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.

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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

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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.

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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 #U3300 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			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	-	0.06				2	0.07	0.05															0.15		0.1		5
	Liver				2			0.4	0.2															67		2		45
	Fat	26	17			1000	1860																	4				8
	Eggs			5		38									3	0.6	0.5		26	30	19	24						
Aroclor 1248	Muscle			0.9	0.7			0.02																		0.1		
	Liver		0.8				50	0.09																0.9				27
	Fat			561	199																					79		
	Eggs	0.7				132						2	0.5	0.1						68	75	36						
Aroclor 1260	Muscle		0.2				11	0.06	0.8															0.09				2
	Liver				6			0.09	0.2																	13		
	Fat	153	15			1520	1159																	10				1000
	Eggs	1		13							1	0.7			13	8												

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TABLE II

IBT #7300 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																			
	Liver				52			14	12															10				
	Fat	30	22			2464	1310																	4				
	Eggs			37		472									53	5	5		345	36	277	360						
Aroclor 1254	Muscle			517	240			2																	61			
	Liver		18				975	4																7				1529
	Fat			872	197																				78			
	Eggs	10				1410						14	6	5						1287	436	832						
Aroclor 1260	Muscle		40				640	5	125															13				867
	Liver				86			6	6																58			
	Fat	183	16			2284	1305																	11				4207
	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 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 1262	Muscle	20	12	195	119	1891	1152																10	6	92	54	901	552
	Liver	12	7	114	69	1104	669																12	7	144	83	1382	806
	Fat	20	12	196	119	1911	1158																10	6	92	56	901	547
	Eggs	3		40		493					2	0.9	0.6	0.4	85	16	10	7	424	362	308	269						
Aroclor 1254	Muscle	68	22	657	213	6315	2044																26	8	249	81	2394	782
	Liver	34	11	326	106	3143	1027																25	8	240	77	2312	739
	Fat	63	22	657	213	6315	2044																26	8	249	81	2394	782
	Eggs	7		92		1141					6	5	4	3	80	66	54	44	1401	801	710	631						
Aroclor 1260	Muscle	128	27	1176	259	10852	2392																42	10	377	90	3479	828
	Liver	57	24	530	221	4894	2040																42	10	366	87	3377	805
	Fat	128	27	1176	259	10852	2392																42	10	377	90	3479	828
	Eggs	11		140		1728					12	13	14	15	137	135	133	131	1515	1330	1170	1029						

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 − 0.0078 (% Cl_2) if rec. − 0.0226 (% Cl_2) if female

Egg Regression Equation log ppm PCB = − 0.7478 − 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 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
		♂♀	♂♀	♂♀	♂♀	♂♀	♂♀	30 Day Recovery Period												♂♀	♂♀	♂♀
Feeds 12%	Muscle			1	0.02	0.04	0.03													0.5		1
	Liver		4		0.3	0.2	0.2														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)

		Routine 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
		♂♀	♂♀	♂♀	♂♀	♂♀	♂♀													♂♀	♂♀	♂♀
Roosters	Muscle			64	5	8	13													19		71
	Liver		30		15	19	17														17	
Hens	Fat			73	3	5	9													14		56
	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
		d ♀	d ♀	d ♀	d ♀	d ♀	d ♀													d ♀	d ♀	d ♀
Female		31	62	123	7	9	11													15	27	58
Female	Liver	18	36	71	15	15	15													17	33	66
Male	Fat	31	62	123	6	6	6													15	29	58
	Eggs	7	15	52				4	2	1	.25	8	5	3	1	19	12	7	4			

Chick Regression Equation - log ppm PCB = a 7486 + a 4385 (if liver) + a 3317 (log ppm fed - if muscle)

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TABLE VII

BIOLOGICAL HALF-LIFE

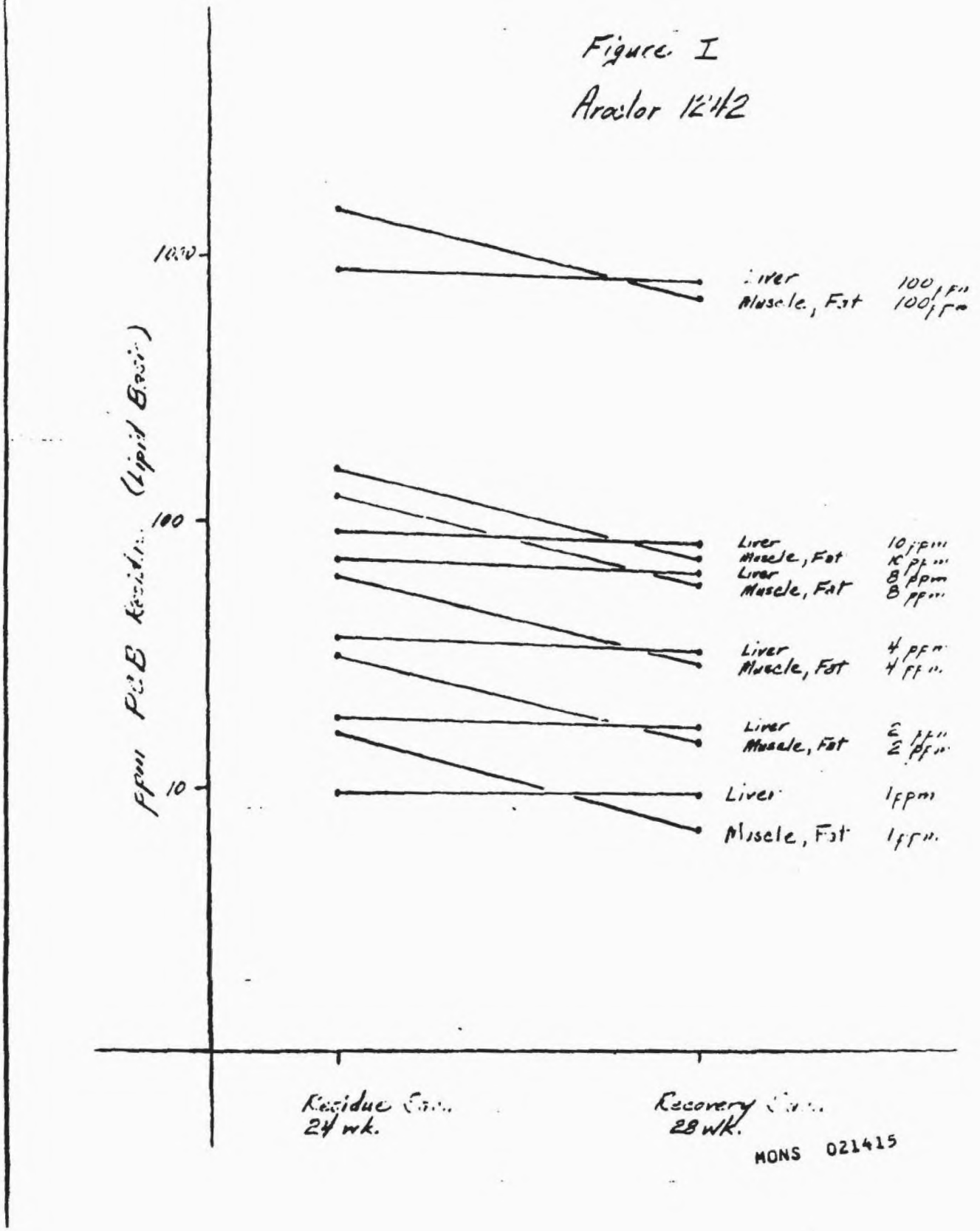
Material	Tissue	Biological Half-Life
Aroclor 1242	Muscle, Fat	27.6 days
" "	Liver	245.4 days
Aroclor 1254	Muscle, Fat	21.4 days
" "	Liver	69.3 days
Aroclor 1260	Muscle, Fat	19.3 days
" "	Liver	51.0 days

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Figure I

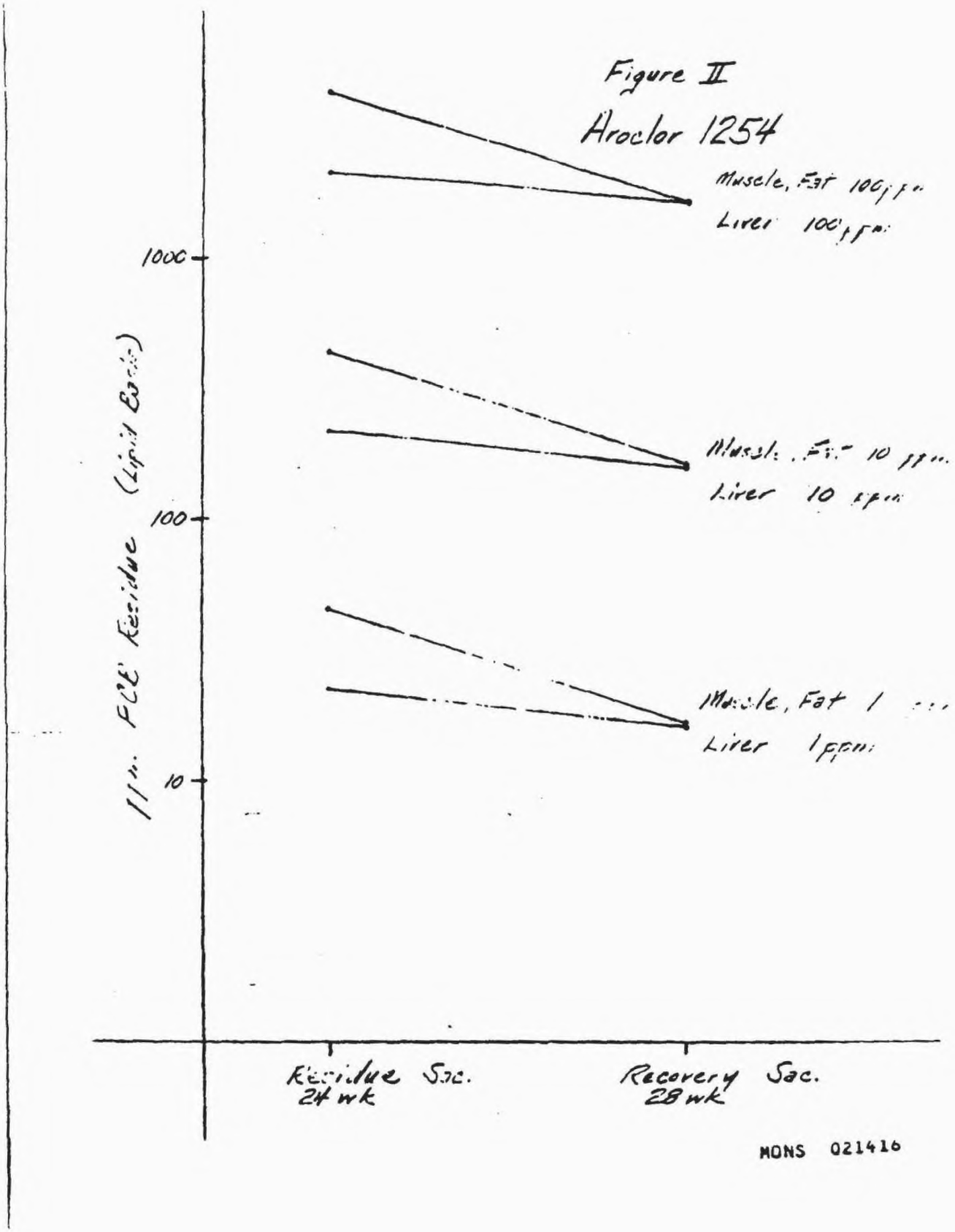
Aroclor 1242



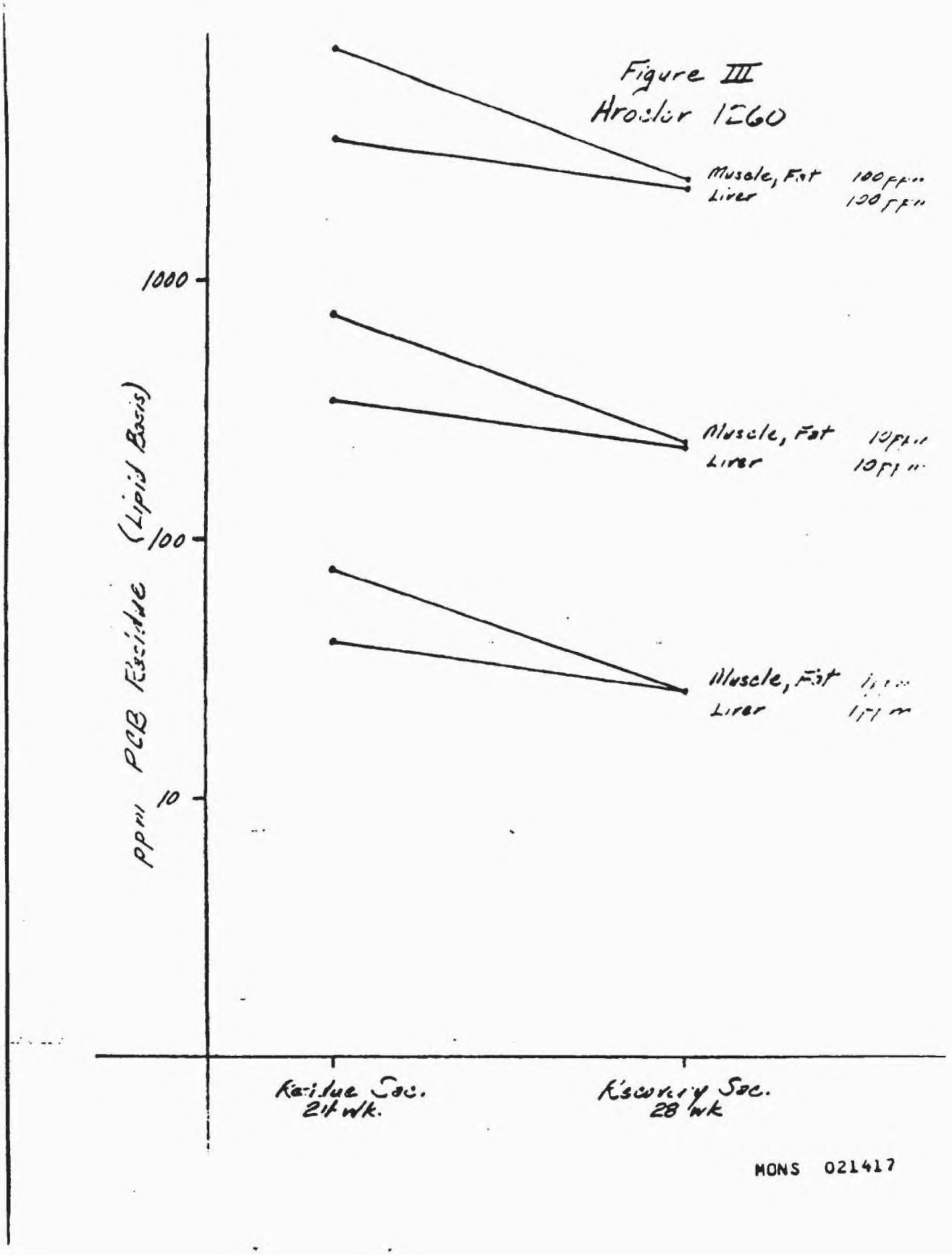
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Figure II

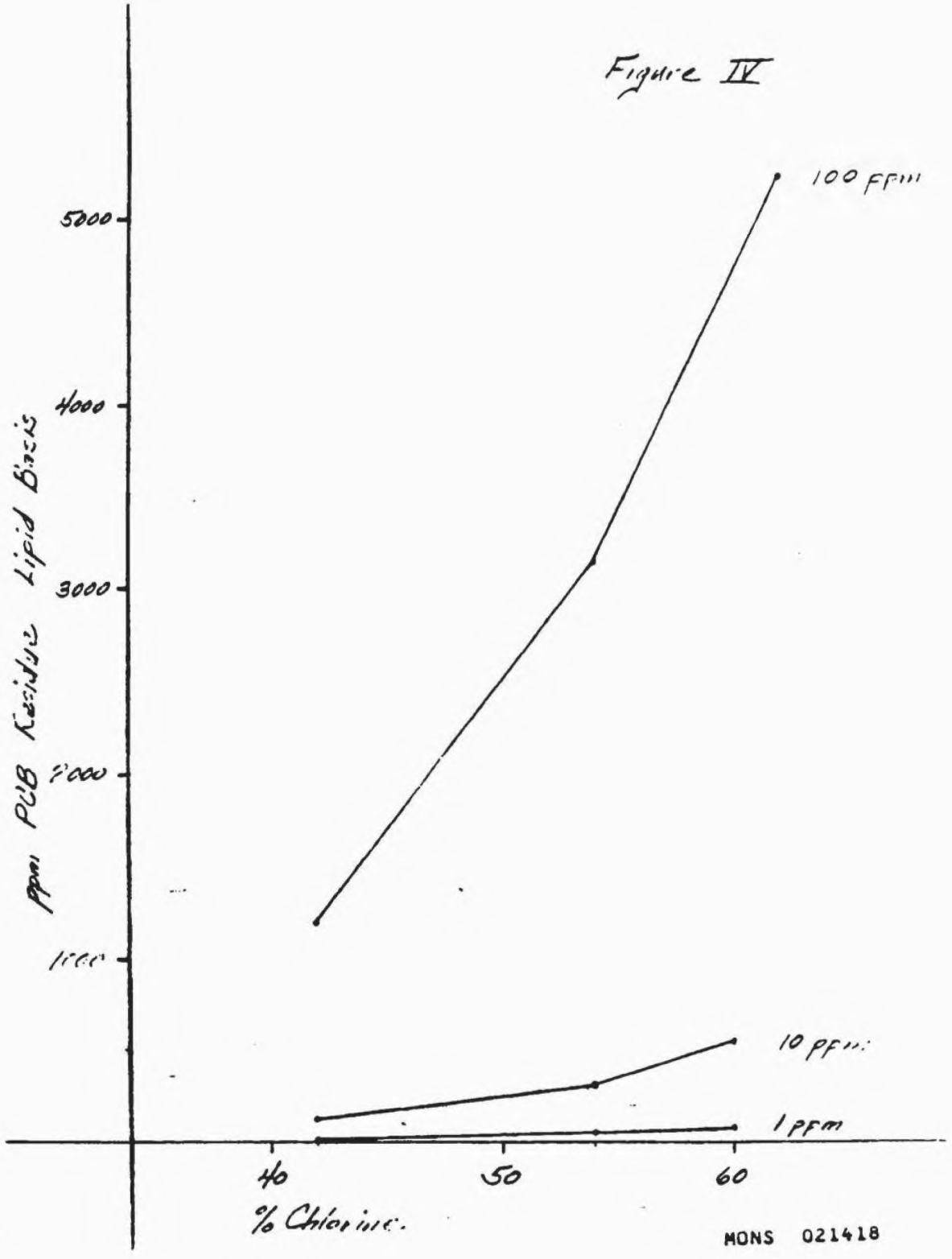


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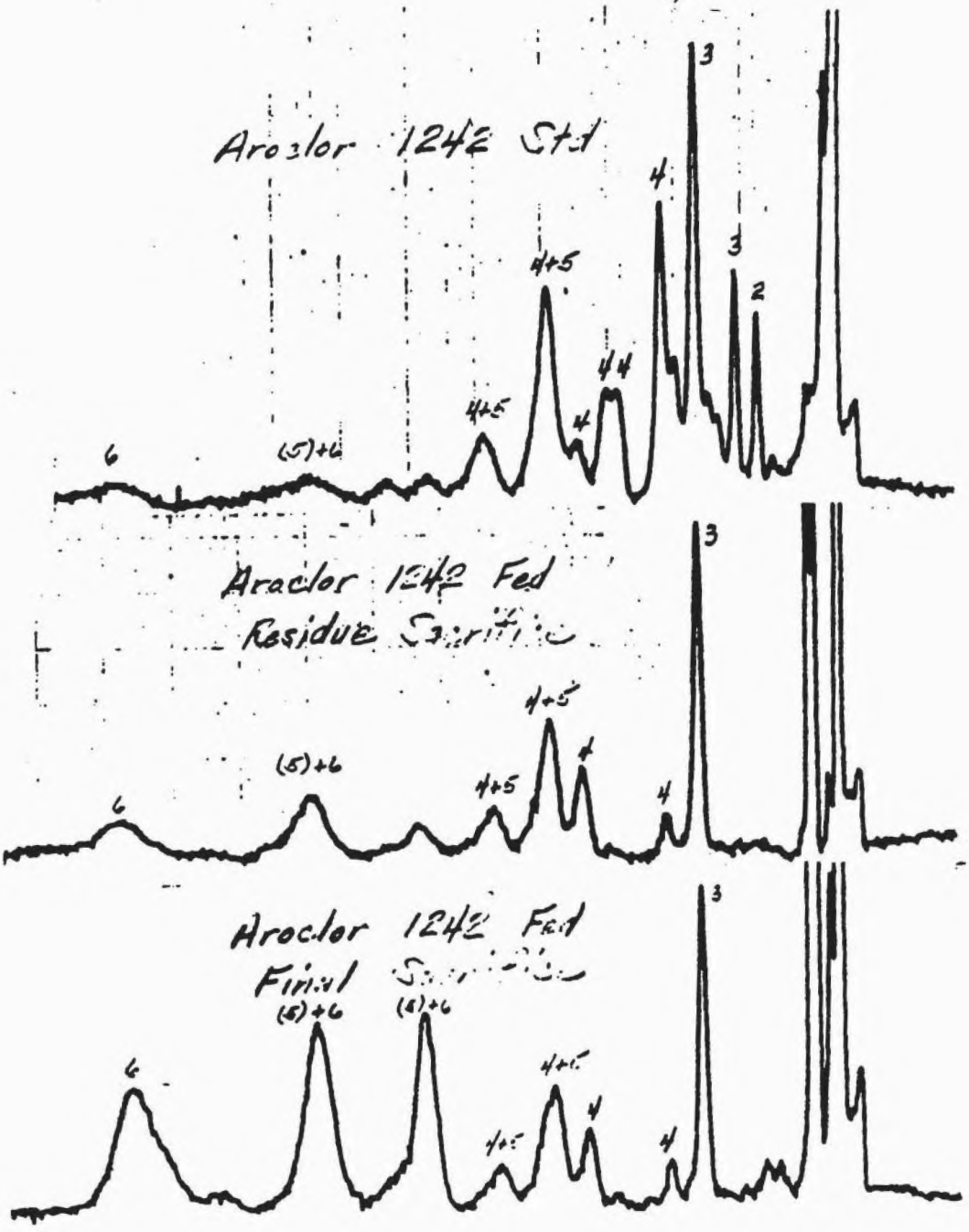
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Figure IV



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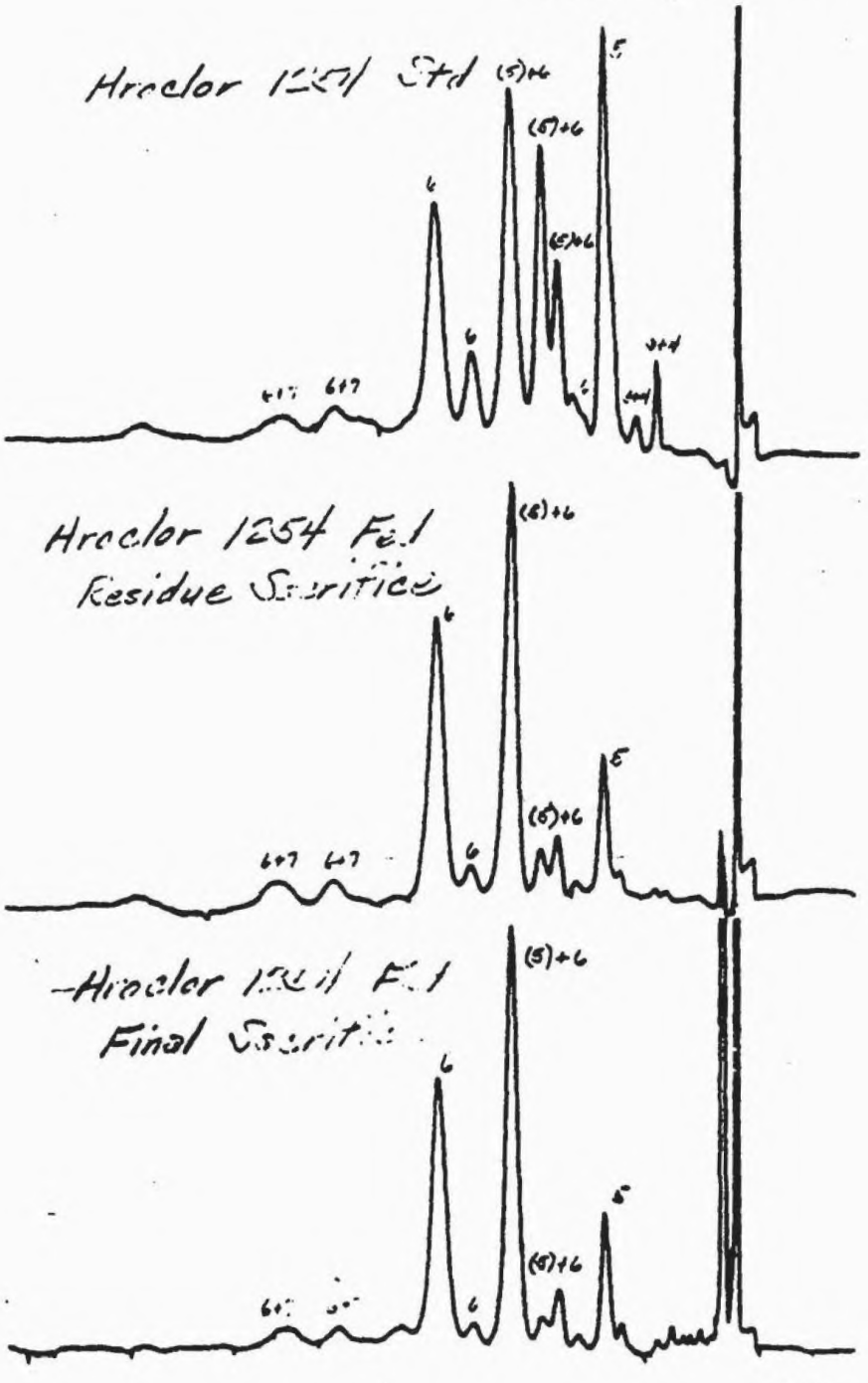
Chromatogram I



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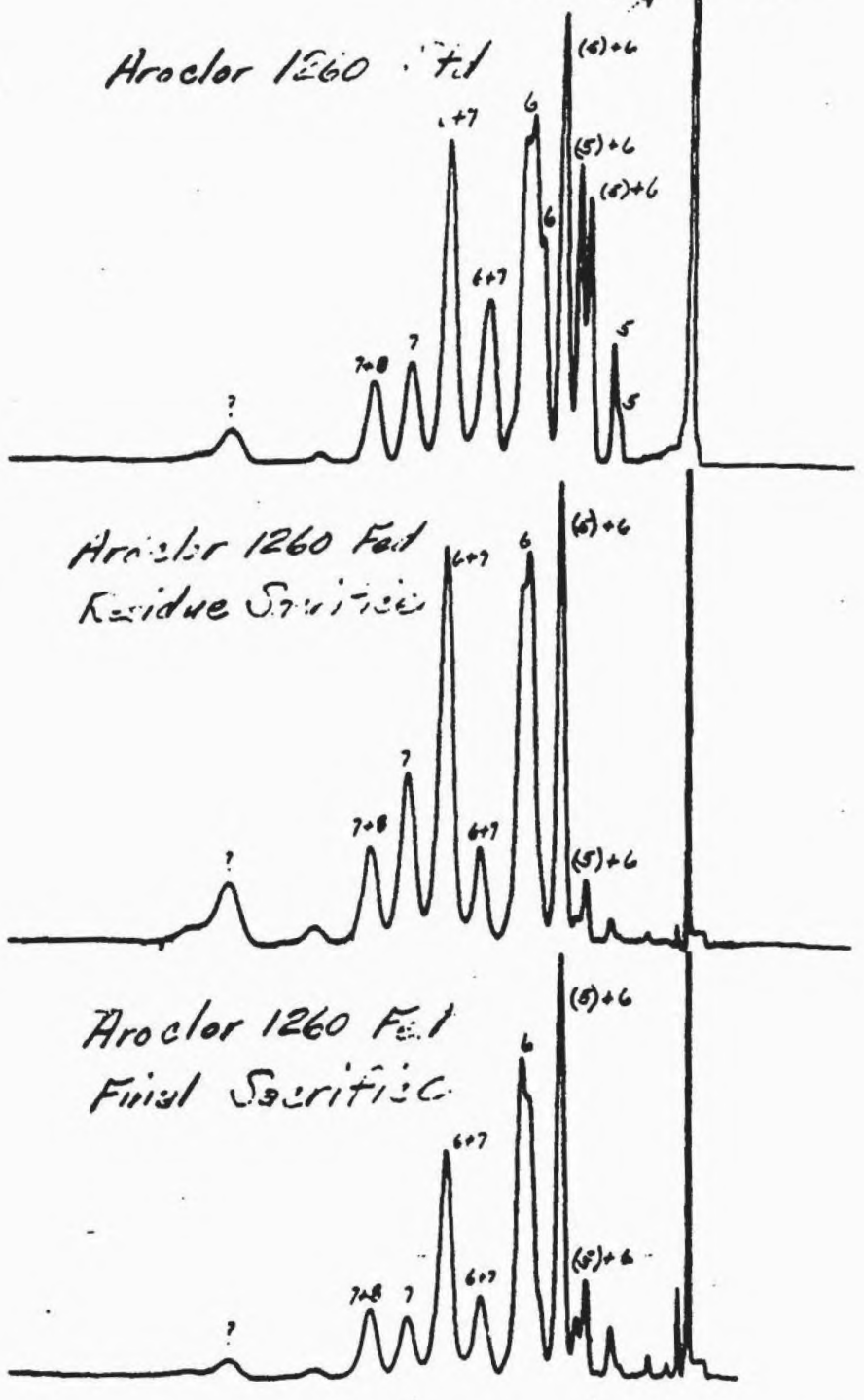
Chromatogram II



