MASTER FILE
Project No. MO-85 9170

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INTRODUCTION

Because the octanol/water partition coefficient indicated that Santicizer 160 has a moderate potential to accumulate in fish and because it is currently under government scrutiny, a fish bioconcentration study was conducted. This study estimates the potential for S-160 to accumulate in fish in the natural environment. The rate and extent of bioconcentration of ^{14}C-residues were measured in bluegill continuously exposed to ^{14}C-labeled S-160 during a definitive 17-day exposure period, a preliminary/kinetic 7-day exposure period and two kinetic 7-day exposure periods. Relative distributions of the residue between wholefish, muscle, and viscera were determined for the fish samples collected during the 17-day definitive study. Following exposure, the rate and extent of clearance or depuration of the residue from the fish were examined after placing exposed fish in a clean water aquarium.

SUMMARY

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coefficient (510). This difference may be [illegible] fish. Based on a BCF of 29 in muscle, the edible portion of the fish, S-160 has a negligible potential to accumulate. The BCF in viscera, although somewhat higher than in wholefish and muscle, is of little concern because the ^{14}C-residue probably represents S-160 metabolites or S-160 adsorbed on food in the gastrointestinal tract.

MATERIALS AND METHODS

Procedures used in the bioconcentration study closely followed those outlined in the ASTM Proposed Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs Draft No. 9 (April 13, 1979). All deviations from these procedures were noted in the following sections.

I. Test Fish

Bluegill (Lepomis Macrochirus) used in the study were received from Osage Catfisheries in Missouri on July 26, 1979. All test fish were held in a culture tank at 18°C and observed for at least 14 days prior to testing. During this period, the fish received a standard fish food (Purina No. 3) ad libitum. Throughout the study, the fish were fed once a day in the morning. On sampling days, the fish were fed after collecting the samples. Mortality during the fourteen days prior to the initiation of the test was less than 1% indicating that the fish were in good condition. Fish were acclimated to the test water temperature of 22°C prior to starting the test. The mean weight for the fish used in this test was 0.915 g.

II. Test System

A continuous flow diluter system described by W. J. Adams, et al., ES-78-SS-21, was used to deliver water to the control aquarium and water plus three nominal concentrations of S-160 (2.22, 22.2, and 222 µg/l) to the test aquaria. Dimethylformamide (DMF) was used as a carrier solvent for S-160. The S-160/DMF stocks for 2.22, 22.2, and 222 µg/l contained 6.66 mg/l, 66.6 mg/l, and 666 mg/l, respectively. They were pumped at rates of 5.2 ml/hr, 2 ml/hr, and 2 ml/hr, respectively, to the mixing cell just beneath the surface of the water. Diluent water, dechlorinated city water (Table 1), entered the mixing cell at rates of 15.6 l/hr, 6 l/hr, and 6 l/hr, respectively. Water and S-160 flowed continuously and were mixed by magnetic stirrers placed under each mixing cell. Water flowed directly from the mixing chamber through glass tubing to glass test aquaria containing 100 l, 30 l, and 30 l, respectively. Flow rates allowed 3.74 tank volume replacements per day for the 2.22 µg/l concentration and 4.8 tank volume replacements per day for the 22.2 µg/l and 222 µg/l test concentrations. The volumes used during exposure allowed loading equal to 1.4 fish/l H_2O.

For the preliminary study, 2.22 µg/l exposure concentration, dilution water flowed at 6 l/hr and toxicant was pumped at 2 ml/hr. There were 4.8 tank volume replacements per day in the 30 liter aquarium. The loading was equal to 1.4 fish/l H_2O as in the definitive and kinetic studies.

III. Test Compound

Ten vials of ^{14}C-labeled S-160 with a total activity of 882.02 µCi, were received from H. Yepez, MIC Applied Sciences on August 7, 1979. Each flask contained 2.0 to 3.6 mg of S-160. The specific activity was 9.5 mCi/mM. All stocks were prepared using DMF. The cold S-160 used in the stocks was Lot No. QK05511. The amount of ^{14}C-labeled S-160 and cold S-160 in each stock is listed below in µCi/l and mg/l, respectively. The ^{14}C-S-160 was uniformly ring labeled on the phthalic ring. The radiochemical purity was 97%. (Hines and Kaelble, 1979).

Exposure Concentration	Stock Concentration mg/l	µCi/l ^{14}C-Labeled S-160	mg/l Cold S-160
2.22	6.66	202.52	0
22.2	66.6	202.52	59.94
222	666	202.52	659.34

IV. Procedure

The preliminary test began on August 8, 1979, by exposing twenty-four bluegill to 2.22 µg/l ^{14}C-labeled S-160 in an aquarium that was equilibrated at 2.22 µg/l. The S-160 concentration in the water was checked on Day 0, August 8, 1979. The concentration of DMF did not exceed 0.33 mg/l, (0.033%). Fish and water were sampled and analyzed for S-160

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based on ^{14}C-measurements over a seven day uptake and 14 day depuration period. Results are summarized in Tables 2 and 3.

The 17 day definitive test (2.22 µg/l) and two 7 day kinetic studies (22.2 and 222 µg/l) began on August 13, 1979. In all 3 tests, the concentration of DMF did not exceed 0.33 ml/l (0.033%). One hundred-twenty fish were placed in the definitive test aquarium. Twenty-four fish were placed into each of the 2 kinetic test aquaria. Fish and water were sampled during the test to monitor the S-160 exposure, accumulation and depuration, based on ^{14}C-residue. Tables 4,5,6,7,8 and 9 summarize the results of water and tissue analysis for S-160 concentration in the definitive, kinetic 1, and kinetic 2 tests, respectively.

V. Sample Schedule

Sampling times were determined based on the predicted time to steady state (SS). Using the octanol/water partition coefficient and the water solubility, an approximate time to steady state (equilibrium) was calculated using the following two formulas: 1) S=3.0/antilog (0.43 log W-2.11); 2) S=3.0/antilog (-0.414 log P + 0.122) where S= time to steady state in hours, W= water solubility, and P= octanol/water partition coefficient (Hamelink and Eaton 1979).

The following sampling points were chosen to monitor fish bioaccumulation.

Concentration	Sampling Points
2.22 Definitive	Uptake 0.5, 1,2,4,7,14,16,18,21 days Depuration 1,2,4,7,14,18,21 days
2.22 Preliminary	Uptake 1,4,7, days Depuration 1,4,7,14 days
22.2 Kinetic 1	Uptake 1,4,7, days Depuration 1,4,7,14 days
222 Kinetic 2	Uptake 1,4,7, days Depuration 1,4,7,14 days

During the definitive study, 6 fish were collected at each sampling time. Fish collected were divided randomly into 2 groups of three fish. One group was analyzed as wholefish. The second group was dissected and muscle and viscera samples were analyzed. Muscle samples were obtained by removing the head, tail, and viscera. Viscera included all organs in the gut region including gastrointestinal track, kidneys, spleen gall bladder, and liver. All samples (wholefish, muscle, and viscera) were weighed to obtain wet weight, placed in marked petri dishes, and dried at 90°C.

For the preliminary test and two kinetic tests, three fish were collected at each sampling time (except day 21 of each test when 5,6, and 4 fish

were removed from the preliminary, kinetic 1 and kinetic 2 tests, respectively). All samples were analyzed individually as wholefish. During uptake, two 2 ml water samples were taken for radioassay at each sampling time. Water samples were also collected on day 0 for all tests and on day 11 for the definitive test. Water samples were placed in scintillation vials containing 16 ml of instagel scintillation cocktail and counted for 10 minutes in a Nuclear Chicago Isocap 300 scintillation counter.

VI. Residue Analysis for Water Samples

Verification of the actual concentration of S-160 in the test aquaria water was determined on day 7, August 20, 1979, by taking two 100 ml grab samples of water from the aquarium and extracting them one time each with 10 ml of nanograde hexane. Hexane extracts were analyzed by the gas chromatographic procedures for analysis of phthalates outlined by Hicks, et al., in ES-78-SS-8.

VII. Residue Analysis for Fish Tissue Samples

Verification of the actual concentration of S-160 in the wholefish tissue samples was made by sampling fish during steady state on day 17, September 3, 1979. Six fish were collected, weighed, placed on a marked petri dish and frozen. Analysis is currently in progress and results will be reported in Environmental Sciences Special Study ES-80-SS-5.

VIII. Radioassay

Triplicate dried samples of viscera, muscle, and wholefish were placed in combusto cones™ together with 0.30 ml of Combustaid™ and were combusted in a Packard Model B306 Tri-Carb™ sample oxidizer. The resulting $^{14}CO_2$ was trapped in Carbo-sorb®, flushed with Permaflour® V into a counting vial, and counted for 10 minutes in the scintillation counter. Oxidizer, chemicals, and supplies were purchased from Packard Instrument Company. Recovery of ^{14}C was 92.8% to 100.8% based on analysis of standards. Memory (^{14}C carryover to next combusted sample) ranged from 0.011% to 0.031%.

