

Temporary
PREFACE

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-system¹ 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 degradation² 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 surfactants³. 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 mixture⁴ 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 state⁵ is achieved and consistent biodegradation rates are obtained. Details of the procedure may be found in the attached Analytical Chemistry Method 71-32.

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

<u>Test Material</u>	<u>% Cl (w/w)</u>	<u>Exposure Period(Hrs)</u>	<u>Mean Per Cent Disappearance Rate</u>
			<u>(Uncorrected)</u>
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

<u>Exposure Period(Hrs)</u>	<u>Aeration Rate(CFH)</u>	<u>Volatility Losses (%)</u>	<u>Mean Per Cent Disappearance Rate</u>	
			<u>Uncorrected</u>	<u>Corrected</u>
24	0.5	16.1	74.9 ± 3.8	58.8
24	0.1	4.5	69.9 ± 16.8	65.4
24	0.1	-	81.0 ± 5.8	-
48	0.1	-	80.6 ± 1.4	-

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

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

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.

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*

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

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.

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 10 and 11, 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

FIGURE 1-1
SCAS BIODEGRADATION DATA
AROCLO 1254

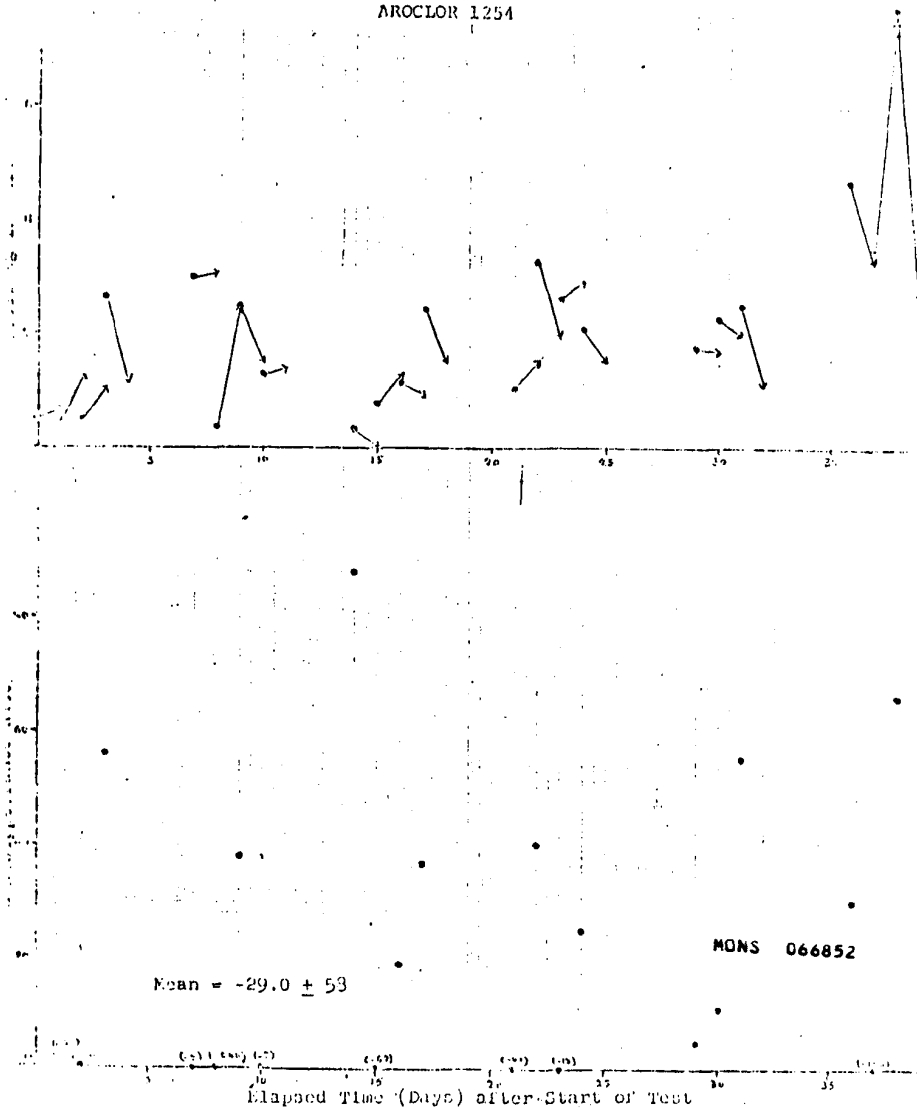


FIGURE 1-1
SCAS BIODEGRADATION DATA
AROCLOR 1254

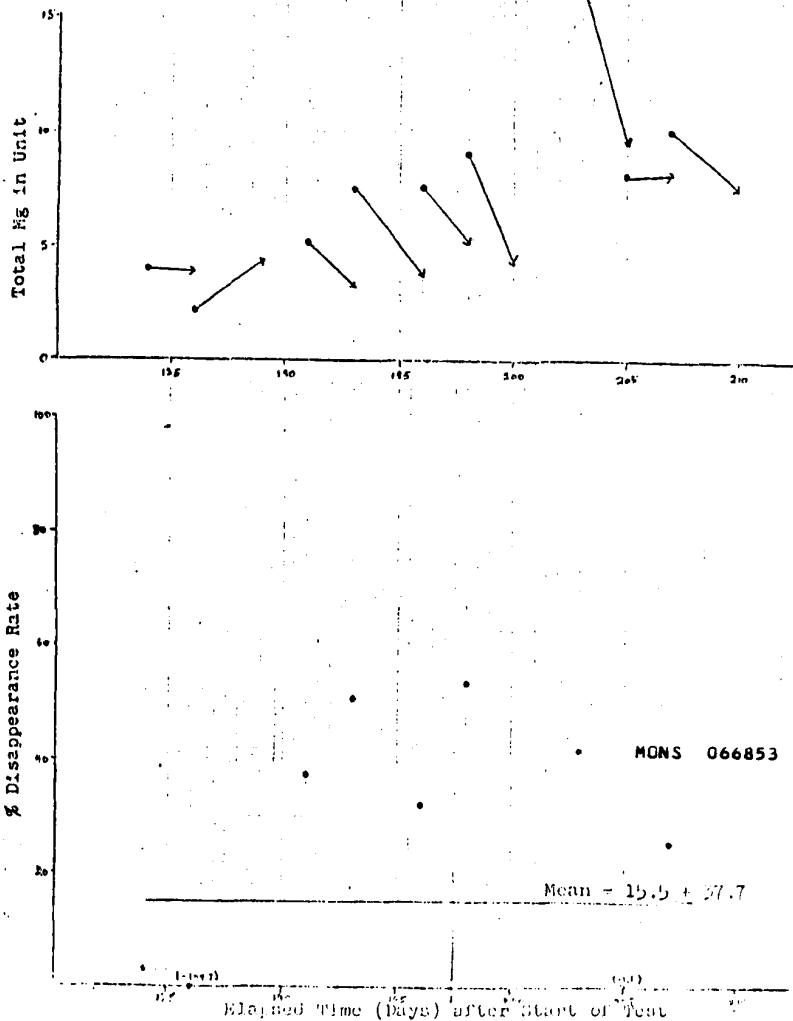


FIGURE 2-1
SCAS BIODEGRADATION DATA
AROCLO 1242

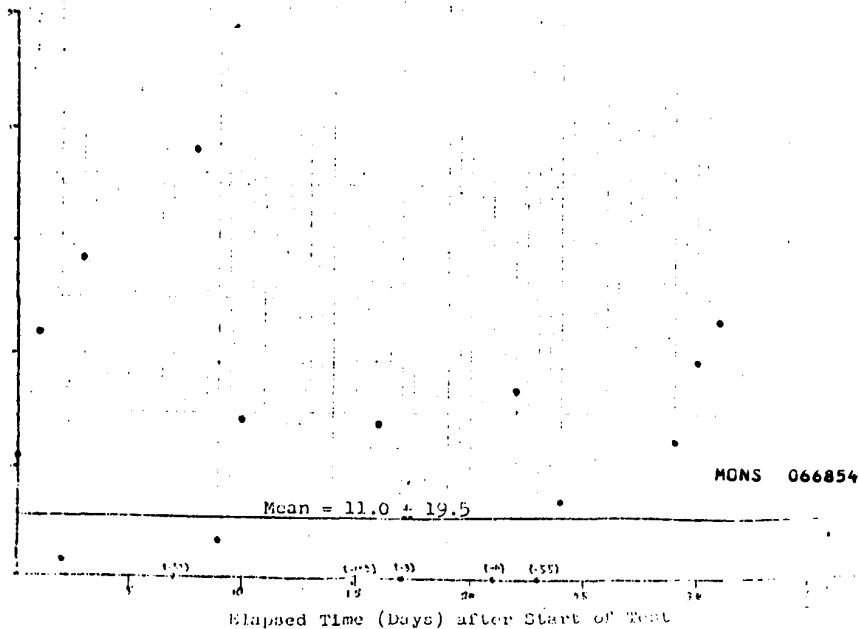
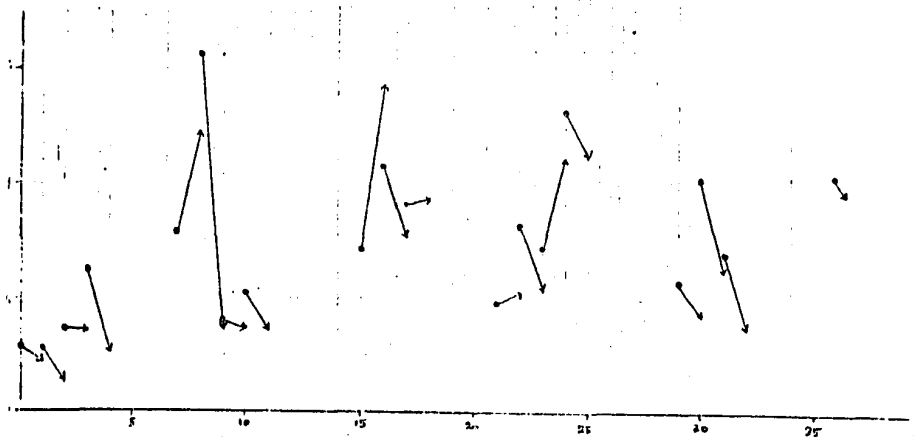
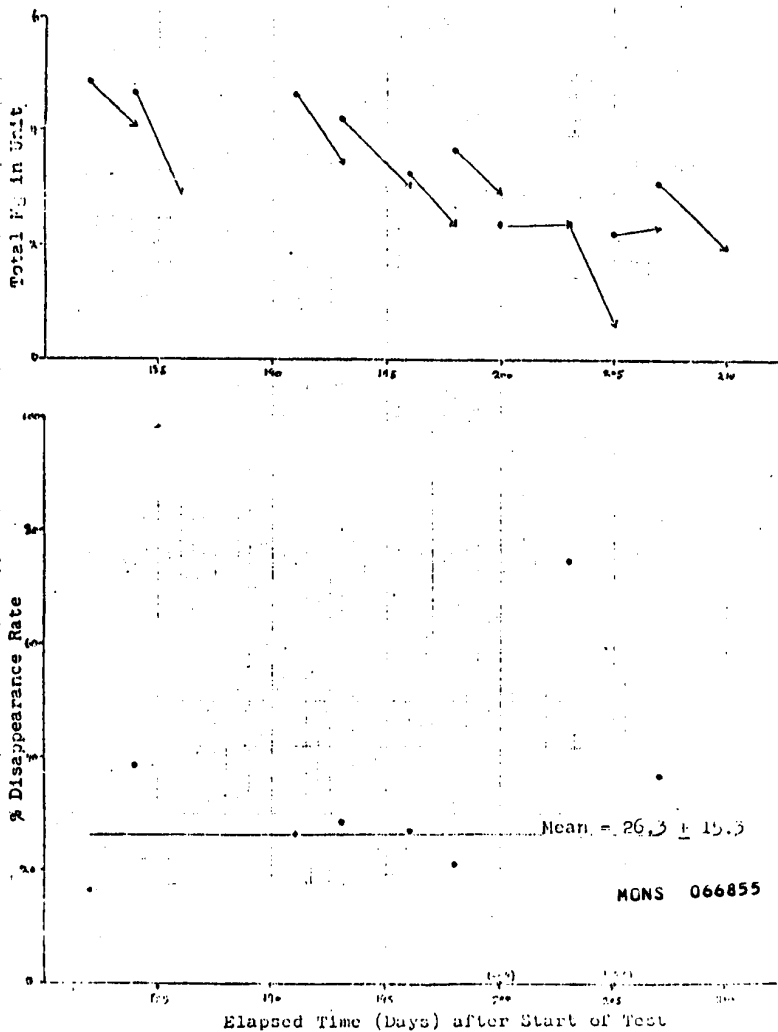
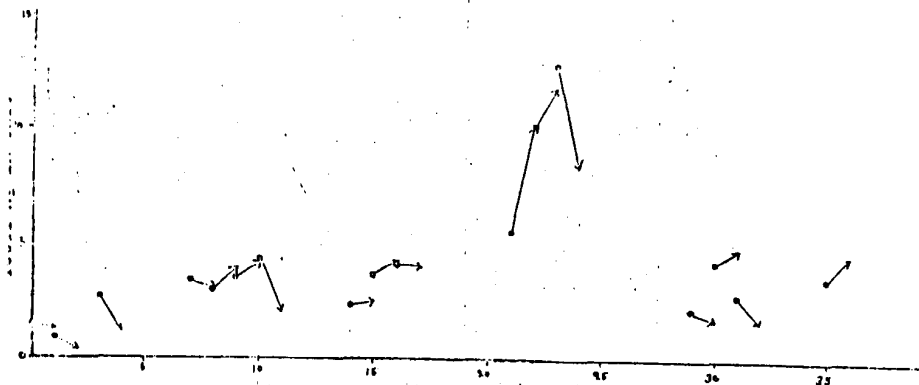


