/2}$ = 34 days), plotted in Review Figure 4. A fat:blood partition coefficient $R_{fat:blood}$ = 11.6 was estimated from 10 day concentrations. The estimated mass of H-24542 in fat at 94 days (0.23 umol) is approximately equal to that in liver (0.26 umol), and 40 times that estimated in the blood (6.3 nmol). The whole body residue can only be approximated, since no carcass or excretion fluoride data are available. At 10 days (final dose) the sum of blood, liver, and fat amounted to about 2 ± 0.5 % of the total dose (see Table 6, below).

Table 6
Fraction of Dose in Tissues at Day 10

Liver	Fat	Blood	Total	
8.7380e-3	0.0165	915.93e-6	0.0259	
6.5375e-3	0.0111	734.72e-6	0.0185	
7.1154e-3	0.0114	876.60e-6	0.0196	
8.2263e-3	0.0193	1.0791e-3	0.0283	
6.2488e-3	8.4037e-3	815.08e-6	0.0150	
7.3732e-3	0.0133	884.28e-6	0.0215	mean
1.0747e-3	4.4164e-3	128.61e-6	5.4893e-3	S.D.

Discussion:

On the basis of computed blood and liver fluoride mass versus time, the half-life for systemic H-24542 -derived fluoride is 17 to 21 days. Disappearance from fat suggests a half-life of 98 days. No data is available from other tissues, nor is any excretion data available.
Time to steady-state with a half-life of 21 days and daily dosing would be in excess of 100 days. If the partition coefficient between blood and fat is constant ($R_{fat:blood}$ = 11.6 at 10 days), it can be expected that fat will dominate the kinetic process at later time points and cause elimination to be delayed, with a consequent increase in time to steady-state. As long as the animals continue to add fat tissue, the test substance will continue to accumulate. The data provided does not allow discrimination between possible telomer alcohol (PFOE) metabolites and the parent ester.

If the normalized data are ignored, and only the fluoride residues in tissues and blood at 10 days are computed, 7.5 % of the total dose of positive control H-24538 (PFOE) could be accounted for in blood, liver and fat. Since the oral absorption of PFOE (@ 5 mg/kg) is about 51 % (Roth, Feb. 28, 2006), most of the dose must have been eliminated by the time tissue measurements were made. This is to be expected since PFOE has a half-life of about 4 days, so the 7.5 % of total dose represents absorption of more than 51 % of the dose from the last day of dosing with PFOE. About 2 % of the total dose of the test substance (H-24542) was recovered in blood, liver and fat. Since the half-life for this chemical appears to be at least 21 days, the fraction of dose absorbed was at least 2 %. It should be noted that the residues of the test substance observed in tissues are very lipophilic. The use of an aqueous

dosing vehicle may have seriously reduced the solubility of the material below what could be expected in a normal food matrix, and consequently underestimated the absorption. Digestible oils and fats should be recommended as vehicles for any future experiments.

References

Brown, R.P., Delp, M.D., Linstedt, S.L., Rhomberg, L.R., Beliles, R.P. (1997) Physiological parameter values for physiologically based pharmacokinetic models. Toxicology and Industrial Health 13(4):407-483

Roth, W.L. (2006) Physiologically Based Pharmacokinetics. In: Green, S. ed. *Toxicology and the Regulatory Process*. Taylor and Francis Publishers, New York. pp. 173-217

Roth, W.L. (Feb. 28, 2006) Review of "8-2 Telomer Alcohol" (DuPont Study # 6997). [redacted] DFCN/OFAS/CFSAN/FDA

