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June 8th, 1976

Monsanto Company
800 North Lindbergh Boulevard
St. Louis, Missouri 63166

SUBJECT: ANIMAL FEEDING STUDIES FOR TISSUE RESIDUE ANALYSIS
In Accordance With Modified Protocols For G. Levinskas, Ph.D., December 7, 1973, and H. O. Tucker, February 19, 1974

MONSANTO PROJECT NUMBERS: T-74-46 - T-74-55

Monsanto Project Number	Test Material	Exposure Level (PPM) Low	High	Number Of Days On Test	Date Sacrificed
PILOT STUDY --					
Test Started: January 30, 1974 (0 Day)					
Animals sacrificed in groups of five; each level and control					
T-74-52	MCB 1863	1	10	2	2/ 1/74
T-74-53	AROCLOR 1262	1	10	5	2/ 4/74
				10	2/ 9/74
D.P. TISSUE RESIDUE STUDY --					
Test Started: February 23, 1974 (0 Day)					
Animals sacrificed in groups of five; each level and control					
T-74-46	MCB 1633	25	100	2	2/25/74
T-74-47	MCB 1623	25	100	5	2/28/74
T-74-48	MCB 1238	25	100	10	3/ 5/74
T-74-49	MCB 1475	25	100	20	3/13/74
T-74-50	MCB 1302	5	25	30	3/25/74
T-74-51	MCB?	25	100	32	3/27/74
T-74-52	MCB 1863	25	100	38	3/ 3/74
T-74-53	MCB 1409	5	25	45	4/ 9/74
T-74-54	ARCLOR 1016	5	25	75	5/ 9/74
T-74-55	ARCLOR 1262	5	25	105	6/ 8/74 *

* T-74-53, T-74-54, T-74-55, and Control only

YOUNGER LABORATORIES, INC.

BY: MELVIN D. BIRCH

MONS 068244

MONSANTO INDUSTRIAL CHEMICALS COMPANY
APPLIED SCIENCES

ST. LOUIS

Date: June 13, 1975

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(Analytical Chemistry Special Study 74-6)

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DETAILS

A. Feeding Protocol

Following a pilot study of the feeding program designed to insure that the feed levels to be administered were not toxic and yet were sufficiently high to allow analysis of the tissue samples, the feeding program for this study was carried out in accordance with established protocol (Appendix A). Feeding and sample sacrifice were carried out by Younger Laboratories, Inc., St. Louis, Missouri.

Sprague-Dawley albino rats were fed (ad libitum) Ralston Purina rat chow impregnated at two levels with one of each of ten fluids for an exposure period of 30 days. Each batch of treated rat chow was sampled and analyzed to insure that proper exposure levels were being maintained.

At predetermined sampling intervals during the exposure period (days 0, 2, 5, 10, 20, and 30) fat, liver, and muscle tissues were excised from five animals for each fluid and feed level. Following the 30-day exposure period, all remaining rats were placed on the basal laboratory diet. At days 30, 35, 45, 75, and 105 of the study (days 2, 5, 15, 35, and 75 of the recovery period), tissues were sampled in a manner identical to that used during the exposure period. A control composite of animals fed only the basal laboratory diet was analyzed at each sampling date during the exposure and recovery periods to assess possible interferences in the analyses of the exposed animals. Samples were composited and quick-frozen in glass containers with foil-lined caps to minimize risk of contamination.

The above steps in the program were carried out at Younger Laboratories, Inc. Then the samples were delivered to the Analytical Chemistry group for sample clean-up and analysis. Animal weights and feed consumption were measured and recorded throughout the study to insure that no anomalies were present during administration of the feeding program.

B. Analytical Methods

The PCB fluids (MCBP, MCS 1043, MCS 1489, Aroclor 1016, and Aroclor 1242) were isolated from the tissues and measured according to Analytical Chemistry Method No. 74-35. The general procedure involved homogenation of the tissues with sodium sulfate and hexane, alumina column clean-up, concentration, and measurement by electron capture gas chromatography.

The non-PCB fluids, specifically MCS 1633, MCS 1622, MCS 1238, MCS 1475, and MCS 1302, were isolated and measured according to Analytical Chemistry Method Nos. 74-1, 74-2, 74-3, 74-4, and 74-16 respectively. These methods generally involve homogenation with sodium sulfate and hexane, alumina column clean-up, gel permation chromatographic separation into components, concentration, and measurement by gas chromatography with flame ionization or flame photometric detection.

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Recoveries from tissues spiked with known amounts of the fluids are documented in the respective methods. Recoveries of added MCS 1302 from tissues using hexane extraction averaged 79.1 ± 11.4%. Later experiments indicated that benzene or 10% diethyl ether in benzene gave essentially quantitative recoveries. Nevertheless, no TKS was detected in selected samples extracted with benzene.

C. Results and Discussion

Five of the dielectric fluids studied were PCB-type fluids. The fluids investigated were monochlorobiphenyl (MCBP), MCS 1043, Aroclor 1242, Aroclor 1016, and MCS 1489 (90% Aroclor 1016 and 10% TKS).

The quantitative data for the PCB fluids are shown in Table 1. These values represent the actual levels of the particular product found in the fat tissue in ppm on a lipid weight basis. In most cases the reported values are averages of replicate determinations.

MCBP was fed at levels of 25 ppm and 100 ppm of MCBP in the feed. Table 1 and Figure 1a show that MCBP reached a relatively stable level of about 9 ppm for the high feed level and 3 ppm for the low feed level when feeding began. These approximate levels were maintained throughout the 30-day exposure period. Upon removal of the impregnated feed, the tissue residue levels dropped in two days to low levels. No MCBP was detected in the fat tissue extracts five days after exposure was stopped. MCBP thus showed no tendency to accumulate or be retained in the fat tissue.

The behavior of MCS 1043 in the tissue residue study was similar to that of MCBP, in that it rapidly reached a plateau level upon initiation of feeding and maintained the level throughout the exposure period. This behavior is shown by Table 1 and Figure 1b. Upon removal of the impregnated feed, the level of MCS 1043 in the tissue extracts dropped rapidly to such an extent that it was not detected after 45 days of the study, i.e., 15 days of the recovery period.

Figure 2 shows the plots of the data for the 5 ppm and 25 ppm feed levels for Aroclor 1242. The curves illustrate that Aroclor 1242 accumulated rapidly in the fat tissue and continued to build up throughout the exposure period. The shape of the buildup curve, however, suggests that a plateau level is being reached. Upon removal of the impregnated feed, the Aroclor 1242 levels in the tissue declined rapidly. However, at day 105 of the study, or day 75 of the recovery period, detectable levels of Aroclor 1242 still remained in the tissue extracts. The residues in the tissues sampled after the exposure period consist of the more highly chlorinated isomers, as discussed below.

