Statistics
Special Study 71-14
Job No. 85050-16

STATISTICAL ANALYSIS OF BIOTEST ORAL TOXICITY STUDY - ALBINO RATS

INTRODUCTION:

Samples of tissues received from Bio Test Laboratories were tested for Aroclor residual and the data submitted for statistical analysis. The experimental design used included as variables three types of Aroclor, three feed levels, three tissues, three feed duration and male versus female rats. The original data are shown in Table I.

The male and female samples were composited prior to test on the animals sacrificed at 12 months. The male and female samples for the 3 month and 24 month duration were averaged to give the data in Table II. These data were analyzed by multiple regression, and the effects of the experimental variables calculated. The residuals (differences between actual results and predictions from the regression) were examined to determine if any "wild" values existed. Four were found, and estimated values were substituted for these and the data resubmitted for regression analysis. The corrected values are shown in Table III. Finally, the data in Table III were analyzed by analysis of variance and the regression results confirmed. The analysis of variance is shown in Table IV.

CONCLUSIONS:

A. Original Data, Ln PPM Residue Found

1. 1242 had significantly lower residue than 1254 and 1260. (Significance 99.9%)
2. Residue in the liver was significantly higher than that found in the other tissues. (Significance 99%)
3. Increasing the level of feed significantly increased the residue found. (Significance 99.99%)
4. The residue found increased significantly with time (99.9%).
5. The rate of residue increase with feed was significantly less for 1242 than for 1254 and 1260. (Significance 99.9%)
6. The increase of residue with time was less between 12 and 24 months, with no significant difference due to type of material.
7. The equation for predicting the amount of residual compound found at a given value of the variables is shown in Table V.

B. Smoothed Data from Table II, Ln PPM Residue Found

The conclusions given in part A were again found, with the following differences.

1. In addition to the 1242 residue being significantly low, 1254 was also found to be significantly lower than 1260. (Significance 99.9%)
6. The increase in residue with time leveled off between 12 months and 24 months for 1242, but continued to increase for the other two materials. (Significance 99%)
7. The equation for predicting the amount of residual compound found fit these data better than the original data. Residual standard deviations (ln scale) for the two sets were: Original data 0.51588; smoothed data 0.36860. (Table V)

C. Analysis of Variance, Smoothed Data (Ln Scale)

1. The analysis of variance confirmed the above results. In addition, experimental error was calculated, using higher order interactions as an estimate of experimental error. The result was a standard deviation of 0.3691, very close to that obtained on the regression approach.
2. 95% confidence limits for a single observation were calculated and are shown on figure 1.

D. Additional Regression on Smoothed Data (Ln Scale)

1. Addition of a 1260 X liver interaction term indicated that the higher concentration of residue in the liver applied only to 1242 and 1254. (Significance 95%)
2. The equation including the new term had a residual standard deviation of 0.34301. (Table V)

E. Miscellaneous Conclusions (Original Data)

1. At the 90% significance level, female rats were found to have higher residual concentrations than male rats. (Sign test)
2. A graphical presentation (Figures 2, 3, and 4) show the build-up with time for the three compounds at 3 feed levels.

DISCUSSION:

The residual standard deviation from the regression analysis, and the square root of the residual mean square from the analysis of variance are legitimate estimates of experimental error. They included all sources of variation in the experiment other than those

specifically calculated.

In the smoothing process used on the data, substitute values were estimated for those points which were more than two standard deviations away from their predicted values. The assumption is that such points represent some major sources of variation not studied in the experiment, or some gross error such as a mislabeled sample. If included in the analysis they might mask the real effects of the variables being studied.

