

Polychlorinated Biphenyls

Storage, Distribution, Excretion, and Recovery: Liver Morphology After Prolonged Dietary Ingestion

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Rats were fed 100 ppm of Aroclor 1242 (6.6 to 3.89 mg/kg/day) or 100 ppm Aroclor 1016 (6.9 to 3.5 mg/kg/day). Plasma, kidneys, urine, brain, liver, and adipose tissue were analyzed for polychlorinated biphenyl (PCB) residues at 0.5, one, two, four, six, eight, and ten months of exposure. In additional groups fed the experimental diet for six months, PCB tissue levels were determined two, four, five, and six months after the exposure to PCBs was discontinued. PCBs were highest in adipose tissue where a steady state was approached in two and reached in 4 months. Little PCB-derived material was excreted in urine.

After discontinuing PCB exposure, Aroclor 1016 was eliminated more rapidly from the organs than Aroclor 1242. Measurable residue levels were still present after five (Aroclor 1016) and six (Aroclor 1242) months of recovery. Microscopic examination of the liver showed enlarged liver cells with vacuolated cytoplasm and inclusions.

The use and replaceability of polychlorinated biphenyls (PCBs) has recently been reviewed.¹ Because of their extensive use in the past and their slow biodegradability, PCBs are now widely distributed in the environment.^{2,3} Recently the use of these materials in the United States has

been restricted to closed systems. A new PCB, Aroclor 1016, which is a good dielectric and heat transfer fluid has been proposed. It contains a lower percentage of the higher PCBs and should prove to be less persistent in the environment. The higher PCBs appear to be more persistent, as evidenced by mass spectral residue analysis of environmental samples.^{4,5}

It is the purpose of this paper to report results of feeding studies conducted in rats with Aroclor 1016 and Aroclor 1242. Aroclor 1242 in its general make-up is very similar to Aroclor 1016, it only lacks the higher PCBs (Papageorge, written communication, June 1973). The Aroclors differ in the percentages of fixed chlorine, $43\% \pm 0.5\%$ (1242) vs $41.3\% \pm 0.5\%$ (1016). Aroclor 1242 contains 9% biphenyls with five or more chlorines and Aroclor 1016 contains 1% biphenyls with five or more chlorines.

MATERIALS AND METHOD

Aroclors 1242 and 1016 were of electrical grade, lot No. KB-05-415 and KB-06-756, respectively. The PCBs were fed in parallel experiments to male Sherman strain rats, 61 to 73 days old. A total of 158 rats was divided into two groups of 27 and two groups of 52 rats each according to a table of random numbers. The rats were group caged and weighed weekly. Their food consumption was measured, one week prior to each scheduled killing. The groups of 52 rats were fed the experimental diets that contained 100 ppm of Aroclor 1016 or Aroclor 1242. The control rats were fed ground laboratory chow ad libitum.

The PCBs were incorporated into the diets by dissolving them in ether and pouring this solution onto 100 gm of corn starch. The ether was evaporated and the PCB cornstarch mixture diluted with increasing amounts of ground chow in an electric mixer.

The following killing schedule was ob-

served (Table 1): one control and four rats fed Aroclor 1016 or 1242 were killed two weeks after onset of the experiment. Four additional rats of each experimental group were killed one and two months after onset of the experiment. The further killing of four experimental rats for each PCB mixture as well as four controls that served for both experimental groups were made four, six, eight, and ten months after onset of the experiment. After six months feeding, the experimental diets were discontinued on 18 randomly selected rats for each PCB mixture. These rats were fed plain chow and four for each PCB mixture were killed together with four control rats at two, four, and five or six months (Table 2) of recovery. Twenty-four hours prior to each killing the rats were housed in individual metabolism cages and urine was collected. During the 24-hour period prior to death, none of the rats in the study was fed a fortified diet. Oxalated blood was obtained by cardiac puncture from rats anesthetized with ether. The animals were killed by exsanguination and liver, fat, kidney, and brain tissues were collected. The liver was weighed and the tissues were frozen for chemical analysis. Liver tissue from the animals killed at four months and at subsequent killings were also fixed in buffered 4% formaldehyde for microscopic examination, and stained with hematoxylin-eosin. Liver tissue at four months of exposure to Aroclor 1016 and at six months of exposure to Aroclor 1242 together with an equal number of controls for electron microscopic examination was fixed in chilled, buffered 5% glutaraldehyde for two hours, postfixed in 1% osmium tetroxide, dehydrated through changes of alcohol and propylene oxide, and embedded in resin. Sections were cut with a glass knife, stained with lead citrate and uranyl acetate, and examined with an electron microscope.