IX. Example Calculations

Computer printout for the scintillation counter gave disintegrations per minute (DPM), corrected for efficiency, and micrograms of S-160 The following equation was used to calculate the water concentration.

$$\mu g\ ^{14}C\text{-S-160/2 ml} \times 1000\ ml/l \times \text{factor} = \mu g\ S\text{-160/l}$$

The factor was based on the mix of hot material to cold material in each stock. The 2.22 µg/l concentration contained only ^{14}C-labeled material; therefore, the factor was 1. The 22.2 and 222 µg/l concentrations contained 10:1 and 100:1 ratios of cold S-160: hot S-160, therefore, the factors were 10 and 100, respectively. Calculations

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for tissue samples used the following equation:

$$\mu g\ ^{14}C\text{-S-160/sample} \times 1/\text{sample weight (mg)} \times 1 \times 10^6 \times \text{Factor} = \mu g\ \text{S-160/kg fish tissue}$$

The same factors were used as for the water sample calculations.

RESULTS AND DISCUSSION

During the uptake portion of the study, the average exposure concentration for each test was as follows:

Concentration	$\bar{X}$ Exposure	Range	Wholefish BCF
2.22 Definitive	2.96 µg/l	2.30→4.19	187.65
2.22 Preliminary	2.62 µg/l	2.23→2.82	135.40
22.2 Kinetic	23.11 µg/l	3.2 →35.3	92.15
222 Kinetic	208.08 µg/l	26→278	96.53

The exposure concentrations were verified using gas chromatography. These results are listed in Table 10 along with the radioanalysis concentrations measured on the same day. Fluctuations in exposure concentrations coincided with malfunctions of the manostat pump. On August 17, day 4, the exposure concentration of S-160 in the definitive test water dropped to 0.72 µg/l. The concentration of S-160 in the fish sampled that day was significantly lower than on previous sample days (61.70 µg/kg fish vs. 1191.04 µg/kg fish). In calculating the BCF and other parameters, day 4 was considered to be day 0 of uptake. The Biofac program was used to calculate the BCF for both sets of data, day 0→21 and day 4(0)→21(17). The derived BCFs were similar, 141.53 and 187.65 for wholefish.

The oxygen, pH, alkalinity, hardness, and temperature ranged from 2.4 to 7.0 mg/l, 6.6 to 7.0, 18 to 30 mg/l, 105 to 120 mg/l, and 22° to 23°C, respectively. These values indicate the water quality was satisfactory throughout the study.

The survival of the controls was excellent. There were no mortalities during the test in the controls or any of the test aquaria.

For the bluegill bioconcentration study with S-160, 17 days was a sufficient amount of time for S-160 to reach steady state in the fish tissues. This exposure period was selected based on the aqueous solubility and the octanol/water partition coefficient for S-160 and a 7 day preliminary exposure of bluegills to 2.22 µg/l S-160. Two additional 7 day studies at concentrations of 22.2 and 222 µg/l were conducted to assess the effect of exposure concentration on bioconcentration of S-160.

Analysis of the wholefish, muscle, and viscera were used to calculate BCF, K_1, K_2, $T_{\frac{1}{2}}$ clearance, and time to 90% SS by entering the derived values in the computer using the Dow Biofac program (Blau and Agin, 1978). All values are listed in the table below. The Biofac program graphs the data, calculates a

for tissue samples used the following equation:

$$\mu g\ ^{14}C\text{-}S\text{-}160/sample \times \frac{1/sample\ weight\ (mg) \times 1 \times 10^6}{\mu g\ S\text{-}160/kg\ fish\ tissue} \times Factor =$$

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best fit line as well as giving the above parameters. An example of the graph, Figures 1, 2, 3, 4, 5 and 6 and printout of data, Figures 7 and 8 are included. Furthermore, the Biofac program can determine rate constants and calculate a BCF from short term exposure data. As a result, kinetic tests can be run at lower cost and can be used to predict BCFs for different exposure concentrations. The rate constants, K_1 and K_2, allow prediction of the concentration of a chemical in fish as related to exposure time and concentration, as well as, how quickly the fish will clear the chemical when exposed to clean water.

Study	Exposure ppb	BCF	K_1	K_2	90% SS	T½ Days
17 Day Def. W.F.	2.22	187.65	143.40	0.76	3.01	0.91
17 Day Def. Mus.	2.22	28.54	17.38	0.61	3.78	1.14
17 Day Def. Vis.	2.22	1693.25	1750.19	1.03	2.23	0.67
7 Day Prelim. W.F.	2.22	136.40	315.07	2.33	0.99	0.30
7 Day Kin 1 W.F.	22.2	92.15	179.09	1.94	1.18	0.36
7 Day Kin 2 W.F.	222	96.53	224.14	2.32	0.99	0.30

K_1 is a rate constant characterizing the uptake of chemical from water by fish in parts water/day/parts fish.

K_2 is a rate constant characterizing the clearance of chemical from fish in day^{-1} units.

The hand calculated steady state BCFs for the 17 day definitive study (189.70 for wholefish, 28.26 for muscle and 1853.26 for viscera) agreed well with the BCFs calculated using the Biofac program. Because of speed, accuracy and the additional information obtained, the Dow Biofac computer program, was used for analysis of the bioconcentration data.

Based on the results of the wholefish analysis, S-160 has a low potential to bioconcentrate in fish. The observed BCF is considerably less than that predicted from the octanol/water partition coefficient (510). This difference may be attributed to metabolism of S-160 by the fish. In the muscle, the consumed portion of the fish, the bioconcentration potential is negligible. The maximum observed concentration in the muscle (121.66 µg/kg) is also insignificant and well below health standards. Although the BCF for viscera was higher than in wholefish and muscle, it is of low concern because most of the ^{14}C-residue found is presumed to be metabolized S-160 or adsorbed on food in the gastrointestinal tract. There was a good correlation between the wholefish BCF of the preliminary test and definitive test. This may allow us to use short term kinetic tests to estimate the bioconcentration potential of other chemicals. The two kinetic tests indicated there was a tendency for the S-160 bioconcentration factor to decrease as the exposure concentration increased.

The BCF calculated at the MIC laboratory (188) is significantly different from a BCF previously calculated at the EG&G Bionomics laboratory in Wareham, Mass. (663) (Barrow et.al.). A greater amount of radiochemical impurities in the

S-160 used in the Bionomics study may have been responsible for this difference. These impurities may have been adsorbed or metabolized more readily by the fish, resulting in a higher apparent S-160 bioconcentration in the Bionomics study. Presently, we are looking more closely at the variables associated with each study in order to explain this discrepancy.

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Table 1. Average water quality characteristics of the dilution (city) water.

Characteristic	City Water Measurement
Alkalinity (mg/l $CaCO_3$)	41
Hardness (mg/l $CaCO_3$)	130
pH (median)	6.90
Aluminum (mg/l Al)	0.031
Amonia-total (mg/l N)	0.35
Amonia-unionized (mg/l NH_4)	<0.001
Antimony (mg/l Sb)	0.010
Barium (mg/l B)	0.021
Beryllium (mg/l Be)	<0.001
Cadmium (mg/l Cd)	0.002
Calcium (mg/l Ca)	55.8
Chloride (mg/l Cl^-)	30.5
Chromium (mg/l Cr)	0.008
Cobalt (mg/l Co)	0.002
Copper (mg/l Cu)	0.004
Fluoride (mg/l F^-)	0.98
Iron (mg/l Fe)	0.025
Lead (mg/l Pb)	0.013
Magnesium (mg/l Mg)	2.80
Manganese (mg/l Mn)	0.001
Molybdenum (mg/l Mo)	0.009
Nickel (mg/l Ni)	0.031
Nitrate + Nitrite (mg/l N)	0.64
Phosphorous (mg/l P)	0.010

Table 1 (Continued)

Characteristic	City Water Measurement
Silicon (mg/l Si)	6.3
Silver (mg/l Ag)	0,005
Sodium (mg/l Na)	83.2
Sulfate (mg/l SO4)	175.4
Strontium (mg/l Si)	0.16
Tin (mg/l Sn)	0.007
Titanium (mg/l Ti)	0.001
Total Organochlorine (ug/l)	≤0.5
Total Organophosphorous (ug/l)	≤0.05
Vanadium (mg/l V)	0.070
Zinc (mg/l Zn)	0.016

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TABLE 2. MEASURED ^{14}C-RESIDUES CALCULATED AS S-160 IN WATER DURING A 7-DAY (PRELIMINARY) CONTINUOUS EXPOSURE OF BLUEGILLS TO A NOMINAL CONCENTRATION OF 2.22 µg/l.

Day	Sample #	Exposure Concentration µg/l Result	Mean
0 Before fish	1	2.37	2.41
	2	2.44	
0.17	1	2.23	2.27
	2	2.30	
1	1	2.53	2.67
	2	2.81	
1.17	1	2.67	2.75
	2	2.82	
4	1	2.81	3.00
	2	3.19	
7*	1	0.31	0.32
	2	0.33	
		$\bar{X}$ = 2.62	
		S = 0.30	

*Day 7 values were not included in calculating the mean because of manostat malfunction.

