Analytical Chemistry
Special Study 71-3
Job No. 1348006

DETERMINATION OF POLYCHLORINATED BIPHENYL RESIDUES IN WHITE LEGHORN CHICKENS FROM A TOXICITY, REPRODUCTION AND RESIDUE STUDY WITH AROCLOR 1242, AROCLOR 1254, AND AROCLOR 1260

INTRODUCTION

An integral part of the overall Aroclor Environmental Program involved obtaining a more complete understanding of the environmental behavior and impact of our polychlorinated biphenyl (PCB) products. In order to accomplish this a number of laboratory feeding studies with beagle dogs, albino rats, and white leghorn chickens were initiated at Industrial Bio-Test Laboratories (IBT).

This report covers the results of the analysis of biological samples from IBT's study #J7300, "Toxicity, Reproduction, and Residue Study with Aroclor 1242, Aroclor 1254 and Aroclor 1260 in White Leghorn Chickens" and a supplementary IBT study #8746 with only Aroclor 1242.

The specific objectives were to establish the relationships between tissue storage levels and exposure period, exposure level, degree of chlorination, and any observed toxic effects as well as determine the homolog distribution of the retained PCB residues. Enough chickens were also included in these studies to determine the rate at which the PCB residues declined when the birds were put on PCB-free diets.

SUMMARY

PCB residues from the Aroclor products fed were observed at all exposure levels (1, 2, 4, 8, 10 and 100 ppm), at each sacrifice period (24 and 28 weeks), and in all of the samples (muscle, liver, fat, egg and chicks) analyzed.

The PCB residue levels found in the tissues decreased as the exposure level was reduced and as the chlorine content of the product fed decreased.

Significant differences in the PCB residue levels were observed between males and females, and between the muscle, liver and fat tissues. Significantly lower PCB residue levels were observed when the birds were put on a recovery diet that contained no PCBs.

The homolog distributions of all of the products fed were altered. The alterations were most pronounced with Aroclor 1242 and least with Aroclor 1254 and Aroclor 1260.

DETAILS

Analyses of the tissues were carried out according to Analytical Chemistry Method No. 71-35 with the exception that the chemical clean-up procedure was necessary only with the chick samples.

Standard solutions of the material fed were used to prepare calibration curves for the quantitation of the PCB residues.

In the initial residue studies all of the samples were analyzed. In subsequent tissue residue studies, including these chicken studies, a statistical approach was employed to minimize the number of samples requiring analysis. In this manner, both the elapsed time and manpower requirements were significantly reduced with little real loss in either accuracy or precision. The approach involved subjecting the results from the residue analysis of a balanced subset of samples, along with the appropriate study variables (tissue type, exposure period, exposure level, sex, and Aroclor fed) and the most probable variable interactions to multiple regression statistics. In this way an interpolation equation was generated for predicting the PCB residues. The initial equation was then smoothed by deleting data more than two standard deviations from the predicted values and the variable interaction terms not significant at the 95% confidence level. The final equations were then used to calculate the PCB residue levels in the unanalyzed tissues.

The validity of each predicting equation was established in the following manner. The respective residual standard deviations of each equation were compared to those obtained for the equations developed for the studies in which all of the tissues were analyzed. The 95% confidence limits calculated for these studies from the log of the residual standard deviations indicated that in all studies the actual values determined by analysis were within ~0.5-2 times the prediced values.

The equations resulting from the statistical analysis used to predict the complete sets of PCB residue levels are shown in Tables III and VI.

Figures I thru III are semi-logarithmic plots of PCB residue levels vs. time for the different exposure levels for each of the Aroclor products tested. The plots clearly illustrate a considerably slower rate of recovery for the liver tissue. Apparently the liver is the primary site for metabolism and/or collection point for elimination of the test material from the animal.

Shown in Table VII are biological half lives for the Aroclor products tested in chicken tissues. Once again significant differences

between tissues were observed. Also apparent are differences in the rate of recovery with the Aroclor product fed.

Calculations based on average body weight, feed consumption and lipid content indicate that only 11% of the total Aroclor 1242 ingested was retained by the animal. The corresponding figures for Aroclor 1254 and Aroclor 1260 were 28 and 49% respectively. Figure IV, which is a plot of PCB residue level vs. degree of chlorination, further illustrates the differences in storage level due to test material fed. It demonstrates that as the degree of chlorination decreases the PCB residue level decreases.

Figure V is a semi-logarithmic plot of PCB residue level vs. exposure level for each sex for the three Aroclor products fed. This figure illustrates the bias, which increases with degree of chlorination, toward higher PCB residue levels in the males. The higher PCB residue levels in males were also observed in the recovery sacrifice samples in the same ratios.

Chromatograms I, II and III compare typical tissue extracts from the residue and final sacrifices with the material fed for Aroclor 1242, Aroclor 1254, and Aroclor 1260. The alterations were greatest with Aroclor 1242 and least with Aroclor 1260. It is apparent upon inspection of the chromatograms that the lower chlorinated isomers are metabolized and/or excreted quite rapidly. Further comparisons between the residue and final sacrifice extracts indicate that some of the higher chlorinated isomers also undergo metabolism and/or excretion but at much slower rates.

Chromatogram IV is comparison of chicken tissue, egg, and chick extracts in the case where Aroclor 1242 was fed. Once again, it is apparent that the lower chlorinated homologs are readily metabolized and/or excreted.

The gas chromatographic conditions employed do not completely resolve the Aroclor products into their component homologs. Nevertheless, the numbers above the chromatographic peaks indicate the dominant homolog(s) in that peak.

TOXICITY

Some toxic effects were observed with the groups fed Aroclor 1242 at 10 ppm and 100 ppm, and Aroclor 1254 at 100 ppm. Consequently a supplementary study with Aroclor 1242 at the 2, 4 and 8 ppm exposure levels was initiated. The toxic effects, primarily related to hatchability, are discussed in detail in the referenced Industrial Biotest Laboratories reports.

REFERENCES

"Toxicity, Reproduction, and Residue Study with Aroclor 1242, Aroclor 1254 and Aroclor 1260 in White Leghorn Chickens," Industrial Bio-Test Laboratories Report (IBT #J7300).

