E. R. Wheeler

Bacterial Degradation of Polychlorinated Biphenyls.I. Identification of Some Metabolic Products from Aroclor[R] 1242

by

KLAUS L. E. KAISER and PAUL T. S. WONG
Environment Canada, Canada Centre for Inland Waters
Burlington, Ontario, Canada

Polychlorinated biphenyls (PCB's) have been recognized as worldwide pollutants with important biological activity and effects upon the aquatic ecosystem (FISHBEIN, 1972). Residual mixtures of the higher chlorinated isomers, i.e. trichloro- to octachloro-biphenyls can be traced in many species of fishes (HANSEN et.al., 1971; DAVIS et.al., 1972) and in fish eating birds within the American and European continents (COOKE, 1973), as well as across the Atlantic (BOGAN and BOURNE, 1972). Though there are several reports on the effects of polychlorinated biphenyls on the metabolism of aquatic and terrestric organisms, only recently has the metabolic degradation of PCB's themselves been found of interest. BAILEY and BUNYAN (1972) found evidence for a rapid metabolism of the less chlorinated isomers of Aroclor 1242 mixtures on feeding it to pigeons and quails. AHMED and FOCHT (1973) described the degradation of PCB's by two species of Achromobacter. The mechanism of the PCB degradation by the affected organisms, however, has not been investigated and therefore, studies were initiated to investigate the biodegradation of PCB's, their kinetic and mechanistic differences with respect to different aquatic organisms.

EXPERIMENTAL

Isolation of Aroclor utilization bacteria

Three 500 ml water samples from Hamilton Harbour, Ontario, were dispersed into three 2 liter Erlenmeyer flasks, capped with rubber stoppers, wrapped in aluminum foil. Ten ml of 5% Aroclor 1242, prepared in acetone were added into each flask to give 0.1% of Aroclor. The samples were incubated for one week at 20°C in a rotary shaker. This procedure would presumably select those bacteria, that could use Aroclor as the sole carbon and energy source for growth. After incubation, aliquots of water samples were plated onto mineral salt agar plates containing 0.1% of Aroclor. Colonies were picked off from the agar plates after one week incubation at 20°C and purified by repeatedly streaking onto Aroclor agar plates. The ability of the purified cultures to use Aroclor for growth was further tested by growing them in Aroclor mineral salt medium. Those bacteria which used agar or contaminants in agar medium would not be able to use Aroclor for growth. The identification and taxonomic studies of these cultures will be reported elsewhere.

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Mineral salt medium

The mineral salt medium used throughout the studies, contained the following salts in distilled water (expressed in g/l): NaCl 0.5; $(NH_4)_2SO_4$ 0.2; $ZnSO_4$ 0.01; H_3BO_4 0.01; $Al_2(SO_4)_3$ 0.01; $MnCl_2$ 0.04; $FeSO_4$ 0.01; $CoSO_4$ 0.002; K_2HPO_4 2.6; KH_2PO_4 1.6. The medium was sterilized at 121°C for 20 minutes.

Extraction and identification of metabolites

A solution containing 0.1% of an Aroclor 1242 mixture in the mineral salt was incubated with the isolated bacteria at 20°C. No bacterial culture was added to the reference flask. After incubation for two months, buildup of metabolites was sufficient for their isolation and identification. One hundred ml samples were extracted with 2 ml of pesticide grade hexane and the extract injected to the GC. For the gas chromatographic determination, a Micro Tek MT-220 gas chromatograph with a 6' x 1/4" stainless steel column, packed with 3% OV 1 on Chromosorb W, HP, 80-100 mesh, carriergas He and an FID detector was employed. Detector and inlet temperatures were at 250°C, the column temperature was programmed from 40 to 250°C at a rate of 20°C/min. For the gas chromatographic mass spectrometric investigation a Finnigan 1015 quadrupole system with a pdp 8/e computer and with a 6' x 1/8" stainless steel column, packed with 3% OV 1 on Chrmosorb W, programmed from 40 to 250°C was used. The computer program was: mass range 40 to 250 amu: integration time 2 ms/amu; threshold 0.25%; samples/amu 1. Mass spectrometer conditions were: electron energy 70 eV; temperatures of ion source and transfer line 175°.

RESULTS AND DISCUSSION

Survey on PCB/microorganism interactions

The Aroclor 1242 mixture consists mainly of monochloro-, dichloro-, trichloro- and tetrachlorobiphenyls. The average content of 42% chlorine of the mixture is equivalent to a trichlorobiphenyl molecule. So far, no attempts have been reported on the bacterial degradation of such a technical mixture of PCB's in water. This is in contrast to quite a few studies on the bacterial metabolism of DDT and other pesticides (MENZIE, 1969). However, AHMED and FOCHT (1973) reported the identification of benzoic acid and p-chlorobenzoic acid from the action of two species of Achromobacter on 4-chlorobiphenyl. Biphenyl and 4-chlorobiphenyl when fed to rabbits were transformed to 4-hydroxy-biphenyl, 4-biphenyl glucosidaromic acid and to 4-(p-chlorophenyl)-phenol, respectively (BLOCK and CORNISH, 1959). These products of mammalian organisms are also believed to be due to bacterial action upon the substrates in the intestinal tracts. The results of these studies show the preferential reaction of the unsubstituted phenyl ring in asymmetric chlorinated biphenyls and also a preferred formation of oxidized derivatives, such as phenols and carboxylic acids.

