12/72

Analytical Chemistry
Special Study 71-9
Job No. 13840

DETERMINATION OF POLYCHLORINATED BIPHENYL RESIDUES IN BEAGLE DOG TISSUES FROM A TWO YEAR ORAL CHRONIC TOXICITY STUDY

INTRODUCTION

This report covers the results of the analysis of tissues from IBT # C7299, "Two Year Chronic Toxicity Study with Aroclor 1242, Aroclor 1254, and Aroclor 1260 in Beagle Dogs", for PCB residues.

The specific objectives of this study were to establish the relationship between tissue storage levels and exposure period, exposure level, degree of chlorination and any observed toxic effects as well as determining the homolog distribution of the retained PCB residues. Enough animals were also included in this study to determine the rate at which the retained residues washed out once oral exposure to the PCB products was ceased.

SUMMARY

The PCB residues found in the dogs were an order of magnitude less than the PCB residues found in a similar study with rats.(1) Material balance calculations indicate that in all cases less than 1% of the total test material ingested was stored in any of the animal tissues.

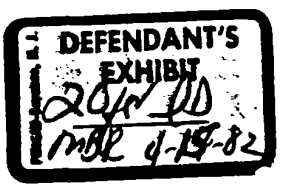
Significant alterations of the homolog distributions of all products were observed. The amount of alteration was greatest for Aroclor 1242 and least for Aroclor 1260.

The estimated biological half lives of the residues retained from each product were: Aroclor 1242 - 47 days; Aroclor 1254 - 108 days; and Aroclor 1260 - 301 days. These half lives were found to be independent of both tissue type and exposure level.

DETAILS

The samples were analyzed as outlined in Analytical Chemistry Method 71-35. The method involves isolation of the PCB's by solvent extraction, followed by chemical and alumina column clean-up of the extract, and detection of the type and level of PCB's retained in the tissues by electron capture gas chromatography (EC/GC).

Standard solutions of the Aroclor fed were used to prepare the calibration curves for quantitation in the cases of Aroclor 1254 and Aroclor 1260. For Aroclor 1242, the Aroclor 1254 standard solutions were used to prepare the calibration curves, since the principal isomers found in the tissue extracts were those that are dominant in production Aroclor 1254.



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The PCB residue levels found in each of the tissue samples are shown in Tables I and II. The values in Table I were calculated using the wet weight of the tissue, those in Table II were calculated on the basis of the hexane soluble lipid weight.

After the 24 month sacrifice samples had been analyzed, the large number of samples (180 total) and hence time and money needed for analysis of the complete set of samples prompted a reevaluation. It was decided that little information would be lost by analyzing only a balanced subset of the recovery samples and using multiple regression statistics to predict the remaining points.(2) The open boxes in Tables I and II represent the samples which were not analyzed. The regression equation obtained indicated that, relative to the rat study completed earlier, there was more scatter in the dog data. This increased variability is attributable to the lower storage levels observed and to the fact that the tissue samples from the recovery sacrifices were from individual animals rather than group composite samples analyzed in the rat study. However, an acceptable regression equation was obtained by eliminating results where the difference between the predicted and actual values exceeded two standard deviations. The predicted PCB residue levels (lipid weight basis) are shown in Table III.

Figures I thru IX are semi-logarithmic plots relating average PCB residue level to exposure period for each Aroclor and for each tissue (muscle, liver, and fat) at three exposure levels (1, 10 and 100 ppm). On these plots solid lines were used for actual data while dashed lines were used for predicted data. These figures illustrate the decrease of PCB residue levels with time in those animals placed on a recovery diet. The figures also show that the recovery was much slower for those animals fed the higher chlorinated products such as Aroclor 1254 and Aroclor 1260. There is little evidence, at least under the conditions employed in this study, that exposure level or tissue differences influenced the residue washout rate.

The significantly higher PCB residue levels found in liver seem to indicate this tissue is a primary storage area and/or site of metabolism.

Chromatograms I, II and III compare the typical homolog distributions of the retained residue to that of the Aroclor fed. Isomeric alterations were observed with each Aroclor at all feed levels. Aroclor 1242 was altered to a greater extent than either Aroclor 1254 or Aroclor 1260.

The chromatographic conditions employed while suitable for quantitation of the PCB residues in the tissues are not ideal for the identification of the specific isomers which are retained. Nevertheless with these limitations, the chlorine number(s) of the principal isomer(s) are shown above each peak on the chromatogram. An inspection

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of the chromatograms reveals that the less chlorinated isomers are metabolized and/or excreted rapidly. Further inspection of the chromatograms from the recovery samples shows that the ratios of the peak heights continue to change which along with the decrease in PCB residue levels, indicates that the higher chlorinated isomers can also be metabolized and/or excreted, albeit more slowly. Chromatogram IV is a comparison of the extracts from the animals after 60 days on a recovery diet free from PCB's. It is evident, that the isomers observed are the same irrespective of the material fed. This again illustrates the much lower wash out rate encountered with the higher chlorinated isomers.

TOXICITY

No untoward behavorial reactions or significant abnormalities were observed during the two year study.(1)

REFERENCES

1. "Two Year Chronic Oral Toxicity Study With Aroclor 1242, Aroclor 1254, and Aroclor 1260 in Beagle Dogs", Industrial Bio-Test Laboratories Report (IBT # C7299).

2. "PCB Residues in Beagle Dogs", Statistics Special Study 71-25.

db

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TABLE

IGT # C7299 Two-Year Chronic Oral Toxicity Study of Aroclor in Beagle Dogs

ppm PCB in Dog Tissues (Wet Weight Basis)

Material	Tissue	1 ppm						10 ppm						100 ppm						Exposure Level
		24 mo.		30 d.		60 d.		24 mo.		30 d.		60 d.		24 mo.		30 d.		60 d.		Sacrifice
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1242	Muscle	0.07	0.08	0.09	0.06			0.3	0.2		0.2		0.04	0.6	0.5	0.3	0.4	0.5		
	Liver		0.08	0.3	0.04		0.04	0.2	0.1		0.1	0.05		0.6		0.4	0.3			
	Fat	2	2	1	0.5	0.5		1	1		2	1	2	4	4	9	2			
Aroclor 1254	Muscle	0.3	0.08	0.2		0.1	0.1	0.7	0.3		0.7			4	3				3	
	Liver	0.1	0.2		0.2			0.4	0.7			0.5		2	2	1				
	Fat	4	3	2			2	5	4		18			95	73				34	
Aroclor 1260	Muscle	0.2	0.1		1		0.2	1	0.4		6		3	26	10		34	26		
	Liver	0.2	0.1	0.1			0.3	2	0.7	2	1	0.9	1	11	4		2	9		
	Fat	11	14		14		4	101	31	40	47		62	93	221		352	175		

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TABLE

IBT #C7299 Two-Year Chronic Oral Toxicity Study of Aroclor in Beagle Dogs

ppm PCB in Dog Tissues (Lipid Weight Basis)

Material	Tissue	1 ppm						10 ppm						100 ppm						Exposure Level
		24 mo.		30 d.		60 d.		24 mo.		30 d.		60 d.		24 mo.		30 d.		60 d.		Sacrifice
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1242	Muscle	2	2	1	2			6	3		2		1	19	20	10	10	6		
	Liver		4	11	2		2	8	8		9	4		29		23	18			
	Fat	2	3	1	1	0.6		1	2		2	2	2	6	7	13	3			
Aroclor 1254	Muscle	10	4	4		2	12	18	18		9			61	85				32	
	Liver	8	6		9			23	40			43		110	130	111				
	Fat	5	3	3			2	6	10		22			128	132				42	
Aroclor 1260	Muscle	9	7		8		3	78	20		66		37	716	100		491	301		
	Liver	17	13	11			21	175	43	112	135	55	120	762	263		81	659		
	Fat	14	18		20		5	159	39	54	72		73	1261	287		137	232		