"Toxicity, Reproduction, and Residue Study with Aroclor 1242 in White Leghorn Chickens," Industrial Bio-Test Laboratories Report (IBT #8746).

Residue Study - Aroclor and White Leghorn Chickens, Statistics Special Study 71-24.

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TABLE I

IBT#U9300 Toxicity Reproduction and Residue Study of Aroclor in White Leghorn Chickens

ppm PCB in Chicken Tissues (Wet Weight Basis)

		Residue Sacrifice						30 Day Chick Sacrifice			1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk	Final Sacrifice					
		1ppm		10ppm		100ppm		1ppm	10ppm	100ppm	1ppm				10 ppm				100 ppm				1ppm		10ppm		100ppm	
		♂	♀	♂	♀	♂	♀	♂ & ♀	♂ & ♀	♂ & ♀	30 Day Recovery Period												♂	♀	♂	♀	♂	♀
Aroclor 1242	Muscle	.	0.06				2	0.07	0.05															0.05		0.1		5
	Liver				2			0.4	0.2															0.7		2		45
	Fat	26	17			[illegible]	[illegible]																	4				8
	Eggs			5		38									3	0.6	0.5		26	30	19	24						
Aroclor 1254	Muscle			0.9	0.7			0.02																		0.1		
	Liver		0.8				50	0.09																0.9				27
	Fat			561	139																					73		
	Eggs	0.7				132						2	0.5	0.4						68	75	56						
Aroclor 1260	Muscle		0.2				11	0.06	0.8															[illegible]				2
	Liver				6			0.09	0.2																	13		
	Fat	153	15			[illegible]	1159																	10				[illegible]
	Eggs	1		13							1	0.7			13	8												

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TABLE II

IBT #U7300 Toxicity, Reproduction and Residue Study of Aroclor in White Leghorn Chickens

ppm PCB in Chicken Tissues (Lipid Weight Basis)

		Residue Sacrifice						30 Day Chick Sacrifice			1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk	Final 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	
		♂	♀	♂	♀	♂	♀	♂ & ♀	♂ & ♀	♂ & ♀	30 Day Recovery Period												♂	♀	♂	♀	♂	♀
Aroclor 1242	Muscle		13				901	9	12															8		30		639
	Liver				52			14	12															10		23		1580
	Fat	30	22			246	1310																	4				9
	Eggs			37		472									33	5	5		383	361	299	368						
Aroclor 1254	Muscle			517	240			2																		61		
	Liver		18				1475	4																7				1529
	Fat			892	197																					78		
	Eggs	10				1410						14	6	5						1334	626	832						
Aroclor 1260	Muscle		40				600	5	125															13				867
	Liver				86			6	6																	58		
	Fat	183	16			2580	1305																	11				1089
	Eggs	12		146							13	7			135	71												

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TABLE III

IBT #J7300 Toxicity, Reproduction and Residue Study of Aroclor in White Leghorn Chickens

Predicted ppm PCB in Chicken Tissues (Lipid Weight Basis)

		Residue Sacrifice						30 Day Check Sacrifice			1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk	Final 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	
		♂	♀	♂	♀	♂	♀	♂ ♀	♂ ♀	♂ ♀	30 Day Recovery Period												♂	♀	♂	♀	♂	♀
Aroclor 1262	Muscle	20	12	193	119	1891	1150																10	6	93	56	894	541
	Liver	12	7	114	69	1124	669																12	7	114	63	1	611
	Fat	20	12	196	119	1911	1148																10	6	92	56	894	541
	Eggs	3		40		493					2	09	06	04	25	16	10	7	104	362	308	263						
Aroclor 1254	Muscle	68	22	657	213	6365	2061																26	8	249	81	2419	782
	Liver	34	11	326	106	3163	1027																25	8	243	79	2358	759
	Fat	63	22	657	213	6374	2061																24	8	249	81	2419	782
	Eggs	7		92		1141					6	5	4	3	80	66	54	44	1049	801	710	601						
Aroclor 1260	Muscle	180	58	1776	580	17662	5772																42	10	377	90	3749	900
	Liver	57	24	558	231	5544	2299																36	10	346	87	3437	865
	Fat	180	58	1776	580	17662	5772																38	10	377	90	3749	900
	Eggs	11		140		1728					12	13	14	15	137	135	133	131	1543	1338	1184	1049						

Chicken Regression Equation: log ppm PCB = − 0.5311 + 0.7858 (log ppm fed) + 0.7344 (if female) + 0.0438 (% Cl_2) + 0.2908 (if rec liver) − 0.0056 (% Cl_2) if liver − 0.0076 (% Cl_2) if rec − 0.0226 (% Cl_2) if female

Egg Regression Equation: log ppm PCB = − 0.7476 − 1.1794 (rec time) + 1.0920 (log ppm fed) + 0.0300 (% Cl_2) + 0.5316 (rec time × log ppm fed) + 0.0202 (rec time × % Cl_2) − 0.0096 (rec time × log ppm fed × % Cl_2)

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TABLE IV

IBT #874 Toxicity, Reproduction and Residue Study of Aroclor in White Leghorn Chickens

ppm PCB in Chicken Tissues (Wet Weight Basis)

		Residue Sacrifice			30 Day Check Sacrifice			30 Day Recovery Period												Final Sacrifice		
		2 ppm	4 ppm	8 ppm	2 ppm	4 ppm	8 ppm	2 ppm				4 ppm				8 ppm				2 ppm	4 ppm	8 ppm
		♂♀	♂♀	♂♀	♂♀	♂♀	♂♀	♂♀				♂♀				♂♀				♂♀	♂♀	♂♀
Hens 12wk	Muscle			1	0.02	0.04	0.03													0.5		1
	Liver		4		0.3	0.2	02														2	
	Fat			56	2	3	5													11		43
	Eggs	1							1		1	2		1			4					

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TABLE V

IBT #8746 Toxicity, Reproduction and Residue Study of Aroclor in White Leghorn Chickens

ppm PCB in Chicken Tissues (Lipid Weight Basis)