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Bacterial effects on Aroclor 1242

The gas chromatographic study of the hexane extract obtained from 2 months incubation of the PCB mixture and bacterial culture revealed an obvious change to the Aroclor 1242 standard, as shown in FIGURE 1. A series of peaks is appearing between the elution of the solvent and that of the PCB mixture. A comparison of the gas chromatograms obtained from the hexane extract and from the Aroclor standard further indicates the preferred degradation of biphenyl (first peak of the PCB pattern) and of the less chlorinated biphenyls. This appears consistent with results on the bacterial degradation of monochlorobiphenyl isomers in lake water (WONG and KAISER, 1973), where kinetic differences were also observed.

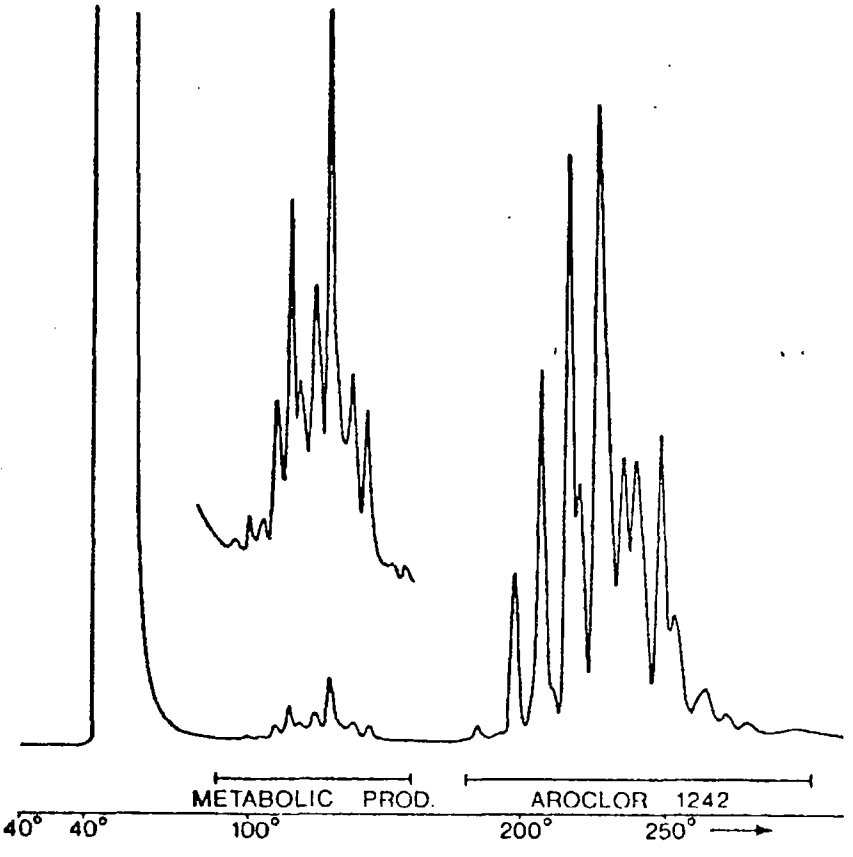

FIGURE 1. Gas chromatogram of a hexane extract of Aroclor 1242, after 2 months incubation with bacteria. Programmed from 40° to 250°C at 20°/min with 1 min initial hold; FID detector; full scale deflection equal to 6.4×10^{-10} amp, for the inset, showing the metabolites only, full scale equal to 6.4×10^{-11} amp.

