POLYCHLORINATED BIPHENYL BIODEGRADATION STUDIES

The threat of continued accumulation of polychlorinated biphenyl (PCB) residues in the environment is responsible for much of the apprehension about the future use of these materials. Examination of the data collected to date from both external and internal PCB residue monitoring programs has confirmed that at their previous rate of release some persistent PCB homologs have begun to accumulate in the eco-system. Conversely, it can also be stated that, with the exception of direct high level controllable contamination, PCB homologs with less than 5 chlorine atoms have not accumulated in the eco-system[1] although they have been released in an unrestricted fashion for many years.

This observation indicates that at the previous rate of release an environmentally compatible PCB product would be one containing no PCB homologs with greater than four chlorines per molecule. As has been previously stated, it is not commercially feasible to produce a product completely free of the persistent homologs. However, it is technically feasible to produce an industrially useful PCB product, Aroclor 1016, which contains significantly (10 to 20 times) lower levels of these persistent homologs.

Two questions remain which must be answered, first to what degree is biological degradation responsible for the disappearance of the "non-persistent" PCB homologs, and secondly, with controlled and restricted usage, will Aroclor 1016 be environmentally compatible.

In an attempt to answer these questions, a number of comparative experimental laboratory studies of the Aroclor products have been carried out to determine the extent to which these materials are degraded by bacterial, avian, mammalian, and aquatic organisms.

Bacterial Degradation - Semi-Continuous Activated Sludge (SCAS) Degradation Studies of Polychlorinated Biphenyls

Introduction

The semi-continuous activated sludge (SCAS) test procedure used to study the primary bacterial degradation[2] of polychlorinated biphenyls is patterned after the test method recommended by the sub-committee on biodegradation test methods of the Soap and Detergent Association for the evaluation of surfactants[3]. This procedure employs sludge from a sewage treatment plant as the source of microorganisms. A specific amount of the material being studied and a synthetic sewage mixture[4] are fed, on a periodic basis to the activated sludge in a specially designed aeration chamber. Aliquots of the mixed liquor (sludge + water) are withdrawn from the chamber shortly after feeding and near the end of the aeration period and analyzed to determine the disappearance rate of the test compound. This cycle is continuously repeated until a steady state[5] is achieved and consistent biodegradation rates are obtained. Details of the procedure may be found in the attached Analytical Chemistry Method 71-32.

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Summary and Conclusions

SCAS testing of Aroclor 1254, Aroclor 1242, Aroclor 1016, MCS 1043, and Aroclor 1221 were carried out over an eight-month period.

Disappearance rate data were obtained during two sampling periods for Aroclor 1254, Aroclor 1242, Aroclor 1016, and MCS 1043. For Aroclor 1221 only one sampling period was employed. In the initial testing of these materials, a feed rate of 1 mg per 24-hour cycle was used. Because the data obtained from the first sampling period for all PCBs studied, except Aroclor 1221, were extremely erratic with little apparent biodegradation, the exposure period between additions of the test material was extended to 48 hours for all materials except Aroclor 1221.

Mean disappearance rates and 95% confidence limits obtained via statistical analysis of the data from the last sampling period are shown in Table I.

TABLE I

Test Material	% Cl (w/w)	Exposure Period (Hrs)	Mean Per Cent Disappearance Rate (Uncorrected)
Aroclor 1221	21	24	64.0 ± 13.6
MCS 1043	30	48	56.2 ± 15.5
Aroclor 1016	41	48	32.9 ± 13.8
Aroclor 1242	42	48	26.3 ± 15.5
Aroclor 1254	54	48	15.2 ± 37.7

It is apparent from inspection of Table I that degradation decreases as the degree of chlorination increases and that because of the uncertainty in the data that it is not possible to differentiate between Aroclor 1242 and Aroclor 1016 with respect to degradability at the 95% confidence level. This is not surprising since Aroclor 1016 constitutes ∿80% of Aroclor 1242 and the resolving power of the test procedure is comparable to this difference.

Subsequent SCAS testing of Aroclor 1221 at a feed level of 1 mg was carried out to establish the significance of volatility losses, the effect of exposure period, and the reproducibility of the test procedure. The results obtained are shown in Table II.

TABLE II
Aroclor 1221

Exposure Period (Hrs)	Aeration Rate (CFH)	Volatility Losses (%)	Mean Per Cent Disappearance Rate Uncorrected	Corrected
24	0.5	16.1	74.9 ± 7.7	58.8
24	0.1	4.5	69.9 ± 17.8	65.4
24	0.1	-	81.0 ± 18.6	-
48	0.1	-	80.6 ± 5.7	-

The data in Table II demonstrate that volatility losses can and do occur and that these losses are a function of the aeration rate and hence also the vapor pressure of the test material in question. It

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is, therefore, reasonable to assume that as the degree of chlorination increases that volatility losses would become insignificant.

The fact that the corrected disappearance rates do not vary with aeration rate also indicates that the volatile components of Aroclor 1221 are subject to degradation and that even if they were not lost in this manner they should not appreciably effect the observed degradation rate.

The mean uncorrected Aroclor 1221 disappearance rates for the 24-hour and 48-hour exposure periods were for all practical purposes equivalent, establishing that when dealing with complex mixtures, a simple linear extrapolation of disappearance rates as a function of exposure period is not valid.

Electron-capture gas chromatography analyses of samples taken at the end of the aeration cycle showed that the lower level of chlorination the greater the changes in the homolog distribution. For Aroclor 1221, the dominant monochloro- and dichlorobiphenyl homologs almost completely disappear after 24 hours of exposure to activated sludge. Similar changes in the ratio of the higher chlorinated homologs to lower ones were noted for the other PCBs studied with the exception of Aroclor 1254. For Aroclor 1254, no significant change in the homolog distribution was observed.

At the feed levels employed, no toxic effects toward the activated sludge were observed for any of the test compounds.

Results and Discussion

The sampling and analytical procedures employed in the disappearance rate determination of a test compound during a cycle were as follows: A 20-ml sample of the mixed liquor (activated sludge + liquor) was withdrawn one hour after feeding and at the end of the aeration period. The sample of mixed liquor was then extracted and the extract concentrated according to the procedure given in Analytical Chemistry Method 71-18. The concentration of test compounds was then determined either by an ultraviolet (UV) spectroscopic or electron-capture gas chromatographic (EC-GC) method. Details of the UV methods are given in Analytical Chemistry Method 71-17 and the EC-GC methods in Analytical Chemistry Method 71-35. The disappearance rate was calculated from the following equation:

$$\% \text{ Disappearance Rate} = \frac{C_o - C_n}{C_o} \times 100$$

Where C_o = level of test material present after feeding

C_n = level of test material present after n hours of exposure

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Tests were carried out to determine if quantitative recovery of the various test materials from the mixed liquor was achieved by the extraction procedure. Details are given in Analytical Chemistry Method 71-17. Although complete recovery was not achieved in all cases, the recoveries were consistent and independent of concentration level. The calculated disappearance rates should, therefore, not be affected significantly.

Since the level of chlorination is the most significant factor in the relative biodegradability of the PCBs, the homolog distribution of the various test materials is given in Table III.

TABLE III
HOMOLOG DISTRIBUTION OF POLYCHLORINATED BIPHENYL PRODUCTS

Homolog No. of Cl Per Biphenyl Molecule	Aroclor 1254 (54% Cl)	Aroclor 1242 (42% Cl)	MCS 1016 (41% Cl)	MCS 1043 (30% Cl)	Aroclor 1221 (21% Cl)
0	0.05	0.01	0.02	0.11	19.00
1	0.06	0.70	1.00	23.4	54.10
2	0.32	15.50	19.70	71.3	20.40
3	0.78	49.40	57.30	5.2	3.90
4	22.99	24.80	21.20		2.30
5	47.38	8.70	0.80		0.25
6	19.75	0.85	0.05		
7	4.62				
8					
9	3.96				
10					

Feeding of the Aroclor 1254, Aroclor 1242, Aroclor 1016, and MCS 1043 units was started on 8-10-70 at a feed level of 1 mg per 24-hr cycle. These units were sampled for approximately one month starting with the first day of feeding. All analyses were carried out by electron-capture gas chromatography. The data obtained were extremely erratic. A statistical analysis of the data (Statistics Special Study 70-22) using the Student's t test indicated that at the 95% significance level, only MCS 1043 showed evidence of biodegradation. Both Aroclor 1016 and Aroclor 1242 showed some evidence of biodegradation, but neither was significant at the 95% level. Aroclor 1254 showed no evidence of biodegradation. In order to detect biodegradation at the 95% level of significance in 20 observations, about one-half of the test materials must be degraded in a cycle. The lack of significant biodegradation during the first sampling period was probably due in part to the need for an acclimation period by the bacterial sludge. Subsequent spot checks on the unit, however, indicated that the data obtained were still erratic. Because of the apparent slow rate of biodegradation of these materials, the cycle time was increased to 48 hours in the hope that larger differences would be observed between the before and after feeding samples.

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Feeding of the Aroclor 1221 unit was started on 10-27-70 at a feed level of 1 mg per 24 hours. Aroclor 1221, because of its higher rate of disappearance was maintained on the 24-hour cycle. Sampling of the unit was started 1-25-71 and completed 1-29-71. Analyses were carried out by electron-capture gas chromatography.

The final sampling period for the Aroclor 1254, Aroclor 1242, Aroclor 1016, and MCS 1043 units at a feed rate of 1 mg per 48-hour cycle was started 2-8-71 and completed 3-8-71. Both UV and EC-GC analyses were carried out; the UV data was used to determine the overall disappearance rate and the EC-GC to monitor the change in homolog distribution.

In Figures 1-1 to 4-2, the observed data from which the mean disappearance rates were calculated are shown for both sampling periods for Aroclor 1254, Aroclor 1242, Aroclor 1016, and MCS 1043. In Figure 5, similar data for Aroclor 1221 is shown for a single sampling period. In the top chart of each figure the total mg found in the unit at the beginning and end of each cycle is plotted versus the elapsed time in days after the start of the test. The after feeding sample is denoted by the solid circle; the before feeding sample by the arrowhead; the connecting line indicates the amount lost during each cycle. In the lower chart of each figure, the disappearance rate obtained is plotted versus elapsed time. In Table IV the mean disappearance rate and 95% confidence limits obtained for the test compound during the various sampling periods are given. Since the analyses for Aroclor 1221 were carried out by EC-GC, the disappearance rate does not include any decrease in the biphenyl component (see Table III for Aroclor 1221 composition).

TABLE IV

SCAS MEAN DISAPPEARANCE RATES FOR POLYCHLORINATED BIPHENYL PRODUCTS*

Sampling Period/ Cycle Time	Mean Disappearance Rate ±95% Confidence Limits				
	Aroclor 1254	Aroclor 1242	MCS 1016	MCS 1043	Aroclor 1221
1/24 Hours	-29.0±58	11.0±19.5	3.6±19.0	4.7±22.0	
2/24 Hours					64.0±13.6
3/48 Hours	15.2±37.7	26.3±15.3	32.9±13.8	56.2±15.5	

*Identification of feed material in SCAS tests

Aroclor 1254	Lot AK-38
Aroclor 1242	Lot AK-255
Aroclor 1016	Sample No. 1, OR 158591
MCS 1043	Sample No. 2, OR 158591
Aroclor 1221	Lot AK-2

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In order to make certain that the observed disappearance rates were not due primarily to volatility losses, scrubbing experiments on the most volatile test material, Aroclor 1221, were carried out in which the off-gases from the units were passed through a series of three hexane scrubbers during a complete time cycle. Data were obtained at both 0.1 and 0.5 SCFH aeration rates. The data established that at the 0.5 SCFH air flow volatility losses were ∿16%, at an aeration rate of 0.1 SCFH the volatility losses were reduced to ∿5%, while the overall disappearance rate was essentially the same as that obtained at 0.5 SCFH. This means that the components lost by volatilization are for all practical purposes completely degradable and the disappearance rate (64.0%) obtained represents actual biodegradation.

Since the volatility of the PCBs decreases with increasing level of chlorination, the volatility losses should likewise decrease. From the volatility losses observed for Aroclor 1221, one would not expect such losses to be a significant factor in test disappearance rates for MCS 1043, Aroclor 1016, Aroclor 1242, and Aroclor 1254.

From the data in Table IV and Figures 1-1, 2-1, 3-1, 4-1, it is quite apparent that the disappearance rate data obtained for Aroclor 1254, Aroclor 1242, Aroclor 1016, and MCS 1043 during the first sampling period was very erratic. The data obtained during the last sampling period as shown in Table IV and in Figures 1-2, 2-2, 3-2, and 4-2 was a significant improvement. From the latter data, the following ranking in terms of biodegradability can be made:

Aroclor 1221>MCS 1043>Aroclor 1016≅

Aroclor 1242>Aroclor 1254

In Figure 6, the mean disappearance rate is plotted versus the per cent chlorine in the test compound. The mean per cent disappearance rate for biphenyl was obtained in a previous study (Statistics Special Study 71-2). It is apparent that the disappearance rate decreases with increasing levels of chlorination.

As was previously noted, selected samples from the last sampling period were analyzed by EC-GC to monitor changes in homolog and isomer distribution. In Figure 7, chromatograms for Aroclor 1221 are shown.

The top trace is a chromatogram of an Aroclor 1221 standard representative of the feed material. The center chromatogram is that of a sample taken at the end of the exposure period or aeration cycle. The bottom chromatogram is that of an Aroclor 1242 standard run under the same GC conditions as the other chromatograms. The numbers above each peak indicate the dominant homolog represented by that peak according to GC-mass spectroscopic determination.

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It is important to note that the electron-capture detector does not have the same response for all components. The sensitivity of the detector generally increases as the degree of chlorination increases. From the chromatograms in Figure 7, it is apparent that the dominant monochlorobiphenyl and dichlorobiphenyl components of Aroclor 1221 have almost completely disappeared after a 24-hour cycle, while there is an apparent buildup of the minor higher chlorinated isomers. These minor components, because of the increase in detector sensitivity with chlorination level, are amplified in the chromatograms compared to their true weight per unit basis. Comparison of the extract chromatogram to that of the Aroclor 1242 standard shows that these minor components in Aroclor 1221, which are more resistant to degradation, comprise the major components of Aroclor 1242.

In Figure 8, similar chromatograms are shown for MCS 1043. Comparison of the extract chromatogram to that of the standard again shows that the lower chlorinated isomers or homologs disappear more rapidly while a buildup of higher chlorinated components occurs.

In Figures 9 and 10, chromatograms for Aroclor 1016 and Aroclor 1242 extracts and standards are shown. With these more highly chlorinated products, the change in homolog distribution are not as dramatic compared to the lower chlorinated products. The buildup of the more refractory penta- and hexachlorobiphenyl homologs is apparent.

For Aroclor 1254, chromatograms have not been included since no significant change in homolog or isomer distribution was observed during the testing period.

At the conclusion of the final sampling periods for Aroclor 1254, Aroclor 1242, Aroclor 1016, and MCS 1043, feeding of the test compound was stopped. The units were then monitored over a period of time to determine how rapidly the residues remaining from the various test compounds disappeared. In Table V, the mg of residue found at various time intervals after the last feeding are shown. As expected, the lower the level of chlorination the more rapidly the residue disappears. For Aroclor 1254, it is apparent that no significant decrease in the residue occurred over the period studied.

TABLE V

Days After Last Feeding of Test Compound	Mg of Residue			
	Aroclor 1254	Aroclor 1242	MCS 1016	MCS 1043
3	5.38	2.62	2.26	1.24
5	4.50	2.29	1.16	1.06
7	4.94	1.47	1.31	0.70
10	7.42	1.76	1.17	0.75

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Residue Studies for Polychlorinated Biphenyls (PCBs) in Fish, Birds and Mammals

Introduction

Early in 1969, after confirmation of Jensen's[6] identification of PCB residues in Swedish birds and fish, Monsanto began initiating a series of Aroclor toxicity-tissue residue studies. The general objective of these studies was to learn more about the environmental behavior and potential effects of PCBs.

As indicated by the title of this appendix, it will deal only with the results of the residue work. Appendix should be consulted for the results of the toxicity work.

The specific objectives of the tissue residue studies with fish, birds and mammals were to learn more about:

1. tissue storage levels as a function of exposure time and exposure level,

2. the character of the PCB residues retained from the various Aroclor products, and

3. the rate at which PCB residues disappear once exposure ceases.

To date, analyses of tissues collected from nine[7] toxicity-tissue residue studies have been completed. The tissue samples were collected, quick frozen, and shipped by the consultant laboratories to Monsanto Industrial Chemicals Company. Upon receipt, the tissues were analyzed for PCB residues by Monsanto personnel in accordance with Analytical Chemistry Method 71-35.

In the first two tissue collection studies all samples were analyzed. In the subsequent studies, a statistical approach was employed because of the large number of samples In this manner, both the elapsed time and manpower requirements were minimized with little real loss in either accuracy or precision. The approach involved analysis of a balanced sub-set (∿25-30%) of the tissues collected. The data from the balanced sub-set of samples, the appropriate study variables (tissue, exposure period, exposure level, sex, and Aroclor fed), and the most probable variable interactions were then used to generate an interpolation equation via multiple regression analysis. 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 un-analyzed tissues.

The validity of each of the predicting equations was established in the following manner. The respective residual standard deviations of each equation was compared to those obtained for the equations developed for the two studies in which all samples 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 analyses would be ∿0.5-2 times the predicted values.

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Summary and Conclusions

The most significant conclusion which can be drawn from these residue studies is that it supports what has and is being observed via residue analysis of environmental samples. That is to say, that from a "residue viewpoint", the bulk of the PCB isomers which have been released to the environment (PCBs with less than 5 chlorines) are subject to degradation. Since degradation of these PCB isomers can and does occur at significant rates they in turn should not be a long term threat to the higher members of the food chain.

In Figure 11, the observed PCB residue levels for rats and dogs exposed to Aroclor 1221, Aroclor 1242, Aroclor 1254 and Aroclor 1260 for 90 days is plotted vs the degree of chlorination of the product fed. It graphically illustrates that PCB storage levels decrease exponentially as the degree of chlorination of the product decreases. This exponential decrease simply reflects the refractory PCB content of each product produced via direct chlorination.

Considerable differences were noted in concentration factors as judged by the ratio of the PCB level found in the extractable lipid to the exposure level. Fish were found to be most efficient PCB concentrators followed by chickens, rats and then dogs. The calculated concentration factors observed for Aroclor 1242 are shown in Table VI.

TABLE VI

	Exposure Level (PPM)	Exposure Period	Max. Observed PCB Level in Lipid	Concentration Factor*
Blue Gills	0.1 in wtr.	21 Days	4.0%	400,000
Catfish	0.1 in wtr.	21 Days	3.2%	320,000
White Leghorn Chickens	1.0 in feed	12 Weeks	20 PPM	20
Albino Rats	1.0 in feed	2 Years	9 PPM	9
Beagle Dogs	1.0 in feed	2 Years	2 PPM	2

*NOTE: Factors do not take into account exposure periods

Calculation of concentration factors in this fashion for chickens, rats, and dogs can be misleading. For example, ratio of total PCBs retained to that orally ingested by chickens, rats and dogs is shown in Table VII.

TABLE VII
Test Material - Aroclor 1242

	Exposure Level		
	1 PPM	10 PPM	100 PPM
White Leghorn Chickens	0.11	0.11	0.11
Albino Rats	0.02	0.01	0.003
Beagle Dogs	0.005	0.001	0.0003

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Inspection of the ratios illustrates that the higher members of the food chain are in fact inefficient PCB concentrators.

Decrease of PCB residues was noted with both chickens and dogs placed on PCB free recovery diets. The dogs remobilized the residues more rapidly than chickens. In both studies, the residue levels fell more rapidly the lower the degree of chlorination of the product fed.

Figure 12 illustrates this point. In it the % total PCBs accumulated in the lipid is plotted vs the recovery periods for the Two Year Beagle Dog Study with Aroclor 1242, Aroclor 1254, and Aroclor 1260.

Figures 13-22 are bar graphs constructed from electron capture gas chromatograms of the product fed and the isolated PCB residues. The bar graphs were constructed by normalization to a convenient peak present in each of the chromatograms being compared. The numbers above each bar indicate the dominant homolog or homologs as determined by mass spectrometry. In no case were the residue fingerprints observed to be a function of the exposure level.

Figures 13-15 compare the distributions observed for residues isolated from fish, chickens, rats and dogs exposed to Aroclor 1242, Aroclor 1254, and Aroclor 1260. Inspection of these figures demonstrates that fish absorbed Aroclor 1242 without altering the isomer distribution. Dogs were found to be most effective at selectively degrading the Aroclor products followed by rats and the chickens. The figures also indicate that, with minor exceptions, the lower chlorinated isomers present in each product are more easily degraded.

Figures 16-18 illustrate the make up of the residues isolated from dogs after 2 years of continuous exposure followed by 30 and 60 day recovery periods on PCB free diets. Inspection of these figures reveals that the bulk of the isomers observed in the dogs sacrificed after two years of continuous exposure rapidly degraded once exposure ceases.

Figures 19-21 illustrate the alterations observed when chickens exposed to these three Aroclor products for 12 weeks were placed on recovery diets for 30 days. Although the degradation was less extensive than observed with dogs, the same general trends were noted.

In Figure 22, the isomer distribution of Aroclor 1242 and the residues isolated from rats fed Aroclor 1242 and Aroclor 1016 for 90 days are shown. Although the difference in the total % chlorine in these products is negligible the isomer distribution of the residues are significantly different. This difference can be explained by the fact that the higher chlorinated biphenyl content of Aroclor 1016 relative to Aroclor 1242 has been reduced by a factor of twenty.

The actual residue levels as determined by analysis and interpolation are shown in Tables VIII - XXXII.

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7. IBT B7009 30-Day Tissue Collection Study In Albino Rats with Aroclors

IBT B7298 Two-Year Chronic Oral Toxicity Study of Aroclor 1242, Aroclor 1254, Aroclor 1260 in Albino Rats

IBT P7297 Three-Generation Reproduction Study in Albino Rats with Aroclor 1242, Aroclor 1254, Aroclor 1260

IBT 9888C 9888D 90-Day Subacute Oral Toxicity Study of Aroclor 1016, Aroclor 1221 in Albino Rats

IBT C7299 Two-Year Chronic Oral Toxicity Study of Aroclor 1242, Aroclor 1254, Aroclor 1260 in Beagle Dogs

IBT 9887 9885 90-Day Subacute Oral Toxicity Study of Aroclor 1016, Aroclor 1221 in Beagle Dogs

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7. Continued

IBT J7300 Toxicity, Reproduction and Residue Study of Aroclor 1242, Aroclor 1254, Aroclor 1260 in White Leghorn Chickens

IBT 8746 Toxicity, Reproduction and Residue Study of Aroclor 1242, Lot #AK-255 in White Leghorn Chickens

The following study was carried out at Bionomics, Inc. of Wareham, Massachusetts.

Acute Toxicity of Aroclor 1016, Aroclor 1242, and DDT to Bluegill and Channel Catfish During 21 Days Continuous Exposure to the Chemicals in Water.

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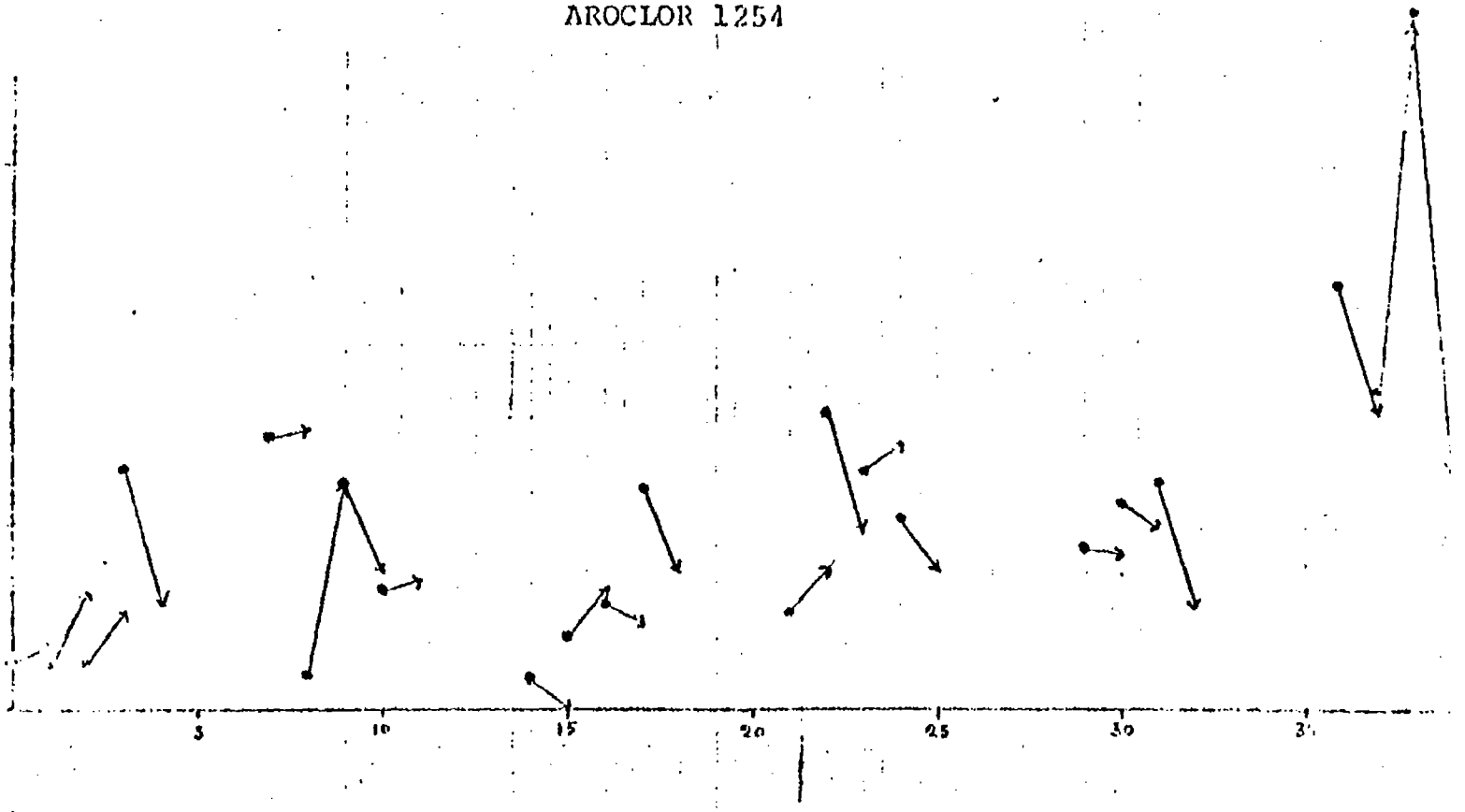
SCAS BIODEGRADATION DATA

AROCLOR 1254



Mean = -29.0 ± 58

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Elapsed Time (Days) after Start of Test

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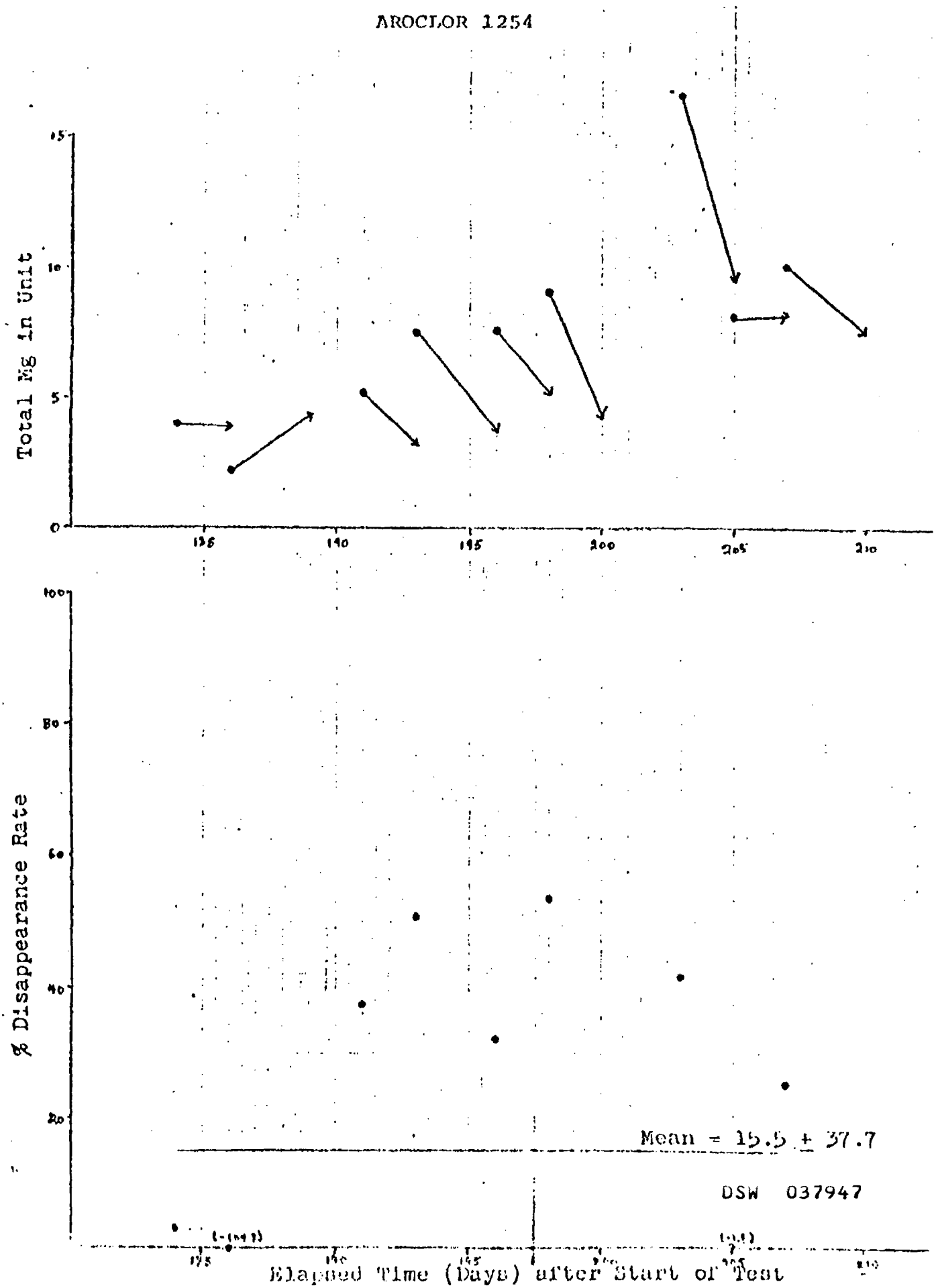
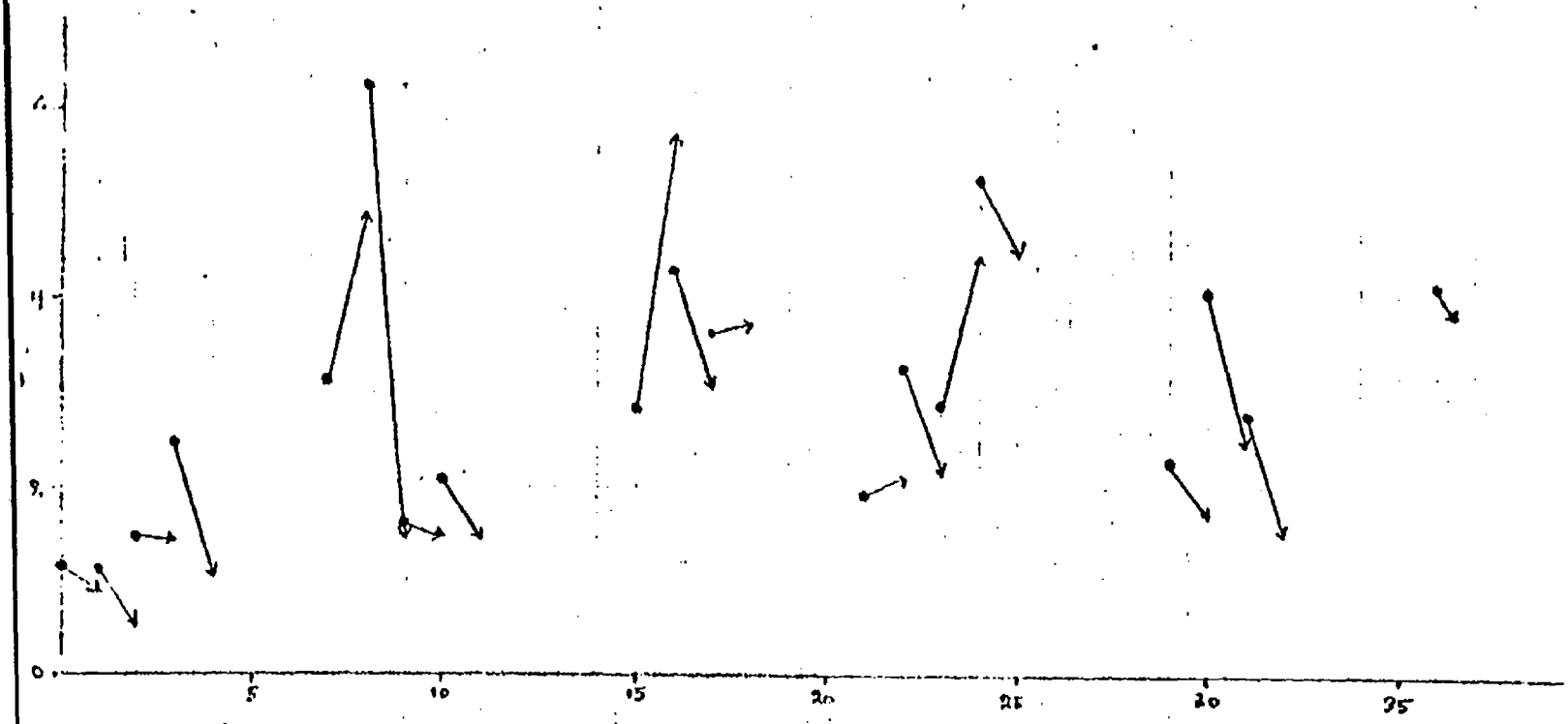
SCAS BIODEGRADATION DATA

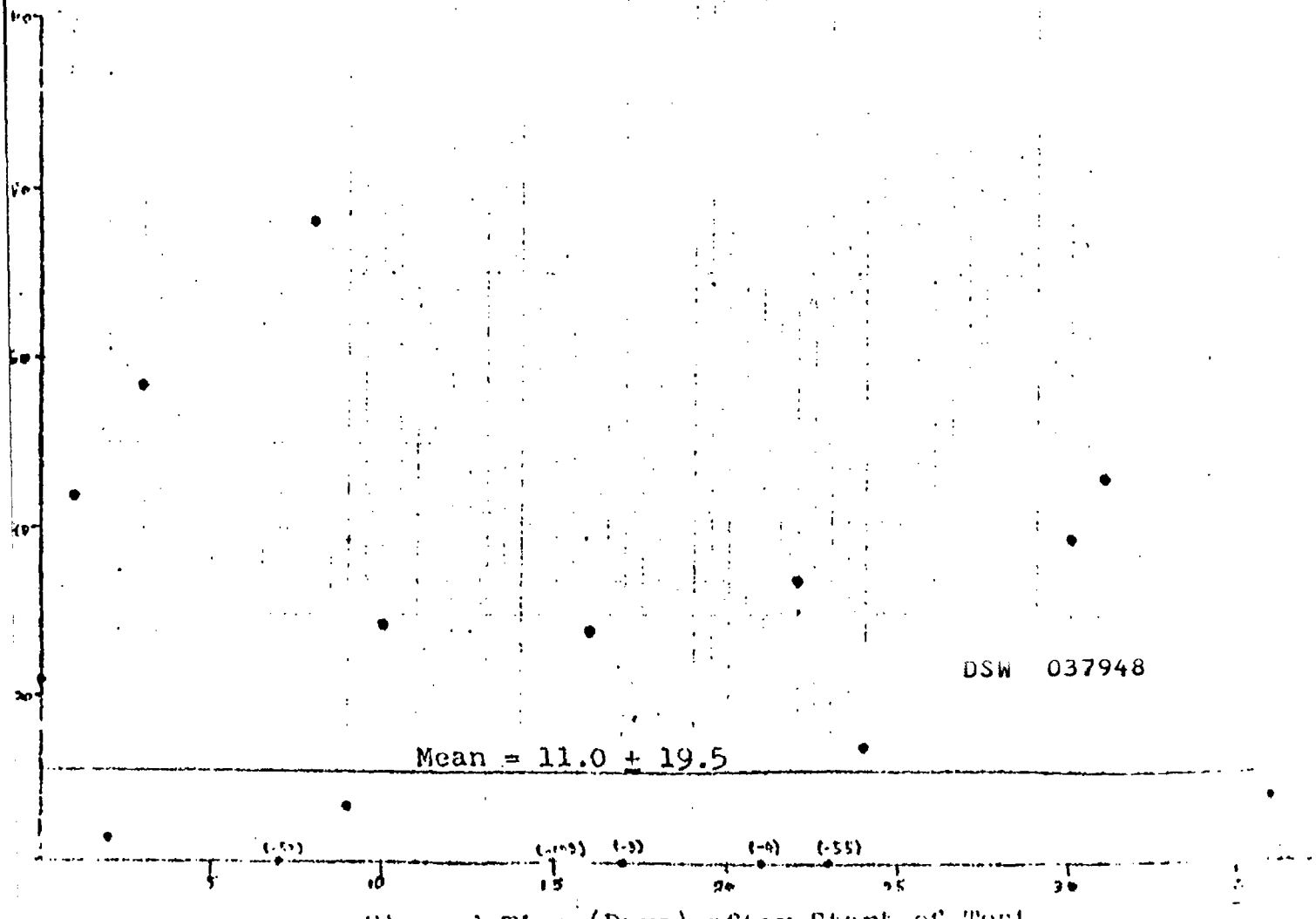
AROCLOR 1254



SCAS BIODEGRADATION DATA

AROCLOR 1242





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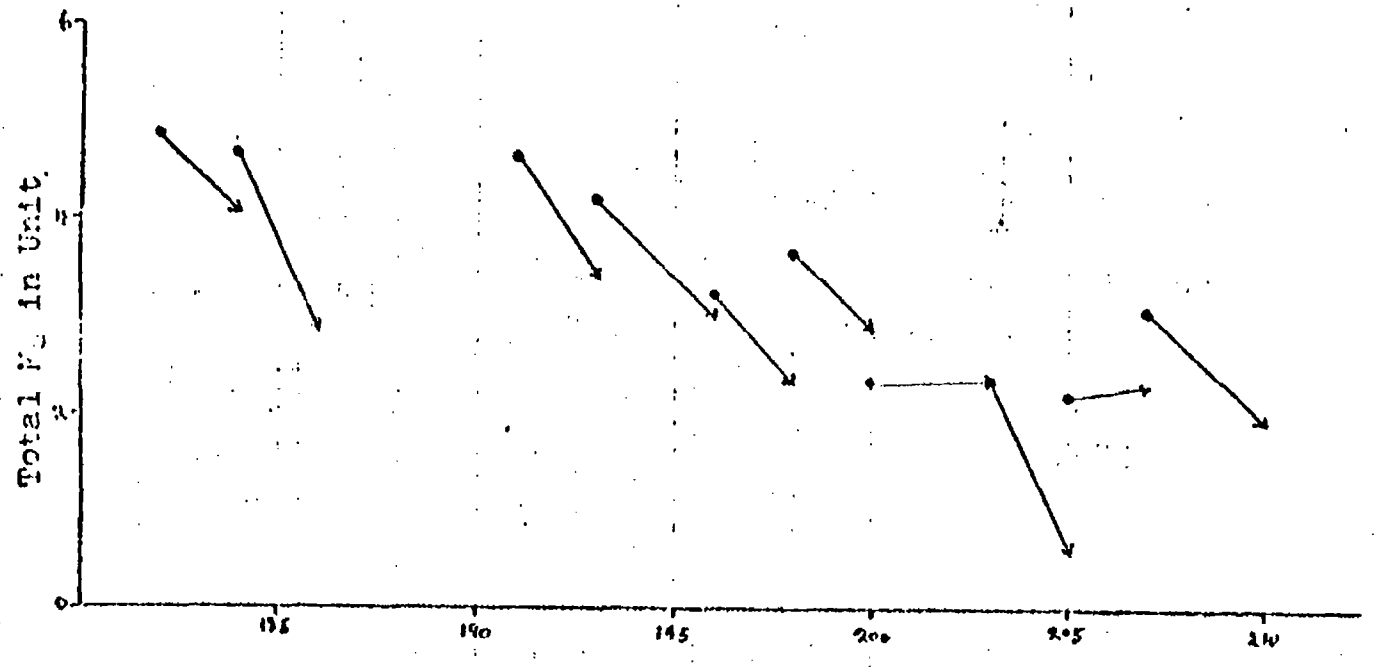
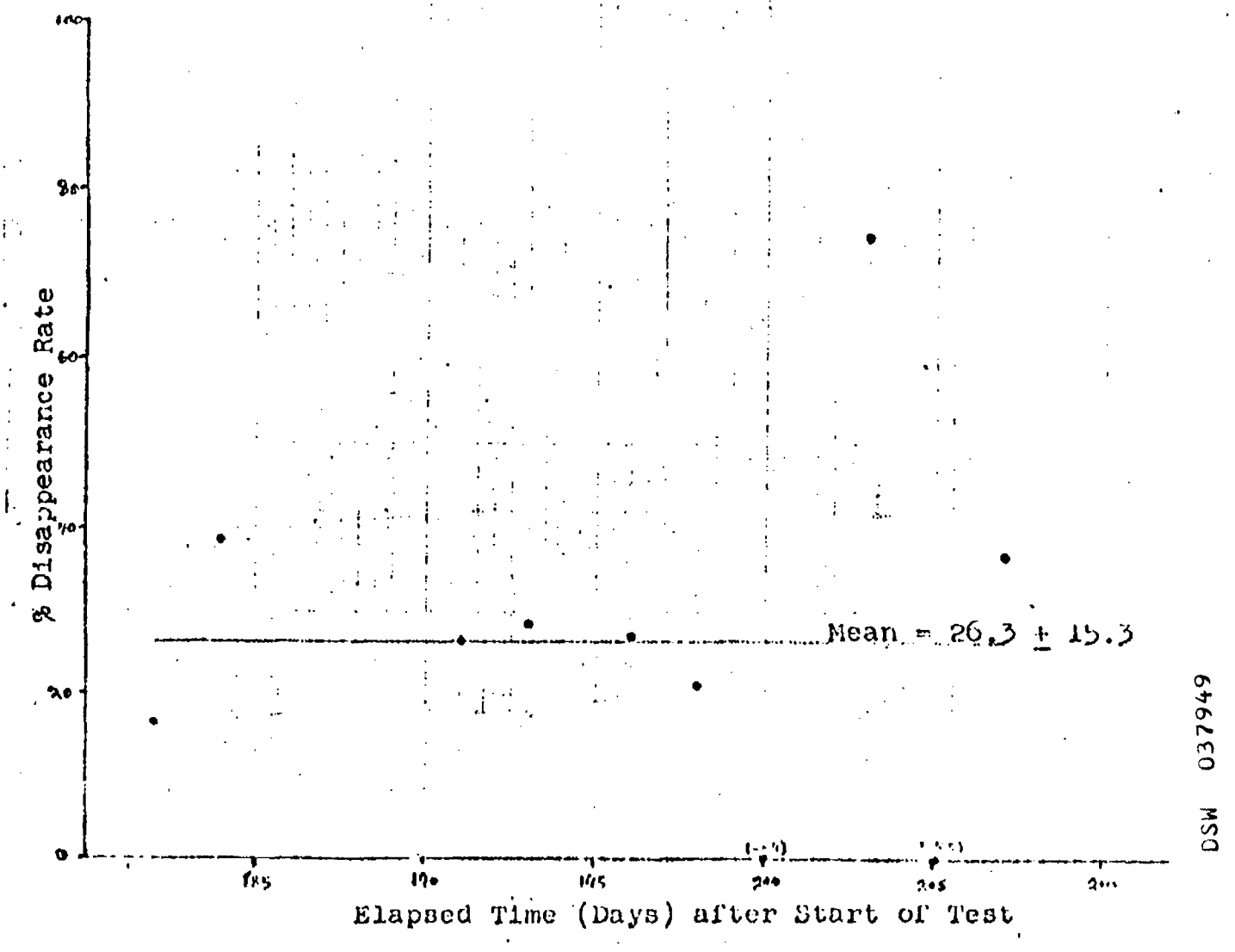
SCAS BIODEGRADATION DATA

AROCLOR 1242





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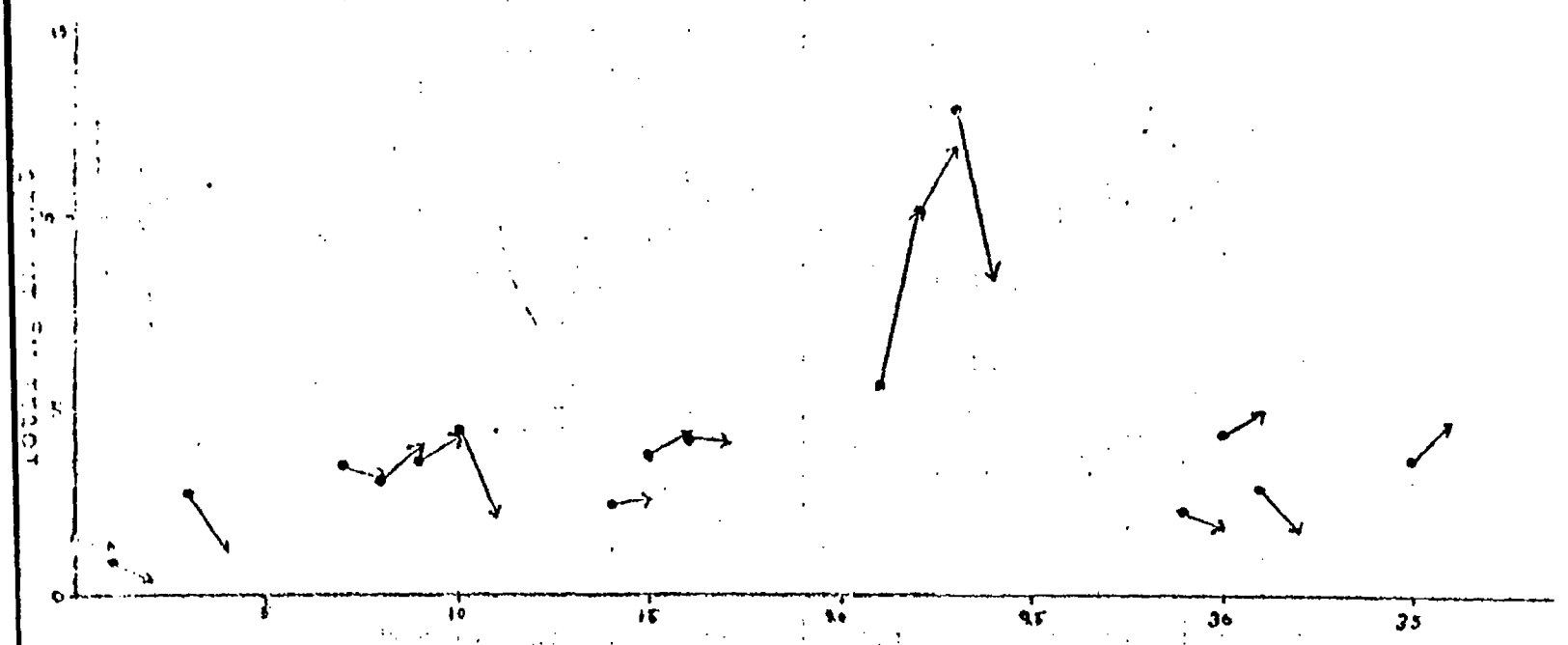
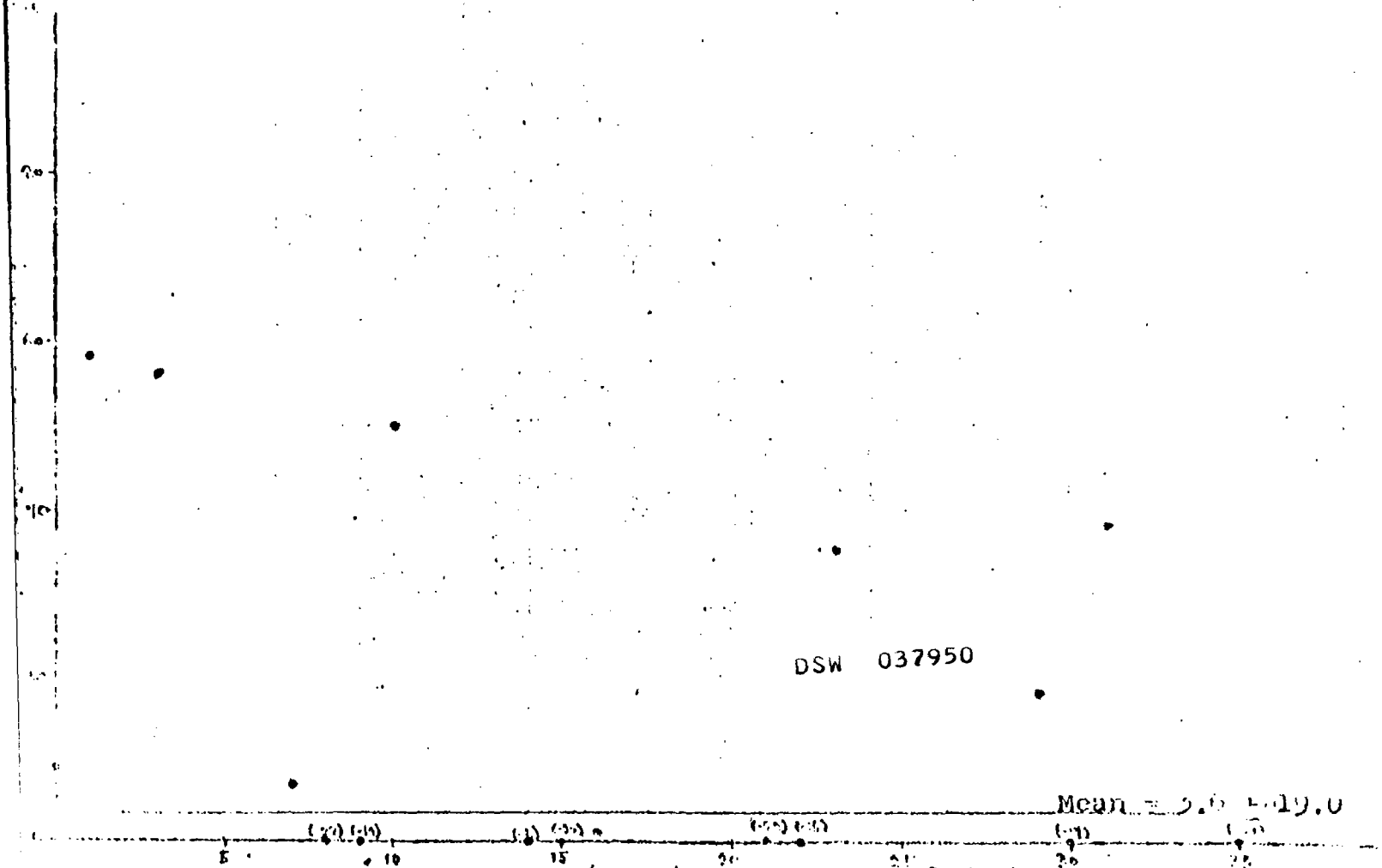
SCAS BIODEGRADATION DATA

MCS 1016





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Mean = 2.6 ± 19.0

Elapsed Time (Days) after Start of Test

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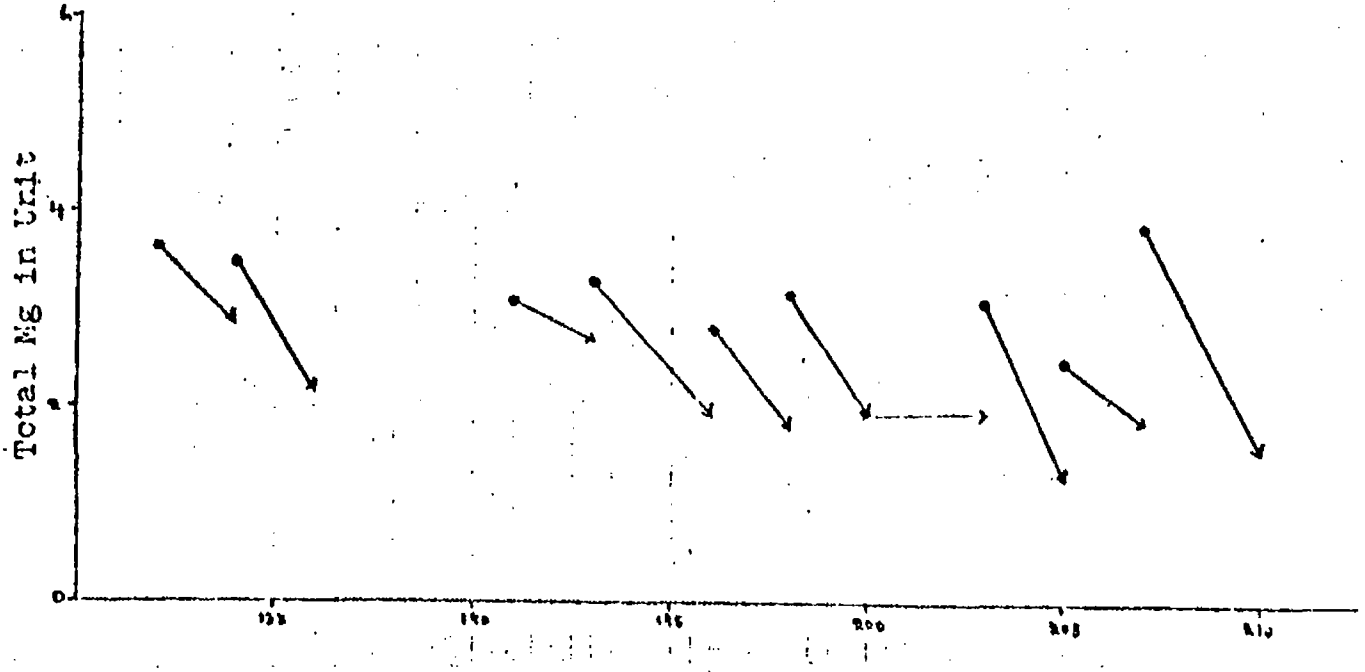
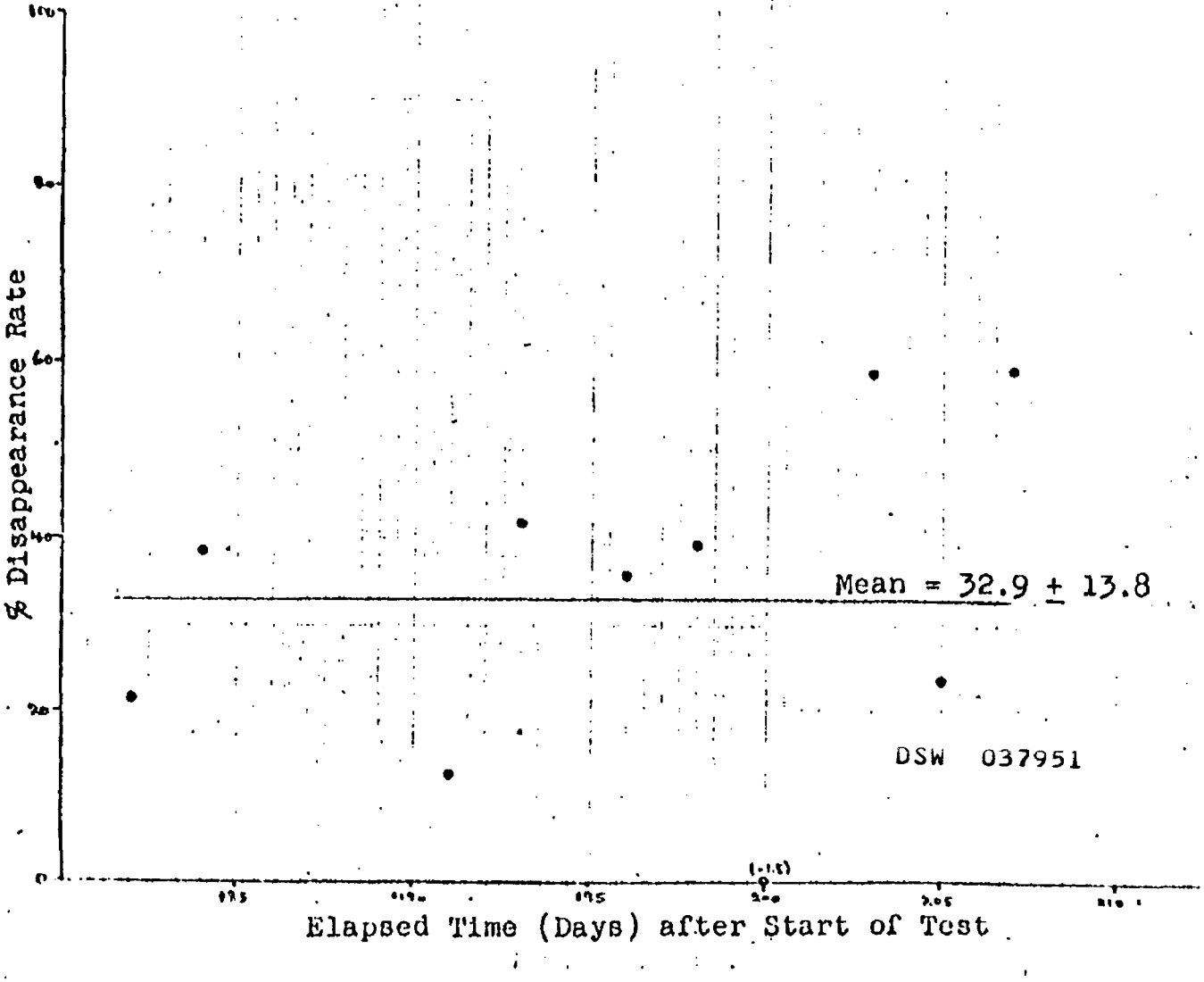
SCAS BIODEGRADATION DATA

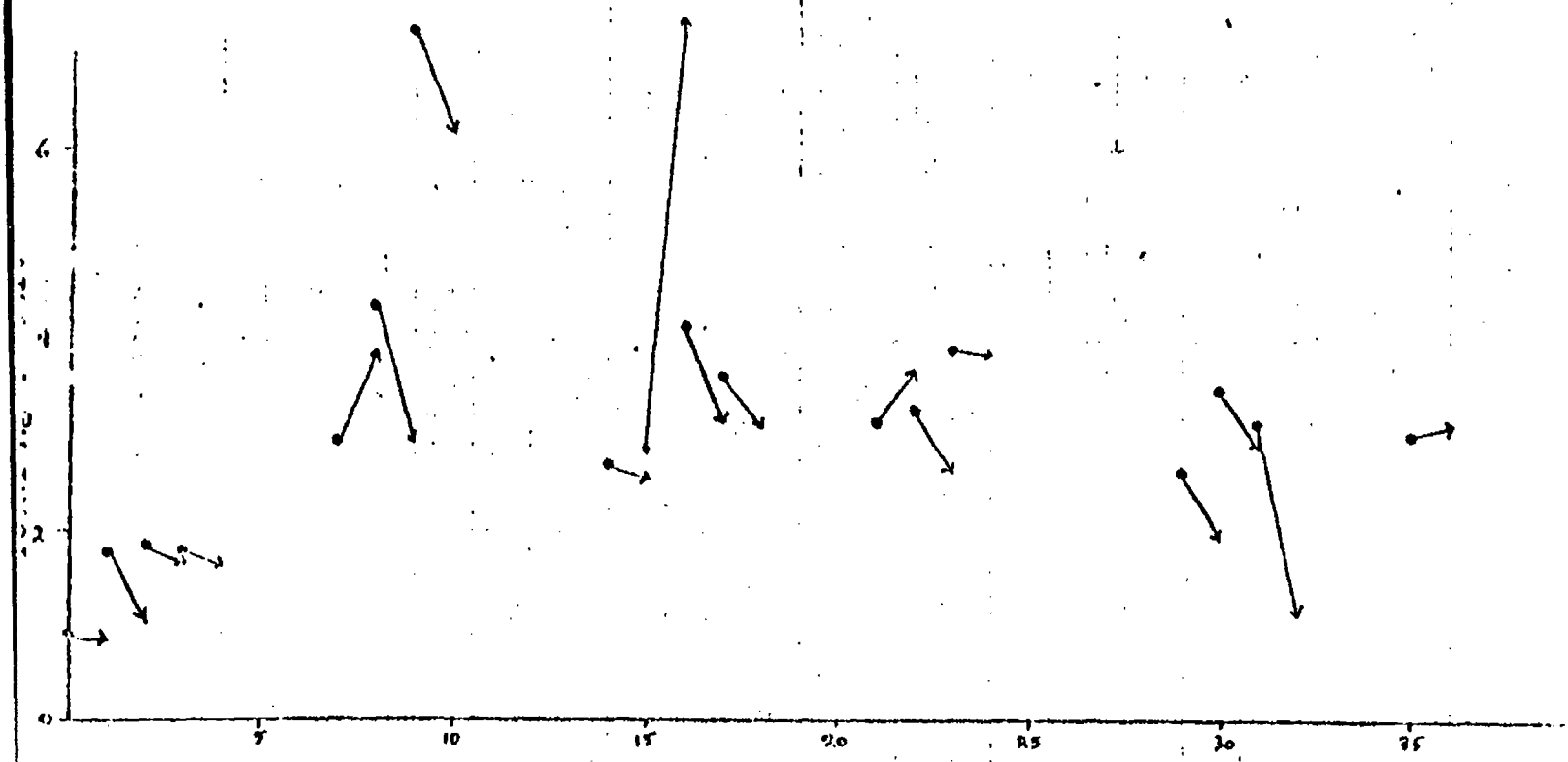
MCS 1016

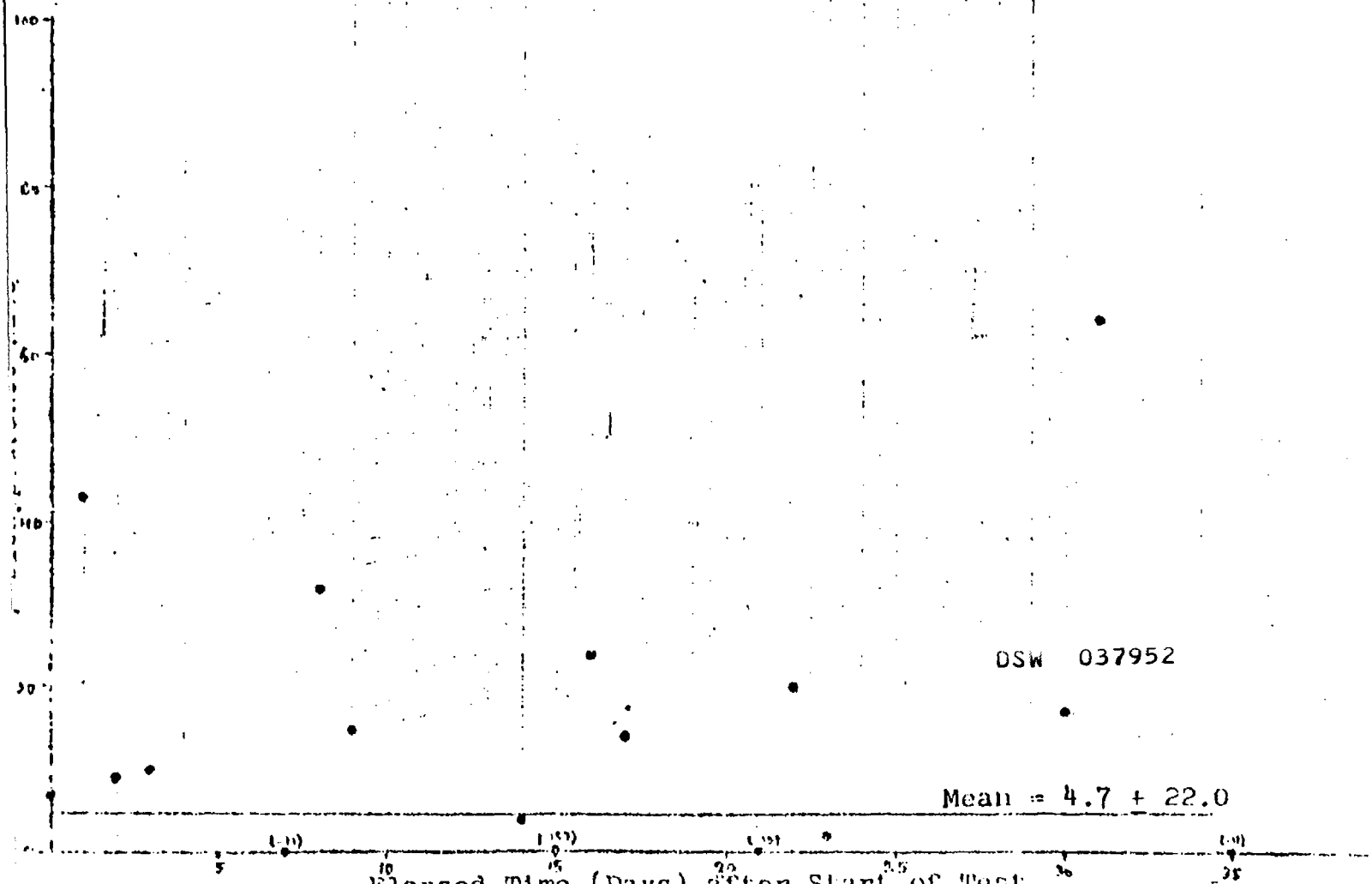




SCAS BIODEGRADATION DATA

MCS 1043



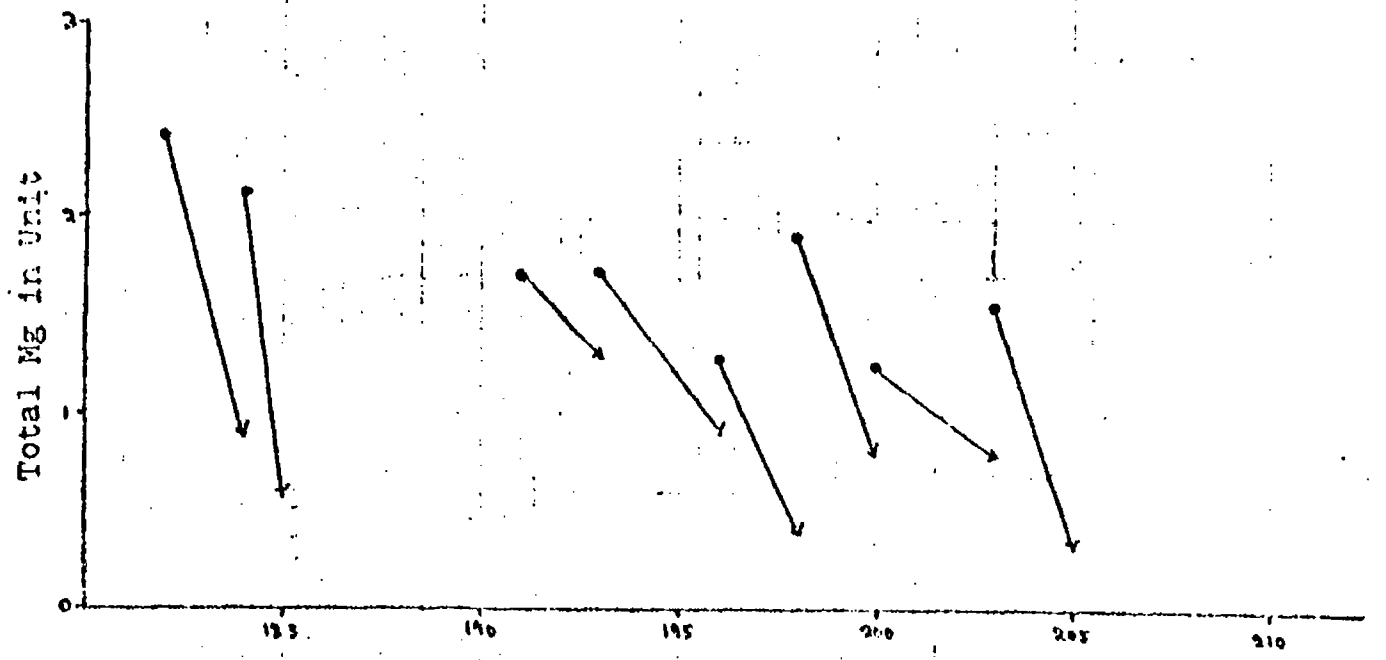


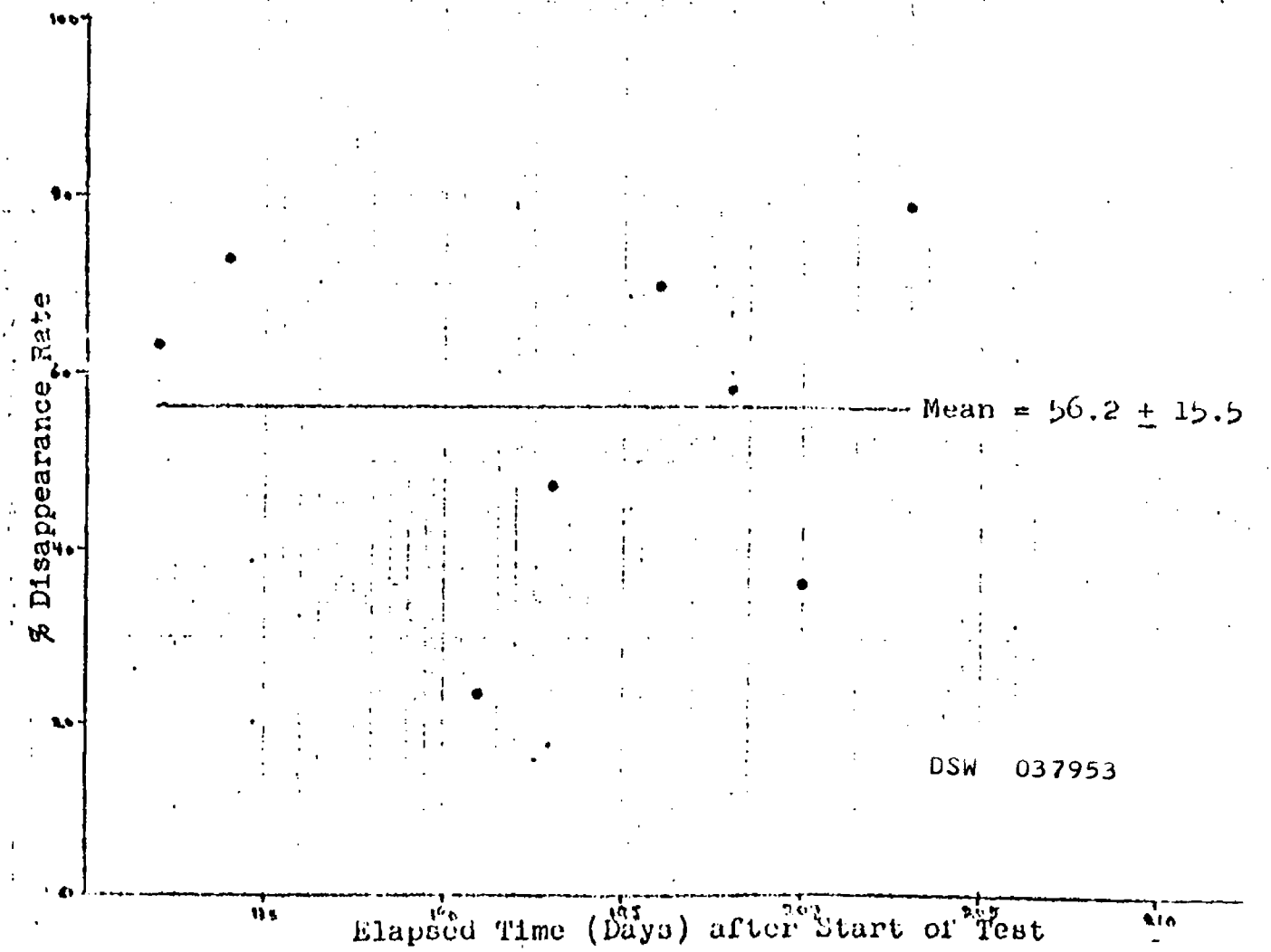
Elapsed Time (Days) after Start of Test

STLCOPCB4021776

SCAS BIODEGRADATION DATA

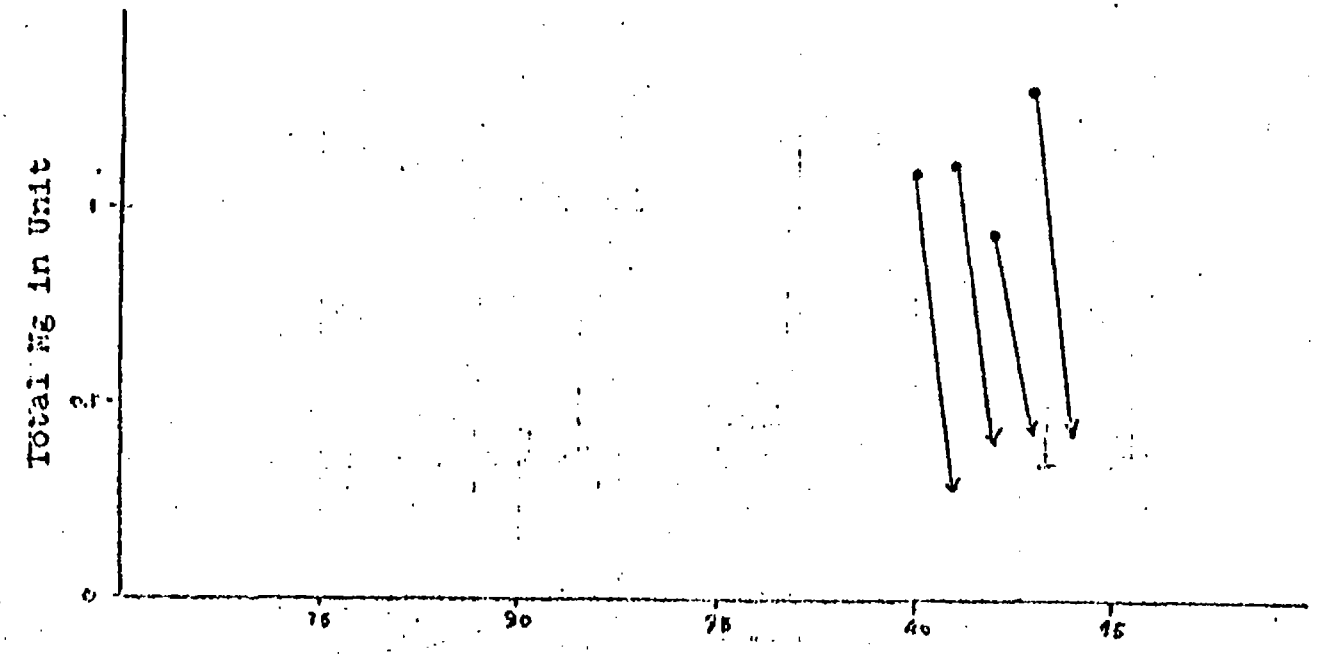
MCS 1043

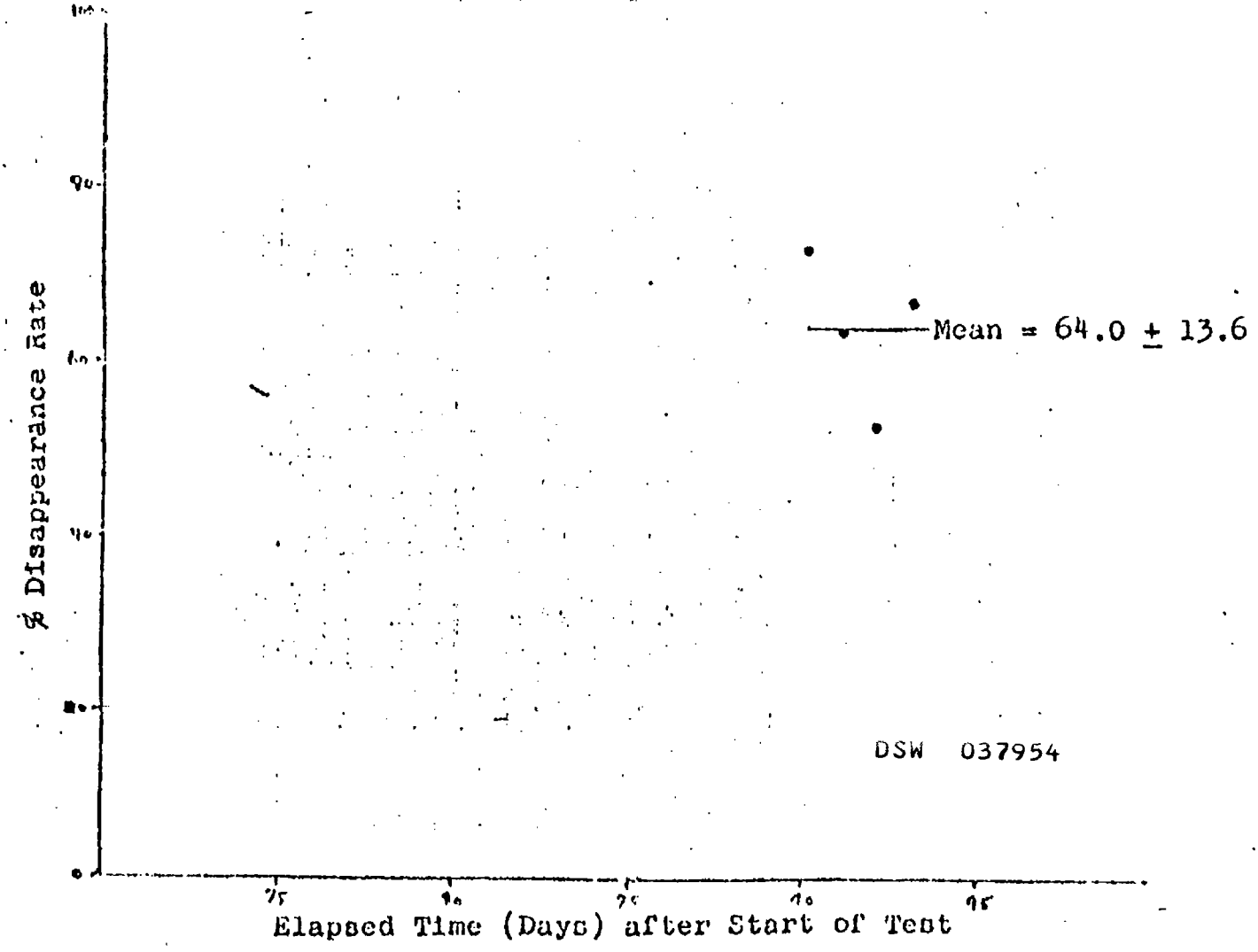




SCAS BIODEGRADATION DATA

AROCLOR 1221

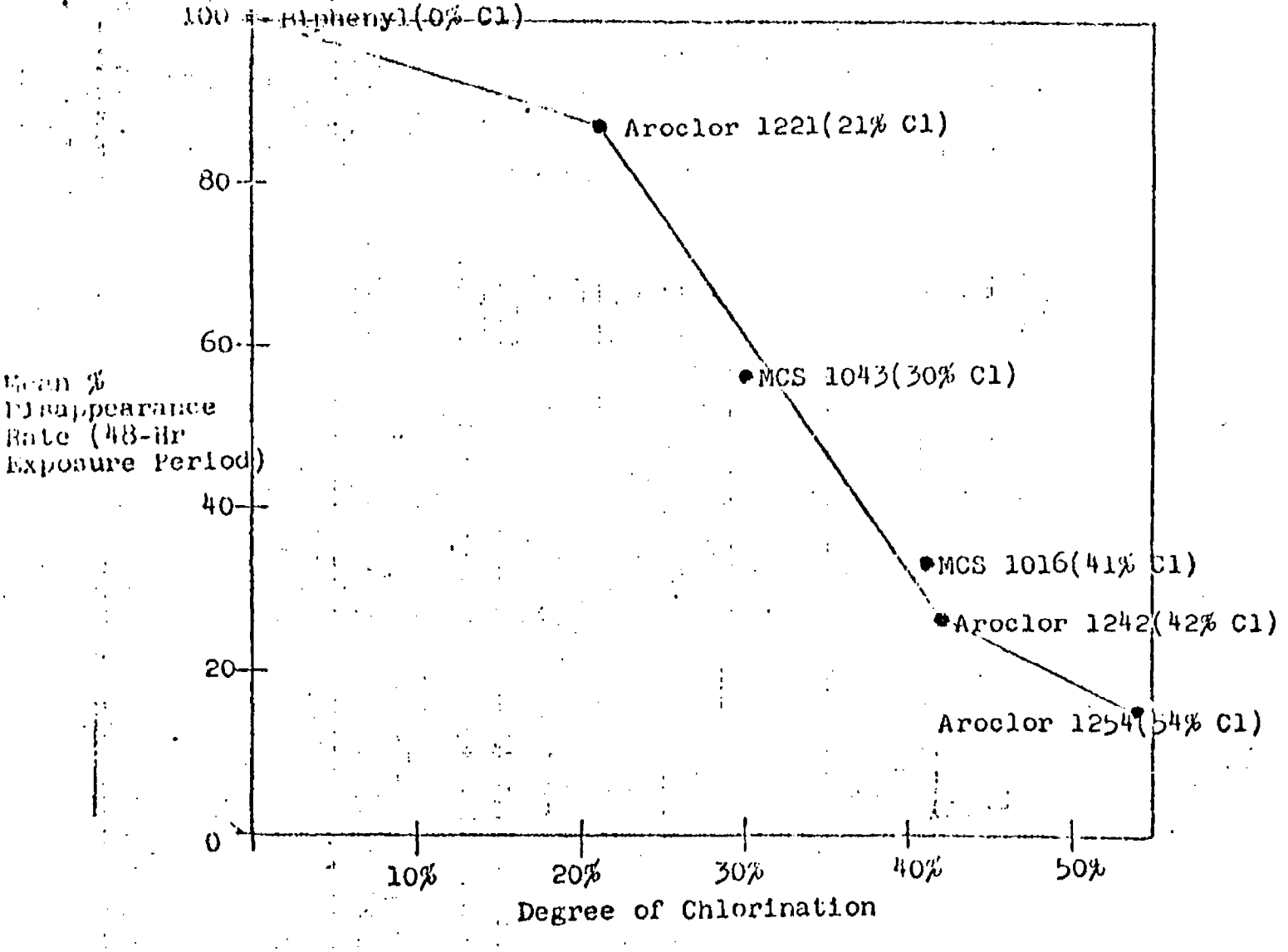




STLCOPCB4021778

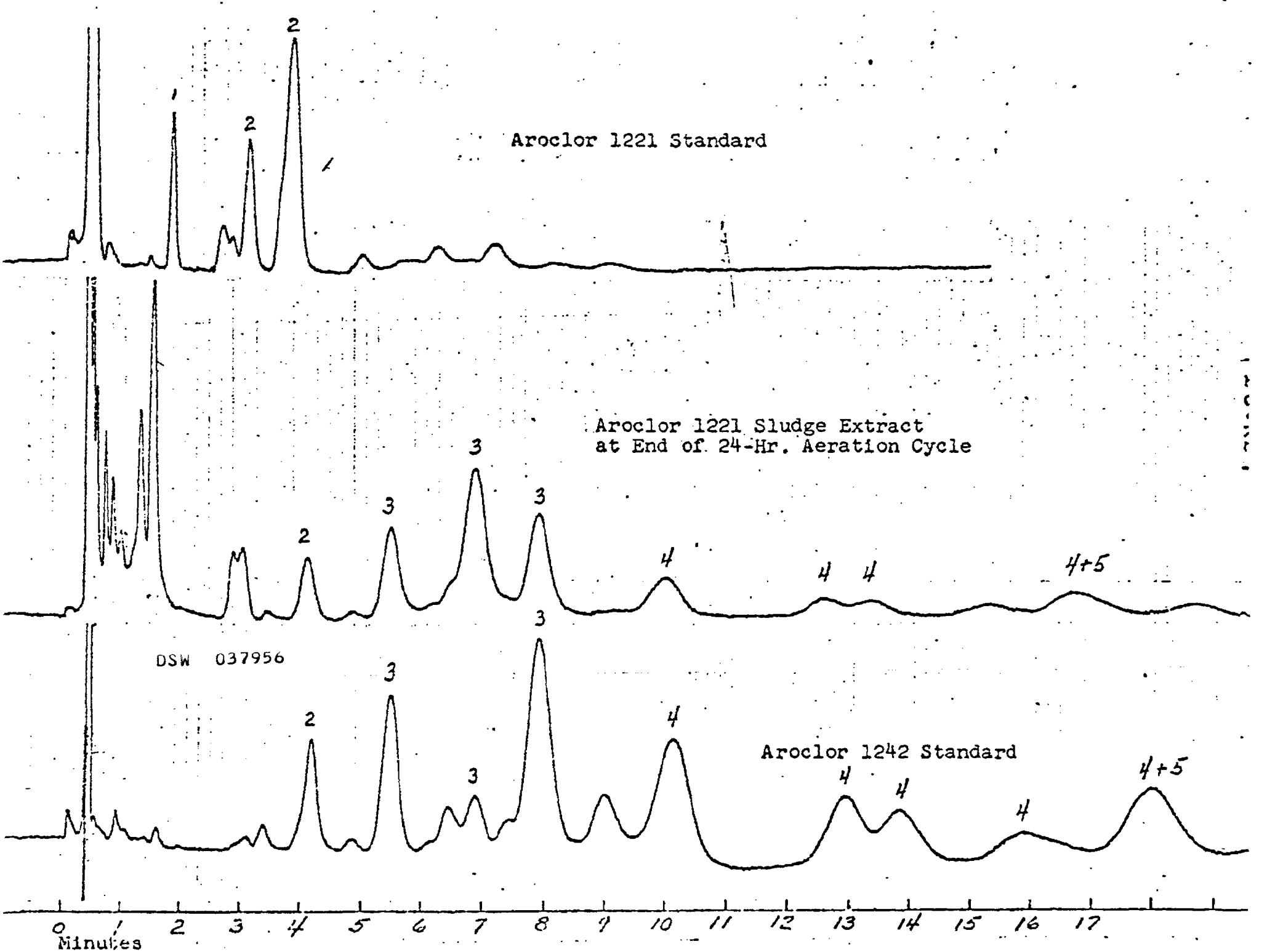
FIGURE 6

SEMI-CONTINUOUS ACTIVATED SLUDGE (SCAS)
DEGRADATION OF POLYCHLORINATED BIPHENYLS



DSW 037955

STLCOPCB4021779



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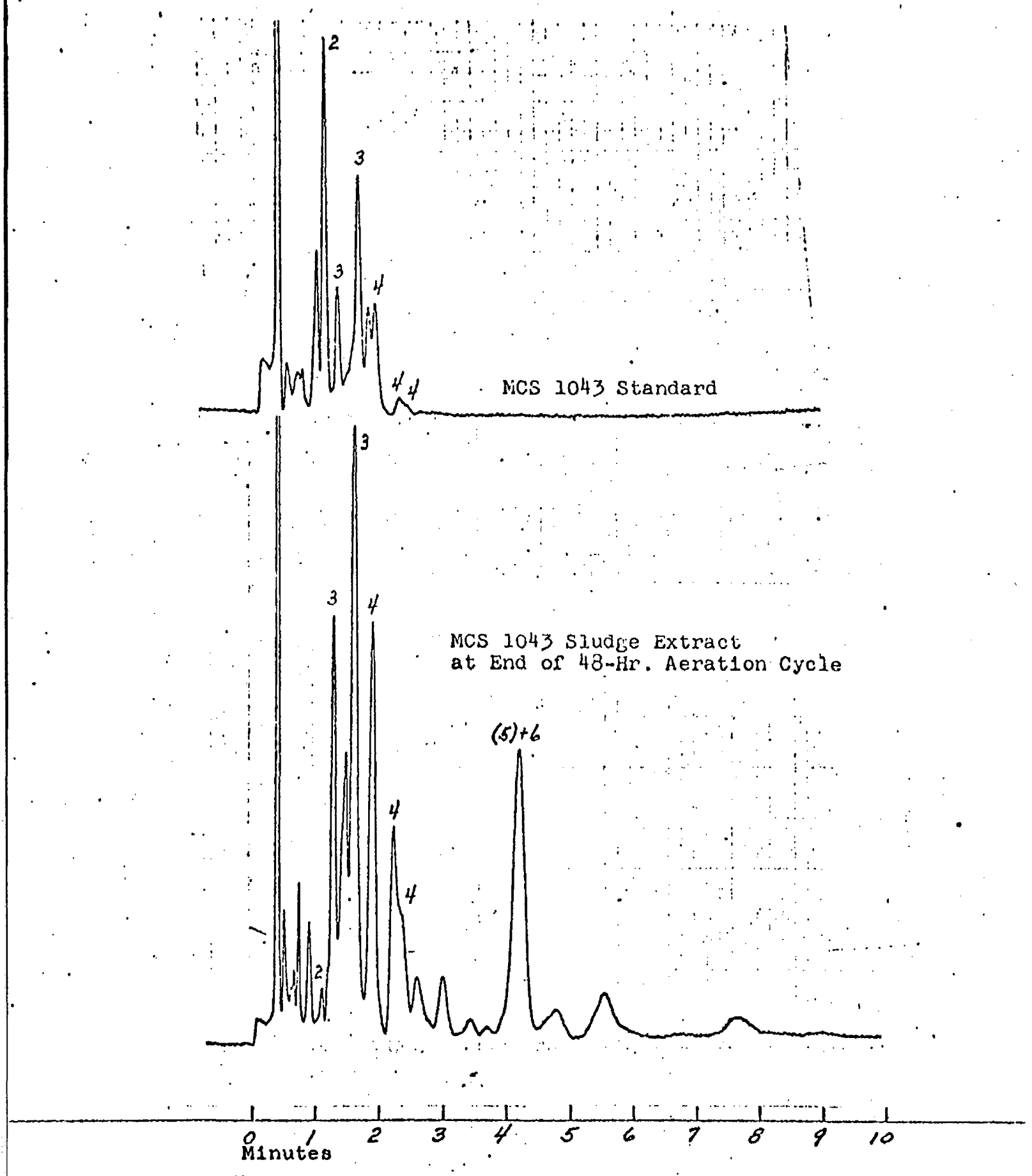

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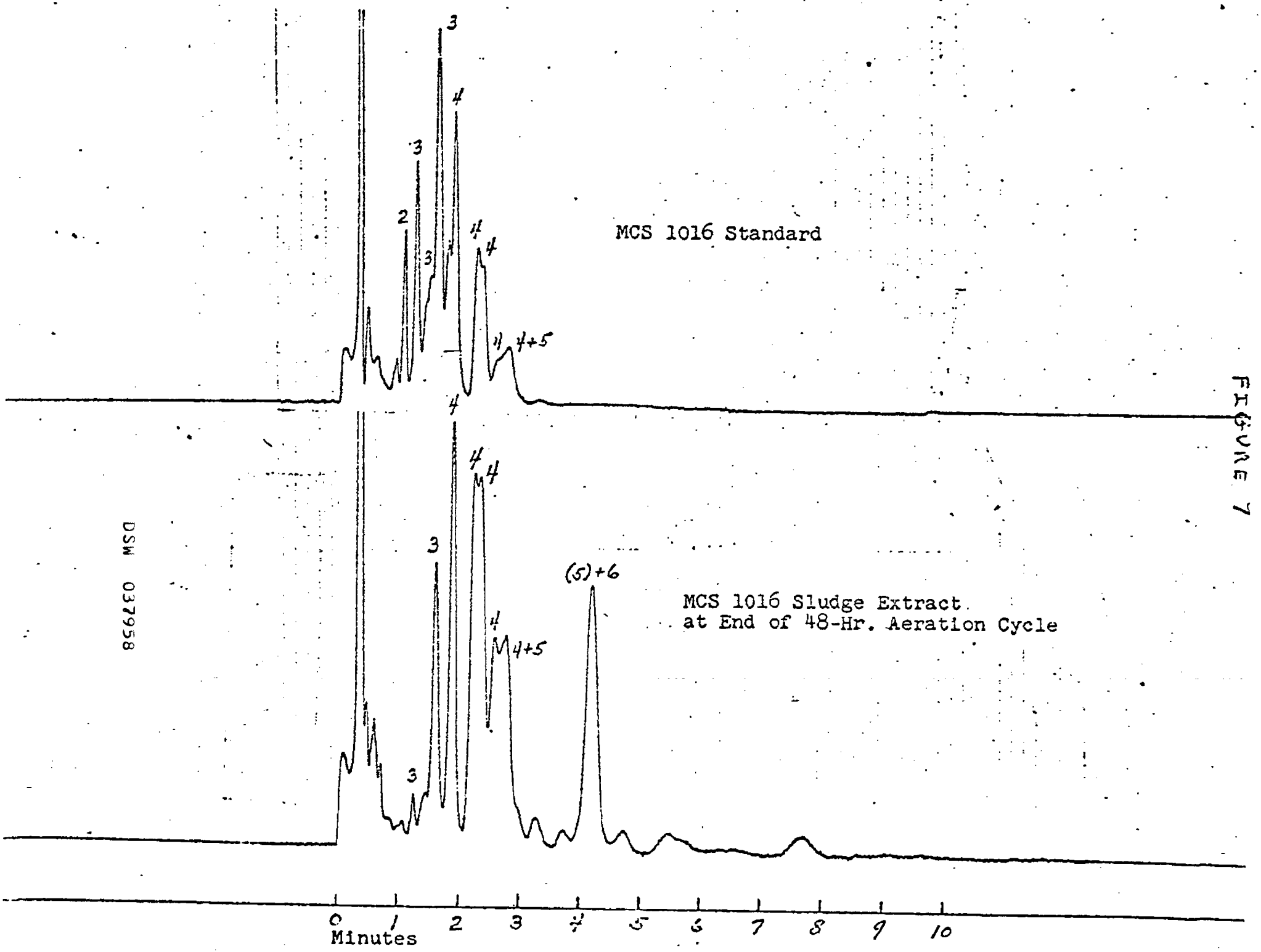
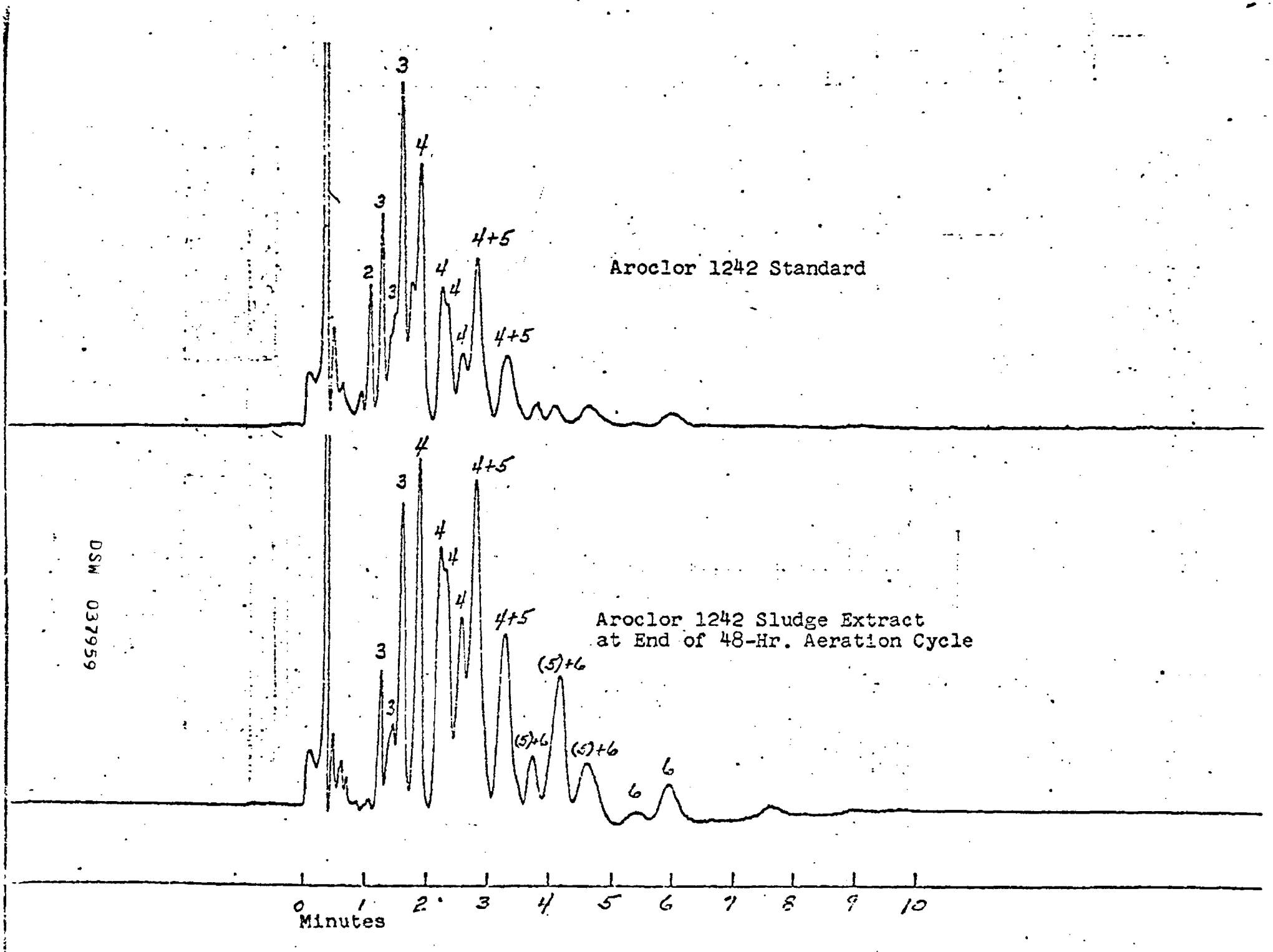


FIGURE 7

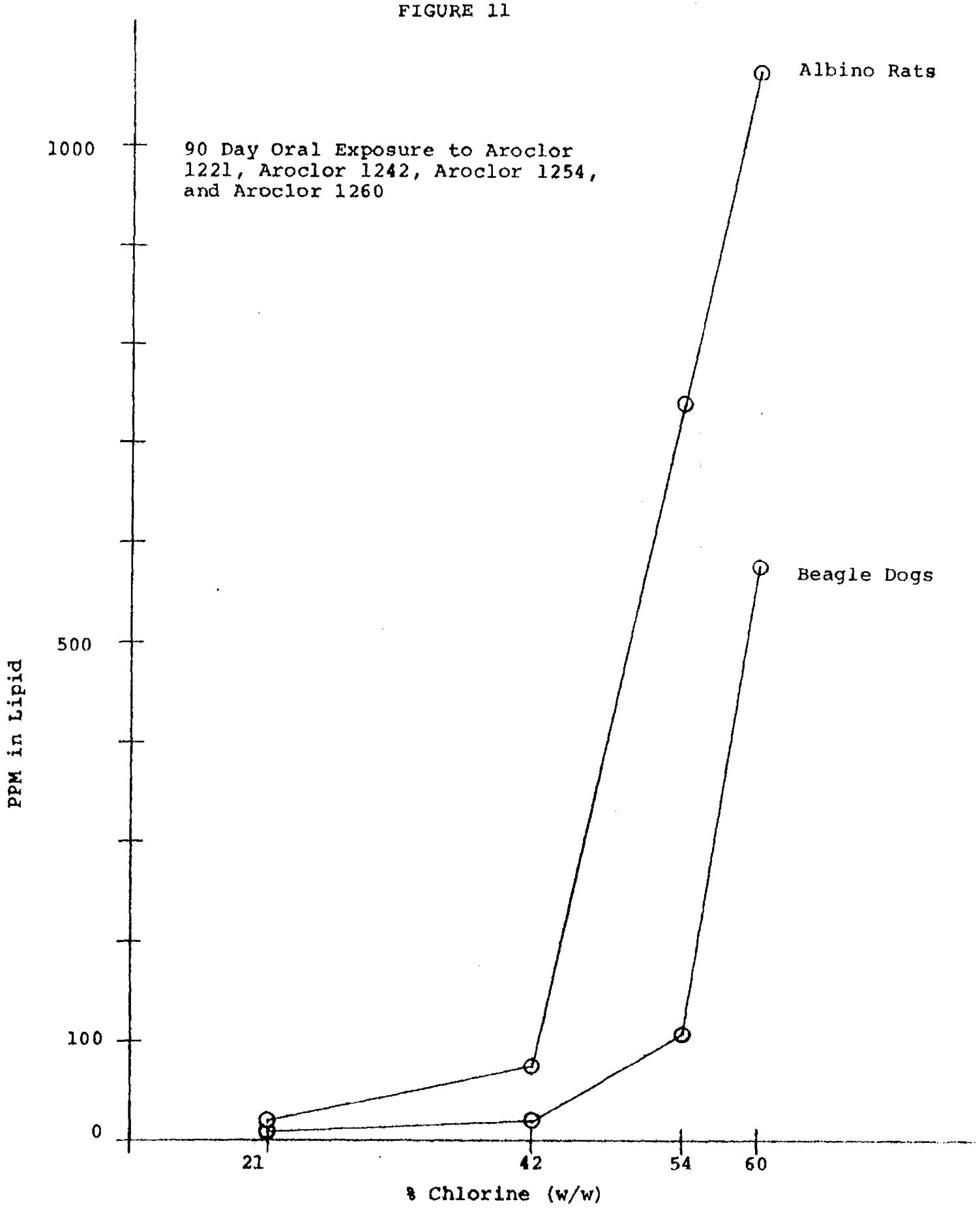
DSM 037958

STLCOPCB4021782



STLCOPCB4021783

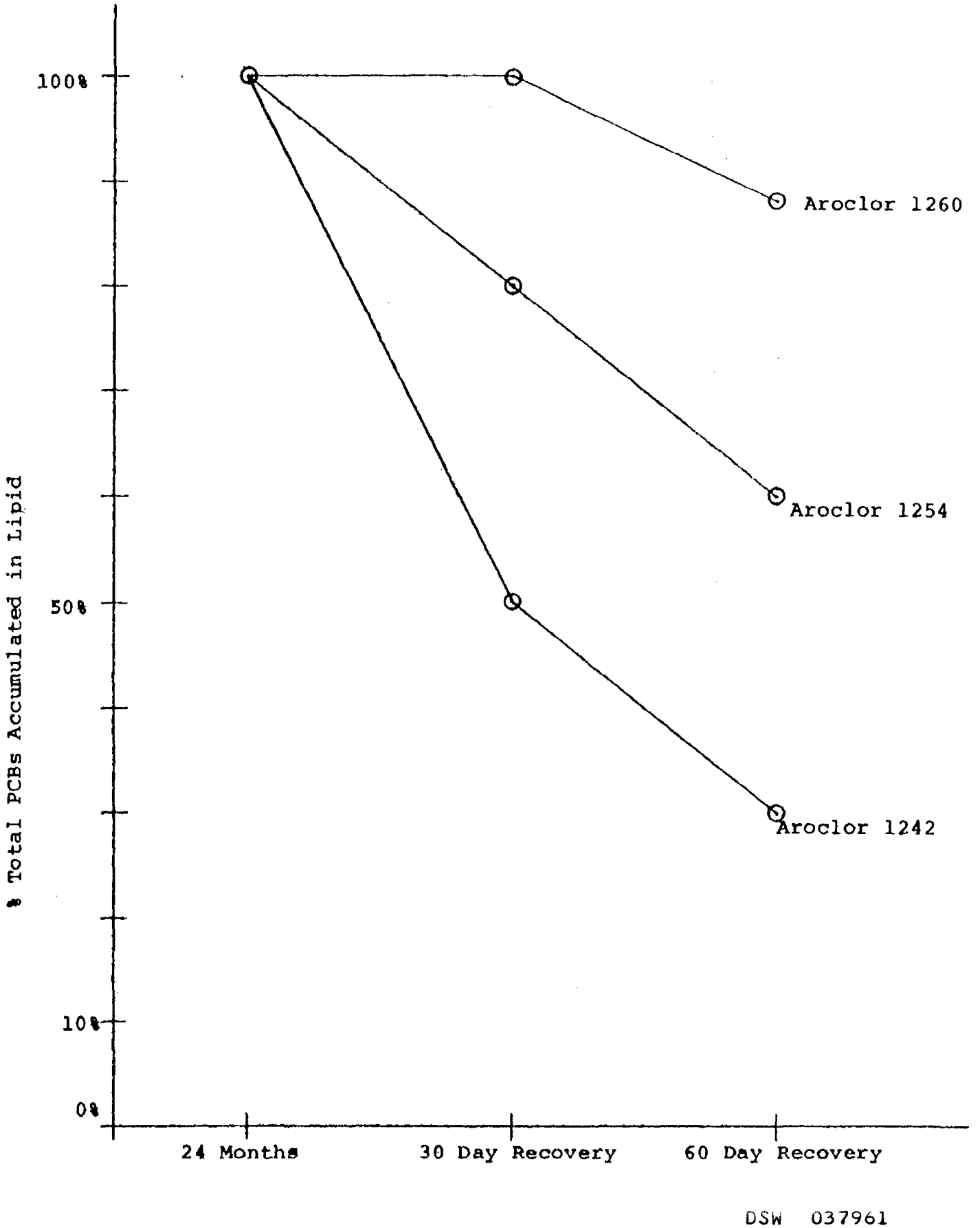
FIGURE 11



DSW 037960

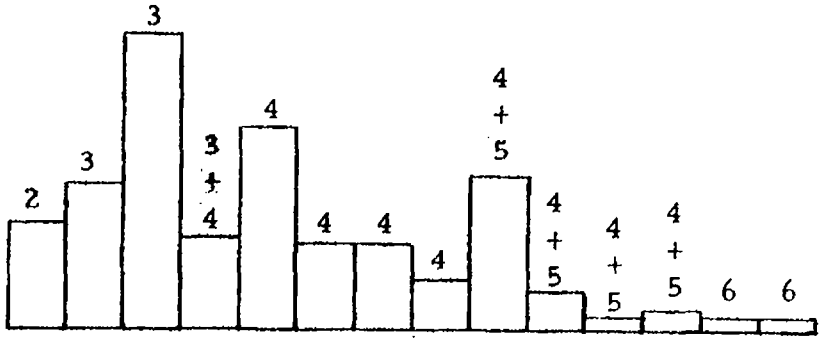
FIGURE 12

Two Year Chronic Oral Toxicity Study of Aroclor 1242, Aroclor 1254 and Aroclor 1260 with Beagle Dogs

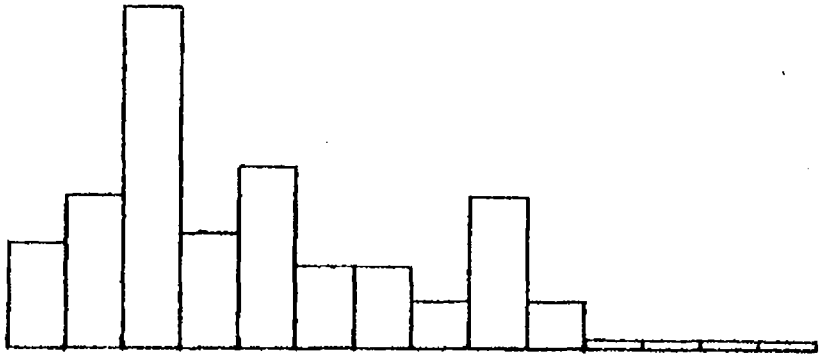


DSW 037961

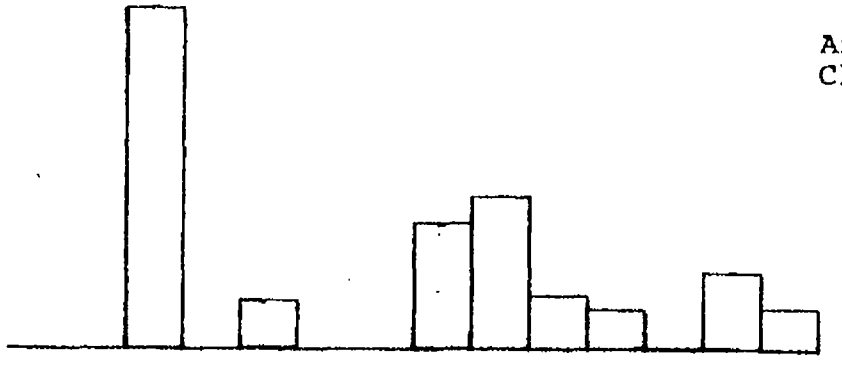
FIGURE 13



Aroclor 1242
Standard



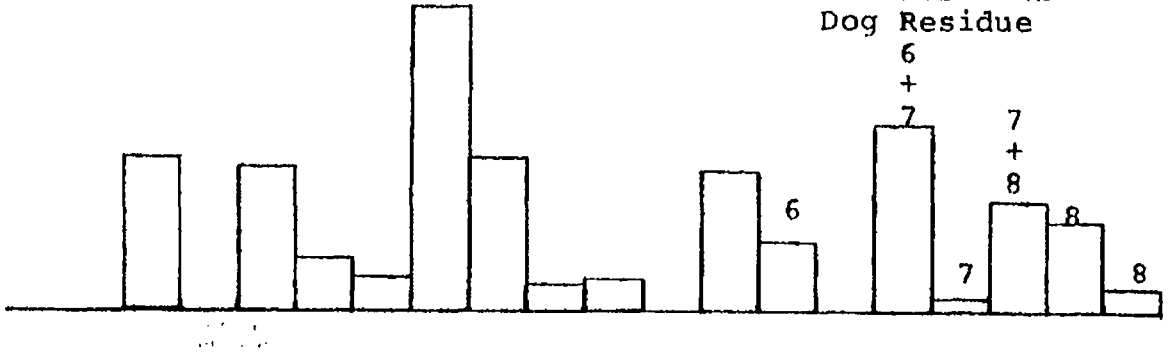
Aroclor 1242
Fish Residue



Aroclor 1242
Chicken Residue



Aroclor 1242
Rat Residue

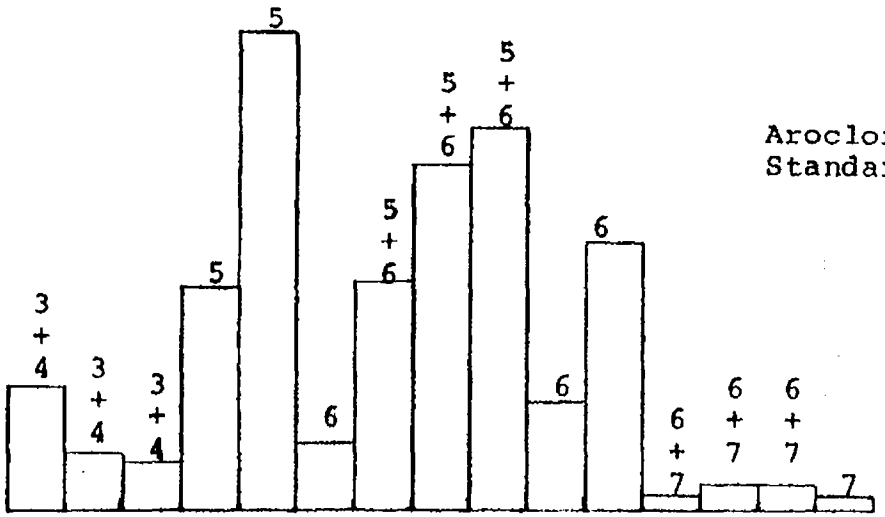


Aroclor 1242
Dog Residue

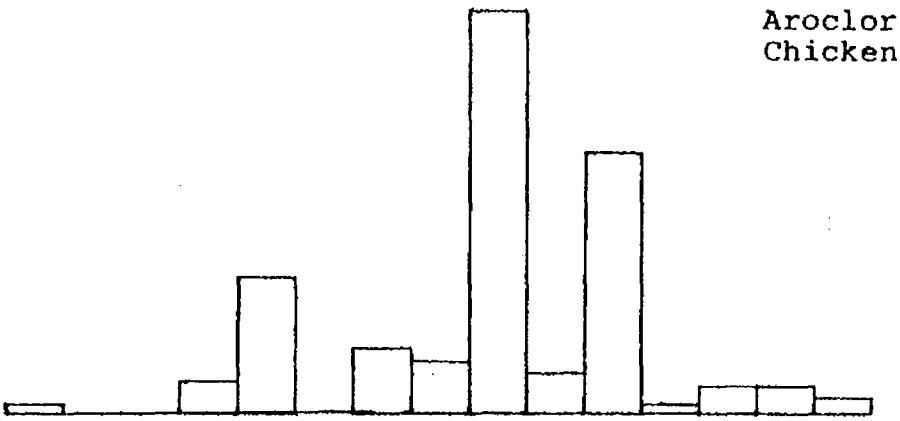
DSW 037962

STLCOPCB4021786

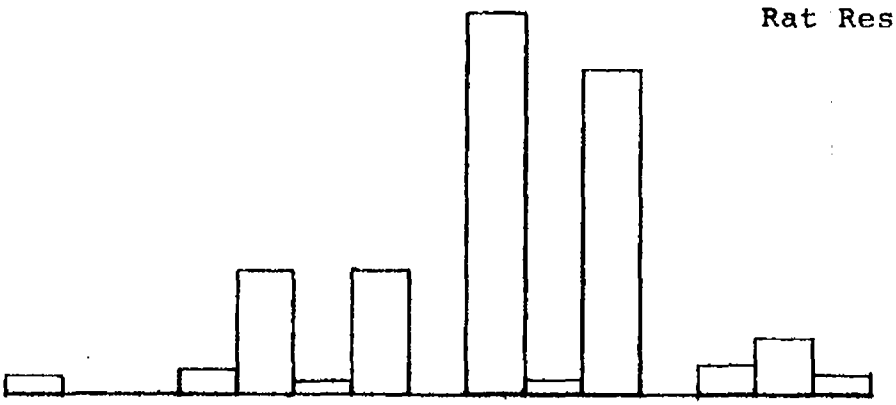
FIGURE 14



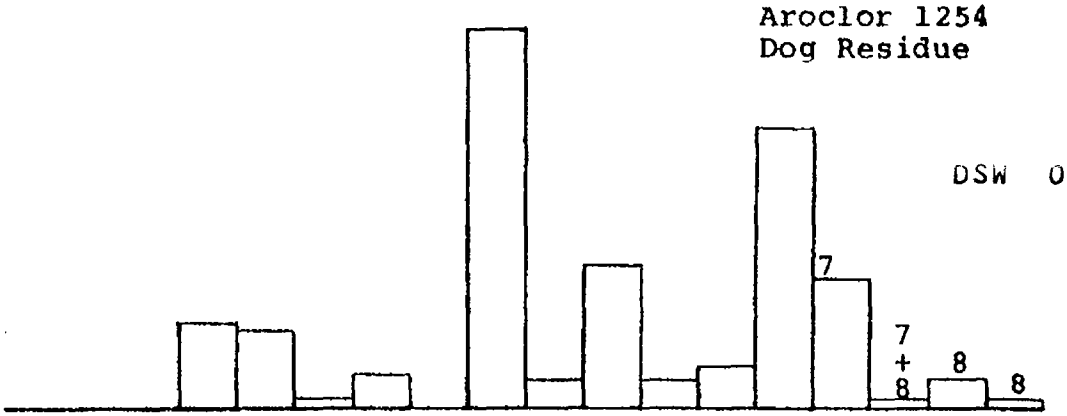
Aroclor 1254
Standard



Aroclor 1254
Chicken Residue



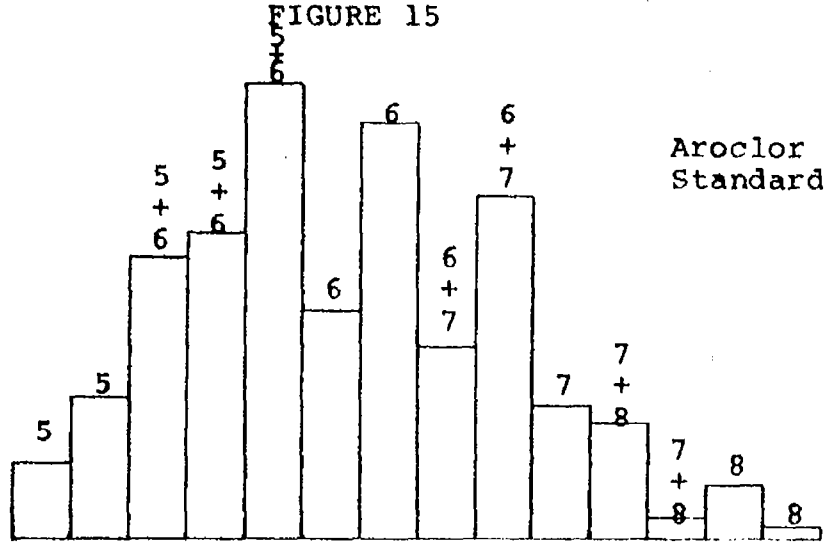
Aroclor 1254
Rat Residue



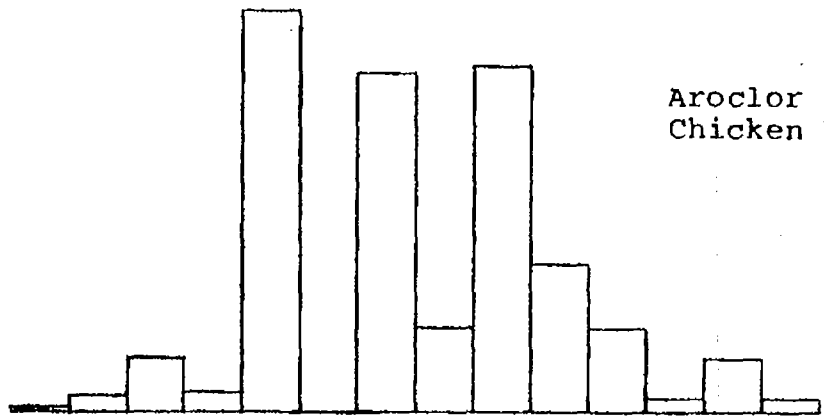
Aroclor 1254
Dog Residue

DSW 037963

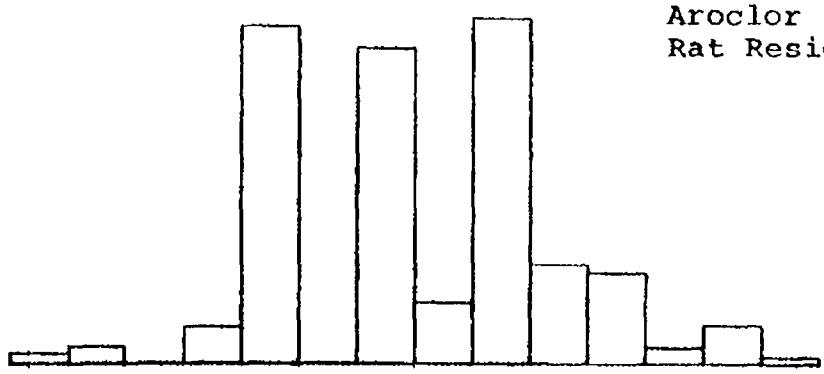
FIGURE 15



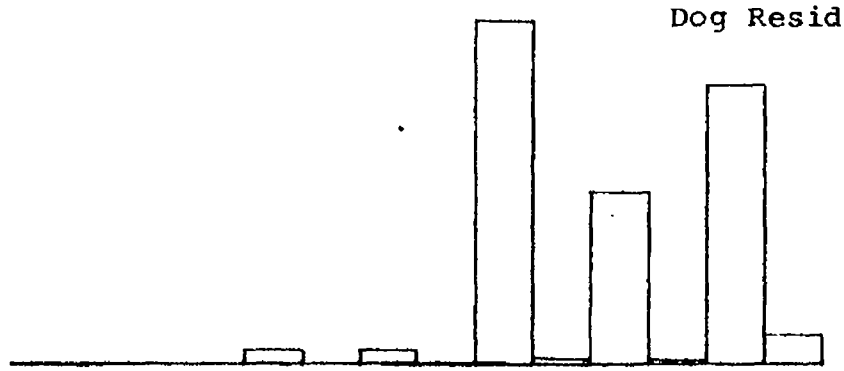
Aroclor 1260
Standard



Aroclor 1260
Chicken Residue



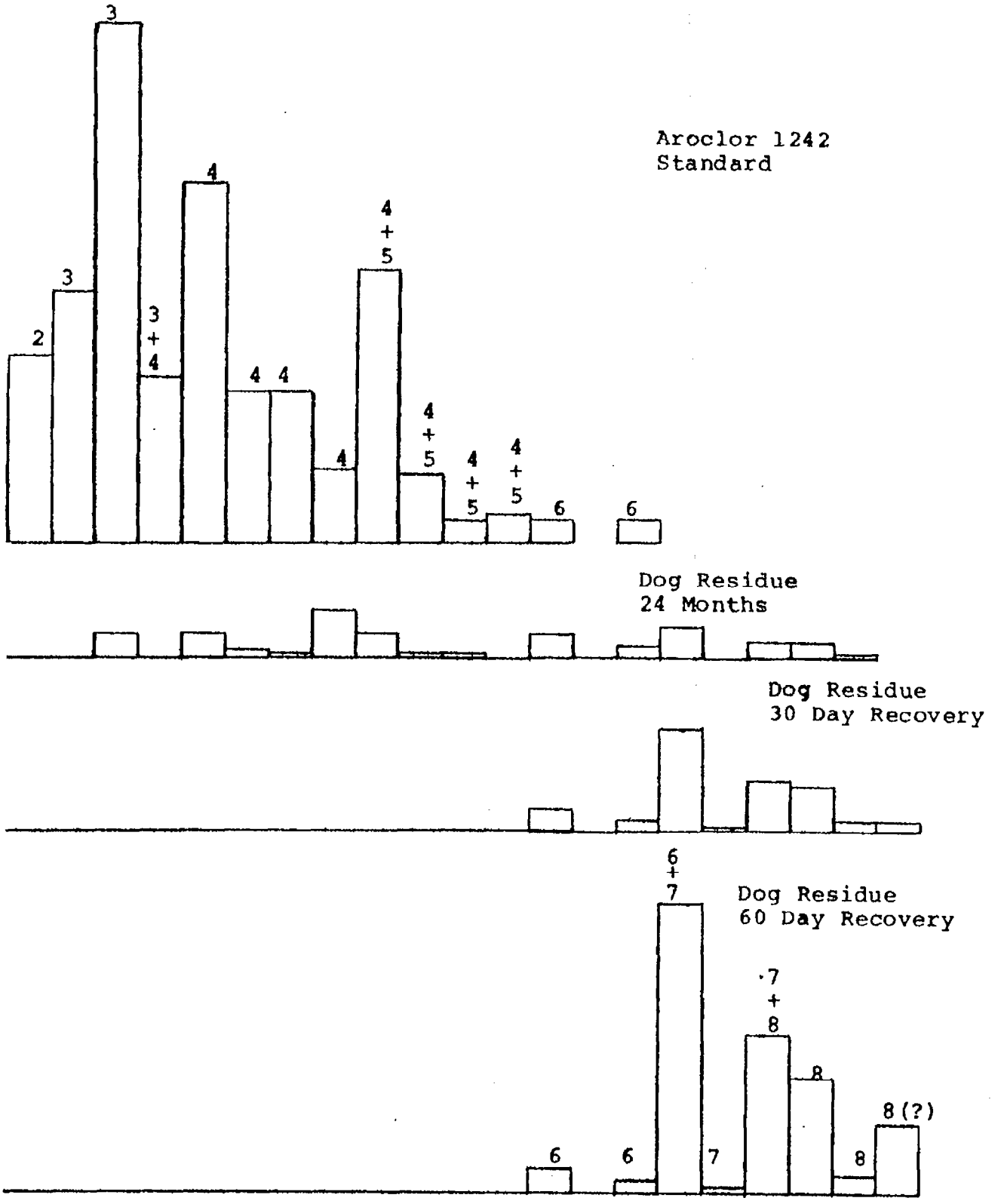
Aroclor 1260
Rat Residue



Aroclor 1260
Dog Residue

DSW 037964

FIGURE 16



DSW 037965

FIGURE 17

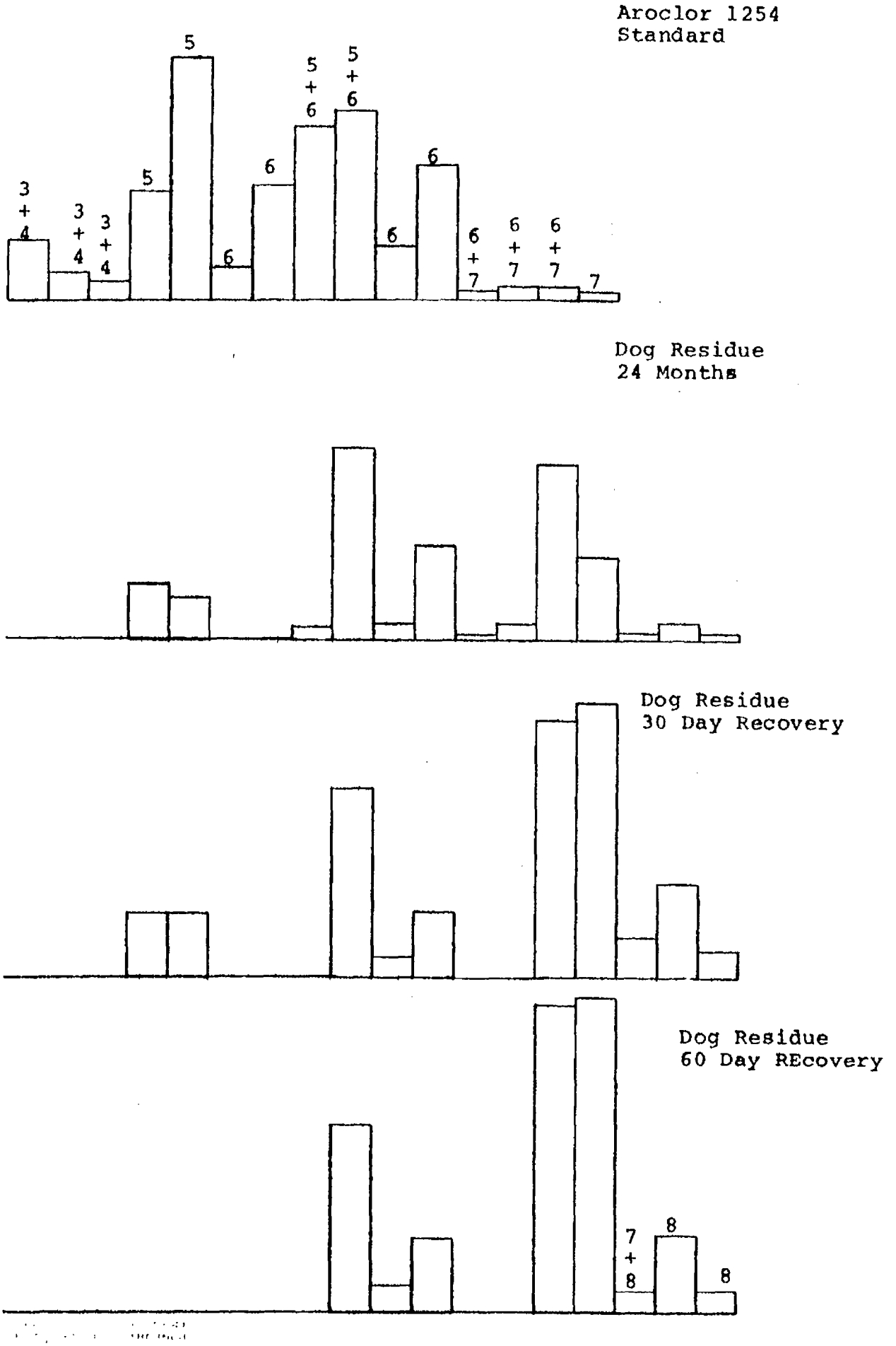
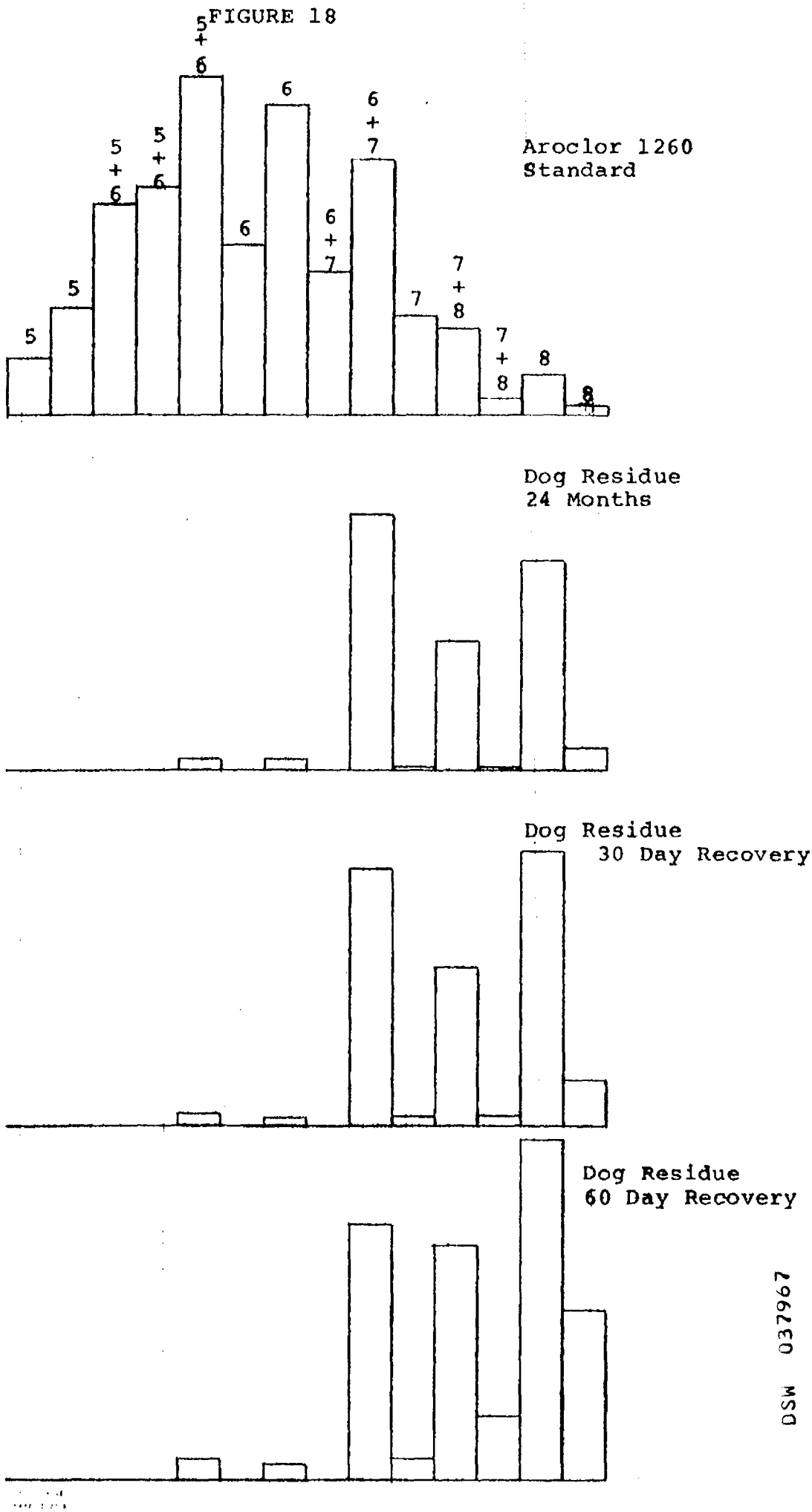
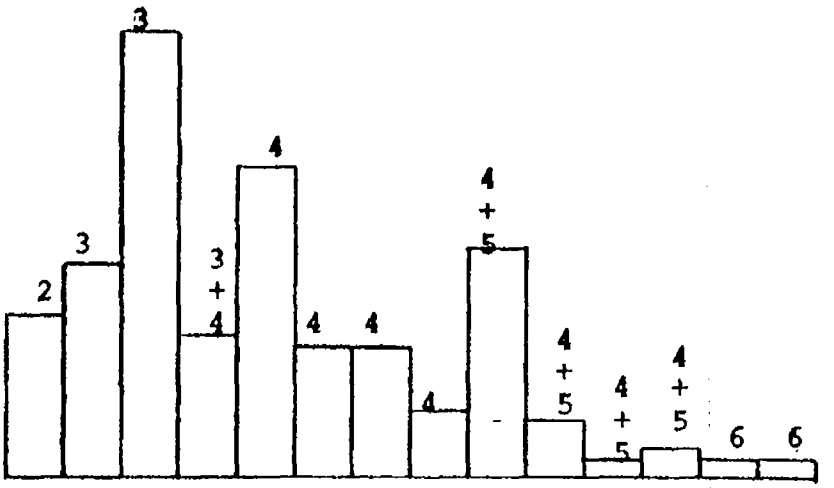

Aroclor 1254
Standard
3 + 4
3 + 4
3 + 4
5
5
6
6
5 + 6
5 + 6
6
6
6 + 7
6 + 7
6 + 7
7
Dog Residue
24 Months
Dog Residue
30 Day Recovery
Dog Residue
60 Day REcovery
7 + 8
8
8
DSW 037966

FIGURE 18

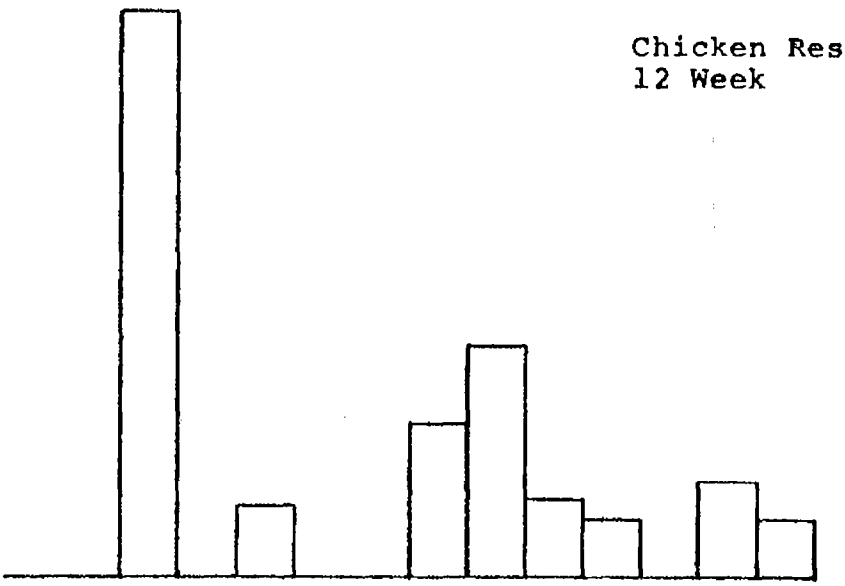


DSW 037967

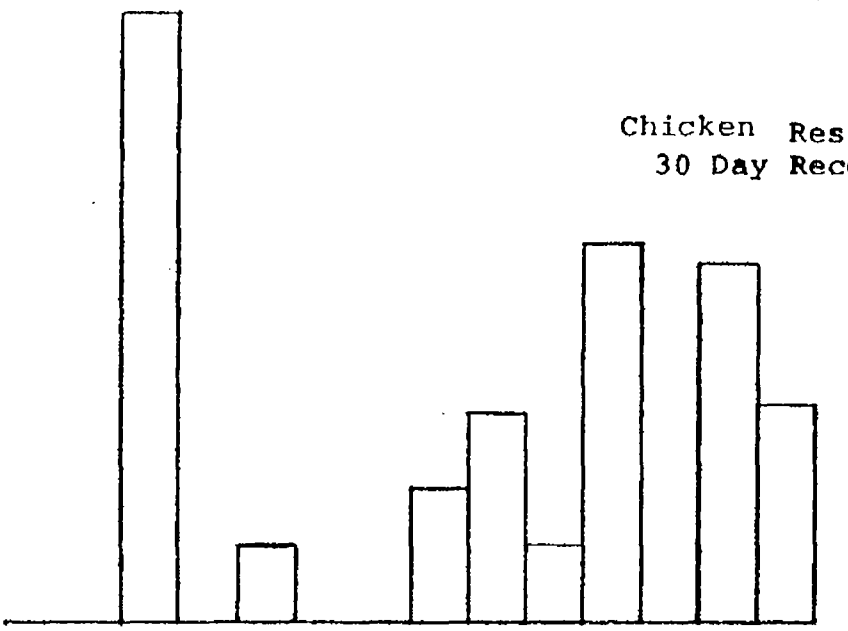
Aroclor 1242
Standard



Chicken Residue
12 Week

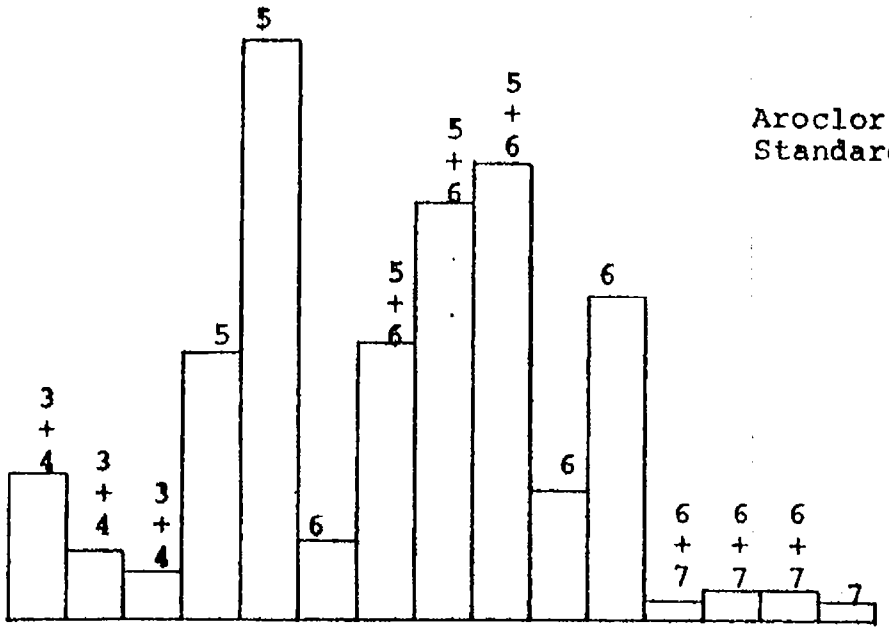


Chicken Residue
30 Day Recovery

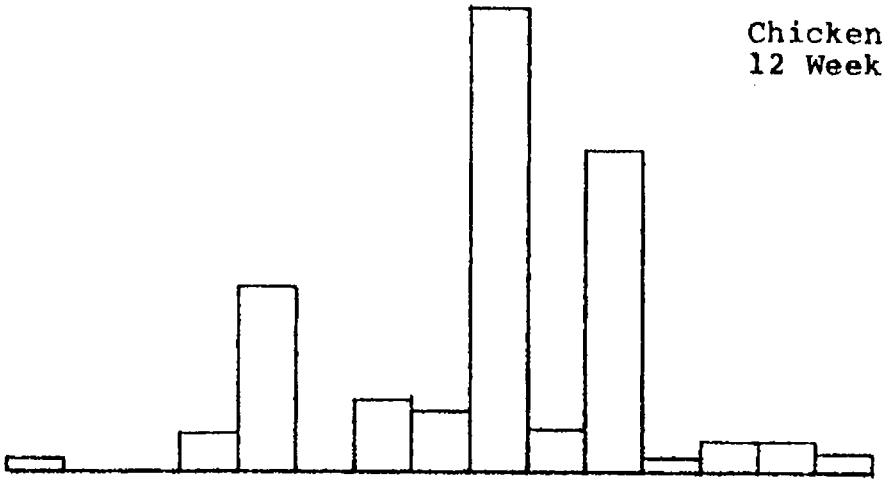


DSW 037968

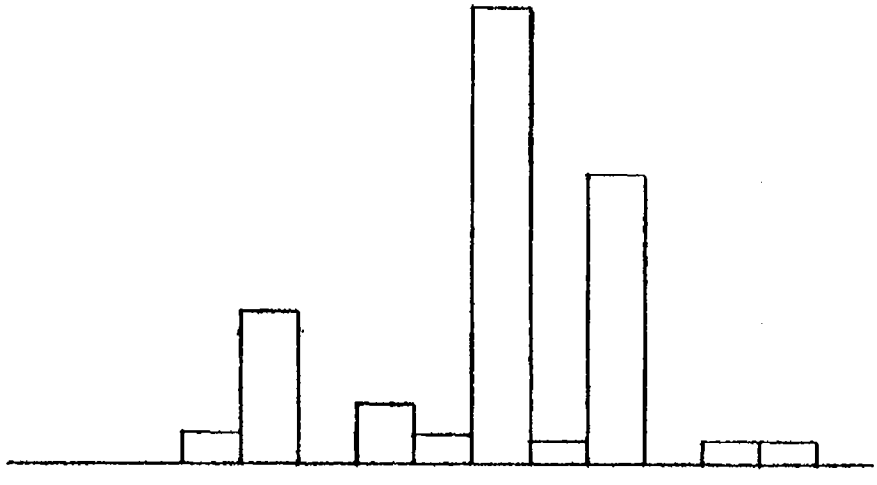
FIGURE 20



Aroclor 1254
Standard

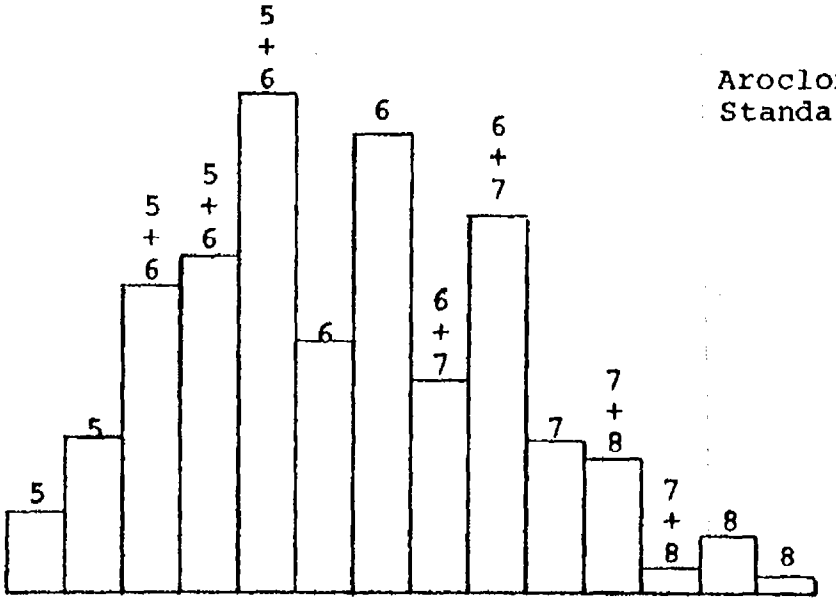


Chicken Residue
12 Weeks

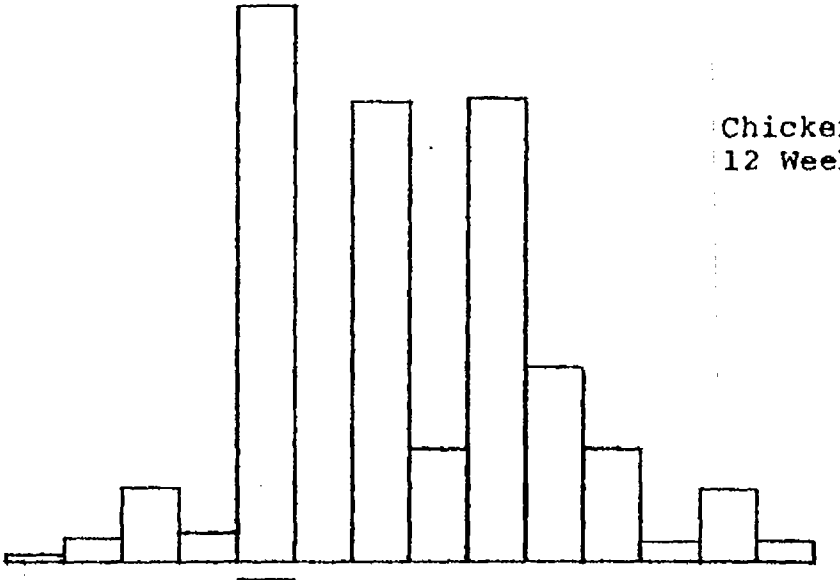


Chicken Residue
30 Day Recovery

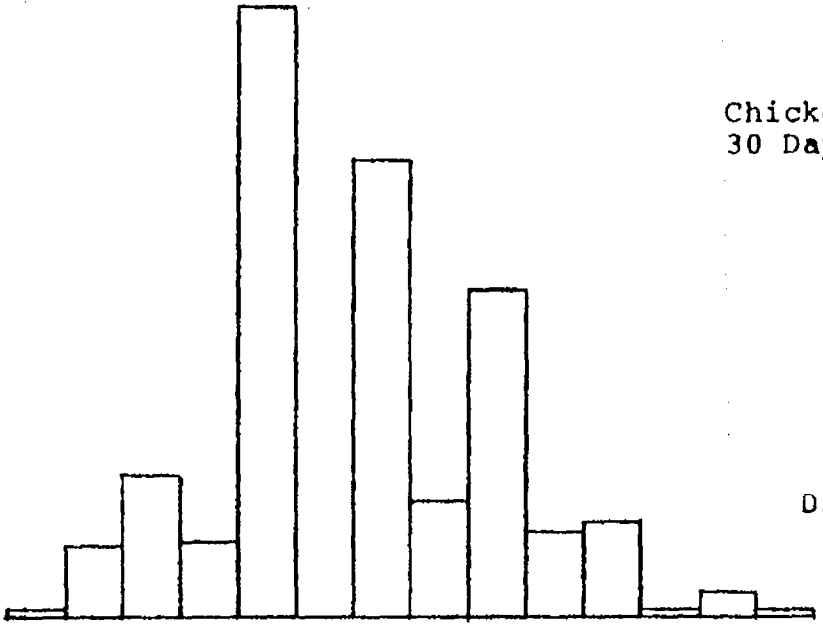
DSW 037969



Aroclor 1260
Standard



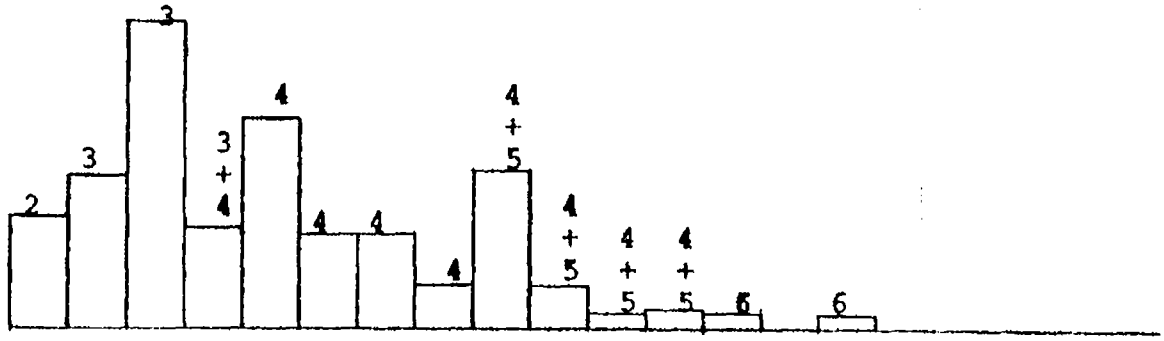
Chicken Residue
12 Weeks



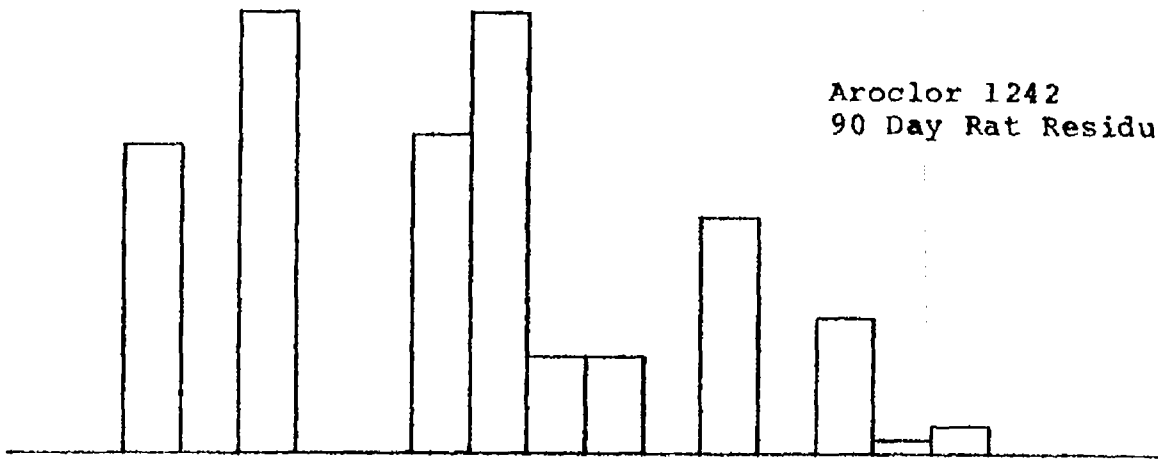
Chicken Residue
30 Day Recovery

DSW 037970

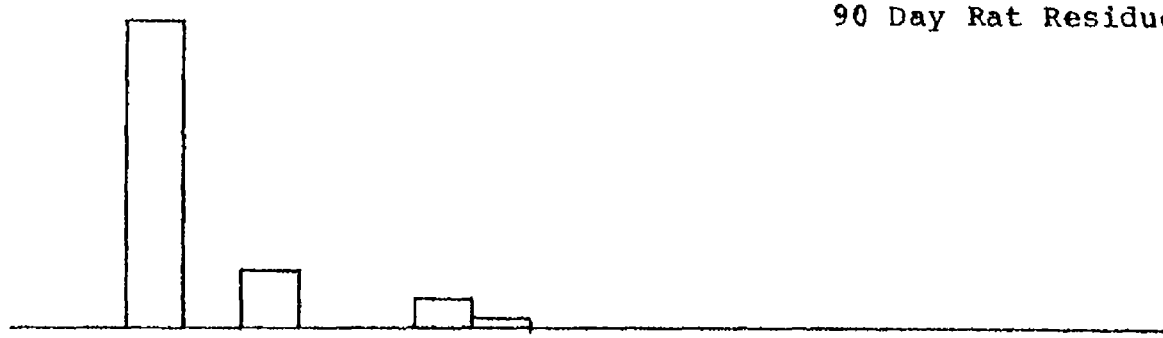
Aroclor 1242
Standard

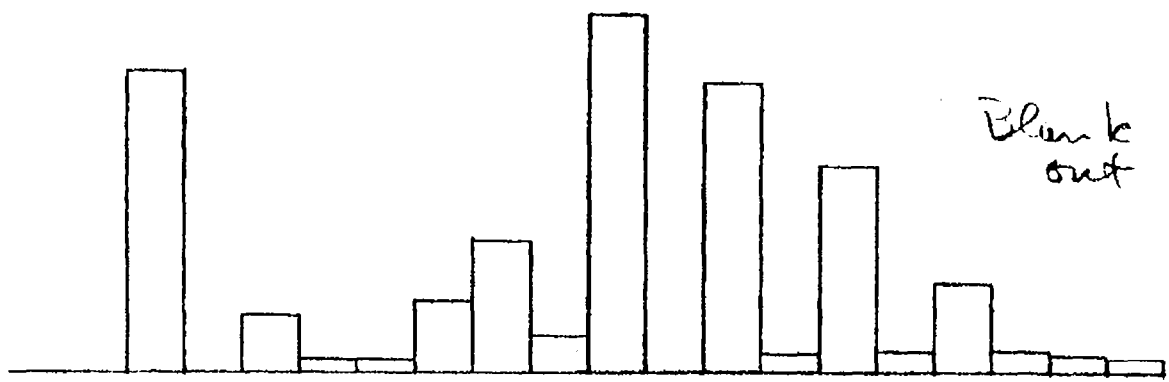


Aroclor 1242
90 Day Rat Residue



Aroclor 1016
90 Day Rat Residue





DSW 037971

TABLE XIII

IBT #B7009 30-Day Tissue Collection Study in Albino Rats with Aroclors

ppm PCB in Rat Tissues (Wet Weight Basis)

Material	Tissue	30 Day Sacrifice				Sacrifice
		300 ppm		1000 ppm		Exposure Level
		♂	♀	♂	♀	Sex
Aroclor 1242	Muscle	5	12	17	29	
	Kidney	1	2	5	2	
	Liver	3	5	10	17	
	Fat	—	276	377	412	
Aroclor 1254	Muscle	56	36	271	167	
	Kidney	8	13	47	156	
	Liver	57	54	176	227	
	Fat	695	660	4660	3430	
Aroclor 1260	Muscle	82	136	266	555	
	Kidney	29	16	72	91	
	Liver	126	125	200	206	
	Fat	2130	2035	9190	11970	

DSM 037972

STLCOPCB4021796

TABLE XIV

IBT #B7009 30 Day Tissue Collection Study in Albino Rats with Aroclors

ppm PCB in Rat Tissues (Lipid Weight Basis)

Material	Tissue	30 Day Sacrifice				Sacrifice
		300 ppm		1000 ppm		Exposure Level
		♂	♀	♂	♀	Sex
Aroclor 1242	Muscle	37	58	736	855	
	Kidney	12	28	276	146	
	Liver	61	220	753	793	
	Fat	—	395	816	722	
Aroclor 1254	Muscle	1396	1274	7735	6202	
	Kidney	655	1895	3325	12972	
	Liver	2370	3358	5318	6678	
	Fat	1094	1289	7208	7544	
Aroclor 1260	Muscle	2470	4849	8879	24111	
	Kidney	2056	2671	5181	7579	
	Liver	3808	6226	6263	10300	
	Fat	3061	3430	13715	19088	

DSM 037973

STLCOPCB4021797

TABLE XV

IBT #B7009 30 Day Tissue Collection Study in Albino Rats with Aroclors

Predicted ppm PCB in Rat Tissues (Lipid Weight Basis)

Material	Tissue	30 Day Sacrifice				Sacrifice
		300 ppm		1000 ppm		Exposure Level
		♂	♀	♂	♀	Sex
Aroclor 1242	Muscle	44	83	608	821	
	Liver	105	199	765	1033	
	Fat	105	199	765	1033	
Aroclor 1254	Muscle	721	1239	5602	6859	
	Liver	1145	1997	4694	5748	
	Fat	1145	1997	4694	5748	
Aroclor 1260	Muscle	2921	4779	17004	19823	
	Liver	3784	6190	11630	13557	
	Fat	3784	6190	11630	13557	

Regression Equation - log ppm PCB = $-0.9861 - 0.2309$ (if muscle) $+ 0.1855$ (% Cl_2) $+ 0.3329$ (log ppm fed) $+ 0.1124$ (if female) $+ 0.1471$ (% Cl_2 if muscle) $+ 0.5306$ (log ppm fed if muscle) $- 0.3994$ (log ppm fed x % Cl_2) $- 0.3552$ (% Cl_2 if female) $- 0.2815$ (log ppm fed if female)

DSM 037974

STLCOPCB4021798

TABLE XVI

IBT # B7298 Two-Year Chronic Oral Toxicity Study of Aroclor in Albino Rats

ppm PCB in Rat Tissues (Wet Weight Basis)

Material	Tissue	1 ppm			10 ppm			100 ppm			Exposure Level
		3 mo.	12 mo.	24 mo.	3 mo.	12 mo.	24 mo.	3 mo.	12 mo.	24 mo.	Sacrifice
Aroclor 1242	Muscle	0.7	1	1.8	1.4	6	14	5	33	73	
	Liver	0.1	0.2	0.5	0.2	0.6	1	0.9	5	5	
	Fat	6	7	6	12	30	43	60	144	220	
Aroclor 1254	Muscle	2	2	13	8	131	98	46	94	352	
	Liver	0.1	0.4	1.2	1	23	6	15	24	133	
	Fat	6	19	45	75	632	315	1208	794	1533	
Aroclor 1260	Muscle	6	8	15	8	50	116	10	419	785	
	Liver	0.2	1	0.8	2	3	5	18	53	107	
	Fat	28	56	50	417	224	298	482	2000	4447	

DSM 037975

STLCOPCB4021799

TABLE XVII

IBT # B7298 Two-Year Chronic Oral Toxicity Study of Aroclor in Albino Rats

ppm PCB in Rat Tissues (Lipid Weight Basis)

Material	Tissue	1 ppm			10 ppm			100 ppm			Exposure Level
		3 mo.	12 mo	24 mo.	3 mo.	12 mo.	24 mo.	3 mo	12 mo.	24 mo.	Sacrifice
Aroclor 1242	Muscle	8	6	5	16	38	74	76	236	239	
	Liver	11	16	18	26	40	52	77	241	234	
	Fat	6	8	9	13	32	47	67	151	227	
Aroclor 1254	Muscle	10	11	38	96	721 (196)	389	756 (800)	1092	2164	
	Liver	18	24	82	115	914 (292)	307	1137 (2000)	869	1810 (4800)	
	Fat	6	19	55	76	688 (231)	348	329 (900)	860	1894	
Aroclor 1260	Muscle	16	47	44	60	259	436	146 (689)	2247	5154	
	Liver	20	50	56	102	189	442	1677	1752	6310	
	Fat	32	60	62	138	262	354	866	2143	5060	

( ) – Smoothed data, used to develop second regression equation

DSM 037976

STLCOPCB4021800

TABLE XVIII

IBT #B7298 Two Year Chronic Oral Toxicity Study of Aroclor in Albino Rats

Predicted ppm PCB in Rat Tissues (Lipid Weight Basis)

Material	Tissue	1 ppm			10 ppm			100 ppm			Exposure Level
		3mo	12mo	24mo	3mo	12mo	24mo	3mo	12mo	24mo	Sacrifice
Aroclor 1242	Muscle	5	8	10	20	32	45	77	133	206	
	Liver	8	12	15	30	48	68	115	199	307	
	Fat	5	8	10	20	32	45	77	133	206	
Aroclor 1254	Muscle	11	21	36	81	163	305	584	1264	2611	
	Liver	17	31	53	121	242	455	870	1884	3893	
	Fat	11	21	36	81	163	305	584	1264	2611	
Aroclor 1260	Muscle	16	30	51	116	234	439	840	1819	3758	
	Liver	19	36	62	140	281	527	1008	2182	4509	
	Fat	20	38	65	147	296	554	1061	2297	4746	

Regression Equation log ppm PCB = 3.3550 − 1.3440 (if 1242) − 0.3696 (if 1254) + 0.2881 (if liver) + 0.8936 (log ppm fed) + 0.0626 (time − 12) − 0.2761 (log ppm fed if 1242) − 0.0246 (time − 12 if 1242)

DSW 037677

STLCOPCB4021801

TABLE XIX

IBT # P7297 Three-Generation Reproduction Study in Albino Rats with Aroclor

ppm PCB in Rat Pups (Wet Weight Basis)

	21 Day Sacrifice			Sacrifice
Material	1 ppm	10 ppm	100 ppm	Exposue Level
Aroclor 1242	0.2	4	32	
Aroclor 1254	2	16	155	
Aroclor 1260	1	8	104	

ppm PCB in Rat Pups (Lipid Weight Basis)

	21 Day Sacrifice			Sacrifice
Material	1 ppm	10 ppm	100 ppm	Exposure Level
Aroclor 1242	67	78	578	
Aroclor 1254	48	170	2328	
Aroclor 1260	21	213	2371	

DSW 037678

STLCOPCB4021802

TABLE XX

IBT # 9888C
9888D

90-Day Subacute Oral Toxicity Study of Aroclor in Albino Rats

ppm PCB in Rat Tissues (Wet Weight Basis)

		90 Day Sacrifice						Sacrifice
		30 ppm		100 ppm		300 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1016	Muscle	14	4	20	15	8	5	
	Liver	5	1	10	16	4	0.5	
	Fat	24	27	138	193	6	88	

		90 Day Sacrifice						Sacrifice
		1 ppm		10 ppm		100 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1221	Muscle	0.5	0.4	3	1	3	2	
	Liver	0.06	0.1	0.06	0.5	0.2	2	
	Fat	2	4	16	5	36	50	

DSW 037979

STLCOPCB4021803

TABLE XXI

IBT # 9888 C
9888 D

90-Day Subacute Oral Toxicity Study of Aroclor in Albino Rats

ppm PCB in Rat Tissues (Lipid Weight Basis)

		90-Day Sacrifice						Sacrifice
		30 ppm		100 ppm		300 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1016	Muscle	28	40	175	16000	4501	4272	
	Liver	160	43	160	376	68	11	
	Fat	30	34	175	294	7	115	

		90-Day Sacrifice						Sacrifice
		1 ppm		10 ppm		100 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1221	Muscle	5	5	29	18	64	74	
	Liver	3	7	4	22	10	65	
	Fat	3	5	19	5	42	56	

DSW 037980

STLCOPCB4021804

TABLE XXII

IBT #9888C 9888D 90-Day Subacute Oral Toxicity Study of Aroclor in Albino Rats

Predicted ppm PCB in Rat Tissues (Lipid Weight Basis)

		* 90-Day Sacrifice						Sacrifice
		30 ppm		100 ppm		300 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1016	Muscle							
	Liver							
	Fat							

		90-Day Sacrifice						Sacrifice
		1 ppm		10 ppm		100 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1221	Muscle	5	5	19	19	76	76	
	Liver	2	8	5	22	14	60	
	Fat	5	5	13	13	36	36	

* Calculation of meaningful regression equation not possible, inconsistent data attributed to sample handling problems.

1221 Regression Equation log ppm PCB = 0.6689 + 0.4427 (log ppm fed) − 0.4186 (if liver) + 0.4628 (if male) + 0.1404 (if female liver)

DSM 037981

STLCOPCB4021805

TABLE XXIII

IBT # 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	0.4		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	936	221		352	173		

DSM 037982

STLCOPCB4021806

TABLE XXIV

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	2	18	18		9			61	95				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	796	100		491	301		
	Liver	17	13	11			21	175	43	112	135	55	120	762	268		81	659		
	Fat	14	18		20		5	159	39	54	72		78	1261	287		439	238		

DSM 037983

STLCOPCB4021807

TABLE XXV

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	38	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 = $-1.3450 - 0.0190$ (day recovery) $- 0.3888$ (log ppm fed) $+ 0.2929$ (if liver) $+ 0.0376$ (% Cl_2) $+ 0.0202$ (log ppm fed × % Cl_2) $- 0.0917$ (log ppm fed – if female) $+ 0.0003$ (day recovery × % Cl_2)

DSM 037984

STLCOPCB4021808

TABLE XXVI

IBT # 9887
9885

90-Day Subacute Oral Toxicity Study of Aroclor in Beagle Dogs

ppm PCB in Dog Tissues (Wet Weight Basis)

		90 Day Sacrifice						Sacrifice
		30 ppm		100 ppm		300 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1016	Muscle	0.3	0.06	2	0.3	7	1	
	Liver	0.1	0.1	0.4	0.1	1	0.2	
	Fat	8	1	9	3	24	4	

		90 Day Sacrifice						Sacrifice
		10 ppm		100 ppm		300 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1221	Muscle	0.04	0.04	0.2	0.2	0.2	0.2	
	Liver	0.07	0.02	0.2	0.1	0.02	0.2	
	Fat	0.7	0.8	5	3	8	9	

DSW 037985

STLCOPCB4021809

TABLE XXVII

IBT #9887
9885

90-Day Subacute Oral Toxicity Study of Aroclor in Beagle Dogs

ppm PCB in Dog Tissues (Lipid Weight Basis)

		90 Day Sacrifice						Sacrifice
		30 ppm		100 ppm		300 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1016	Muscle	8	1	30	5	69	20	
	Liver	10	6	27	10	90	18	
	Fat	11	2	13	8	36	7	

		90 Day Sacrifice						Sacrifice
		10 ppm		100 ppm		300 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1221	Muscle	0.8	0.8	2	5	27	7	
	Liver	6	2	20	10	1	18	
	Fat	0.9	1	6	6	11	12	

DSM 037986

STLCOPCB4021810

TABLE XXVIII

IBT #9887
9885

90-Day Subacute Oral Toxicity Study of Aroclor in Beagle Dogs

Predicted ppm PCB in Dog Tissues (Lipid Weight Basis)

		90 Day Sacrifice						Sacrifice
		30 ppm		100 ppm		300 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1016	Muscle	8	2	20	5	48	12	
	Liver	13	4	35	9	82	22	
	Fat	8	2	20	5	48	12	

		90 Day Sacrifice						Sacrifice
		10 ppm		100 ppm		300 ppm		Exposure Level
Material	Tissue	♂	♀	♂	♀	♂	♀	Sex
Aroclor 1221	Muscle	1	1	5	5	11	11	
	Liver	5	2	25	10	60	21	
	Fat	1	1	5	5	11	11	

1016 Regression Equation $\log \text{ppm PCB} = -0.2759 + 0.7885(\log \text{ppm fed}) + 0.2382$ (if liver) $- 0.5815$ (if female)

1221 Regression Equation $\log \text{ppm PCB} = -0.7730 + 0.7299(\log \text{ppm fed}) + 0.7178$ (if liver) $- 0.4252$ (if female liver)

DSW 037987

STLCOPCB4021811

TABLE XXIX

IBT #J7300 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			1430	1231																	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	189																					73		
	Eggs	0.7				132						2	0.5	0.4						68	75	36						
Aroclor 1260	Muscle		0.2				11	0.06	0.8															0.04				2
	Liver				6			0.09	0.2																	13		
	Fat	153	15			6295	1159																	10				1010
	Eggs	1		13							1	0.7			13	8												

DSM 037988

STLCOPCB4021812

TABLE XXX

IBT #17300 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		28		1580
	Fat	30	22			2460	1310																	4				9
	Eggs			37		472									33	5	5		383	361	247	368						
Aroclor 1254	Muscle			517	240			2																		61		
	Liver		18				1475	4																7				1529
	Fat			892	199																					78		
	Eggs	10				1410						14	6	5						1324	626	832						
Aroclor 1260	Muscle		40				6080	5	125															13				827
	Liver				86			6	6																	56		
	Fat	183	16			7580	1308																	11				1084
	Eggs	12		146							13	7			135	71												

DSM 037989

STLCOPCB4021813

TABLE XXXI

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 1242	Muscle	20	12	196	119	1899	1152																10	6	92	56	893	541
	Liver	12	7	114	69	1104	669																12	7	104	63	1013	614
	Fat	20	12	196	119	1899	1152																10	6	92	56	893	541
	Eggs	3		40		498					2	0.9	0.6	0.4	25	16	10	7	424	362	308	263						
Aroclor 1254	Muscle	68	22	657	213	6358	2064																26	8	249	81	2409	782
	Liver	34	11	326	106	3163	1027																25	8	243	79	2338	759
	Fat	68	22	657	213	6358	2064																26	8	249	81	2409	782
	Eggs	7		92		1141					6	5	4	3	80	66	54	44	1007	881	775	681						
Aroclor 1260	Muscle	124	29	1196	284	11643	2762																42	10	379	90	3949	938
	Liver	57	24	552	131	5366	1275																42	10	366	87	3557	845
	Fat	124	29	1196	284	11643	2762																42	10	379	90	3949	938
	Eggs	11		140		1728					12	13	14	15	137	135	133	131	1543	1378	1231	1099						

Chicken Regression Equation $\log \text{ppm PCB} = -0.5311 + 0.9858 (\log \text{ppm fed}) + 0.7344 (\text{if female}) + 0.0438 (\%Cl_2) + 0.2908 (\text{if rec. liver}) - 0.0056 (\%Cl_2) \text{ if liver} - 0.0078 (\%Cl_2) \text{ if rec.} - 0.0226 (\%Cl_2) \text{ if female}$

Egg Regression Equation $\log \text{ppm PCB} = -0.7478 - 1.1794 (\text{rec. time}) + 1.0920 (\log \text{ppm fed}) + 0.0300 (\%Cl_2) + 0.5316 (\text{rec. time} \times \log \text{ppm fed}) + 0.0202 (\text{rec. time} \times \%Cl_2) - 0.0096 (\text{rec. time} \times \log \text{ppm fed} \times \%Cl_2)$

Chick Regression Equation – insufficient data

DSM 037990

STLCOPCB4021814

TABLE XXXII

IBT #8746 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												♂♀	♂♀	♂♀
Aroclor 1242	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					

DSM 037991

STLCOPCB4021815

TABLE XXXIII

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		
		2ppm	4ppm	8 ppm	2ppm	4ppm	8 ppm	2 ppm				4 ppm				8 ppm				2ppm	4ppm	8 ppm
		♂♀♀	♂♀♀	♂♀♀	♂♀♀	♂♀♀	♂♀♀													♂♀♀	♂♀♀	♂♀♀
Aroclor 1242	Muscle			84	5	8	13													19		71
	Liver		30		15	19	17														17	
	Fat			73	3	5	9													14		56
	Eggs	14							14		15	22		25			42		28			

DSW 037992

STLCOPCB4021816

TABLE XXXIV

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		
		2ppm	4ppm	8ppm	2ppm	4ppm	8ppm	2ppm				4ppm				8ppm				2ppm	4ppm	8ppm
		♂♀♀	♂♀♀	♂♀♀	♂♀♀	♂♀♀	♂♀♀													♂♀♀	♂♀♀	♂♀♀
Aroclor 1242	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	0.5	8	5	3	1	19	12	7	4			

Chick Regression Equation – log ppm PCB = 0.7436 + 0.4385 (if liver) + 0.3317 (log ppm fed – if muscle)

DSM 037993

STLCOPCB4021817

TABLE XXXV

Bionomics Inc. Acute Toxicity of Aroclor to Bluegill and Channel Catfish

% PCB in Fish Tissues (Wet Weight Basis)

		21 Day Sacrifice							Sacrifice
Material	Species		0.02 ppm	0.04 ppm	0.06 ppm	0.11 ppm	0.18 ppm	0.30 ppm	Exposure Level
Aroclor 1016	Bluegill		0.01	0.005	0.007	0.04	0.04		
	Channel Catfish		0.01	0.01	0.05	0.06	0.1	0.2	

		21 Day Sacrifice							Sacrifice
Material	Species	0.014 ppm	0.023 ppm	0.038 ppm	0.064 ppm	0.108 ppm	0.180 ppm	0.300 ppm	Exposure Level
Aroclor 1242	Bluegill	0.004	0.01	0.03	0.02	0.07			
	Channel Catfish	0.009	0.02	0.03	0.09	0.2	0.3	0.3	

DSM 037994

STLCOPCB4021818

TABLE XXXVI

Bionomics Inc. Acute Toxicity of Aroclor to Bluegill and Channel Catfish

% PCB in Fish Tissues (Lipid Weight Basis)

		21 Day Sacrifice							Sacrifice
Material	Species		0.02ppm	0.04ppm	0.06ppm	0.11ppm	0.18ppm	0.30ppm	Exposure Level
Aroclor 1016	Bluegill		0.8	0.5	0.7	3.8	3.2		
	Channel Catfish		0.8	0.3	3.8	3.3	5.3	7.5	

		21 Day Sacrifice							Sacrifice
Material	Species	0.014ppm	0.023ppm	0.038ppm	0.064ppm	0.108ppm	0.180ppm	0.300ppm	Exposure Level
Aroclor 1242	Bluegill	0.5	0.8	1.6	1.6	5.6			
	Channel Catfish	0.2	0.5	1.0	2.0	4.2	5.6	6.6	

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TABLE XXXVII

Bionomics Inc. Acute Toxicity of Aroclor to Bluegill and Channel Catfish

Predicted % PCB in Fish Tissues (Lipid Weight Basis)

		21 Day Sacrifice							Sacrifice
Material	Species		0.02 ppm	0.04 ppm	0.06 ppm	0.11 ppm	0.18 ppm	0.30 ppm	Exposure Level
Aroclor 1016	Bluegill		0.5	0.9	1.6	3.0	5.6		
	Channel Catfish		0.3	0.6	1.2	2.4	4.6	9.0	

		21 Day Sacrifice							Sacrifice
Material	Species	0.014 ppm	0.023 ppm	0.038 ppm	0.064 ppm	0.108 ppm	0.180 ppm	0.300 ppm	Exposure Level
Aroclor 1242	Bluegill	0.5	0.8	1.3	2.3	4.0			
	Channel Catfish	0.3	0.5	0.9	1.7	3.2	5.8	10.6	

Regression Equation $\log \%\ PCB = 1.6355 + 1.0635\ (\log ppm\ PCB\ in\ H_2O) + 0.1063$ (log ppm PCB in H_2O if channel catfish) + 0.1332 (log ppm PCB in H_2O if 1016)

DSM 037996

STLCOPCB0021820

Figures must be re-numbered

Statistics
Special Study 70-22
Job No. 8908116

ANALYSIS OF BIODEGRADATION DATA

Data collected in the biodegradation test program prior to November 16, 1970 were submitted for statistical analysis. The numbers used in the analysis represented the amount of test compound degraded in a 21 hour period. ~~Six~~ materials representing varying degrees of molecular complexity were tested. The conditions for biodegradation were similar for all, except that smaller quantities of the less degradable materials were used.

Student's t Test was used to check for significant evidence of biodegradation. Control charts were plotted on the amount of the test compound present at the beginning and the end of the 21 hour period.

CONCLUSIONS

1. Significant evidence of biodegradability was found for ~~MIPB, HB-40 and~~ MCS 1043. Significance level used - 95%.
2. MCS 1016 and MCS 1242 showed some evidence of biodegradation, but neither was significant at 95%.
3. Aroclor 1254 showed no evidence of biodegradation.
4. The compounds studied ranked from the most to the least degradable were: ~~MIPB, HB-40~~, MCS 1043, MCS 1242, MCS 1016, Aroclor 1254. (See Table I)
5. In order to detect biodegradability at the 95% level of significance in 20 observations, approximately one-half of the amount of test material added must be degraded. For MCS 1016 this would require about one week; for Aroclor 1242, about 2 days.
6. The day to day variation in amount of test compound found both before and after the 21 hour degradation period is shown in Figures 1 thru 6.

DISCUSSION

This was a preliminary analysis of existing data. This will be followed by a designed experiment to investigate several variations in experimental technique. The designed experiment is intended to lead to experimental conditions which will provide more consistent results.

db

Monsanto Company
Organic Chemicals Division
Applied Sciences Section
St. Louis, Missouri

11/70 - J. D. Hinchen, R. E. Keller

DSW 037997

STLCOPCB4021821

TABLE I

COMPARISON OF TEST MATERIALS

DIFFERENCE BETWEEN FEED SAMPLE + 21 HOUR SAMPLE

Material	Ave. Diff. (mg)	Std. Dev. of Diff.	S/D	Significance
MIPB	8.7	1.95	0.22	***
HB-40	7.0	8.80	1.26	*
MCS 1016	0.11	ca 1.0	10	NS
Aroclor 1242	0.30	1.07	3.56	NS
Aroclor 1254	-0.04	ca 1.0	25.0	NS
MCS 1043	0.55	0.95	1.73	*

* = 95% Significance

*** = 99.9% Significance

NOTE: 10 mg of MIPB and HB-40 were added every 24 hours. For the other compounds, the addition rate was 1 mg every 24 hours.

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FIGURE I

MIPB

O: FEED SAMPLE

X = 21 HR SAMPLE

DELETE

Maj. Compound Found

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

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FIGURE 2
H.B. 40

O = FEED SAMPLE
X = 21 HR SAMPLE

DELETE

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FIGURE 3
MCS 1043

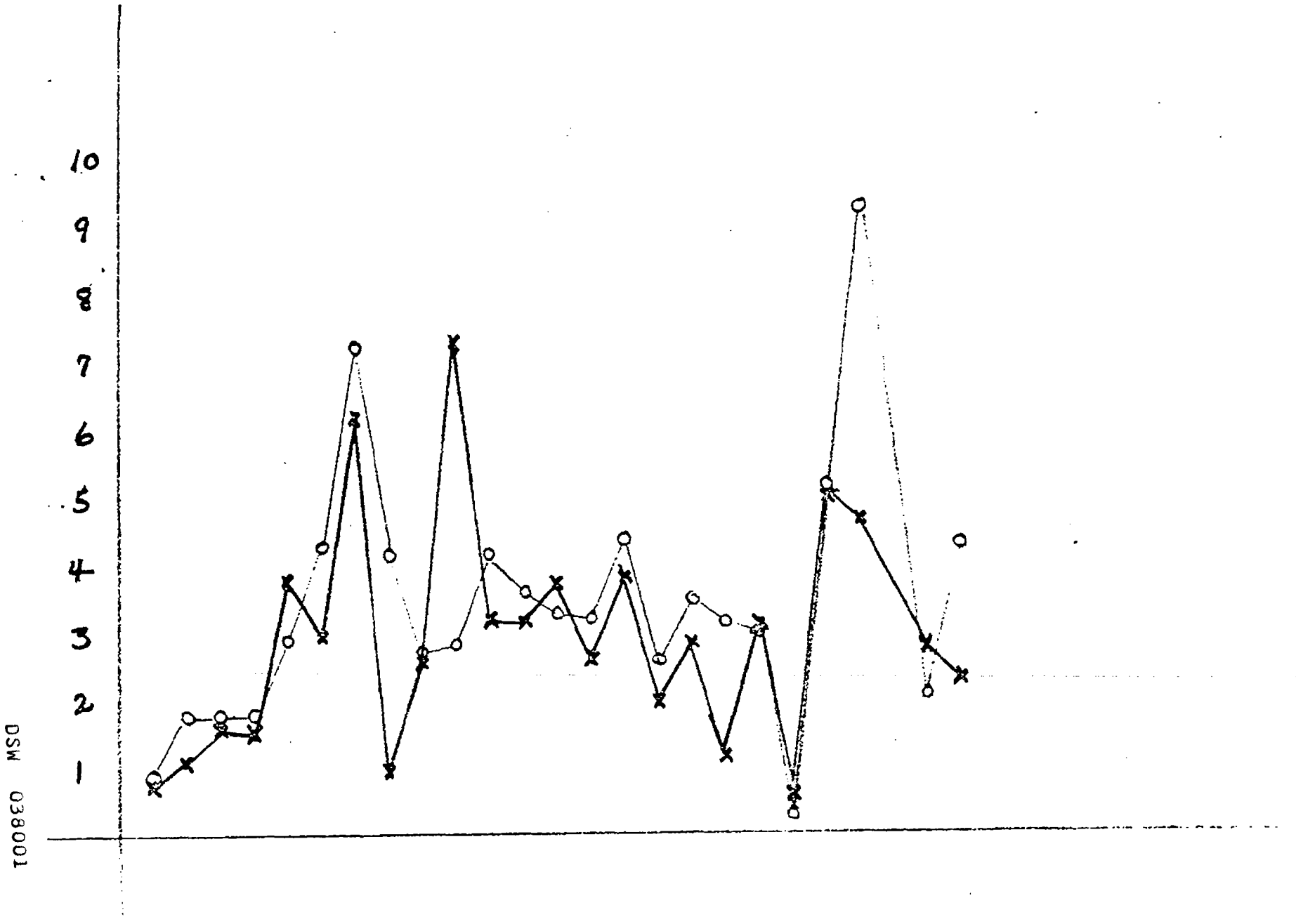

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FIGURE 4
AROCLOR 1242
AMOUNT FOUND

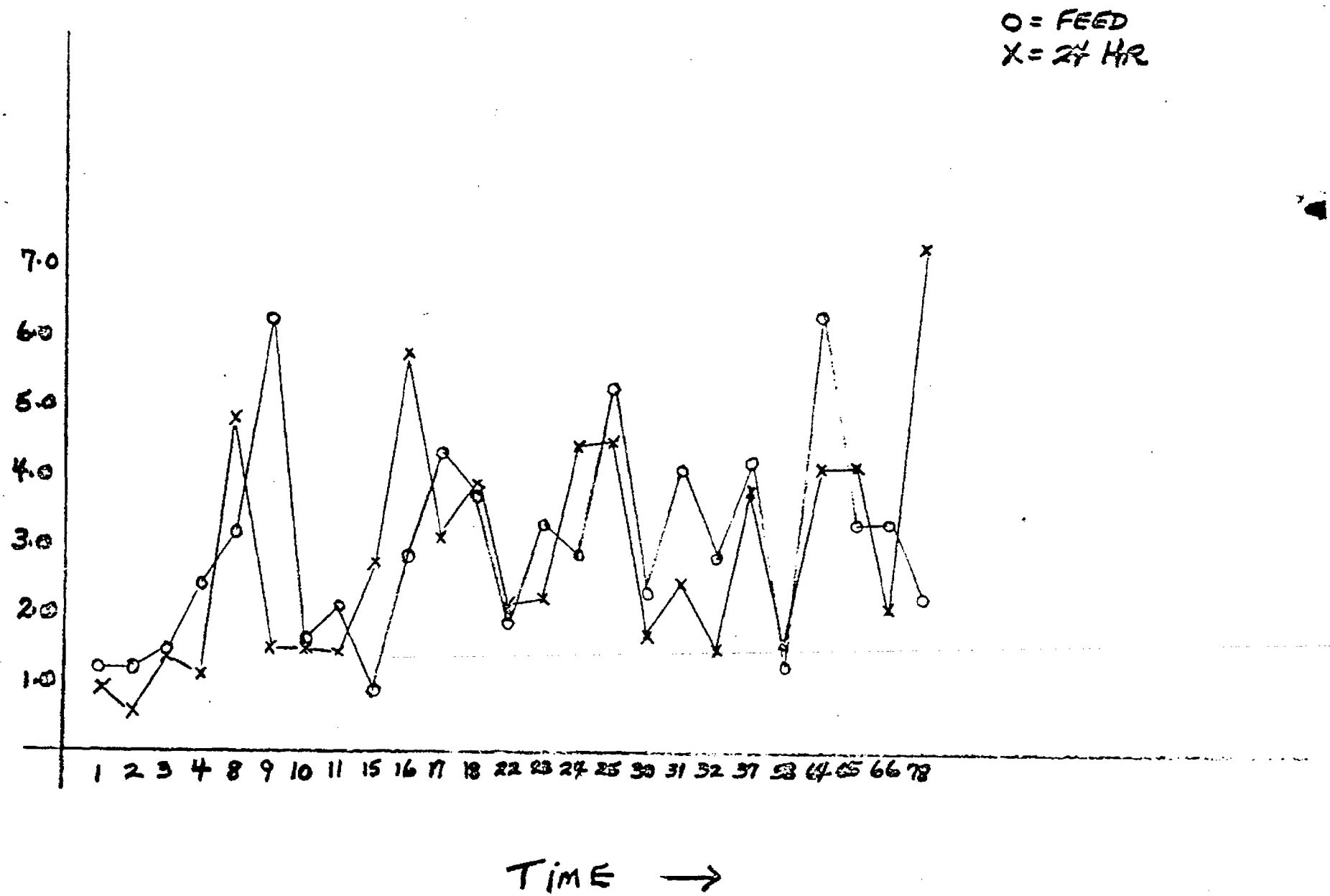

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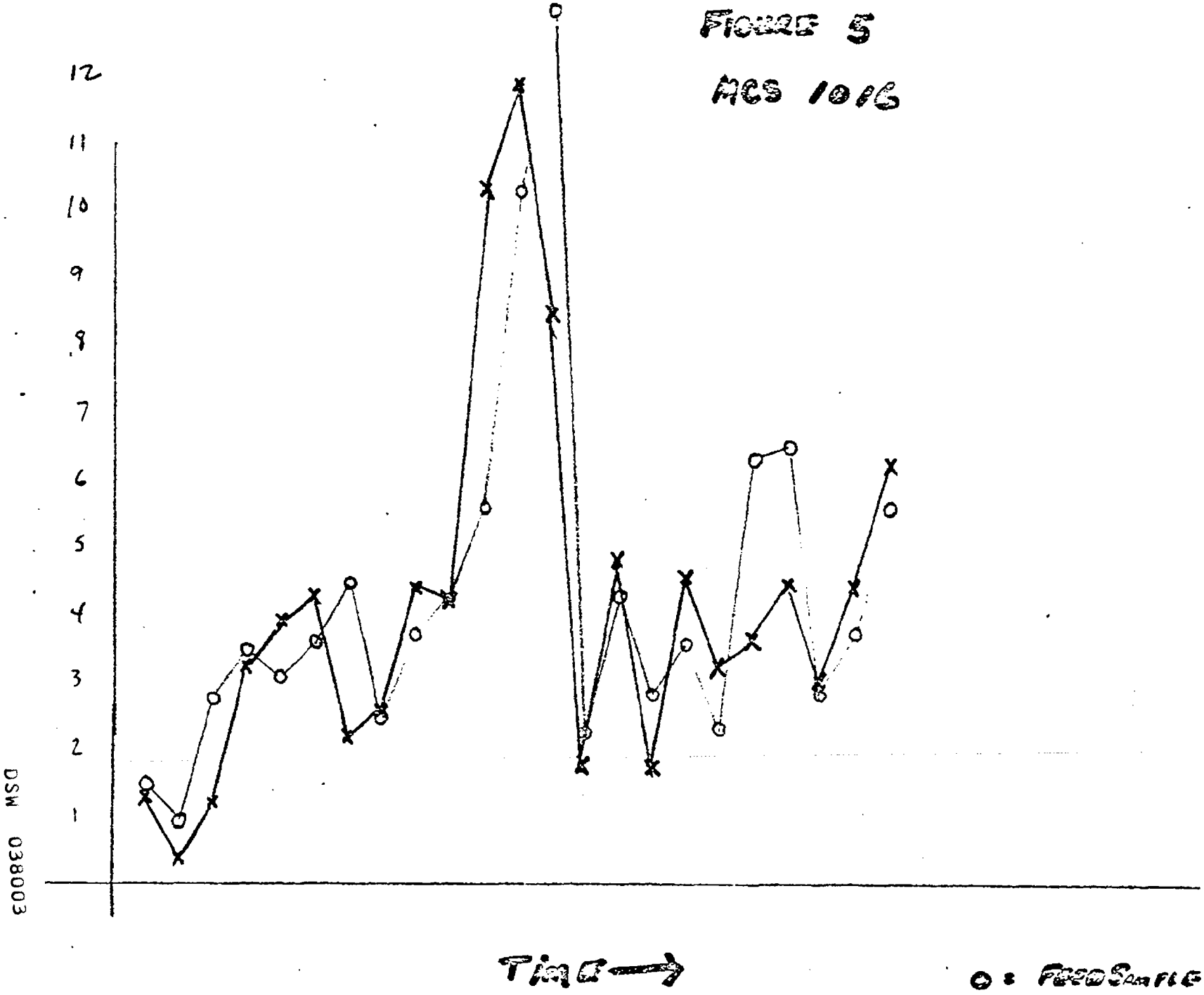
FIGURE 5

ACS 1016



○ = FEED SAMPLE

X = 21 HRS.

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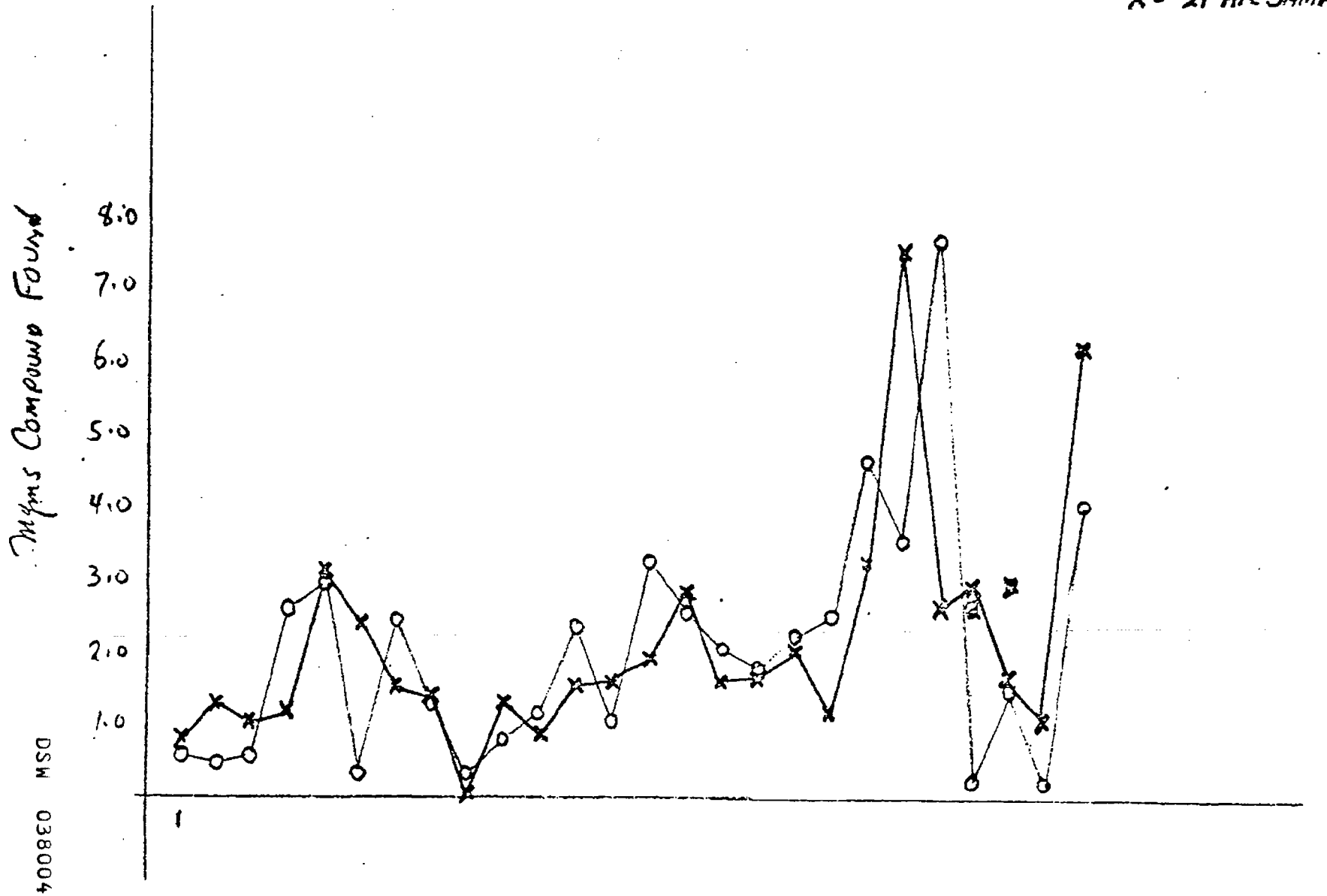
FIGURE 6

AROCLOR 1254

O = FEED SAMPLE

X = 21 HR SAMPLE



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STLCOPCB4021828

Statistics
Special Study 71-2
Job No. 85050-16

STUDY OF OPERATING VARIABLES IN THE ACTIVATED SLUDGE BIODEGRADEABILITY TEST

INTRODUCTION

In order to provide more meaningful information on the biodegradation of Monsanto products, several of the operating variables in the activated sludge test procedure were studied. These variables were time cycle, suspended solids, amount of test material fed, method of feeding, aeration rate, method of sampling and concentration of test material in the solvent. The effects of these variables on the percent disappearance of the test material during the time period involved were sought. Also of interest was the variability of the calculated percent disappearance. Three test materials (biphenyl, 2,4'-polychlorinated biphenyl and 2,2',4,4',6,6'-polychlorinated biphenyl) were used.

CONCLUSIONS

1. Higher percent disappearance was obtained at longer time cycles, lower suspended solids, higher amount of test material fed, surface feeding and sidearm sampling (biphenyl and 2,4'-PCB).

2. On the hexachlorinated material deep injection feeding and low concentration in solvent gave higher results.

3. At the levels studied, aeration rate has no effect.

4. The longer time cycle and lower suspended solids gave more reproducible result.

DATA AND OBSERVATIONS

The experimental design used was a Plackett and Burman screening design which permits study of seven main effects in eight experimental runs. The factors and levels were as follows:

Factor	Low Level	High Level
Time Cycle	24 hrs.	48 hrs.
Suspended Solids	1250 g	2500 g
Amt. of Material Fed	1 mg	5 mg
Method of Feed	Surface	Deep Injection
Aeration Rate	1/2 cu ft/min	1 cu ft/min
Method of Sampling	Sidearm	Surface
Concentration in solvent	1/2 max.	max.

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The experimental design is shown below:

Run No.	Time Cycle	Suspended Solids	Amt. of Material	Method of Feed	Aeration Rate	Method of Sampling	Concentration in Solvent
1	24	1250	1 mg	Surface	1 cu ft/min	Sidearm	1/2 max
2	24	1250	5 mg	Surface	1/2 "	Surface	max
3	24	2500	1 mg	Injection	1 "	Surface	max
4	24	2500	5 mg	Injection	1/2 "	Sidearm	1/2 max
5	48	1250	1 mg	Injection	1/2 "	Sidearm	max
6	48	1250	5 mg	Injection	1 "	Surface	1/2 max
7	48	2500	1 mg	Surface	1/2 "	Surface	1/2 max
8	48	2500	5 mg	Surface	1 "	Sidearm	max

The differences between factor levels were calculated for each test material by subtracting the results of the runs at the low level of each factor from the results at the high level of each factor. The response was % disappearance of the test material during the time period under study. Runs 1 through 4 had four results each and runs 5 through 8 had two results each. The differences given below are based on the average percent disappearance for each run.

Differences Between Factor Levels

	Biphenyl	2,4'-PCB	[1] 2,2',4,4',6,6'-PCB	Total
Time Cycle	5.6	39.5***	3.3	48.4***
Suspended Solids	-11.4**	-34.5***	-2.3	-43.6***
Amt. Material	10.2*	- 2.2	8.1	16.1
Method of Feed	3.6	33.1***	-12.1**	24.6
Aeration Rate	- 3.5	1.2	-1.7	- 4.0
Method of Sampling	- 8.4*	- 3.4	6.1	- 5.7
Conc. in Solvent	- 1.6	- 3.3	-15.6***	-20.5

[1] (One wild value in run 8 was replaced by a more reasonable result.)

For each material an analysis of variance was run on the factors giving the three highest differences as shown above. The asterisks show statistical significance at 90% (one asterisk) and 95% (two asterisks).

Reproducibility of the data, expressed as estimated standard deviation, was calculated for each run as shown below.

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Run No.	Biphenyl	2,4'-PCB	2,2',4,4',6,6'-PCB	Ave.
1	4.0	0.8	17.3	9.8
2	0.3	8.7	9.3	6.1
3	5.3	10.7	23.9	13.3
4	6.7	8.1	16.1	10.3
5	0.0	0.1	0.2	0.1
6	0.0	0.0	6.6	2.2
7	10.6	3.3	6.4	6.8
8	1.2	1.2	11.2	4.5

Ave. Std. Deviation

Factor	Low Level	High Level
Time	9.9	3.4
Suspended Solids	4.3	8.7
Sample Size	7.5	5.8

RECOMMENDATIONS

The following conditions were recommended for adoption in routine degradation testing: 48 hr cycle, low suspended solids, surface feed, 5 mg sample, sidearm sampling and low concentration in solvent.

MONSANTO COMPANY
Organic Chemicals Division
Applied Sciences Section
St. Louis, Mo.

3-71 J. D. Hinchen

STLCOPCB4021831

Analytical Chemistry
Method 71-32
Job No. 1348006, 8581006

DETERMINATION OF BIODEGRADABILITY OF ORGANIC COMPOUNDS BY THE SEMI-CONTINUOUS ACTIVATED SLUDGE (SCAS) METHOD

SCOPE

This method is intended for the determination of the biodegradability of organic compounds or mixtures thereof. It is a revision of Physical Chemistry Method 70-16 which was patterned after the test method for surfactants set forth by the Subcommittee on Biodegradation Test Methods of the Soap and Detergent Association [JAOCS 42, 986 (1965)].

PRINCIPLE

Biodegradability is the susceptibility of a compound to degradation by a mixed bacterial population in the presence of a natural energy source, e.g., waste water. In the semi-continuous activated sludge test, biodegradability is measured using activated sludge from a sewage treatment plant as the source of micro-organisms. A given level of test compound and a synthetic sewage as an energy source are fed on a periodic basis to the activated sludge contained in a specially-designed stirred aeration chamber (Figure 1). Aeration of the mixed liquor (activated sludge and liquor) is carried out for (n-1) hours of a n hour cycle. Cycle times generally employed are 24, 48, or 72 hours. Representative samples of the mixed liquor are taken shortly after feeding and near the end of the aeration period to determine the disappearance rate of the test compound during the cycle. The cycle is repeated for as long as needed to obtain consistent biodegradation rate data. The SCAS test is intended to simulate a secondary sewage treatment facility.

APPARATUS AND REAGENTS

1. Pyrex glass aeration chamber (see Figure 1).
2. Magnetic stirrer (Fisher Catalog No. 14-511-1A)
3. Flow meter suitable for 0.5 cubic foot per hour (CFH) air flow (Brooks-Mite Model 2001-V flow indicator, 0.20-2.0 CFH range).
4. Compressed air - cylinder air or other source of pure air.
5. 25 ml. graduated cylinders for mixed liquor sampling.
6. Gooch crucibles (Fisher Catalog No. 8-195D), fibre-glass filters (Millipore Catalog No. AP 2502200), and 5-ml wide-bore pipettes for suspended solids determination.
7. Hellige A.B.S. Testing Outfit No. 367-DO for LAS determination.
8. pH meter.
9. Nutrient Broth Dehydrated (Difco Laboratories Cat. No. 0003-01).

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10. Dextrose (Difco Laboratories Cat No. 0155-17).
11. Dipotassium hydrogen phosphate (Fisher Catalog No. P-288).
12. Linear alkylbenzene sulfonate reference (e.g. dodecene-1 derived Reference LAS #2).

PROCEDURE

Activated sludge obtained from the Sugar Creek treatment plant of the Metropolitan St. Louis Sewer District is used in this test. The mixed liquor as obtained from the sewage treatment plant is filtered through 20-mesh stainless steel screen to remove any extraneous particulate matter. After adjustment with tap water to a suspended solids content of 2500 mg per liter, 1500 milliliters of the mixed liquor is charged to the aeration chamber shown in Figure 1. The aeration chamber is then connected to the compressed air source and the mixture aerated at a 0.5 cubic foot per hour (SCFH) flow rate. During the aeration, agitation of the mixed liquor using a magnetic stirrer is also provided. The compound to be tested in the form of either an absolute ethanol or aqueous solution and 10 milliliters of a synthetic sewage used as an energy source for the sludge micro-organisms are fed to the chamber at the beginning of each cycle. For materials which have an appreciable disappearance rate, a 24-hour cycle is employed together with a 72-hour cycle on weekends. For the more refractory material, a basic 48-hour cycle is employed. At the end of the aeration period or cycle, the sludge is allowed to settle and one liter of supernatant liquid removed. The unit is re-fed, the mixed liquor volume adjusted to 1500 milliliters with tap water, and the aeration cycle repeated. Samples of mixed liquor (e.g. 20 ml) are taken through the side-arm stopcock with 25 ml graduated cylinders one hour after feeding and at the end of the aeration cycle and analyzed for the compound or compounds of interest. A maintenance schedule for the SCAS units is shown in Figure 2.

The compounds to be tested are generally quite insoluble in water. Except for the very limited amount which may be dissolved in the aqueous phase, the test compound is adsorbed on the activated sludge. In order to disperse the insoluble test compound on feeding, it is fed in the form of an absolute ethanol solution (generally 200 µl of solution). The feed level of test compound is dictated both by the sensitivity of the analytical method available and the disappearance rate of the material. A feed level of one milligram of test compound per cycle has been used for refractory compounds which can be analyzed by electron-capture gas chromatography. For compounds analyzed by UV spectroscopy or flame-ionization gas chromatography, a higher feed level is generally necessary (e.g. 5 to 10 mg).

The pH of the supernatant liquid removed during each cycle is measured as a check on the activity of the activated sludge. If toxic effects of the test compounds on the activated sludge are suspected, 20 mg (5 ml of a 4 mg/ml aqueous solution) of linear alkylbenzene sulfonate (LAS) are fed along with the test compound and synthetic sewage. The LAS content of the supernatant liquid is then determined at the end of the cycle. An LAS disappearance rate of greater than 99 percent should be observed in the absence of any toxic effects.

The suspended solids content of the mixed liquor is maintained between 2000 and 3000 mg per liter by periodic removal (generally once a week) of mixed liquor. The suspended solids content is determined from a 5 milliliter aliquot of the mixed liqour by filtration through a fibre glass filter in a Gooch crucible. The collected solids are then dried in an oven and weighed.

The synthetic sewage stock solution is prepared by dissolving 30 g of dextrose, 20 g of nutrient broth, and 13 g of dipotassium hydrogen phosphate in a liter of water. The solution is heated to just below the boiling point before the final volume adjustment. The solution is then stored in a refrigerator.

MIXED LIQUOR ANALYSES

The method of analyses will vary with the test compound. Electron capture and flame ionization gas chromatography and UV and visible spectroscopy are presently being utilized. In general, water insoluble test compounds have to be extracted from the mixed liquor with a suitable immiscible solvent (e.g. hexane). The solvent extracts are then concentrated before the analysis. The general extraction and concentration procedure employed is described in Analytical Chemistry Method 71-18. The biodegradability of the test compound is expressed in terms of the disappearance rate.

$$\text{Disappearance Rate (\%)} = \frac{\text{Mg Test Cmpd (AF Sample)} - \text{Mg Test Cmpd (BF Sample)} \times 100}{\text{Mg Test Compound (AF Sample)}}$$

where, AF sample is the sample taken directly after feeding and
BF sample at the end of the cycle before the next feeding.

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Monsanto Company
Organic Chemicals Division
Applied Sciences Section
St. Louis, Mo.

8/71 - V. W. Saeger, E. S. Tucker

STLCOPCB4021834

FIGURE I

SCAS AERATION CHAMBER

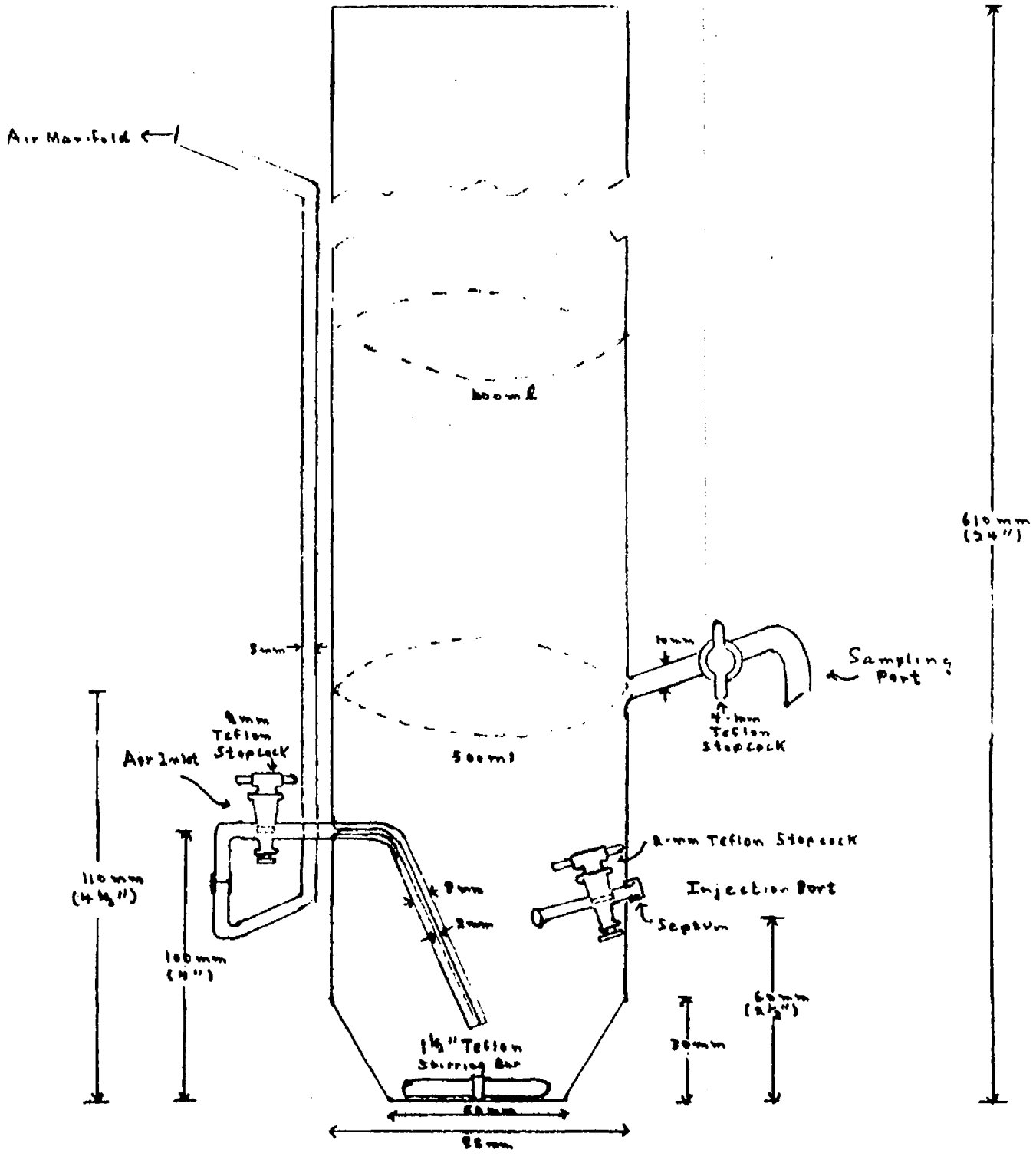

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FIGURE II

MAINTENANCE SCHEDULE FOR SCAS UNITS

8:00 A.M.	- Increase stirring rate to near maximum.
8:10-8:30 A.M.	- Before feeding sampling of specified units; note time of sampling for each unit.
8:30 A.M.	- Close air-inlet stopcocks on units and close master valve to air supply; shut off magnetic stirrers.
9:00 A.M.	- Read sludge volumes and record.
9:00-9:45 A.M.	- Drain units to 500 ml taking sample of supernatant liquid for pH measurement.
9:45 A.M.	- Open master valve to air supply and then open air-inlet stopcocks.
9:45-10:15 A.M.	- Fill units with tap water from hose to slightly below the 1500 ml mark; feed 10 ml of nutrient solution to each unit specified; feed 5 ml of LAS solution to each unit specified; adjust volume to 1500 ml mark with tap water. Turn on stirrers to near maximum rate.
10:15-11:15 A.M.	- Syringe feeding of test materials: note injection time of those units which are to be sampled after feeding.
11:15-12:15 P.M.	- After feeding, sample specified units; one hour after the injection of the test compound.
12:15 P.M.	- Return stirring rate to normal value.

STLCOPCB4021836

GENERAL EXTRACTION PROCEDURE FOR RECOVERY OF ORGANIC TEST MATERIALS IN MIXED LIQUOR SAMPLES FROM SEMI-CONTINUOUS ACTIVATED SLUDGE UNITS

SCOPE

This procedure is intended for the extraction of various organic test materials from mixed liquor samples taken from semi-continuous activated sludge (SCAS) units for the purpose of measuring the rate of biodegradation of those materials.

PRINCIPLE

The method is based on the principle that most organic test materials can be extracted from SCAS mixed liquor samples with an appropriate organic solvent. The concentrated extract can then be measured for the test material by a suitable detection procedure.

REAGENTS AND APPARATUS

1. 25 ml. graduated cylinders
2. 250 ml. separatory funnels
3. 100 mm. 58° filtering funnel - Fisher Cat. No. 10-325E.
4. Kuderna-Danish evaporative concentrator - Ace Glass Cat. No. 6707 - 15 ml. graduated conical centrifuge tubes may be modified for use with this equipment.
5. Anhydrous sodium sulfate - Fisher Cat. No. S-421.
6. Solvent of choice (spectrograde or nanograde depending upon technique used for measurement).
7. Glass wool.
8. Carborundum boiling chips.
9. A steam heated water bath - a 16" diameter battery jar, 10" high, equipped with an overflow valve 1" from the top is filled with water and heated with steam for concentration of low boiling solvents in Kuderna-Danish evaporative concentrators.

STLCOPCB4021837

PROCEDURE

1. Record the volume of the SCAS mixed liquor sample. (A description of the SCAS units and sampling instructions are found in Analytical Chemistry Method No. 71-32.)

2. After agitating, transfer the entire mixed liquor sample from the 25 ml. graduate cylinder to a 250 ml. separatory funnel.

3. Rinse the graduate cylinder several times with a total of 25 ml. of solvent, adding each solvent rinse to the separatory funnel.

4. Stopper the separatory funnel and shake vigorously for at least 1 minute. Allow the layers to separate and transfer the lower aqueous phase to a second separatory funnel.

5. Extract the aqueous layer a second time with a 25 ml. portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous layer to the original separatory funnel.

6. Repeat the extraction with a third 25 ml. portion of solvent. Discard the aqueous layer and combine the extracts.

7. Filter the combined extracts through a 4" funnel plugged with glass wool which is covered with sodium sulfate, washing with sufficient solvent to insure a quantitative transfer. Collect the filtrate in a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the solvent volume to less than the receiver volume by heating the apparatus in a 80-90°C. water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

8. Remove the receiver, dilute to the desired volume (or evaporate with a stream of nitrogen to reduce volume) for the measurement technique to be applied and cap tightly.

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Monsanto Company
Organic Chemicals Division
Applied Sciences Section
St. Louis, Missouri

DSW 038014

6/71 - O. Hicks, E. S. Tucker

STLCOPCB4021838

Analytical Chemistry
Method No. 71-17
Job No. 1348006

DETERMINATION OF AROCLORS AND RELATED PRODUCTS IN MIXED LIQUOR SAMPLES FROM SEMI-CONTINUOUS ACTIVATED SLUDGE UNITS BY ULTRAVIOLET ABSORPTION

SCOPE

This procedure is intended for the extraction and measurement of Aroclor 1242, Aroclor 1142 residue, ~~Arobromchlor 1232~~, MCS 1016, MCS 1043, Aroclor 1221, ~~2,2'-dichlorodiphenylether~~ and ~~trichlorodiphenylether~~ from activated sludge mixed liquor samples. The data are used to determine the rate of biodegradation of the above components in semi-continuous activated sludge (SCAS) units.

PRINCIPLE

The compound of interest is extracted into spectrograde hexane and the level present measured by ultraviolet absorption. The wavelength(s) of the absorption maxima and absorptivities in liters/g. cm. are as follows:

Aroclor 1242	245 nm.	37.0 ± 0.7 l/g cm
Aroclor 1142 Residue	248	40.2 ± 0.6
~~Arobromchlor 1232~~	~~240~~	~~59.8 ± 0.7~~
MCS-1016	245.5	36.1 ± 0.3
MCS-1043	244	48.9 ± 0.5
Aroclor 1221	245	66.4 ± 1.1
~~2,2'-Dichlorodiphenylether~~	~~273.5~~	~~8.50 ± 0.09~~
Trichlorodiphenylether	280 & 227.5	7.47 ± 0.07 & 63.1 ± 0.6

REAGENTS AND APPARATUS

1. Spectrograde hexane - Fisher Cat. No. H-334 or Mallinckrodt Cat. No. 5188.

2. Standard test materials -

Aroclor 1242	- Lot AK-255
Aroclor 1142 Residue	- OR-159809
~~Arobromchlor 1232~~	- OR-16882
MCS-1016	- OR-158591
MCS-1043	- OR-158591
Aroclor 1221	- Lot AK-2
2,2'-Dichlorodiphenylether	- OR-150661
~~Trichlorodiphenylether~~	- OR-174019

3. Undenatured absolute ethyl alcohol.

4. Cary Model 14 Recording Spectrophotometer with matched sets of 1.0, 2.0 and 5.0 cm. quartz cells, or equivalent.

STLCOPCB4021839

PROCEDURE

1. Extract the sample and a blank according to Analytical Chemistry Method No. 71-18 using spectrograde hexane concentrating the extracts to a final volume of 10 ml. as described.

2. Measure the absorption from 400 to 210 nm. of the concentrated blank and sample extracts from step 1 in suitable path length cells using spectrograde hexane as a reference.

3. Record the absorbance of the sample and blank at the maximum wavelength for the component of interest (listed in Principle section).

CALCULATIONS

$$\text{Milligrams of Component in SCAS unit} = \frac{(\text{Sample absorbance} - \text{blank absorbance})(0.01)(10^3)(1500)}{(\text{absorptivity})(\text{cell path, in cm.})(\text{Sample volume, in ml.})}$$

$$\text{where absorptivity} = \frac{\text{absorbance}}{(\text{conc. in g/l})(\text{cell path, in cm.})}$$

Absorptivities for specific components are listed in Principle section.

RECOVERY DATA

Samples of activated sludge were spiked in duplicate at two levels with each component in undenatured absolute alcohol solution and analyzed by the above procedure.

Aroclor 1242	- 83.8 ± 1.3% recovered
Aroclor 1142 Residue	- 75.5 ± 2.4% recovered
~~Arobromchlor 1232~~	~~- 92.4 ± 1.7% recovered~~
MCS-1016	- 91.6 ± 1.2% recovered
MCS-1043	- 94.7 ± 1.0% recovered
Aroclor 1221	- 95.8 ± 1.4% recovered
~~2,2'-Dichlorodiphenylether~~	~~- 87.3 ± 4.2% recovered~~
~~Trichlorodiphenylether~~	~~- 95.1 ± 6.3% recovered~~

The levels selected were on either side of the level found in the SCAS units.

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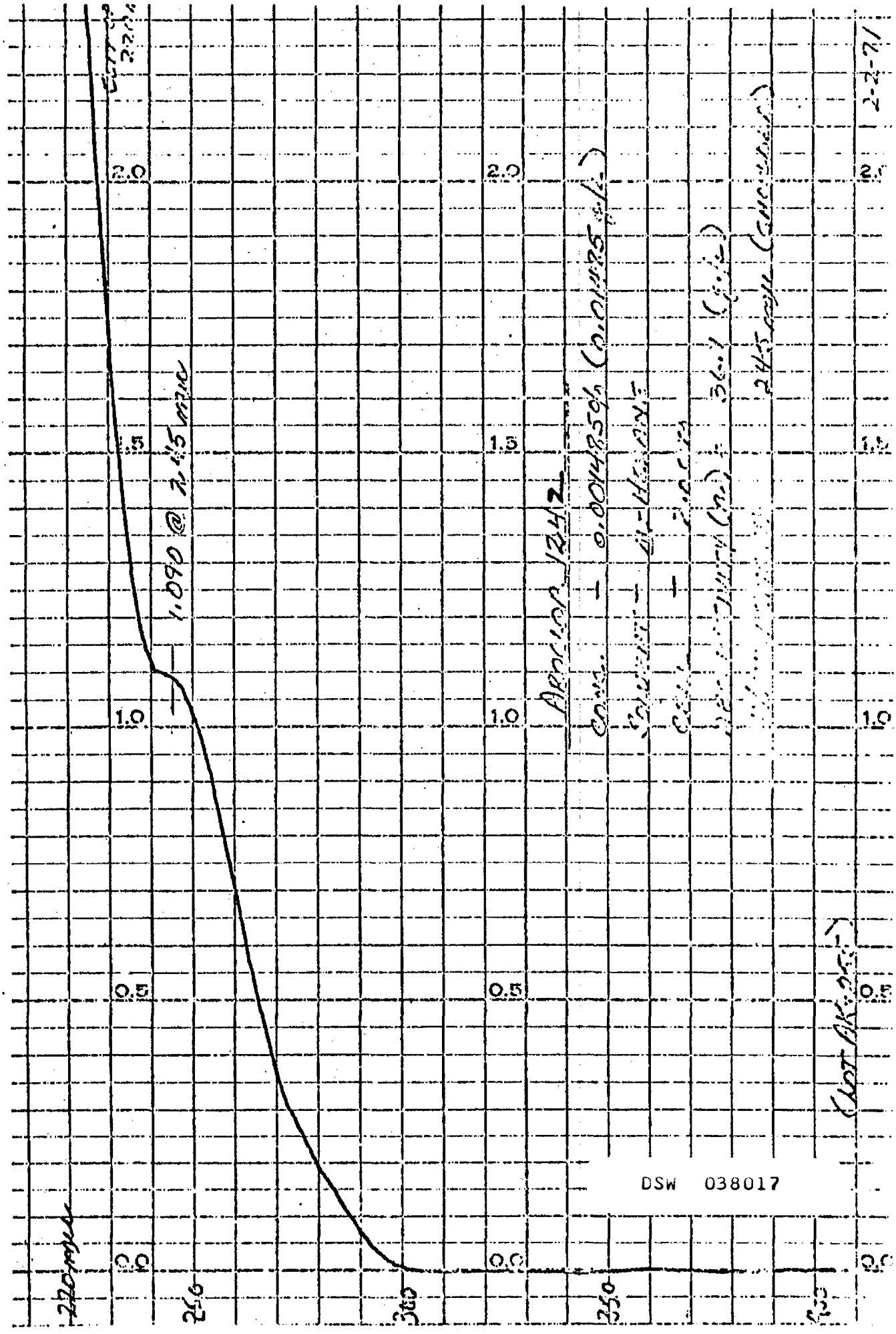
Monsanto Company
Organic Chemicals Division
Applied Sciences Section
St. Louis, Missouri

6/71 - O. Hicks, V. Saeger, E. S. Tucker

DSW 038016

STLCOPCB4021840



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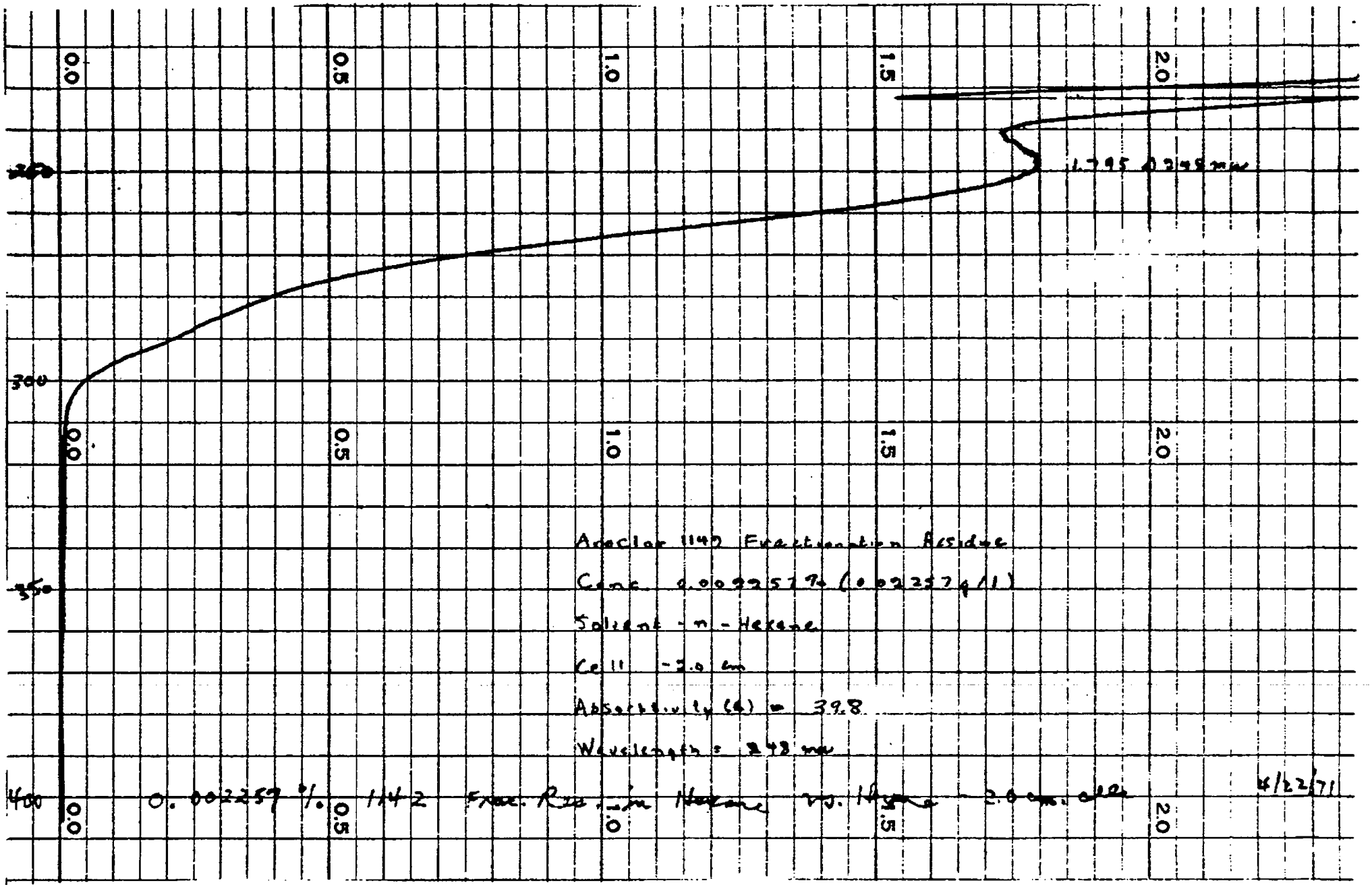

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DSW 038019

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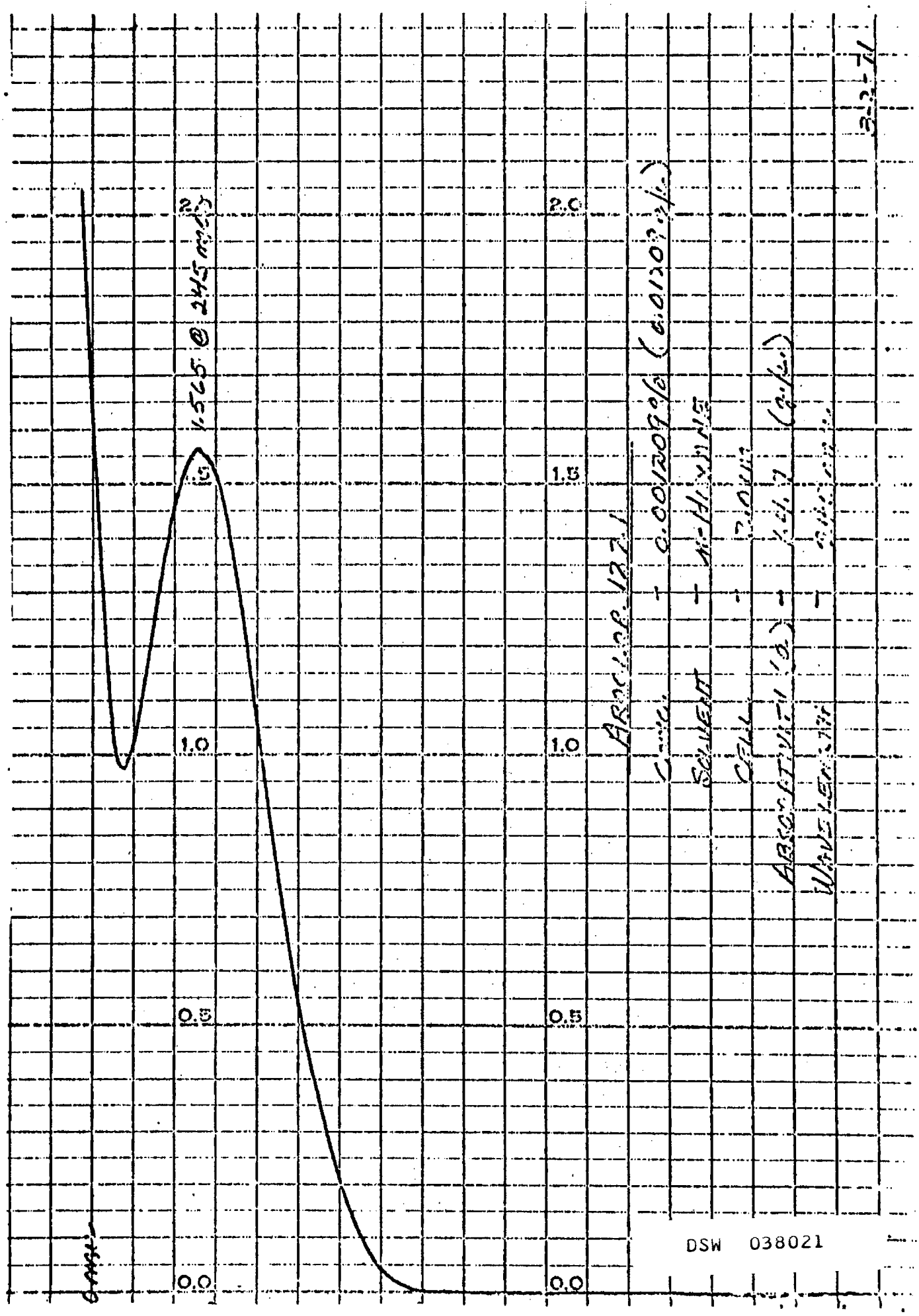

2.0
1.5
1.0
0.5
0.0
1.565 @ 245 mg/l
ABSOLUTE 1221
SOLVENT
CELL
ABSORPTIVITY (a)
WAVELENGTH

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DSW 038022

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DSW 038023

STLCOPCB4021847

DSW 038024

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Analytical Chemistry
Method 71-35

(Consolidated Revision of
Analytical Chemistry
Methods 69-13 and 70-1)

ANALYSIS OF ENVIRONMENTAL MATERIALS FOR POLYCHLORINATED BIPHENYLS

SCOPE

This methodology was developed for the determination of the amount and type of polychlorinated biphenyls (PCB's) in environmental materials. Absolute confirmation of PCB structures is not obtained with this method. Structure proof can be obtained using additional techniques such as mass spectrometry to further identify the GC fractions.

PRINCIPLE

The PCB's present in environmental and laboratory exposed samples are isolated from the bulk of the sample matrix by solvent extraction. Interfering matrix components and endogenous residues are then removed from the sample by a suitable combination of clean up procedures and the amount and type of PCB's present determined by electron capture gas chromatography (EC/GC).

REAGENTS

Hexane	Nanograde, Mallinckrodt Chemical Works, Catalog No. 4159.
N,N-Dimethyl Formamide	Certified, Fisher Scientific Company, Catalog No. D-119. (Saturated in a separatory funnel with hexane.)
Ethyl Ether	Anhydrous, AR grade, Mallinckrodt Chemical Works, Catalog No. 0848.
Acetonitrile	Nanograde, Mallinckrodt Chemical Works, Catalog No. 2442.
Sodium Sulfate	Anhydrous, granular: AR grade, Mallinckrodt Chemical Works, Catalog No. 8042. Heat at 400°C for one hour prior to use.
Alumina Adsorption	(For chromatographic analysis) 80/200 mesh, Fisher Scientific Co., Catalog No. A540. Heat at 400°C for a minimum period of 4 hours and deactivate with 5% (w/w) distilled water. Alumina column preparation: Fill a chromatographic column with hexane up to the point where the reservoir joins the column and push a glass wool plug to the bottom with a glass rod. In a 50 ml beaker measure 35 ml of deactivated alumina (∿30g), and pour this

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REAGENTS (Cont'd)

Alumina Adsorption (Cont'd)	slowly into the column. Tap or vibrate the column to settle the alumina and top the alumina with 2-3 cm of anhydrous sodium sulfate. Wash the column with 50-100 ml of hexane prior to the addition of the sample.
Distilled Water	Extracted with hexane to remove hexane soluble electron capturing impurities.
Sulfuric Acid	Analytical Reagent Grade, SG = 1.84.
Potassium Oxalate	Analytical Reagent Grade.
Potassium Hydroxide	Analytical Reagent Grade.
Ethanol	Formula 2B.
2.5% (w/v) Alcoholic Potassium Hydroxide	Dissolve ∿12.5 grams of AR grade KOH in 500 ml of ethanol.
5% (w/v) Aqueous Potassium Oxalate	Dissolve ∿5 grams of AR grade potassium oxalate in 100 ml of distilled water.
PCB Standards	Production Grade - Aroclor 1221, Aroclor 1242, Aroclor 1248, Aroclor 1254 and Aroclor 1260.

APPARATUS

1. Separatory funnels equipped with ground glass stoppers and Teflon stopcocks: 125, 250, 500, 1000 and 2000 ml capacities.
2. Kunderna-Danish Evaporative Concentrators, 500 ml capacity equipped with 3-ball Snyder columns and graduated 5 ml capacity vials: Ace Glassware Company, Catalog No. 6707.
3. Chromatographic columns, glass, 10" X 20 mm (OD) with a 5" X 50 mm (OD) reservoir at the top, equipped with Teflon stopcocks.
4. Sintered glass filter funnels, 600 ml capacity, 90M.
5. Flat bottomed boiling flasks, 125 ml capacity: Ace Glassware Company, Catalog No. 6896, Code - 04.
6. Liebig Condenser, 200 mm in length: Ace Glassware Company, Catalog No. 5915, Code - 12.
7. Hot plates, Corning PC-100: Fisher Scientific Company.
8. Water bath, Thelco, Precision Scientific, Model No. 84, Fisher Scientific Company.

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APPARATUS (Cont'd)

9. Reciprocating variable speed shaker, Eberbach Corporation, Fisher Scientific Company.

10. 10 µℓ Hamilton Syringes, Catalog No. 701 RN.

11. 32 oz. all glass mortars and pestles.

12. 8 X 12 X 2" (2-1/2 qt.) Pyrex baking dishes.

13. U.S. Standard Sieve, No. 30, Fisher Scientific Company.

14. Polytron Tissue Homogenizer, PT 10-20-3500, Brinkman Instruments Co.

15. Grinding Mills, Catalog No. 8-450, and Grinding Unit, Catalog No. 8-455, Fisher Scientific Company.

16. Usual laboratory glassware.

PROCEDURES

Sampling

It is to be assumed that a rather wide variety of sampling techniques were employed in collecting the samples submitted to us for analysis. In general the procedures used were probably selected for ease of adaptation to the local situation. For this reason, a brief description of the manner in which we treat the samples submitted will usually precede the sample preparation and extraction sections.

Sample Preparation and Extraction

Water

Where possible, the entire water sample, including the container in which it was collected, is extracted with hexane. With larger samples, where this is not physically possible, the containers are simply agitated and a 250 ml portion used for analysis.

Extraction of Water Samples

1. Extraction of water samples - after agitating, transfer the entire aqueous sample or a 250 ml aliquot into a graduated glass cylinder. Record the volume of the sample and quantitatively transfer it to a separatory funnel with distilled water.

2. Rinse the sample container and graduated cylinder with two 50 ml portions of hexane and add each to the separatory funnel.

3. Stopper the separatory funnel and shake vigorously for at least 1 minute. Allow the layers to separate and transfer the lower aqueous phase to a second separatory funnel.

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PROCEDURES (Cont'd)

4. Extract the water sample a second time with a 50 ml portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous layer to the original separatory funnel.

5. Repeat the extraction with a third 50 ml portion of hexane. Discard the aqueous layer and combine the hexane extracts.

6. Filter the combined extracts through a 4" funnel plugged with glass wool which is covered with sodium sulfate. Collect the filtrate in a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

Sediment and Soil

Any excess water is decanted and the entire sediment or soil sample transferred to a glass baking dish to air dry at room temperature. The dried material is transferred from the dish into a mortar and pestle and ground. The ground sediment is sieved, remixed, and a 250g portion taken for analysis.

Extraction of Sediment and Soil Samples

1. Weigh 250g (to the nearest 0.01g) into a 16 oz. narrow neck screw cap (aluminum foil liner) glass bottle.

2. Moisten the soil with water (~10 ml) and add 150 ml of acetonitrile. Cap the bottle tightly, and mechanically shake for a minimum period of 1 hour.

3. Quantitatively transfer the acetonitrile extract into a 600 ml sintered glass filter funnel containing a 1/4" layer of anhydrous sodium sulfate. Collect the filtrate in a 600 ml beaker (vacuum filtration may be necessary).

4. After the acetonitrile has completely drained into the beaker, wash the bottle twice with 50 ml portions of acetonitrile, adding each wash to the funnel after the previous has completely percolated through the sediment.

5. Quantitatively transfer the extract to a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the solvent volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED INTO A HOOD.)

6. Carefully evaporate the acetonitrile extracts to dryness with the aid of a gentle stream of dry nitrogen. Re-dissolve the residue in maximum of 5 ml of nanograde hexane.

PROCEDURES (Cont'd)

Whole Animal, Poultry and Fish and Individual Tissue (Muscle, Liver, Fat, Kidney, Heart, Spleen, Lungs, Fat, Etc.) Samples

All individual and composite tissue samples exceeding 75 grams (25 grams for liver) must initially be homogenized by passing through a meat grinder. The tissue mass is re-ground, with mixing between grindings, until the tissue appears homogeneous. A 50-60 gram portion of the ground tissue (25-30 grams for liver) is transferred to a tared 500 ml Erlenmeyer flask for extraction.

Samples weighing less than 75 grams (25 grams for liver) are cut into small chunks (1-2 cm^3) and the entire sample transferred to a tared 500 ml Erlenmeyer flask for extraction.

Extraction of Tissue Samples

1. Weigh the tared Erlenmeyer flask containing the tissue to be extracted and record sample weight. Add ∿100g of anhydrous sodium sulfate and ∿150 ml of hexane to the flask. Insert the Polytron tissue homogenizer probe and blend, while rotating the flask, until the mixture is well homogenized (∿60-90 seconds).

2. Carefully decant the supernate (hexane extract) through a 4" funnel plugged with glass wool and covered with a layer of anhydrous sodium sulfate, collecting the supernate in a 500 ml Kunderna-Danish evaporative concentrator or a 250 ml volumetric flask.

3. Re-extract the homogenate with a second portion of hexane, and again decant the supernate into the filtering funnel.

4. Wash the Polytron* shaft with several small portions of hexane and transfer the homogenate and washings into the filtering funnel with the aid of several small portions of hexane. Press the homogenate with a spatula to remove as much hexane as possible and complete the filtration by washing the funnel and tissue cake with several small portions of hexane.

5. (a) If the extracts are collected in a volumetric flask, dilute to volume with hexane and mix.

   (b) Transfer a suitable aliquot to a tared 50 ml beaker and evaporate the solvent with the aid of heat and a gentle stream of clean dry nitrogen. Re-weigh the beaker and record the lipid weight.

*The Polytron tissue homogenizer is cleaned between each sample by operating it with the shaft immersed in distilled water followed by nanograde acetone and hexane.

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PROCEDURES (Cont'd)

6. (a) If the extracts are collected in a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place and reduce the solvent volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED INTO A HOOD.)

   (b) After cooling, remove the 5 ml graduated tube, dilute to volume with hexane, cap and mix thoroughly. The entire concentrate or an aliquot is used to determine the lipid weight in manner described above.

Milk

1. Weigh ∿50 grams of whole milk in a 150 ml beaker and quantitatively transfer it to a 500 ml separatory funnel with the aid of several portions of water.

2. Add 5 ml of the 5% aqueous potassium oxalate solution and swirl to mix.

3. Add 100 ml of diethyl ether, shake for several minutes, add 50 ml of hexane and shake for several more minutes.

4. Allow the mixture to stand for 10-15 minutes and discard the lower aqueous layer.

5. Filter the remaining solvent layer through a 4" funnel plugged with glass wool covered with anhydrous sodium sulfate into a tared 400 ml beaker.

6. Evaporate the solvent, allow the beaker to cool, re-weigh, and record the lipid weight.

Sample Clean Up

DMF/Hexane Partition, Chemical Treatment

1. Place an appropriately sized aliquot of the hexane extracts, containing a maximum of 3.5g of fat and not exceeding 25 ml, in a 500 ml separatory funnel. If the aliquot taken is some volume less than 25 ml, add enough additional hexane to yield a final volume of 25 ml.

2. Add 50 ml of DMF (hexane saturated) to the separatory funnel, stopper the funnel, and shake vigorously for at least 1 minute. Allow the layers to separate and transfer the lower DMF layer to a second separatory funnel.

3. Repeat the extraction of the hexane layer with two more 50 ml portions of DMF (hexane saturated) collecting each in turn in the second separatory funnel. Discard the remaining hexane layer.

PROCEDURES (Cont'd)

4. Add 100 ml of distilled water to the combined DMF extracts, swirl to mix, without excessive agitation, allowing the hexane to layer out.

5. Transfer the lower aqueous-DMF layer to another 500 ml separatory funnel.

6. Extract the aqueous DMF sample with a 50 ml portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous-DMF layer to the original separatory funnel.

7. Re-extract the aqueous-DMF with two more 50 ml portions of hexane. Discard the aqueous-DMF layer and combine the hexane extracts.

8. Filter the combined extracts through a 4" funnel plugged with glass wool which is covered with sodium sulfate. Collect the filtrate in a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

Saponification with Alcoholic Potassium Hydroxide and Extraction with Sulfuric Acid

1. Quantitatively transfer the concentrated extracts to a 125 ml extraction flask with the aid of several small portions of solvent.

2. After cooling, remove the 5 ml graduated tube and transfer the concentrate of extracts to a 125 ml extraction flask with the aid of several small portions of solvent.

3. Evaporate the extract just to dryness with a gentle stream of dry filtered air and add 25 ml of 2.5% alcoholic potassium hydroxide.

4. Add a boiling chip, put a water condenser in place, and allow the solution to reflux for 45 minutes.

5. After cooling, transfer the solution to a 250 ml separatory funnel with the aid of 25 ml of distilled water.

6. Rinse the extraction flask with 25 ml of hexane and add it to the separatory funnel.

7. Stopper the separatory funnel and shake vigorously for at least 1 minute. Allow the layers to separate and transfer the lower aqueous phase to a second separatory funnel.

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PROCEDURES (Cont'd)

8. Extract the saponification solution with a second 25 ml portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous alcohol layer to the original separatory funnel.

9. Repeat the extraction with a third 25 ml portion of hexane. Discard the saponification solution and combine the hexane extracts.

10. CAREFULLY add 25 ml of concentrated sulfuric acid, in small portions, to the hexane extracts.

11. Stopper the separatory funnel and shake vigorously for at least one minute. Allow the layers to separate and discard the lower aqueous -acid layer. Repeat this step until the acid layer is colorless.

12. Wash the hexane with 25 ml portion of water. Discard the water wash.

13. Filter the hexane extract through a 4" funnel plugged with glass wool which is covered with a layer of sodium sulfate. Collect the filtrate in a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

Liquid/Solid Chromatographic Clean-Up With Alumina

1. After preparation of the alumina column, described in the "Reagents" section, quantitatively transfer the hexane extracts (5 ml or less) on to the column with the aid of several 5 ml portions of hexane (reducing the solvent level to the adsorbant level after each addition of solvent).

2. Carefully add 125 ml of hexane to the column reservoir and collect the total eluent in a Kunderna-Danish evaporative concentrator.

3. Add a small boiling chip to the Kunderna-Danish evaporative concentrator, put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

Electron Capture Gas Chromatography

Instrument(s): Hewlett-Packard 5750
Research Chromatograph and/or 402 High Efficiency Gas Chromatograph

Detector: High Temperature Ni^{63} Electron Capture Cell

Column: Glass, Spiral and/or "U", 2M x 4mm, 4% XE-60 on 80/100 Mesh Chromosorb W, HP, AW-DMCS

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Column Temperature: 170°C-220°C (specific temperatures employed for each Aroclor are shown on attached chromatograms)

Detector Temperature: 250°-300°C

Injection Port: 220°C

Pulse: 50 µsec

Flow Rates

Carrier: ∿60 ml/min He

Purge: ∿120 ml/min AR/CH_4 (10% AR)

The electron capture gas chromatographic system can be effectively employed to guide the isolation, separation and clean up procedures. Sample extracts should be checked for the presence of PCB's and/or matrix and other chlorinated hydrocarbon interferences by injecting microliter portions of the extracts at various points in the extraction and concentration scheme. In this manner, it can be determined if the sample must be concentrated or diluted and what, if any, confirmatory and/or clean-up procedures are needed. The approaches routinely employed in our laboratory are shown schematically in Figure I.

Absolute confirmation of the presence of PCB's in a sample is obtained by GC/Mass analysis of the residue. Alternate chemical and physical confirmatory techniques not routinely employed in this laboratory are listed in the reference section.

Quantitation

Quantitative determinations employing the electron capture detector are non-stoichiometric measurements made by comparing peak heights or areas for known concentrations with those for unknown compositions. Four variations of the peak height or area quantification procedures have been employed.

Case I EC gas chromatogram of PCB unknown unchanged with respect to standard PCB with no evidence of interferences.

Case II EC gas chromatogram of PCB unknown altered with respect to standard PCB with no evidence of interference.

Case III EC gas chromatogram of PCB unknown unchanged with respect to standard PCB with evidence of interference.

Case IV EC gas chromatogram of PCB unknown altered with respect to standard PCB with evidence of interference.

The PCB level in a Case I sample can be determined by comparison of the height of the major peak in the unknown with a calibration curve prepared by plotting the height of the major peak in the electron capture chromatogram of the corresponding standard vs the number of ng of the standard injected. With a Case II sample because of the alteration, the maximum PCB level is estimated by comparison of the total area of the electron capture envelope of the unknown to a calibration curve prepared by plotting the total area of the electron capture envelope of the most similar standard vs the number of ng of the standard

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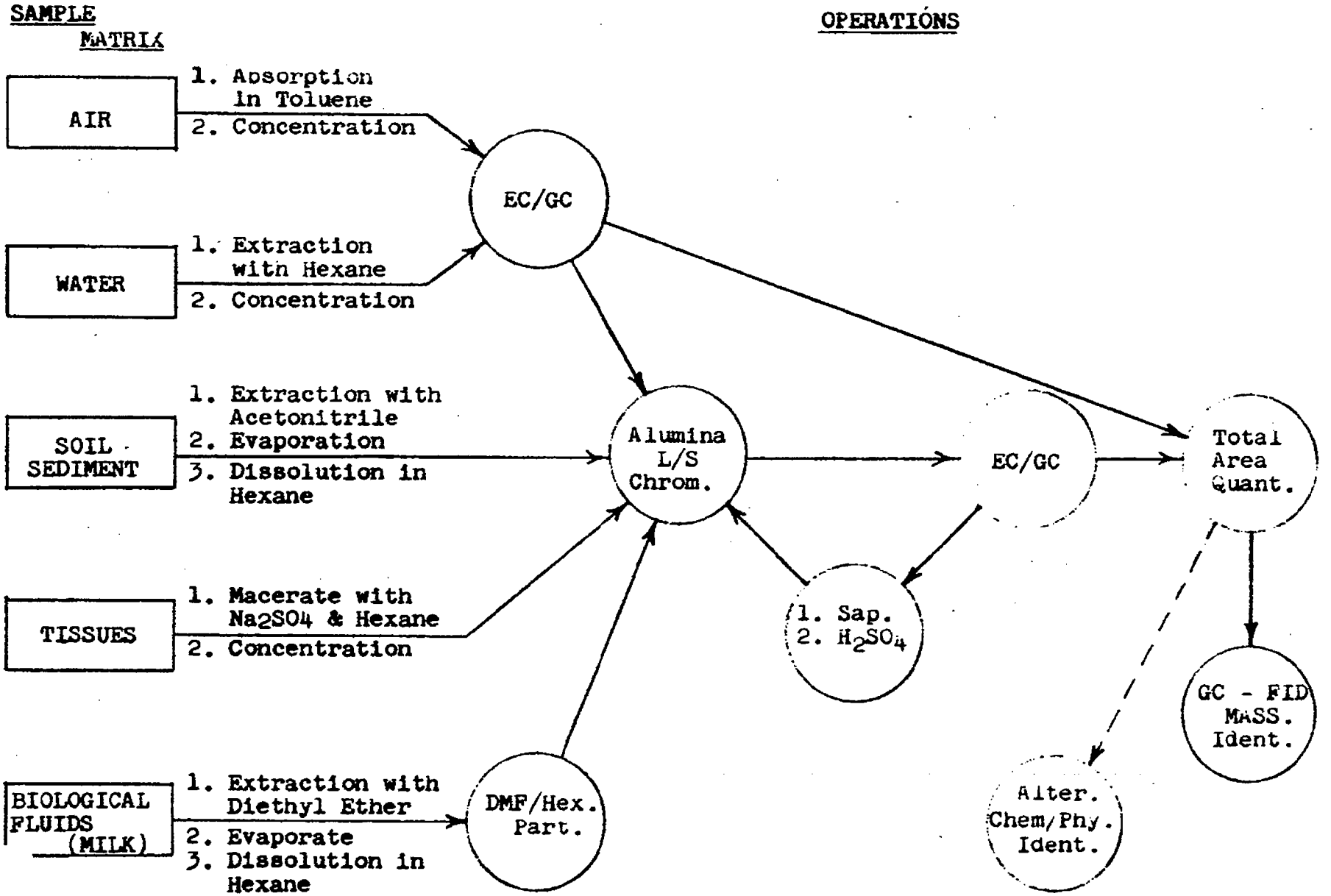

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PROCEDURES (Cont'd)

injected. Case III and IV samples are initially subjected to the chemical clean-up procedure followed by chromatography as outlined on alumina. If the interferences are removed by this treatment, the PCB levels are calculated for Case III and IV in the same manner as Case I and II, respectively. If dominant interference(s) is (are) still present, then the height of a different analytical peak, free from obvious interference, is employed to calculate the PCB level in Case III. With Case IV samples, the maximum PCB level is estimated as in Case II after correcting the total area for that of the interfering peak(s).

In all cases, the response of the electron capture must be linear for quantitative analysis.

Detection Limits

Sample Matrix	Sample Size	Final Extract Volume	Injection Volume	Detection* Lim
Water	250 ml	5 ml	5 µl	2 ppb
Tissues	25-50g	5 ml	5 µl	10-20 ppb
Milk	50g	5 ml	5 µl	20 ppb

*Greater sensitivity can be achieved as needed by increasing the sample size, decreasing the final extract volume, or increasing the injection volume.

DISCUSSION

Extraction

The extraction of PCB's from water employing hexane as the extractant was found to be quantitative and to be sufficiently simple and rapid for use as a routine procedure.

The evaluation of this method was based on spiking water samples with standard acetone solutions of PCB's. The spiking method consisted of adding the PCB's in acetone (25-50 µl) to 500 ml of tap water in a 32 oz. narrow neck screw cap jar. After thoroughly mixing, duplicate 225-250 ml aliquots were taken and subjected to the proposed sample preparation and work up as outlined. The results were quantified by preparing a calibration curve using standard hexane solutions of the PCB's used to spike the water samples. The total area of the electron capture chromatogram was used to construct the calibration plot.

The average recovery and deviation achieved substantiated the applicability of the method for the quantitative recovery and analysis of PCB's from water at the ppb-ppm level.

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DISCUSSION (Cont'd)

No PCB recovery experiments from spiked sediment and soil samples have been performed. Instead, several of the residual solids representative of some of the types of sediment or soil analyzed were re-extracted with hexane/acetone (40/60) in a soxhlet extractor to test for the efficiency of the acetonitrile extraction step. The hexane, after isolation by dilution with distilled water was then carried through the purification steps. Recoveries by soxhlet extraction have indicated that the acetonitrile extraction of PCB's was essentially quantitative in the cases checked.

Based on the assumption that fat or fatty organs generally yield the lowest recoveries a series of experiments were conducted with hexane solutions of chicken fat fortified with known amounts of Aroclor. The spiked fat samples were cleaned up (DMF/Hexane partition and alumina chromatography) and the amount of PCB determined by EC/GC. All recoveries fell in the range of 98-102%; for this reason recovery experiments were not carried out with each individual tissue. While a more realistic evaluation of the overall recoveries might produce recoveries in the range of 70-90%, it was felt that this would be more than sufficient for our purposes, especially in light of the quantitation problems which exist.

Sample Concentration

Concentration of sample extracts is necessary, prior to clean up by chromatographic or chemical means, to reduce sample size and increase sensitivity. The preferred method of concentrating allows minimum loss through volatilization or chemical decomposition and requires a minimum time. The three methods of solvent volume reduction most commonly used are evaporation by exposure to a stream of air, evaporation employing a Kunderna-Danish evaporative concentrator equipped with a Snyder column, and evaporation under reduced pressure. We have used all three techniques and have not encountered any significant losses from volatilization or chemical alternation. However, the Kunderna-Danish evaporative concentrator and the stream of air were employed because of the ease of use.

Column Adsorption Chromatography and Chemical Clean-Up

Silica gel, Florisil and Alumina deactivated with 0, 1.0, 1.5, 2.0 and 5% water were investigated as adsorbants for the elimination of interferences. Alumina (5% water) was found to be effective and more reproducible than either silica gel or Florisil. The activity of alumina varies with age and lot, therefore, 5% water was added to the alumina, after heating for a minimum of 4 hours at 400°C, to insure a reproducible activity.

Saponification and subsequent extraction of the sample with sulfuric acid is an effective way to remove a number of chlorinated hydrocarbon interferences as well as other matrix interferences. Nevertheless, several pesticides survive the chemical clean-up procedures (e.g. strobane, toxaphene, DDE, chlordane, etc.) demanding careful interpretation of the EC chromatograms or alternate chemical and separation procedures referenced in the appendix. PCB's are not affected.

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DISCUSSION (Cont'd)

Electron Capture Gas Chromatography

Columns

Column performance is the key to effective gas chromatographic analysis and as such the choice of column materials is particularly important. Ideally, the support employed should be inert, mechanically strong, and of high surface area. For these reasons, Chromosorb W, HP, AW-DMCS was used in all of our work.

A variety of polar and non-polar liquid phases were investigated. The following columns were found to provide adequate separation, etc., for use in PCB analysis by electron capture: 4% (w/w) DC-200, SF-96, OV-17, SE-30, SE-54, XE-60, Apiezon L, and 6% QF-1. DC-200 and XE-60 or QF-1 have been found to be the most suitable of these liquid phases.

Another important consideration when working with an extremely sensitive detector and consequently low levels of materials is column conditioning. With polar phases such as XE-60 and QF-1, we have found that operating a new column overnight at a temperature 25-50°C higher than to be used during analysis results in a more stable column. A no-flow conditioning technique is employed to condition non-polar columns. The column is purged with carrier gas, heated for 30 minutes at an elevated temperature without carrier flow and then cooled to room temperature. At the end of this cycle, the carrier flow is resumed and the conditioning is completed as in the case of the polar liquid phase. Two precautions: During conditioning, the column should not be connected to the detector and one should not exceed the maximum safe temperature of the liquid phase.

Since all liquid substrates bleed to one degree or another and columns eventually degrade, we characterize all new columns with two column performance indicators - the number of theoretical plates (N) and a tailing factor (T). p,p'-DDT is employed to check these parameters because it is known to degrade on "poor" columns. In this manner, we can determine if the performance of a new column is satisfactory and when the column performance begins to fall off. We consider a column good if the number of theoretical plates per foot is on the order of 400-500 with tailing factors of 1.0-1.3. Calculation of these parameters is shown in the appendix. Additionally, there should be no significant extraneous peaks upon injection of a pure p,p-DDT standard.

Other chromatographic conditions that can be adjusted are column temperature and flow rates. Although resolution of a mixture increases with decreasing temperature, a temperature should be chosen that allows the elution of all components within a convenient time period. The flow rates are optimum for our instrument, column and detector system and, of course, should be adjusted if better results can be achieved.

Two gas chromatographic systems have been used for PCB analysis - HP Model 402 and 5750. We find that any system of instrument and column suitable for chlorinated pesticides is satisfactory for PCB analysis.

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DISCUSSION (Cont'd)

The bulk of analyses in our laboratories was carried out using the systems outlined. The use of the high temperature Ni^{63} electron capture cell is highly recommended. The ability to operate at higher temperatures prevents maintenance problems due to contamination from high boiling components.

CONTAMINATION

In determining PCB's by electron capture gas chromatography, laboratory sources of contamination can be a major problem. The samples and extracts should never be allowed to come in contact with materials other than glass, Teflon or metal. Laboratory glassware should be thoroughly washed with hot, soapy water, rinsed with distilled water, acetone and then hexane. All equipment should also be rinsed again with hexane just prior to use and blanks should be frequently carried through all steps of the procedures to insure against the possibility of contamination.

db

Monsanto Company
Organic Chemicals Division
Applied Sciences Section
St. Louis, Missouri

10/71 - W. J. Litschgi, W. M. Mees, E. S. Tucker

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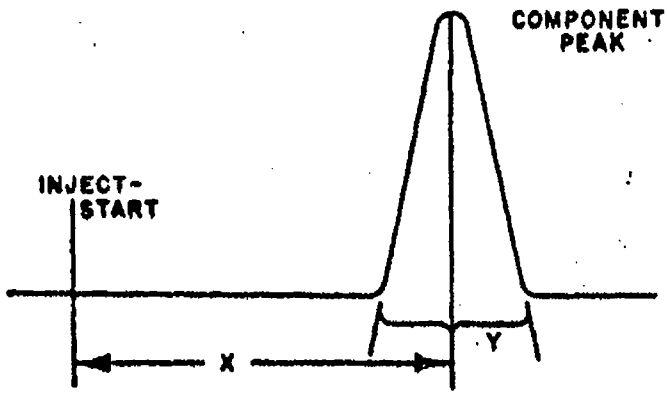
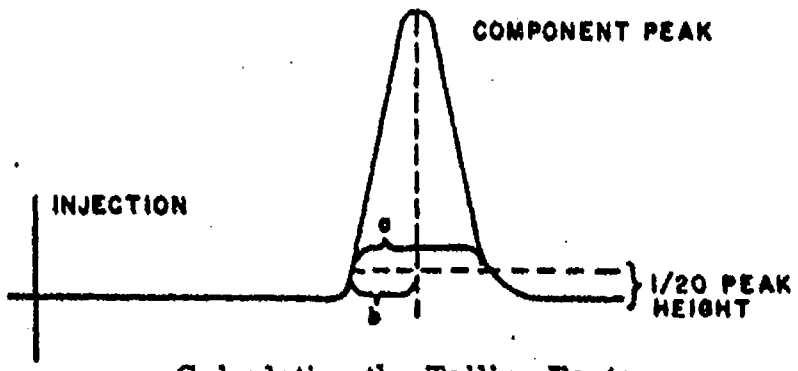
COLUMN PERFORMANCE INDICATORS



Calculating Column Efficiency

1. Theoretical Plates, N

$$N = 16(x/y)^2$$



Calculating the Tailing Factor

2. Tailing, T

$$T = a/2b$$

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FIGURES

Figure I	Composite electron capture chromatograms - demonstrating overlap regions for technical Aroclor products.
Figure II	Aroclor 1221 electron capture reference chromatogram at analysis conditions.
Figure III	Aroclor 1242 electron capture reference chromatogram at analysis conditions.
Figure IV	Aroclor 1248 electron capture reference chromatogram at analysis conditions.
Figure V	Aroclor 1254 electron capture reference chromatogram at analysis conditions.
Figure VI	Aroclor 1260 electron capture reference chromatogram at analysis conditions.
Figure VII	Support Coated Open Tubular flame ionization chromatogram - demonstrating the complexity of the isomer distribution of Aroclor 1242.
Figure VIII	Typical alteration of Aroclor 1242 observed after metabolism and/or excretion by Albino rats.
Figure IX	Typical alteration of Aroclor 1254 observed after metabolism and/or excretion by Albino rats.
Figure X	Typical alteration of Aroclor 1260 observed after metabolism and/or excretion by Albino rats.
Figure XI	Typical alteration of Aroclor 1242 after metabolism and/or excretion by White Leghorn chickens.
Figures XII - XIX	Electron capture chromatograms of some commercial pesticide mixtures before and after treatment on XE-60 and DC-200.

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ALTERNATE CHEMICAL/PHYSICAL SEPARATION AND IDENTIFICATION PROCEDURES

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FIGURE I

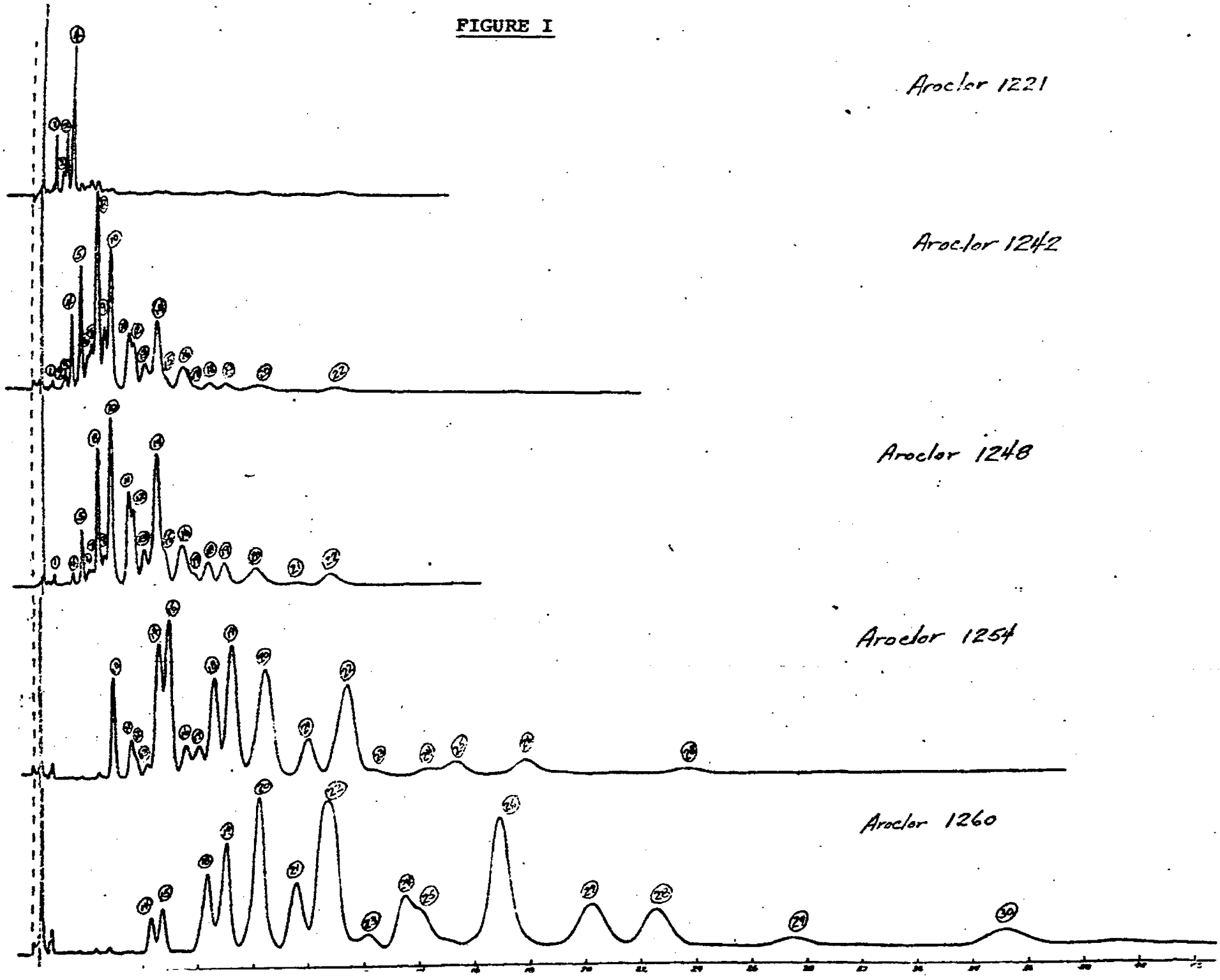

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FIGURE II

AROCLOR 1221

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 170°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min.
Purge Gas - 10% CH4/Argon 120 ml/min.
Pulse Interval - 50 µsec
Amt. Injected - 5 µl
Std. Conc. - 1.48 X 10^{-6} g/ml
Range - 10
Attn. - 4

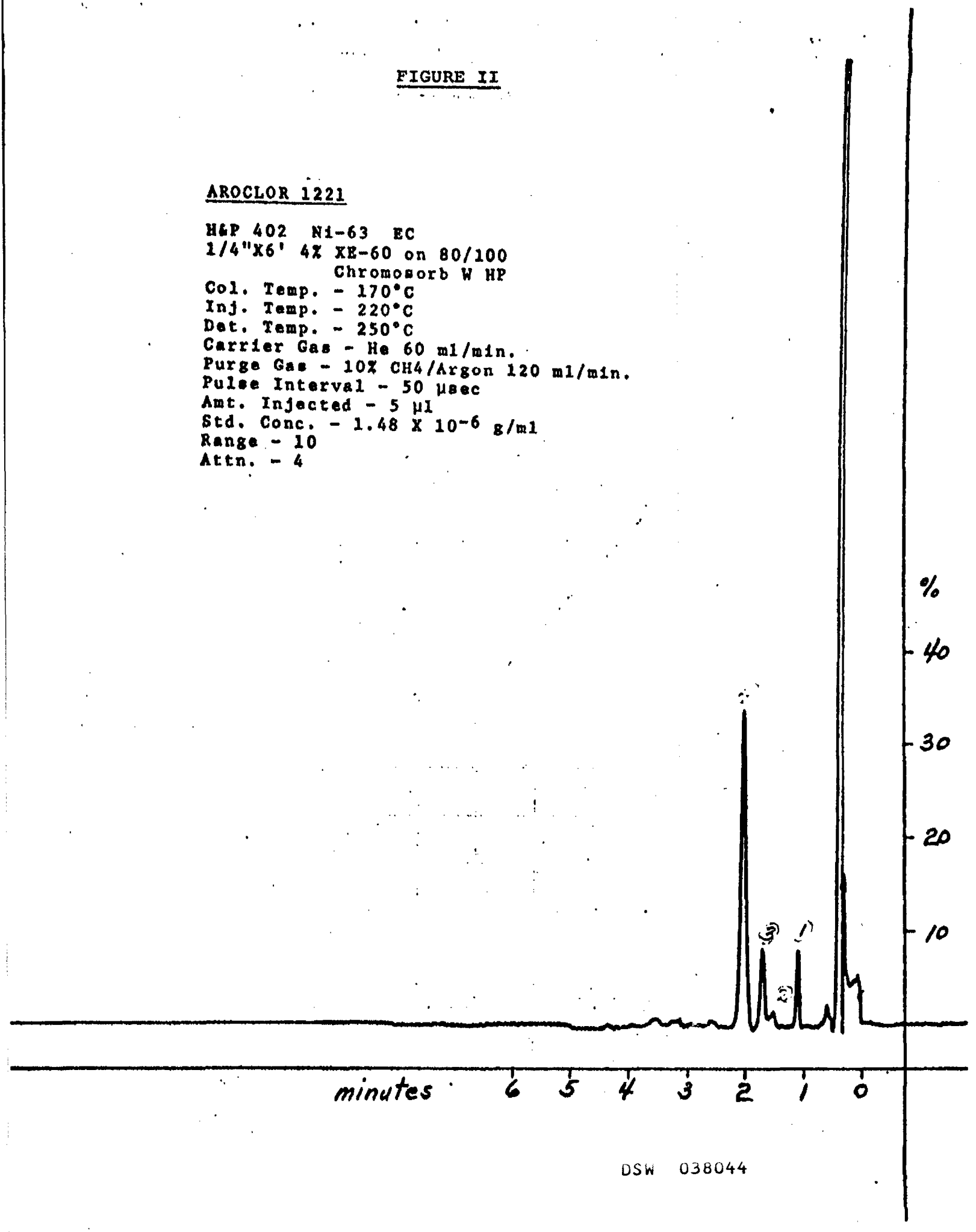

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FIGURE III

AROCLOR 1242

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 190°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min.
Purge Gas - 10% CH4/Argon 120 ml/min.
Pulse Interval - 50 µsec
Amt. Injected - 5 µl
Std. Conc. - 1.03×10^{-6} g/ml
Range - 10
Attn. - 8

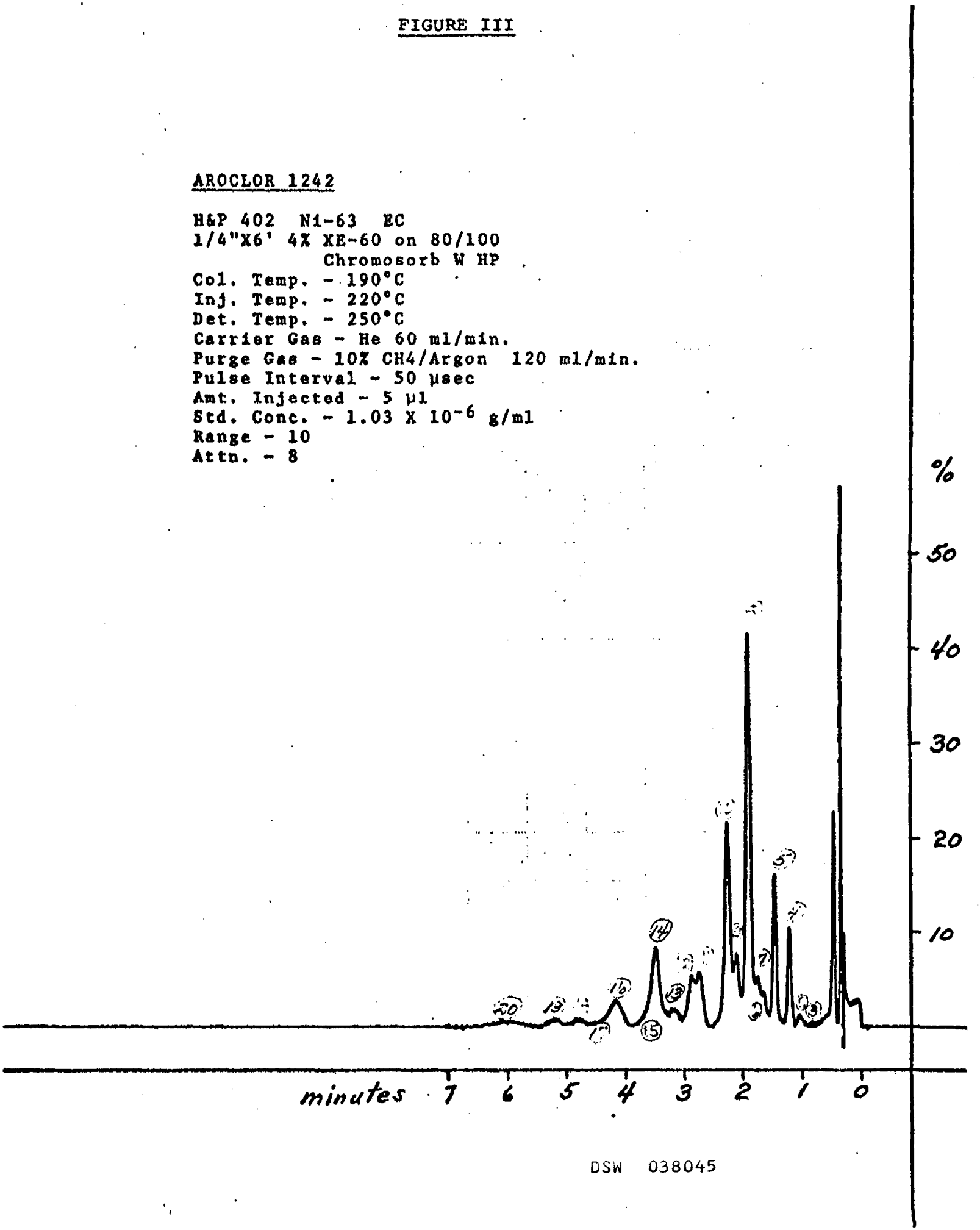

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FIGURE IV

AROCLOR 1248

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 190°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min.
Purge Gas - 10% CH4/Argon 120 ml/min.
Pulse Interval - 50 µsec
Amt. Injected - 5 µl
Std. Conc. - 1.31 X 10^{-6} g/ml
Range - 10
Attn. - 4

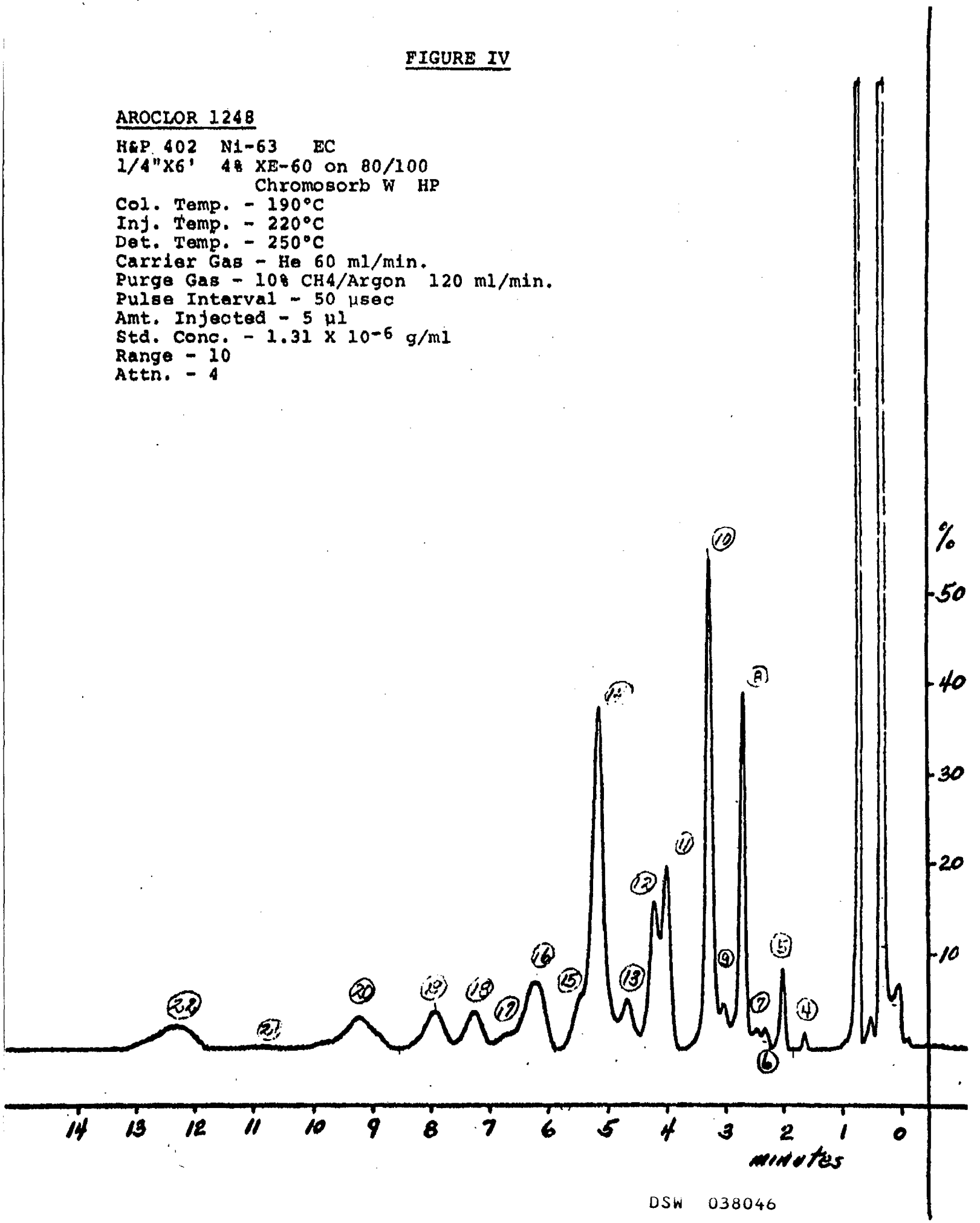

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FIGURE V

AROCLOR 1254

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 205°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min
Purge Gas - 10% CH_4/Argon 120 ml/min.
Pulse Interval - 50 µsec
Amt. Injected - 5 µl
Std. Conc. - 1.02 X 10^{-6} g/ml
Range - 10
Attn. - 8

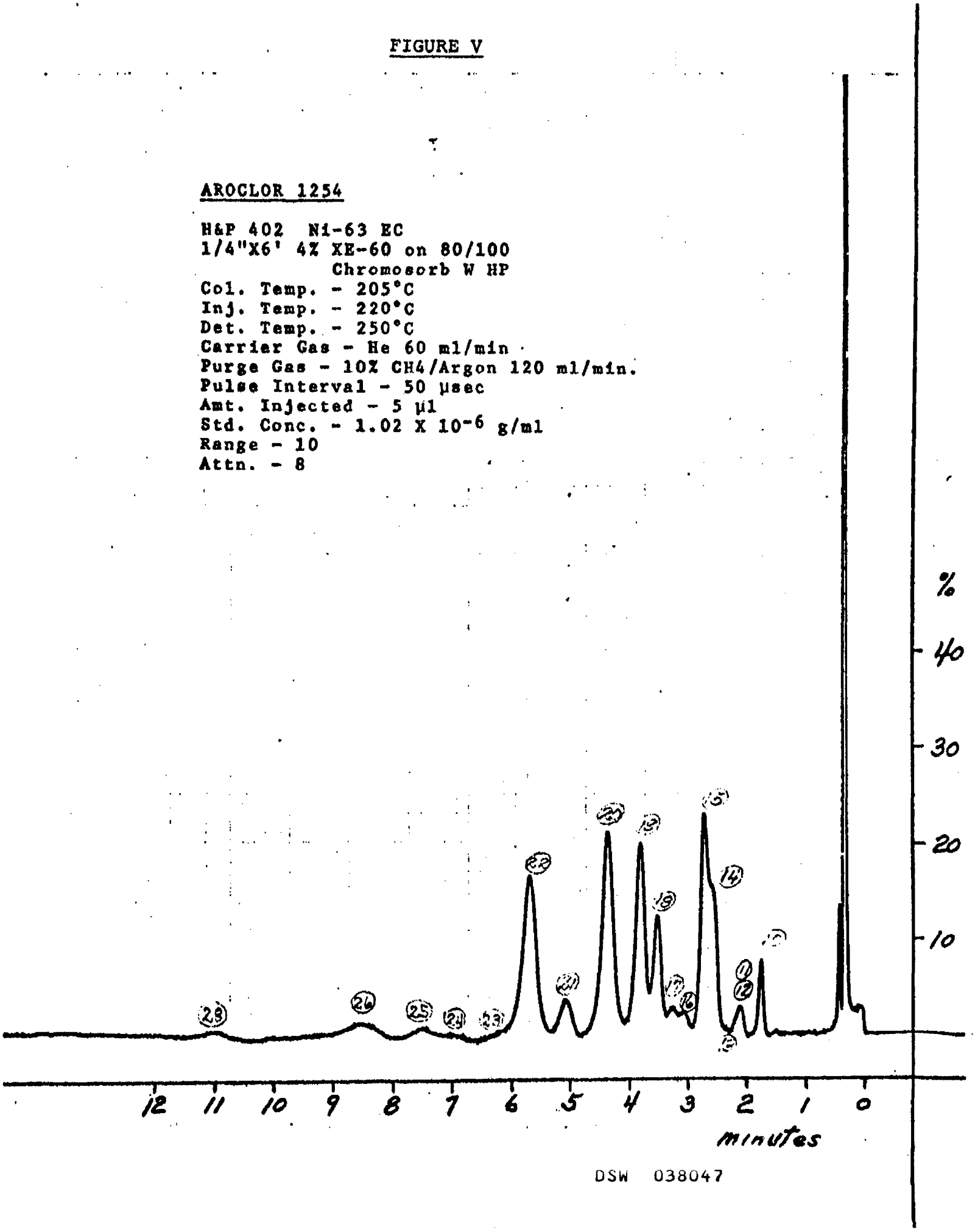

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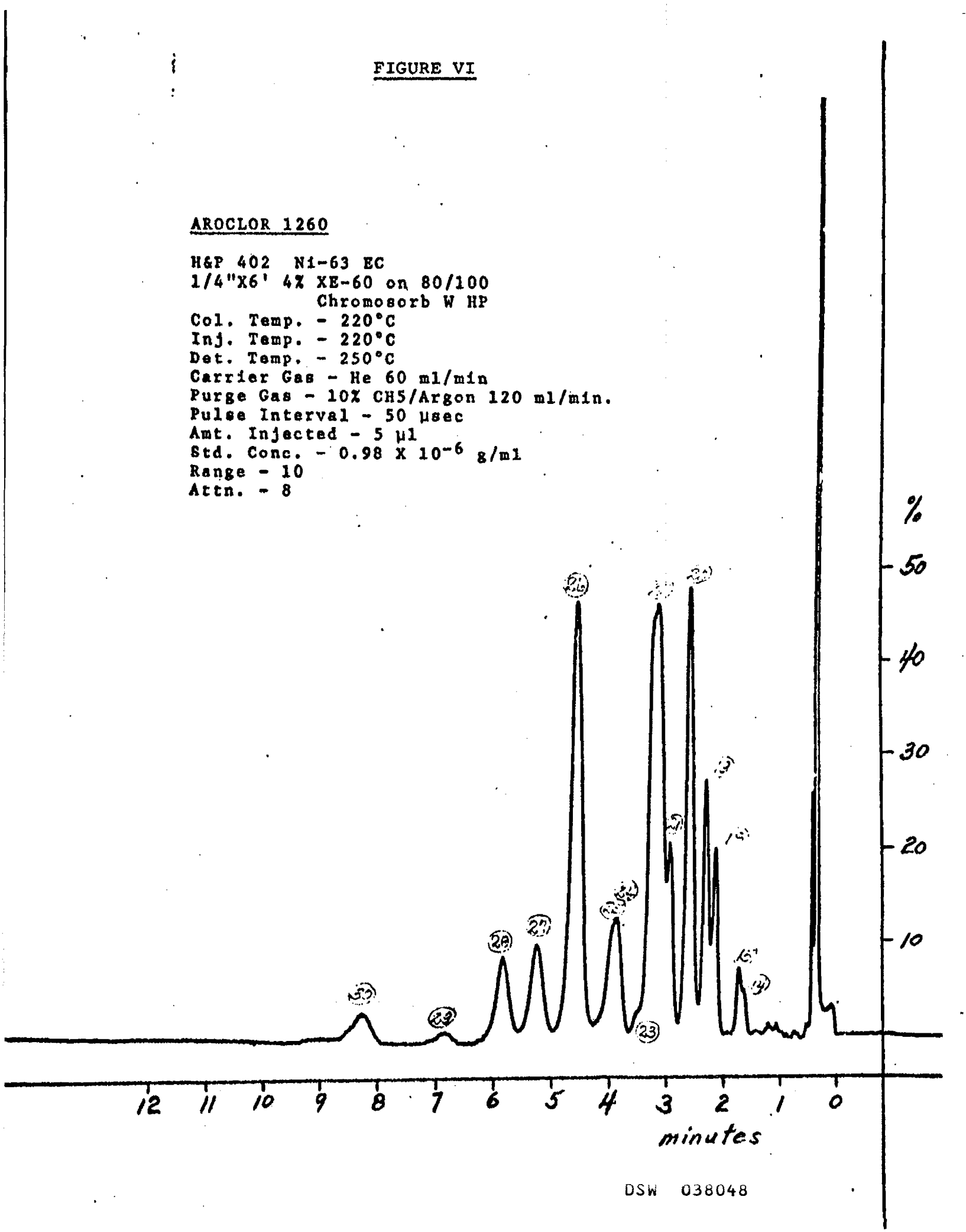
FIGURE VI

AROCLOR 1260

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 220°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min
Purge Gas - 10% CH5/Argon 120 ml/min.
Pulse Interval - 50 µsec
Amt. Injected - 5 µl
Std. Conc. - 0.98 X 10^{-6} g/ml
Range - 10
Attn. - 8



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CHROMATOGRAPHIC CONDITIONS

FIGURE VII

Instrument: Perkin-Elmer Model 800

Type Detector: Flame Ionization

Column: 100' X 0.02" I.D. FFAP S.C.O.T. (Support Coated Open Tubular) Capillary (Perkin-Elmer Part No. 008-0804 for each 50' length)

Column Temperature: 230°C

Detector Block Temperature: 285°C

Injection Port Temperature: 330°C

Sample Volume Injected: 0.002 ml
Split 30 to 1

Flow Rates

Column Helium: 5 ml/min
(7 psig)

Auxiliary Helium: 50 ml/min

Hydrogen: 50 ml/min

Air: 400 ml/min

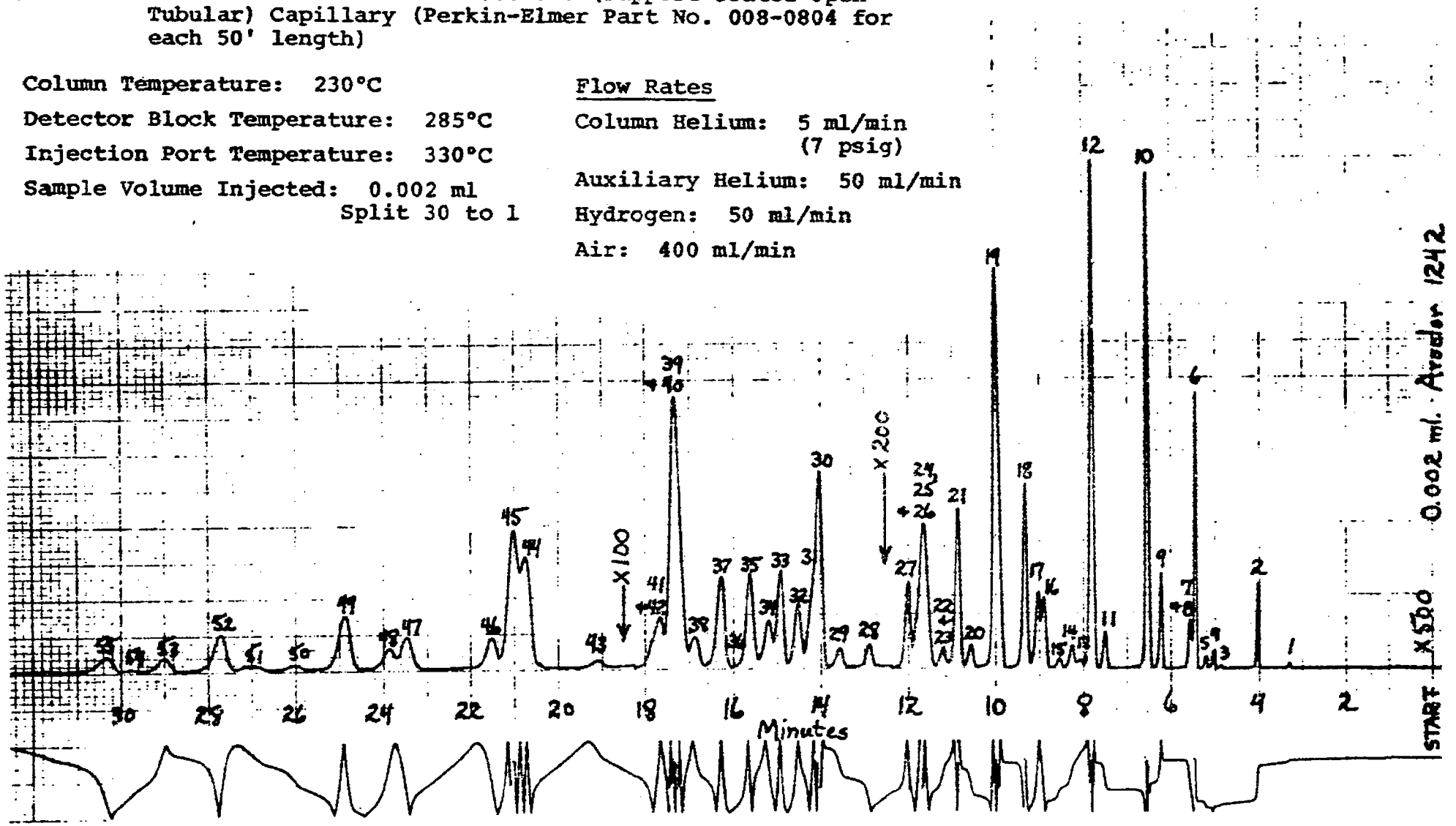

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FIGURE VIII

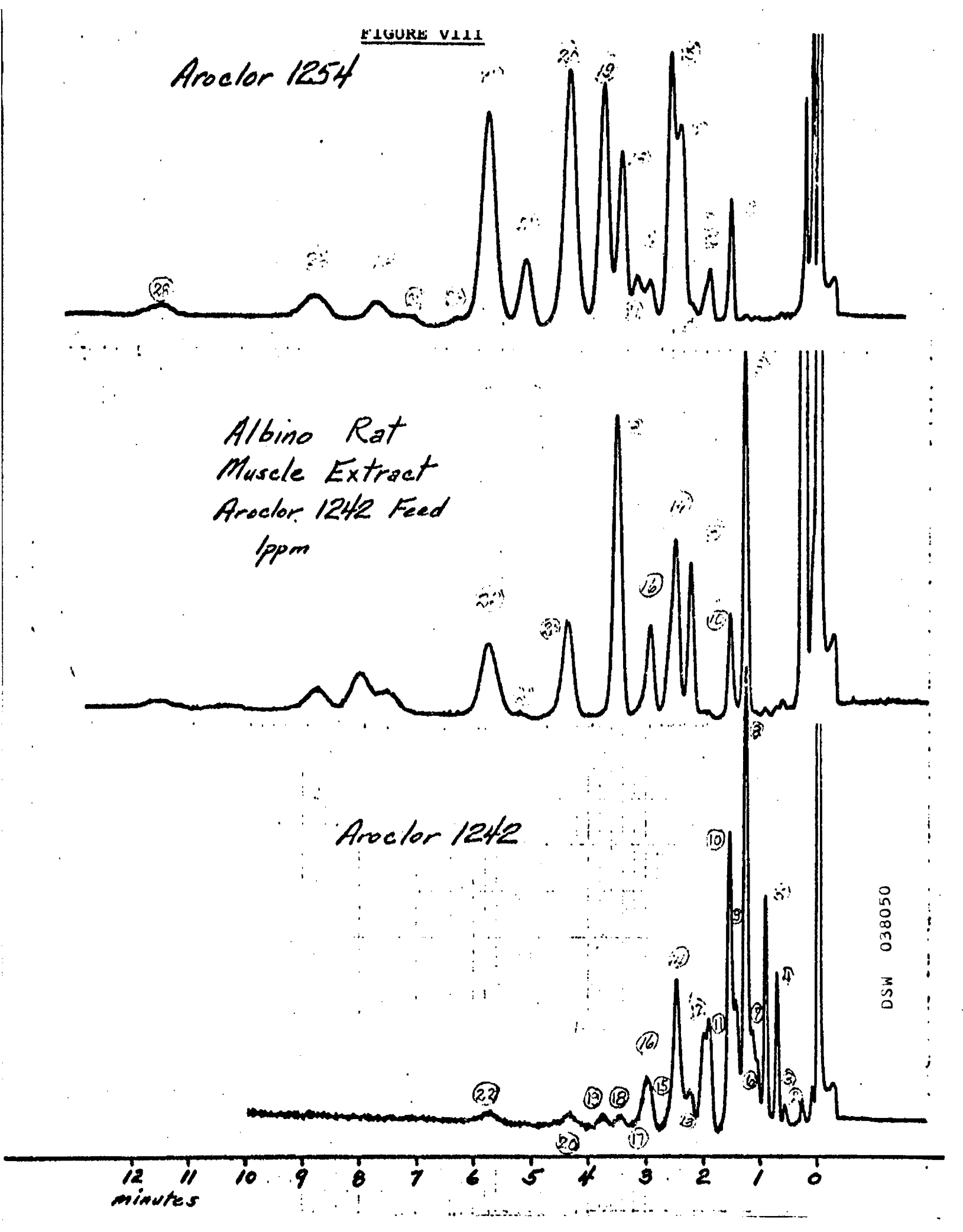

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FIGURE IX

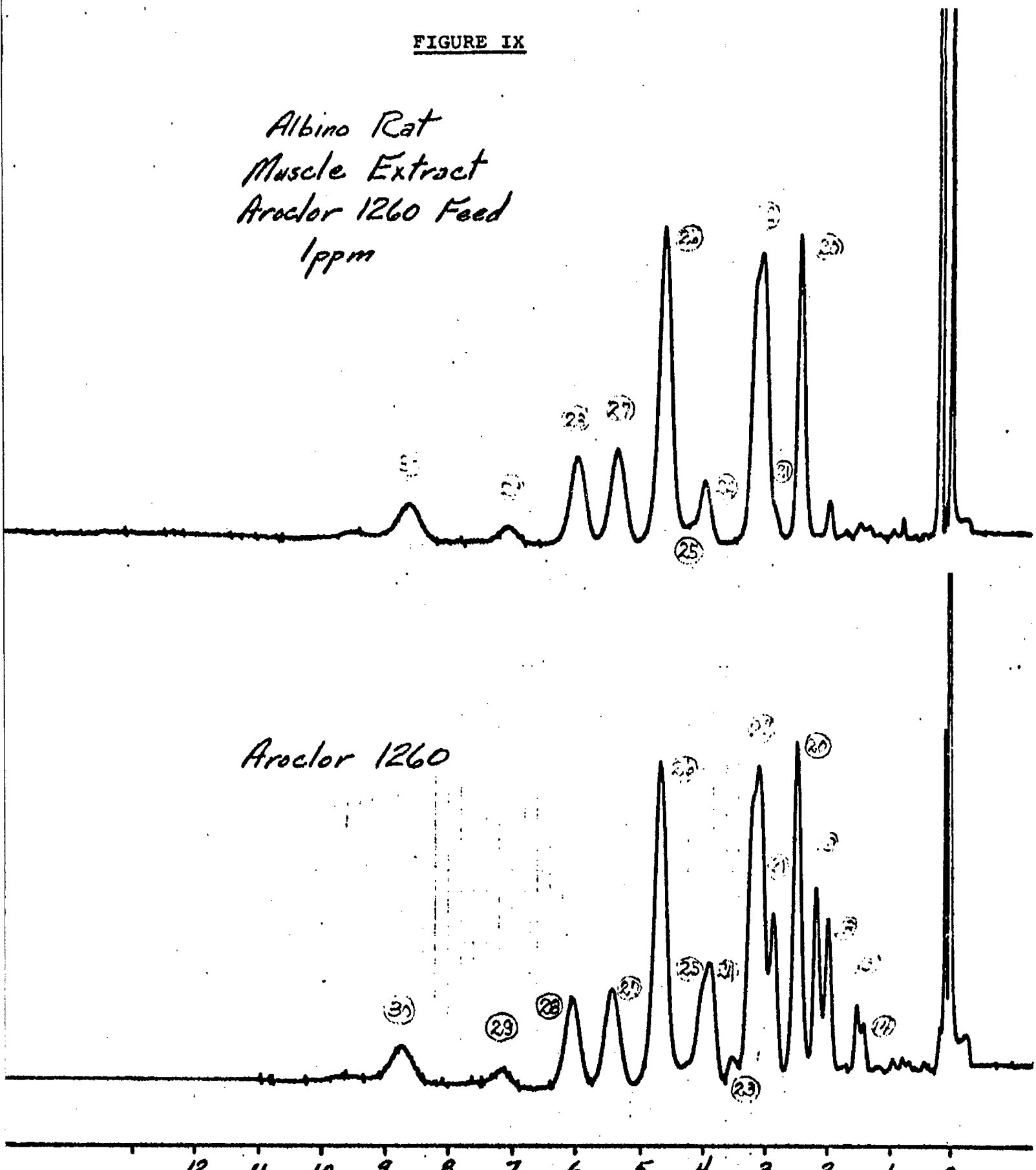

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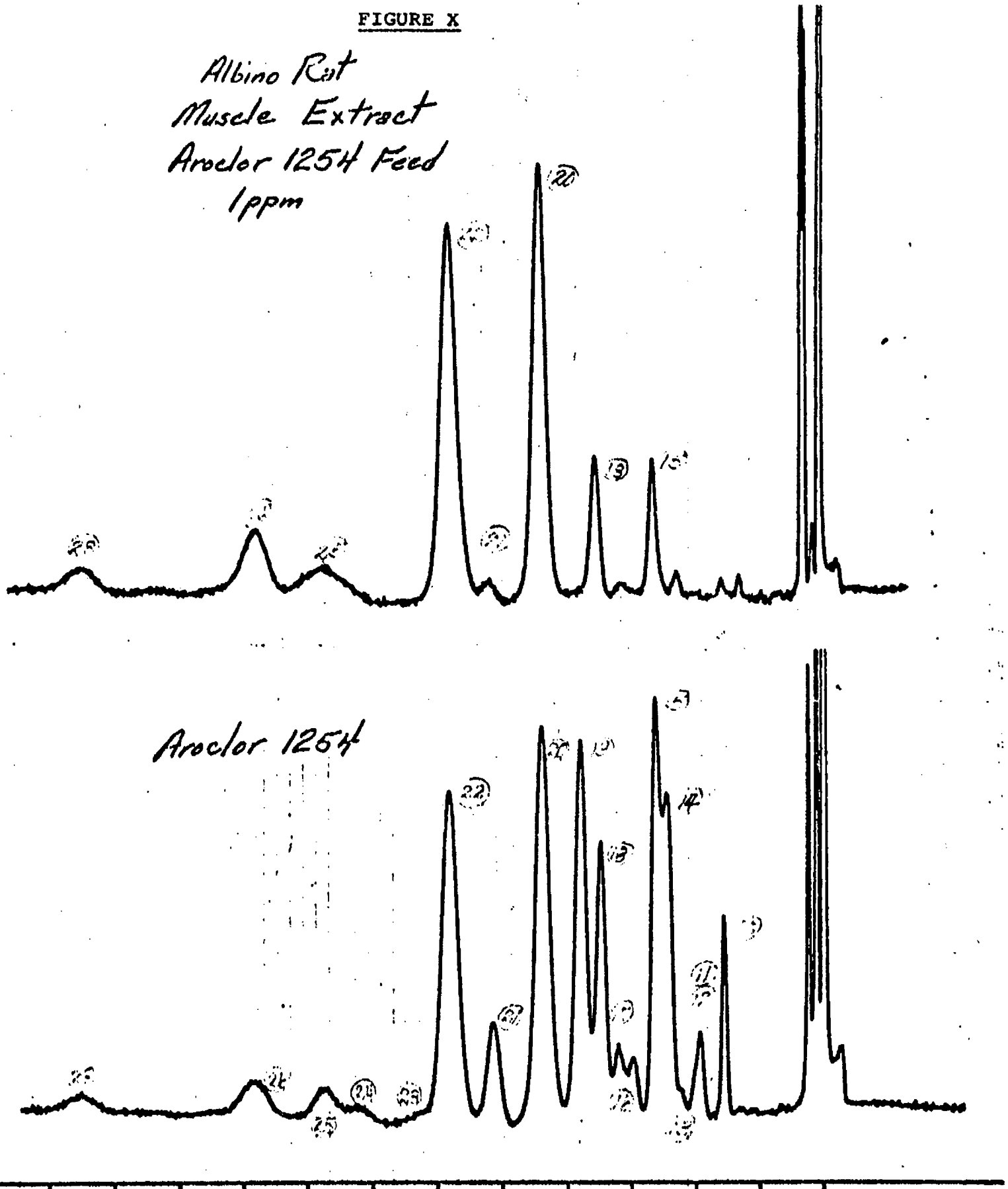
FIGURE X



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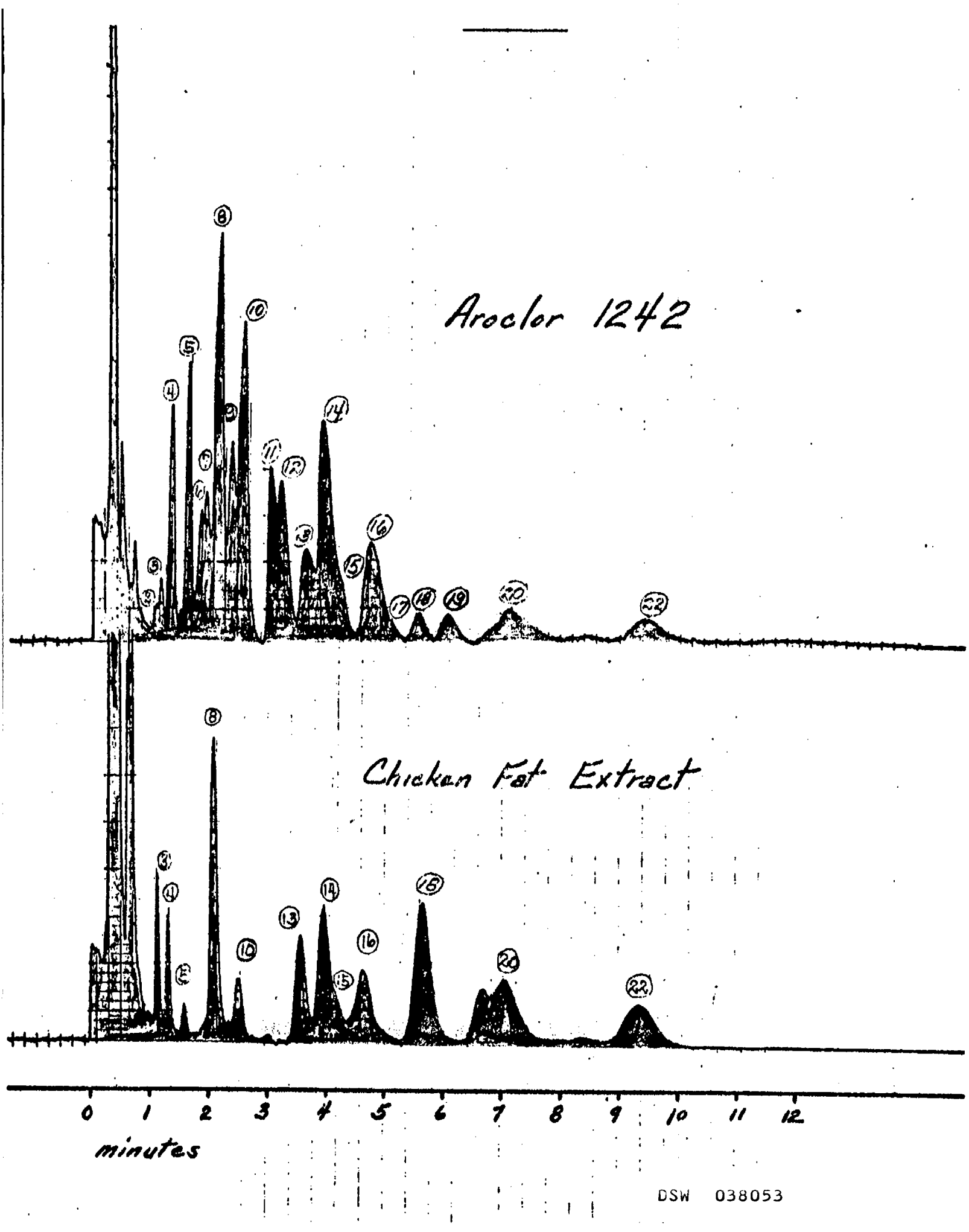

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FIGURE XII

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FIGURE XIII

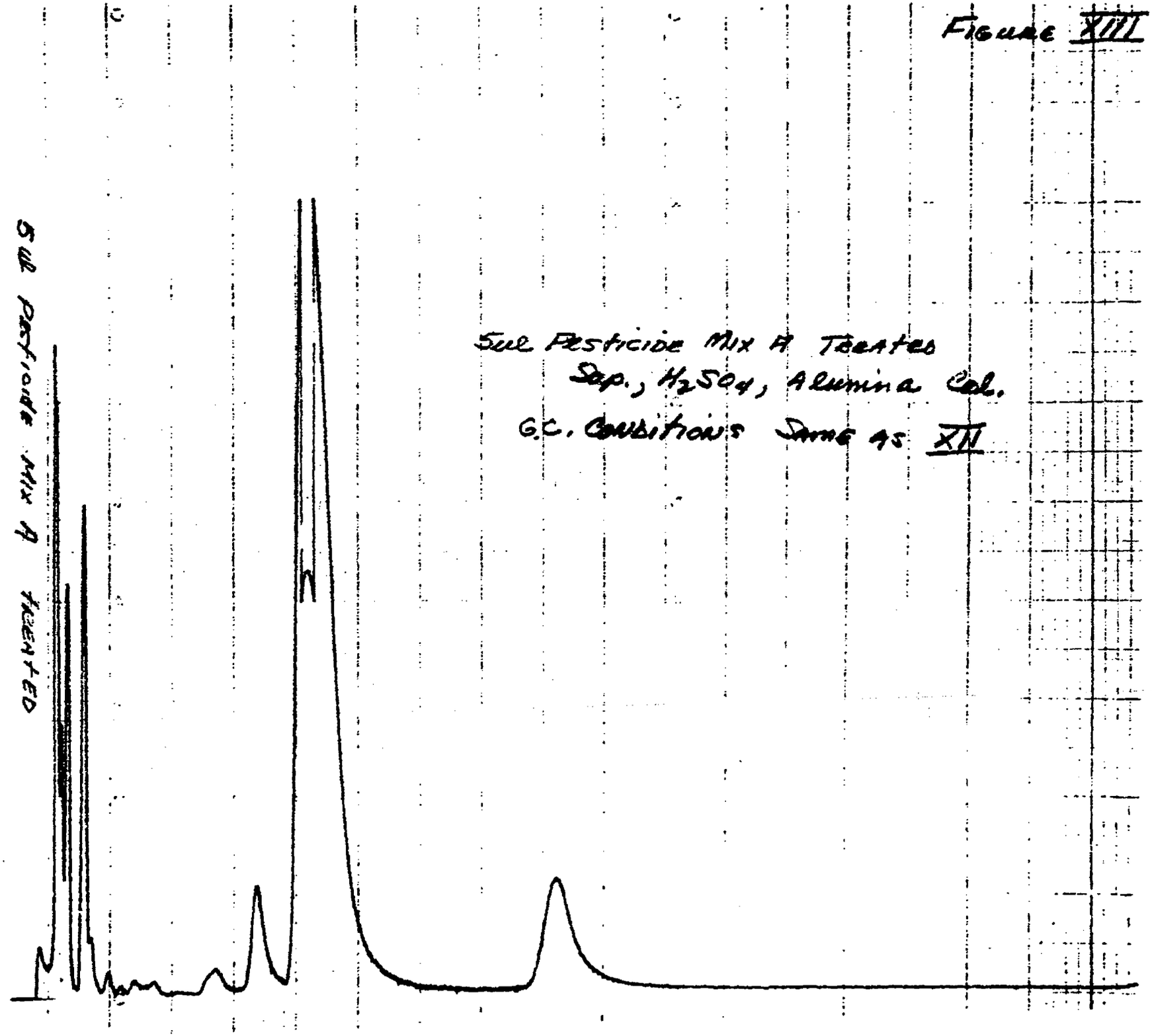

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Figure XIV

G.C. Conditions - Same as XIII

5ug Pesticide Mix B

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Figure XII

5ul Pesticide Mix B Treated

5ul Pesticide Mix B Treated
Sep., H_2SO_4, Alumina Cl
G.C. conditions - Same as XII

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FIGURE XVI

DSW 038058

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FIGURE XVII

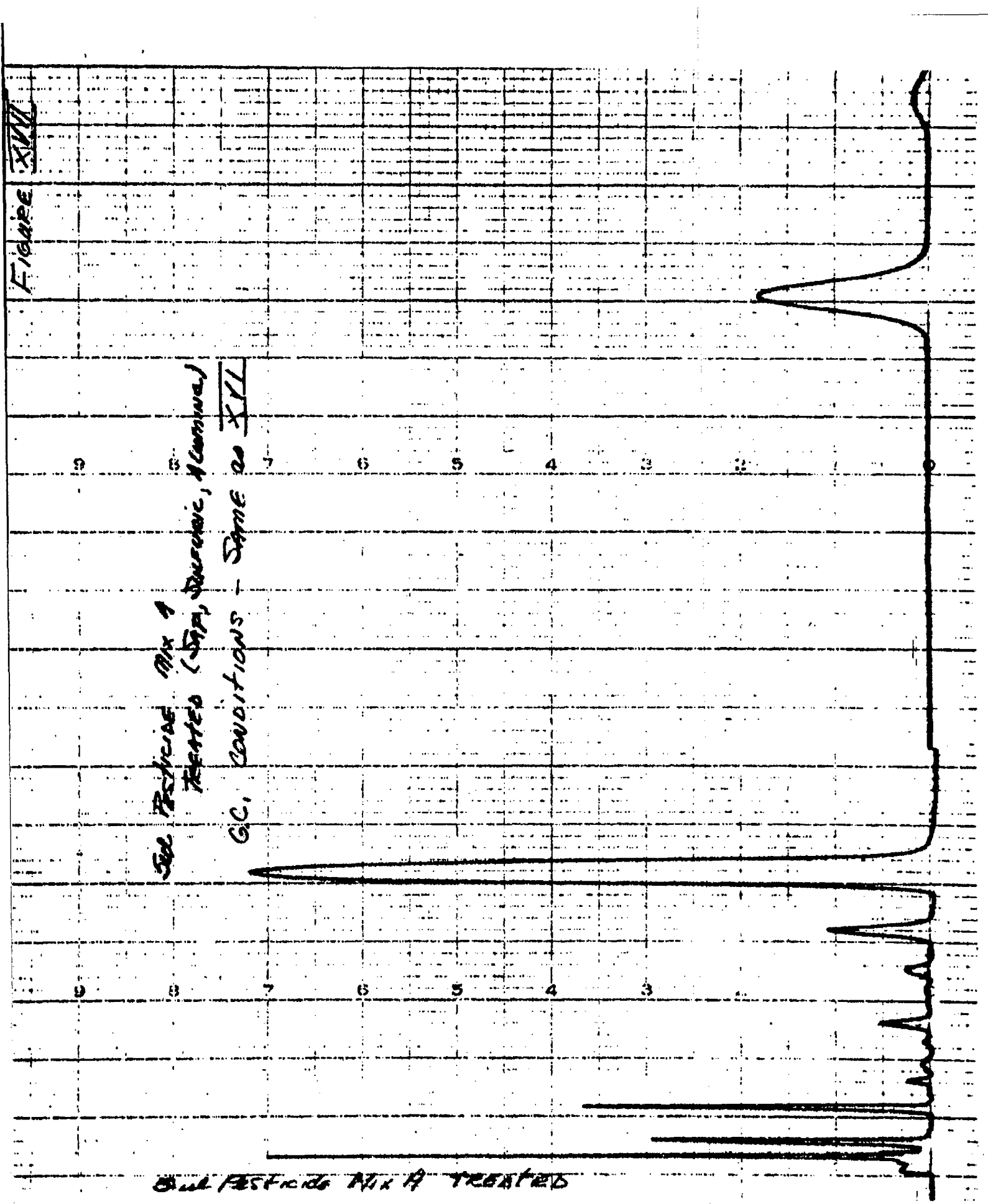

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Figure XIX

DSW 038060

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