Analytical Chemistry
Special Study 71-6
Job No. 1348006

BIODEGRADATION TESTING OF POLYCHLORINATED BIPHENYLS

INTRODUCTION

Because of mounting evidence that the polychlorinated biphenyls (PCB's), particularly the more highly chlorinated homologs, constitute an environmental hazard due to a combination of liquid solubility, stability and biological activity, biodegradation testing on a series of polychlorinated biphenyl products and potential products was undertaken. Biodegradability data could provide guidance in determining whether a particular PCB product is more or less environmentally compatible. The specific test utilized in this study was the semi-continuous activated sludge test which simulates a secondary sewage treatment plant.

SUMMARY AND CONCLUSIONS

Semi-continuous activated sludge (SCAS) testing of Aroclor 1254, Aroclor 1242, MCS 1016, Arobromchlor 1232, MCS 1043 and Aroclor 1221 has been carried out over an eight-month period. Disappearance rate data was obtained during two sampling periods for Aroclor 1254, Aroclor 1242, MCS 1016 and MCS 1043. For Arobromchlor 1232 and Aroclor 1221, only one sampling period was employed. In the initial testing of these materials, a feed level of one mg. per 24-hr. cycle was used. Because the data obtained from the first sampling period for Aroclor 1254, Aroclor 1242, MCS 1016, and MCS 1043 was extremely erratic with little apparent biodegradation, the time cycle was extended subsequently to 48 hours for all of the materials except Aroclor 1221.

Statistical analysis of the disappearance rate data from the last sampling period yielded the following mean disappearance rates and 95% confidence limits: Aroclor 1254 - 15.2 ± 37.7 (48-hr. cycle); Aroclor 1242 - 26.3 ± 15.3 (48-hr. cycle); MCS 1016 - 32.9 ± 13.8 (48-hr. cycle); MCS 1043 - 56.2 ± 15.5 (48-hr. cycle); Arobromchlor 1232 - 54.1 ± 19.3 (48-hr. cycle); Aroclor 1221 - 64.0 ± 13.6 (24-hr. cycle). On the basis of these data, these materials would be classified in the partially biodegradable to refractory range. The biodegradability decreases going from Aroclor 1221 to Aroclor 1254 or with increasing levels of chlorination. Because of the uncertainty of the data, it was not possible to differentiate between Aroclor 1242 and MCS 1016 with respect to biodegradability. This is not surprising since 1016 constitutes 80% of 1242 and the resolving power of the method is comparable to the difference. Similarly there was no statistically significant difference between MCS 1043 and Arobromchlor 1232.

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Electron-capture gas chromatography analyses of samples taken at the end of the aeration cycle show that the lower the 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. There is, however, a buildup of the higher chlorinated homologs compared to the distribution in the starting material. For Aroclor 1254, there is no significant change in the homolog distribution after 48 hours exposure compared to the starting material.

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

RESULTS AND DISCUSSION

The SCAS test method used is patterned after the standard test method for surfactants [JAOCS 42, 986 (1965); JAOCS 46, 432 (1969)]. A description of the test method as applied to our work is given in Analytical Chemistry Method 71-32.

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:

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

where AF Sample = sample taken 1 hour after feeding.
BF Sample = sample taken at end of cycle before the next feeding.

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, the recoveries were consistent and independent of level. The calculated disappearance rates should, therefore, not be affected significantly.

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Since the level of chlorination is the most significant factor in the relative biodegradability of the PCB's, the homolog distribution of the various test materials is given in Table I.

Feeding of the Aroclor 1254, Aroclor 1242, MCS 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 MCS 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 was 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.

Feeding of the Arobromchlor 1232 and Aroclor 1221 units was started on 10/27/70 at a feed level of 1 mg. per 24 hours. The cycle time for Arobromchlor 1232 was subsequently increased to 48 hours. The unit was then sampled along with the Aroclor 1254, Aroclor 1242, MCS 1016, and MCS 1043 units during their final sampling period. 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, MCS 1016, MCS 1043, and Arobromchlor 1232 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.

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TABLE I

HOMOLOG DISTRIBUTION OF POLYCHLORINATED BIPHENYL PRODUCTS

Homolog No. of Cl Per Biphenyl Molecule	Aroclor[1] 1254 (54% Cl)	Aroclor[2] 1242 (42% Cl)	MCS[2] 1016 (41% Cl)	MCS[3] 1043 (30% Cl)	Aroclor[1] 1221 (21% Cl)	Arobromchlor 1232 (21% Cl, 11% Br)
0	0.05	0.01	0.02	0.11	19.00	Data not available
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						

1 - Spectroscopy Special Study

2 - Memo, E.M. Emery to R.E. Keller, 1/22/71.

3 - Data from G. Gasser, Batch #1, 3/9/71.

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In Figure 1-1 to Figure 4-2, the observed data from which the mean disappearance rates were calculated are shown for both sampling periods for Aroclor 1254, Aroclor 1242, MCS 1016, and MCS 1043. In Figures 5 and 6, similar data for Arobromchlor 1232 and Aroclor 1221 are shown from their 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 II 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 I for Aroclor 1221 composition).

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. The data obtained indicates that for Aroclor 1221 the disappearance rate due to volatility was a maximum of 25%, compared to the overall rate of 64%. Since the volatility of the PCB's decreases with increasing level of chlorination, the effect of volatility losses on the disappearance rate should likewise decrease. The vapor pressure of Aroclor 1242 and MCS 1016 are about a factor of five less than Aroclor 1221.

From the data in Table II and Figures 1-1, 2-1, 3-1, and 4-1, it is quite apparent that the disappearance rate data obtained for Aroclor 1254, Aroclor 1242, MCS 1016, and MCS 1043 during the first sampling period was very erratic. The data obtained during the last sampling period as shown in Table II and in Figures 1-2, 2-2, 3-2, 4-2, 5, and 6 was a significant improvement. From the latter data, the following ranking in terms of biodegradability can be made:

Aroclor 1221 > MCS 1043 $\cong$ Arobromchlor 1232 > MCS 1016 $\cong$

Aroclor 1242 > Aroclor 1254

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In Figure 7, the mean disappearance rate is plotted versus the percent chlorine in the test compound. The 48-hour value for Aroclor 1221 was obtained by extrapolation of the 24-hour cycle data; the value 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 8, 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.

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 8, 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 cent 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 9, 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 Figure 10, Arobromchlor 1232 chromatograms are shown. No homolog identification for this mixed chloro-and bromo-product is available. The disappearance of the peaks with lower elution times is apparent.

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TABLE II

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	Arobromchlor 1232	Aroclor 122
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	54.1 ± 19.3	

* Identification of feed material in SCAS tests

Aroclor 1254:	Lot AK-38
Aroclor 1242:	Lot AK-255
MCS 1016:	Sample No.1, OR 158591
MCS 1043:	Sample No. 2, OR 158591
Arobromchlor 1232:	OR 16882
Aroclor 1221:	Lot AK-2

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TABLE III

DISAPPEARANCE OF TEST COMPOUND RESIDUES AT END OF SCAS TEST

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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In Figures 11 and 12, chromatograms for MCS 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 in 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, MCS 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 III, 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.

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12/71 - V.W. Saeger, E.S. Tucker