The analytical methods used for the determination of PCB-derived materials have been described previously.⁶ Briefly, 100 to 200 mg of fat, brain, kidney, and liver or 1 to 2 ml of plasma were extracted with hexane. Brain was extracted with acetone, the solution evaporated to dryness

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Correction

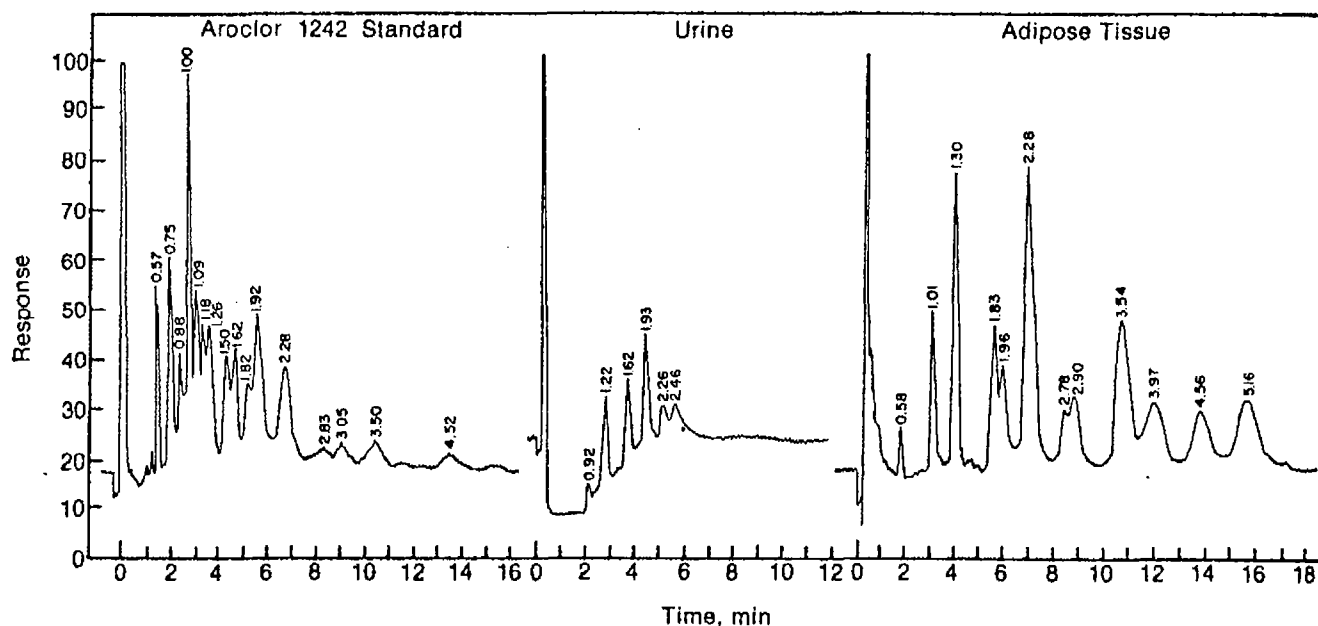


Fig 1.—Chromatograms of Aroclor 1242: 3.66 ng, an extract of urine: 4.5 mg, and adipose tissue: 0.027 mg from a male rat fed a diet containing 100 ppm Aroclor 1242 for eight months.

Table 1.—Mean Concentration and Standard Error of PCB-Derived Materials in Tissues of Four Rats Per Killing Fed 100 ppm Aroclor 1242 or 1016

