

TRADE SECRET

Study Title

H-23961: Biopersistence Screening
10-Dose Oral Gavage Study in Rats

Supplement No. 1

Laboratory Project ID: DuPont-2923

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CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

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STUDY INFORMATION

TEST SUBSTANCE:

Substance Tested: [REDACTED]

Synonyms/Codes: • H-23961
[REDACTED]

Haskell Number: 23691

Composition: [REDACTED]

Known Impurities: [REDACTED]

POSITIVE CONTROL:

Substance Tested: [REDACTED]

Synonyms/Codes: • H-24019
[REDACTED]

Haskell Number: 24019

STUDY INFORMATION (Continued)

Composition:

[REDACTED]

Known Impurities: Unknown

POSITIVE CONTROL:

Substance Tested:

[REDACTED]

Synonyms/Codes: • H-24020

[REDACTED]

Haskell Number: 24020

Composition:

[REDACTED]

Known Impurities: Unknown

Sponsor: E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: April 30, 1999 / (see report cover page)

In-Life Initiated/Completed: May 30, 1999 / August 30, 1999

STUDY PERSONNEL

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REASON FOR SUPPLEMENT NO. 1

The purpose of this supplement is to present the results from analysis of selected liver samples from rats treated with the test substance (H-23961), 2 positive controls (H-24019 and H-24020), and 2 negative controls (corn oil and corn oil/acetone).

SUMMARY

The objective of this study was to evaluate the potential for H-23961, when administered by gavage, to be absorbed and to accumulate in a mammalian system. Six groups of 5 male Crl:CD[®](SD)IGS BR rats each were given 1000 mg/kg/day of H-23961. The test substance was administered to one group of 5 rats for 5 consecutive days and to 5 groups for 10 days. On selected days (5, 10, 13, 24, 52, 94) 5 rats per group were euthanized and the blood and livers were collected. Livers were weighed and those collected on study days 10, 13, and 94 were analyzed for total fluorine. This supplement includes liver weight data and the results of total fluorine analyses in livers. Additionally, 2 negative controls, corn oil and corn oil/acetone (80:20), and 2 positive controls, H-24019 (10 mg/kg/day) and H-24020 (20 mg/kg/day), were tested and analyzed as described for H-23961.

The mean relative liver weights (liver/body weight) of rats dosed with the test substance, H-23961, were similar to the negative controls during the dosing and recovery periods. On day 10, the mean relative liver weight of rats dosed with the positive control H-24019 was up to 27% higher than the negative controls. By day 94, the mean relative liver weight was up to 28% higher than the negative controls. In rats given H-24020, the mean relative liver weight was up to 75% higher at day 10 than the negative controls. By day 94, the weights were similar.

The concentration of fluorine in the livers from rats dosed with the test substance, H-23961, was 18.43 μ M equivalents on day 10 and 3.50 μ M equivalents on day 94. On day 10, mean μ M equivalent concentrations of fluorine in the livers from rats dosed with the positive control materials were approximately 275-fold (H-24019) and 60-fold (H-24020) greater than the fluorine concentration in livers from rats treated with the test substance. By day 94, the concentrations were approximately 340x and 3x the fluorine concentration in rats treated with H-23961.

The μ M equivalents of fluorine in the livers from rats dosed with the test substance were higher than levels in the blood.

Under the conditions of this study, there was some absorption and retention of fluorine in the liver from rats dosed with H-23961. However, fluorine levels in the liver were significantly lower than levels in rats dosed with the positive control materials, H-24019 and H-24020. Liver weights were not affected in rats dosed with the test substance.

MATERIALS AND METHODS

Blood and livers were collected on days 5, 10, 13, 24, 52, and 94. The livers were weighed. Only the test day 10, 13, and 94 livers were analyzed for total fluorine. These time points provided sufficient data to determine the accumulation and persistence of fluorine in the liver. See the original report for details of the analysis.

RESULTS AND DISCUSSION

A. Liver Weights

(Table 1, Figures 1-3, Appendix A)

As with body weights, comparison of absolute liver weights was complicated by the fact that there was a difference in age on test day 1 between rats dosed with the test substance and those dosed with the positive or negative controls. The rats dosed with the test substance, H-23961, were older and heavier in weight than the corn oil negative control and H-24020 positive control rats, but younger and lighter in weight than the corn oil/acetone negative control and H-24019 positive control rats. Therefore, liver weights relative to body weight are presented for comparison.

1. Test Substance

The mean relative liver weights of rats dosed with the test substance, H-23961, were similar to the negative controls during the dosing and recovery periods.

2. Positive Controls

The mean relative liver weight of rats dosed with one of the positive controls, H-24019, was 17% higher on day 10 than the liver weight of rats dosed with the test substance, H-23961. On days 13, 24, and 52 (recovery), the mean relative liver weights of rats dosed with H-24019 were 13-36% higher than the liver weights of rats dosed with H-23961. By test day 94 (end of recovery), the mean relative liver weight of rats dosed with H-24019 was 21% higher than the liver weight of rats dosed with H-23961. The mean relative liver weight of rats dosed with H-24020, the other positive control, was 60% higher on day 10 than the liver weight of rats dosed with H-23961. On days 13 and 24 (recovery), the mean relative liver weights of rats dosed with H-24020 were 60 and 33% higher than the liver weights of rats dosed with H-23961. On days 52 and 94, the weights were similar.

The mean relative liver weight of rats dosed with H-24019 was up to 27% higher at day 10 than the negative control groups. By day 94, the mean relative liver weight of rats dosed with H-24019 was up to 28% higher than the negative controls. The mean relative liver weight of rats dosed with H-24020 was up to 75% higher at day 10 than the negative controls. The mean

relative liver weights of rats dosed with H-24020 began to decrease during the recovery period and were similar by test day 94 to the negative controls.

Therefore, liver weights of rats dosed with the test substance, H-23961, were not affected.