TABLE 3. MEASURED ^{14}C-RESIDUE AS S-160 IN BLUEGILL DURING A 7-DAY PRELIMINARY CONTINUOUS EXPOSURE OF 2.22 µg/l (DAYS 1-7) AND DURING ELIMINATION (DAYS 9-21)

Day	Fish #	µg/kg	Mean
1	1	313.23	306.70
	2	287.01	
	3	319.86	
4	1	368.37	441.29
	2	543.15	
	3	412.36	
7	1	267.71	267.32
	2	198.14	
	3	336.12	
8	1	28.95	40.85
	2	45.63	
	3	47.96	
11	1	9.90	11.84
	2	14.34	
	3	11.29	
14	1	9.83	23.73
	2	30.42	
	3	30.95	
21	1	7.72	11.68
	2	27.10	
	3	5.75	
	4	10.35	
	5	7.49	

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8	1	28.95	40.85
	2	45.63	
	3	47.96	
11	1	9.90	11.84
	2	14.34	
	3	11.29	
14	1	9.83	23.73
	2	30.42	
	3	30.95	
21	1	7.72	11.68
	2	27.10	
	3	5.75	
	4	10.35	
	5	7.49	

TABLE 4. MEASURED ^{14}C-RESIDUES CALCULATED AS S-160 IN WATER DURING A 17-DAY (DEFINITIVE) CONTINUOUS EXPOSURE OF BLUEGILLS TO A NOMINAL CONCENTRATION OF 2.22 µg/l

Day	Sample #	Exposure Concentration µg/l Result	Mean
0 Before fish	1	5.81	6.34
	2	6.87	
0 After fish were added	1	6.06	5.93
	2	5.80	
0 Adjusted H_2O flow	1	6.13	6.00
	2	5.86	
0.5	1	5.77	5.69
	2	5.61	
1	1	6.14	6.05
	2	5.95	
2	1	7.07	7.27
	2	7.46	
4 (0)	1	0.73	0.72
	2	0.70	
7 (3)	1	4.06	4.13
	2	4.19	
11 (7)	1	2.52	2.62
	2	2.72	
14 (10)	1	2.59	2.59
	2	2.59	
16 (12)	1	2.30	2.49
	2	2.67	
18 (14)	1	3.17	3.04
	2	2.90	
21 (17)	1	2.84	2.88
	2	2.92	

$\bar{X}$ = 2.96
S = 0.59

The values for the days in parentheses were used to calculate the BCF. The mean is calculated for those days only.

TABLE 6. MEASURED ^{14}C-RESIDUES AS S-160 IN BLUEGILL DURING A 17-DAY DEFINITIVE CONTINUOUS EXPOSURE OF 2.22 ug/l (DAYS 0.5-21) AND DURING ELIMINATION (DAYS 22-42).

Day	Wholefish	Mean	Muscle	Mean	Viscera	Mean
0.5	535.04	505.89	71.57	64.98	7519.94	8490.66
	531.68		51.03		5612.81	
	450.95		72.33		12339.23	
1	765.07	858.82	82.53	91.59	11147.33	10728.37
	809.84		94.47		*	
	1001.54		97.78		10309.40	
2	1193.97	1191.04	126.64	220.77	11026.45	11247.04
	1009.26		268.14		*	
	1369.89		267.53		11465.63	
4 (0)	40.14	61.70	14.35	17.70	272.25	239.27
	35.68		10.77		131.22	
	109.31		27.99		314.35	
7 (3)	480.36	659.16	85.43	121.66	1888.38	5670.37
	409.24		179.33		7148.07	
	487.87		100.22		7974.66	
14 (11)	503.68	601.29	69.60	65.52	9262.66	7169.97
	661.84		51.44		6900.18	
	638.36		*		5347.07	
16 (12)	526.25	558.34	143.48	91.99	3496.81	3987.55
	520.39		60.21		2472.13	
	628.38		72,28		5993.70	
18 (14)	454.67	550.50	73.28	60.50	3139.39	3659.69
	572.38		50.14		3687.78	
	624.46		58.08		4151.91	
21 (17)	587.21	638.25	69.16	78.65	7990.98	6940.62
	645.02		92.87		*	
	682.53		73,92		5890.26	
22 (18)	131.97	196.34	25.46	36.93	750.85	875.03
	358.73		44.27		566.12	
	98.32		41.05		1318.12	
23 (19)	39.86	69.46	14.92	21.06	69.97	209.20
	93.90		28,43		134.92	
	44.62		19.82		423.11	
25 (21)	46.63	54.10	25.63	24.21	64.86	54.46
	30.77		31.99		66.19	
	84.91		15.00		32,34	
28 (24)	36.96	35.82	20.75	18.26	38.06	41.16
	40.96		16.05		40.31	
	39.52		17.99		45,07	
35 (31)	27.06	29,91	14.29	17.02	*	29,56
	13.97		21.93		28.58	
	48.70		14.85		24,54	
39 (35)	*	29.88	*	10.58	11.00	16.83
	29.56		8.91		22.65	
	30.20		12.25		*	
42 (38)	29.13	26.59	6.42	9.75	*	33.96
	24.72		14.37		*	
	22.93		8.46		33.96	

*No Sample

Values for days in parentheses were used to calculate the BCF.

TABLE 5. MEASURED ^{14}C-RESIDUES AS S-160 IN BLUEGILL DURING A 17-DAY DEFINITIVE CONTINUOUS EXPOSURE OF 2.22 ug/l (DAYS 0.5-21) AND DURING ELIMINATION (DAYS 22-42).

Day	Wholefish	Mean	Muscle	Mean	Viscera	Mean
0.5	635.04	505.89	71.57	64.98	7519.94	8490.66
	531.68		51.03		5612.81	
	450.95		72.33		12339.23	
1	766.07	858.82	82.53	91.59	11147.33	10728.37
	809.84		94.47		*	
	1001.54		97.78		10309.40	
2	1193.97	1191.04	128.64	220.77	11028.45	11247.04
	1009.26		268.14		*	
	1369.89		267.53		11466.63	
4 (0)	40.14	61.70	14.35	17.70	272.25	239.27
	35.66		10.77		131.22	
	109.31		27.99		314.35	
7 (3)	480.36	459.16	85.43	121.66	1888.38	5670.37
	409.24		179.33		7148.07	
	487.87		100.22		7974.66	
14 (11)	503.68	601.29	69.60	65.52	9262.66	7169.97
	661.84		61.44		6900.18	
	638.36		*		5347.07	
16 (12)	526.25	558.34	143.48	91.99	3495.81	3987.55
	520.39		60.21		2472.13	
	628.38		72.28		5993.70	
18 (14)	454.67	550.50	73.28	60.50	3139.39	3659.69
	572.38		50.14		3687.78	
	624.46		58.08		4151.91	
21 (17)	587.21	638.25	69.16	78.65	7990.98	6940.62
	645.02		92.87		*	
	682.53		73.92		5890.26	
22 (18)	131.97	196.34	25.46	36.93	750.85	875.03
	358.73		44.27		566.12	
	98.32		41.05		1318.12	
23 (19)	39.86	59.46	14.92	21.06	69.97	209.20
	93.90		28.43		134.52	
	44.62		19.82		423.11	
25 (21)	46.63	54.10	25.63	24.21	64.86	64.46
	30.77		31.99		66.19	
	84.91		15.00		32.34	
28 (24)	36.98	35.82	20.75	18.26	38.06	41.15
	40.96		16.05		40.31	
	39.52		17.99		45.07	
35 (31)	27.06	29.91	14.29	17.02	*	29.56
	13.97		21.93		29.58	
	48.70		14.85		24.54	
39 (35)	*	29.88	*	10.58	11.00	16.83
	29.56		8.91		22.65	
	30.20		12.25		*	
42 (38)	29.13	25.59	6.42	9.75	*	33.96
	24.72		14.37		*	
	22.93		8.46		33.96	

*No Sample

Values for days in parentheses were used to calculate the BCF.

TABLE 6. MEASURED ^{14}C-RESIDUES CALCULATED AS S-160 IN WATER DURING A 7-DAY (KINETIC 1) CONTINUOUS EXPOSURE OF BLUEGILLS TO A NOMINAL CONCENTRATION OF 22.2 µg/l.

Day	Sample #	Exposure Concentration µg/l Result	Mean
0 Before fish	1	30.0	29.2
	2	28.4	
0 After fish were added	1	35.3	34.6
	2	34.0	
2	1	3.2	3.7
	2	4.2	
2.33	1	23.0	23.4
	2	23.8	
4	1	16.3	15.2
	2	14.0	
7	1	32.1	32.6
	2	33.0	

$\bar{X}$ = 23.11
S = 11.30

TABLE 7. MEASURED ^{14}C-RESIDUES CALCULATED AS S-160 IN BLUEGILL DURING A 7-DAY (KINETIC 1) CONTINUOUS EXPOSURE OF 22.2 µg/l AND DURING ELIMINATION (DAYS 8-21)

Day	Wholefish	Mean
1	2043.15 1733.44 2498.25	2091.61
4	1641.37 1670.68 1785.82	1699.29
7	1627.54 3694.81 1527.28	2283.21
8	789.64 394.82 268.74	484.40
11	534.64 139.32 106.14	260.03
14	96.86 103.98 186.53	129.12
21	39.19 132.43 61.82 172.41 63.65 53.43	87.16

TABLE 7. MEASURED ^{14}C-RESIDUES CALCULATED AS S-160 IN BLUEGILL DURING A 7-DAY (KINETIC 1) CONTINUOUS EXPOSURE OF 22.2 µg/l AND DURING ELIMINATION (DAYS 8-21)

Day	Wholefish	Mean
1	2043.15 1733.44 2498.25	2091.61
4	1641.37 1670.68 1785.82	1699.29
7	1627.54 3694.81 1527.28	2283.21
8	789.64 394.82 268.74	484.40
11	534.64 139.32 106.14	260.03
14	96.86 103.98 186.53	129.12
21	39.19 132.43 61.82 172.41 63.65 53.43	87.16

TABLE 8. MEASURED ^{14}C-RESIDUES CALCULATED AS S-160 IN WATER DURING A 7-DAY (KINETIC 2) CONTINUOUS EXPOSURE OF BLUEGILLS TO A NOMINAL CONCENTRATION OF 222 µg/l.