db

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TABLE I

PPM Aroclor found in Tissue

Fiotest Study #67938

Two Year Chronic Oral Toxicity Study Of Aroclor In Albino Rats

		3 Mo. Sacrifice						12 Mo. Sacrifice			24 Mo. Sacrifice						Recovery Sacrifice		
		1 ppm		10 ppm		100 ppm		1 ppm	10 ppm	100 ppm	1 ppm		10 ppm		100 ppm		1 ppm	10 ppm	100 ppm
		♂	♀	♂	♀	♂	♀	Composite ♂ ♀	Composite ♂ ♀	Composite ♂ ♀	♂	♀	♂	♀	♂	♀	Composite ♂ ♀	Composite ♂ ♀	Composite ♂ ♀
Aroclor 1242	Muscle	5	10	13	18	64	89	6	38	236	6	2	10	39	187	289			
	Kidney	7	72	21	16	41	40												
	Liver	10	12	20	31	82	72	16	40	241	18	19	64	39	238	321			
	Fat	6	6	13	13	53	81	8	32	151	6	12	49	47	219	235			
Aroclor 1254	Muscle	9	12	85	107	486	1026	11	721	1092	42	46	237	450	1171	2402			
	Kidney	11	12	54	41	233	323												
	Liver	15	20	108	122	3239	1137	24	914	869	125	57	2250	2484	1516	1[illegible]			
	Fat	8	3	86	66	329	2362	19	688	860	59	21	2251	2251	2097	1152			
Aroclor 1260	Muscle	98	16	44	76	137	156	47	259	2247	19	42	1[illegible]	1[illegible]	7043	3266			
	Kidney	7	22	66	142	606	831												
	Liver	20	—	112	93	1539	1815	50	189	1752	55	5	335	2[illegible]	7034	5587			
	Fat	—	32	138	760	1060	672	60	262	2143	3[illegible]	2[illegible]	2[illegible]	2[illegible]	4700	5420			
Control	Muscle			*					*				*					*	
	Kidney																		
	Liver																		
	Fat																		

* Common controls, one per sacrifice

1. Calculated on the basis of wet tissue
2. Chromatogram of muscle 1254
3. Average (Av.)
4. Eliminated, see text for explanation

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TABLE II

PPM AROCLOR FOUND IN TISSUE - COMPOSITE DATA

Aroclor ↓Type	Tissue ↓	1 PPM			10 PPM			100 PPM			Amount ← Fed
		3 Mo.	12 Mo.	24 Mo.	3 Mo.	12 Mo.	24 Mo.	3 Mo.	12 Mo.	24 Mo.	Duration of ← Feed
1242	M	8	6	5	16	38	39	76	236	239	
	L	11	16	18	26	40	52	77	241	234	
	F	6	8	9	13	32	47	67	151	227	
1254	M	10	11	38	96	721	389	800	1092	2164	
	L	18	24	82	115	914	307	2000	869	4800	
	F	6	19	55	76	688	348	900	860	1894	
1260	M	16	47	44	60	259	436	146	2247	4950	
	L	20	50	56	102	189	442	1677	1752	6310	
	F	32	60	62	138	262	354	850	2143	5060	

M = Muscle
L = Liver
F = Fat

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TABLE III

COMPOSITE DATA CORRECTED FOR "WILD" VALUES

Aroclor Type	Tissue	1 PPM			10 PPM			100 PPM			← Amount Fed
		3Mo	12Mo	24Mo	3Mo	12Mo	24Mo	3Mo	12Mo	24Mo	← Duration of Feed
1242	M	8	6	5	16	38	39	76	236	239	
	L	11	16	18	26	40	52	77	241	234	
	F	6	8	9	13	32	47	67	151	227	
1254	M	10	11	38	96	196*	389	800	1092	2164	
	L	18	24	82	115	292*	307	2000	869	4800	
	F	6	19	55	76	231*	348	900	860	1894	
1260	M	16	47	44	60	259	436	689*	2247	4950	
	L	20	50	56	102	189	442	1677	1752	6310	
	F	32	60	62	138	262	354	850	2143	5060	