B. Liver Fluorine Data

(Tables 2-3, Figures 3-5, Appendix B)

1. Positive Controls

The positive controls were H-24019 and H-24020. The concentrations of fluorine in the livers on day 10 from rats dosed with the positive control materials were 5045.85 and 1127.25 μM equivalents for H-24019 and H-24020, respectively. At day 13, the liver concentrations were 4135.69 and 334.49 μM equivalents. By day 94, the concentrations were 1190.15 and 10.72 μM equivalents.

2. Test Substance

Levels of total fluorine in livers from rats dosed with the test substance, H-23961, were lower than the levels in livers from rats dosed with the positive control materials. The total fluorine concentration in the liver from rats dosed with H-23961 was 18.43 μM equivalents at day 10; 11.89 μM equivalent at day 13, and 3.50 μM equivalents at day 94. For the positive control H-24019, the liver concentrations were approximately 275x higher (day 10) and approximately 340x higher (end of study) than H-23961. For H-24020, the liver concentration was approximately 60x higher (day 10) and approximately 3x higher (end of study).

The μM equivalents of fluorine in the livers from rats dosed with the test substance were higher than levels in the blood.

CONCLUSIONS

Under the conditions of this study, there was some absorption and retention of fluorine in the liver from rats dosed with H-23961. However, fluorine levels in the liver were significantly lower than levels in rats dosed with the positive control materials, H-24019 and H-24020. Liver weights were not affected in rats dosed with the test substance.

TABLES

TABLE 1

MEAN BODY AND LIVER WEIGHTS (g)

CORN OIL (NEGATIVE CONTROL)

Test Days	Absolute		Mean Relative
	Body Weight	Liver Weight	Liver Weight(% of Body Weight)
5	259.3	12.620	0.049
10	311.1	15.861	0.051
13	322.9	14.906	0.046
24	391.2	16.635	0.043
52	464.7	17.240	0.037
94	557.8	20.118	0.036

CORN OIL:ACETONE (NEGATIVE CONTROL)

Test Days	Absolute		Mean Relative
	Body Weight	Liver Weight	Liver Weight(% of Body Weight)
5	313.4	13.632	0.043
10	356.5	15.597	0.044
13	377.6	17.715	0.047
24	423.5	17.172	0.041
52	527.6	21.275	0.040
94	567.8	18.447	0.032

H-24019 (POSITIVE CONTROL)

Test Days	Absolute		Mean Relative
	Body Weight	Liver Weight	Liver Weight(% of Body Weight)
5	306.6	15.456	0.050
10	310.1	17.336	0.056
13	344.8	22.049	0.064
24	387.9	21.313	0.055
52	438.1	19.826	0.045
94	597.9	24.455	0.041

TABLE 1 (Continued)

MEAN BODY AND LIVER WEIGHTS (g)

H-24020 (POSITIVE CONTROL)

Test Days	Absolute		Mean Relative
	Body Weight	Liver Weight	Liver Weight(% of Body Weight)
5	244.8	17.052	0.070
10	284.4	21.944	0.077
13	289.1	21.780	0.075
24	370.3	20.619	0.056
52	456.7	17.713	0.039
94	538.7	18.205	0.034

H-23961 (TEST SUBSTANCE)

Test Days	Absolute		Mean Relative
	Body Weight	Liver Weight	Liver Weight(% of Body Weight)
5	264.9	11.821	0.045
10	306.4	14.729	0.048
13	336.7	15.488	0.046
24	381.7	15.991	0.042
52	502.0	19.891	0.040
94	561.7	18.957	0.034

TABLE 2
MEAN LIVER FLUORINE LEVELS

Test Days	<u>Negative Controls</u>		<u>Positive Controls</u>		<u>Test Substance</u>
	Corn Oil (ppm)	Corn Oil:Acetone (ppm)	H-24019 (ppm)	H-24020 (ppm)	H-23961 (ppm)
10	<0.2	<0.5	328.18 (19.3) ^b	155.76 (29.2)	20.84 (2.7)
13	-	-	269.02 (10.1)	46.36 (10.7)	13.52 (1.5)
94	0.6 ^a	1 ^a	77.56 (10.1)	1.68 (0.3)	4.12 (4.0)

a One value. Four of the values were below the level of detection (LOD).

b Standard deviation is in parentheses.

- Indicates no value for this day.

TABLE 3
MEAN LIVER FLUORINE CONCENTRATION
NORMALIZED TO DOSE

Test Days	<u>Positive Controls</u>		<u>Test Substance</u>
	H-24019 μM F Equivalents	H-24020 μM F Equivalents	H-23961 μM F Equivalents
10	5045.85 (296.91) ^a	1127.25 (211.37)	18.43 (2.4)
13	4135.69 (155.38)	334.49 (77.70)	11.89 (1.4)
94	1190.15 (156.05)	10.72 (2.14)	3.50 (3.6)

a Standard deviation is in parentheses.

