

PCB Metabolism in Rats Following Prolonged Exposure to Aroclor 1242 and Aroclor 1016

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Determination of the level of metabolic products present in urine has been utilized as an indicator of type and degree of exposure to chemicals. Two of the most widely studied urinary metabolites, namely DDA, the carboxylic acid metabolite of DDT and the para-nitrophenol metabolite of parathion have been used to gain insight into the degree of exposure to these parent compounds (LAWS, et al. 1967; ELLIOTT, et al. 1960).

Polychlorinated biphenyls (PCB's) were first reported as environmental contaminants in 1966 (JENSEN). The scientific literature now abounds with reports concerning the analysis and toxicology of these highly stable and persistent materials.

For many years it was generally believed that PCB's were not metabolized to any appreciable extent by mammalian systems (REYNOLDS, 1969). However, as early as 1959, BLOCK and CORNISH (1959) reported on the conversion of biphenyl and 4-chlorobiphenyl to monohydroxylated compounds in the rabbit. WEST, et al. (1956) isolated pure compounds resulting from the metabolism of biphenyl in the rat. Recently JENSEN, et al. (1974) described the separation and identification of individual isomers of PCB's in the technical material and in human adipose tissue. Based on the percentages of various isomers stored in adipose tissue, he indicated that two adjacent unsubstituted carbon atoms were required for rapid metabolism. This supported the contention of previous investigators who were working with chlorobenzenes (JONDORF, et al. 1955). KAISER and WONG (1974) reported that microbial degradation of Aroclor 1242 resulted in non-chlorinated aromatic and aliphatic products. GRANT and his group (1974) demonstrated that Aroclor 1254 was metabolized in rats. He noted significant differences in GLC peak patterns for the PCB standard and the PCB which was extracted from the tissues. Similar finds were reported for Aroclor 1254 by CURLEY, et al. (1971) using Electron Capture-Gas Chromatography in their examinations of rat tissues and urine. No metabolites were isolated or identified.

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This paper reports on metabolic products observed in rat urine, through Coulson Conductivity-Gas Chromatography (CC-GC) and combined Gas Chromatography-Mass Spectrometry (GC-MS) analyses, following a prolonged diet of Aroclor 1016 or Aroclor 1242.

EXPERIMENTAL

Aroclor 1016 and 1242 were of electrical grade, lot No. KB-06-756 and KB-05-415, respectively. They were supplied by Monsanto Chemical Company, St Louis, Missouri. The Aroclors were fed in parallel experiments to male Sherman strain rats, 61 to 73 days old at a dietary level of 100 ppm each. Twenty-four hour urine was collected from four experimental rats fed Aroclor 1242 or Aroclor 1016 and one control under the following schedule: 2 weeks after onset of the experiment; one and 2 months after onset of experiment; 4, 6, 8 and 10 months after onset of experiment. After 6 months on the diet, some rats were allowed to recover by removal of PCB diet, for time periods of 2, 4 and 6 months. Urine was collected at these intervals. A more detailed accounting of the experimental protocol can be found in another publication (BURSE, et al. 1974). The amount of Aroclor 1242 consumed ranged from 6.6 mg/kg bodyweight/day to 3.89 mg/kg bodyweight/day while Aroclor 1016 ranged from 6.9 - 3.5 mg/kg bodyweight/day.

The twenty-four hour urine samples were combined from four rats in each experimental group. Total urine volume ranged from 40 to 80 ml. The urine was refluxed in an equal volume of concentrated HCl for three hours and extracted 3 times with 50 ml of benzene. The benzene extract was washed with 20 ml of 5% NaOH w/v followed by 20 ml water. The aqueous phases were combined and acidified with 20 ml of 1.2 N HCl. The acidic aqueous phase was extracted 3 times with 10 ml of benzene and dried over sodium sulfate. To each sample was added diazomethane (STANLEY, 1966). Each sample in a volume of 0.5 ml was eluted from a micro column containing 3% w/w deactivated silica gel using 10 ml of a 1:1 benzene: hexane mixture.

A Microtek-2000 gas chromatograph, equipped with a Coulson Conductivity Detector was used for preliminary screening for halogen in the sample extracts. Pyrex glass columns (1.83m X 4mm i.d.), packed with 5% OV-210 on 80/100 mesh Supelcoport, and 3% OV-1 on 70/80 mesh Chromosorb G were operated at 165° and 170° respectively.