The plots of the residue levels for Aroclor 1016 and MCS 1489 are shown in Figure 3. The behavior of Aroclor 1016 was qualitatively similar to that of Aroclor 1242, in that it did accumulate in the tissues throughout the feeding period and decreased in concentration fairly rapidly during the recovery period. The quantitative differences between Aroclor 1016 and Aroclor 1242 will be discussed below.

Comparison of the curves of Figure 3 clearly demonstrates that Aroclor 1016 as a 90% constituent of MCS 1489 behaves in an identical manner to Aroclor 1016 administered as a pure product. TRS at the 10% level thus exhibited no synergistic effect on the quantitative behavior of Aroclor 1016.

Since MCS 1489 is 90% Aroclor 1016, it would be expected that the ratio of the Aroclor 1016 residue levels of MCS 1489 to those of the pure Aroclor 1016 should be 0.9. The composite experimental ratio for the data of this study was calculated to be 0.85, which is within the precision limits of the method used to determine the residues. This agreement further substantiates the lack of a synergistic effect of the TRS on Aroclor 1016.

Figure 4 shows the composite plots of the 25 ppm feed level curves for Aroclor 1242, Aroclor 1016 and MCS 1043. The behavior of each product has been described above. However, this set of curves emphasizes the relationships in the behavior among the products at the same feed level. The difference in the behavior of MCS 1043 is obvious, since it did not accumulate and it declined to less than the detection limit rapidly.

Aroclor 1242 and Aroclor 1016 behave similarly. However, the levels of Aroclor 1016 were lower than those of Aroclor 1242. A test was made to determine whether this difference was statistically significant based on the estimated precision of the determinations. Using the range of logarithms estimation of test error, the 95% confidence level limits for the determination of Aroclor 1016 and Aroclor 1242 were calculated to be 79% to 126% of the average value of a data point. In order to give an estimation of the significance of the difference between Aroclor 1016 and Aroclor 1242, these test error limits were superimposed on theoretical build-up and recovery plots (based on multiple regression analysis of the actual data) for the two products (see Appendix B). These plots are shown in Figures 5 and 6. The difference in residue levels for the two products is significantly different where the superimposed error limits on the regression curves do not overlap. As can be seen from the graphs, at day 17 of the study the difference between the levels became statistically significant. After day 17, there is no overlap of limits throughout the remainder of the exposure period and the entire recovery period. Thus, there is a significant difference in the tissue residue levels of Aroclor 1016 and Aroclor 1242.

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Figures 7, 8, and 9 are chromatograms of the impregnated feed and fat tissue extracts (30-day exposure samples) for Aroclor 1242, Aroclor 1016, and MCS 1043, respectively. Figure 7, the chromatograms for Aroclor 1242, shows that significant differences existed between the material fed and the material retained in the fat tissue. Most of the early peaks in the extract of the feed have disappeared or decreased in relative size, indicating that the corresponding components were not retained in the tissue. These peaks correspond to PCB isomers with a low degree of chlorination, i.e., tri- and dichloro-biphenyls. Some peaks corresponding to more highly chlorinated isomers increased in relative size, indicating a greater tendency to accumulate in the tissue. However it should be noted that since relative electron capture detector response increases with increasing chlorine substitution on the biphenyl molecule, a peak corresponding to a tetra-isomer, for example, results from a much smaller absolute amount of PCB than a peak of equal area corresponding to a di- isomer.

Chromatograms for Aroclor 1016 are shown in Figure 8. There is exact correspondence of peaks in the early portions of these chromatograms with peaks in the Aroclor 1242 chromatograms. The products differ markedly, however, in the regions where more highly chlorinated isomers elute. Examination of the 30-day exposure chromatograms of the tissue residue of Aroclor 1016 shows that there was no accumulation of the more highly chlorinated isomers analogous to that noted for Aroclor 1242.

The feed and residue chromatograms for MCS 1043 are shown in Figure 9. These two chromatograms are not significantly different with respect to isomer distribution. However, MCS 1043 did not rapidly or significantly accumulate in the fat tissue, indicating that all of the isomers present in MCS 1043 were subject to rapid depletion.

Chromatograms are not shown for the other two PCB-type fluids. Since the analyte in MCS 1489 was Aroclor 1016, the chromatograms exactly correspond with respect to isomer distribution. This correspondence indicates that THB at the 10% level in MCS 1489 exhibited no notable synergistic effect on the qualitative behavior of Aroclor 1016 in the feeding study.

Since the chromatogram of MCBP is a single peak and MCBP did not accumulate in the tissues, chromatograms of MCBP are not particularly informative and are not included.

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The second part of this oral feeding study involved the determination of the tissue residue levels of five hydrocarbon-based non-PCB dielectric fluid candidates. The products studied were MCS 1302 (ethylxylylsulfone (EXS)), MCS 1238 (75% EXS, 75% monoisopropylbiphenyl (MIPB)), MCS 1475 (90% Santicizer 711 (S-711), 10% EXS), MCS 1623 (50% Santocel 100 (S-100), 25% MCS01, 25% EXS), and MCS 1633 (75% EXS, 75% 27DA still bottoms (25% hydrogenated)). The principal analytes were EXS (MCS 1302), S-711 (MCS 1475), 27DA 25% hydrogenated still bottoms (MCS 1633), MIPB (MCS 1238), and S-100 (MCS 1623). The EXS fractions of several samples were analysed to see if synergistic effects were present.

The data for the determination of the components of the non-PCB fluids are shown in Table 2. The behavior of MCS 1623, MCS 1633 and MCS 1238 was similar with respect to the levels of the fluids found as residues in the fat tissues. For all three fluids, the residue levels for the high (100 ppm) feed level rapidly reached a plateau level of about 2 ppm on a lipid weight basis, which was maintained throughout the exposure period. When oral exposure was ceased after 30 days, the levels in the tissue declined to below the detection limit for MIPB and 27DA 25% hydrogenated still bottoms and to near the detection limit for S-100.

Only the 30-day high level exposure sample for S-711 in MCS 1475 contained detectable S-711. Since the 10, 20, and 32-day samples had no detectable S-711, no further samples were analysed.

None of the MCS 1302 samples analysed had detectable EXS in the tissue extracts. Thus, no samples were analysed beyond those from the 30-day exposure period.