FIGURE 2-2
SCAS BIODEGRADATION DATA
AROCOR 1242



SCAS BIODEGRADATION DATA

MCS 1016



MONS 066856

Elapsed Time (Days) after Start of Test

Mean = 5.0 ± 10.0

FIGURE 3-2
SCAS BIODEGRADATION DATA
MCS 1016

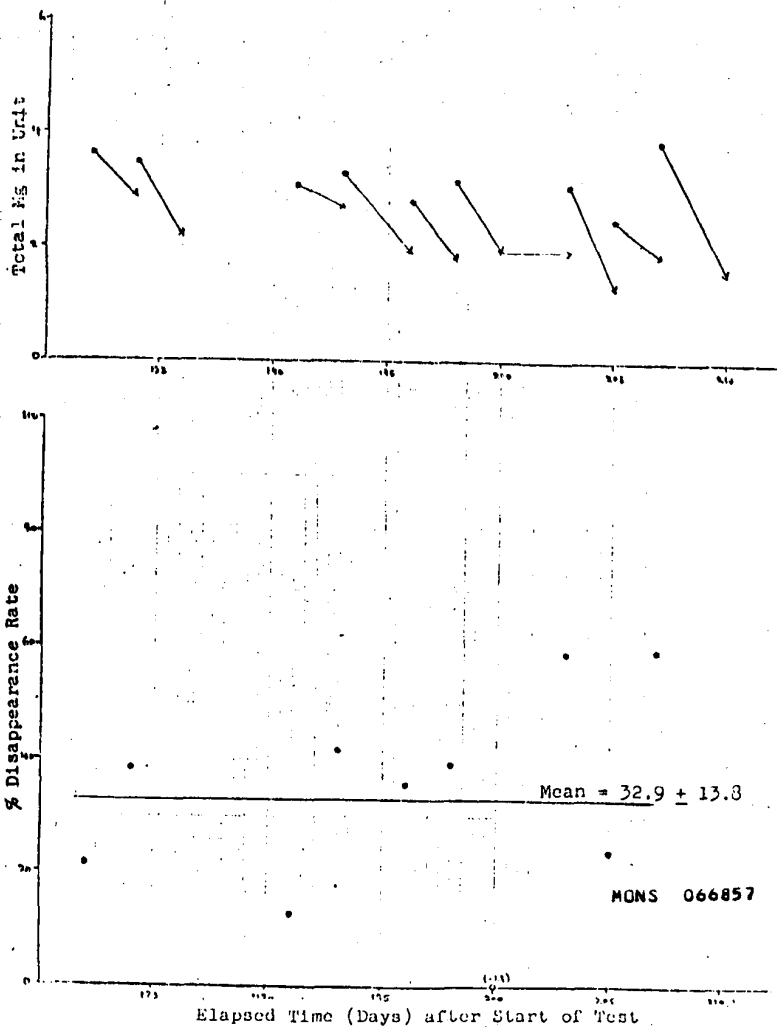