Composited urine sample extracts were adjusted to 0.5 ml with pesticide grade hexane. Eight microliters

were injected into a Hewlett Packard 5700 A gas chromatograph containing a 1.22m x 2mm i.d. stainless steel column packed with 3% OV-1 on 80/100 mesh Gas Chrom Q. The initial column temperature was 150°, and the oven was programmed at 2°/min. to 210°. The injection port, transfer line and silicone membrane separator were kept at 200°, 220°, and 200° respectively. Helium flow rate past the membrane was 32 ml/min. Compounds transmitted through the membrane separator entered the ion source of a Hewlett Packard 5930A dodecapole mass spectrometer. Parameters of the mass spectrometer were as follows: ion source, 200°; mass filter, 110°; electron impact source at 70 eV; filament emission current, 250µ amps; target current, 220µ amps, scan rate 100 amu/sec from 45 - 450 amu. Ions were detected with a Bendix Continuous Dynode Electron Multiplier. Data were acquired, stored and plotted using the Hewlett Packard 5932A Data System.

RESULTS AND DISCUSSION

Recently HUTZINGER and co-workers (1972) demonstrated the metabolism of pure chlorobiphenyl isomers in pigeons and rats. The 4-chloro-, 4,4'-dichloro-, and 2,2',5,5'-tetrachlorobiphenyl isomers were metabolized to monohydroxy compounds. A dihydroxy monochloro metabolite was also reported. GARDNER, et al. (1973) fed 2,5,2',5'-tetrachlorobiphenyl to rabbits and identified 3-hydroxy-2,5,2',5'-tetrachlorobiphenyl, 4-hydroxy-2,5,2',5'-tetrachlorobiphenyl and trans-3,4-dihydro-3,4-dihydroxy-2,5,2',5'-tetrachlorobiphenyl in urine samples.

In this study we have identified at least six hydroxylated biphenyl metabolites (Table I). Five of these compounds were found in both treatment groups. Of the three dihydroxylated metabolites, two were previously unreported. These compounds, a dichlorodihydroxy and a trichlorodihydroxy biphenyl, were found in both groups of animals. The only significant difference noted between the two treatment groups was the absence of a tetrachlorodihydroxy biphenyl in Aroclor 1242.

Significant quantities of hydroxylated chlorobenzenes were found in the urine extracts of both treatment groups (Table II). The source of these metabolites has not been determined but there is reason to suspect that they may have arisen from hexachlorobenzene which was found in the adipose tissue samples of the experimental animals.

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TABLE I

PCB Metabolites Found in Urine of Rats
 Fed Aroclor 1016 or Aroclor 1242 (as
 Methyl Ether Derivatives)

Composition	Molecular Ion (M ⁺)	Aroclor 1016	Aroclor 1242
C ₁₂ H ₇ Cl ₂ OCH ₃	252	yes	yes
C ₁₂ H ₆ Cl ₂ (OCH ₃) ₂	282	yes	yes
C ₁₂ H ₆ Cl ₃ OCH ₃	286	yes	yes
C ₁₂ H ₅ Cl ₃ (OCH ₃) ₂	316	yes	yes
C ₁₂ H ₅ Cl ₄ OCH ₃	320	yes	yes
C ₁₂ H ₄ Cl ₄ (OCH ₃) ₂	350	yes	no

TABLE II

Phenolic Metabolites Found in Urine of
 Rats Fed Aroclor 1016 or Aroclor 1242
 (as Methyl Ether Derivatives)

Composition	Molecular Ion (M ⁺)
C ₆ Cl ₅ OCH ₃	278
C ₆ HCl ₄ OCH ₃	244
C ₆ Cl ₄ (OCH ₃) ₂	274

Quantitative estimation and exact identification
 of positional isomers of the observed metabolites was
 precluded because of the lack of authentic standards.
 However, GARDNER, et al. (1973) established positional
 identity for a specific tetrachlorohydroxylated bi-
 phenyl. By analogy, based on GLC retention and MS
 fragmentation data, it appears that more than one
 isomer of some of the hydroxylated biphenyls were pre-
 sent in these samples. Total ion current reconstructed
 chromatograms are presented in Figures 1 and 2.