Although the non-PCB fluids did have minor differences in behavior, the hydrocarbon-based products showed no tendency to accumulate in the fat tissue during oral exposure at 25 ppm and 100 ppm feed levels. Nor were the residues retained in the fat tissue for an extended period of time after feeding of impregnated chow was stopped.

In addition to the complete sets of samples run to determine residue levels of MIPB, S-711, S-100, and 27DA still bottoms from the fluids, the EXS fraction of selected samples were analysed to determine if any of the components had a synergistic effect resulting in tissue retention of EXS, a component of all the fluids of the non-PCB-type and of MCS 1489. As shown by the data in Table 2 for MCS 1475, MCS 1238, MCS 1623, MCS 1633, and MCS 1489, no synergistic effect was evident, since no EXS was detected at the 30-day high feed level data points.

Since non-TXS-containing samples of the fluid mixtures were not fed during this study, it was not possible to determine if TXS affected retention of the other components, except in the case of Aroclor 1016 (MCS 1489) which was discussed above. However, since the levels of the other components are quite low, no effect would be expected.

Figure 10 shows a composite graph of the 25 ppm feed level residues of the non-PCB-type fluids compared to the 25 ppm feed level Aroclor 1242 data. The behavior of the hydrocarbon products was obviously significantly different than that of the typical PCB product. The non-PCB fluids did not accumulate as Aroclor 1242 did, nor were they retained to any extent during the recovery period. These fluids were much more readily metabolized and/or excreted than Aroclor-type fluids containing PCB isomers of four or more chlorine atoms per biphenyl molecule.

Figures 11, 12, 13, and 14 show chromatograms of feed extracts and 30-day or 30-day exposure tissue extracts for MIPB, S-711, 27DA 25% hydrogenated still bottoms, and S-100, respectively. No chromatograms are shown for MCS 1302 since the chromatogram of the substance is a simple two-peak chromatogram and no TXS was detected in any of the samples analyzed.

The chromatograms of MIPB in Figure 11 show that the first two peaks, attributable to mono-substituted isopropylbiphenyl, are greatly reduced in intensity with respect to the second group of peaks, attributable to di-substituted species, in the 30-day exposure sample. Further, there is some isomer distribution alteration in the di-substituted biphenyl peaks. These observations indicate that the mono- and certain of the di-substituted species were more readily metabolized.

Figure 12 is the chromatograms for S-711. The peaks in the 30-day exposure residue are so small that little information can be inferred, other than the fact that some of the components of S-711 were retained to an appreciable extent in the fat tissue.

Figure 13, the chromatograms of 27DA 25% hydrogenated still bottoms, shows that the lower boiling components were retained to a lesser extent than those corresponding to the later-eluting peaks.

Based on the chromatograms of Figure 14, it can be seen that S-100 behaved in an analogous manner to MIPB, in that the mono-substituted species were not retained to any measurable extent in the fat tissue, while the di-substituted species were retained to some extent.

Finally, in the cases of both types of dielectric fluids, complete analysis of tissue samples was carried out for the fat tissues of the animals. Additionally, the 30-day high feed level samples of the liver and muscle tissues were also analyzed. The data for these analyses are shown in Table 3. Since the levels were not significantly different than those in the fat tissue, it was not necessary to analyze any further liver and muscle tissue samples.

Monsanto Industrial Chemicals Co.
Applied Sciences
St. Louis, Missouri

5/75 - R. G. Haley, G. Hicks, W. H. Rees, E. S. Tucker, J. P. Moore

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Special Study 74-6
Job No. 16250

TABLE I

[illegible] BOILING LEVELS OF PCB-TYPE FLUIDS

Analyte	Days	Fraction Values									
		0	5	10	20	30	32	35	45	75	105
MCBP	25 ppm	3.8	3.8	8.2	8.8	0.5	0.1				
	100 ppm	6.9	9.8	9.4	9.8	6.4	1.1				
MCB 1043	25 ppm	3.8	3.6	8.3	8.6	8.0	1.3	0.5	0.1		
	100 ppm	16.0	7.8	8.4	7.1	11.5	4.0	0.9	0.2		
Aroclor 1242	5 ppm	8.6	4.1	6.9	9.7	10.4	11.9	11.1	8.5	3.2	1.2
	25 ppm	6.4	25.4	30.4	31.9	37.3	34.9	32.2	30.4	8.1	4.2
Aroclor 1016	5 ppm	2.2	2.1	3.4	6.6	6.5	4.9	3.5	2.7	0.7	0.5
	25 ppm	8.1	13.8	17.2	28.5	22.7	20.3	17.5	14.3	4.9	1.9
MCB 1089 (1016 Fraction)	5 ppm	1.6	2.3	2.8	6.8	4.3	4.3	4.2	3.2	0.8	0.5
	25 ppm	7.9	11.3	12.3	28.2	21.0	20.4	15.6	9.5	2.3	2.1

MONS 068253

FIGURE 1A
Fat tissue residue level vs
time plots for mono-chlorobiphenyl

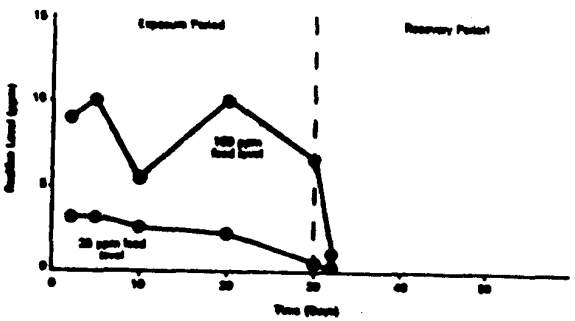

FIGURE 1B
Fat tissue residue level vs
time plots for MCB 1243

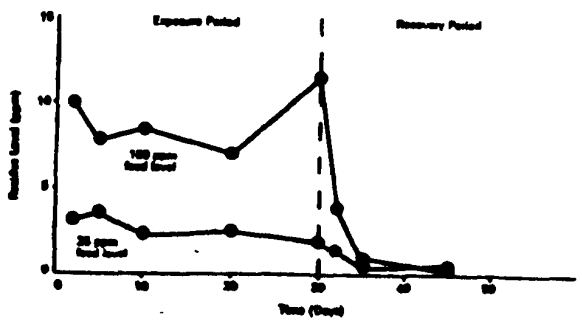

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FIGURE 2
Fat tissue residue level
vs time plots for Aroclor 1242