Months on Diet	Plasma		Kidneys		Urine*		Brain		Liver		Adipose Tissue	
	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016
0.5	0.081 ±0.000	0.182 ±0.017	0.68 ±0.15	0.63 ±0.08	<0.02 ...	0.075 ±0.042	<0.25 ...	1.52 ±0.75	0.55 ±0.08	1.46 ±0.24	35 ± 2.4	69 ± 4
1	0.180 ±0.030	0.175 ±0.028	0.51 ±0.13	1.58 ±0.11	<0.01 ...	0.008 ±0.000	<0.25 ...	3.05 ±0.13	1.46 ±0.10	3.05 ±0.26	85 ± 10	103 ± 8
2	0.310 ±0.057	0.331 ±0.010	1.44 ±0.18	2.74 ±0.19	<0.02 ...	0.162 ±0.038	<0.25 ...	3.40 ±0.13	3.24 ±0.30	3.86 ±0.36	92 ± 1.5	182 ± 12
4	0.219 ±0.028	0.372 ±0.040	1.84 ±0.27	4.76 ±0.24	0.030 ±0.02	0.259 ±0.083	0.56 ±0.08	3.18 ±0.23	3.94 ±0.72	7.40 ±0.79	115 ± 11	214 ± 18
6	0.528 ±0.150	0.384 ±0.024	1.89 ±0.47	3.21 ±0.48	0.028 ±0.000	0.275 ±0.112	1.69 ±0.25	2.98 ±0.44	4.21 ±0.57	7.86 ±0.75	110 ± 13	236 ± 9
8	0.240 ±0.052	0.394 ±0.014	2.14 ±0.41	2.07 ±0.06	0.168 ±0.04	0.057 ±0.017	1.63 ±0.24	2.64 ±0.36	4.56 ±0.61	5.97 ±0.94	143 ± 6	221 ± 10
10	0.172 ±0.014	0.334 ±0.040	3.13 ±0.56	1.84 ±0.38	<0.01 ...	0.021 ±0.000	2.05 ±0.08	2.70 ±0.36	6.39 ±0.68	6.70 ±0.34	133 ± 15	188 ± 9

* Confirmed qualitatively by Coulson conductivity.

Table 2.—Mean Concentration and Standard Error of PCB-Derived Material in Tissues of Four Rats Two, Four, Five, or Six Months After They Had Been Fed 100 ppm Aroclor 1242 or 1016 for Six Months

Months Recovery	Plasma		Kidneys		Urine		Brain		Liver		Adipose Tissue	
	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016
2	0.438 ±0.091	0.154 ±0.034	2.07 ±0.40	1.08 ±0.27	0.02 ±0.01	0.033 ±0.004	1.06 ±0.24	1.40 ±0.07	4.32 ±0.72	2.25 ±0.32	60 ± 3.7	104 ± 13
4	0.149 ±0.000	0.193 ±0.050	1.84 ±0.27	1.82 ±0.44	<0.01 ...	0.008 ±0.002	1.22 ±0.53	1.35 ±0.21	3.46 ±1.04	3.13 ±0.78	44 ± 9	36 ± 6
5*	...	0.062 ±0.011	...	0.44 ±0.14	...	0.006 ±0.002	...	0.38 ±0.06	...	1.06 ±0.09	...	28 ± 1
6*	0.122 ±0.026	...	0.34 ±0.06	...	0.004 ±0.000	...	0.51 ±0.06	...	0.97 ±0.35	...	24 ± 5	...

* Experiment had to be prematurely terminated.