FIGURES

FIGURE 1

COMPARISON OF MEAN RELATIVE LIVER WEIGHTS:
TEST SUBSTANCE AND NEGATIVE CONTROLS

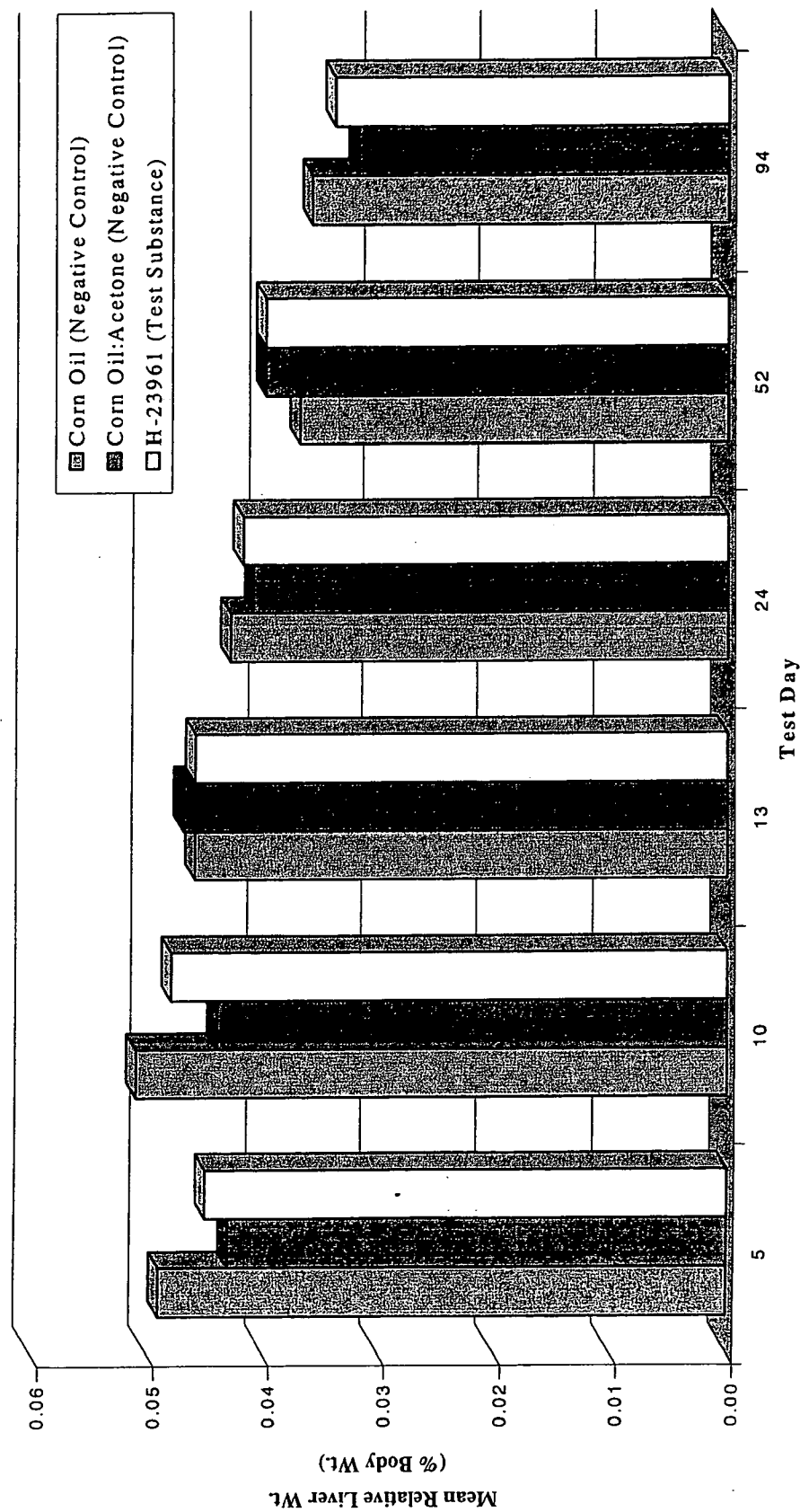


FIGURE 2

COMPARISON OF MEAN RELATIVE LIVER WEIGHTS:
TEST SUBSTANCE AND POSITIVE CONTROLS

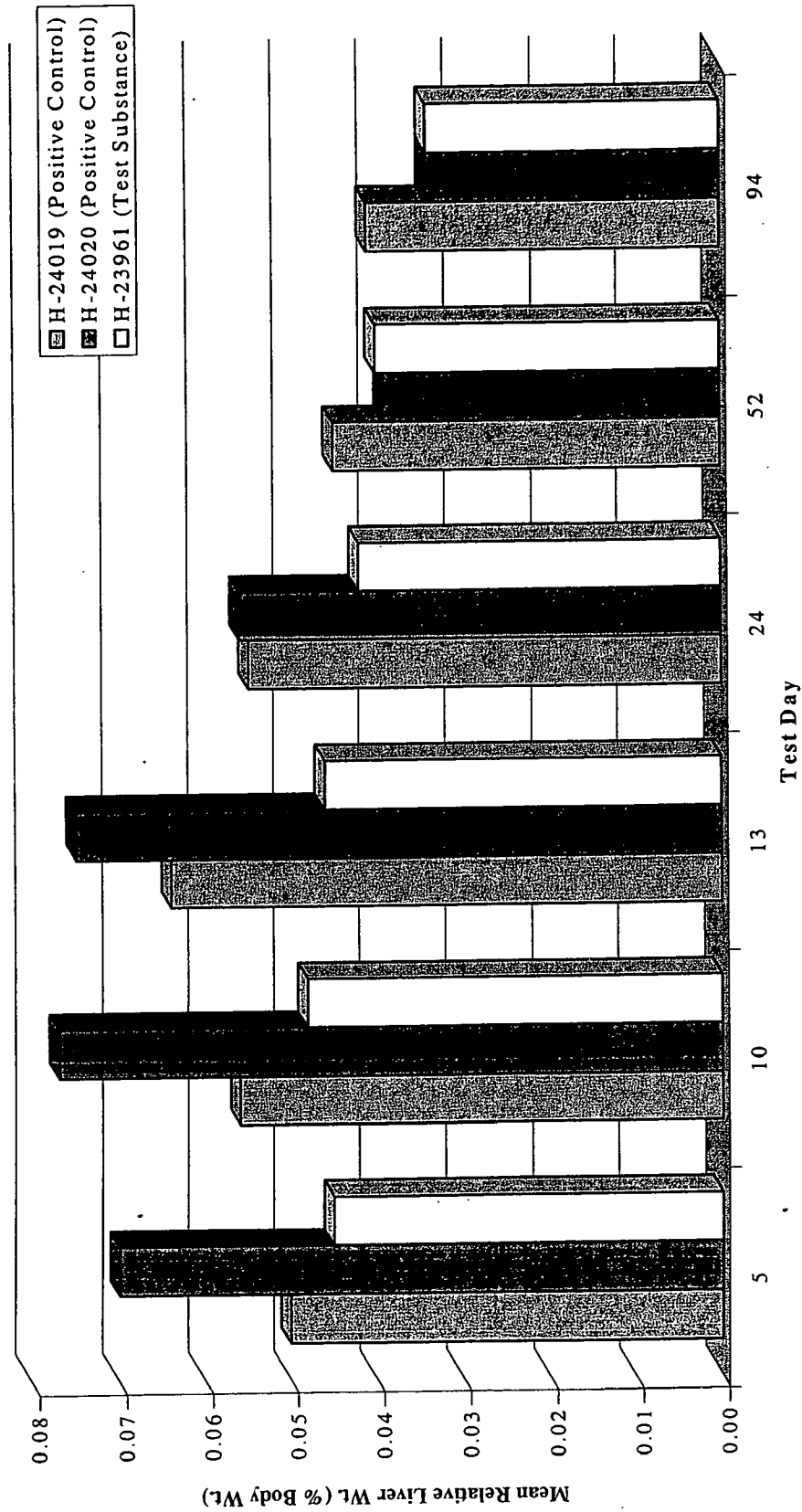


FIGURE 3

COMPARISON OF RELATIVE LIVER WEIGHT AND MEAN LIVER FLUORINE CONCENTRATION
FOR H-23961 AND NEGATIVE CONTROLS