Assignment of the molecular formulae of the
 observed metabolites was based on numerous mass spectra
 obtained during GLC separation for each component. In

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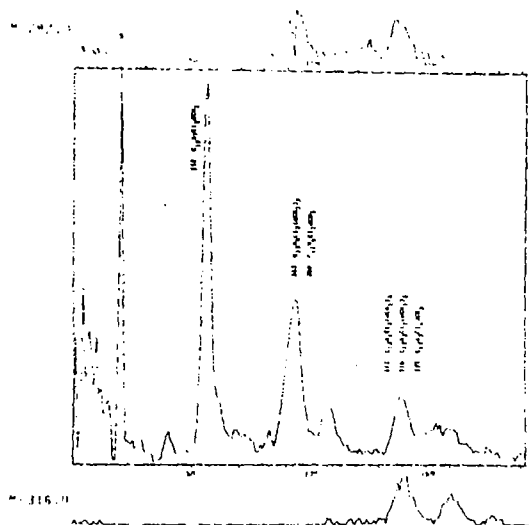


Figure 1. Reconstructed chromatograms of urine extracts from rats fed a diet of Aroclor 1242. 3% OV-1 column programmed from 150° to 210° at 2° per minute.

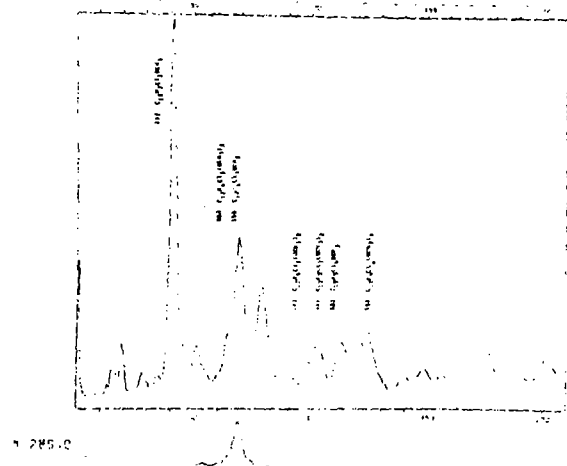


Figure 2. Reconstructed chromatograms of urine extracts from rats fed a diet of Aroclor 1016. 3% OV-1 column programmed from 150° to 210° at 2° per minute.

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OV-1 column programmed from 150° to 210° at 20 per minute.

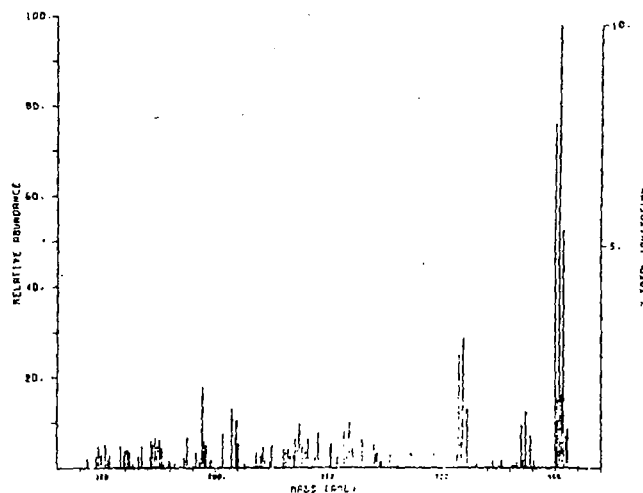


Figure 3. Mass spectrum of a methylated dihydroxytetrachlorobiphenyl ($M^+=350$) metabolite found in urine of rats fed a diet of Aroclor 1016.

a few instances, overlap of two or more components yielded spectra with more than one molecular ion.

The structural assignment of the methoxy derivatives of polychlorinated biphenyls rests upon the following: (1) The presence of intense molecular ions of the correct masses, (2) The correct chlorine "isotope clusters", to establish the number of chlorine substituents and (3) rational fragments, yielding the correct mass ions and appropriate chlorine "isotope clusters" for the fragments.

Figure 3 illustrates the typical features of the mass spectra of the methylated ether derivatives of the phenolic PCB metabolites. The intense molecular ion at m/e 350 and four chlorine "isotope cluster" are consistent with the assigned composition of a dimethoxytetrachlorobiphenyl, $C_{12}H_{10}O_2Cl_4$. Loss of methyl radical leads to the fragment at 335 amu containing four chlorines. Loss of methyl radical and of carbon monoxide from the aromatic ring, produces the largest fragment of M^+-43 with four chlorines at 307 amu. The loss of methyl radical and extrusion of carbon monoxide from aromatic rings is well documented for methoxy and dimethoxy aromatic ethers (BUDZIKIEWICZ, et al. 1967).

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