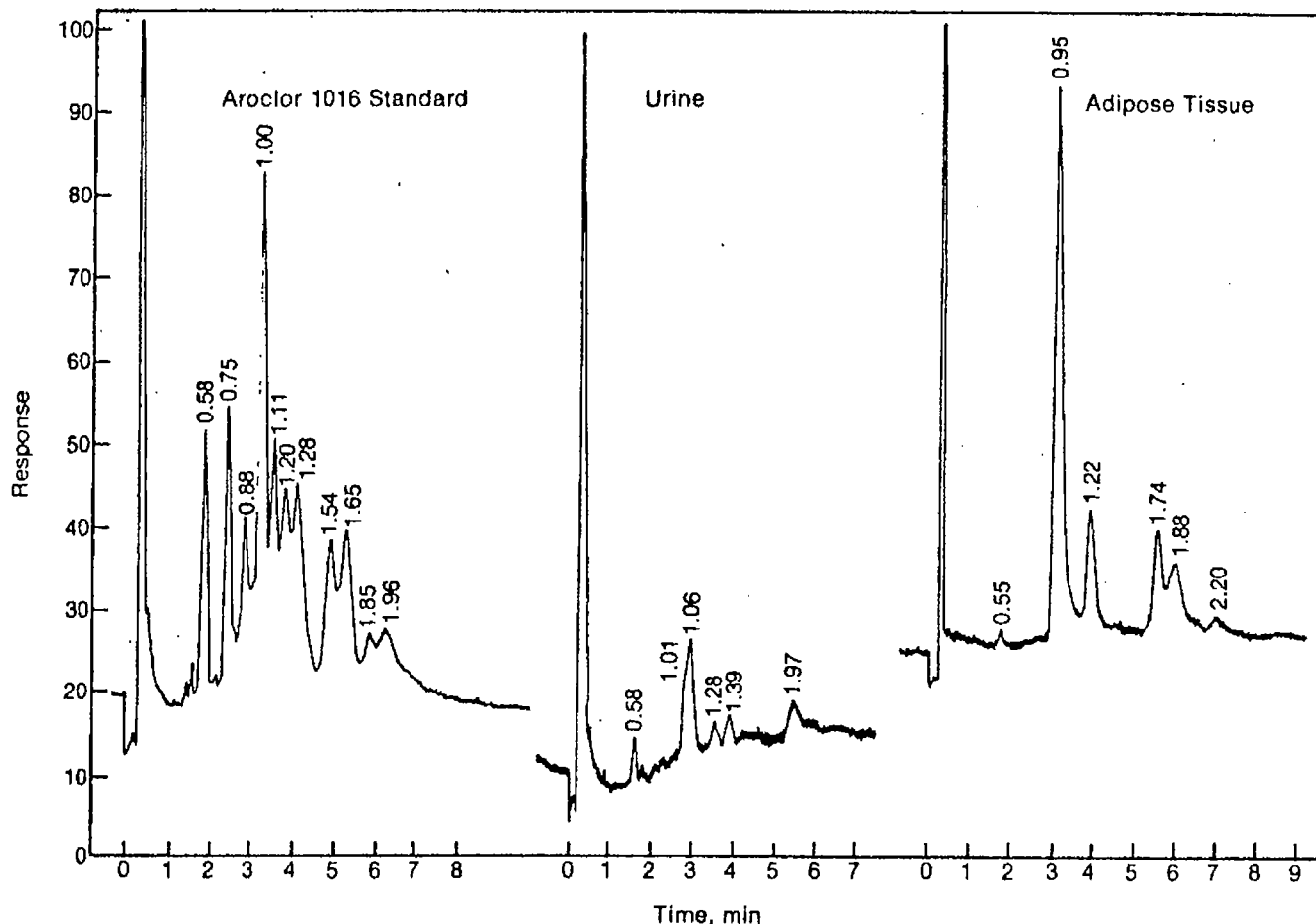


Fig 2.—Chromatograms of Aroclor 1016: 2.84 ng, extract of urine: 5.4 mg, and adipose tissue: 0.008 mg from a male rat fed a diet containing 100 ppm Aroclor 1016 for eight months.

Table 3.—Incidence of Microscopic Changes Observed in Livers of Rats Fed Aroclor 1242 or 1016

Length of Exposure	Enlarged Hepatocytes		Vacuolated Cytoplasm		Inclusions		Brown Pigment		Foamy Cytoplasm		Foci of Necrosis Hemorrhage	
	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016
4	2/4	4/4	...	1/4	...	...	...	...	...	...	...	1/4
6	4/4	4/4	3/4	1/4	3/4	3/4	...	...	...	...	...	...
8	4/4	3/4	3/4	...	3/4	...	2/4	...	...	...	1/4	...
10	4/4	4/4	4/4	1/4	1/4	...	2/4	...	2/4	...	...	...
6 mo exp 2 mo rec	2/4	1/4	3/4	1/4	1/4	...	...	...	...	...	...	...
6 mo exp 4 mo rec	3/3	3/3	1/3	1/3	1/3	1/3	...	...	...	...	...	...
6 mo exp* 6 mo rec	3/4	...	...	...	2/4	...	...	...	...	...	...	...

* Only rats fed Aroclor 1242 were examined microscopically.

and the solute taken up in hexane. Urine, 10 to 20 ml, was extracted at approximately pH 7 with hexane. Each sample in a volume of 0.5 ml was eluted from a micro-silica gel column using a 1:1 benzene-hexane mixture.

Samples were analyzed with a tritium source electron capture detector operated in the direct current mode at 12 v. Quantitation of the standard and of the different

kinds of samples was based in each instance on total response in peak heights. However, as reported more fully below, there were qualitative differences in the peaks for the standard, the urine, the tissues collected during dosing, and the tissues collected after dosing was discontinued. Under these circumstances exact quantitation is impossible, but the use of total response in peak heights is consid-

ered to offer the best estimate of quantitation.