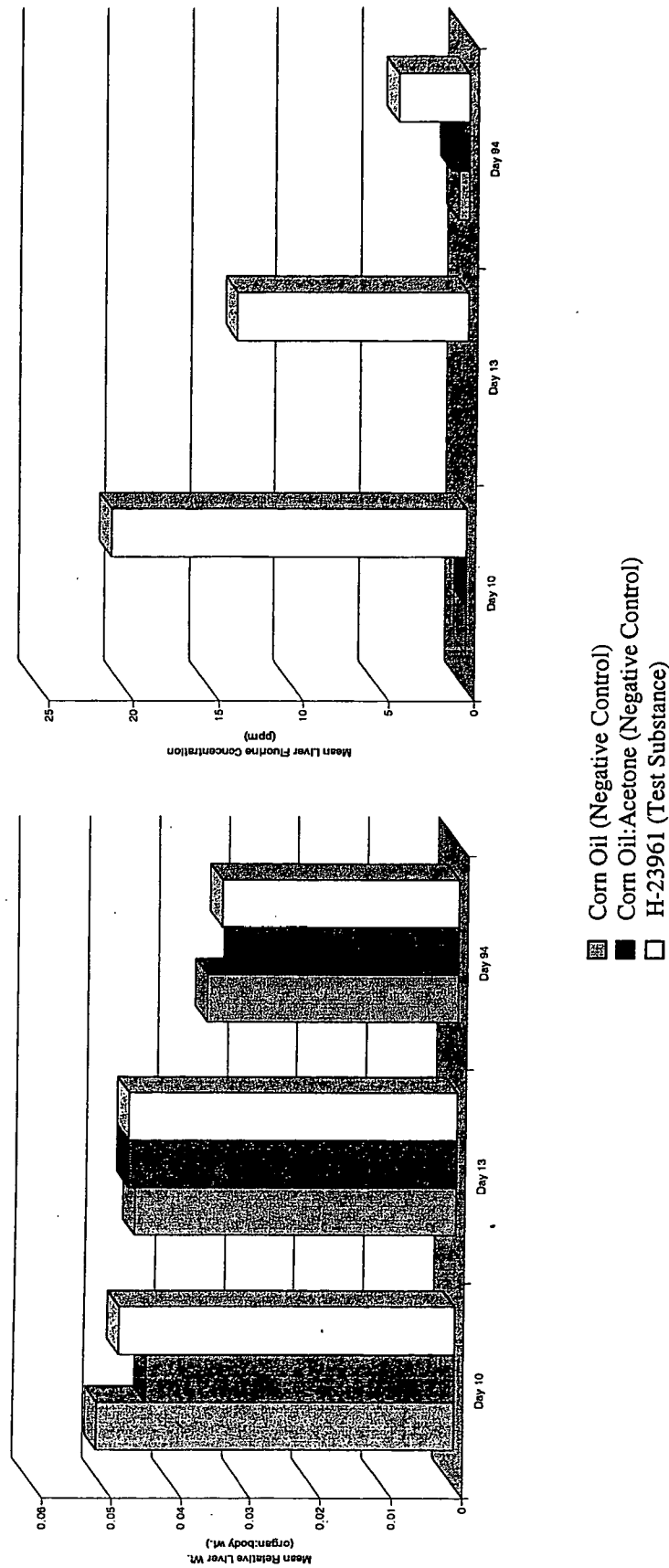


FIGURE 4

MEAN LIVER FLUORINE CONCENTRATION NORMALIZED TO DOSE

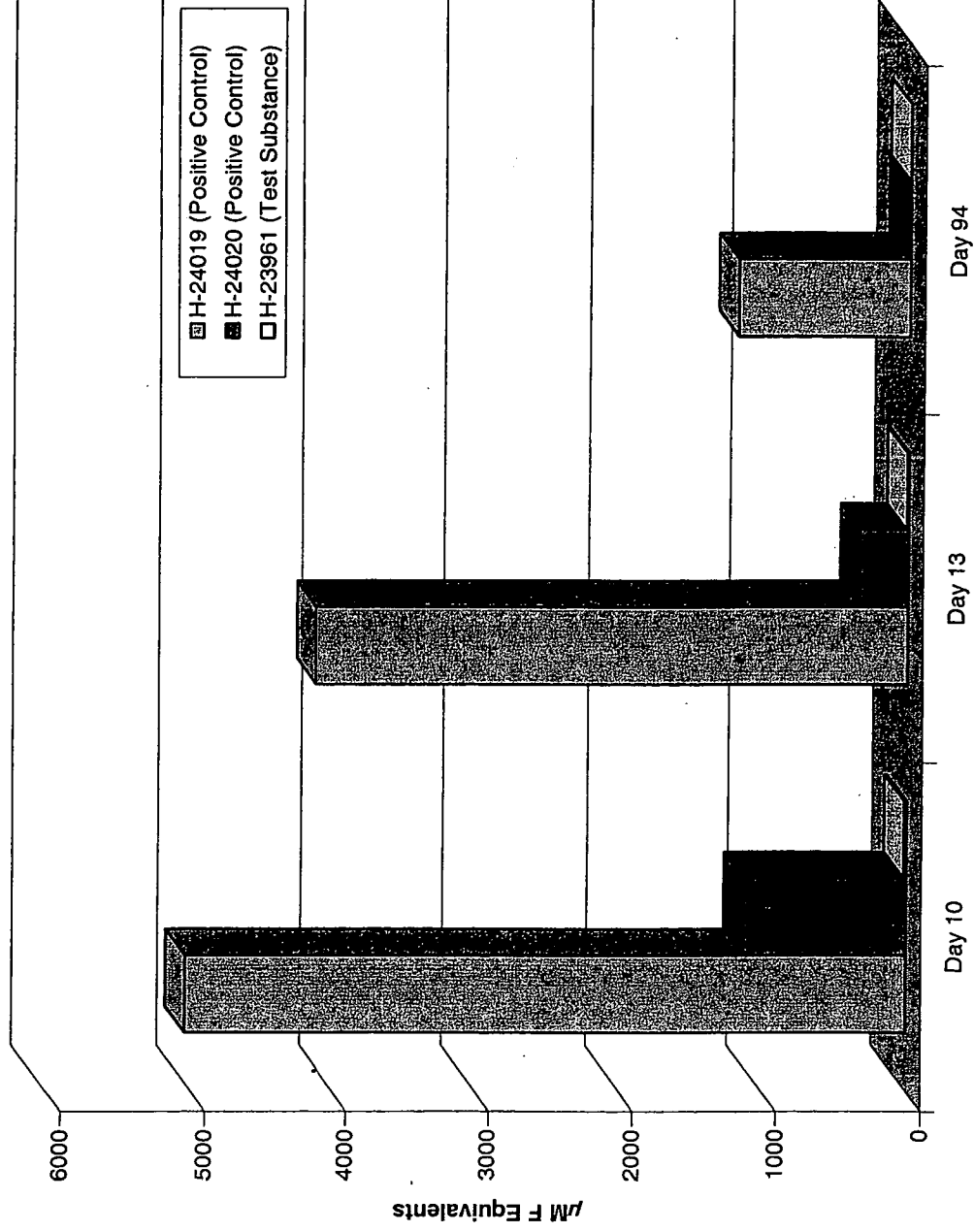
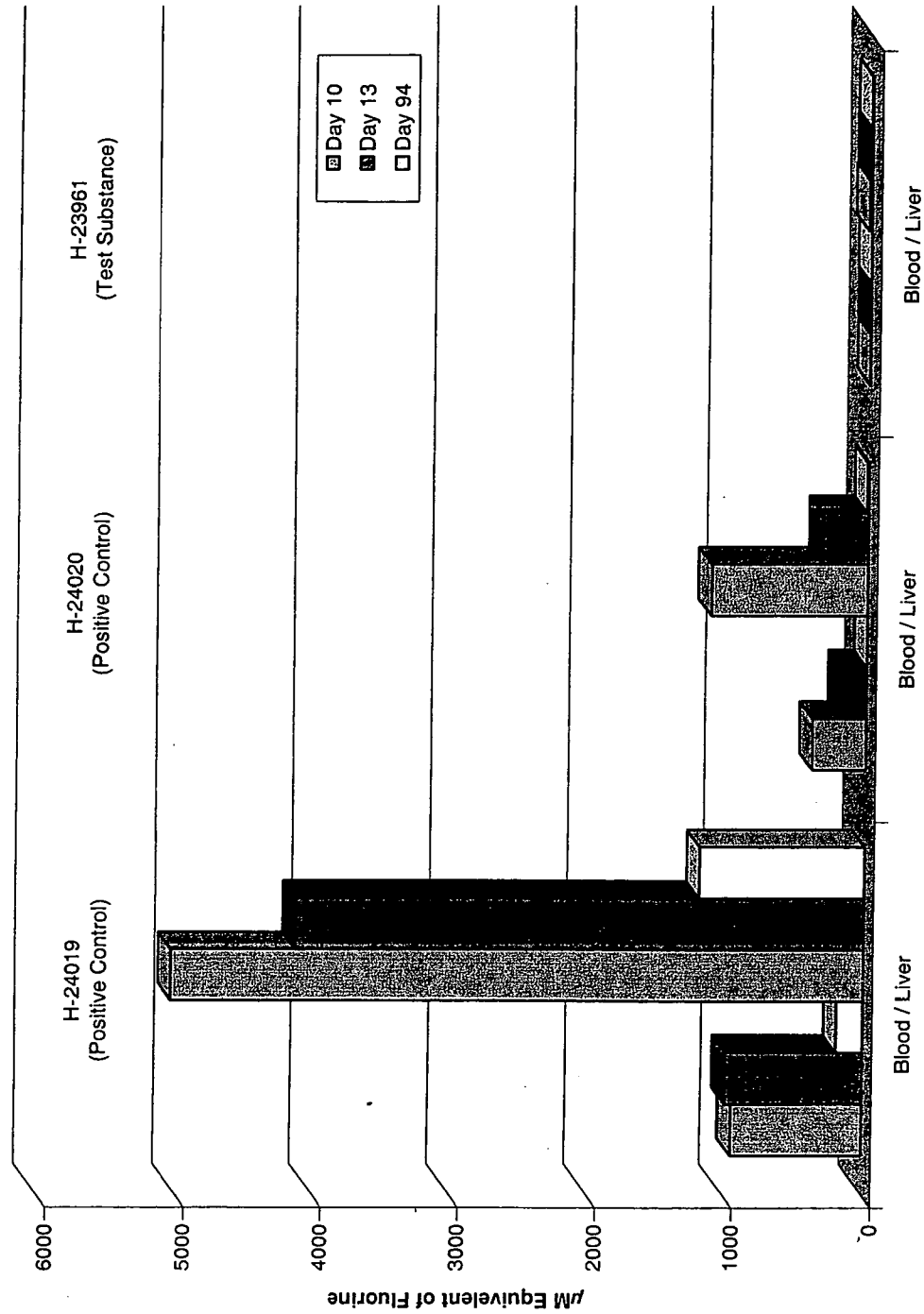


FIGURE 5

COMPARISON OF MEAN BLOOD AND MEAN LIVER FLUORINE CONCENTRATION NORMALIZED TO DOSE



APPENDICES

APPENDIX A

Individual Final Body Weights, Liver Weights, and Relative Liver to Body Weight

CORN OIL (NEGATIVE CONTROL)

INDIVIDUAL FINAL BODY WEIGHTS (g), LIVER WEIGHTS (g), AND
RELATIVE LIVER TO BODY WEIGHT OF MALE RATS

GROUP I

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627789	247.7	11.428	0.046	5
627801	240.1	12.216	0.051	5
627805	283.7	14.081	0.050	5
627810	265.0	13.395	0.051	5
627818	260.2	11.981	0.046	5

GROUP II

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627793	283.5	15.292	0.054	10
627797	324.2	15.803	0.049	10
627799	294.7	14.285	0.048	10
627800	331.3	16.344	0.049	10
627808	321.8	17.580	0.055	10

GROUP III

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627796	322.7	13.404	0.042	13
627806	320.3	15.413	0.048	13
627807	305.2	14.935	0.049	13
627809	336.2	14.967	0.045	13
627816	330.2	15.811	0.048	13

CORN OIL (NEGATIVE CONTROL)

INDIVIDUAL FINAL BODY WEIGHTS (g), LIVER WEIGHTS (g), AND
RELATIVE LIVER TO BODY WEIGHT OF MALE RATS

GROUP IV

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627791	365.6	15.371	0.042	24
627794	416.7	19.105	0.046	24
627804	378.1	15.326	0.041	24
627812	388.4	16.775	0.043	24
627817	407.3	16.600	0.041	24

GROUP V

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627792	496.8	16.359	0.033	52
627798	517.3	19.293	0.037	52
627813	416.8	15.234	0.037	52
627814	386.9	14.358	0.037	52
627815	505.9	20.958	0.041	52

GROUP VI

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627795	485.0	16.649	0.034	94
627811	511.6	17.405	0.034	94
627819	653.1	21.828	0.033	94
627820	618.0	26.766	0.043	94
627821	521.5	17.943	0.034	94

CORN OIL:ACETONE (NEGATIVE CONTROL)

INDIVIDUAL FINAL BODY WEIGHTS (g), LIVER WEIGHTS (g), AND
RELATIVE LIVER TO BODY WEIGHT OF MALE RATS

GROUP I

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625361	349.7	16.800	0.048	5
625363	286.7	11.236	0.039	5
625364	312.0	13.845	0.044	5
625380	305.5	13.253	0.043	5
625383	313.1	13.026	0.042	5

GROUP II

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625355	333.6	14.409	0.043	10
625362	332.9	14.363	0.043	10
625367	363.8	16.139	0.044	10
625368	386.7	17.745	0.046	10
625378	365.4	15.328	0.042	10

GROUP III

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625357	405.6	20.091	0.050	13
625358	340.7	12.954	0.038	13
625370	407.0	21.654	0.053	13
625384	357.1	16.520	0.046	13
625385	377.6	17.355	0.046	13

CORN OIL:ACETONE (NEGATIVE CONTROL)

INDIVIDUAL FINAL BODY WEIGHTS (g), LIVER WEIGHTS (g), AND
RELATIVE LIVER TO BODY WEIGHT OF MALE RATS

GROUP IV

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625354	396.1	16.383	0.041	24
625365	459.6	18.313	0.040	24
625371	447.3	17.859	0.040	24
625376	432.9	19.168	0.044	24
625381	381.8	14.135	0.037	24

GROUP V

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625356	533.7	20.498	0.038	52
625359	530.3	22.901	0.043	52
625360	555.4	23.312	0.042	52
625372	506.2	21.287	0.042	52
625374	512.3	18.378	0.036	52

GROUP VI

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625353	565.3	18.655	0.033	94
625369	514.8	17.379	0.034	94
625373	673.7	20.073	0.030	94
625375	588.8	18.869	0.032	94
625377	496.6	17.261	0.035	94

H-24019 (POSITIVE CONTROL)

INDIVIDUAL FINAL BODY WEIGHTS (g), LIVER WEIGHTS (g), AND
RELATIVE LIVER TO BODY WEIGHT OF MALE RATS

GROUP I

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625322	329.0	16.409	0.050	5
625323	298.0	14.830	0.050	5
625336	318.1	16.545	0.052	5
625343	294.0	13.809	0.047	5
625349	293.7	15.685	0.053	5

GROUP II

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625320	312.1	18.845	0.060	10
625330	310.1	16.343	0.053	10
625332	318.3	17.528	0.055	10
625341	325.6	18.254	0.056	10
625350	284.3	15.709	0.055	10

GROUP III

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625321	371.2	22.322	0.060	13
625327	355.3	22.259	0.063	13
625342	324.4	20.718	0.064	13
625345	336.9	24.403	0.072	13
625348	336.4	20.541	0.061	13

H-24019 (POSITIVE CONTROL)

INDIVIDUAL FINAL BODY WEIGHTS (g), LIVER WEIGHTS (g), AND
RELATIVE LIVER TO BODY WEIGHT OF MALE RATS

GROUP IV

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625325	381.3	21.479	0.056	24
625328	423.9	22.100	0.052	24
625338	339.9	18.018	0.053	24
625340	419.0	23.131	0.055	24
625352	375.3	21.835	0.058	24

GROUP V

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625324	447.4	20.900	0.047	52
625329	498.1	20.911	0.042	52
625333	427.4	20.508	0.048	52
625334	426.5	19.144	0.045	52
625344	391.0	17.666	0.045	52

GROUP VI

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
625326	577.5	23.749	0.041	94
625335	579.5	21.548	0.037	94
625346	556.9	23.945	0.043	94
626347	638.9	27.416	0.043	94
625351	636.8	25.615	0.040	94

H-24020 (POSITIVE CONTROL)