FIGURE 7-1
SCAM BIODEGRADATION DATA

MCS 1043

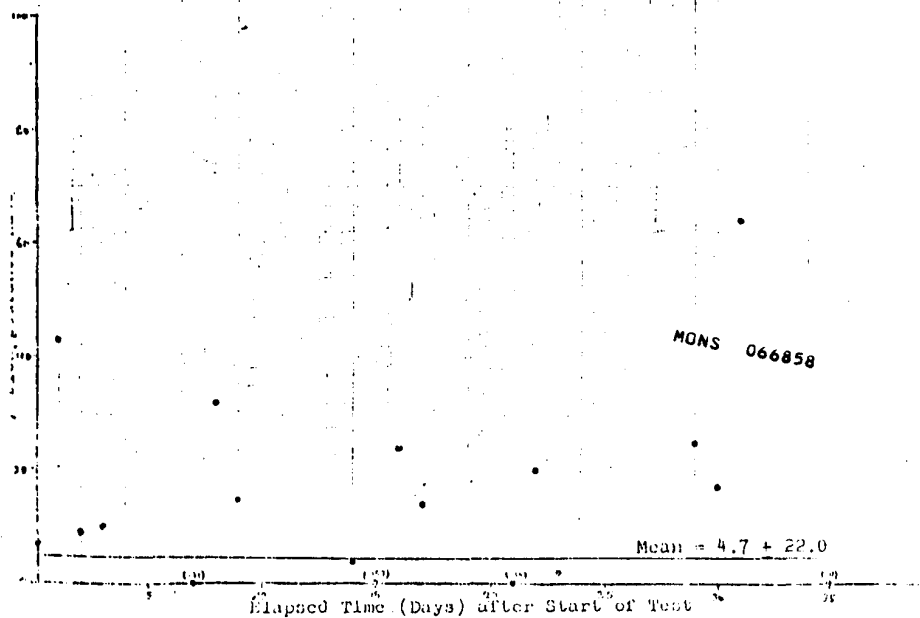
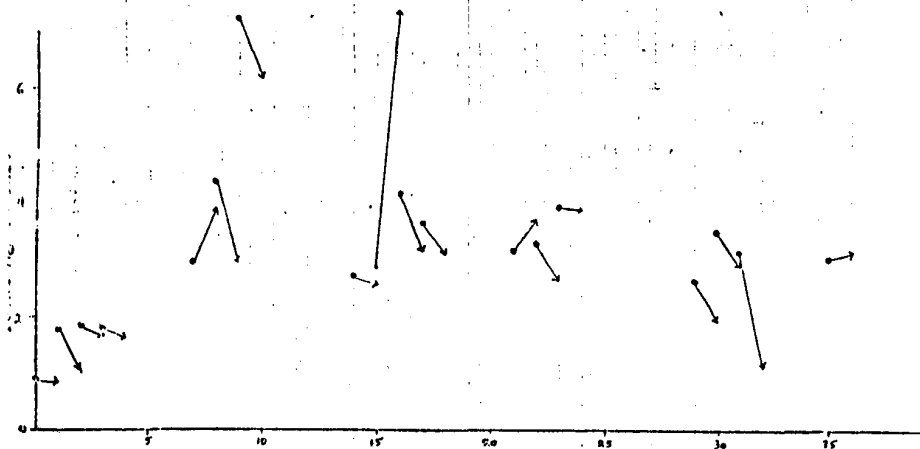
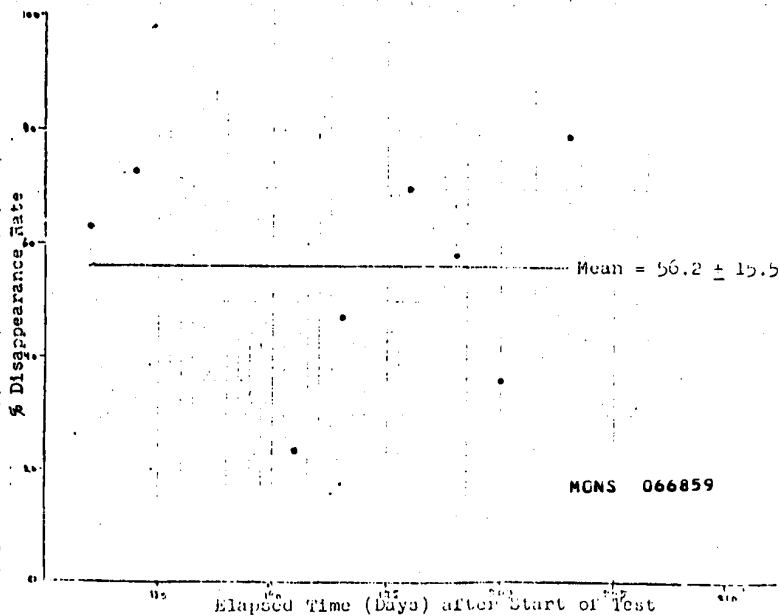
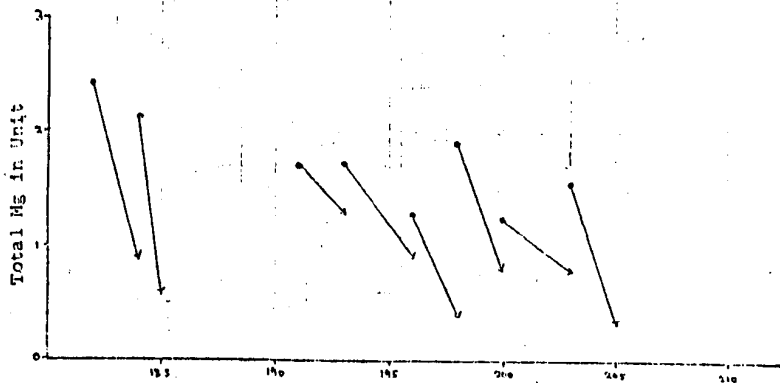