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FIGURE 1-1

SCAS BIODEGRADATION DATA

AROCLOR 1254

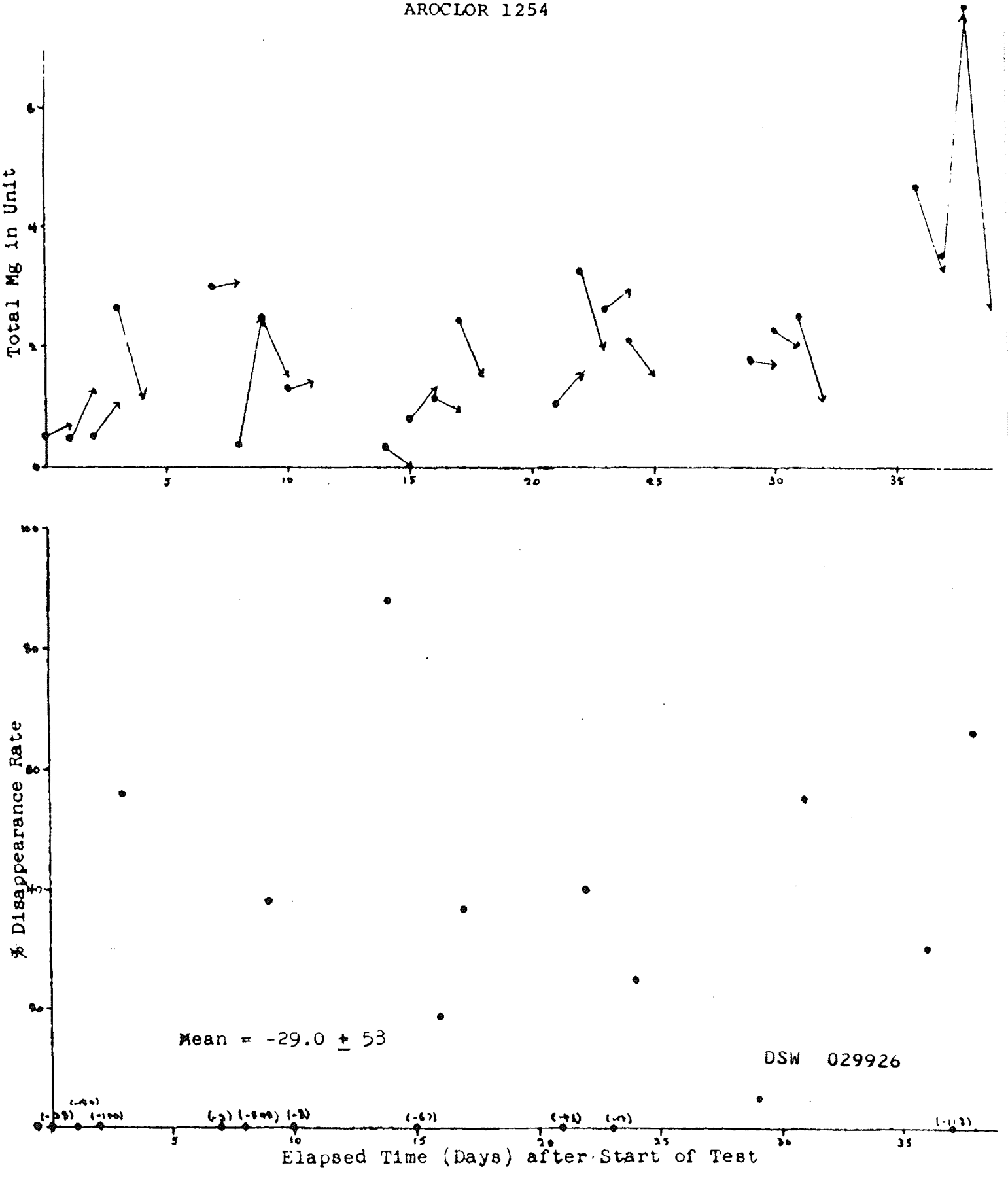

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FIGURE 1-2

SCAS BIODEGRADATION DATA

AROCLOR 1254

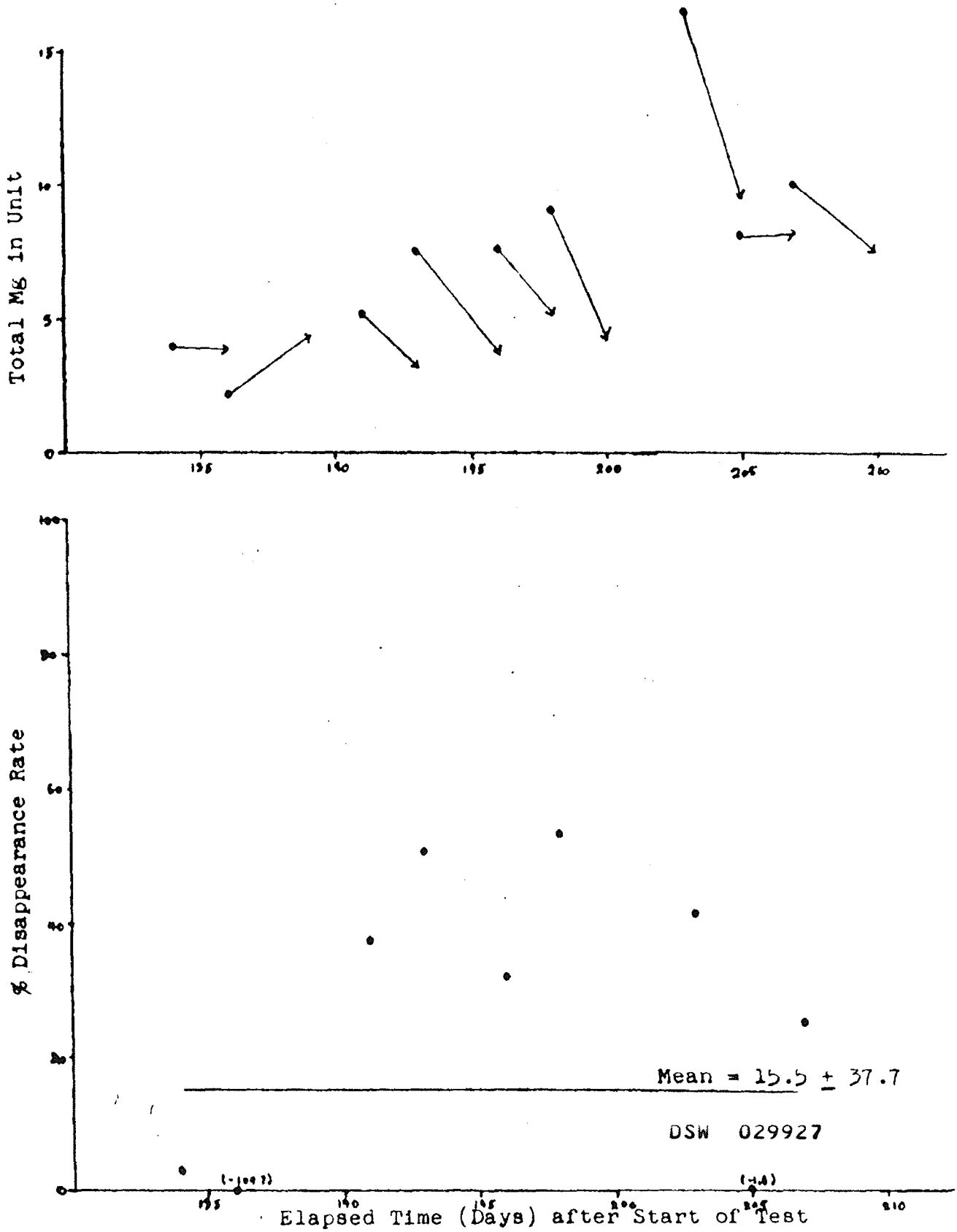

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FIGURE 2-1

SCAS BIODEGRADATION DATA

AROCLOR 1242