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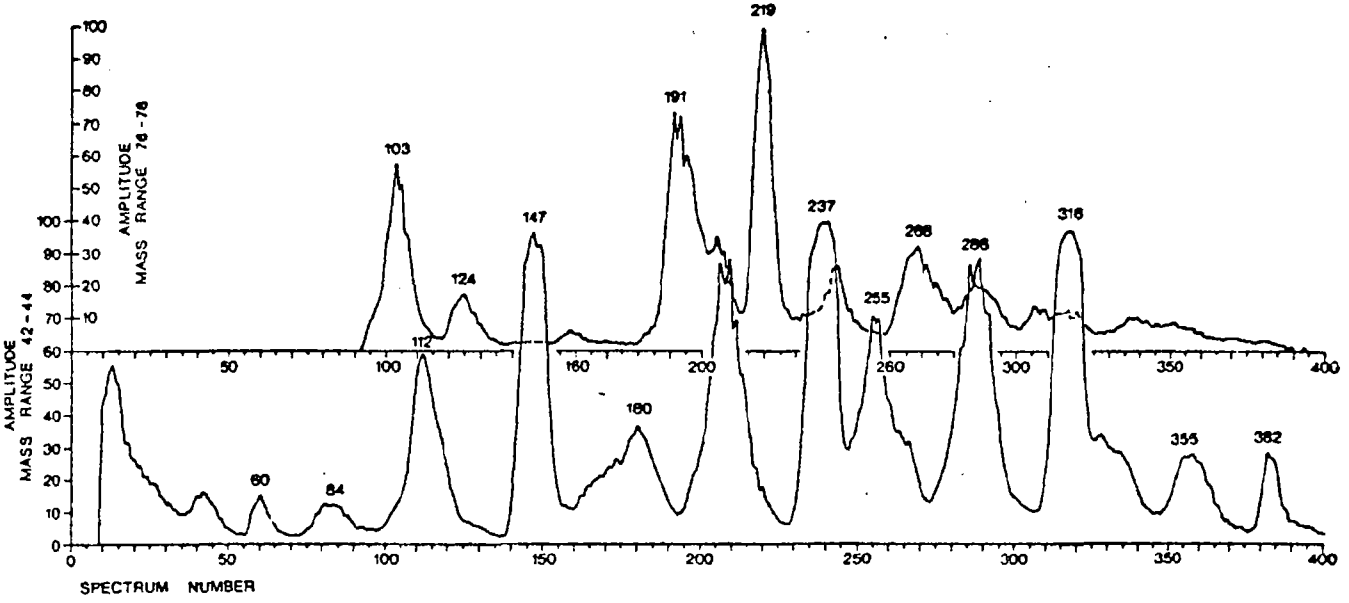

FIGURE 2. Computer drawn gas chromatogram of a single injection of Aroclor 1242 metabolites. Lower trace of mass range m/e 42–44, upper trace showing mass range m/e 76–78.

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upper trace showing mass range m/e 76-78.

Determination of metabolic products

For the identification of the metabolites, the growth media and culture were extracted with hexane and the solution subjected to the combined gas chromatographic/mass spectrometric analysis. FIGURE 2 shows the resulting computer printout of a gas chromatogram of the low temperature part (metabolites only) with the ion monitor as GC detector. In order to facilitate differentiating of the aromatic and aliphatic peaks, the spectrum shows the monitoring of two mass ranges by way of superimposed printouts of a single gas chromatogram. The lower trace comprises the mass range m/e 42-44, equal to $C_4H_9^+$ and the upper trace the mass range m/e 76-78, equal to $C_6H_5^+$. As can be seen from FIGURE 2, peaks of both traces are mostly alternating and indicate a series of alkyl substituted aromatic hydrocarbons and the presence of purely aliphatic hydrocarbons. A detailed investigation of the mass spectra of these compounds and their comparison with mass spectra in the NIH library (HELLER, 1972) reveals the nature of most of the major peaks of the obtained gas chromatogram. For the confirmation of their identity, several of the proposed hydrocarbons were compared with respect to their GC retention times and were found to be in good agreement.

TABLE 1

Metabolites of Aroclor 1242. Spectrum numbers referring to those in FIGURE 2.

Spectrum number	Molecular weight	Formula	Structure
60	86	C_6H_{14}	iso-Hexane
84	114	C_8H_{18}	iso-Octane
103	106	C_8H_{10}	Ethyl-benzene[a]
147	100	C_7H_{16}	iso-Heptane
180	114	C_8H_{18}	iso-Octane
191	120	C_9H_{12}	iso-Propyl-benzene[a]
219	120	C_9H_{12}	n-Propyl-benzene[a]
237	114	C_8H_{18}	iso-Octane
246	134	$C_{10}H_{14}$	iso-Butyl-benzene
286	134	$C_{10}H_{14}$	n-Butyl-benzene[a]
316	128	C_9H_{20}	iso-Nonane
382	128	C_9H_{20}	iso-Nonane

[a] Compounds with identity confirmed by GC retention times comparison.

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TABLE I gives a compilation of the hydrocarbons identified in the gas chromatogram of FIGURE 2. As shown, the identity of all major metabolites has been established as exclusively aliphatic and aromatic hydrocarbons. No evidence has been found of any metabolite containing chlorine; such would have been easily detected by their isotope peaks in the mass spectra. Apparently, neither phenols, alcohols nor other oxidized derivatives of the PCB's were produced in traceable amounts. The bacterial degradation of pesticide substrates usually proceeds via the hydroxylation of energy sources (MENZIE, 1969). In this case of bacteria growing on PCB's, one must assume the rapid reduction of intermediate metabolites to the observed hydrocarbons.

SUMMARY

A bacterial culture, isolated from lake water was shown to degrade polychlorinated biphenyls at a relative high concentration of a commercial mixture (Aroclor® 1242). Several of the metabolites were identified by their mass spectral and gas chromatographic data. None of the metabolites seem to contain any chlorine.

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