FIGURE 4-2
SCAS BIODEGRADATION DATA
MCS 1043



MCNS 066859

FIGURE 5
SCAS BIODEGRADATION DATA

AROCLOR 1221

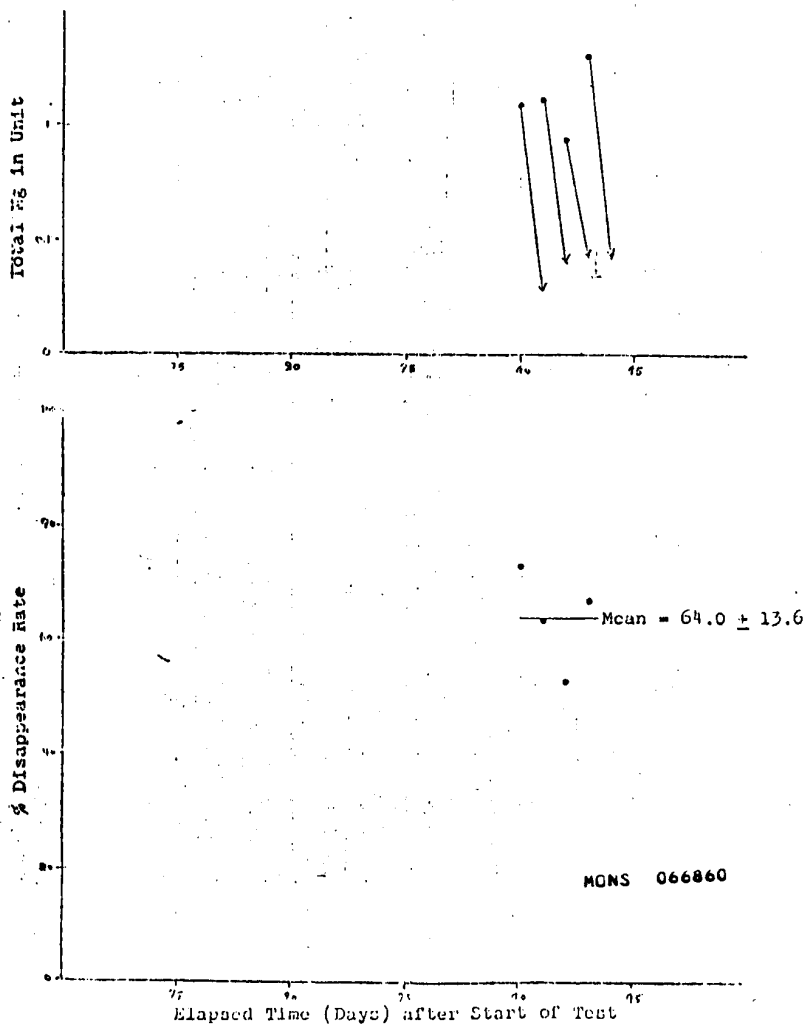
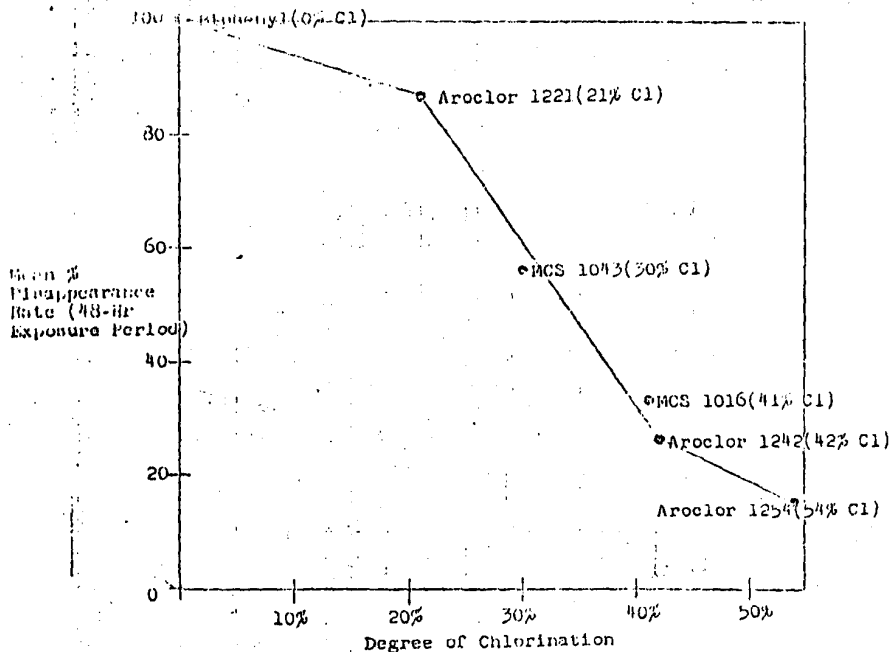