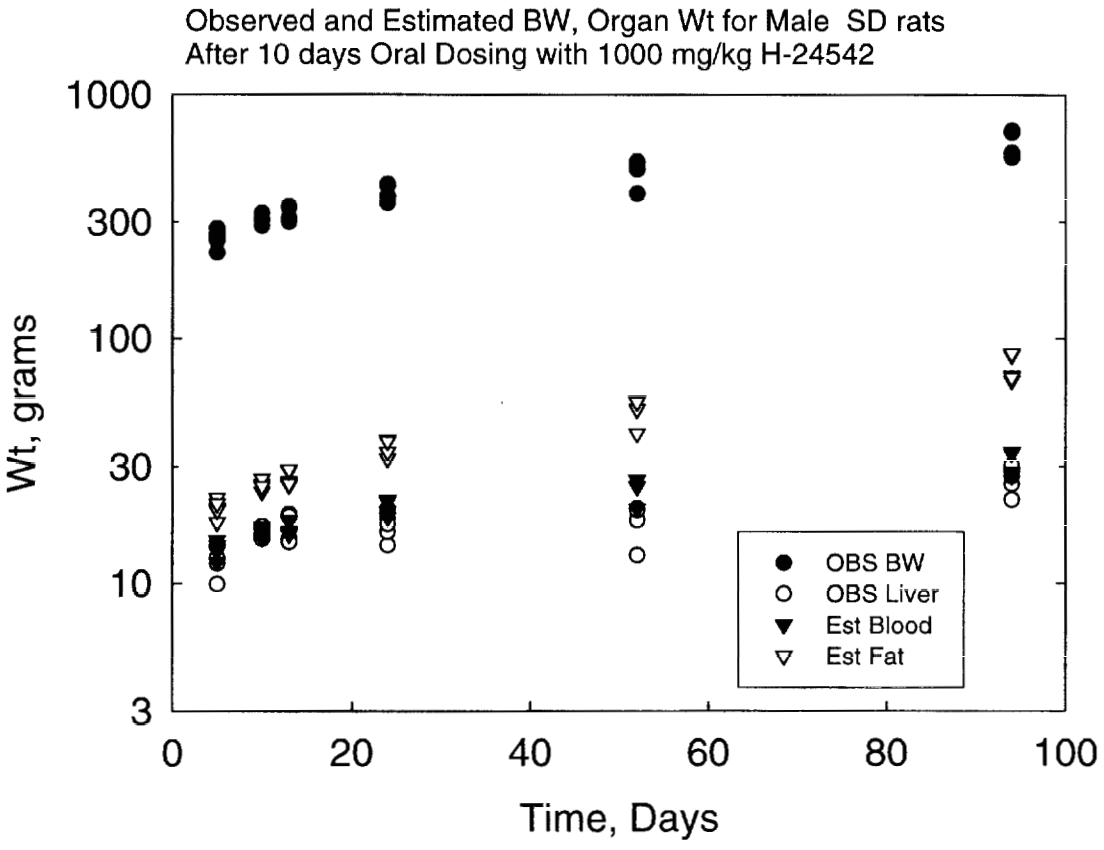
Figure 1 (next page) Logarithmic (top) and linear(bottom) plots of observed (BW and liver) and estimated (blood and fat) volumes of male rats treated with 1000 mg/kg BW H-24542. Blood volume was estimated from body weight (BW) using the formula

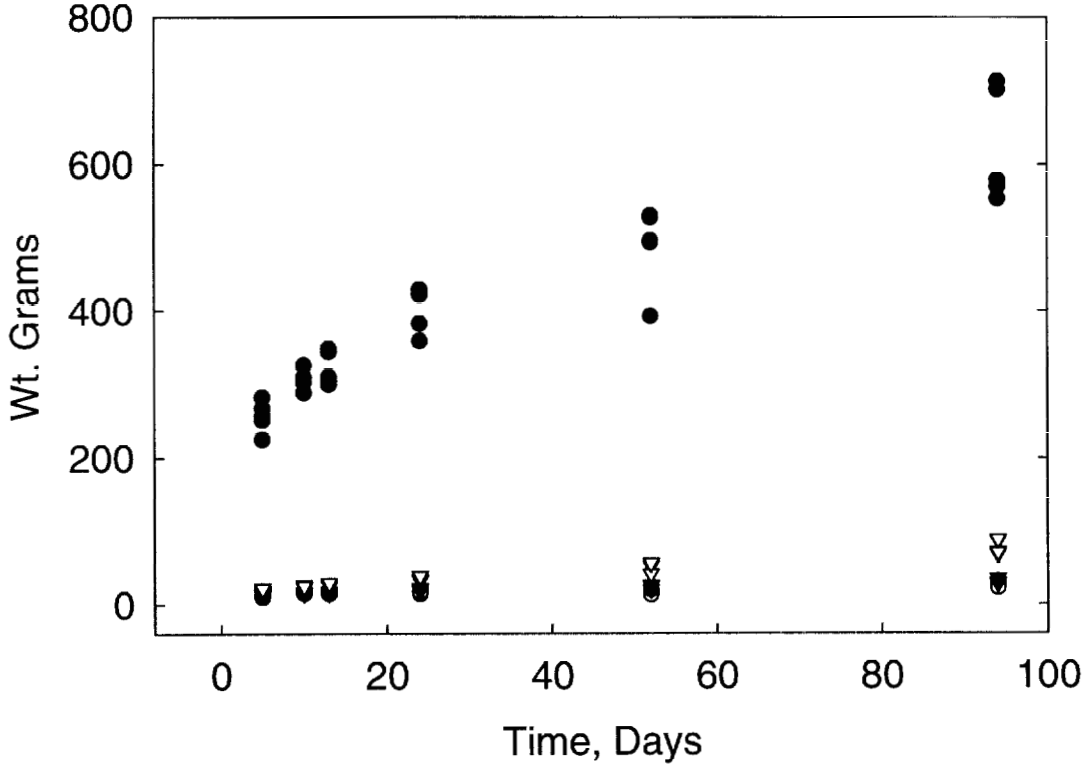
$$V_{blood}(BW,Age) = 0.0935(BW)^{0.9}$$