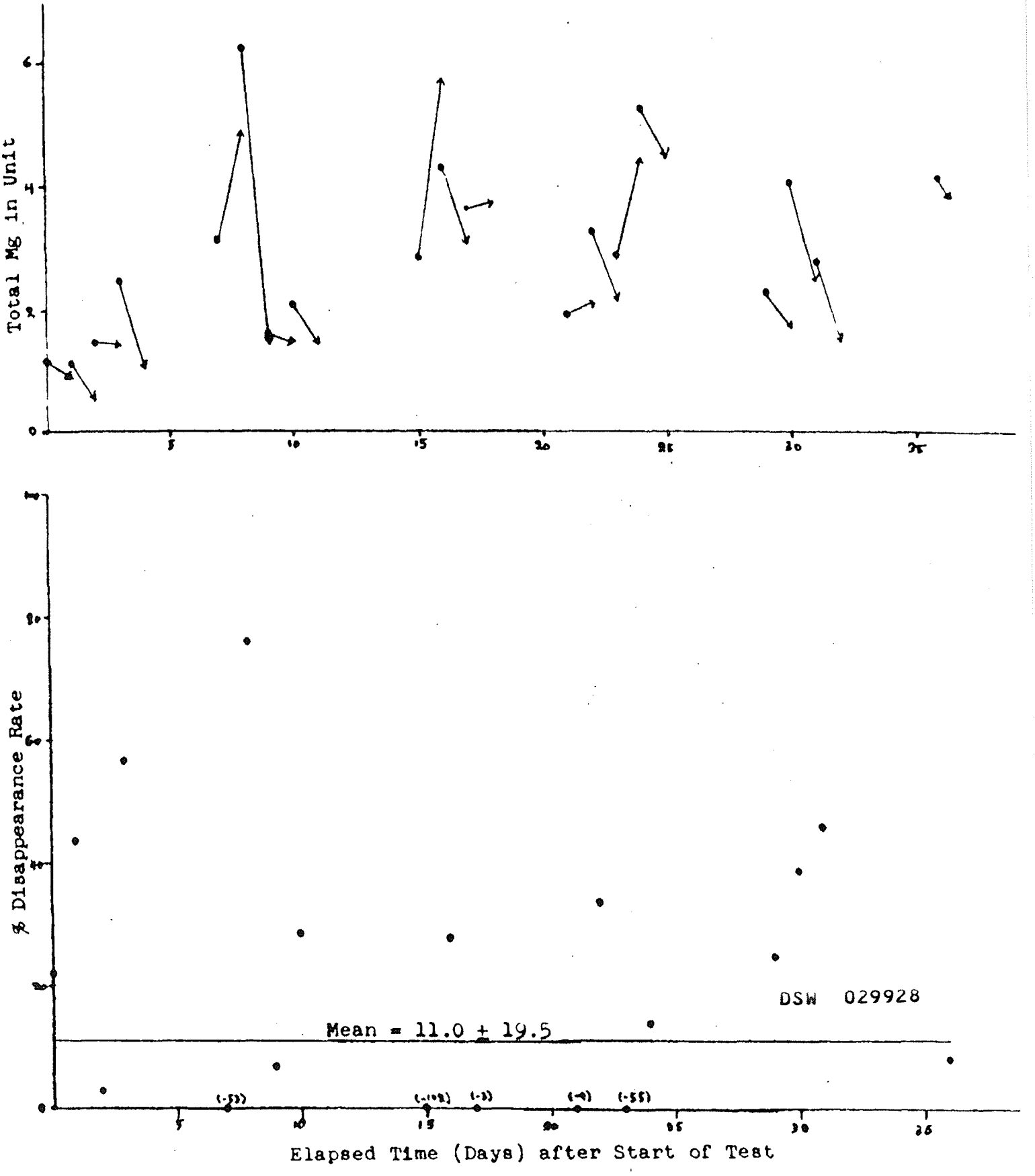

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FIGURE 2-2

SCAS BIODEGRADATION DATA

AROCLOR 1242

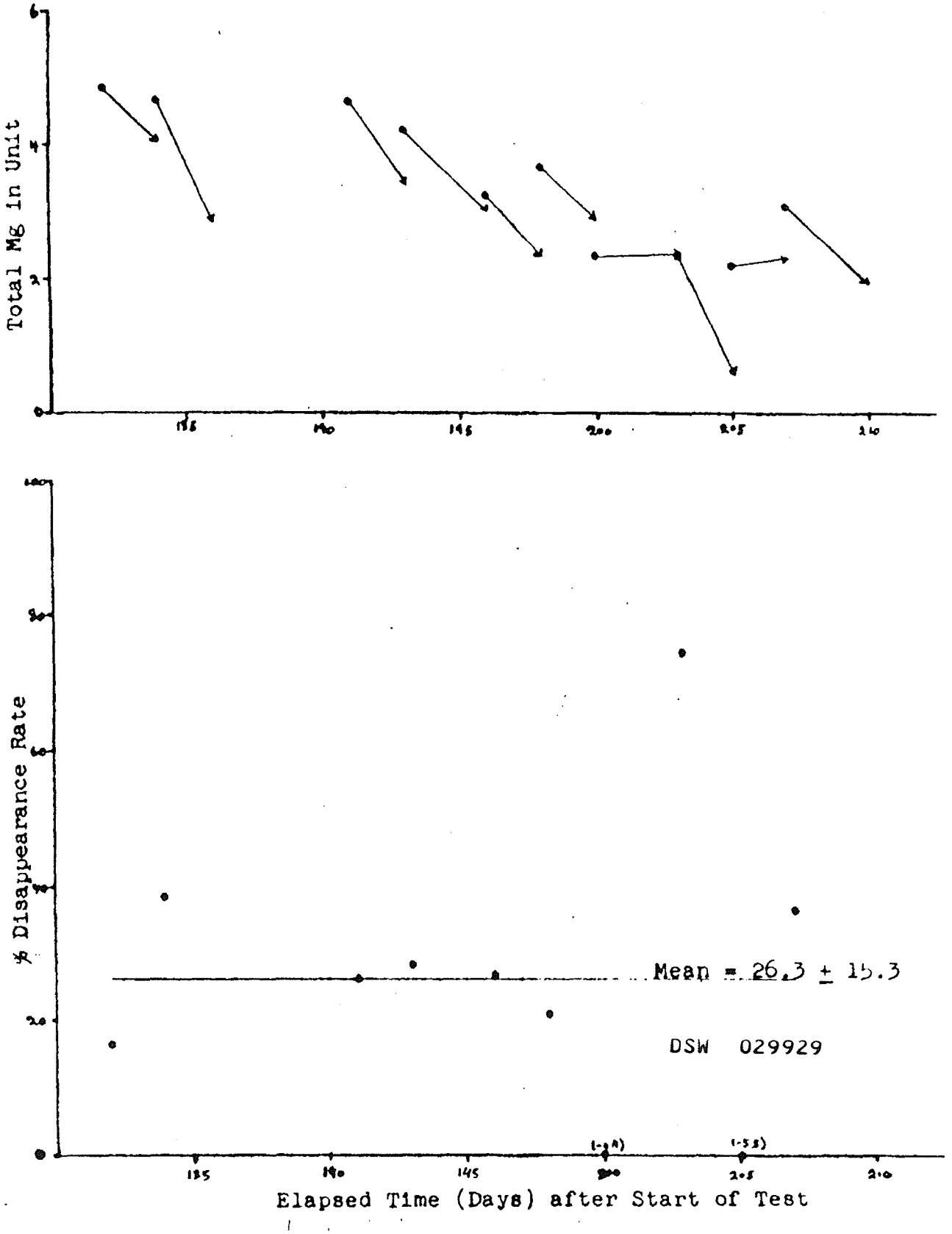

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FIGURE 3-1

SCAS BIODEGRADATION DATA

MCS 1016

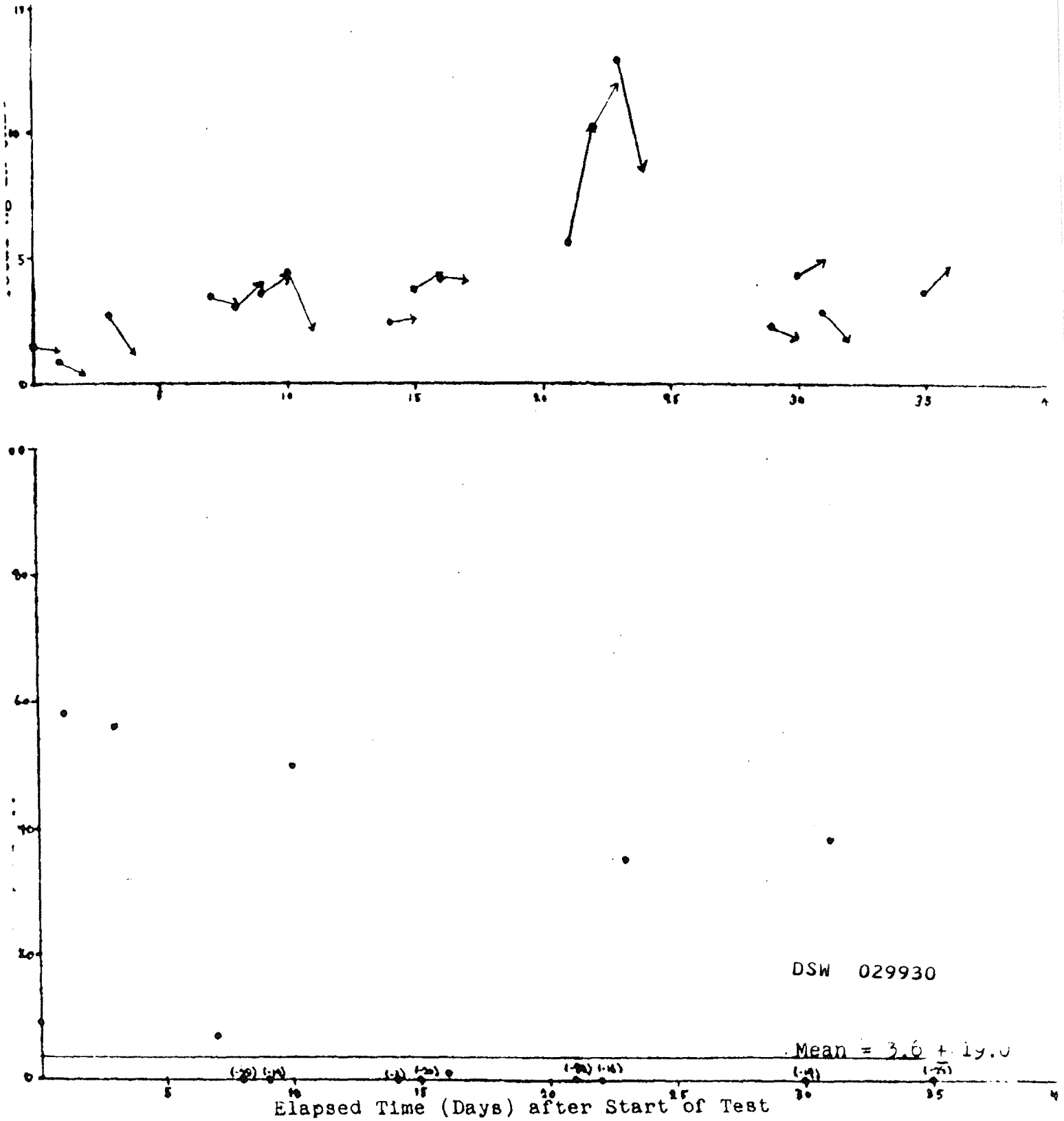

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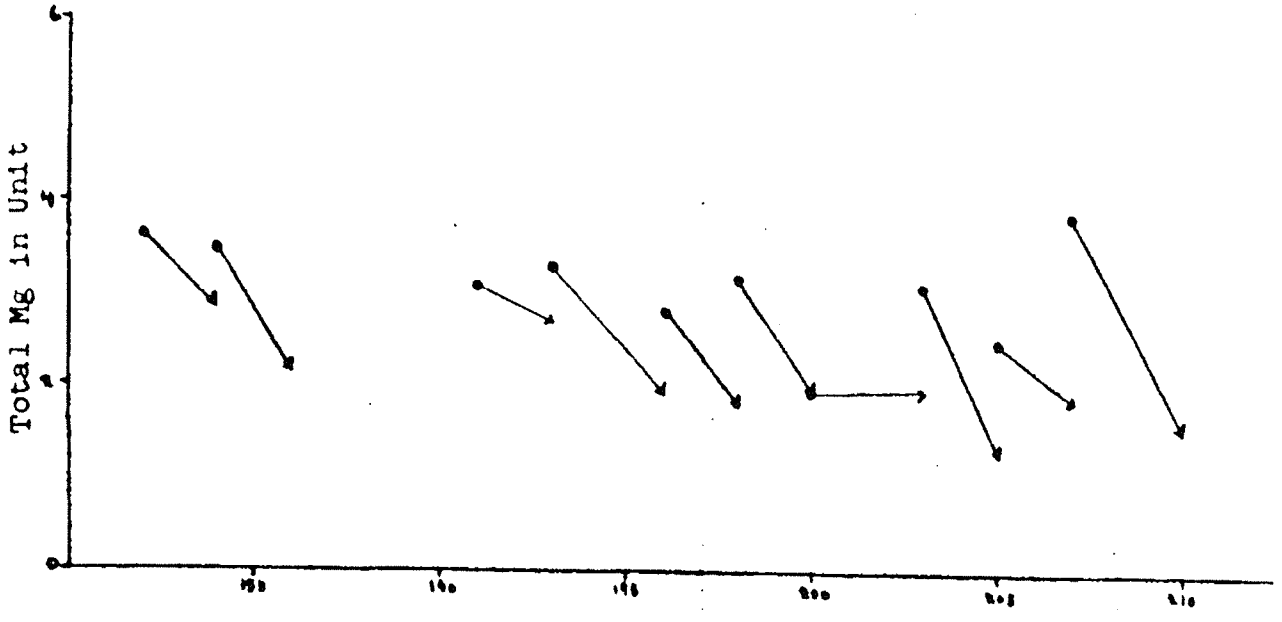
FIGURE 3-2