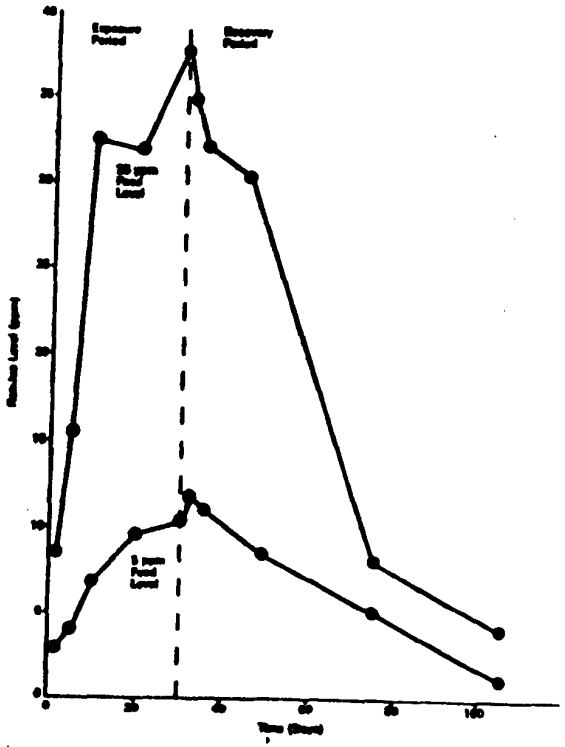

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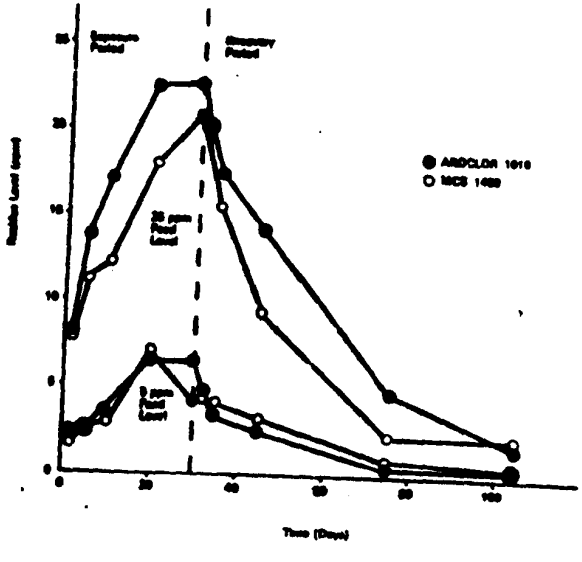
FIGURE 3
FAT TISSUE RESIDUE LEVEL
VS TIME PLOTS FOR AROCLOR 1016
AND MCS 1489 (AROCLOR 1016 FRACTION)



and MCS 1953 at 25 ppm feed level for rats

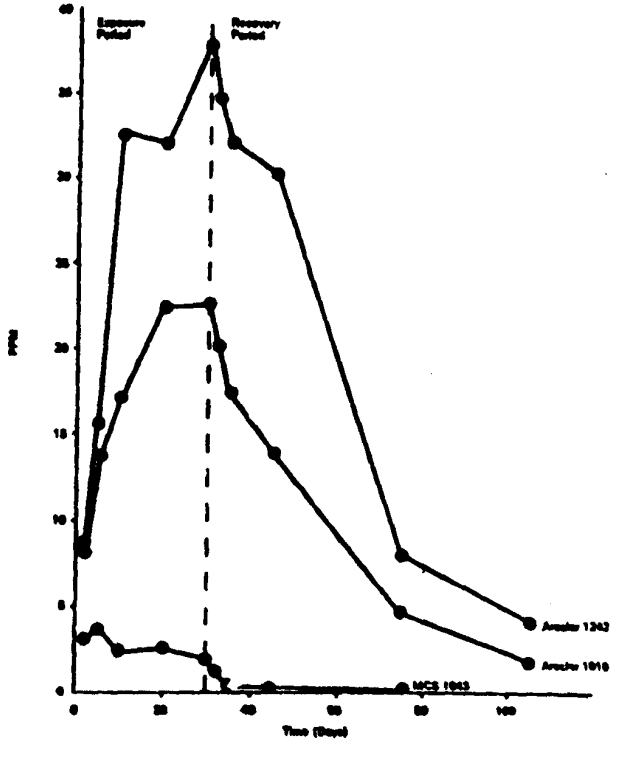


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FIGURE 6

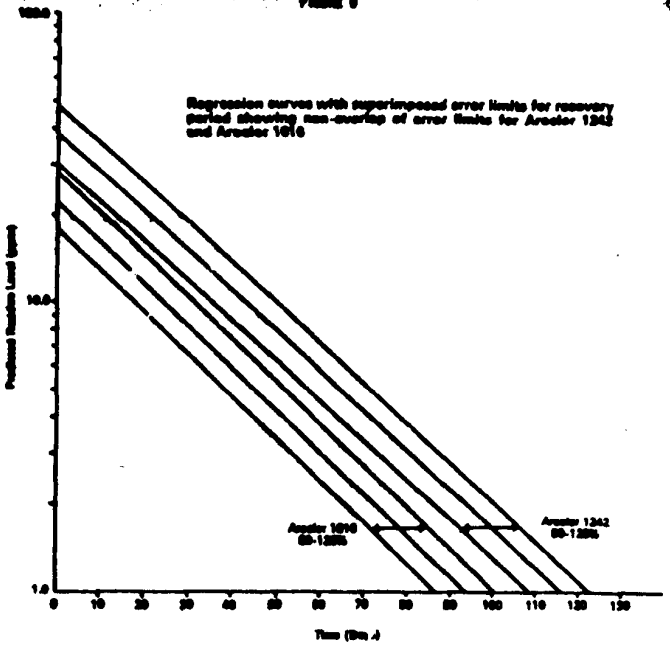

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FIGURE 7

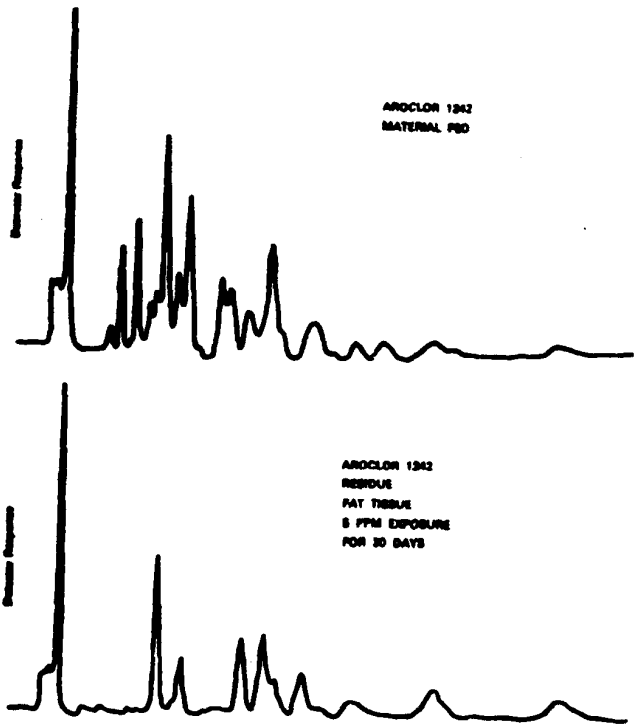
AROCLOR 1242
MATERIAL FED
AROCLOR 1242
RESIDUE
FAT TISSUE
5 PPM EXPOSURE
FOR 30 DAYS
Retention Time

FIGURE 8

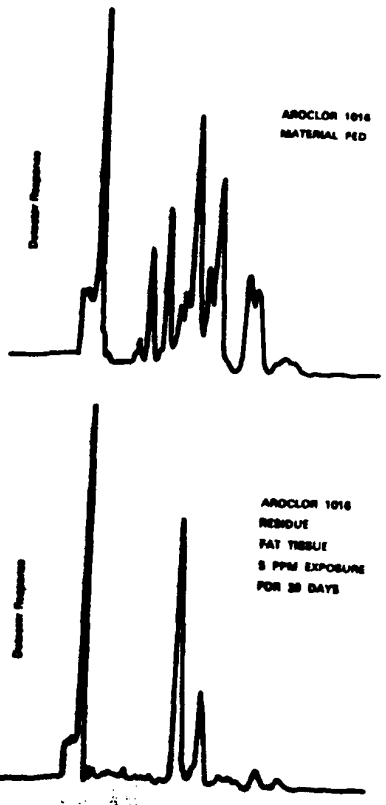

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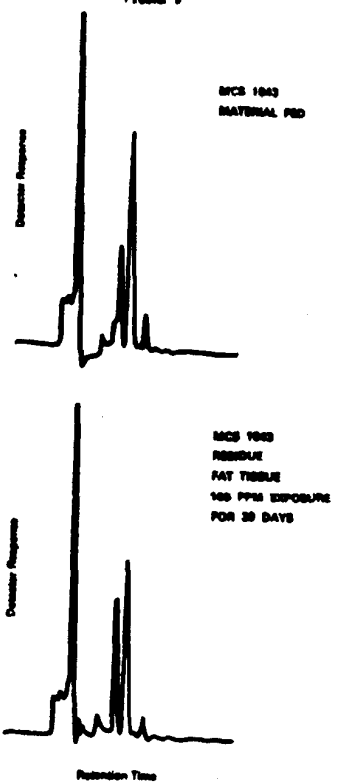
FIGURE 9



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[redacted]

TABLE 2 FAT TISSUE RESIDUE LEVELS OF NON-PCB-TYPE FLUIDS

Analyte	Day	2	6	10	20	30	32	35	65	75		
MCS 1633 (27 0426 Fraction)	25 ppm	1.0	0.5	0.6	0.8	<3*	<3*	<3*	<3*	[redacted]0.4		*Possible Presence of 27 0426 Masked By Solvent Impurities
	100 ppm	2.6	0.9	1.2	1.3	<3*	<3*	<3*	<3*	<0.4		
MCS 1682 (S-100 Fraction)	25 ppm	<1	0.5	0.4	0.6	0.8	0.6	0.7	0.4	0.3		
	100 ppm	0.9	2.0	1.0	2.2	2.7	1.6	1.8	1.2	0.6		
MCS 1230 (RIPB Fraction)	25 ppm	0.4	0.5	0.9	0.9	0.8	1.2	0.5	0.4	<0.4		
	100 ppm	2.0	2.2	2.0	3.0	2.3	1.0	2.1	0.7	<0.4		
MCS 1476 (S-711 Fraction)	25 ppm											
	100 ppm			<1.5	<1.0	1.2	<1.5					
MCS 1302	25 ppm	<0.5	<0.5	<0.25								
	100 ppm	<1	<0.5	<0.25	<0.25	<0.25						
MCS 1476 (TXS Fraction)	100 ppm					<0.25						
MCS 1230 (TXS Fraction)	25 ppm	<0.1		<0.1								
	100 ppm	<0.1	<0.1	<0.1		<0.25						
MCS 1683 (TXS Fraction	100 ppm					<0.25						
MCS 1680 (TXS Fraction)	100 ppm					<0.25						
MCS 1681 (TXS Fraction)	100 ppm					<0.25						

[redacted]

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FIGURE 10

Fat tissue residue level vs time plots comparing Aroclor 1262 and non-PCB type fluids

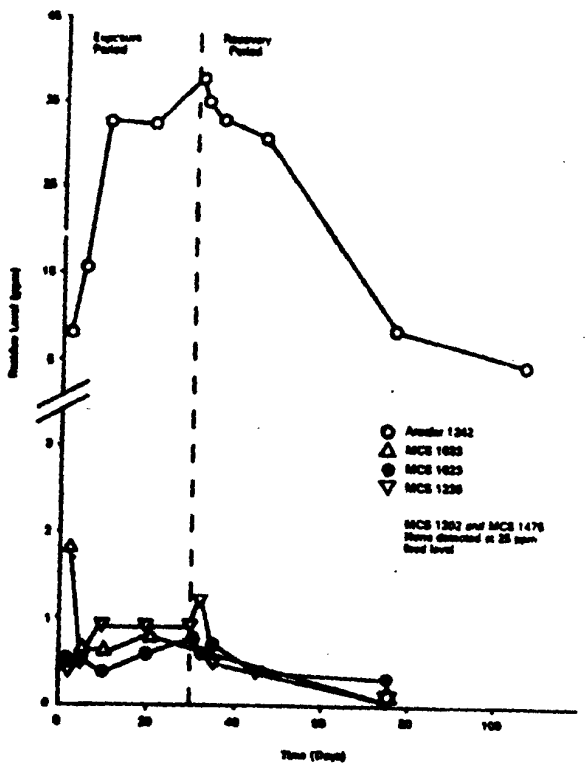

FIGURE 12

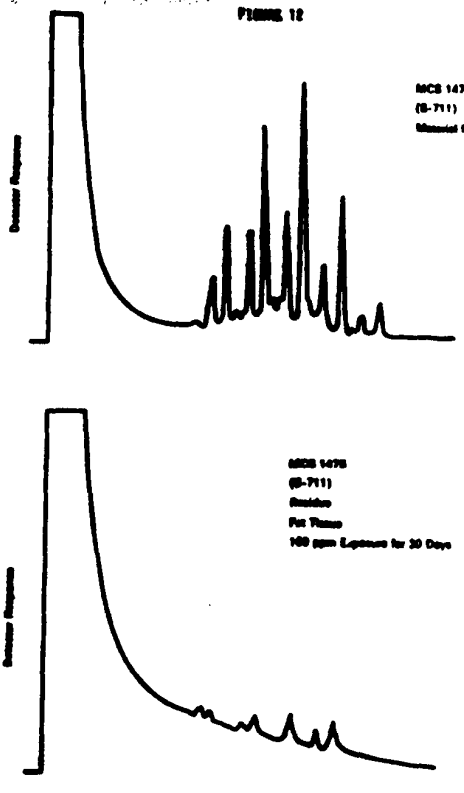

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FIGURE 10

Rat tissue residue level vs time plots comparing Aroclor 1242 and non-PCB type fluids

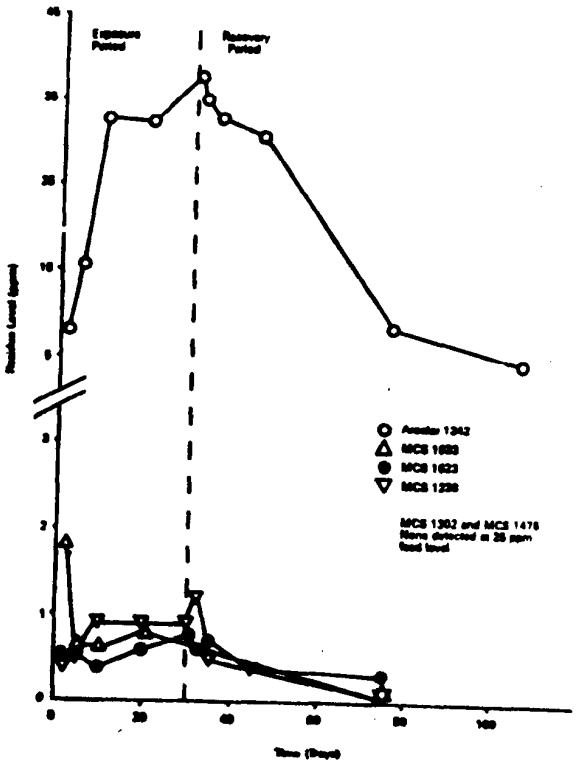

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FIGURE 11

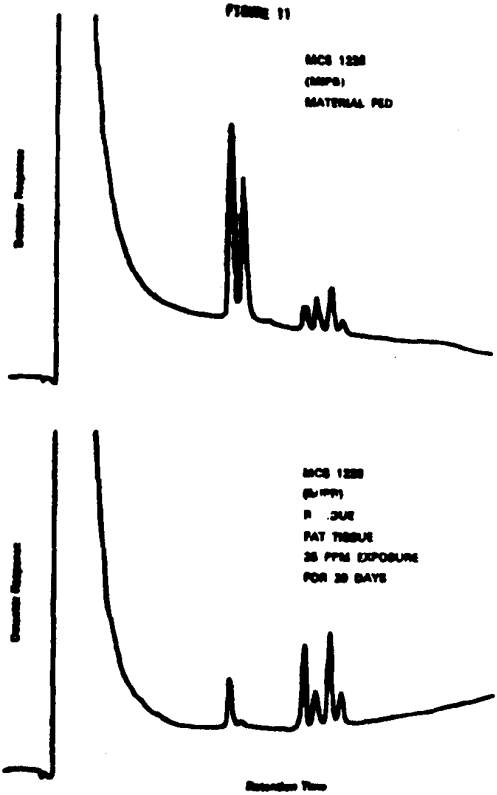

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FIGURE 13

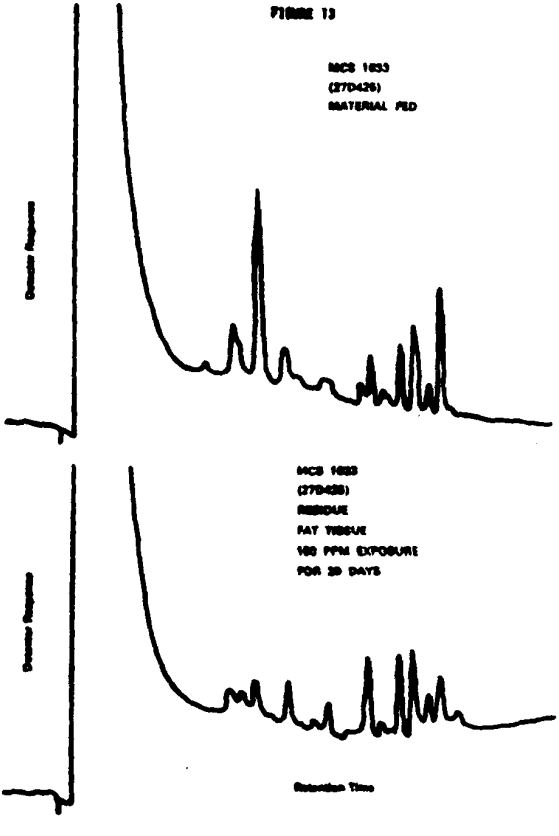
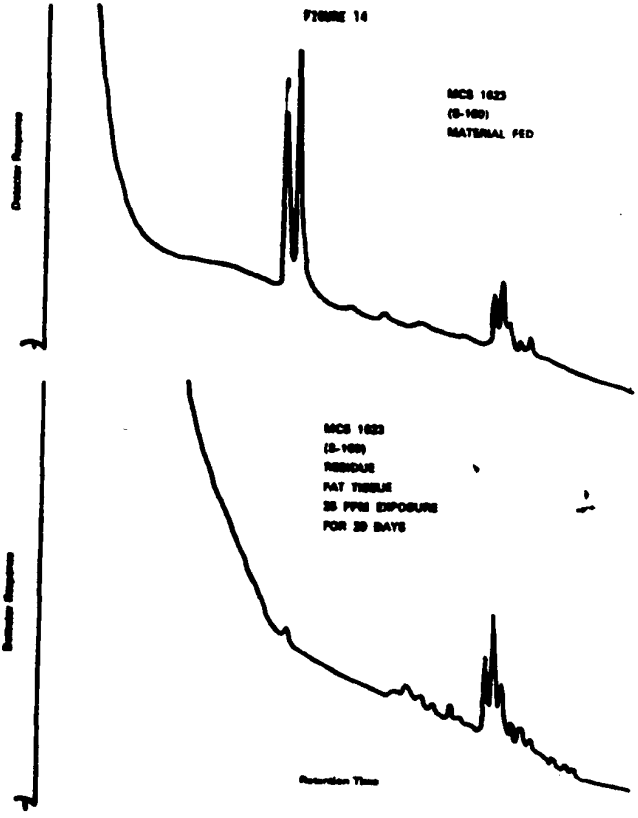

MCS 1653
(27D426)
MATERIAL FED
Detector Response
MCS 1653
(27D428)
RESIDUE
FAT TISSUE
100 PPM EXPOSURE
FOR 30 DAYS
Detector Response
Retention Time

FIGURE 14


Detector Response
MCS 1623
(S-169)
MATERIAL FED
MCS 1623
(S-169)
RESIDUE
FAT TISSUE
35 PPM EXPOSURE
FOR 30 DAYS
Detector Response
Retention Time

Special Study 74-6
Job No. 16250

TABLE 3

30-DAY EXPOSURE LIVER, MUSCLE, AND FAT
RESIDUE LEVELS (LIPID WEIGHT BASIS)

	Muscle (ppm)	Liver (ppm)	Fat (ppm)	Feed Level (ppm)
MCS 1633 (2704 25 Fraction)	<4	<4	<3	100
MCS 1623 (5-100 Fraction)	5.6	3.6	2.7	100
MCS 1238 (HIPB Fraction)	2.2	<3	2.3	100
MCS 1502	<1	<1	<.25	100
MCBP	7.4	9.1	6.4	100
MCS 1043	8.4	15.9	11.5	100
Aroclor 1242	42.0	60.4	37.3	25
Aroclor 1016	31.3	46.3	22.7	25
MCS 1486 (1016 Fraction)	23.4	40.0	21.0	25

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APPENDIX A

PILOT STUDY

2 compounds

70 male rats

Experimental setup -

For each compound:

15 rats fed 1 ppm for up to 10 days

15 rats fed 10 ppm for up to 10 days

After 2, 5 and 10 days, 5 rats from each group with each compound (total of 30 rats at each interval) to be killed. Fat, liver and muscle to be taken from each animal, packed in dry ice, and sent to Dr. R. S. Tucker for analysis. The same tissues from each of the 5 animals in a given group are to be pooled, i. e., 5 livers from low level of compound 1 grouped; 5 livers from high level of compound 1 grouped, etc. There is to be no pooling of all tissues from any one animal and no pooling across group lines.

Ten rats will be control animals. Fat, liver and muscle will be taken from 5 of these animals killed at the start of the study. The second group of 5 will be killed and tissues taken at the end of the study.

FINAL STUDY

8 compounds plus control group

850 male rats

Experimental setup -

For each compound:

50 rats fed low level of compound for up to 30 days after which time remaining animals are returned to a control diet for up to 75 days, perhaps longer.

50 rats fed high level of compound for up to 30 days after which time remaining animals are returned to a control diet for up to 75 days, perhaps longer.

After 2, 5, 10 and 30 days on test, 5 rats from each group with each compound (total of 85 rats at each interval) are to be killed. Fat, liver and muscle to be taken from each animal, packed in dry ice, and sent to Dr. R. S. Tucker for analysis. The same tissue from each of the 5 animals in a given group

are to be pooled, i. e., 5 livers from control grouped; 5 livers from low level of first compound grouped, etc. There is to be no pooling of all tissues from any one animal and no pooling across group lines.

After 30 days on the test diets, all animals are to be given the basal laboratory diet. Only the 32nd, 35th, 45th and 75th days of the experimental period (the 2nd, 5th, 15th and 45th days after they have been returned to the basal diet), 5 rats from each group with each compound (total of 85 rats at each interval) are to be killed. Fat, liver and muscle from each animal to be taken as before, pooled by tissue and group, packed in dry ice and shipped to Dr. E. S. Tucker. Again, the same precautions as before are to be observed.

The preceding schedules will account for 680 animals. There are a total of 170 animals (10 in each group) remaining. These remaining animals may be inserted at some other interval during the feeding or withdrawal periods, or they may be used to extend the observations beyond 75 days. They can also be used to replace individual animals which may have died before the scheduled time for sacrifice of that group.

If the unused rats are carried through the end of the study, there will be 37,740 rat days on test. On the assumption that a rat will eat 25 grams of diet per day, this would require 943.5 kilograms (approx. 1 ton) of food. Therefore, select as many bags of food as you can conveniently mix and mix these thoroughly before the test. This will become the basal ration for these animals and will be used as long as it lasts when a new batch should be prepared. This is necessary to insure that we know the degree of interference, if any, which the diet would have on the analytical procedure. A portion of each such batch (0.5 kg) is to be sent to Dr. E. S. Tucker for analysis. Each time that diets are mixed, a sample (0.5 kg) of each test diet also is to be taken and sent to Dr. Tucker for analysis.

During the study, food consumption is to be recorded during the first 30-day period while animals are eating diets containing the test materials. This is to be done for all rats to insure that they are eating as well as the control and also to estimate their intake of the test compounds. Animals may be housed in groups of 5 and the intake of individual animals can be averaged from the group intake of food. Food intake can be measured periodically, i.e., it does not have to be determined daily.

At time of sacrifice, individual body weights should be recorded for each animal. No organ or tissue weights are to

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Table 1

Pilot Study

Group	Test diet conc. ppm	Day of Test * 0	5	10
Control	0	x		x
Compound 1	1	x	x	x
	10	x	x	x
Compound 2	1	x	x	x
	10	x	x	x

* 5 animals sacrificed and fat, liver, muscle taken for analysis

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be recorded. However, fat, liver and muscle are to be retained for analysis as mentioned earlier.

The attached tables outline these studies in a schematic form.

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Table I

Final Study

Group	Test diet conc. ppm	Days of Test*							
		Exposure Period				Recovery Period			
		2	5	10	30	32	35	45	75
Control	0	x	x	x	x	x	x	x	x
Compound 1	1	x	x	x	x	x	x	x	x
	10	x	x	x	x	x	x	x	x
Compound 2	1	x	x	x	x	x	x	x	x
	10	x	x	x	x	x	x	x	x
Compound 3	1	x	x	x	x	x	x	x	x
	10	x	x	x	x	x	x	x	x
Compound 4	1	x	x	x	x	x	x	x	x
	10	x	x	x	x	x	x	x	x
Compound 5	10	x	x	x	x	x	x	x	x
	100	x	x	x	x	x	x	x	x
Compound 6	10	x	x	x	x	x	x	x	x
	100	x	x	x	x	x	x	x	x
Compound 7	10	x	x	x	x	x	x	x	x
	100	x	x	x	x	x	x	x	x
Compound 8	to be set	x	x	x	x	x	x	x	x
	to be set	x	x	x	x	x	x	x	x

*5 animals sacrificed and fat, liver, muscle taken for analysis.
170 animals (10 pen/group)to be allocated as seem fit.

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APPENDIX B

Summary of Statistical Treatment of Data

(1) Test Error Calculated by Range of Logarithms Estimation

a) All samples for which replicates were run:

95% Confidence Level Limits—67% to 150% of the average value

b) Aroclor 1016 and Aroclor 1242 samples for which replicates were run:

95% Confidence Level Limits—73% to 126% of the average value

(2) Regression Analysis of Data for Aroclor 1016, Aroclor 1242, and NBS 1589

a) Equations shown in Table A-1

b) Plotted build-up and recovery curves shown in Figures A-1 and A-2

Acknowledgment is made to J.D. Hinshaw for the statistical treatment of the data from this study.

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INCREASE

Log Amt 1016	= 0.133 + 0.463 log time	(5 PPM)
Log Amt 1016	= 0.837 + 0.308 log time	(.5 PPM)
Log Amt 1242	= 0.307 + 0.516 log time	(5 PPM)
Log Amt 1242	= 0.805 + 0.309 log time	(25 PPM)
Log Amt 1489	= 0.070 + 0.457 log time	(5 PPM)
Log Amt 1489	= 0.793 + 0.309 log time	(25 PPM)

DECREASE

Log Amt 1016	= 0.695 - 0.0147 time	(5 PPM)
Log Amt 1016	= 1.366 - 0.0143 time	(25 PPM)
Log Amt 1242	= 1.066 - 0.0130 time	(5 PPM)
Log Amt 1242	= 1.288 - 0.0126 time	(25 PPM)
Log Amt 1489	= 0.857 - 0.0137 time	(5 PPM)
Log Amt 1489	= 1.258 - 0.0149 time	(25 PPM)

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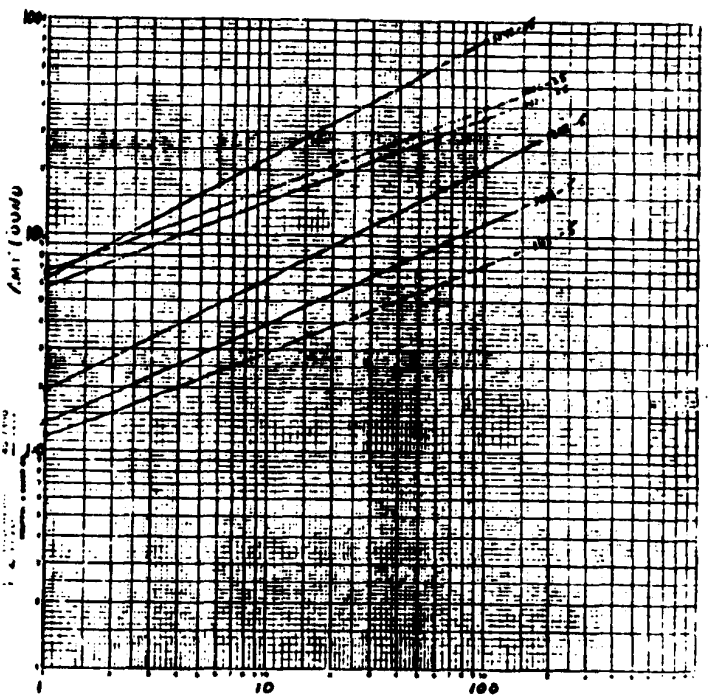
PREDICTED LEVEL OF MATERIAL
FOUND VS. DAYS FED



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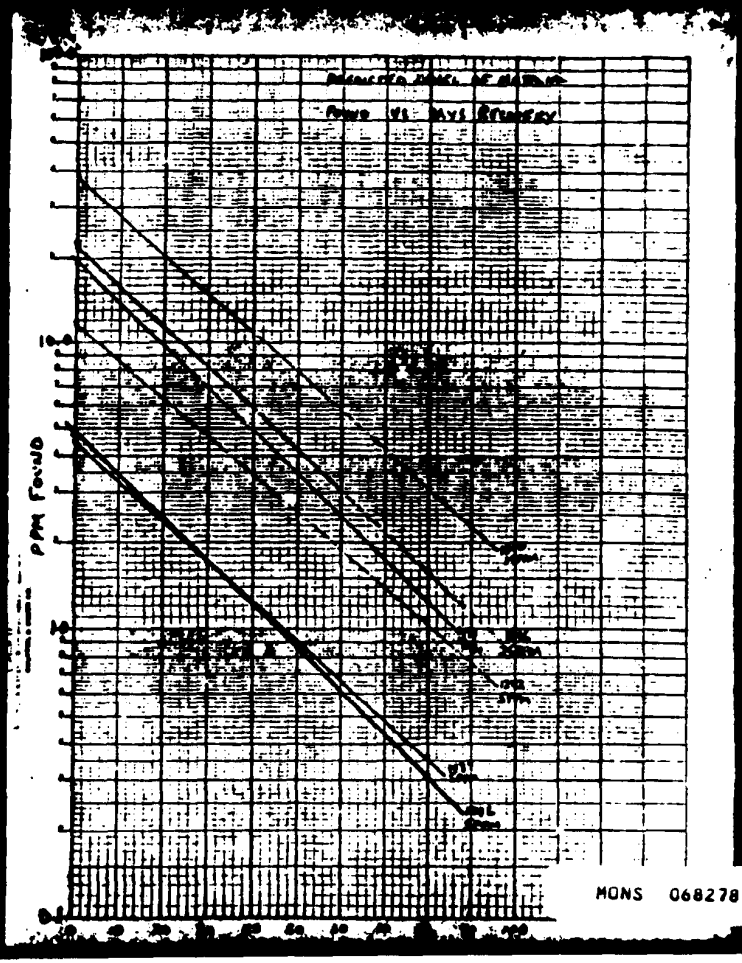
PPM Found
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