M = Muscle
L = Liver
F = Fat

* Adjusted Values

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TABLE IV

LOGARITHMS OF PPM FOUND

		1 PPM			10 PPM			100 PPM		
		3MO	12MO	24MO	3MO	12MO	24MO	3MO	12MO	24MO
1242	M	1.10	1.79	1.61	2.77	3.64	4.30	4.33	5.46	5.48
	L	2.40	2.77	2.89	3.26	3.69	3.95	4.34	5.48	5.46
	F	1.79	2.08	2.20	2.56	3.47	3.85	4.20	5.02	5.42
1254	M	2.30	2.46	3.64	4.56	5.28	5.96	6.68	7.100	7.68
	L	2.89	3.18	4.41	4.74	5.68	5.73	7.60	6.77	8.48
	F	1.79	2.94	4.01	4.33	5.44	5.85	6.80	6.76	7.55
1260	M	2.77	3.85	3.78	4.09	5.56	6.08	6.54	7.72	9.51
	L	3.00	3.91	4.03	4.62	5.24	6.09	7.42	7.47	8.75
	F	3.49	4.09	4.13	5.86	5.57	5.87	6.75	7.67	8.53

TWO WAY TABLES

FEED X TIME

	1	10	100	
3MO	21.51	36.79	54.66	112.96
12MO	27.01	43.57	59.35	129.93
24MO	30.70	47.68	65.96	144.24
	79.22	128.04	179.87	387.13

FEED X MATERIAL

	1	10	100	
1242	18.63	31.49	45.19	95.31
1254	27.56	47.57	65.32	140.45
1260	33.03	48.98	69.36	151.37
	79.22	128.04	179.87	387.13

FEED X TISSUE

	1	10	100	
MUSCLE	23.24	42.24	59.40	124.88
LIVER	29.48	43.00	61.77	134.25
FAT	26.50	42.80	58.70	128.00
	79.22	128.04	179.87	387.13

TIME X MATERIAL

	3MO	12MO	24MO	
1242	26.75	33.40	35.16	95.31
1254	41.69	45.45	53.31	140.45
1260	44.52	51.08	55.77	151.37
	112.96	129.93	144.24	387.13

TIME X TISSUE

	3MO	12MO	24MO	
MUSCLE	35.14	42.70	47.04	124.88
LIVER	40.27	44.19	49.79	134.25
FAT	37.55	43.04	47.41	128.00
	112.96	129.93	144.24	387.13

MATERIAL X TISSUE

	1242	1254	1260	
MUSCLE	30.48	45.50	48.90	124.88
LIVER	34.24	49.48	50.53	134.25
FAT	30.59	45.47	51.94	128.00
	95.31	140.45	151.37	387.13

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TABLE IV (cont)

ANALYSIS OF VARIANCE

		DF	MEAN SQUARE	
SS IND = 2138.2389 − 1850.2424 = 287.9965		80		
SS FEED = 2037.8788 − 1850.2424 = 187.6564		2	93.8282	NS
SS TIME = 1868.4053 − 1850.2424 = 18.1629		2	9.0809	NS
SS MATERIAL = 1915.6695 − 1850.2424 = 65.4271		2	32.7136	NS
SS TISSUE = 1851.9288 − 1850.2424 = 1.6864		2	0.8432	6.19 **
SS FEED X TIME = 2056.4021 − 1850.2424 − 187.6564 − 18.1629 =	0.3403	4	0.0851	NS
SS FEED X MAT = 2107.8694 − 1850.2424 − 187.6564 − 65.4271 =	4.5435	4	1.1359	8.34 **
SS FEED X TISSUE = 2040.6732 − 1850.2424 − 187.6564 − 1.6864 =	1.0880	4	0.2720	NS
SS TIME X MAT = 1934.9503 − 1850.2424 − 18.1629 − 65.4271 =	1.1179	4	0.2795	2.05 NS
SS TIME X TISSUE = 1870.4776 − 1850.2424 − 18.1629 − 1.6864 =	0.4079	4	0.1019	NS
SS MAT X TISSUE = 1918.3635 − 1850.2424 − 65.4271 − 1.6864 =	1.0276	4	0.2569	NS
SS RESIDUAL	6.5385	48	0.1362	

$F_{2,48} = 3.19$

$F_{4,48} = 2.56$

EST STD DEV = $\sqrt{.1362}$ = 0.37

95% LIMITS: ± 0.74

at ave = 10, 95% limit are 9.18 to 10.0

100 95% limits 98 to 100

1000 95% limit 998 to 1000 etc.