Fat volume was estimated from BW and age using the formula

$$V_{fat}(BW,Age) = 0.04 * BW + 0.15(1 - e^{-0.0055 * Age})$$

Note that the animals nearly tripled their BW and doubled their blood and liver volumes during the course of this experiment. These values were multiplied (by this reviewer) by concentrations of F in the corresponding tissues and blood to obtain the total mass of F in each compartment.


Observed and Estimated BW, Organ Wt for Male SD rats
After 10 days Oral Dosing with 1000 mg/kg H-24542
Wt, grams
1000
300
100
30
10
3
0
20
40
60
80
100
Time, Days
OBS BW
OBS Liver
Est Blood
Est Fat


Wt. Grams
800
600
400
200
0
0
20
40
60
80
100
Time, Days

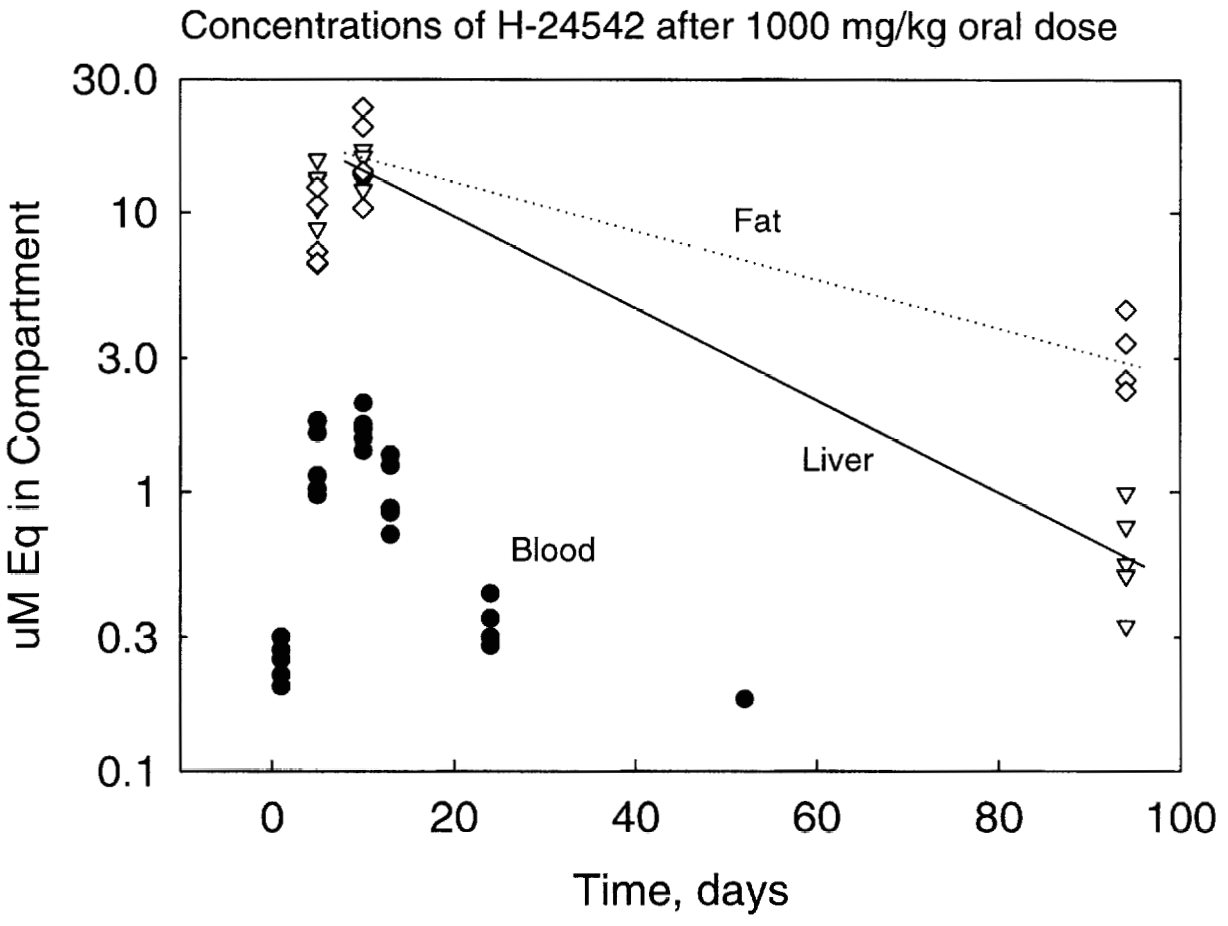


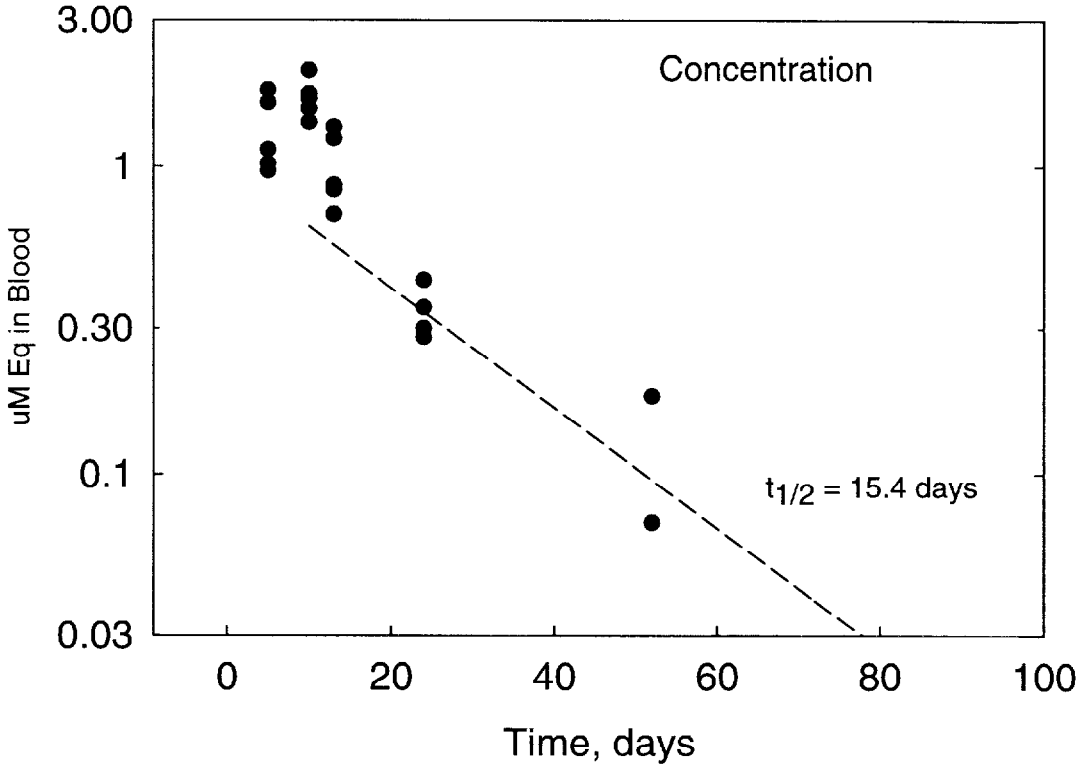
Figure 2 Comparison of concentrations of F derived from H-24542 in each measured compartment during this experiment. Symbols are measurements, lines represent linear regression lines for disappearance based on days 10 and 94.