SCAS BIODEGRADATION DATA

MCS 1016



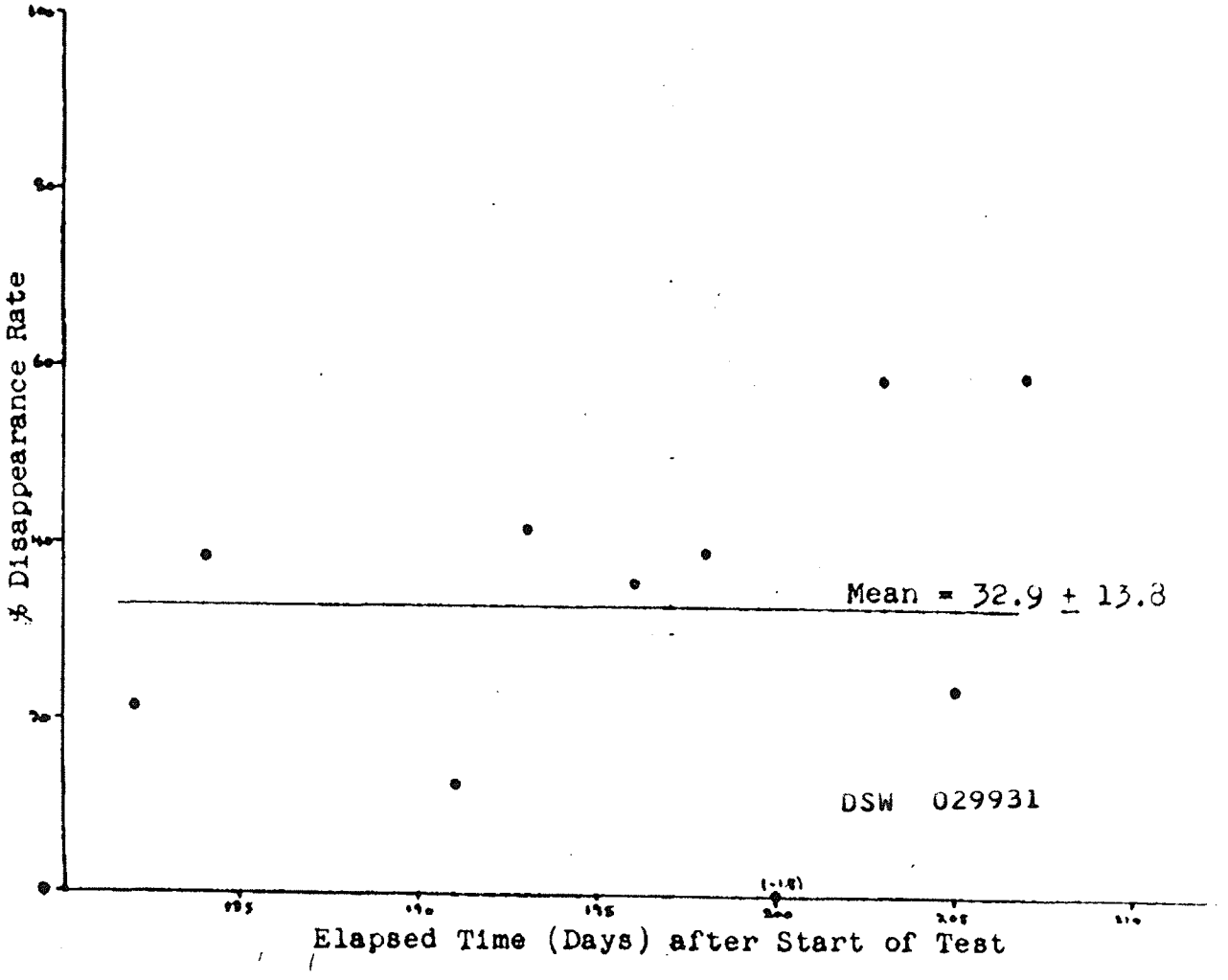


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FIGURE 4-1

SCAS BIODEGRADATION DATA

MCS 1043

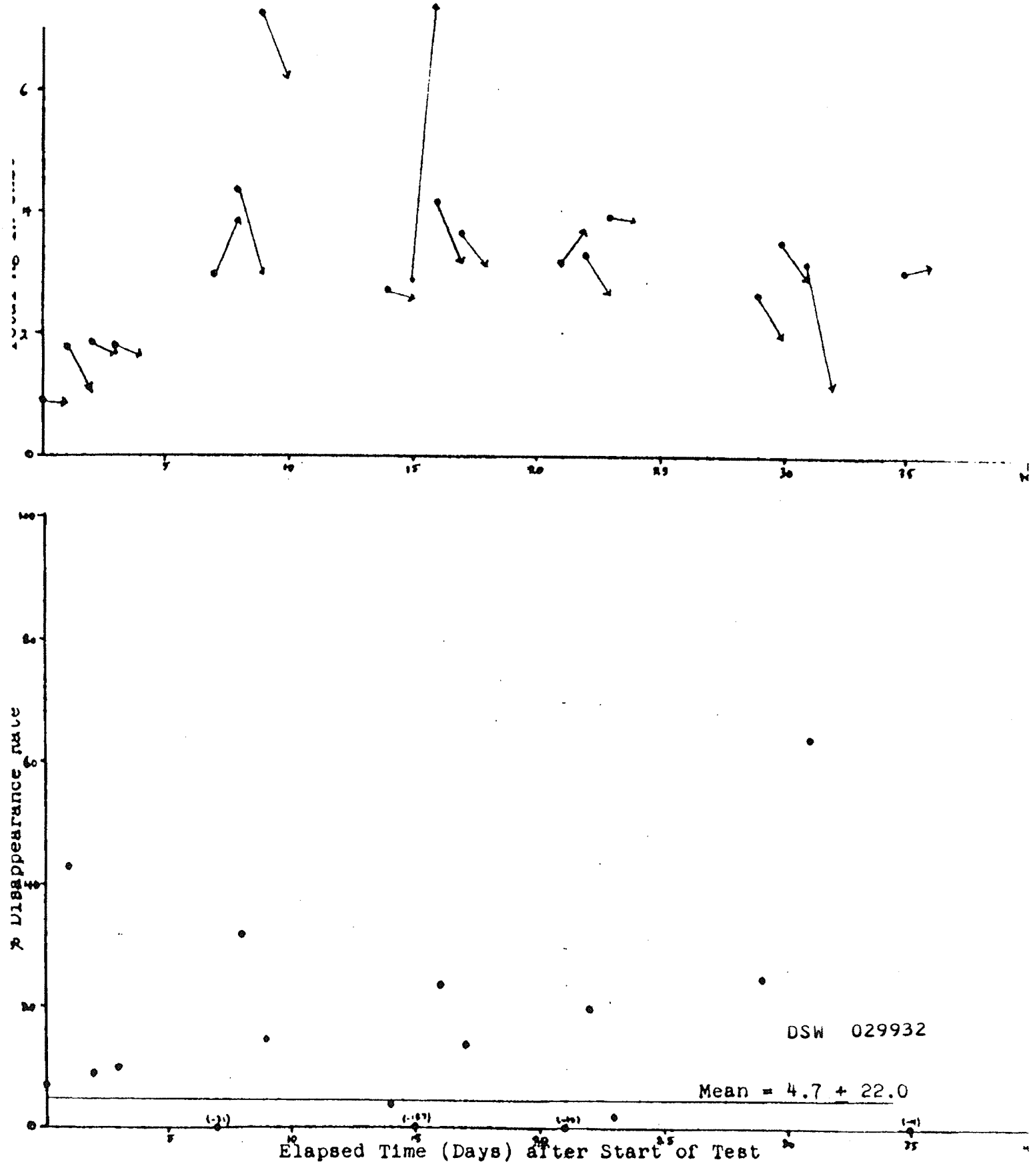

FIGURE 4-2

SCAS BIODEGRADATION DATA

MCS 1043

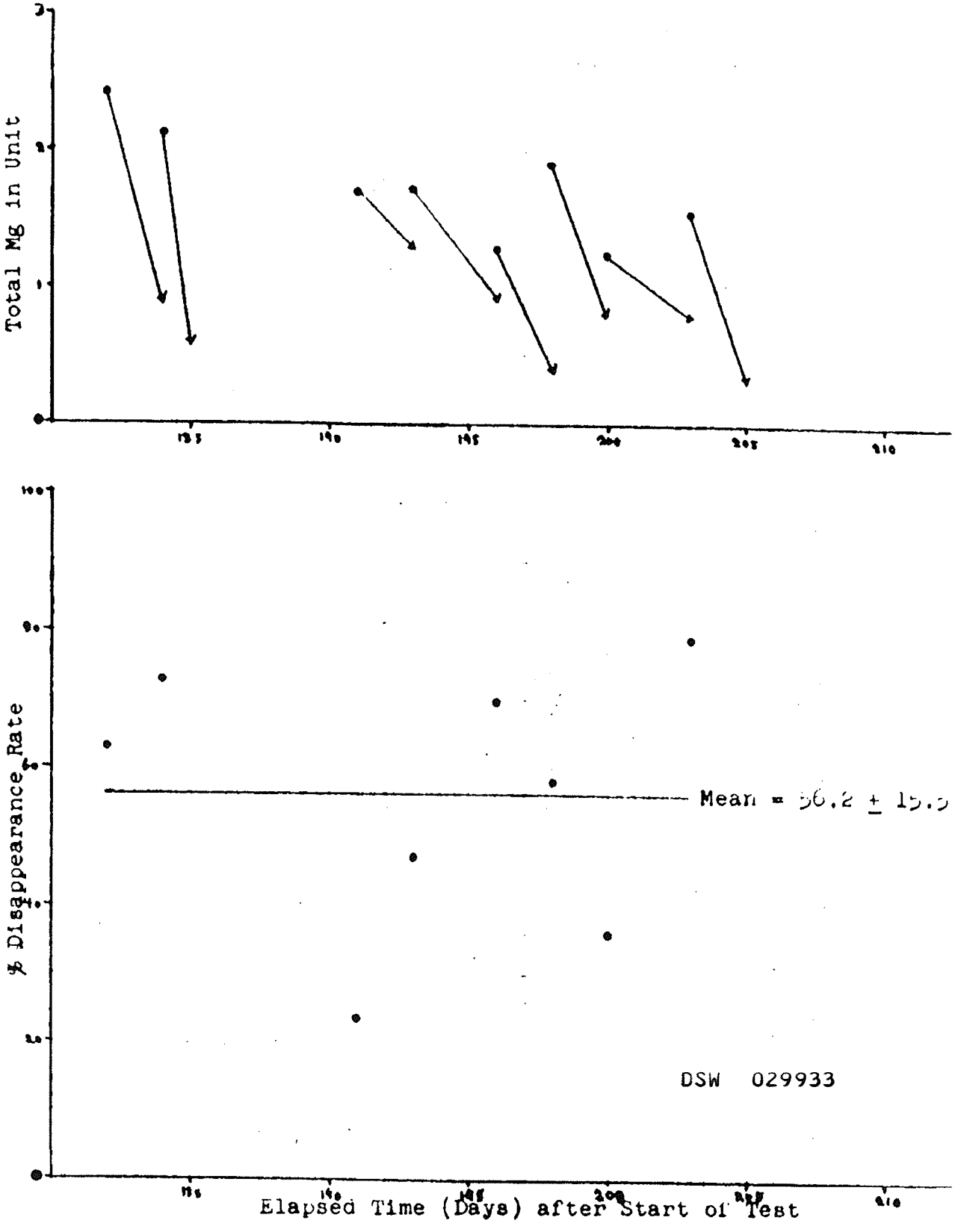

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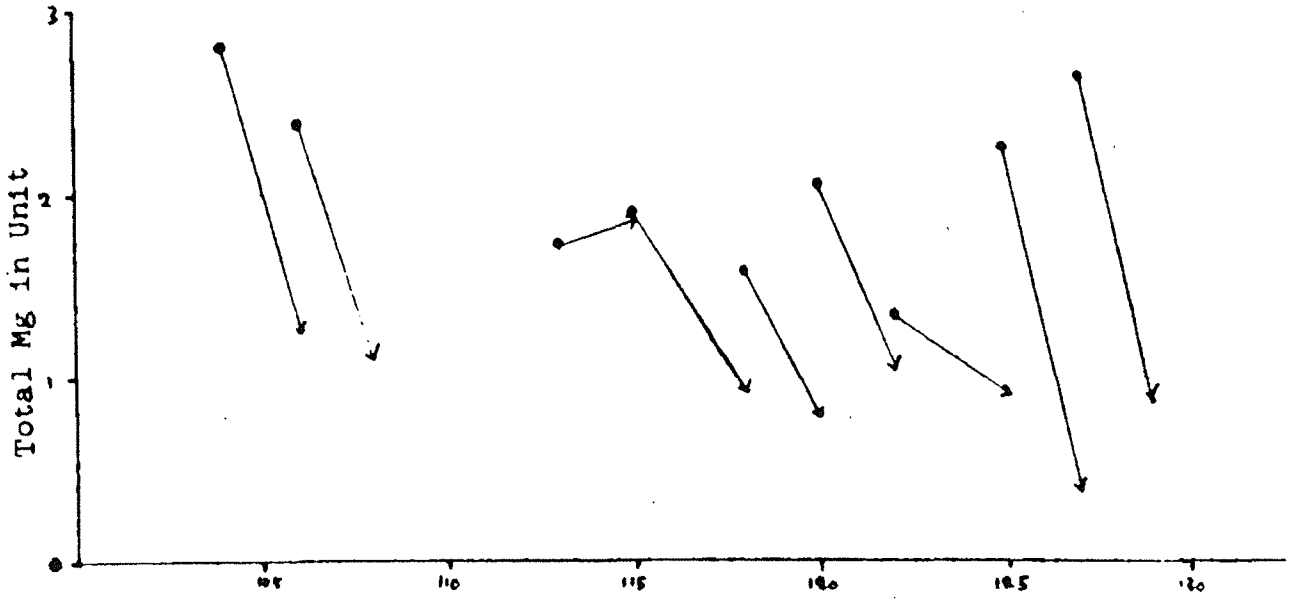
FIGURE 5

SCAS BIODEGRADATION DATA

AROBROMCLOR 1232



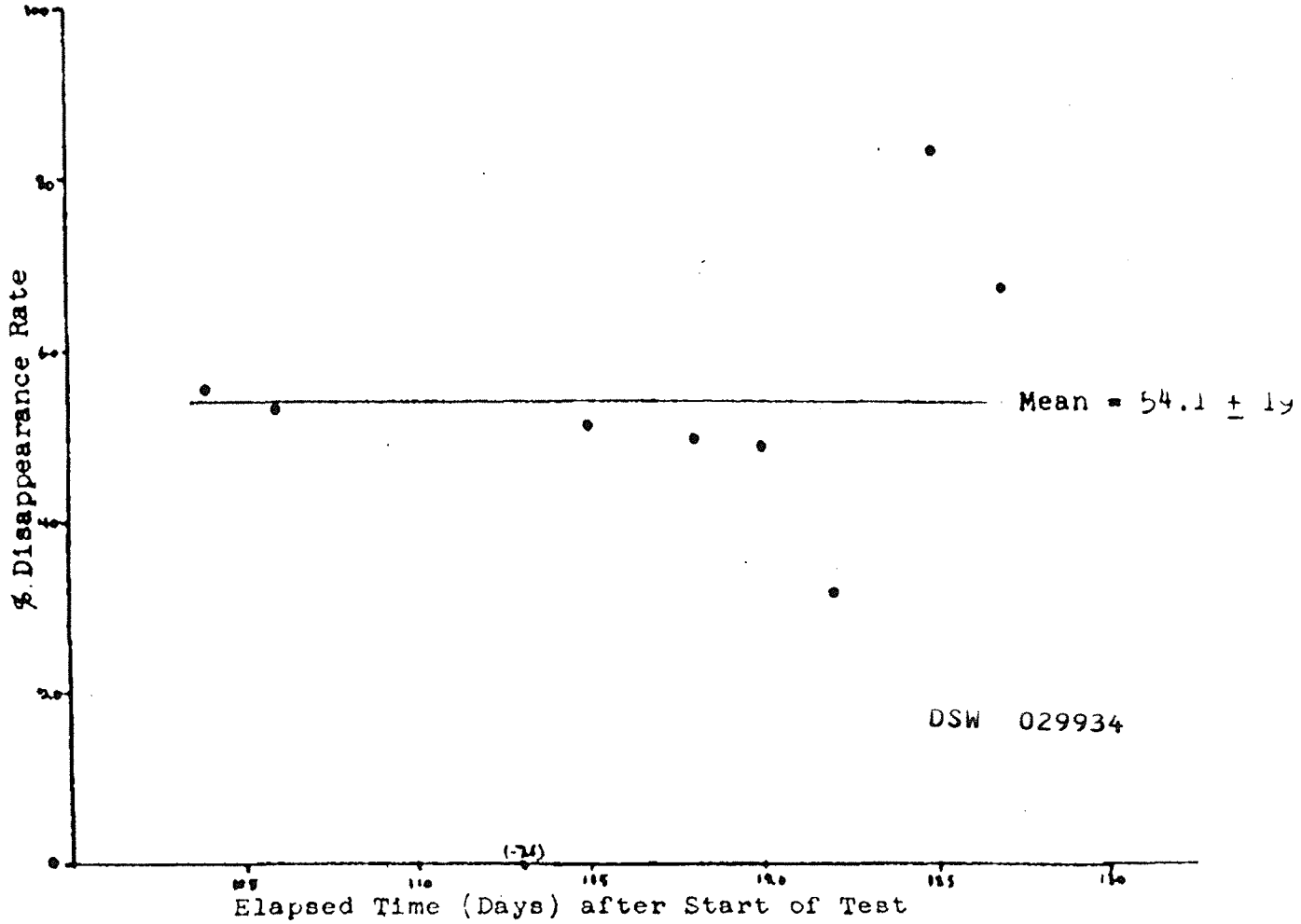


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FIGURE 6

SCAS BIODEGRADATION DATA

AROCLOR 1221

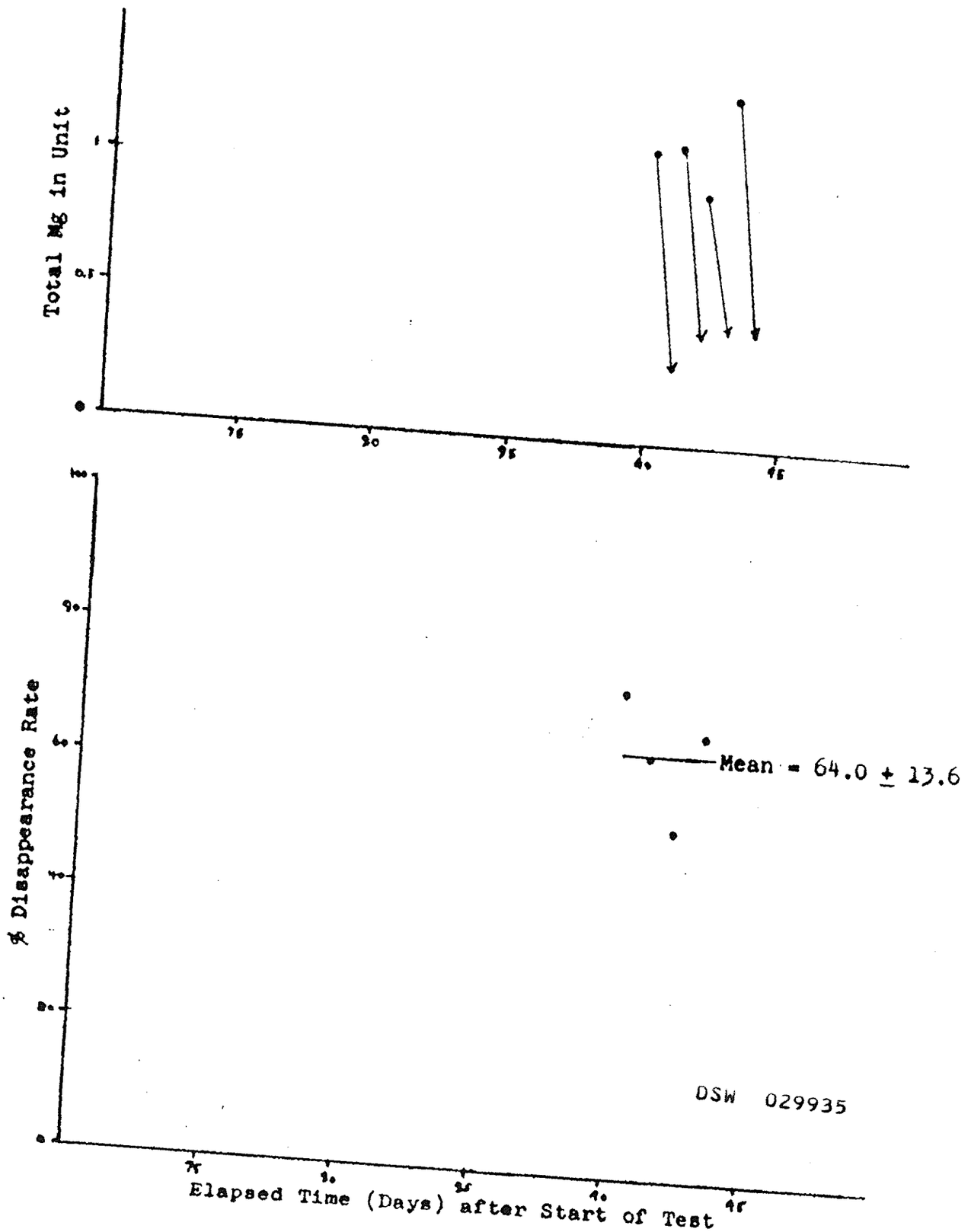

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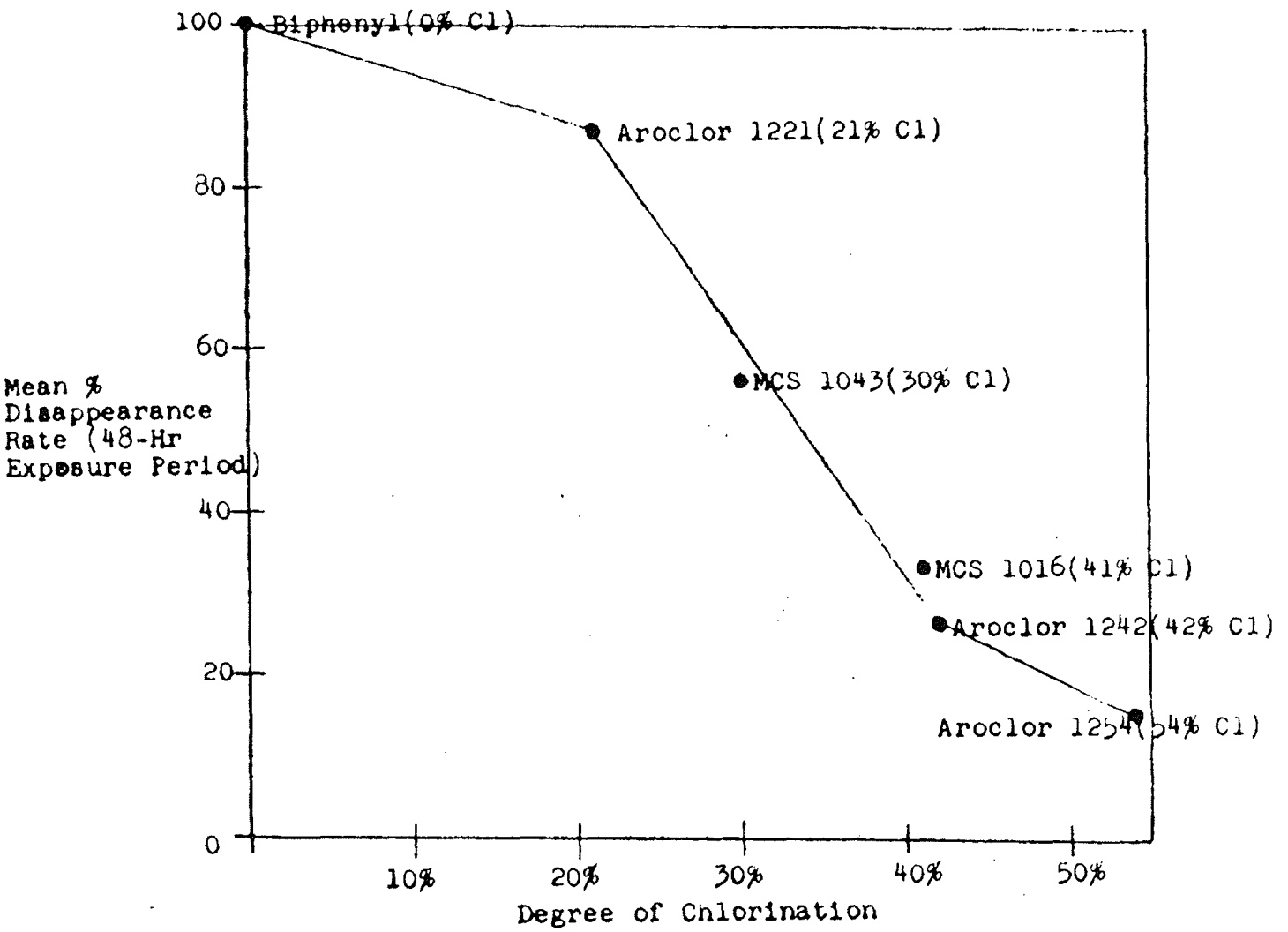
FIGURE 7

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



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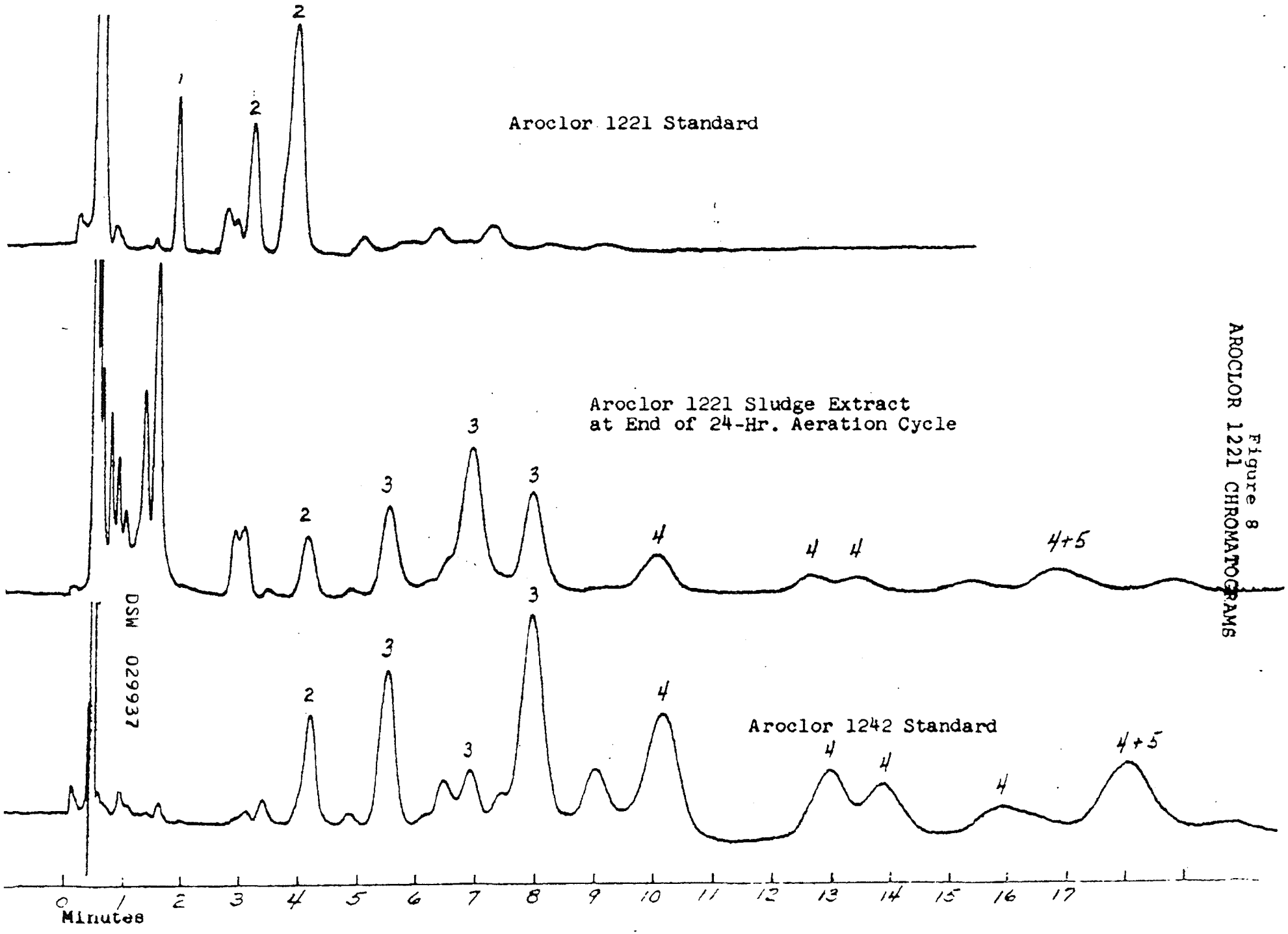

Figure 8
AROCLOR 1221 CHROMATOGRAMS

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Figure 9
MCS 1043 CHROMATOGRAMS

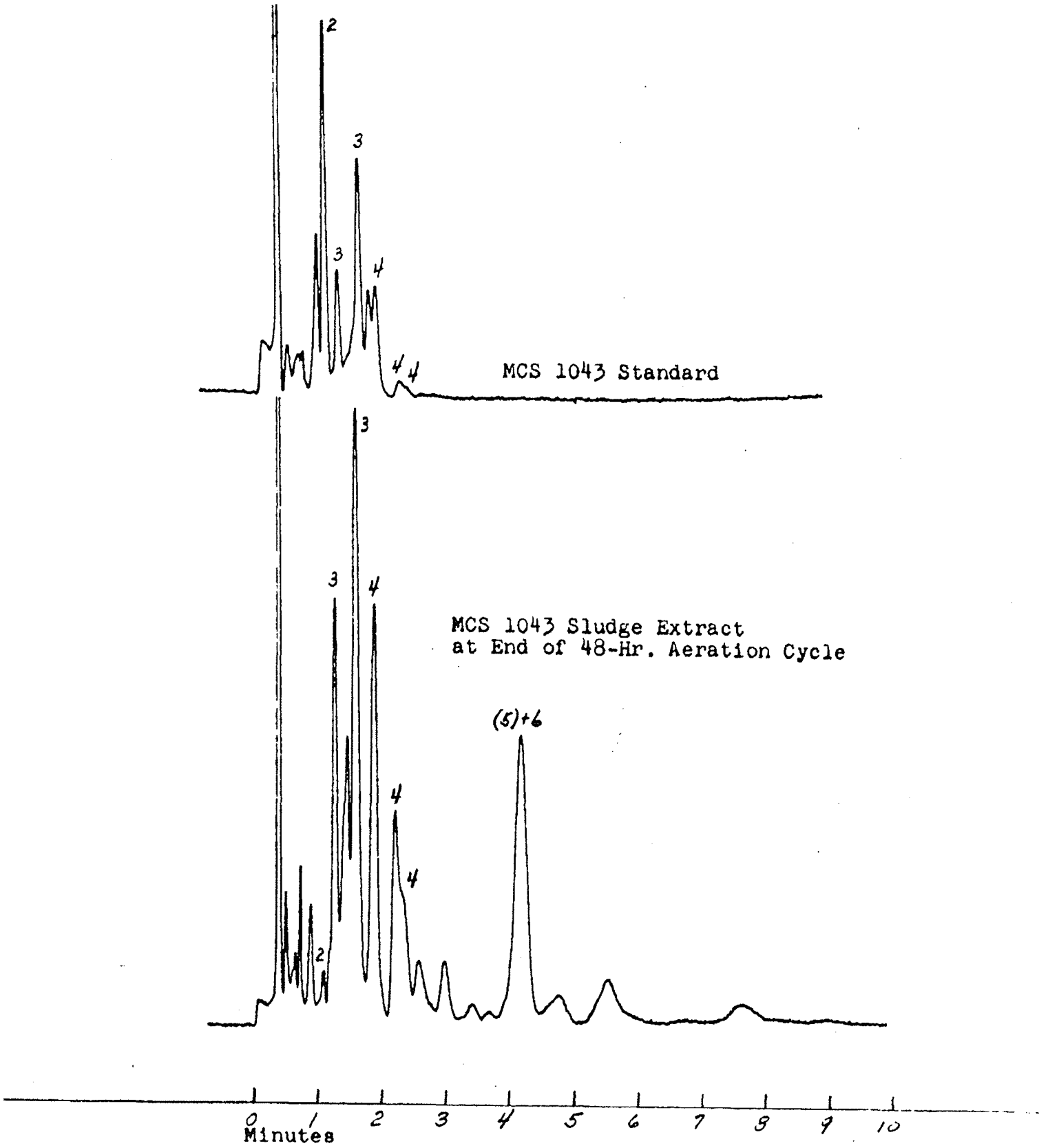

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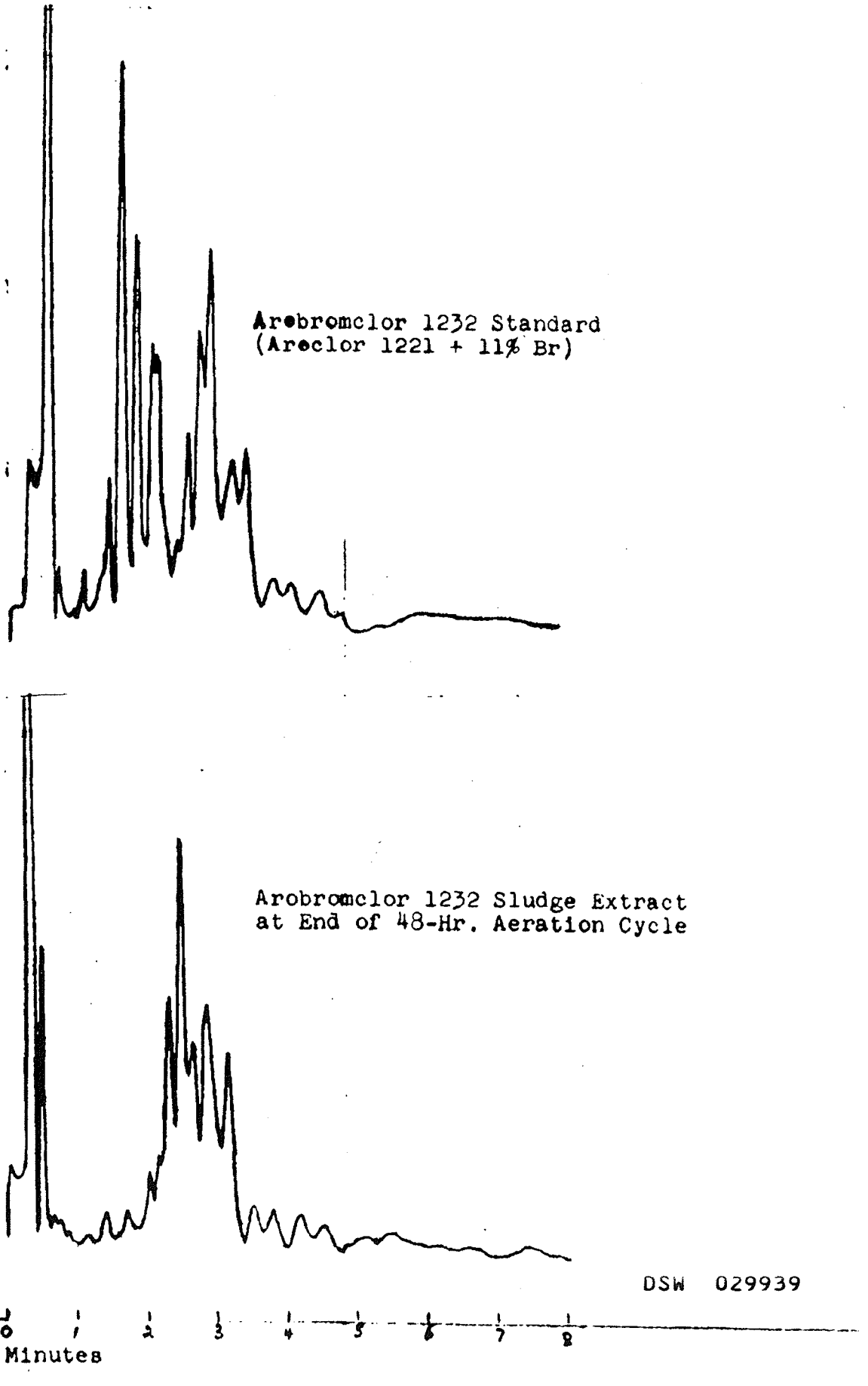
Figure 10
AROBROMCLOR 1232 CHROMATOGRAMS