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TABLE V

EQUATIONS FOR PREDICTING RESIDUE FOUND

Ln(PPM RESIDUE)
Only Coefficients Significant at 95% Included

	Regression A	Regression B	Regression D
CONSTANT	3.4596	3.3933	3.6265
IF 1242	-1.3717	-1.2331	-1.5799
IF 1254	—	-0.4040	-0.7620
IF LIVER	0.3157	0.2891	0.4267
Ln AMT FED	0.8749	0.8936	0.8936
(TIME-12.0)	0.0660	0.0585	0.0609
LnAmtFed (IF1242)	-0.2338	-0.2525	-0.2525
(TIME-12.0) (IF1242)	—	—	—
$(TIME-12.0)^2$	-0.0027	—	-0.0026
$(TIME-12.0)^2$ (IF1242)	—	-0.0035	—
$(TIME-12.0)^2$ (IF1254)	—	—	0.0036
LIVER (IF1260)	—	—	-0.3613
%VARIANCE EXPLAINED	93.28%	96.54%	97.08%
RESIDUAL STD. DEV.	0.5159	0.3686	0.3430

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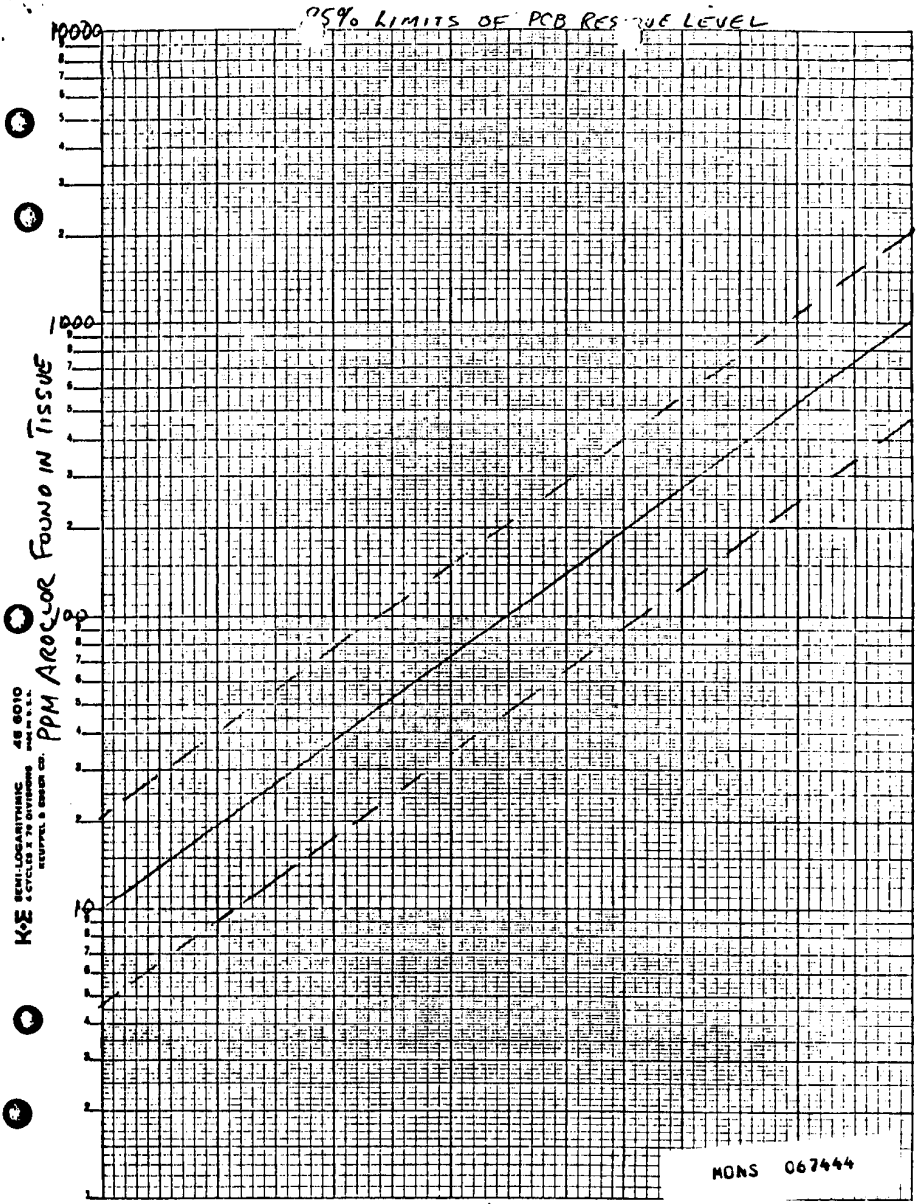