RESULTS

The rats did not suffer from any overt clinical symptoms of poisoning. During the course of the experiment five rats that were fed Aroclor 1016 and one rat that was fed Aroclor 1242

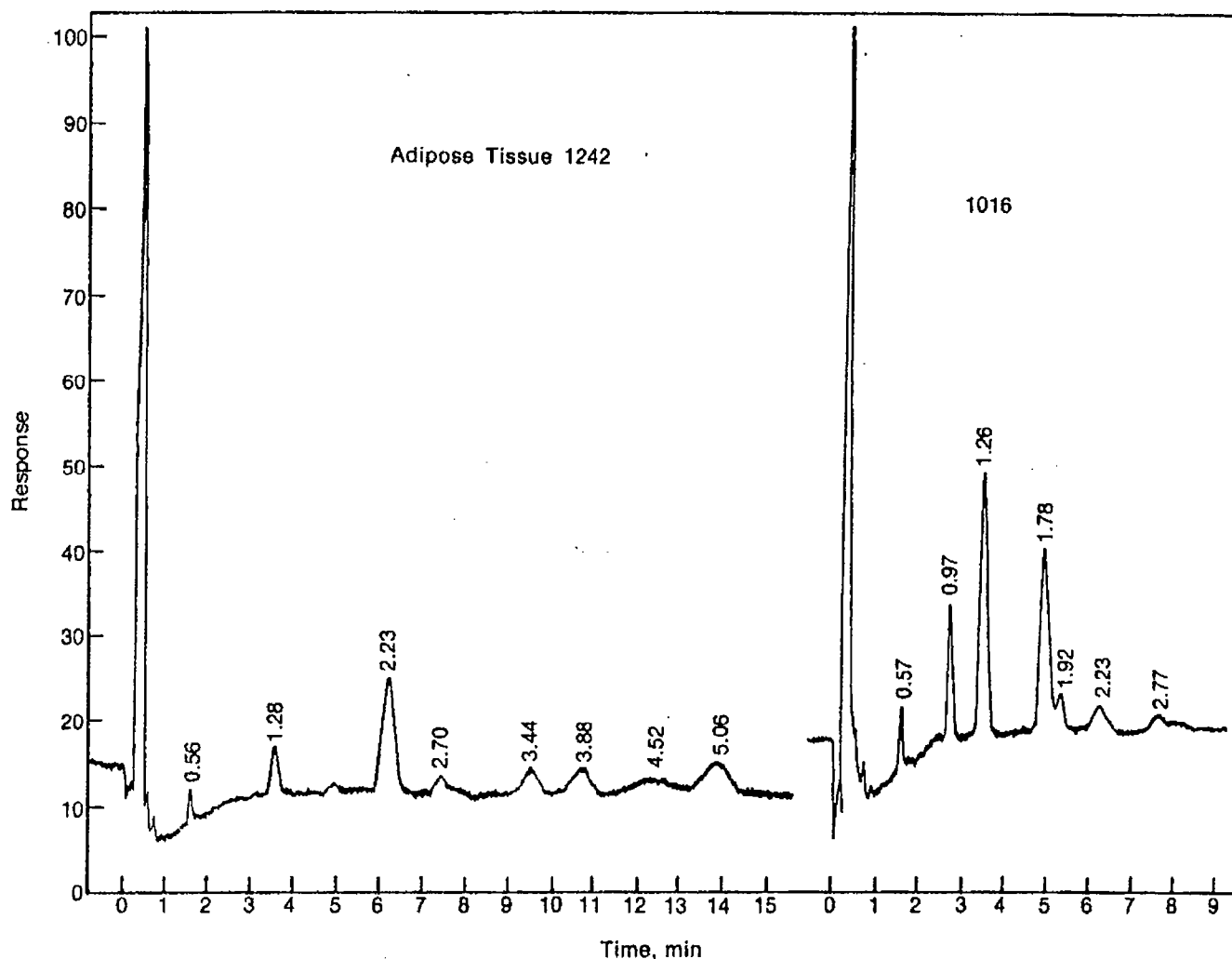


Fig 3.—Chromatograms of adipose tissue: 0.011 mg from a rat fed a diet containing 100 ppm Aroclor 1242 for six months and six months of a control diet; 0.016 mg from a rat fed a diet containing 100 ppm Aroclor 1016 for six months and a control diet for five

months. (Relative retention times were computed relative to the most intense electron capturing peak present in Aroclor 1016 or 1242. All gas chromatographic columns were composed of 1.5% OV-17/1.95% QF-1 on 100/120 Gas Chrom Q.)

died. The cause of death was not established. No difference between the experimental and the control rats was observed in the body weight gain and the food consumption. As the rats aged food consumption decreased relative to their body weight. The amount of Aroclor 1242 consumed by the rats ranged from 6.6 mg/kg body weight/day to 3.89 mg/kg body weight/day, and the amount of Aroclor 1016 consumed by the rats ranged from 6.9 mg/kg body weight/day to 3.5 mg/kg body weight/day.