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TABLE 11

IBT #C7299 Two-Year Chronic Oral Toxicity Study of Aroclor in Beagle Dogs

Predicted ppm PCB in Dog Tissues (Lipid Weight Basis)

Material	Tissue	1 ppm						10 ppm						100 ppm					
Exposure Level		24 mo		30 d.		60 d.		24 mo.		30 d.		60 d.		24 mo.		30 d.		60 d.	
Sacrifice																			
Sex		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Aroclor 1242	Muscle	2	2	1	1	0.7	0.7	5	4	4	3	2	2	20	10	9	6	6	4
	Liver	3	3	2	2	1	1	10	8	7	5	5	4	28	18	18	12	11	8
	Fat	2	2	1	1	0.7	0.7	5	4	4	3	2	2	20	10	9	6	6	4
Aroclor 1254	Muscle	5	5	4	4	3	3	24	20	20	16	16	13	121	80	100	65	80	54
	Liver	10	10	8	8	6	6	47	39	39	31	33	26	238	156	195	128	157	103
	Fat	5	5	4	4	3	3	24	20	20	16	16	13	121	80	100	65	80	54
Aroclor 1260	Muscle	8	8	8	8	7	7	54	44	50	40	47	38	355	233	333	217	308	202
	Liver	16	16	14	14	14	14	106	84	98	80	91	74	698	457	654	426	605	397
	Fat	8	8	8	8	7	7	54	44	50	40	47	38	355	233	333	217	308	202

Regression Equation – log ppm PCB = –[illegible]

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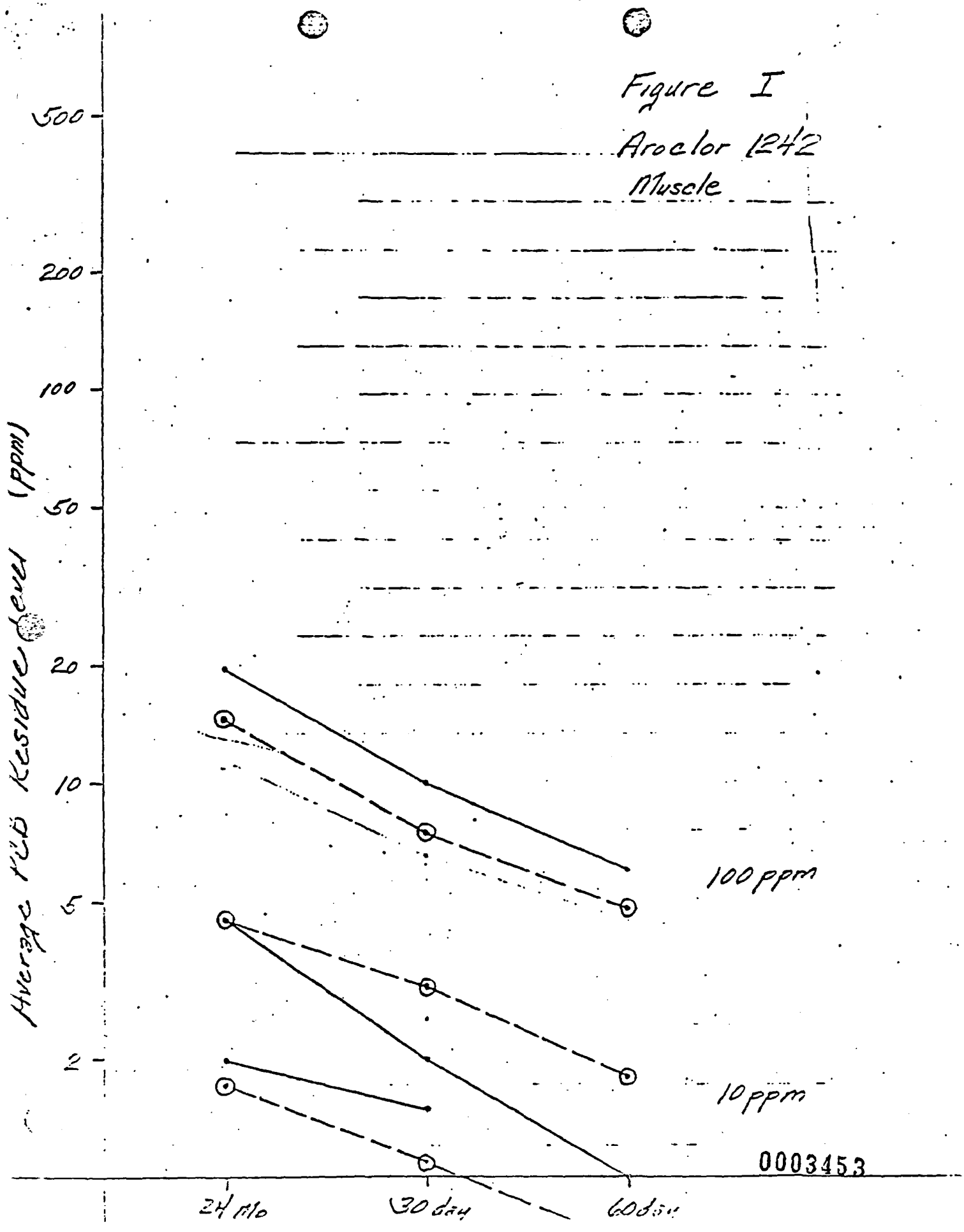

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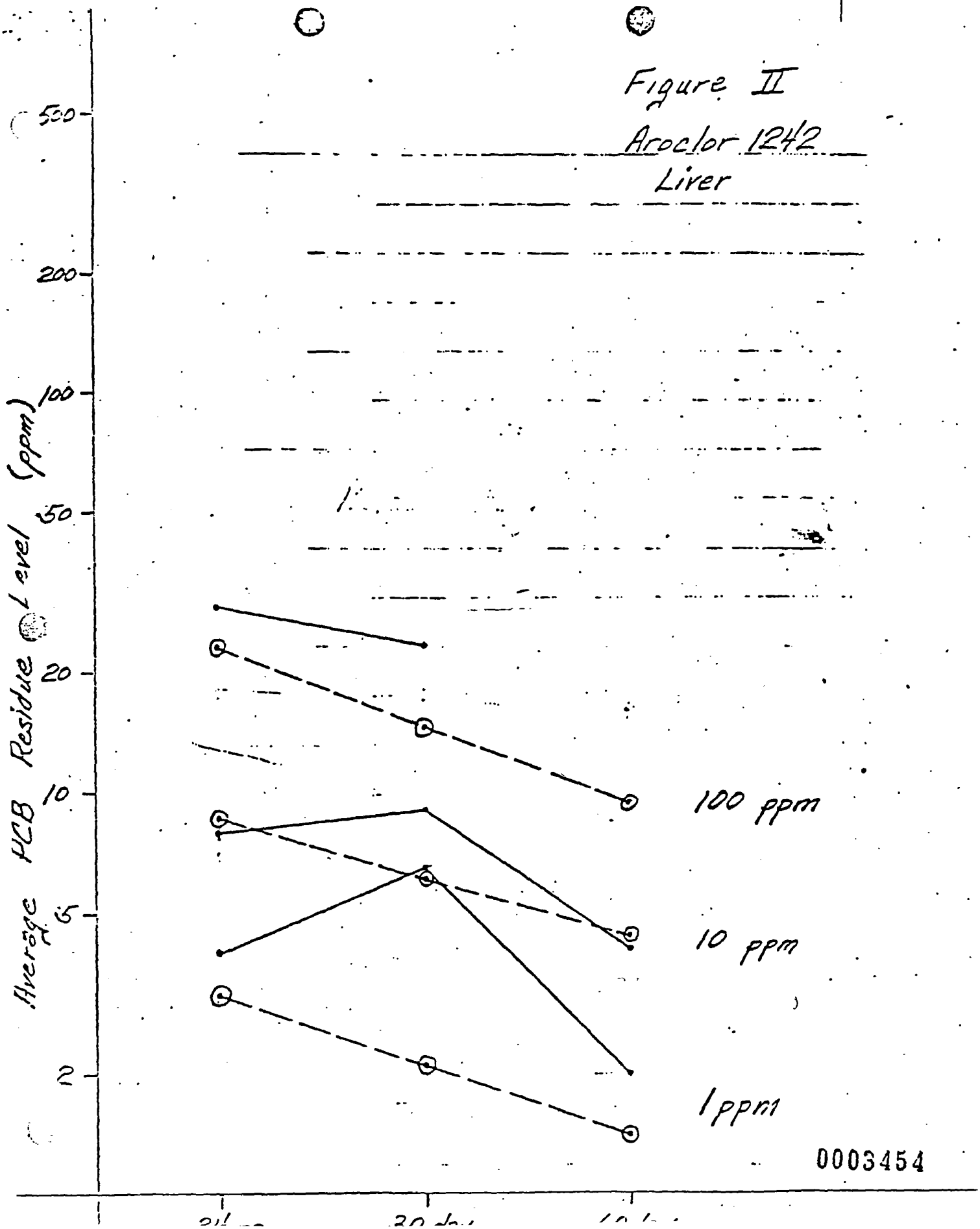

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Figure III

Aroclor 1242

Fat

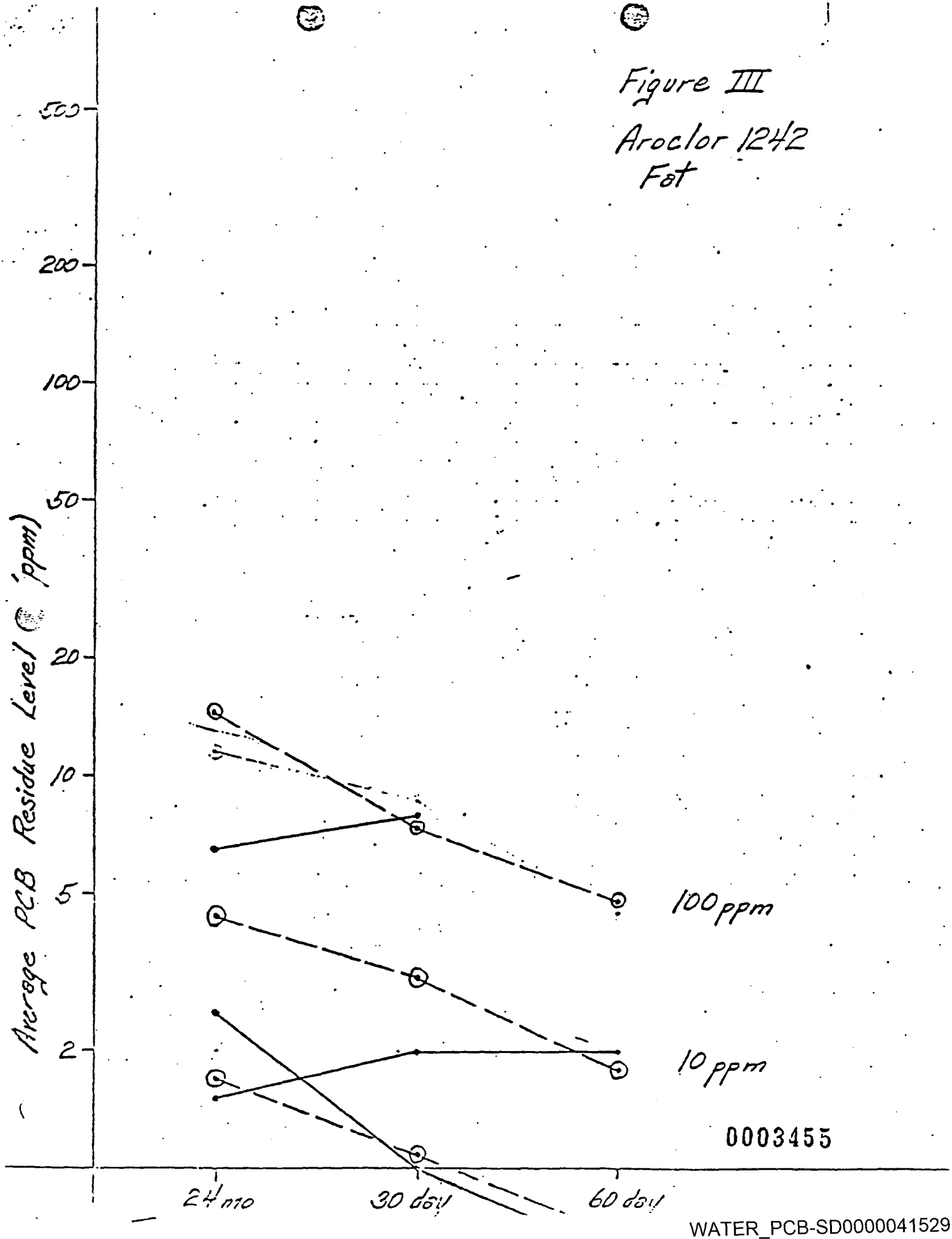

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Figure IV

Aroclor 1254

Muscle

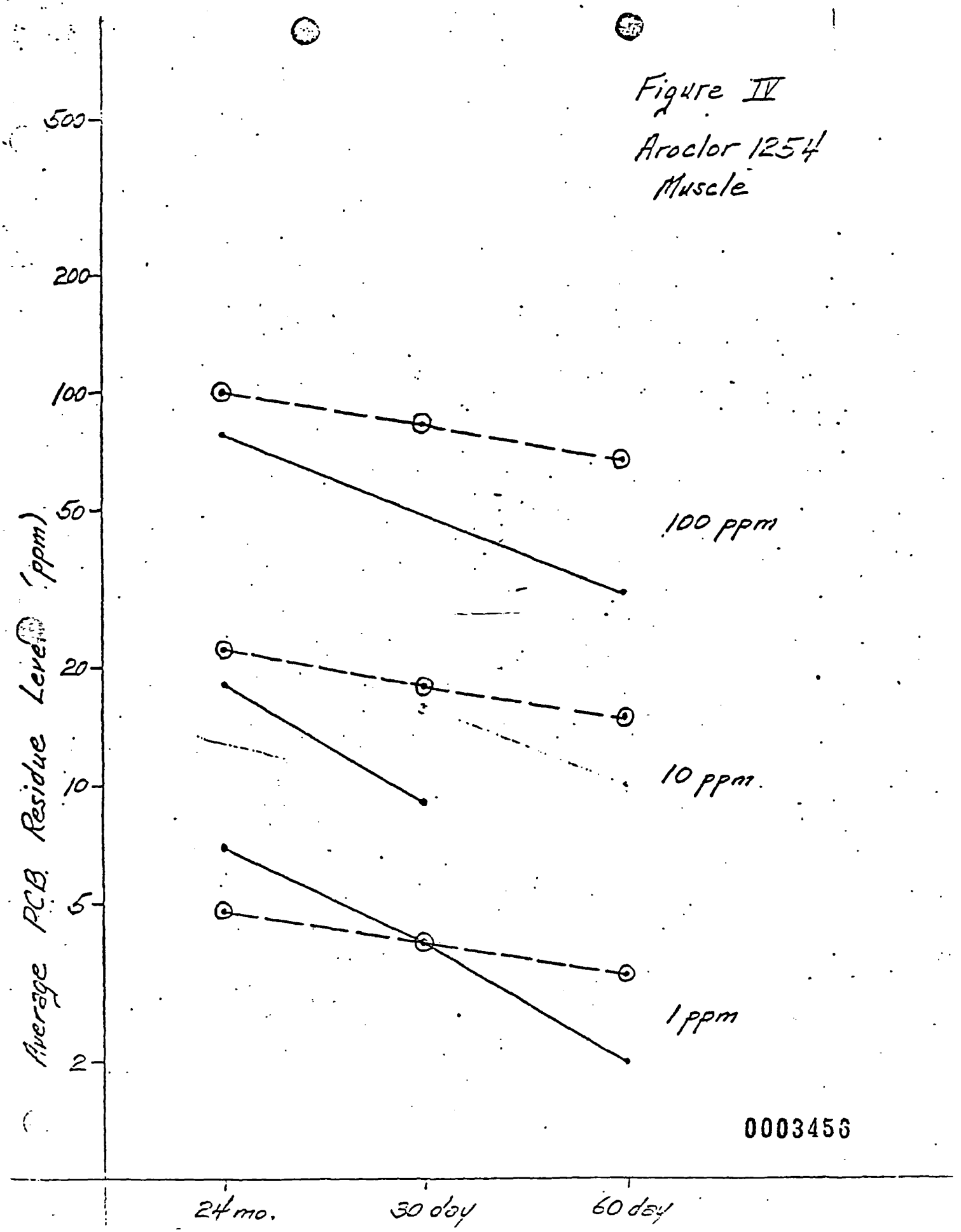

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Figure II

Aroclor 1254

Liver

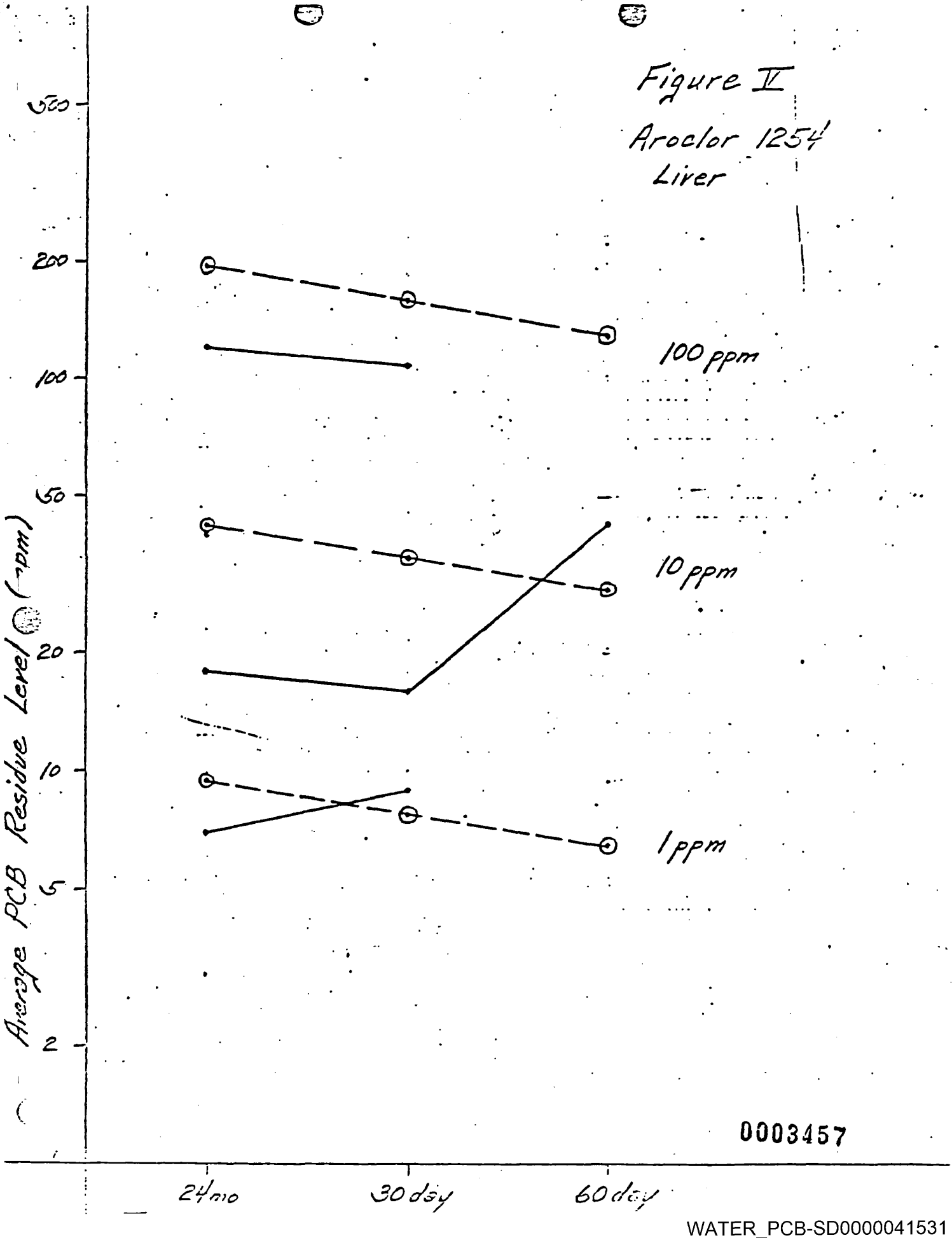

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Figure VI

Aroclor 1254

Fat

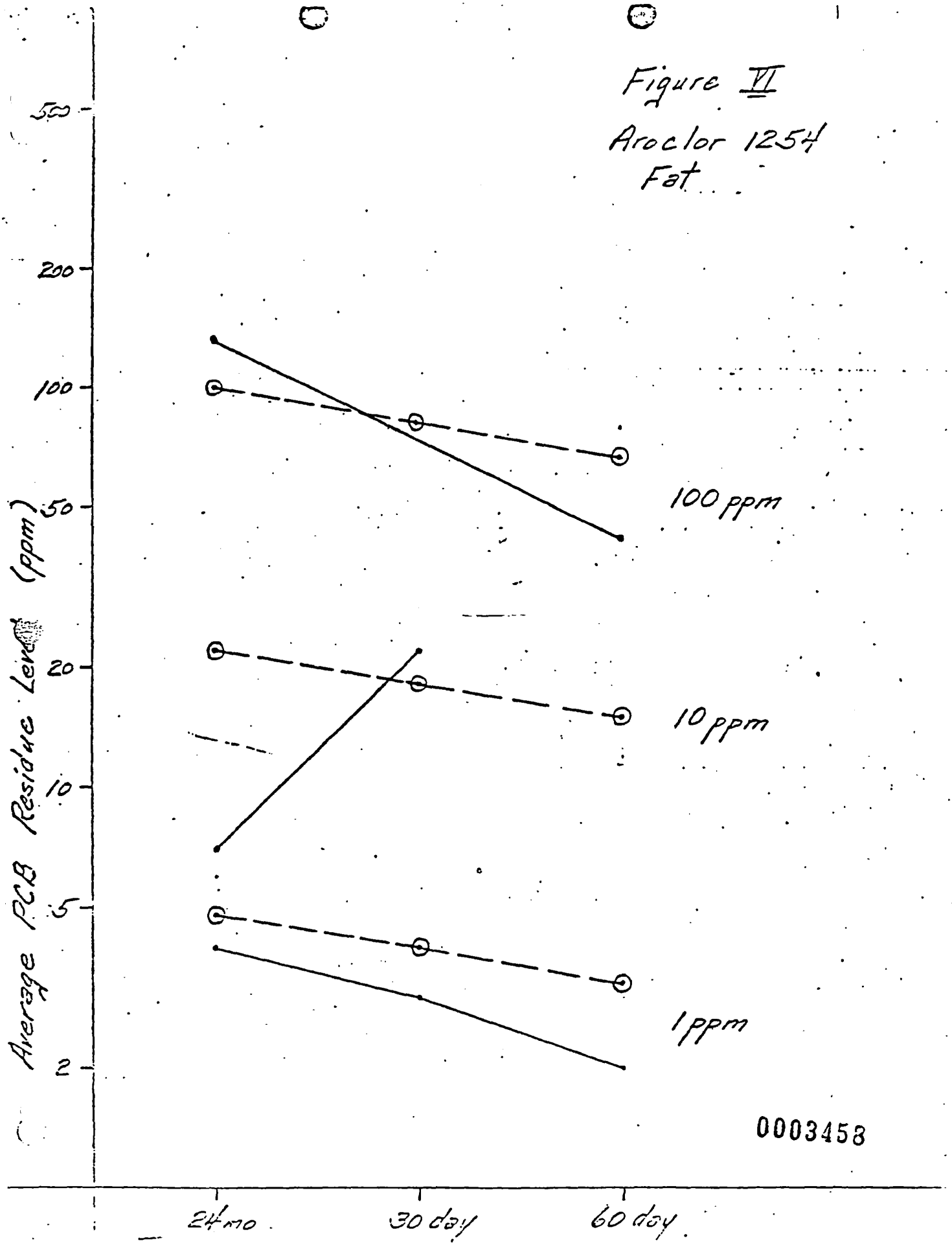

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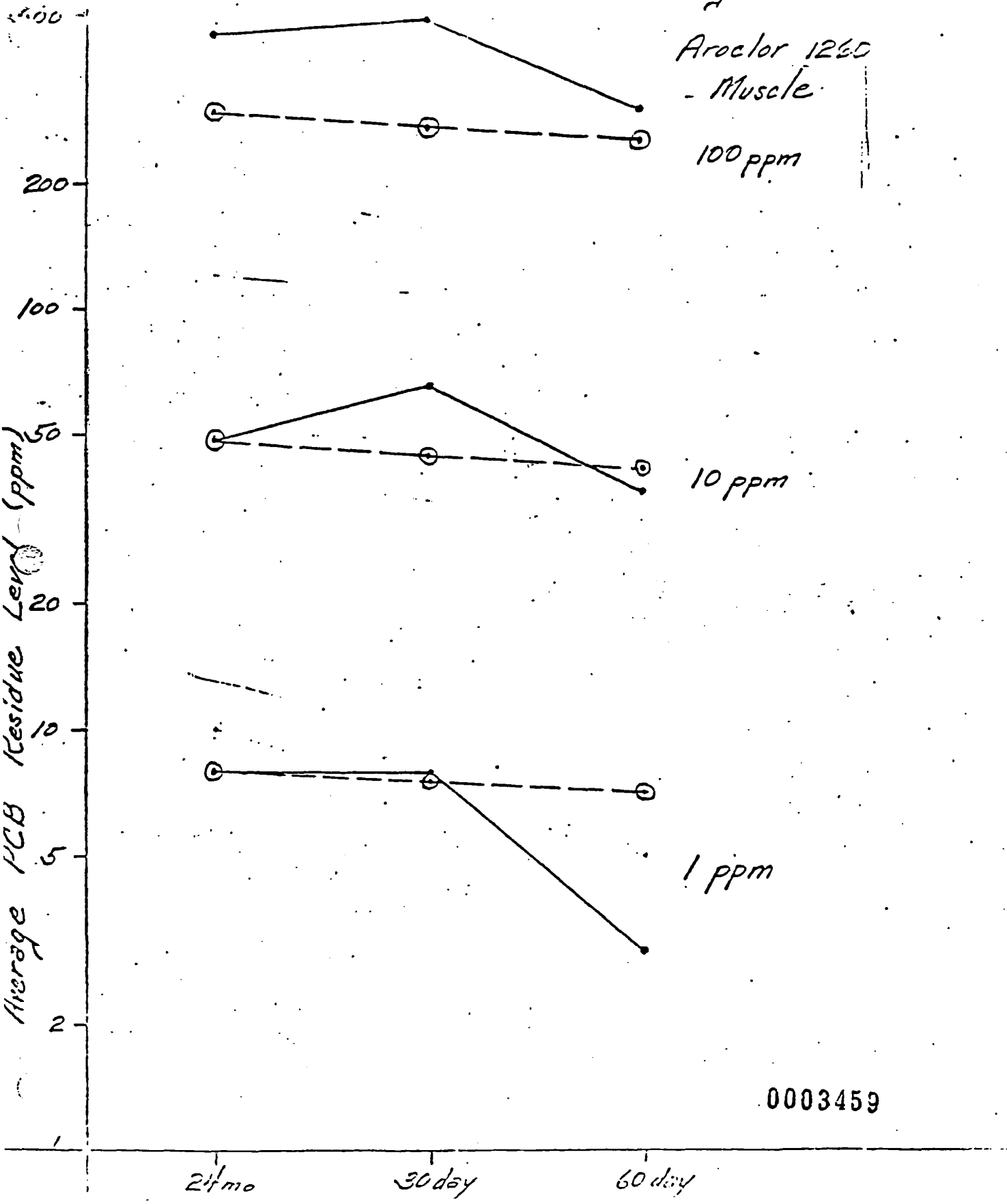
Figure II



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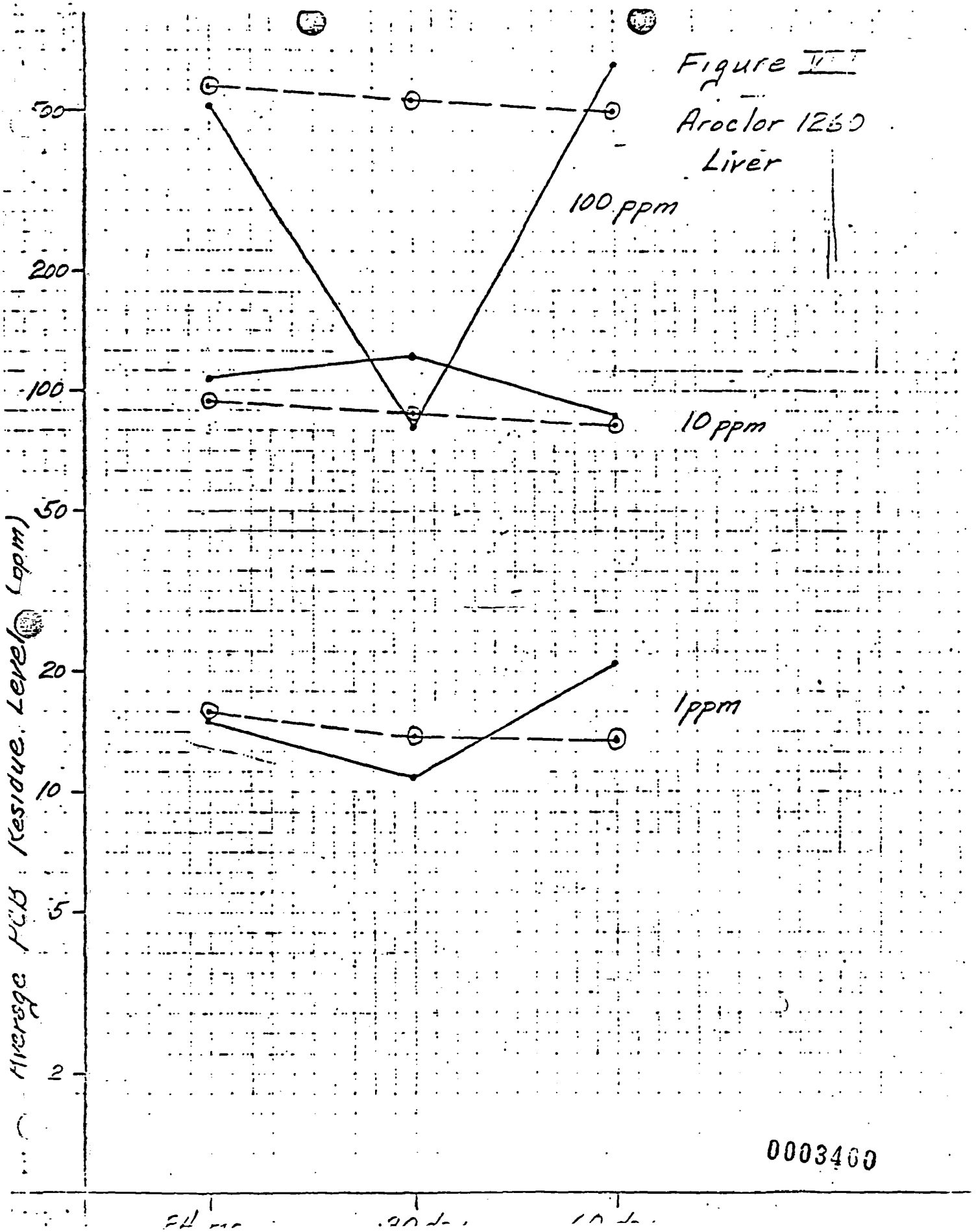

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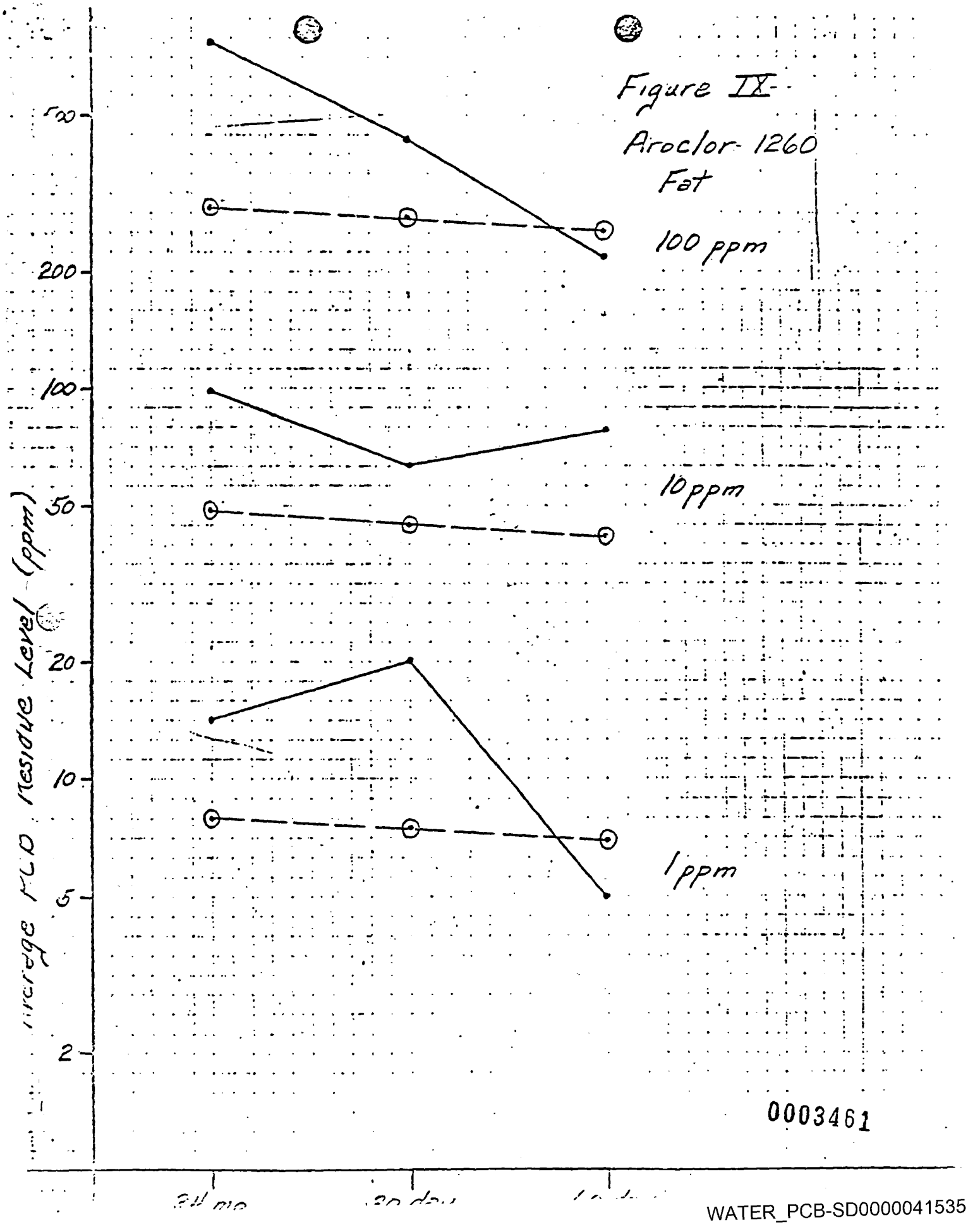

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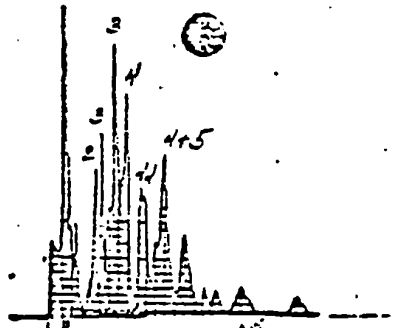
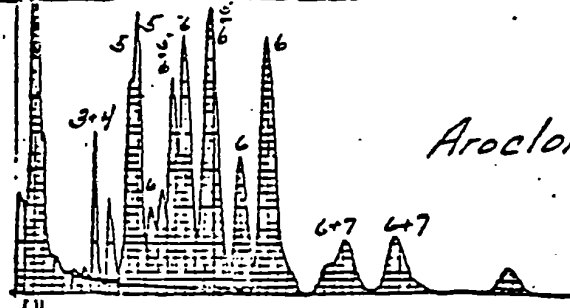
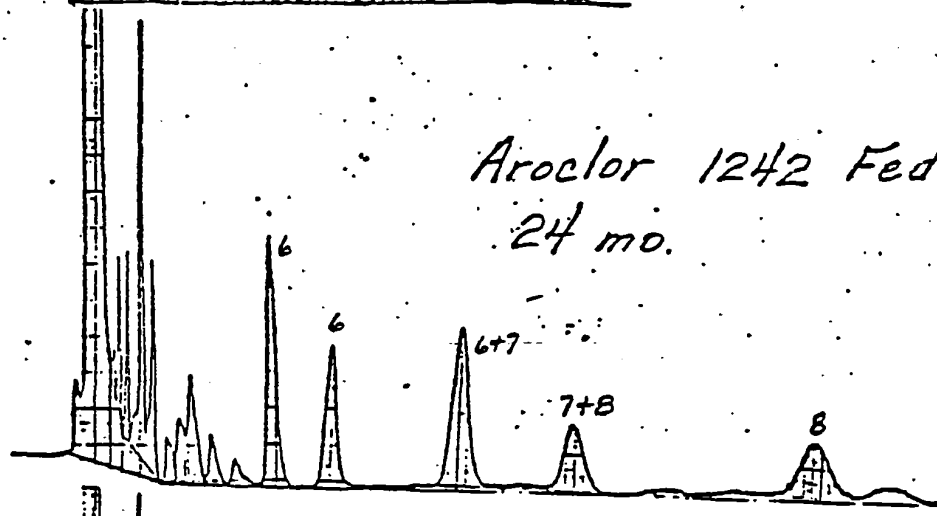
Chromatogram I



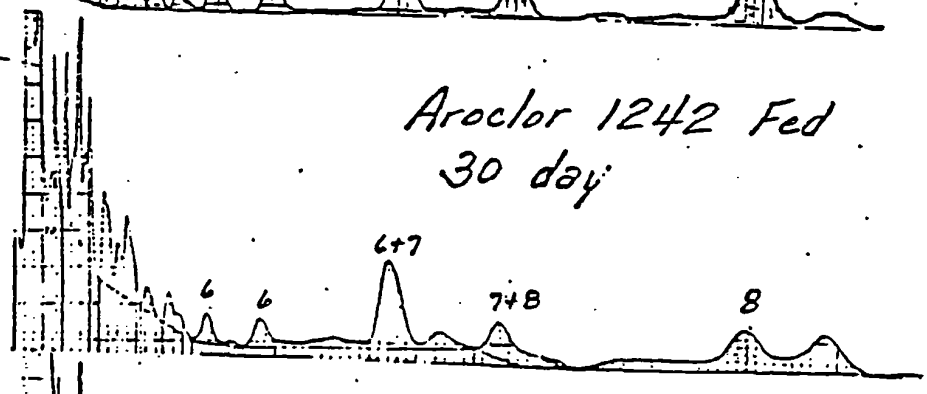
Aroclor 1242 Std.



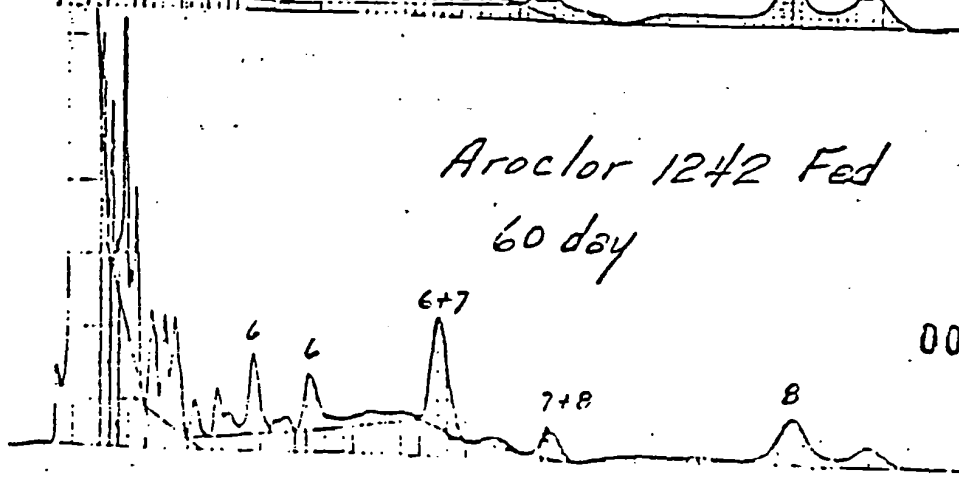
Aroclor 1254 Std.



Aroclor 1242 Fed
24 mo.



Aroclor 1242 Fed
30 day



Aroclor 1242 Fed
60 day

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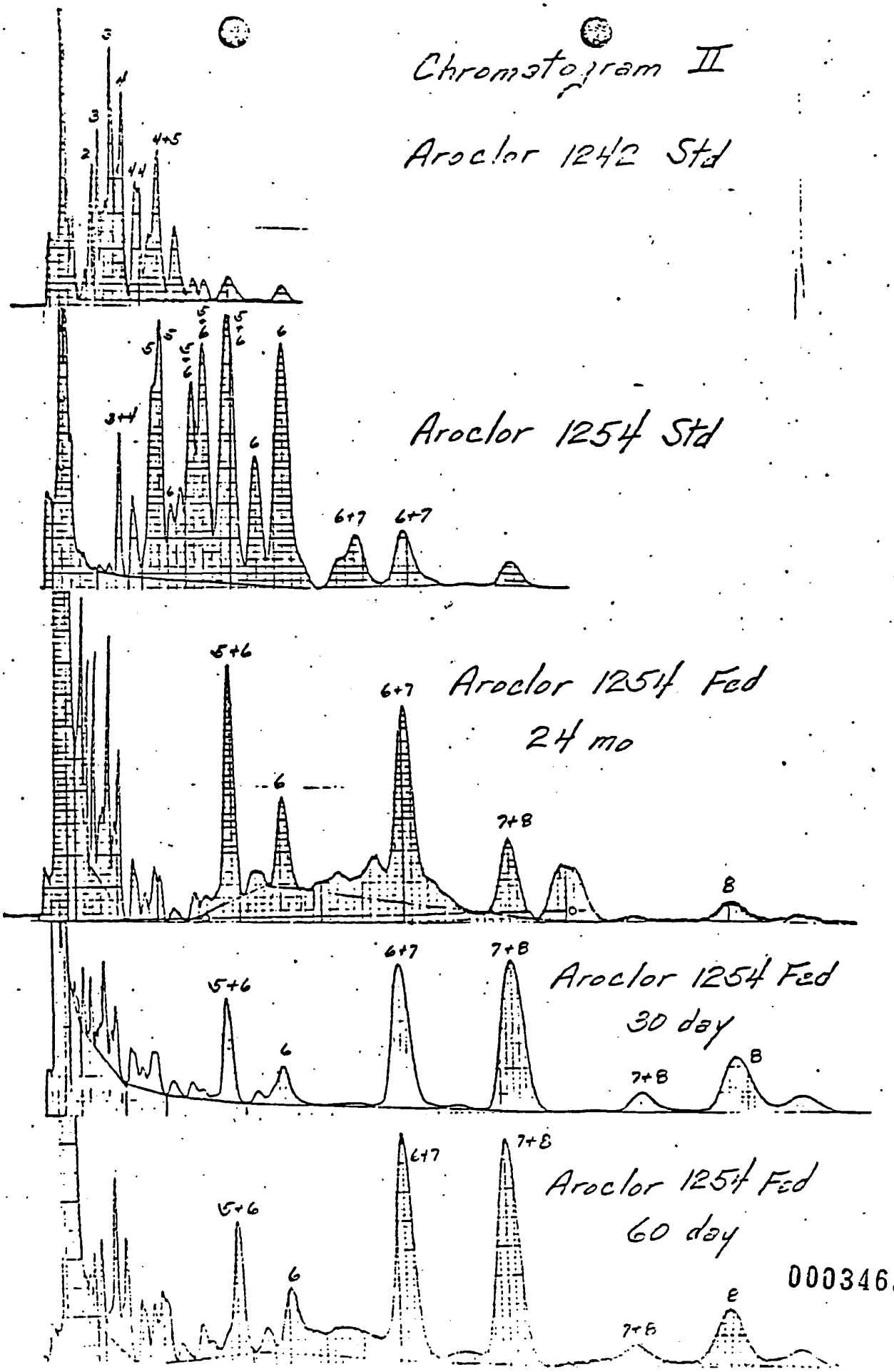

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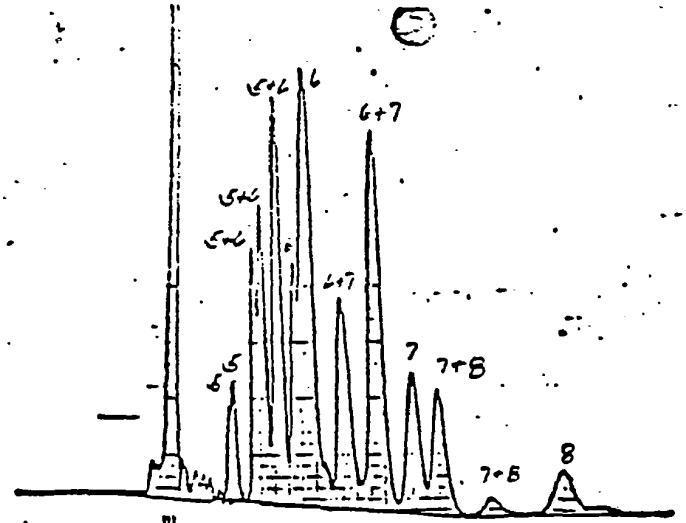
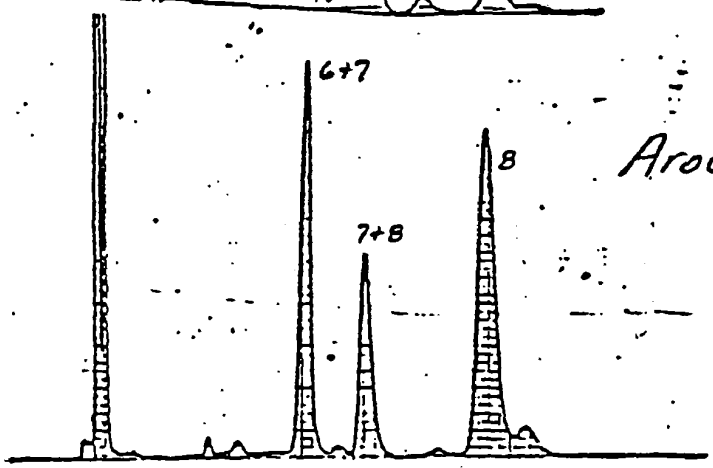
Chromatogram III

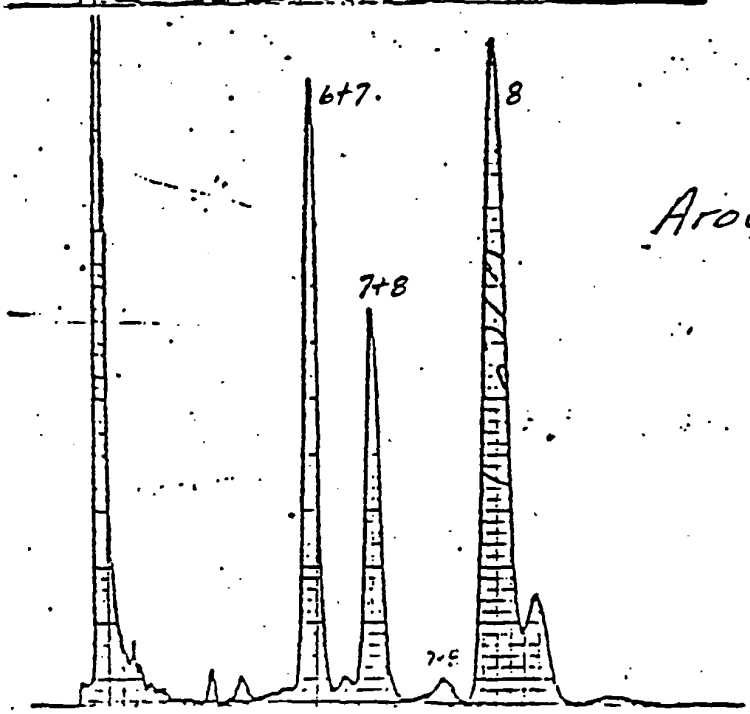


Aroclor 1260 Std



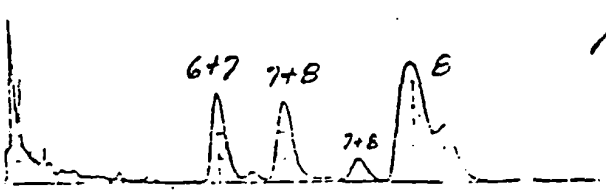
Aroclor 1260 Fed

24 mo



Aroclor 1260 Fed.

30 day



Aroclor 1260 Fed

60 day

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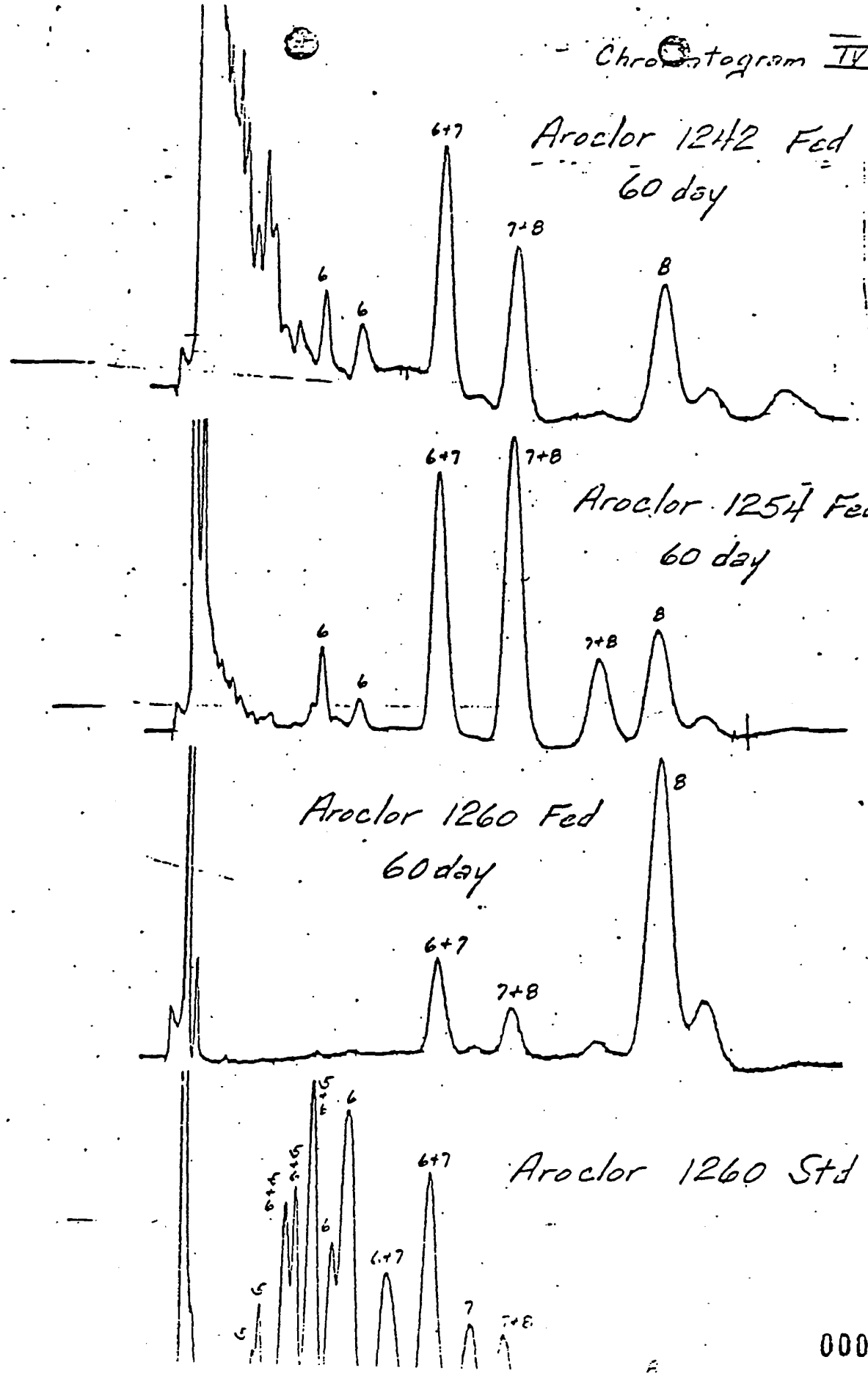
Chromatogram IV



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