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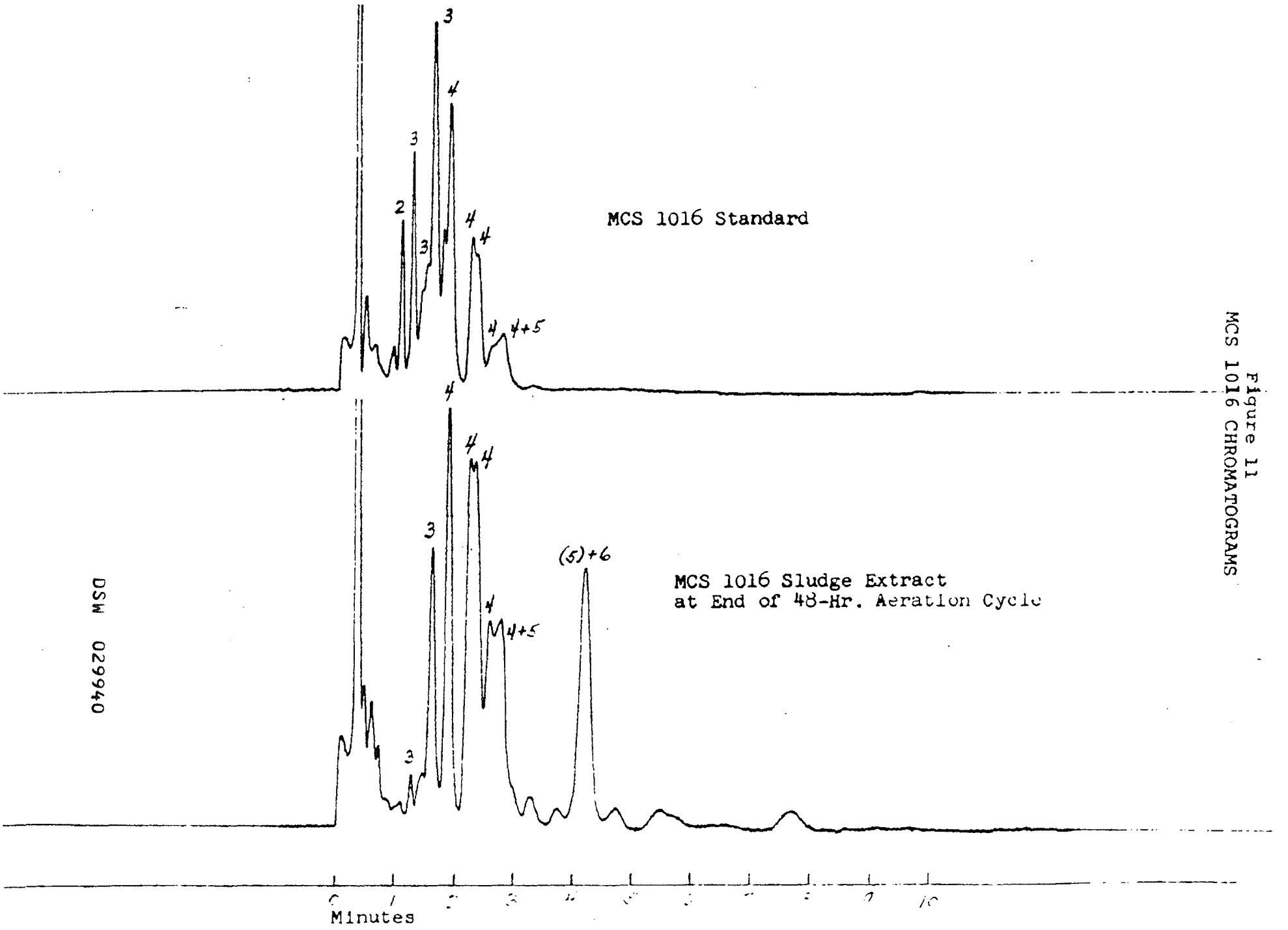

Figure 11
MCS 1016 CHROMATOGRAMS

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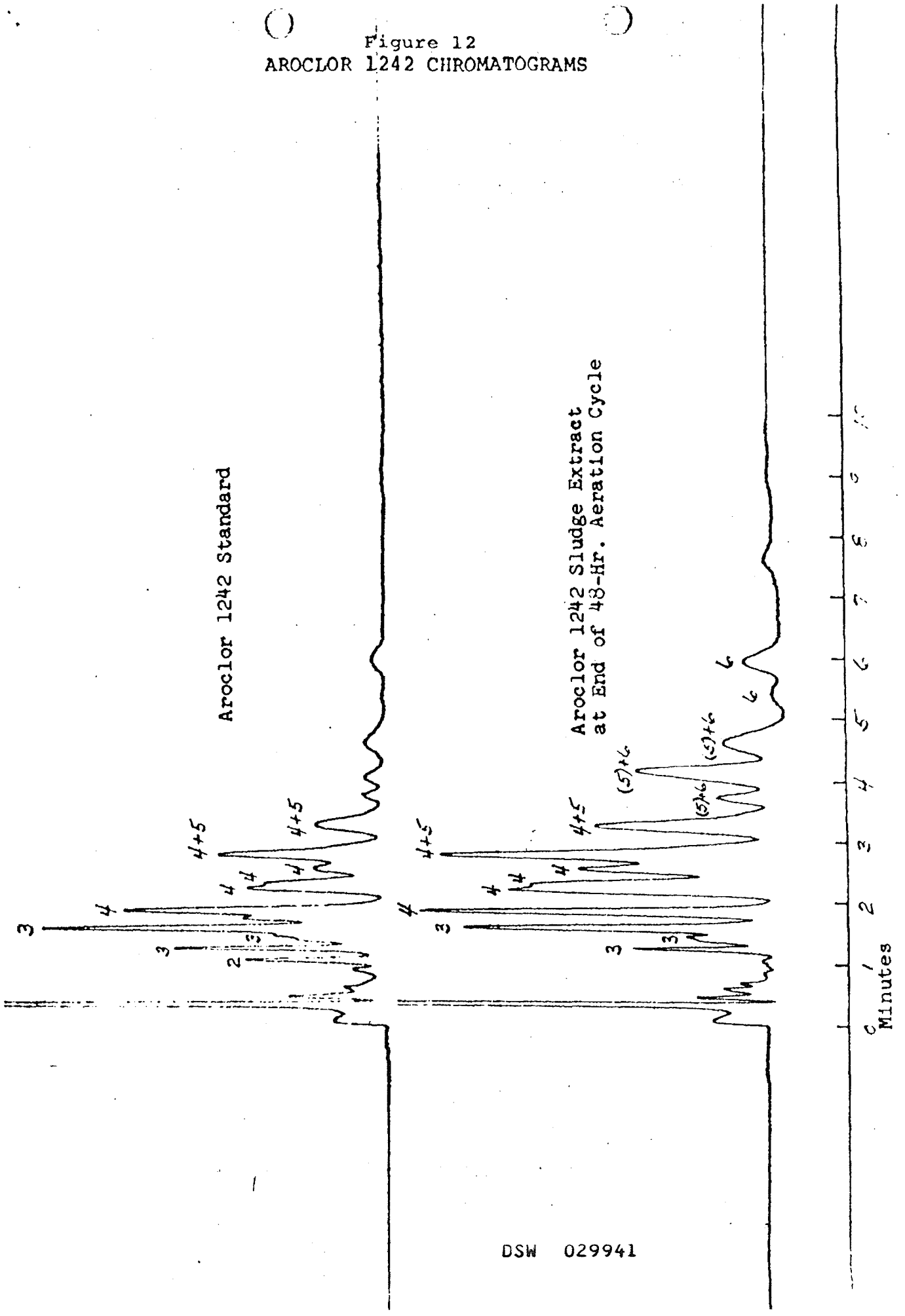
Figure 12
AROCLOR 1242 CHROMATOGRAMS



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