		Residue Sacrifice			30 Day Chick Sacrifice			1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk	Final Sacrifice		
		2 ppm	4 ppm	8 ppm	2 ppm	4 ppm	8 ppm	2 ppm				4 ppm				8 ppm				2 ppm	4 ppm	8 ppm
		♂♀	♂♀	♂♀	♂♀	♂♀	♂♀													♂♀	♂♀	♂♀
Adult Male	Muscle			64	5	8	13													19		71
	Liver		30		15	19	17														19	
	Fat			73	3	5	9													14		56
Adult Female	Eggs	14							14		15	22		25			42		28			

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TABLE VI

IBT #8746 Toxicity, Reproduction and Residue Study of Aroclor in White Leghorn Chickens

Predicted ppm PCB in Chicken Tissues (Lipid Weight Basis)

		Residue Sacrifice			30 Day Chick Sacrifice			1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk	Final Sacrifice		
		2 ppm	4 ppm	8 ppm	2 ppm	4 ppm	8 ppm	2 ppm				4 ppm				8 ppm				2 ppm	4 ppm	8 ppm
		♂♀	♂♀	♂♀	♂♀	♂♀	♂♀													♂♀	♂♀	♂♀
Aroclor 1016	Muscle	31	62	123	7	9	11													15	29	58
	Liver	18	36	71	15	15	15													17	33	66
	Fat	31	62	123	6	6	6													15	29	58
	Eggs	7	15	32				4	2	1	.45	8	5	3	1	19	12	7	4			

Chick Regression Equation - log ppm PCB = 0.7486 + 0.4385 (Cf liver) + 0.3317 (log ppm fed - if muscle)

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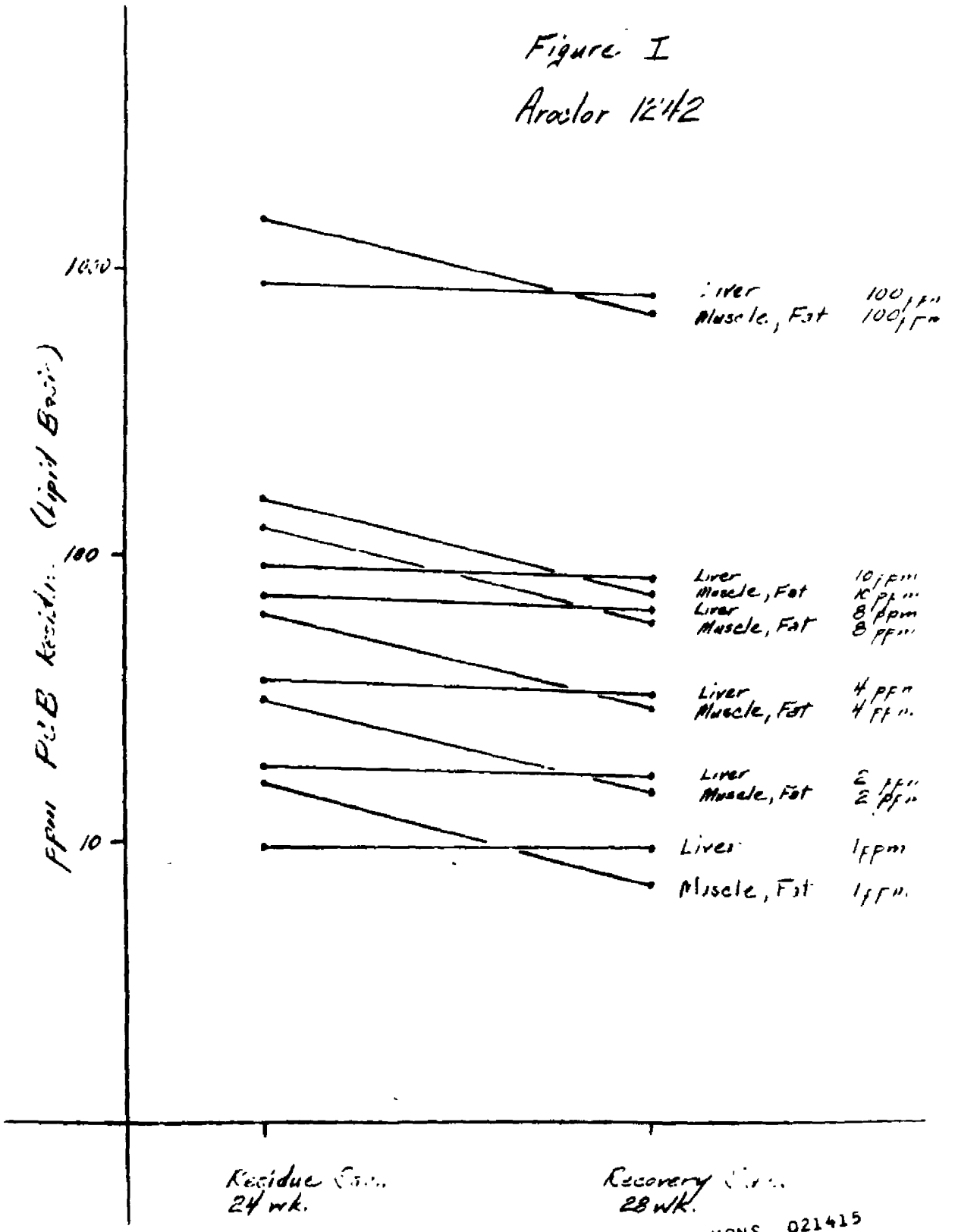
TABLE VII

BIOLOGICAL HALF-LIFE

Material	Tissue	Biological Half-Life
Aroclor 1242	Muscle, Fat	27.6 days
" "	Liver	245.4 days
Aroclor 1254	Muscle, Fat	21.4 days
" "	Liver	69.3 days
Aroclor 1260	Muscle, Fat	19.3 days
" "	Liver	51.0 days