At autopsy the organs appeared grossly normal except for one rat fed Aroclor 1016 that had leukemia (a disease that occurs occasionally spontaneously in our colony). The liver

weights of the experimental animals did not differ from those of the controls.

The concentrations of the PCB-derived materials are given in Table 1. The observed levels in adipose tissue are also shown in Fig 1. The highest concentrations for both compounds were found in adipose tissue, ranging from 35 to 143 ppm for Aroclor 1242 and 69 to 236 ppm for Aroclor 1016. The levels for brain, liver, and kidney were lower. The concentrations in urine and plasma were always less than 1 ppm. In adipose tissue a steady state of storage of both compounds was approached in about two months and essentially achieved in four months.

Gas chromatographic traces for all tissues were similar to those obtained from adipose tissue. The traces obtained from urine analysis had an entirely different appearance. The different profiles obtained from adipose tissue, urine, and the PCB standards are illustrated in Fig 1 to 3. When the peaks in the PCB standards were compared with the peaks obtained from PCB-derived material stored in the adipose tissue, it was found that some of the early peaks were absent, the intensity of others was enhanced, and peaks also emerged that were observed only as "shoulders" in the standard. This observation suggests either preferential absorption from the gastrointestinal tract, or prefer-

ential metabolism, distribution, and excretion in the rats or both.

The urine samples show a very different pattern. This suggests that certain isomers are excreted by the kidneys, that the products excreted in the urine are polar metabolites, or that nonchlorinated moieties responded to the electron capture detector. However, a positive response was obtained with a conductometric detector (Coulson).

Residue levels arising from Aroclor 1016 ingestion were generally found to be higher than for Aroclor 1242,

Fig 4.—Residues resulting from a continuous diet of a 100 ppm concentration of Aroclor 1016 (●) or Aroclor 1242 (○) for a period of ten months. Residues following a continuous diet for only six months and recovery for five months for Aroclor 1016 (□) and for six months for Aroclor 1242 (○) are illustrated by the broken lines. Each point represents the mean and standard error (1) of four rats.

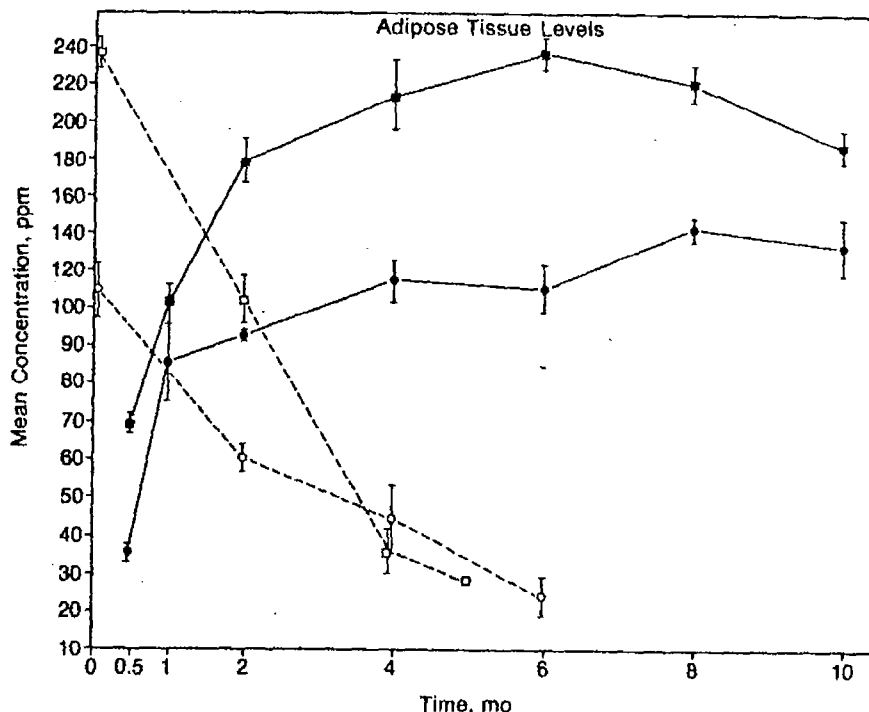


Fig 5.—Liver of rat fed Aroclor 1016 for four months. Note dilated vesicles of smooth endoplasmic reticulum (SER). Some of the mitochondria are degenerated and others show an increased number of parallel arranged cristae (lead citrate, uranyl acetate, $\times 35,000$).



with the exception of the residues observed in the kidneys. However, this was not true for all organs at every killing. After a ten-month feeding period the concentration of both PCBs in the liver was about the same, while at four months almost twice as much Aroclor 1016 as Aroclor 1242 was found. The levels found in adipose tissue indicate a very sharp rise during the first month for Aroclor 1242, while Aroclor 1016 also maintains a sharp rise through the second month. After two months of exposure no appreciable increase was observed in adipose tissue of rats exposed to either Aroclor 1016 or 1242.

Very little PCB-derived material was excreted in the urine, and the excretion was very erratic. When rats were fed Aroclor 1242 they only excreted measurable amounts during four to eight months of exposure. Rats fed Aroclor 1016 excreted measurable amounts at each urine collection. The variation in urinary excretion of PCB-derived material has been observed previously in this laboratory¹ and by other workers in the field.²

The data resulting from the analysis of tissues from rats that were fed the respective PCB-containing diets for six months and were then continued on plain chow for various intervals up to six months are given in Table 2. The concentrations found in adipose tissue are also illustrated in Fig 4. Storage of material as detected by gas chromatography decreased more rapidly following discontinuation of dosing in rats previously fed Aroclor 1016 than in those previously fed Aroclor 1242. Following a recovery period of six months the residue levels in adipose tissue were 21.8% of those observed after a six-month exposure period to Aroclor 1242. Following a recovery period of five months the residue levels in adipose tissue of rats fed Aroclor 1016 were only 11.8% of the concentration found after six months of ingestion of this material. The gas chromatographic traces obtained from the adipose tissue following recovery from exposure to the PCB mixtures are shown in Fig 3. (Relative retention times were computed relative to the most intense

electron capturing peak present in Aroclor 1016 or 1242. All gas chromatographic columns were composed of 1.5% OV-17/1.95% QF-1 on 100/120 Gas Chrom Q.) If these results are compared with those obtained earlier from adipose tissue, a slight decrease in the number of peaks as well as a difference in the relative heights of the peaks is noticed.

Composite urine and fat samples from rats fed Aroclor 1242 and 1016 continuously for varying time periods were analyzed by gas chromatography-mass spectrometry. Spectra resulting from the composite adipose tissue analysis showed primarily tri- and tetrachlorobiphenyls, for both PCB mixtures. Trace amounts of penta- and hexachlorobiphenyls were found in the Aroclor 1016 composites, while significant amounts of pentachlorobiphenyls and hexachlorobiphenyls were found in the composites obtained from rats that were fed Aroclor 1242.

Spectra from urine composites of rats fed Aroclor 1016 revealed the presence of the same molecular ions observed in the adipose tissue composites, with the exception of hexachlorobiphenyl. Dichlorohydroxybiphenyls (DCHB) and trichlorohydroxybiphenyls (TCHB) were also observed. Dichlorohydroxybiphenyl was more abundant.

Spectra from urine composites of rats fed Aroclor 1242 revealed the presence of the same molecular ions observed in adipose tissue, with the noted absence of hexachlorobiphenyl. In contrast to Aroclor 1016, hydroxylated chlorobiphenyls were not observed in any of the spectra resulting from Aroclor 1242 urine composite analysis.

The incidence of the various microscopic changes observed in the liver is given in Table 3. Light microscopic examination of the liver of rats fed the two PCBs showed enlarged hepatocytes predominantly in the center of the lobules. Occasional hepatocytes had coarsely vacuolated cytoplasm or contained inclusions within the cytoplasm. The cytoplasm of some of the hepatocytes was finely vacuolated and a brown pigment was noticed in the Kupffer cells and macrophages of

some of the livers of the experimental rats fed Aroclor 1242 but not in rats fed Aroclor 1016.

Vacuolation of the cytoplasm of the liver cells was more consistently observed in the livers of rats fed Aroclor 1242. Generally not much difference was seen in the incidence of the various changes between the two experimental groups. All of the observed changes have to be considered as mild alterations that have been observed with a number of chlorinated cyclic chemicals.

Four and six months after exposure to the PCBs was discontinued the hepatocytes were still larger than those of the controls, but only one out of three rats showed vacuolated cytoplasm or inclusions within the cytoplasm. Since the number of animals was small, these results have to be interpreted with reservation; however, it appears that the morphological changes encountered following exposure to Aroclor 1242 and 1016 are reversible and disappear gradually after dosing is stopped. Except for the hemorrhage and necrosis observed in one animal in each experimental group no evidence of serious liver damage was encountered.