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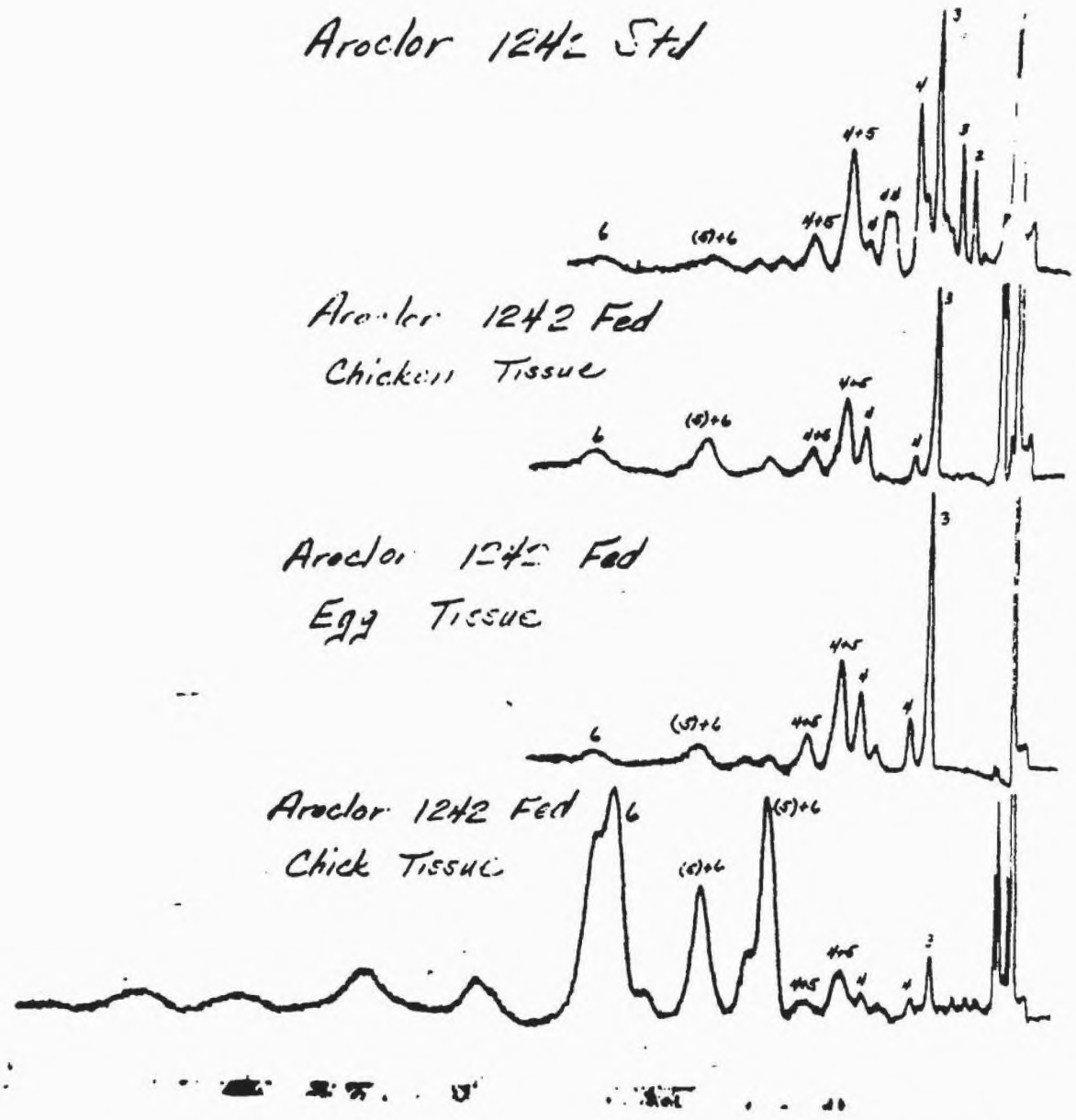
Chromatogram III



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Chromatogram IV



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