FIGURE 6

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



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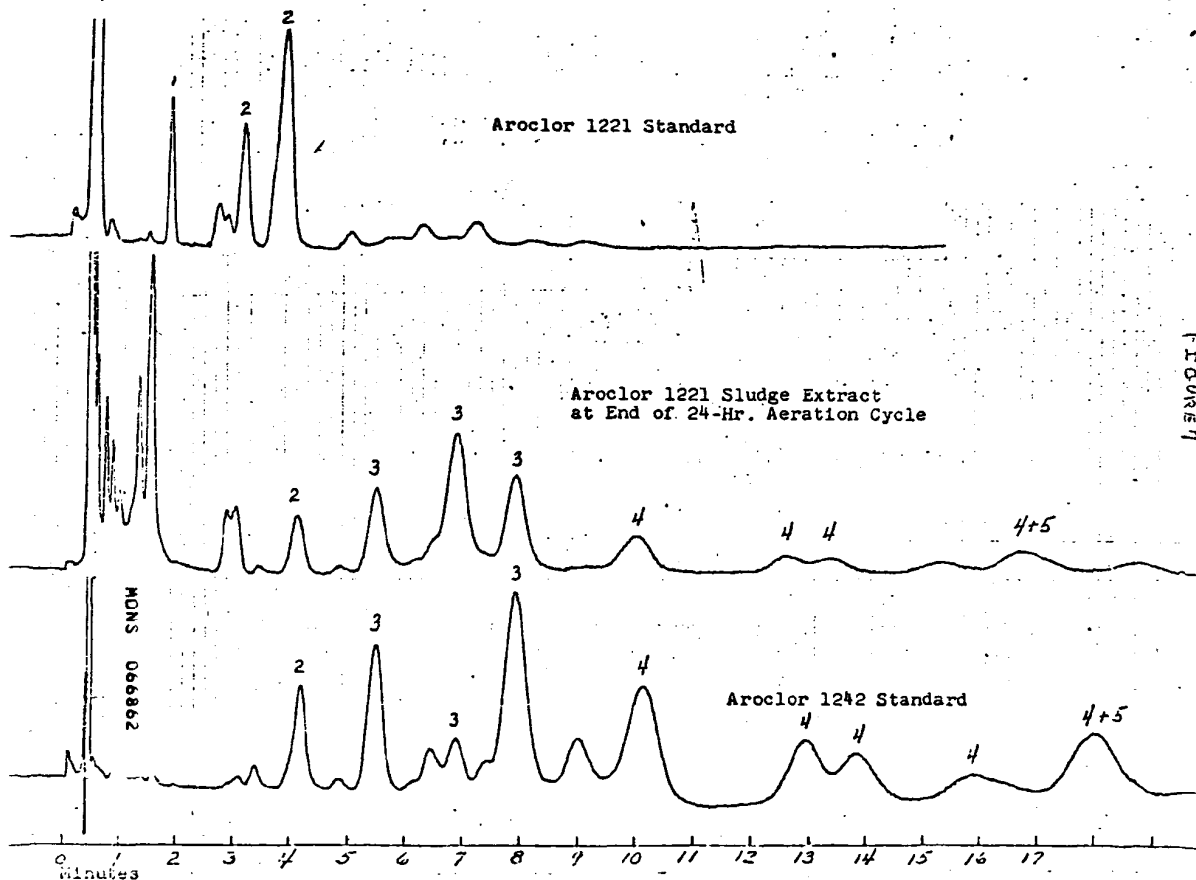
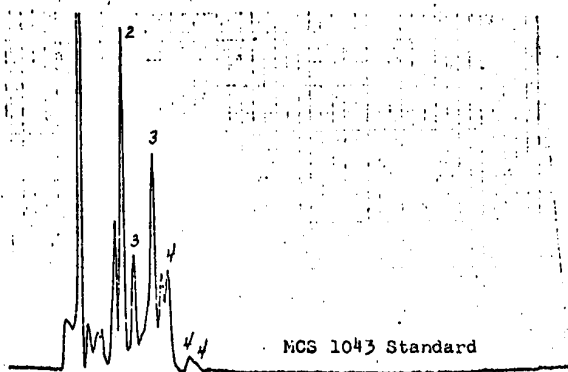
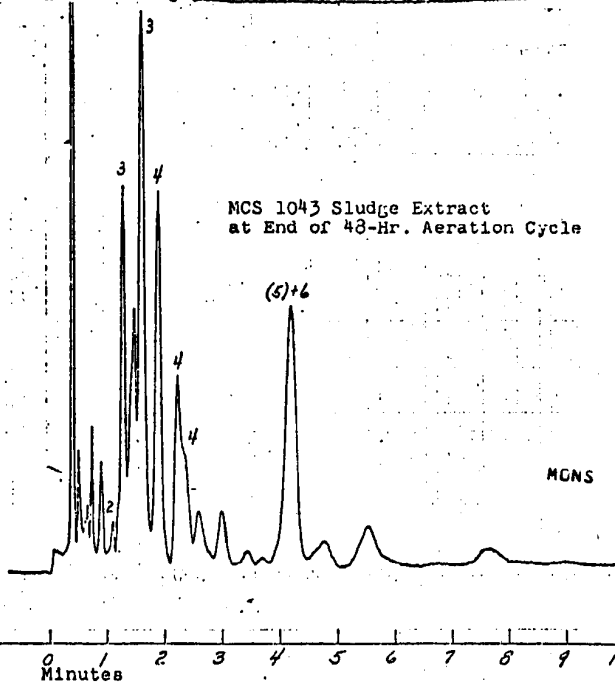