Figure I

Aroclor 1242



MONS 021415

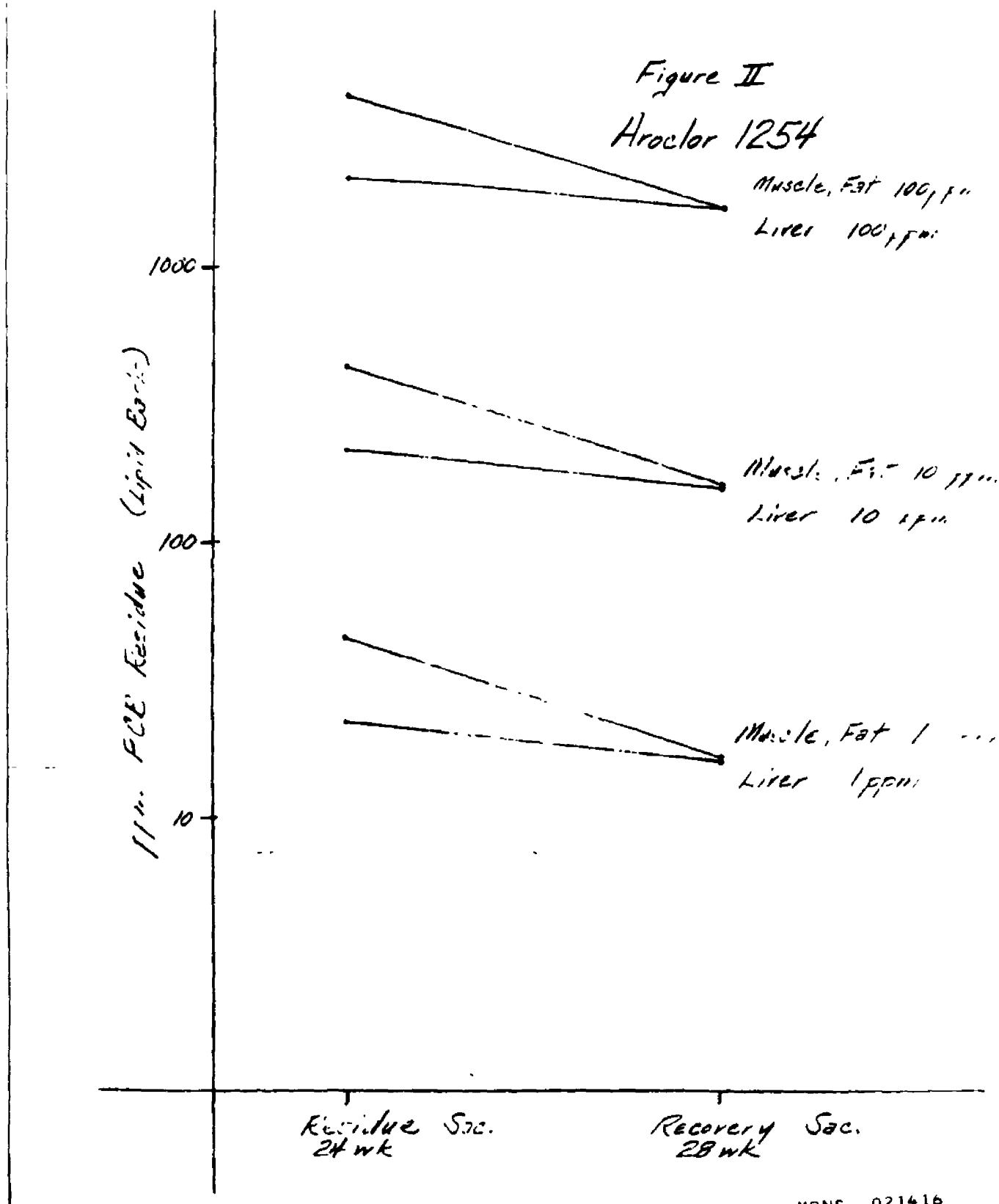


Figure II
Aroclor 1254
Muscle, Fat 100 ppm
Liver 100 ppm
Muscle, Fat 10 ppm
Liver 10 ppm
Muscle, Fat 1 ppm
Liver 1 ppm
1000
100
10
1 ppm PCB Residue (Lipid Basis)
Residue Sac. 24 wk
Recovery Sac. 28 wk
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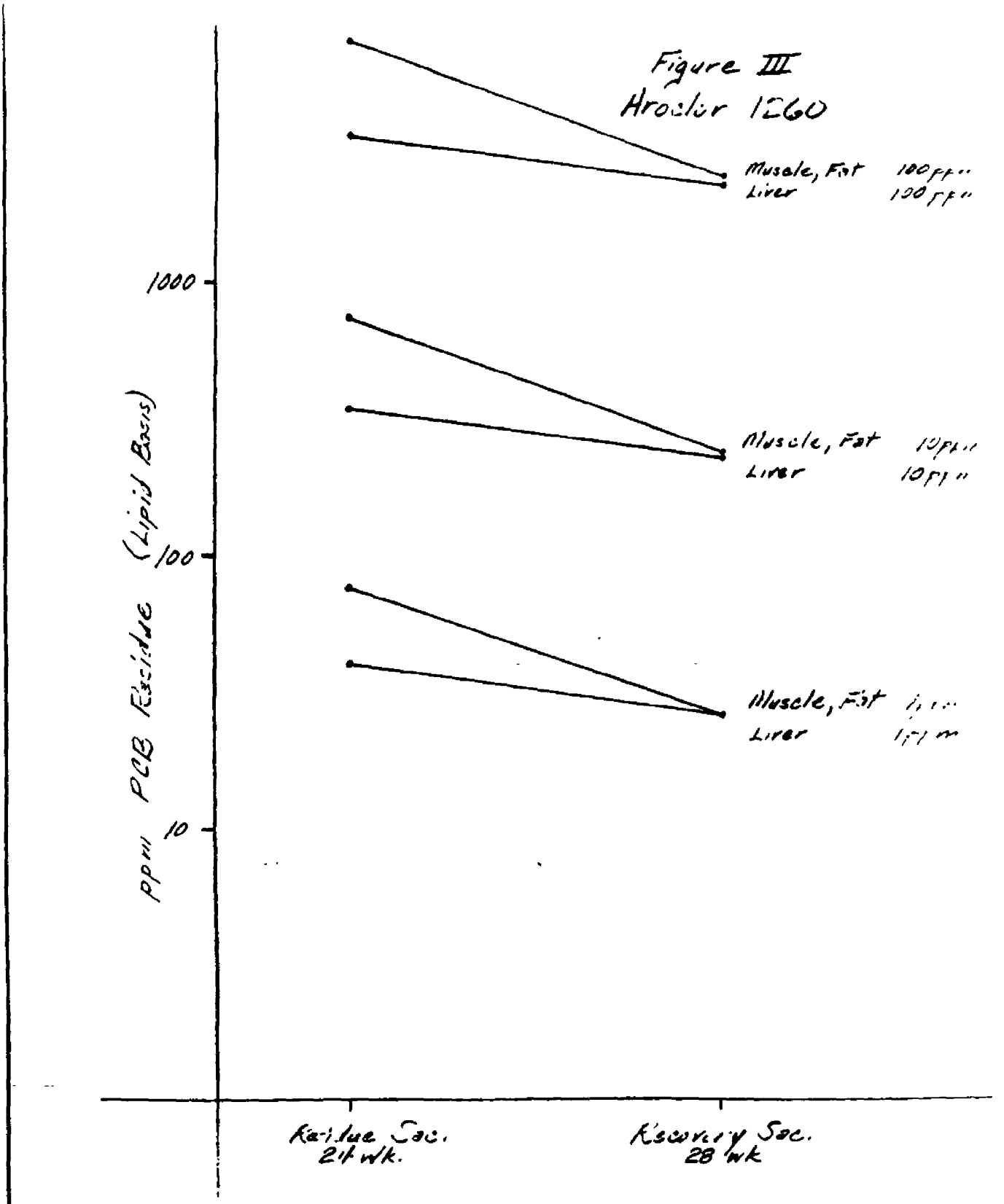
Figure III
Aroclor 1260
Muscle, Fat
Liver
1000
100
10
ppm PCB Residue (Lipid Basis)
Residue Sac.
21 wk.
Recovery Sac.
28 wk