95% LIMITS OF PCB RESIDUE LEVEL
10000
1000
100
10
PPM AROCLOR FOUND IN TISSUE
K&E SEMI-LOGARITHMIC 46 6010
MONS 067444

FIGURE 1

FIG 2

AROCLOR 1242

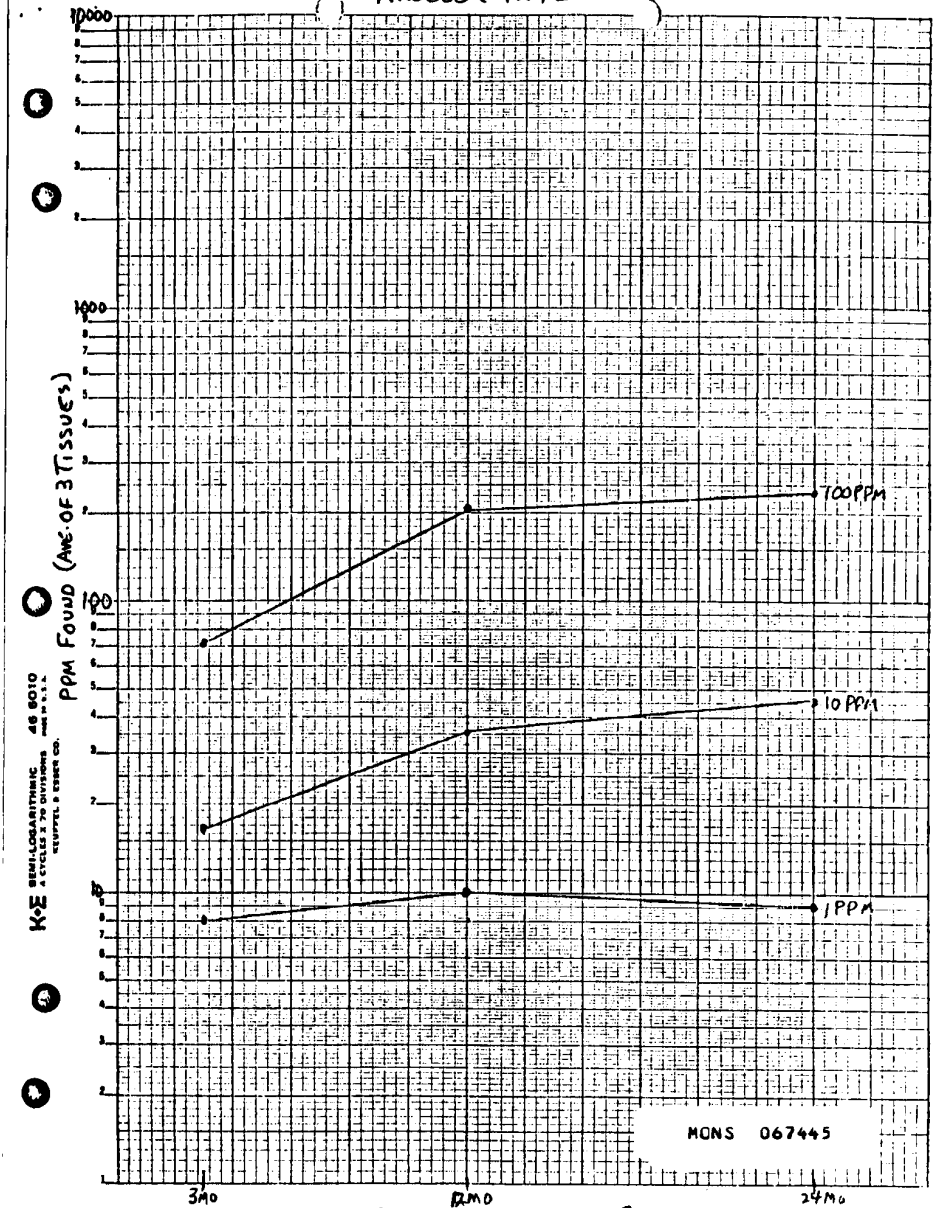


FIG 3

AROCLOR 1254

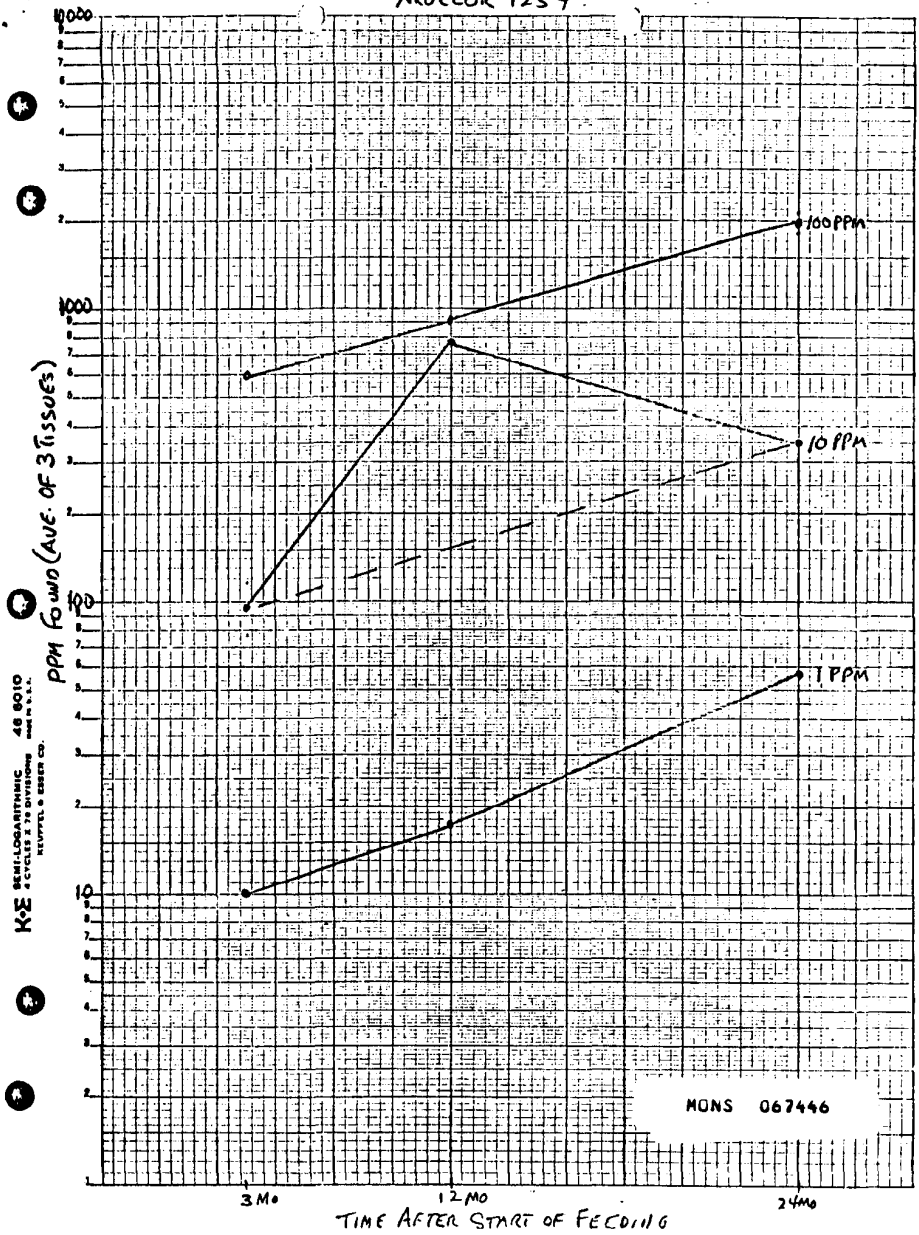
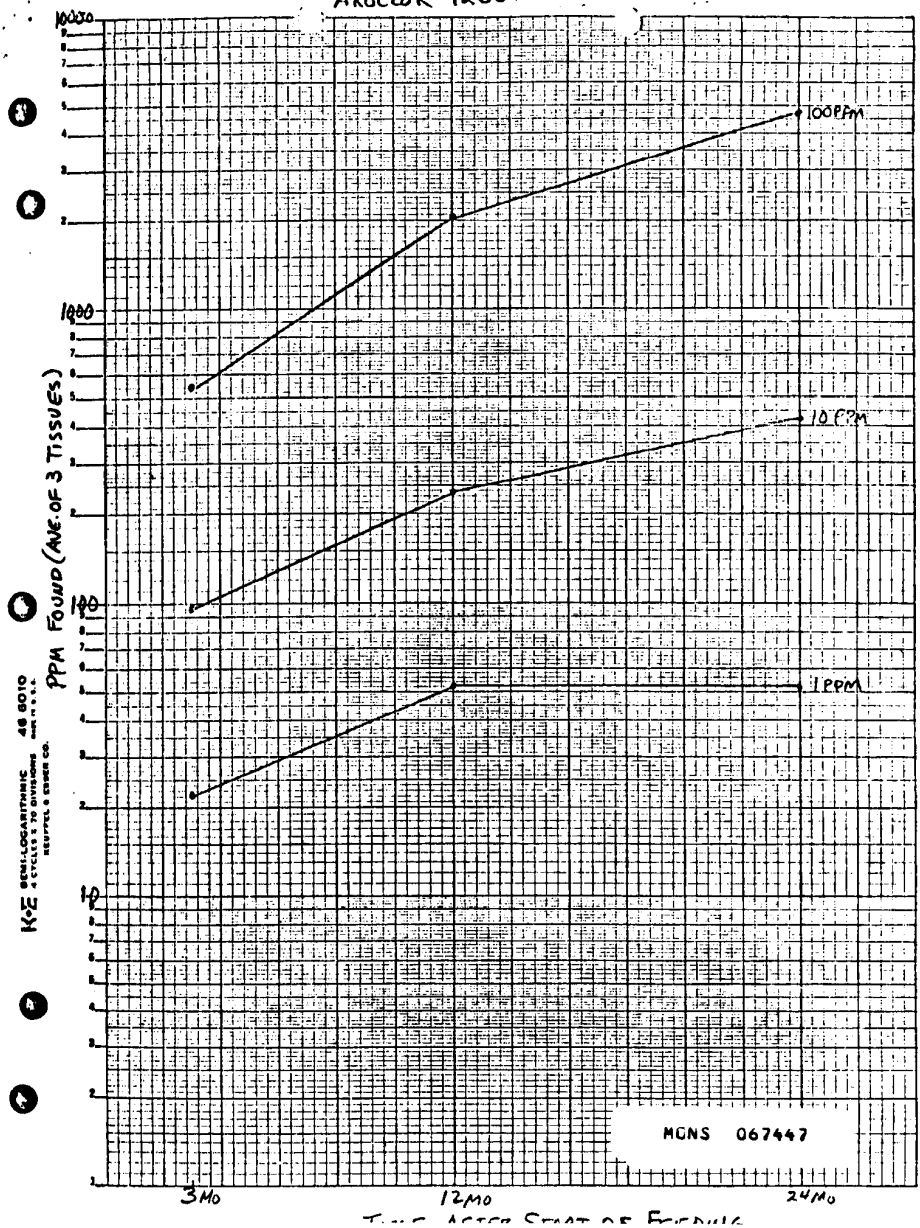


FIG 4


AROCLOR 1260
PPM FOUND (AVE. OF 3 TISSUES)
10000
1000
100
10
1
100PPM
10PPM
1PPM
3MO
12MO
24MO
MGNS 067447
K+E SEMI-LOGARITHMIC 46 6010
4 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO.