Tissue Residues from Subacute Oral Feeding of Polychlorinated Biphenyl Dielectric Fluids

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Since the identification of polychlorinated biphenyl (PCB) residues as environmental contaminants, numerous studies have investigated the uptake, distribution, and depuration of PCBs by various organisms (HUTZINGER et al. 1974). It has been established that the lower chlorinated PCBs are readily biodegraded and that resistance to biodegradation increases with increasing degree of chlorination (BAXTER et al. 1975, METCALF et al. 1974, TUCKER et al. 1975). Monsanto Company, a producer of PCB fluids, marketed as Aroclor* products, has restricted sales to use as dielectric fluids in sealed systems such as capacitors and transformers. Recently a new product, Aroclor 1016, has been introduced. Aroclor 1016 contains only about one tenth the level of the more resistant penta- and hexachlorobiphenyls as the functionally similar product Aroclor 1242.

The purpose of the present study was to assess the tissue residue accumulation and depuration of Aroclor 1242 and Aroclor 1016 during a 30-day subacute feeding to albino rats. Monochlorobiphenyl (MCBP) and a 30%-chlorinated biphenyl, MCS 1043, were included in this feeding study.

During the course of this study, other workers (BURSE et al. 1974) reported that Aroclor 1242 accumulated to a lesser extent than Aroclor 1016 in rat tissues. This result was somewhat unexpected since, as mentioned above, the only difference in the products is the level of the more resistant, more highly chlorinated biphenyl homologs, as shown in Table I. It is these more highly chlorinated species which have been consistently reported as environmental residues in wildlife (POLYCHLORINATED BIPHENYLS AND THE ENVIRONMENT 1972).

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TABLE I
TYPICAL % COMPOSITION OF
POLYCHLORINATED BIPHENYL PRODUCTS

HOMOLOG Cl/BIPHENYL	MCBP	MCS 1043	AROCLOR 1016	AROCLOR 1242
0	ND	0.1	<0.1	<0.1
1	100	22	1	1
2	ND	72	20	16
3	ND	6	57	49
4	ND	ND	21	25
5	ND	ND	1	8
6	ND	ND	<0.1	1
7	ND	ND	ND	<0.1
8	ND	ND	ND	ND

PERCENT (W/W) BY GC/MS USING AREA CORRECTION FACTORS BY HOMOLOG RESPONSE ND = NONE DETECTED, <0.01%

Experimental

Eight batches of feed were prepared by mixing the four products at two levels each into Ralston Purina rat chow. The treated chow was fed ad libitum to adult male Sprague-Dawley albino rats for an exposure period of 30 days. At predetermined intervals during the exposure period (days 2, 5, 10, 20, and 30), five rats from each set were sacrificed. Fat, liver, and muscle were excised for analysis and composited by tissue for each group. Following the 30-day feeding period, all remaining rats were placed on the basal laboratory diet. At days 32, 35, 45, 75, and 105 of the study (days 2, 5, 15, 45, and 75 of recovery), tissues were sampled in a manner identical to that used during exposure. Samples were composited and quick-frozen in glass containers with aluminum foil-lined caps to minimize risk of contamination. A control composite of tissues from rats fed only the basal laboratory diet was collected at each sampling date to determine possible interferences in the analyses of tissues from the exposed animals. During the period that rats were fed PCB-containing rat chow, their food intake and body weight gains were similar to those of rats fed the basal laboratory diet alone.

Feeding, sacrifice, and tissue excision were carried out at Younger Laboratories, Inc., St. Louis, Mo. The samples were delivered to our laboratories for sample clean-up and analysis.

The PCB residues were isolated from the tissues by solvent extraction. A weighed amount (approximately 10 g) of each composited tissue was placed in an Erlenmeyer flask and homogenized three times with 25 ml of

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pesticide grade hexanes and anhydrous sodium sulfate using a Polytron ultrasonic homogenizer (Brinkman Instruments, Westbury, N.Y.). The combined supernatants and washings were filtered through anhydrous sodium sulfate and diluted to 100 ml with pesticide grade hexanes.

A 5 ml aliquot of the hexanes solution was pipetted onto an alumina column (TUCKER et al. 1975) and the PCBs were eluted with 125 ml of pesticide grade hexanes. The column eluate was collected in a Kuderna-Danish evaporative concentrator, a 3-ball Snyder condenser was attached, and the solution was concentrated for electron capture gas chromatographic analysis. The gas chromatographic conditions are listed in Table II.

TABLE II

Instrument:	Hewlett-Packard 5753A Research Gas Chromatograph (Ni-63 Pulsed Electron Capture Detector)
Column:	2 m X 1/4" glass column packed with 4% XE-60 on 80/100 mesh Chromosorb W-HP
Temperatures:	
Injection Port:	230° C
Detector:	300° C
Column:	140° C Isothermal for MCBP and MCS 1043
	170° C Isothermal for Aroclor 1016 and Aroclor 1242
Carrier Gas:	Helium, 60 ml/min
Purge Gas:	10% Methane in Argon, 120 ml/min
Pulse Interval:	50 microsec

A second 5 ml aliquot of the extract solution was pipetted into a tared 50 ml beaker. After evaporation of the solvent under a stream of nitrogen, the beaker and residue were reweighed to obtain the lipid weight of the aliquot. All residue levels are reported as parts per million (ppm) on a lipid weight basis.

Calibration curves for each product were prepared by plotting detector response (total peak area) versus nanograms of material injected. Residue levels were determined by summation of the total area of the PCB peaks in the sample and use of the appropriate calibration curve. The calculations were done as follows:

$$\text{Residue (ppm)} = \frac{(N)(V_F)}{(V_I)(W)}$$

Where N = amount of PCB from calibration curve (ng)

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V_F = volume of final concentrate (ml)

V_I = volume injected (ul)

W = lipid weight of original sample (g).

Results and Discussion

Plots of the data for the PCB adipose tissue residues for the chlorinated biphenyl products are shown in Figure 1. The values plotted represent the levels of each product found in the adipose tissue, calculated on a lipid weight basis.

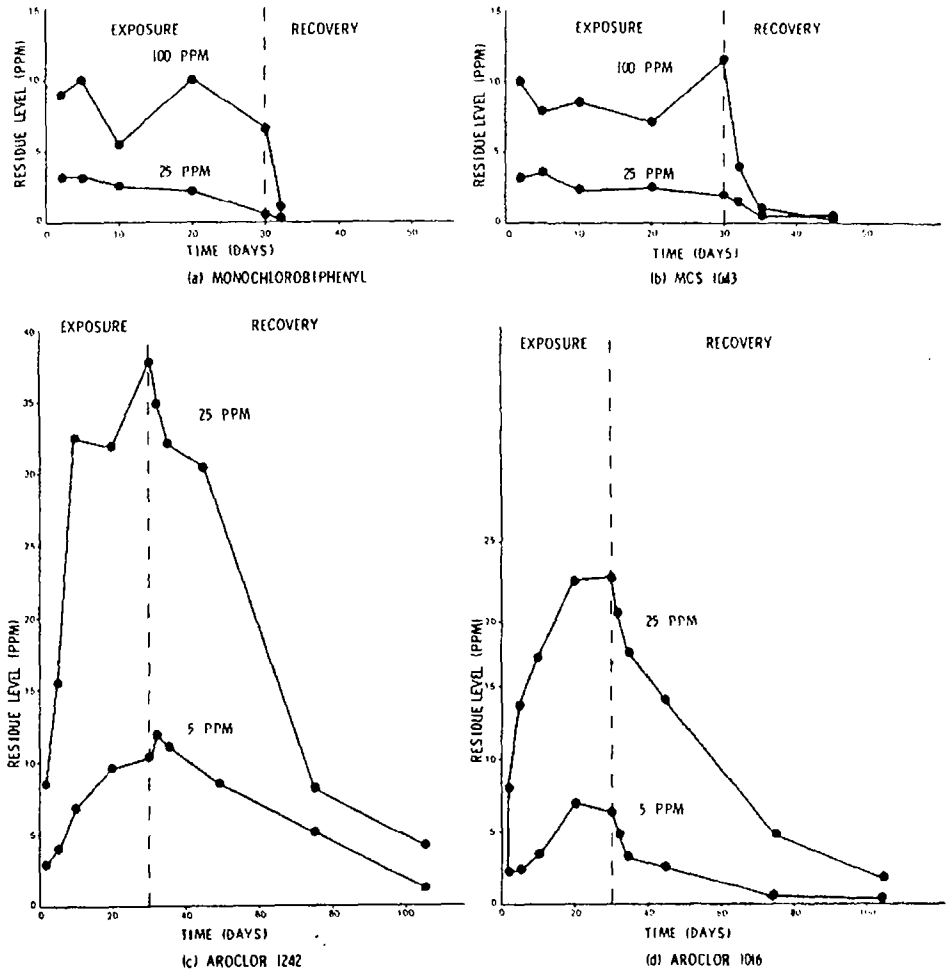


Figure 1: Adipose Tissue Residue Level Versus Time Plots for Products Investigated

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The test error associated with the data points of Figure 1 was evaluated using the logarithms of the data points for which replicate determinations were performed. The 95% confidence limits for the determination of Aroclor 1016 and Aroclor 1242 were calculated to be 79% to 126% of the observed value of a data point. In the case of MCBP and MCS 1043 the test error limits were calculated to be 63% to 160% of the observed value.

MCBP was fed at levels of 25 and 100 ppm. Figure 1a shows that MCBP reached a relatively stable level of about 3 ppm for the low dietary level and 9 ppm for the high dietary level immediately after feeding began. These levels were maintained throughout the 30-day exposure period. Upon removal from the test diets, the tissue residue levels dropped to such low levels that no MCBP was detected in fat extracts five days after cessation of exposure. MCBP thus showed no tendency to accumulate or be retained in fat.

When fed to rats at the same levels as MCBP, the behavior of MCS 1043 was similar to that of MCBP. It reached a plateau in fat immediately upon initiation of feeding and maintained that level throughout the exposure period. This behavior is shown in Figure 1b. Upon cessation of exposure the level of MCS 1043 in fat rapidly dropped to such an extent that it was not detected after 15 days of the recovery period.

Figure 1c is the plots of the fat residue levels for the 5 and 25 ppm feeding levels for Aroclor 1242. The curves indicate that Aroclor 1242 accumulated in the fat and continued to build up throughout the exposure period. The shape of the build-up curve suggests, however, that a plateau level was being approached. Upon removal of the test diet, the Aroclor 1242 level in the tissues declined rapidly. However, at day 75 of the recovery period, detectable levels of Aroclor 1242 remained in the tissues. The residues in the tissues sampled after the exposure period consisted of the more highly chlorinated homologs, as discussed below.

The plots of the fat residue levels for Aroclor 1016 are shown in Figure 1d. Aroclor 1016 accumulated more slowly than Aroclor 1242 during exposure, and declined rapidly during recovery. Low levels of Aroclor 1016 remained in the tissues through day 75 of the recovery period.

Figure 2 emphasizes the differences in response to the feeding of the products at the same dietary level. The difference in the behavior of MCS 1043 is obvious, since it did not accumulate in fat and rapidly declined

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to below the detection limit when the animals were returned to the basal laboratory diet.