Day	Sample #	Exposure Concentration µg/l Result	Mean
0 Before fish	1	265	268
	2	271	
0 After fish	1	278	272
	2	265	
2	1	26	29
	2	31	
2.33	1	172	174
	2	175	
4	1	246	246
	2	245	
7	1	267	262
	2	256	

$\bar{X}$ = 208.08
S = 90.86

TABLE 9. MEASURED ^{14}C-RESIDUES CALCULATED AS S-160 IN BLUEGILL TISSUES DURING A 7-DAY (KINETIC 2) CONTINUOUS EXPOSURE OF 222 µg/l AND DURING ELIMINATION (DAYS 8-21).

Day	Wholefish	Mean
1	19075.41 18023.06 25353.05	20817.17
4	20843.32 20925.46 15691.61	19153.43
7	18091.65 19428.64 17791.45	18439.25
8	1840.37 4289.24 6065.12	4064.91
11	954.74 530.72 396.03	627.16
14	619.37 720.03 371.13	570.18
21	224.42 384.90 1528.99 257.16	598.87

TABLE 9. MEASURED ^{14}C-RESIDUES CALCULATED AS S-160 IN BLUEGILL TISSUES DURING A 7-DAY (KINETIC 2) CONTINUOUS EXPOSURE OF 222 µg/l AND DURING ELIMINATION (DAYS 8-21).

Day	Wholefish	Mean
1	19075.41 18023.06 25353.05	20817.17
4	20843.32 20925.45 15691.61	19153.43
7	18091.65 19428.64 17791.45	18439.25
8	1840.37 4289.24 6065.12	4064.91
11	954.74 530.72 396.03	627.16
14	619.37 720.03 371.13	570.18
21	224.42 384.90 1528.99 257.16	598.87

TABLE 10. CONCENTRATIONS OF S-160 DETERMINED FOR THE EXPOSURE WATER OF THREE BLUEGILL BIOCONCENTRATION STUDIES ON DAY 7. ANALYSES WERE PERFORMED BY GAS CHROMATOGRAPHY AND BETA SPECTROSCOPY.

Exposure Concentration µg/l			
Nominal	Study	Measured	Radioanalysis
2.22	17-Day Definitive	2.29 1.75	4.06 4.19
22.2	7-Day Kinetic 1	14.9 14.9	32.1 33.0
222	7-Day Kinetic 2	85.4 92.7	267 256

FIGURE 1. BIOFAC COMPUTER PROGRAM GRAPH OF 17-DAY DEFINITIVE WHOLEFISH BIOCONCENTRATION

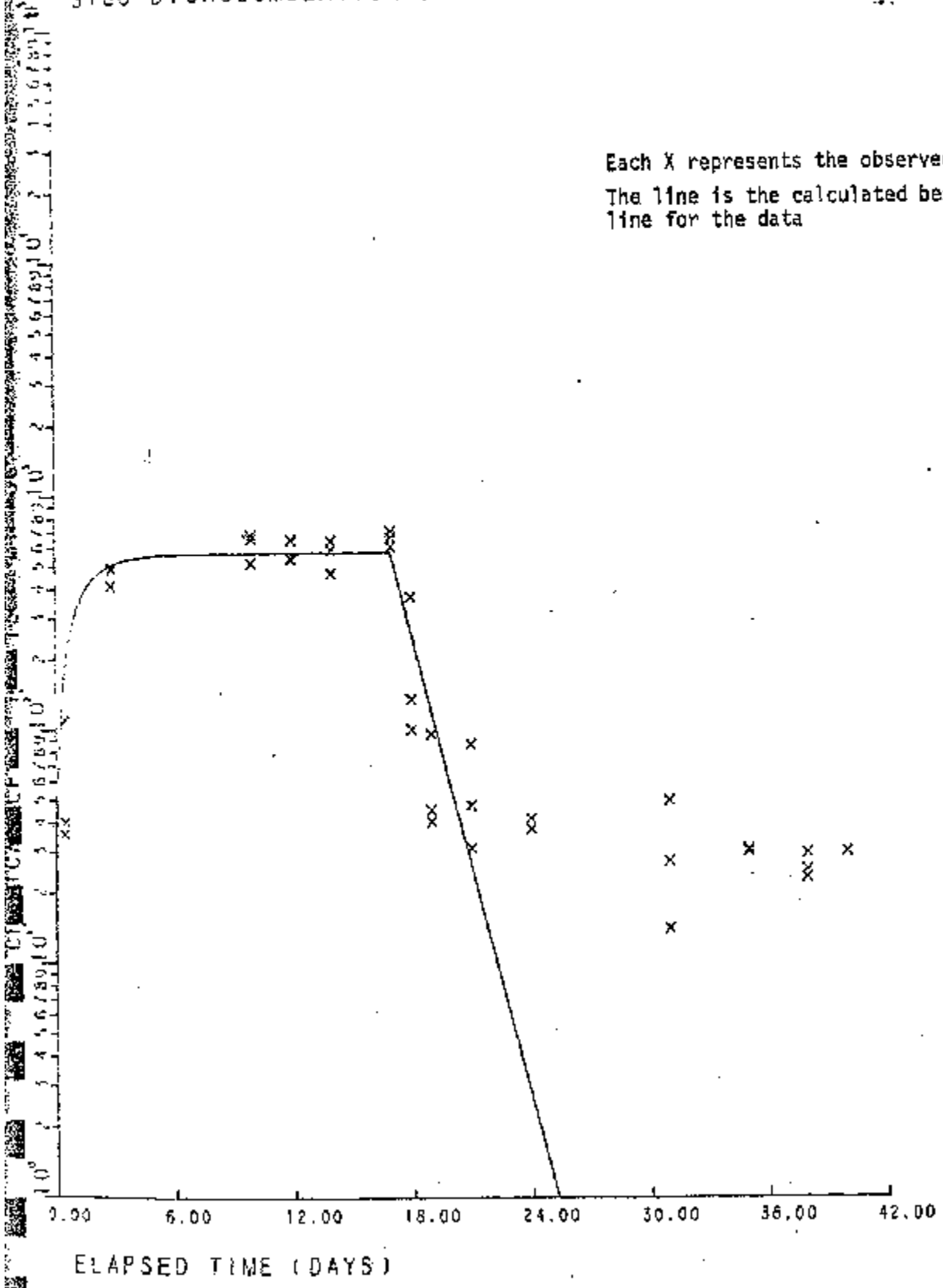


FIGURE 1. BIOFAC COMPUTER PROGRAM GRAPH OF 17-DAY DEFINITIVE WHOLEFISH BIOCONCENTRATION

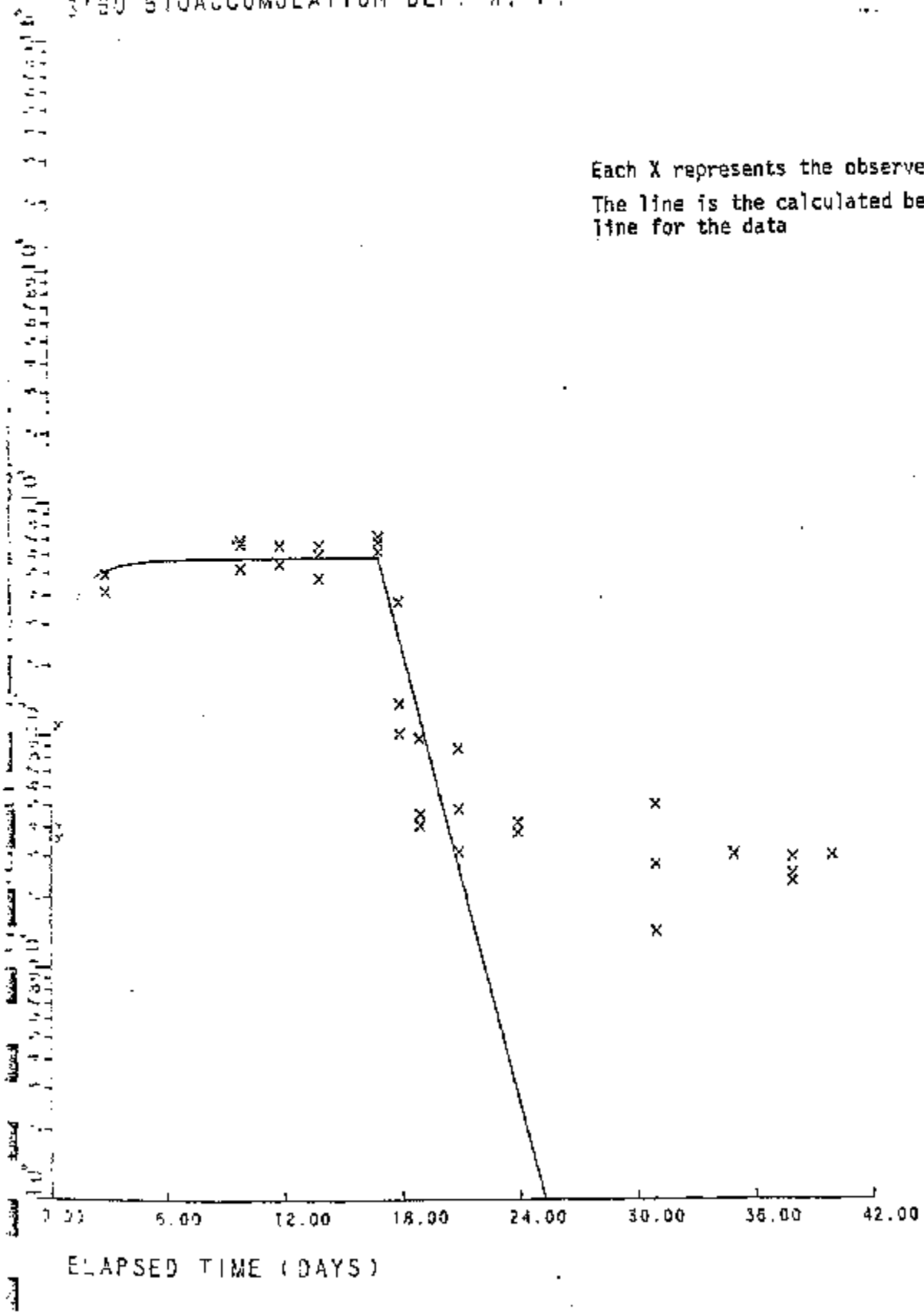


FIGURE 2. BIOFAC COMPUTER PROGRAM GRAPH OF 17-DAY DEFINITIVE MUSCLE BIOCONCENTRATION STUDY

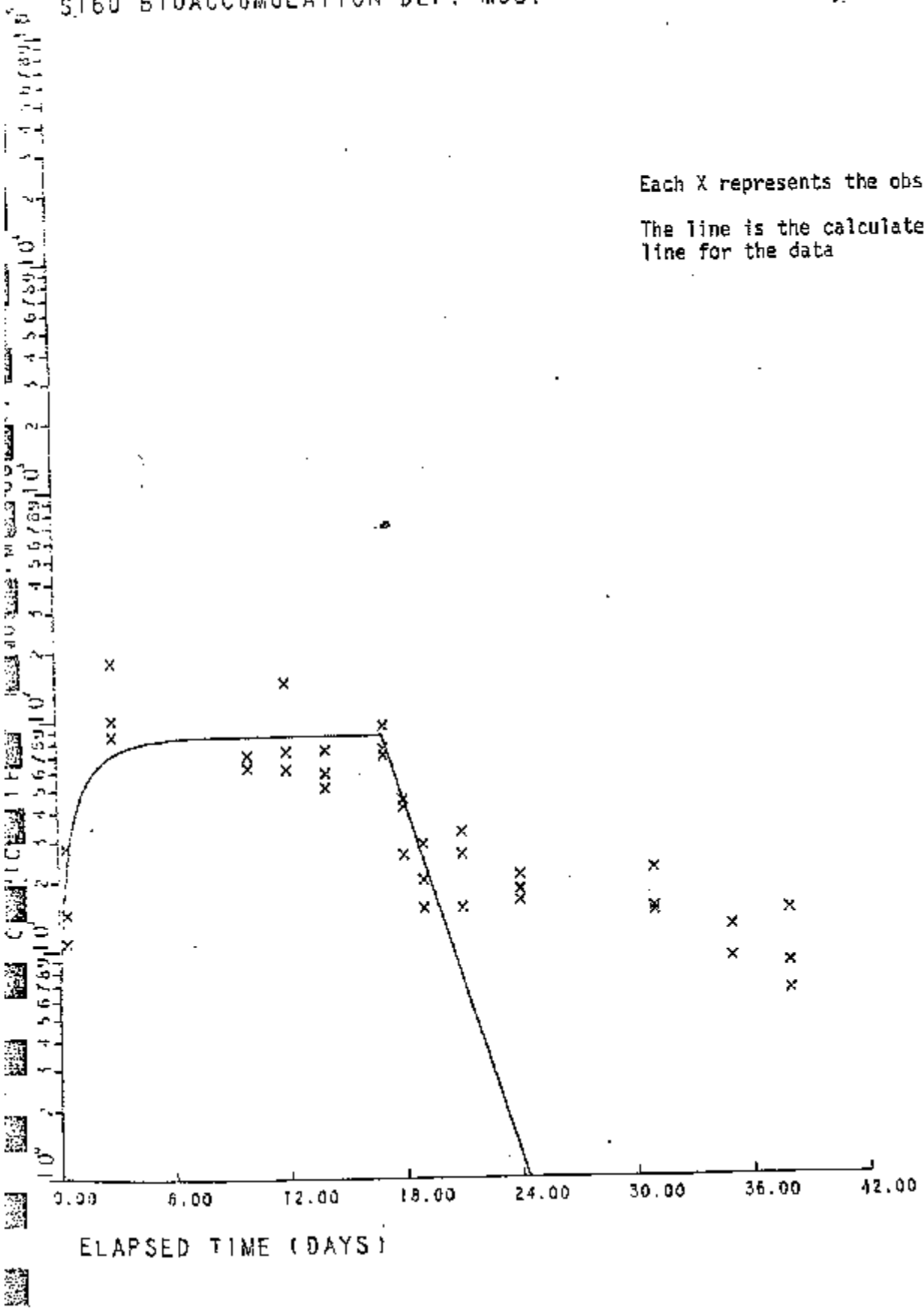


FIGURE 3. BIOFAC COMPUTER PROGRAM GRAPH OF 17-DAY DEFINITIVE VISCERA BIOCONCENTRATION STUDY

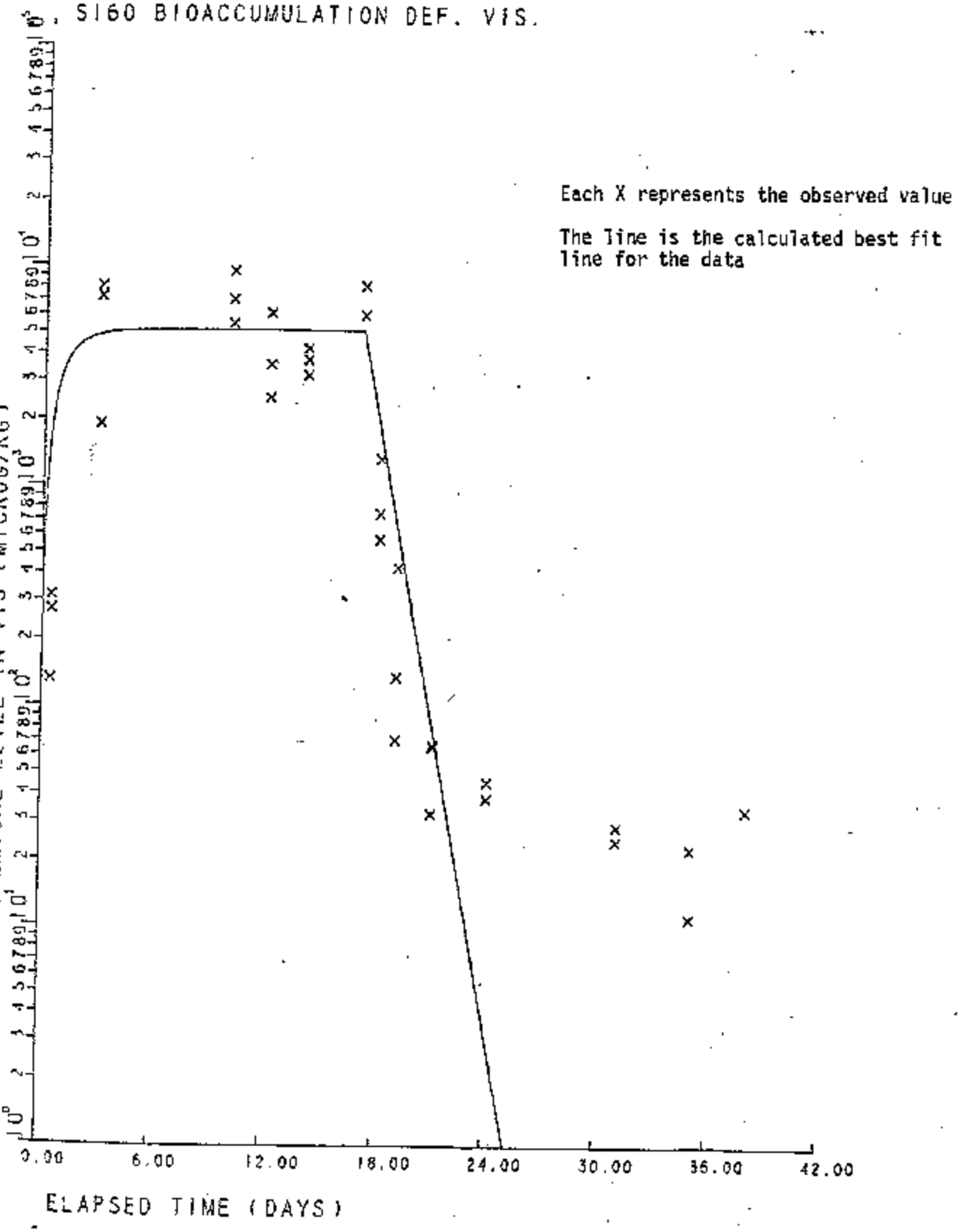


FIGURE 3. BIOFAC COMPUTER PROGRAM GRAPH OF 17-DAY DEFINITIVE VISCERA BIOCONCENTRATION STUDY

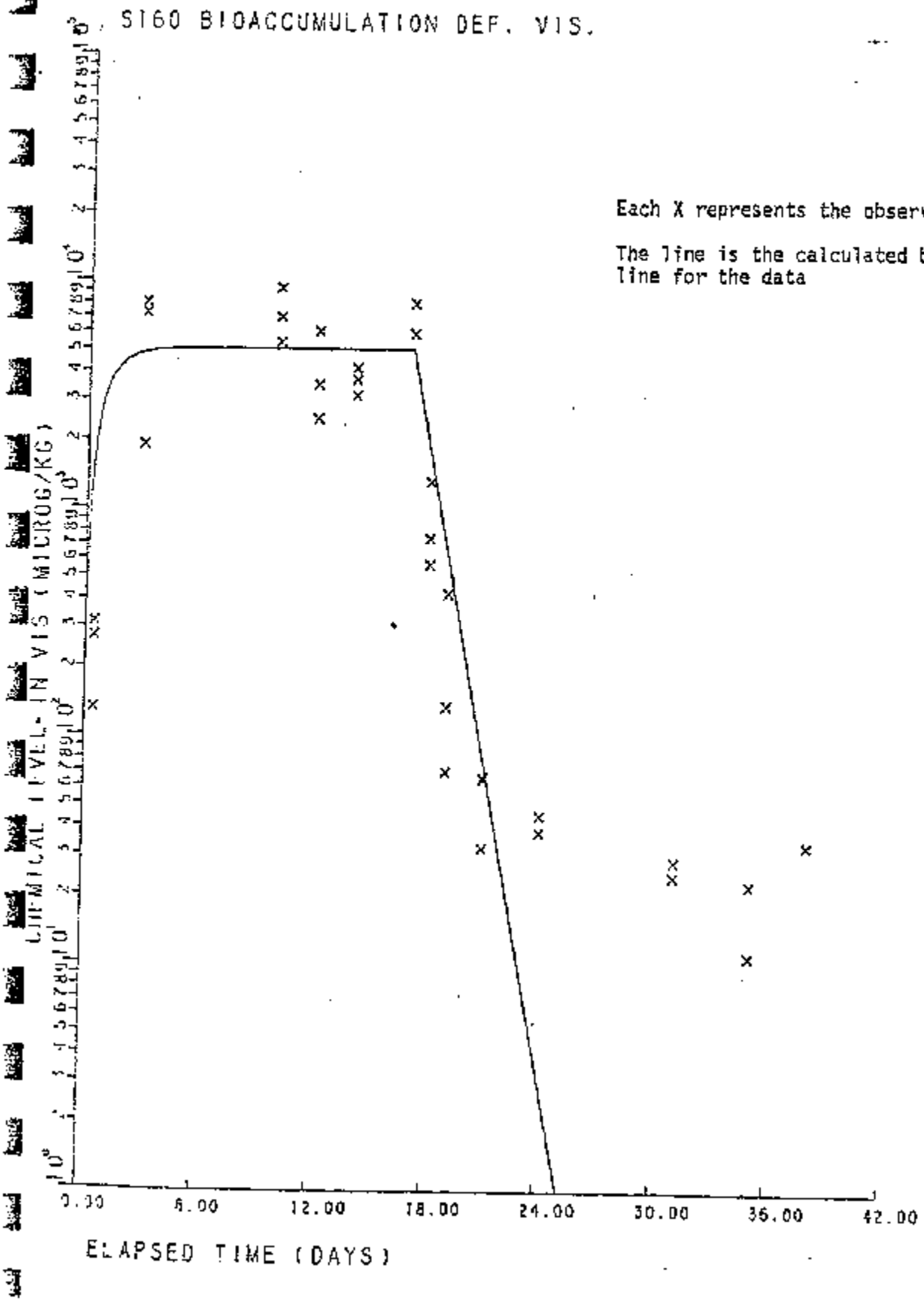


FIGURE 4. BIOFAC COMPUTER PROGRAM GRAPH OF 7-DAY PRELIMINARY WHOLEFISH BIOCONCENTRATION STUDY.

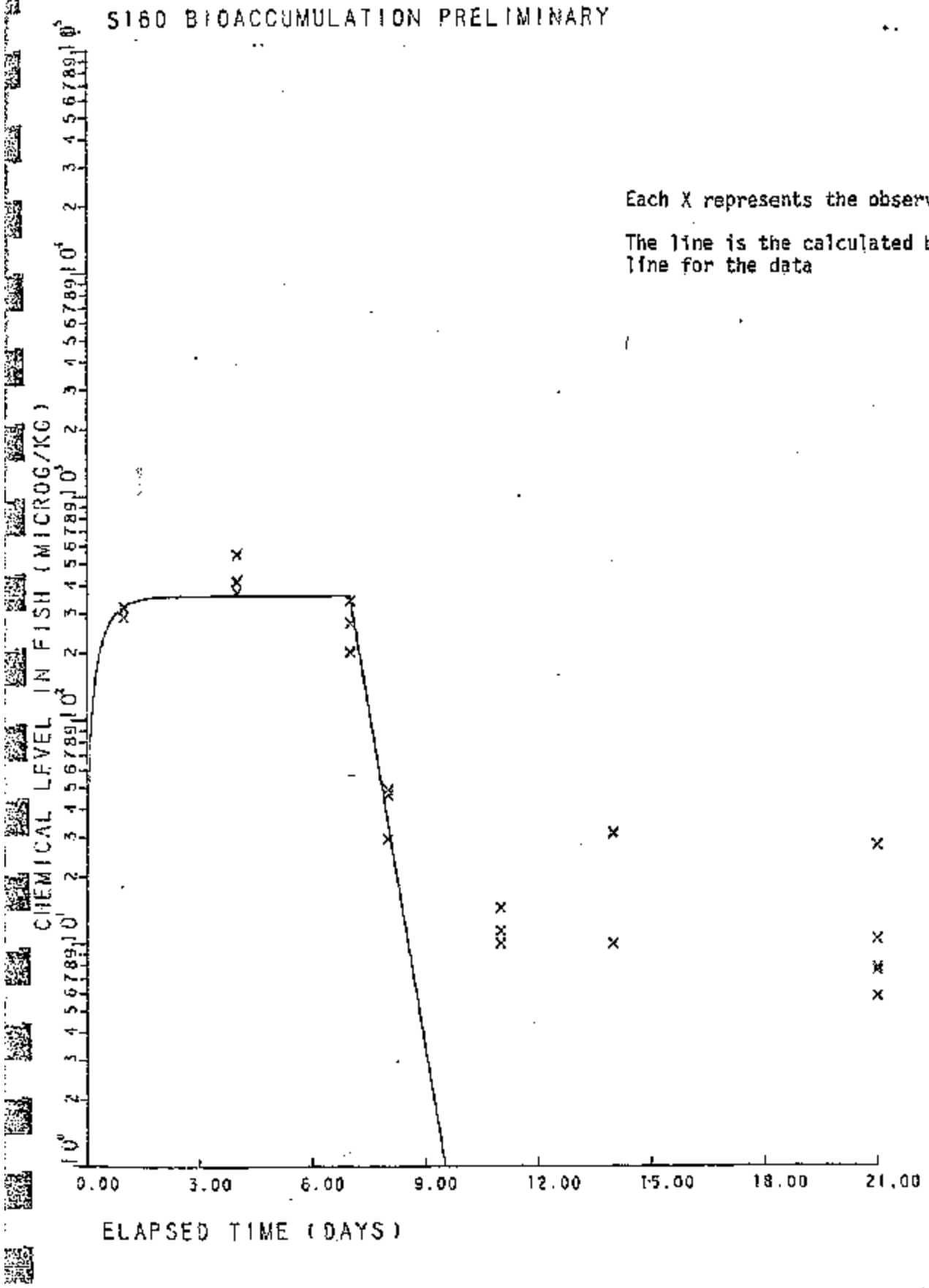


FIGURE 5. BIOFAC COMPUTER PROGRAM GRAPH OF 7-DAY KINETIC 1 WHOLEFISH BIOCONCENTRATION STUDY.

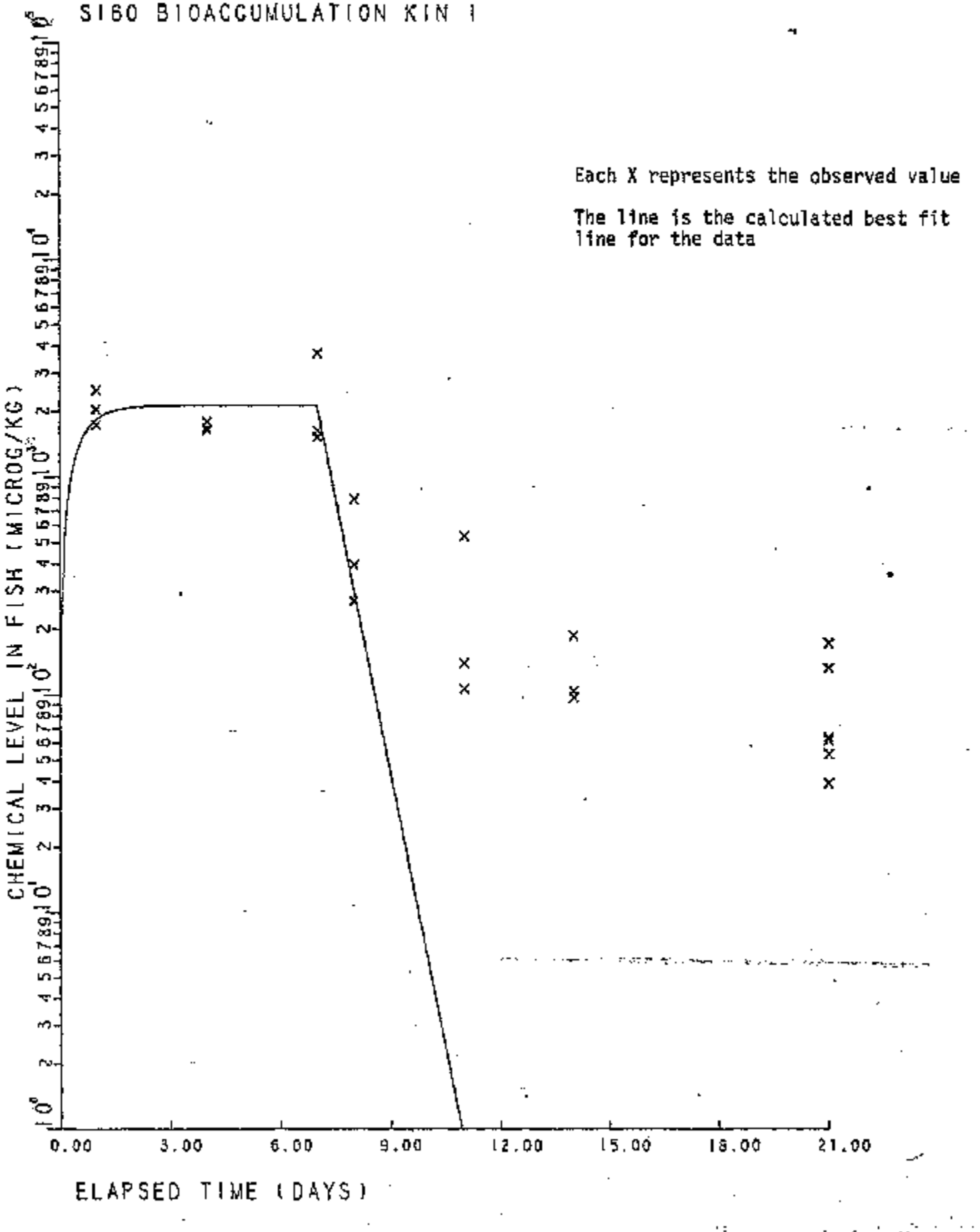


FIGURE 5. BIOFAC COMPUTER PROGRAM GRAPH OF 7-DAY KINETIC 1 WHOLEFISH BIOCONCENTRATION STUDY.

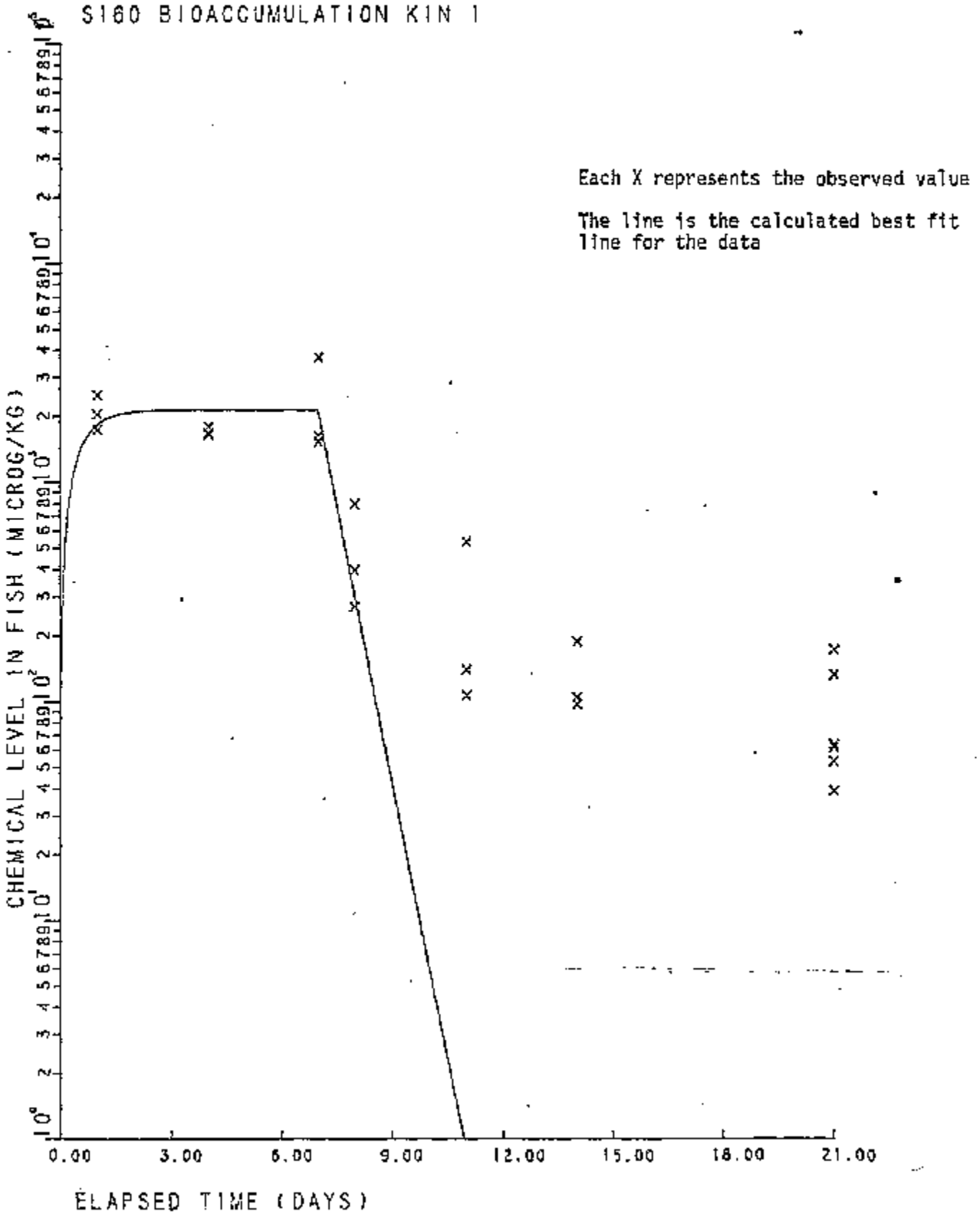
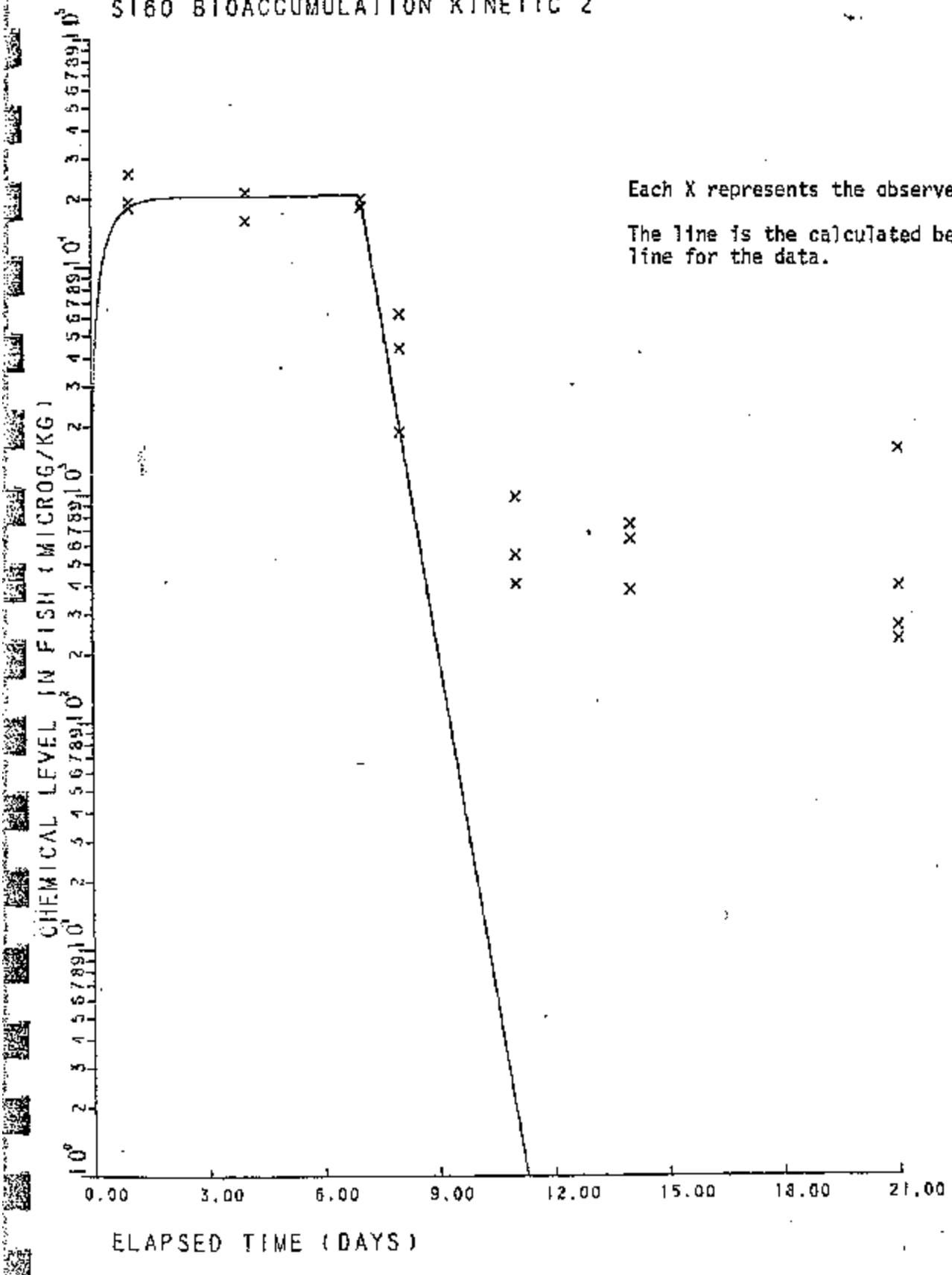


FIGURE 6. BIOFAC COMPUTER PROGRAM GRAPH OF 7-DAY KINETIC 2 WHOLEFISH BIOCONCENTRATION STUDY.



Same additions as 5711

5160 BIOACCUMULATION DEFINITIVE W. F.

Day of Observation in Exp. Form OBSERVATIONS		Observed value µg S-160/kg fish in Exp. Form
1	0.500000E+00	0.461400E+02
2	0.500000E+00	0.356600E+02
3	0.500000E+00	0.109370E+03
4	0.300000E+01	0.480360E+03
5	0.300000E+01	0.409240E+03
6	0.300000E+01	0.487870E+03
7	0.100000E+02	0.503680E+03
8	0.100000E+02	0.661840E+03
9	0.100000E+02	0.658360E+03
10	0.120000E+02	0.526250E+03
11	0.120000E+02	0.520390E+03
12	0.120000E+02	0.628480E+03
13	0.140000E+02	0.454670E+03
14	0.140000E+02	0.572380E+03
15	0.160000E+02	0.624460E+03
16	0.170000E+02	0.587210E+03
17	0.170000E+02	0.645020E+03
18	0.170000E+02	0.682530E+03
19	0.180000E+02	0.131970E+03
20	0.180000E+02	0.358730E+03
21	0.180000E+02	0.985200E+02
22	0.190000E+02	0.398600E+02
23	0.190000E+02	0.939000E+02
24	0.190000E+02	0.646200E+02
25	0.210000E+02	0.466300E+02
26	0.210000E+02	0.387700E+02
27	0.210000E+02	0.849100E+02
28	0.240000E+02	0.369800E+02
29	0.240000E+02	0.409600E+02
30	0.240000E+02	0.295200E+02
31	0.310000E+02	0.270600E+02
32	0.310000E+02	0.139700E+02
33	0.310000E+02	0.487000E+02
34	0.350000E+02	0.295600E+02
35	0.350000E+02	0.302000E+02
36	0.380000E+02	0.291300E+02
37	0.380000E+02	0.247200E+02
38	0.380000E+02	0.229300E+02

TIME OF CHANGEOVER TO FRESH WATER = 17.0
EXPOSURE WATER CONCENTRATION DURING UPTAKE = 2.96000

PARAMETER INITIAL ESTIMATES

Heteroscedacity γ	Uptake K_1	Clearance K_2
0.190000D+01	0.196000D+02	0.700000D-01

Some additions-as 5711

S160 BioAccumulation Definitive Data

Day of Observation in Exp. Form OBSERVATIONS	Observed value in S-160/kg fish in Exp. Form
1 0.500000E+00	0.401400E+02
2 0.500000E+00	0.356600E+02
3 0.500000E+00	0.709370E+03
4 0.300000E+01	0.480360E+03
5 0.300000E+01	0.409240E+03
6 0.300000E+01	0.487670E+03
7 0.100000E+02	0.503080E+03
8 0.100000E+02	0.661840E+03
9 0.100000E+02	0.638360E+03
10 0.120000E+02	0.526250E+03
11 0.120000E+02	0.520390E+03
12 0.120000E+02	0.626480E+03
13 0.140000E+02	0.454670E+03
14 0.140000E+02	0.572380E+03
15 0.140000E+02	0.624480E+03
16 0.170000E+02	0.587210E+03
17 0.170000E+02	0.645020E+03
18 0.170000E+02	0.682530E+03
19 0.180000E+02	0.131970E+03
20 0.180000E+02	0.358730E+03
21 0.180000E+02	0.985200E+02
22 0.190000E+02	0.398600E+02
23 0.190000E+02	0.939000E+02
24 0.190000E+02	0.446200E+02
25 0.210000E+02	0.466300E+02
26 0.210000E+02	0.307200E+02
27 0.210000E+02	0.869100E+02
28 0.240000E+02	0.369800E+02
29 0.240000E+02	0.409600E+02
30 0.240000E+02	0.295200E+02
31 0.310000E+02	0.270600E+02
32 0.310000E+02	0.139700E+02
33 0.310000E+02	0.487000E+02
34 0.350000E+02	0.295600E+02
35 0.350000E+02	0.302000E+02
36 0.380000E+02	0.291300E+02
37 0.380000E+02	0.247200E+02
38 0.380000E+02	0.229300E+02

TIME OF CHANGEOVER TO FRESH WATER= 17.0
EXPOSURE WATER CONCENTRATION DURING UPTAKE 2.96000
PARAMETER INITIAL ESTIMATES
0.190000D+01 0.196000D+02 0.700000D-01

Heteroscadacity γ Uptake K_1 Clearance K_2

TIME OF CHANGEOVER TO FRESH WATER= 17.0

OBSERVED AND PREDICTED VALUES FOR CHEMICAL CONCENTRATION IN FISH (ug S-160/kg Fish)

TIME	OBSERVED	CALCULATED
0.50	40.1400	176.3930
0.50	35.6600	176.3930
0.50	109.3100	176.3930
3.00	480.3601	499.3334
3.00	409.2600	499.3334
3.00	487.8701	499.3334
10.00	503.6799	555.1649
10.00	661.8401	555.1649
10.00	638.3601	555.1649
12.00	526.2500	555.3736
12.00	520.3899	555.3736
12.00	628.4800	555.3736
14.00	454.6699	555.4189
14.00	572.3799	555.4189
14.00	624.4600	555.4189
17.00	587.2100	555.4302
17.00	645.0200	555.4302
17.00	682.5300	555.4302
18.00	131.9700	256.6635
18.00	358.7300	256.6635
18.00	98.3200	256.6635
19.00	39.8600	120.4594
19.00	93.9000	120.4594
19.00	64.8200	120.4594
21.00	46.6300	26.1247
21.00	30.7700	26.1247
21.00	84.9100	26.1247
24.00	36.9800	2.6386
24.00	40.9600	2.6386
24.00	29.5200	0.0000
31.00	27.0600	0.0125
31.00	13.9700	0.0125
31.00	48.7000	0.0125
35.00	29.5600	0.0004
35.00	30.2000	0.0004
38.00	24.1300	0.0001
38.00	24.7200	0.0001
38.00	22.0300	0.0001

WEIGHTED RESIDUAL SUM OF SQUARES = 0.1322253E+06
STANDARD DEVIATION = 0.8342197E+02
WEIGHTED SUM OF RESIDUALS = -757.831055

OPTIMAL PARAMETER ESTIMATES AND LINEARIZED STANDARD DEVIATIONS

UPTAKE RATE CONSTANT--K1--	143.40171	16.86215
CLEARANCE RATE CONSTANT--K2--	0.76422	0.09184
T(1/2) FOR CLEARANCE	0.90681	0.10897
BIOCONCENTRATION FACTOR--KB--	187.6645	23.442
TIME TO REACH 90% OF SS	3.01301	0.36208
HETEROSCEDASTICTY PARAMETER--GAMMA--	0.15307	0.02430

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