FIGURE 8



NCS 1043 Sludge Extract
at End of 48-Hr. Aeration Cycle



MCNS 066863

MONS 066864

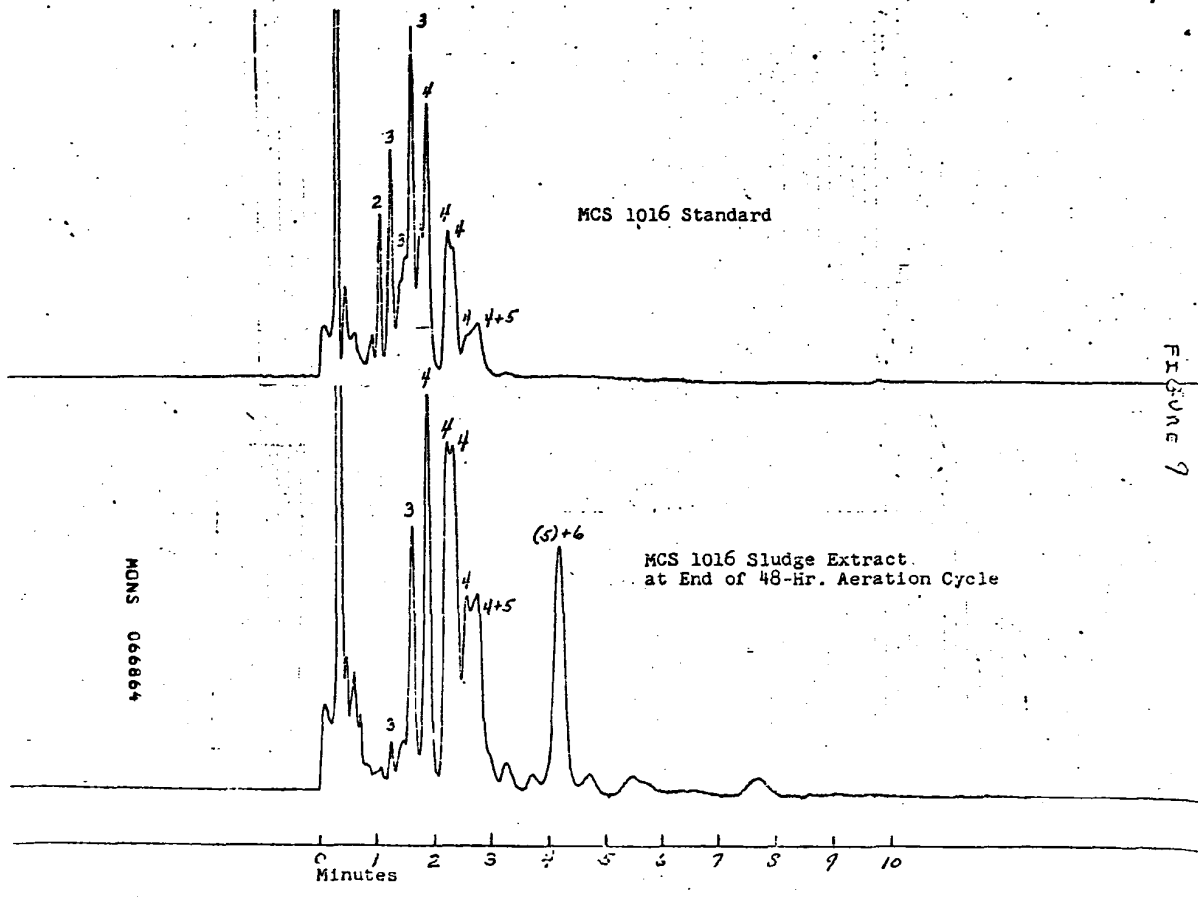
MCS 1016 Standard

MCS 1016 Sludge Extract
at End of 48-Hr. Aeration Cycle

FIGURE 9

Minutes

0 1 2 3 4 5 6 7 8 9 10



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