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Figure IV

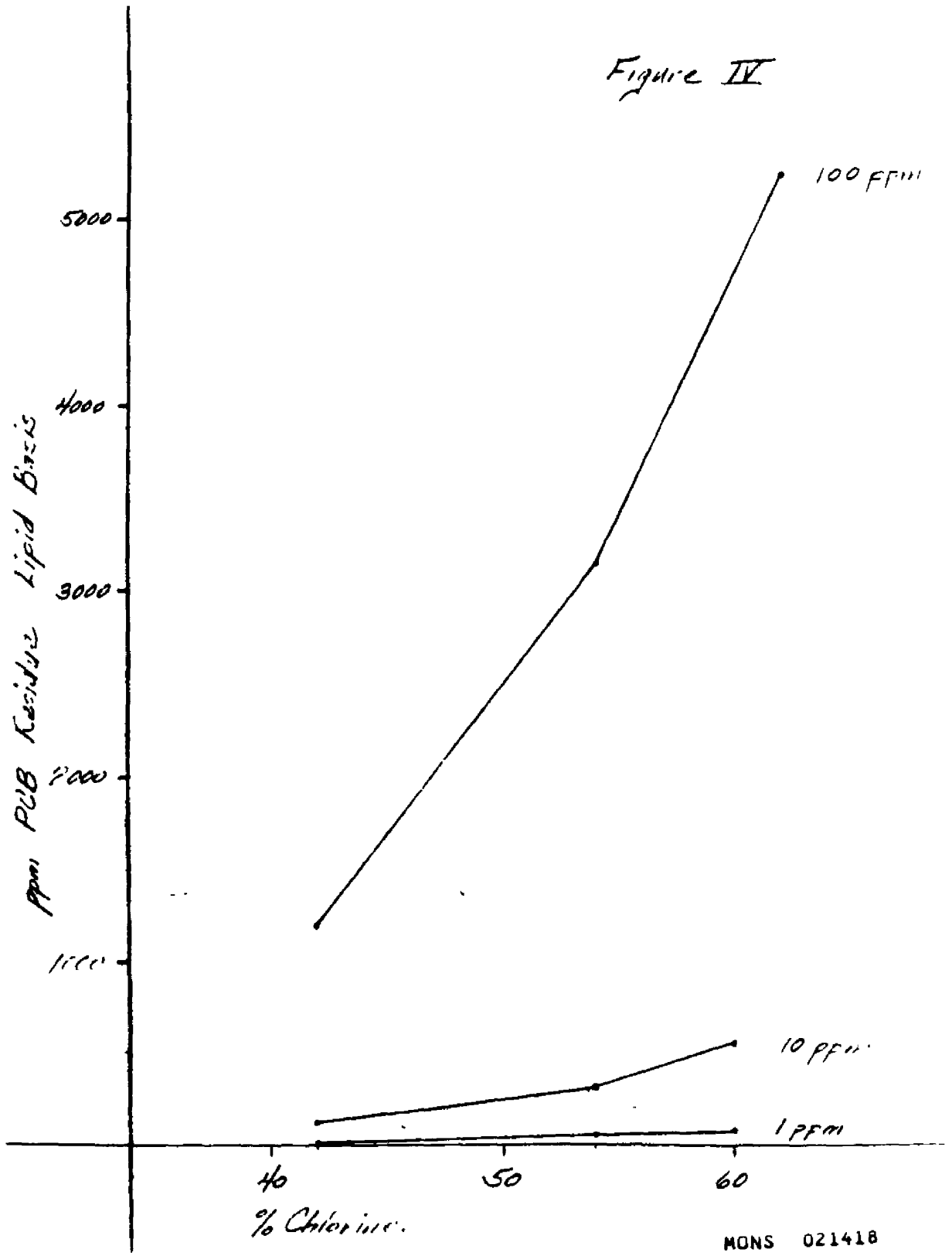
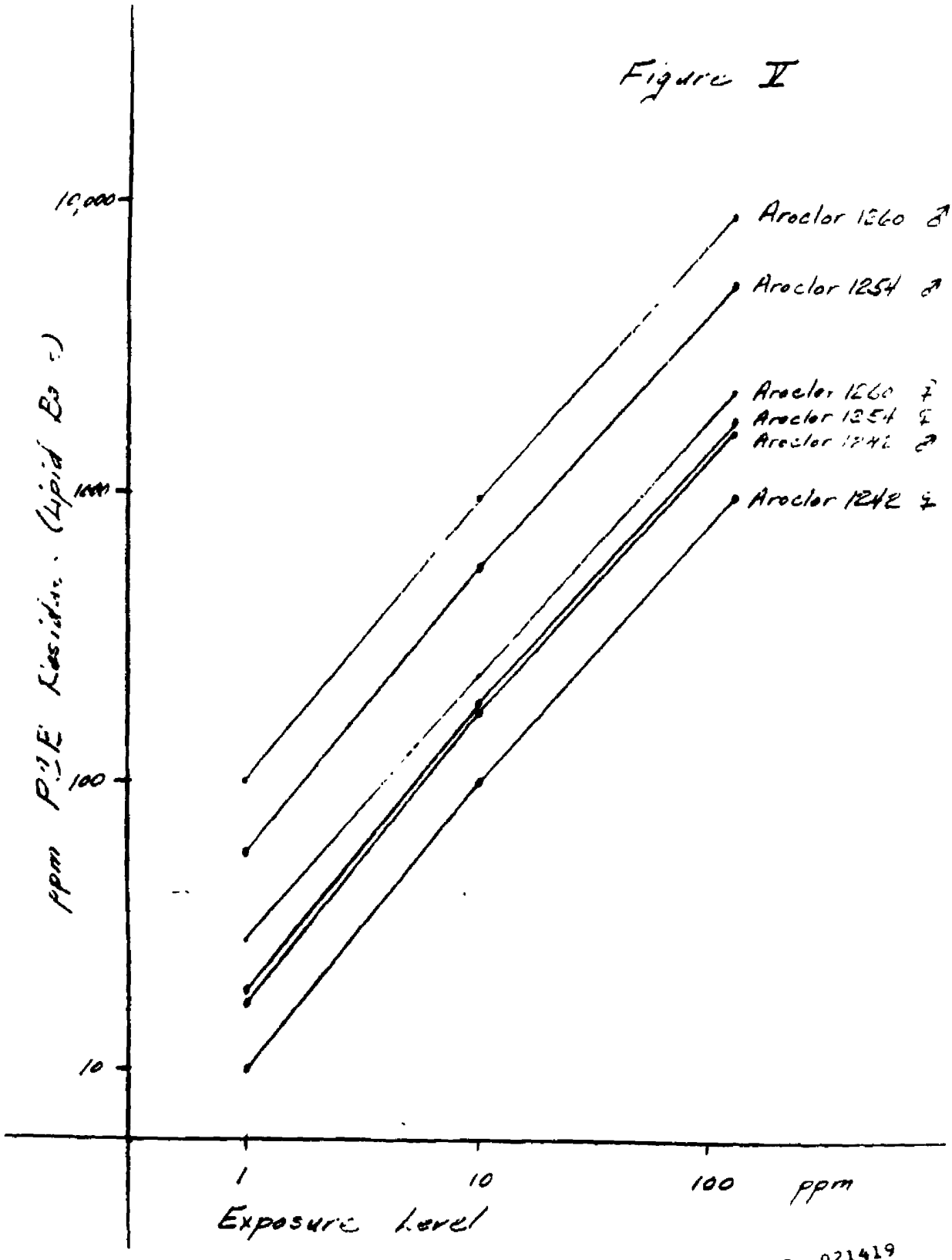

100 ppm
5000
4000
3000
2000
1000
ppm PCB Residue Lipid Basis
10 ppm
1 ppm
40
50
60
% Chlorine
MONS 021418

Figure I



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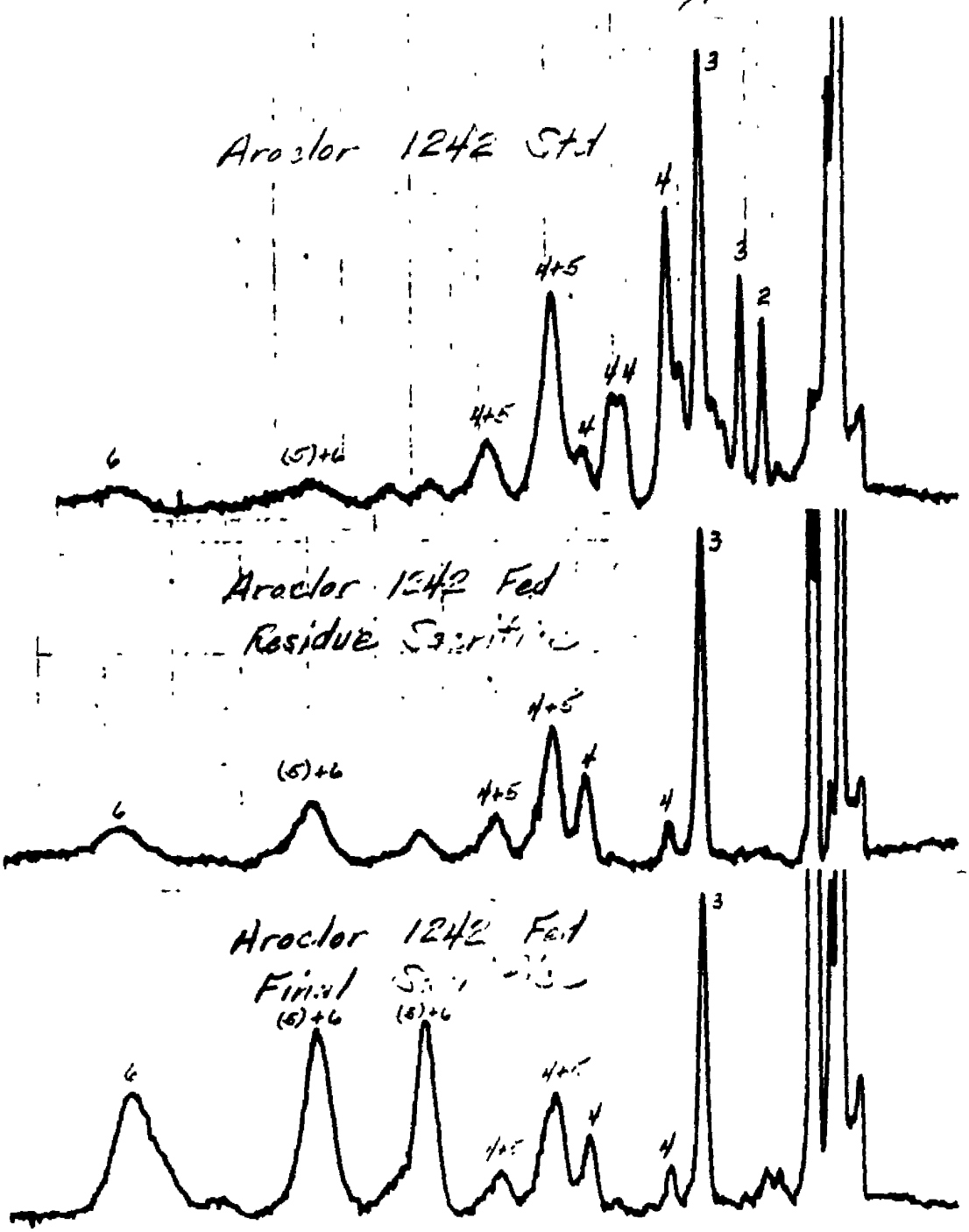
Chromatogram I



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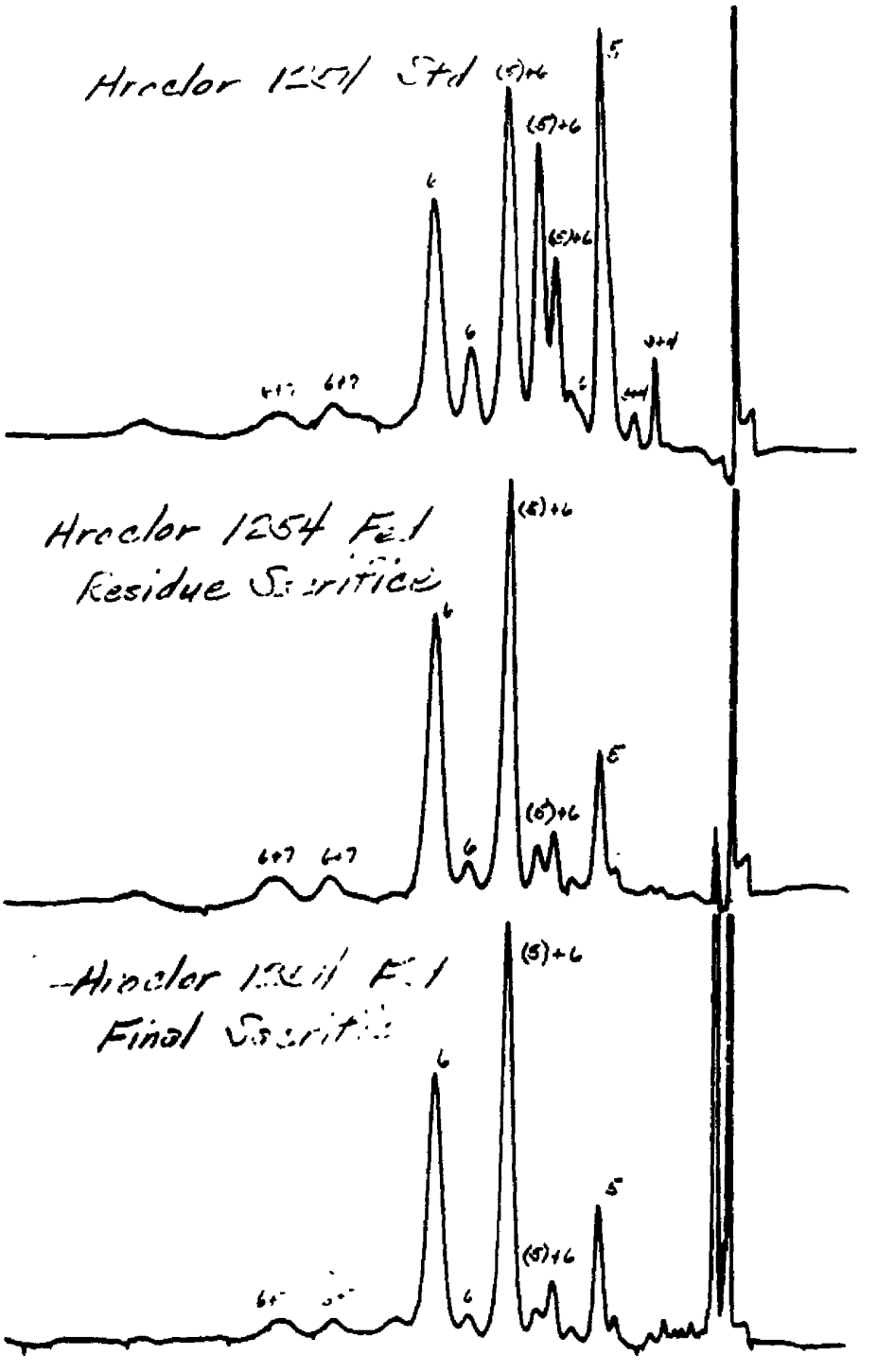
Chromatogram II