Figure 3 Recomputed concentration (top) and estimated total mass (bottom) of H-24542 in blood during the course of this experiment. Concentrations were recomputed from net ppm F^- via the formula:

uM "S" = (ppm F^- [mg/kg])(mol "S"/mol F^-) / (fluoride F.W. [=19])(1000 [mg/g])

The total mass "S" was estimated by multiplying the concentration by an estimated blood volume derived from a standard allometric formula (see text and Figure 1). Dashed lines represent linear regression slopes between the two elimination phase measurements.

1000 mg/kg oral dose H-24542



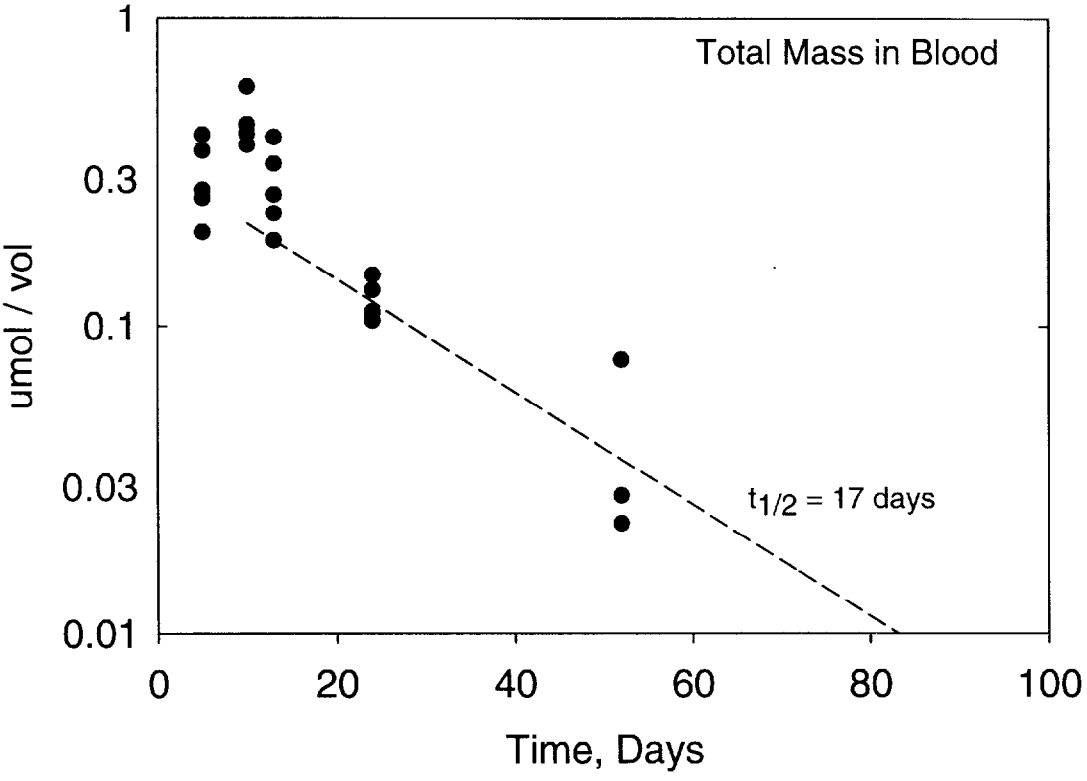
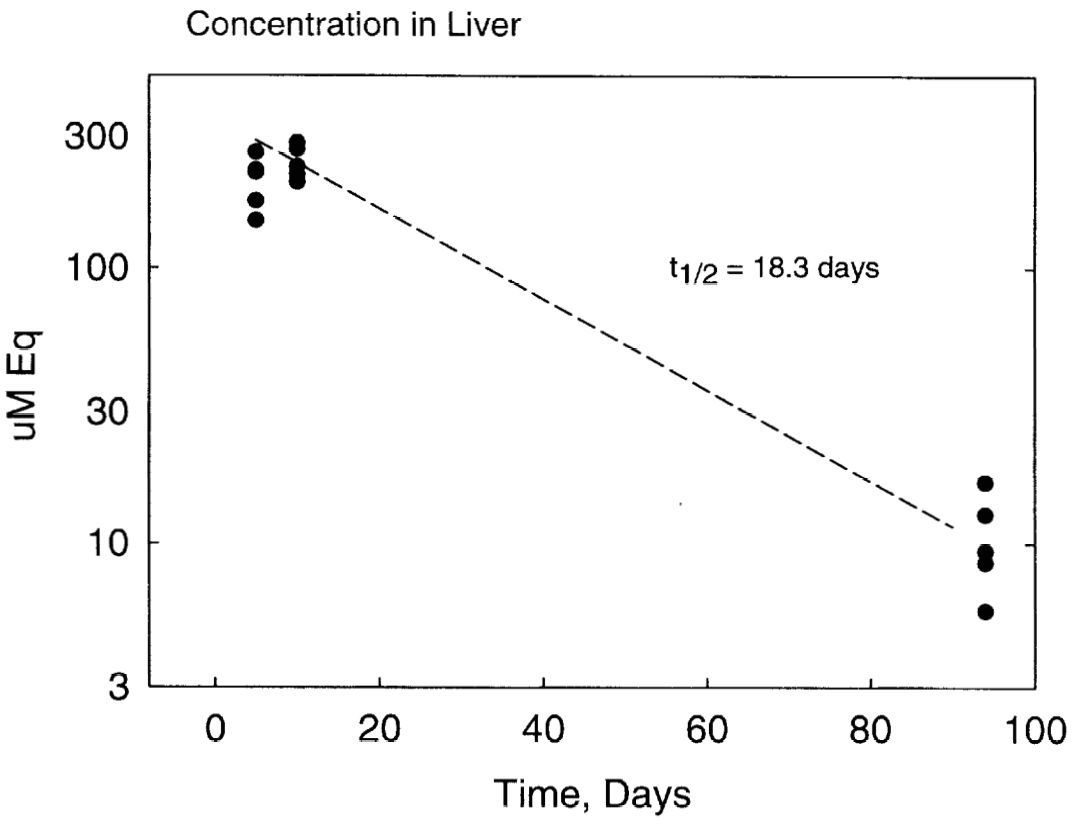