INDIVIDUAL FINAL BODY WEIGHTS (g), LIVER WEIGHTS (g), AND
RELATIVE LIVER TO BODY WEIGHT OF MALE RATS

GROUP I

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627756	237.5	15.360	0.065	5
627767	248.6	19.033	0.077	5
627771	239.6	16.968	0.071	5
627773	262.3	17.629	0.067	5
627777	236.0	16.268	0.069	5

GROUP II

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627768	293.5	22.536	0.077	10
627774	338.2	27.332	0.081	10
627776	254.4	18.821	0.074	10
627782	268.2	20.172	0.075	10
627785	267.9	20.857	0.078	10

GROUP III

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627758	308.7	26.021	0.084	13
627763	285.8	22.123	0.077	13
627764	306.7	21.526	0.070	13
627787	234.9	16.498	0.070	13
627788	309.4	22.730	0.073	13

H-24020 (POSITIVE CONTROL)

INDIVIDUAL FINAL BODY WEIGHTS (g), LIVER WEIGHTS (g), AND
RELATIVE LIVER TO BODY WEIGHT OF MALE RATS

GROUP IV

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627759	380.1	22.707	0.060	24
627761	359.4	19.579	0.054	24
627770	379.8	19.540	0.051	24
627779	406.2	24.858	0.061	24
627786	326.1	16.410	0.050	24

GROUP V

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627757	436.7	16.899	0.039	52
627760	456.5	18.240	0.040	52
627765	455.6	16.505	0.036	52
627772	513.5	20.225	0.039	52
627781	421.0	16.695	0.040	52

GROUP VI

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
627762	528.6	17.732	0.034	94
627769	609.6	19.324	0.032	94
627775	552.8	17.296	0.031	94
627778	445.3	15.628	0.035	94
627783	557.2	21.044	0.038	94

H-23961 (TEST SUBSTANCE)

INDIVIDUAL FINAL BODY WEIGHTS (g), LIVER WEIGHTS (g), AND
RELATIVE LIVER TO BODY WEIGHT OF MALE RATS

GROUP I

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
626876	262.6	12.535	0.048	5
626877	278.2	12.834	0.046	5
626885	245.9	10.960	0.045	5
626889	268.9	11.468	0.043	5
626890	268.9	11.306	0.042	5

GROUP II

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
626871	309.0	16.809	0.054	10
626872	318.1	15.772	0.050	10
626875	315.1	15.102	0.048	10
626882	325.1	14.461	0.044	10
626888	264.8	11.499	0.043	10

GROUP III

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
626868	366.9	16.841	0.046	13
626879	320.6	15.748	0.049	13
626886	319.9	14.966	0.047	13
626887	329.9	13.393	0.041	13
626891	346.3	17.845	0.052	13

H-23961 (TEST SUBSTANCE)

INDIVIDUAL FINAL BODY WEIGHTS (g), LIVER WEIGHTS (g), AND
RELATIVE LIVER TO BODY WEIGHT OF MALE RATS

GROUP IV

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
626864	390.7	16.059	0.041	24
626865	351.8	14.379	0.041	24
626873	424.7	18.570	0.044	24
626894	380.8	15.501	0.041	24
626895	360.6	15.449	0.043	24

GROUP V

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
626867	478.7	17.371	0.036	52
626880	470.8	19.473	0.041	52
626881	533.9	21.007	0.039	52
626884	507.8	21.287	0.042	52
626892	518.8	20.319	0.039	52

GROUP VI

Animal Number	Final Body Wt.	Liver Wt.	Relative Liver to Body Wt.	Test Day
626866	565.5	19.198	0.034	94
626869	507.0	17.216	0.034	94
626874	654.5	22.444	0.034	94
626893	539.5	16.143	0.030	94
626896	541.8	19.786	0.037	94

APPENDIX B

Individual Fluorine Levels

TERMS AND CALCULATIONS

Terms:

Active

% Active

Mol Wt Active

Formulation Dose

% F in Active

Mol Wt F

Compound Calculations:

Dose Active (mg/kg)	The mg of fluorine containing compound administered per kg of animal body weight. $= (\% \text{ active}/100) \times \text{Formulation Dose}$
Dose Active (mmole/kg)	The mmole of fluorine containing compound administered per kg of animal body weight $= \text{dose [mg/kg]} / \text{Mol Wt Active [mg/mmol]}$
Dose F (mg/kg)	The mg Fluorine administered per kg of animal body weight $= (\% \text{ F in active}/100) \times \text{Dose Active [mg/kg]}$
Dose F (mmol/kg)	The mmole of fluorine administered per kg of animal body weight $= \text{Dose F [mg/kg]} / \text{Mol Wt F [mg/mmol]}$
Molar Ratio (Active/F)	The moles of fluorine containing compound per mole of fluorine $= \text{Dose Active [mmol/kg]} / \text{Dose F [mmol/kg]}$
Formulation Dose Normalization Factor	The formulation dose that would be required to administer the amount of active needed to achieve the normalized dose $= (\text{Normalized dose of Active [mmol/kg]} / \text{Dose Active [mmol/kg]}) \times \text{Formulation Dose}$

TERMS AND CALCULATIONS

Individual Animal Measurement:

ppm F in liver The ppm fluoride measured in liver

Individual Animal Calculations:

ppm F in Liver minus
Bkg 0.2 ppm The ppm fluoride measured in liver minus the background fluoride
measured in control animal liver. In this case the value was
established at 0.2 ppm.

ppm F in Liver
normalized to
0.1 mmol/kg Dose The ppm fluoride minus background that would be expected if the
active dose was 0.1 mmol/kg instead of the actual active dose. This
assumes linearity between administered dose and liver fluorine levels,
but is needed because different doses of active were used in the study.
$$= (0.1 \text{ [mmol/kg]} / \text{Active dose [mmol/kg]}) \times (\text{ppm F in liver} \\ \text{minus background})$$