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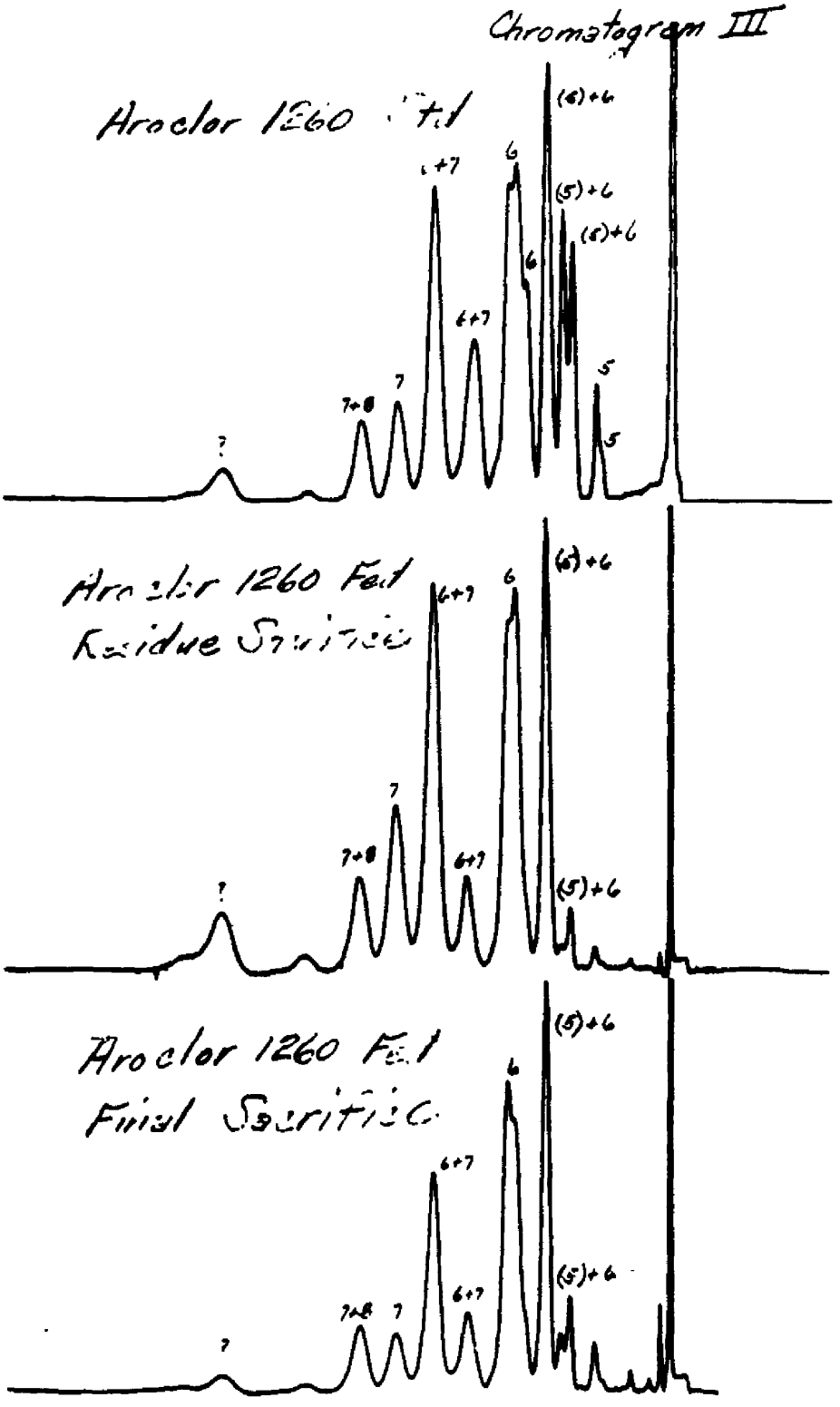
Chromatogram III



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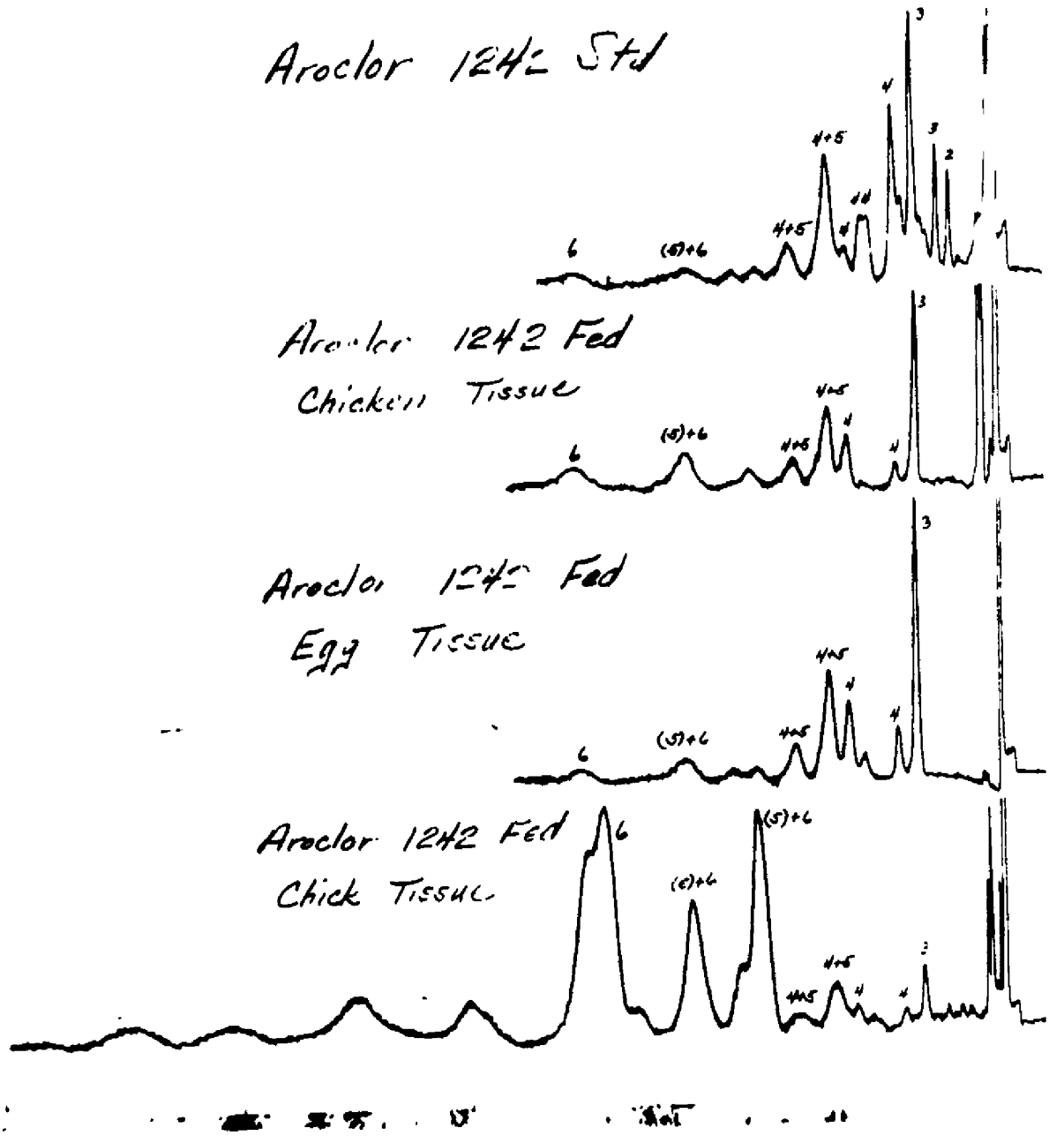
Chromatogram IV



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