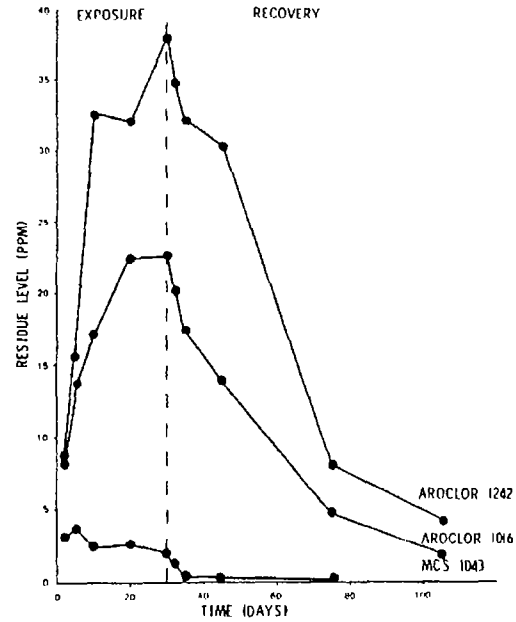


Figure 2: Adipose Tissue Residue Level Versus Time Plots for Aroclor 1242, Aroclor 1016 and MCS 1043 at 25 ppm Feeding Level

As shown in Figure 2, the residue levels of Aroclor 1016 were lower than those of Aroclor 1242. A test was made to see if this difference was statistically significant based on the estimated precision of the determinations. The 95% confidence limits for Aroclor 1242 and Aroclor 1016 (79-126%) were superimposed on theoretical accumulation and recovery plots (based on multiple regression analysis of the actual data) for the two products. These plots are shown in Figures 3a and 3b. The difference in residue levels for the two products is statistically significant at the 95% confidence level 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 residue levels of Aroclor 1016 and Aroclor 1242 became significant and remained so throughout the remainder of the exposure period and the entire recovery period. Thus, this study shows a significant difference in the tissue residue levels of Aroclor 1016 and Aroclor 1242.

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These findings differ from those reported by Burse et al.(1974). The feed levels in our study, 5 and 25 ppm, were chosen to be high enough to permit precise and accurate determination of residue levels but low enough to have minimal effect on the health of the rats. Burse et al. did report some morphological effect in the livers of the rats at the 100 ppm exposure level used in their study.

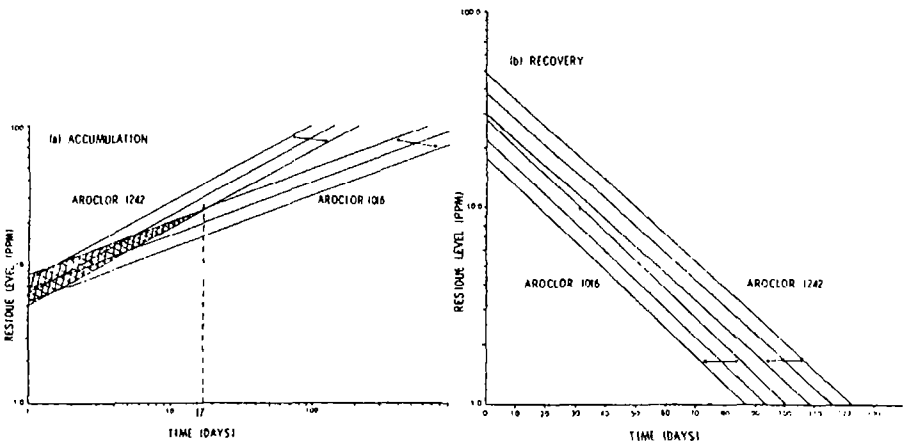


Figure 3: Regression Curves for Accumulation and Recovery with Superimposed Error Limits for Aroclor 1242 and Aroclor 1016

Figures 4a and 4b are chromatograms of the extracts of the feed and fat tissue (30-day exposure samples) for Aroclor 1242 and Aroclor 1016, respectively. The chromatograms for Aroclor 1242 show that most of the early peaks seen in the extract of the feed have disappeared or decreased in relative size in the fat extracts, indicating that the corresponding components were not retained in the tissue. These peaks correspond to PCB homologs with a low degree of chlorination, i.e., di- and trichlorobiphenyls. Some peaks corresponding to more highly chlorinated homologs increased in relative size, indicating a greater tendency for these homologs to accumulate. 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-homolog, for example, results from a much smaller absolute amount of PCB than a peak of equal area corresponding to a di-homolog.

In the chromatograms for the Aroclor 1016 extracts shown in Figure 4b, there is exact correspondence of

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peaks in the early portions of the feed and fat chromatograms with peaks in the Aroclor 1242 chromatograms. The chromatograms differ markedly, however, in the region where the more highly chlorinated homologs elute. Examination of the 30-day exposure chromatogram for Aroclor 1016 shows that there was no accumulation of more highly chlorinated homologs analogous to that noted for Aroclor 1242.

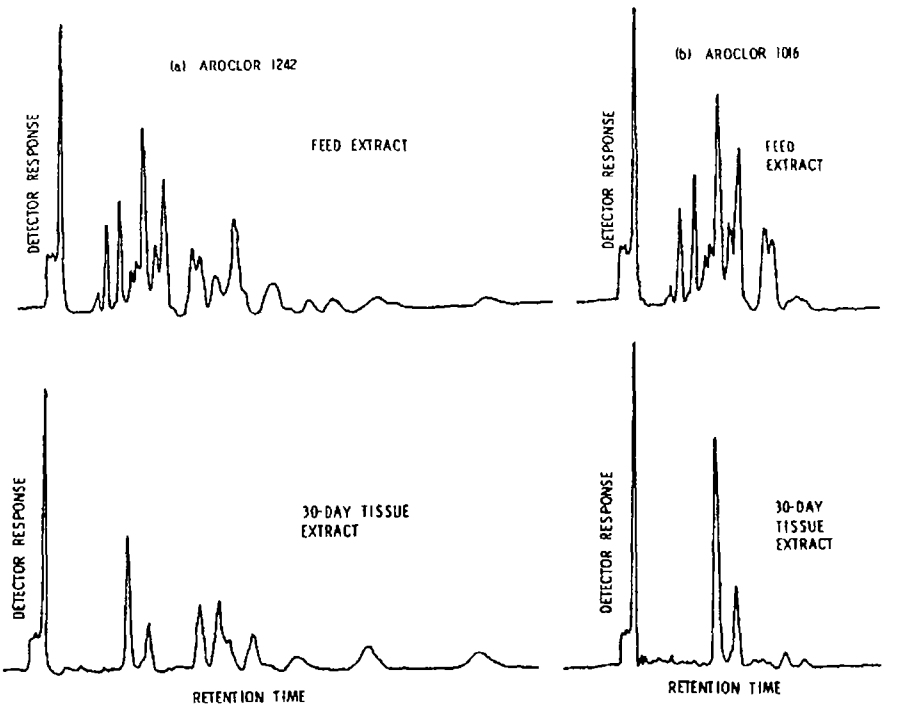


Figure 4: Chromatograms of Feed and Tissue Extracts of Aroclor 1242 and Aroclor 1016

Since no persistent residues of MCBP or MCS 1043 were detected in fat during this study, no chromatograms for these products are shown.

Liver and muscle tissues from the 30-day high feeding level of each material were also analyzed. The data for these analyses are shown in Table III.

Summary

The results of this study can be summarized as follows:

(1) Monochlorobiphenyl and the 30%-chlorinated biphenyl MCS 1043 did not accumulate in the lipid reservoir of the rats when fed at 25 ppm and 100 ppm in the diet.

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

Product	Dietary Level (ppm)	Muscle (ppm)	Liver (ppm)	Fat (ppm)
MCBP	100	7.4	9.1	6.4
MCS 1043	100	8.4	15.9	11.5
Aroclor 1242	25	42.0	60.4	37.3
Aroclor 1016	25	31.3	46.3	22.7

This result indicates that mono-, di-, and trichlorobiphenyls are readily metabolized and/or excreted under the conditions of this study.
(2) Although a fraction of the ingested Aroclor 1242 and Aroclor 1016 was stored in the rats' lipid reservoir, most of this residue was depleted after the rats had been on the basal laboratory diet for several weeks.
(3) Residues of Aroclor 1016 accumulated more slowly and to a significantly lesser extent than those of Aroclor 1242. During the recovery period these PCB residues decreased to lower values for Aroclor 1016. This result indicates that a product containing reduced amounts of the more highly chlorinated PCBs should have improved environmental compatibility.

Acknowledgment

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