μ molar equivalents of
active in liver The μ molar [μ mol/L] concentration of fluorine containing compound
in liver based on the ppm fluorine in liver normalized to 0.1 mmol/kg
active dose. This assumes that all liver fluorine is derived from the
fluorine-containing component in the formulation. Note: 1 ppm –
1 mg/L
$$= (\text{Normalized ppm [mg/L] fluorine in liver} / \text{Mol Wt F} \\ \text{[mg/mmol]}) \times \text{molar ratio active/F [mmol active/mmol F]} \times \\ 1000 \mu\text{mol/mmol}$$

FACTORS INFLUENCING INTERPRETATION OF KINETIC ANALYSIS

Considerations:

- The data used for kinetic analysis was from a limited screen and extrapolation should be done cautiously.
- Sample size is low
- Analytical data used without validation
- Steady-state not reached
- Terminal phase may not be reached
- Some compounds are mixtures of fluorinated compounds
- Different active and formulation doses were used
- Different vehicles were used to deliver formulations
- Each compound may have very different potency for producing toxicity

Assumptions: (May or may not be justified in all cases)

- Liver fluorine concentrations are linear with respect to dose
- Analytical method is appropriate for all types of compounds
- Elimination kinetics can be determined based on total fluorine rather than on concentrations of individual components
- Background Fluorine is 0.2 ppm
- % F data is the % Fluorine of the active (Fluorine containing component(s) in the formulation)
- Molecular weight is the molecular weight of the active component in the formulation

Data for H- 24019

Given:

Mol Wt. Active (g/mole):	497	% F in Active:	65
Formulation Dose (mg/kg):	10	Mol Wt. F (g/mol):	19
% Active (F Containing) in Formulation:	100		

Calculated Values:

Dose Active (mg/kg):	10	Dose F (mmol/kg):	0.342
Dose Active (mmole/kg):	0.020	Molar Ratio (Active/F):	0.059
Formulation Dose Normalization Factor:	49.7	Dose F (mg/kg):	6.5

Rat Number	Test Day Sample	ppm F in Liver	ppm F in Liver Minus Bkg 0.2 ppm	ppm F in Liver Normalized to 0.1 mmoles/kg Dose	μmolar Equivalents of Active in Liver
Group II					
625320	10	319.4	319.2	1586.42	4910.77
625330	10	355.4	355.2	1765.34	5464.62
625332	10	338.4	338.2	1680.85	5203.08
625341	10	305.0	304.8	1514.86	4689.23
625350	10	322.7	322.5	1602.83	4961.54
Group III					
625321	13	255.9	255.7	1270.83	3933.85
625327	13	275.3	275.1	1367.25	4232.31
625342	13	277.9	277.7	1380.17	4272.31
625345	13	260.4	260.2	1293.19	4003.08
625348	13	275.6	275.4	1368.74	4236.92
Group VI					
625326	94	79.8	79.6	395.61	1224.62
625335	94	89.6	89.4	444.32	1375.38
625346	94	73.8	73.6	365.79	1132.31
625347	94	62.5	62.3	309.63	958.46
625351	94	82.1	81.9	407.04	1260.00

Data for H- 24020

Given:

Mol Wt. Active (g/mole):	426	% F in Active:	69
Formulation Dose (mg/kg):	20	Mol Wt. F (g/mol):	19
% Active (F Containing) in Formulation:	100		

Calculated Values:

Dose Active (mg/kg):	20	Dose F (mmol/kg):	0.726
Dose Active (mmole/kg):	0.047	Molar Ratio (Active/F):	0.065
Formulation Dose Normalization Factor:	42.6	Dose F (mg/kg):	13.8

Rat Number	Test Day Sample	ppm F in Liver	ppm F in Liver Minus Bkg 0.2 ppm	ppm F in Liver Normalized to 0.1 mmoles/kg Dose	μmolar Equivalents of Active in Liver
Group II					
627768	10	182.5	182.3	388.30	1321.01
627774	10	109.0	108.8	231.74	788.41
627776	10	174.0	173.8	370.19	1259.42
627782	10	147.5	147.3	313.75	1067.39
627785	10	165.8	165.6	352.73	1200.00
Group III					
627758	13	61.5	61.3	130.57	444.20
627764	13	40.5	40.3	85.84	292.03
627787	13	33.3	33.1	70.50	239.86
627763	13	51.3	51.1	108.84	370.29
627788	13	45.2	45.0	95.85	326.09
Group VI					
627778	94	1.9	1.7	3.62	12.32
627783	94	1.2	1.0	2.13	7.25
627762	94	1.9	1.7	3.62	12.32
627775	94	1.6	1.4	2.98	10.14
627769	94	1.8	1.6	3.41	11.59

Data for H-23961

Given:

Mol Wt. Active (g/mole):	27500	% F in Active:	40
Formulation Dose (mg/kg):	1000	Mol Wt. F (g/mol):	19
% Active (F Containing) in Formulation:	28		

Calculated Values:

Dose Active (mg/kg):	280	Dose F (mmol/kg):	5.895
Dose Active (mmole/kg):	0.010	Molar Ratio (Active/F):	0.002
Formulation Dose Normalization Factor:	9821.4	Dose F (mg/kg):	112

Rat Number	Test Day Sample	ppm F in Liver	ppm F in Liver Minus Bkg 0.2 ppm	ppm F in Liver Normalized to 0.1 mmoles/kg Dose	μmolar Equivalents of Active in Liver
Group II					
626871	10	19.6	19.4	190.54	17.32
626872	10	22.8	22.6	221.96	20.18
626875	10	20.8	20.6	202.32	18.39
626882	10	17.1	16.9	165.98	15.09
626888	10	23.9	23.7	232.77	21.16
Group III					
626868	13	15.7	15.5	152.23	13.84
626879	13	13.4	13.2	129.64	11.79
626886	13	12.4	12.2	119.82	10.89
626887	13	14.3	14.1	138.48	12.59
626891	13	11.8	11.6	113.93	10.36
Group VI					
626866	94	11.3	11.1	109.02	9.91
626869	94	2.5	2.3	22.59	2.05
626874	94	1.6	1.4	13.75	1.25
626893	94	2.4	2.2	21.61	1.96
626896	94	2.8	2.6	25.54	2.32