The livers of rats from the controls were normal when studied with the light microscope. Sections of four livers of rats fed Aroclor 1242 for six months and of four rats fed Aroclor 1016 for four months were also studied under the electron microscope, together with the same number of controls. The livers of the control animals showed occasional lipid vacuoles but no abnormal changes. The livers of the experimental rats fed both PCB compounds showed an increase in smooth endoplasmic reticulum, an increase in the number of lipid vacuoles, and atypical mitochondria. Stacking of membranes within the mitochondria was also observed (Fig 5). Except for one animal that showed lymphocytic leukemia, no other abnormal findings were made on gross inspection of the organs at autopsy. It must be emphasized, however, that the only organ examined microscopically was the liver, since it was this organ that was primarily affected by Aroclor 1254 and Aroclor 1260 as pre-

viously
tory.^{3,4}

Levels found in level of 9 182 ppm months' containing crease the sumption both group higher re Aroclor 10 the gastr. clor 1242. storage v: pounds. 1 Aroclor 10 attributal is known tion and substitution iron capt compound electron proper An alter PCB deri complete phenyls Whether factors difference at similar ent not the other for Arocl that were creased exposure ued.

From Aroclor 10 reached exposure more rat 1016.

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viously reported from our laboratory.^{4,5}

COMMENT

Levels of PCB-derived components found in adipose tissue reached a level of 92 ppm (Aroclor 1242) and of 182 ppm (Aroclor 1016) after two months' exposure of rats to a diet containing 100 ppm, with little increase thereafter. Although food consumption of the rats was the same in both groups, rats on Aroclor 1016 had higher residues. It is possible that Aroclor 1016 is absorbed better from the gastrointestinal tract than Aroclor 1242. Another possibility is that storage varies between the two compounds. The higher residue levels of Aroclor 1016 vs Aroclor 1242 could be attributable to PCB quantitation. It is known that the degree of chlorination and the position of the chlorine substitution contribute to the electron capturing properties of these compounds. In some instances their electron capture responses are not proportional to their concentration.^{10,11} An alternative to quantitating the PCB derivatives in tissue could be complete chlorination of the biphenyls to decachlorobiphenyl.¹² Whether a combination of the various factors outlined contributed to the difference in the PCB concentration at similar exposure times is at present not known. The concentrations in the other organs were often higher for Aroclor 1016, and the residues that were built up in the tissues decreased faster for this compound once exposure to the PCBs was discontinued.

From previous experience with Aroclor 1254⁶ equilibrium seems to be reached sooner, and elimination after exposure has been discontinued is more rapid with Aroclors 1242 and 1016.

Mass spectral identification of PCBs in composite fat and urine samples was based on: (1) the presence of the correct molecular ion, (2) characteristic chlorine isotopic clusters, and (3) observed fragmentation patterns. These were all in agreement with

previously published data.

The presence of DCHB and TCHB in Aroclor 1016 urine composite is in keeping with published data on metabolic products for these compounds.¹³ However, one would generally not expect the method of extraction employed here (hexane at pH 7) to extract these metabolic products since they are generally found bound in the urine as glucosiduronides,¹⁴ and are detected following hydrolysis of the urine. It is feasible that small amounts of hydroxylated compounds could be found as free phenols and could then be chromatographed under selected conditions. As could be expected, the concentrations of the PCBs were much lower in the liver, kidneys, and brain than in adipose tissue, and even lower in plasma. Plasma concentration probably is not a very good indicator of the amount of PCBs stored in adipose tissue.

The morphological changes observed in the liver are similar to those observed with other chlorinated hydrocarbons.¹⁵ They were generally mild, particularly when compared with findings made earlier with Aroclor 1254 and 1260.^{6,7} Neither the pronounced lipid accumulation nor the adenofibrosis was observed in this study. However, some brown pigment was observed in the macrophages of the livers of some rats fed Aroclor 1242 but not Aroclor 1016. It was not determined whether the pigment represented ceroid or whether part of it was caused by an accumulation of hemosiderin and porphyrin in the liver. After feeding rats Aroclor 1242 and 1016 for six months, Goldstein et al.⁶ found an increase in liver uroporphyrin for Aroclor 1242 at this dietary level but not Aroclor 1016 at the same dietary level. Unfortunately the number of rats was not large enough to determine if the occasional liver cell necrosis and hemorrhage that was seen was related to the exposure to Aroclor 1242. From this study it may be concluded that both Aroclor 1242 as well as Aroclor 1016 are less persistent in the rat and have also less of an effect on the liver than either Aroclor 1254 or 1260.

Aroclors 1242 and 1016 were supplied by Monsanto Chemical Company, St. Louis. J. Curney, R. Moore, J. Coule, and J. M. Jack gave technical assistance.

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