Figure 4 Observed concentration (top) and total mass (bottom) of H-24542 in liver during the course of this experiment. The total mass was obtained by multiplying the concentration by measured liver weight. Dashed line represent linear regression slopes between the two elimination phase measurements.


Concentration in Liver
300
100
30
10
3
uM Eq
$t_{1/2}$ = 18.3 days
0
20
40
60
80
100
Time, Days

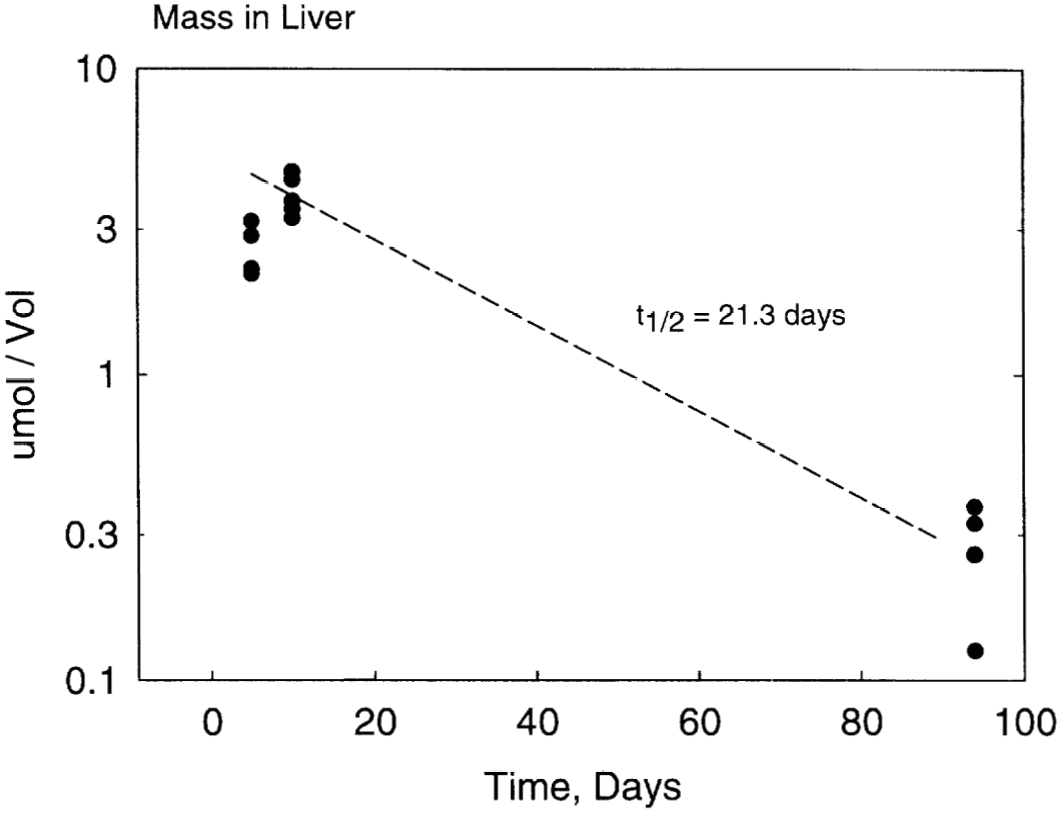
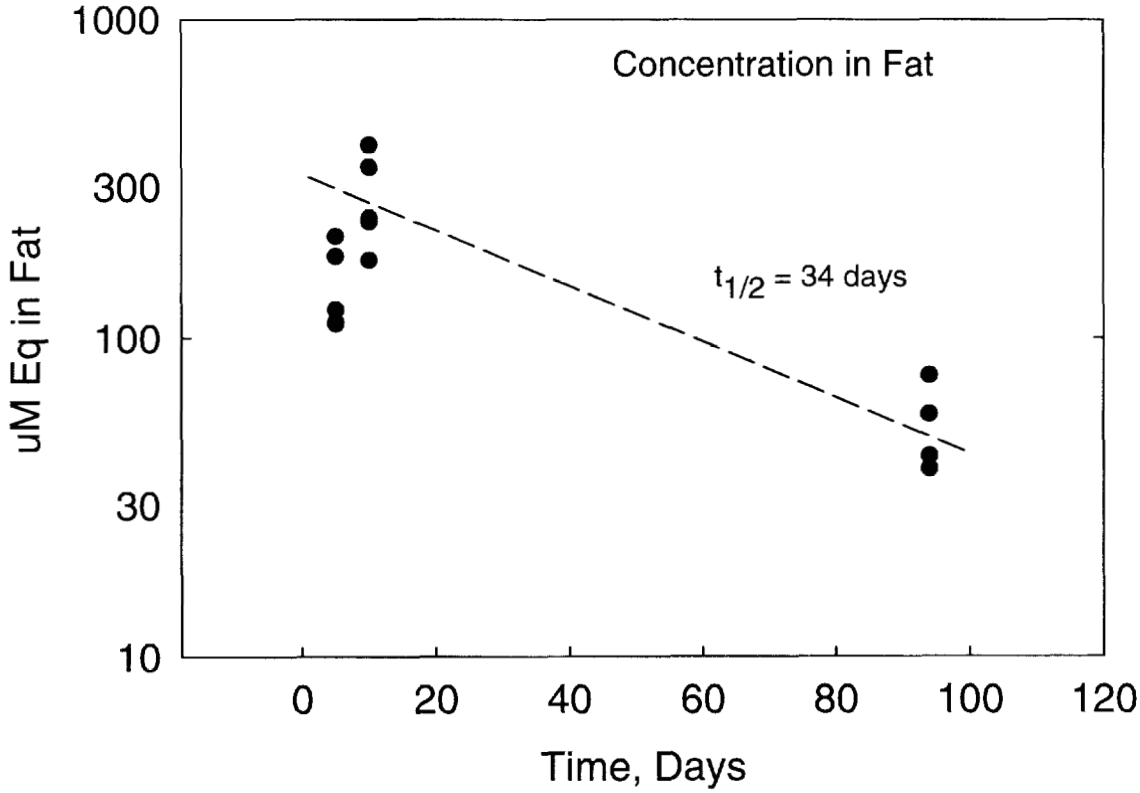
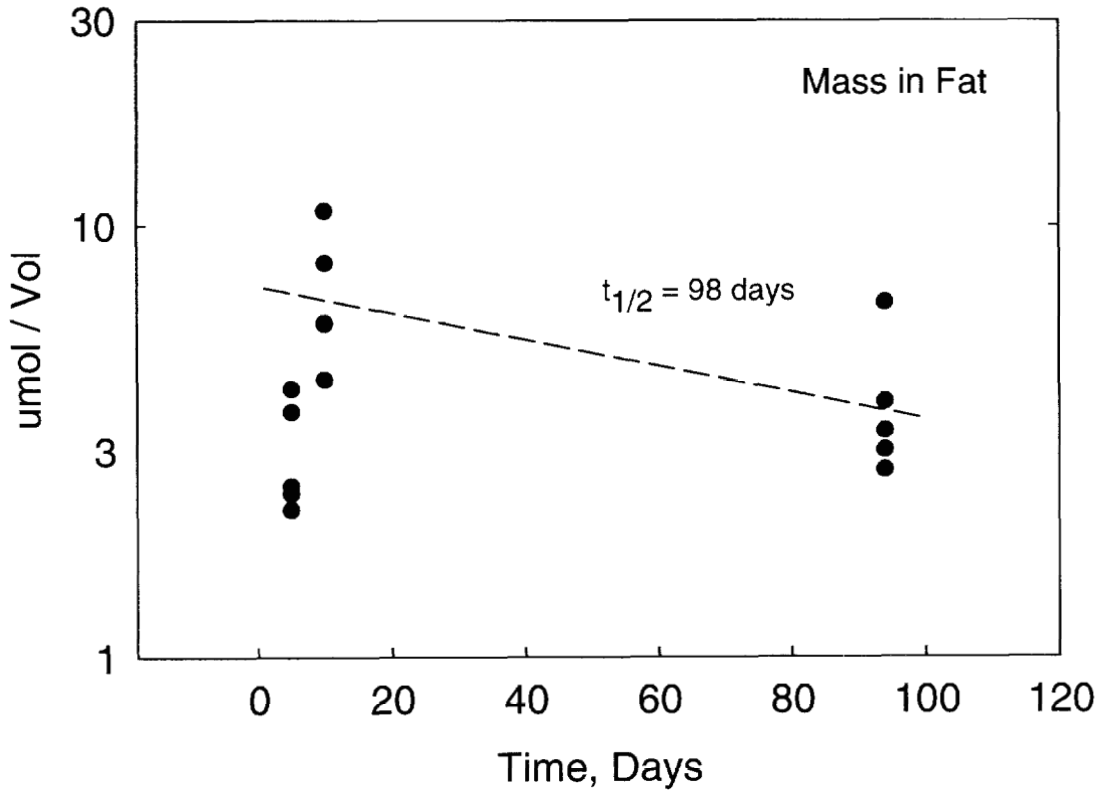

Mass in Liver
10
3
1
0.3
0.1
umol / Vol
$t_{1/2}$ = 21.3 days
0
20
40
60
80
100
Time, Days

Figure 5 Observed concentration (top) and estimated total mass (bottom) of H-24542 in fat during the course of this experiment. The total mass was estimated by multiplying the concentration by an estimated blood volume derived from a standard allometric formula for total fat (see text and Figure 1). The total body fat composition changes from about 7 % at 50 days of age to about 12 % at the end of the experiment. This change causes a dramatic change in the actual elimination half-life. Dashed lines represent linear regression slopes between the two elimination phase measurements.


Concentration in Fat
$t_{1/2}$ = 34 days
uM Eq in Fat
1000
300
100
30
10
0
20
40
60
80
100
120
Time, Days


Mass in Fat
$t_{1/2}$ = 98 days
umol / Vol
30
10
3
1
0
20
40
60
80
100
120
Time, Days