Bull. Environ. Contam. Toxicol. (1985) 34:96–105

Bulletin of Environmental Contamination and Toxicology

PCB Congener Analysis of Water and Caddisfly Larvae (Insecta:Trichoptera) in the Upper Hudson River by Glass Capillary Chromatography

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The separation and analysis of the constituents of industrially produced polychlorinated biphenyls (PCBs) has been almost exclusively performed using techniques involving packed gas chromatographic columns. However, the packed column chromatograms proved to be inadequate with regard to the separation of the many congeners which compose the complex PCB mixtures (Duinker and Hillebrand 1983). Unlike the packed column methods, a separation technique based upon gas capillary technology such as that developed by Bush et al. 1983 produces chromatograms with sharp peaks, and hence accurate analysis is possible according to individual congener concentrations. By using this technique 74 PCB congeners, p,p'-DDE, hexachlorobenzene, octachlorostyrene and mirex may be determined in samples as small as 5 mg of tissue (Bush and Barnard 1982). This capability permitted more precise analysis of macroinvertebrates -- specifically aquatic insects in the polluted water of the upper Hudson River (Simpson et al. 1977).

Macroinvertebrates are relatively immobile, most have life cycles of 1 year or less, and consequently reflect recent levels of contamination at a specific site. Laboratory studies have shown various species to rapidly bioconcentrate PCBs to levels several thousand times higher than in the surrounding water (Mayer 1977; Sanders and Chandler 1972). These organisms are also important because they occupy an intermediate position between microorganisms and vertebrates, being one link in the transport of toxic substances through the food web.

This study examines the presence and relative concentration of 74 congeners in water and caddisfly larvae collected from 3 sites in the Hudson River (Fig. 1). Caddisfly larvae (Insecta:Trichoptera) usually dominate the macroinvertebrate communities in riffle habitats of the study area. Their relatively large size and abundance facilitated the acquisition of sufficient biomass for analysis (200 mg wet weight). The sampling was carried out on July 6, 1983 and August 15, 1983.

Table 1. Mean water concentration (ng/l) of PCB congeners at Roger's Island (RI), Thompson's Island (TI) and Stillwater (ST).

Congener	Aroclor	JULY			AUGUST		
		RI	TI	ST	RI	TI	ST
2	1221	.5†	145†*	5.5*	38	20	2.6
2,2'	21/42	2.8†	147†*	73*	4‡	44‡	37
2,6	21/42	3	150 *	73*	4	50	40
2,3	21/42	0.6†	3.6†*	1.1*	ND‡	0.5‡	ND
2,4'	21/42	1.2†	33†*	17*	.8‡	9.7‡	8.9
2,2'5'	21/42	2.0†	14†*	4.9*	2.1	4.7	ND[1]
2,2'4'	1242	3.1	11	8	2.6‡	6.4‡	5.6
2,2'3'+3,2'6'	1242	.95†	6.2†	4.3	0.6‡	2.1‡	2.2
4,2'6'	1242	1.0	3.3	2.3	1.0‡	6.0‡	1.9
4,4'	1242	5.1	17	15	1.6‡	5.5‡	6.7
2,2'4'6'	21/42	ND	10	13	1.8	3.6	2.1
3,2'5'	1242	0.6†	1.1†*	2.6*	0.4	1.6	1.5
2,4,2'6'	1242	0.8†	7.5†	6.7	0.5	15	3.8
3,2'4'	1242	2.5†	13†	9.7	1.8	11	2.4
3,2'3'+4,2'4'	1242	3.3	8.1	5.8	5.8	6.7	10
4,2'3'	1242	2.2	5.0	3.2	1.0‡	2.6‡	3.1
2,5,2'5'	42/54	2.3†	10†	7.0	2.9‡	6.3‡	7.4
2,4,2'5'	42/54	2.0†	8.1†	6.4	1.5	2.3	2.2
2,3,2'5'	42/54	2.1	1.6	3.3	3.5‡	9.5‡	11
2,4,2'4'	42/54	1.2†	7.4†	4.9	0.9	4.5	1.6
CL4C	54/60	2.2	6.8	4.9	2.2	3.9	3.9
CL4D	54/60	2.6	9.2	6.1	2.7	4.8	5.3
2,3,2'3'6'	54/60	0.5	1.0	0.8	ND	3.6	0.7
2,5,3'4'	54/60	2.0	3.7	2.6	1.2	5.9	3.5
2,4,3'4'	54/60	1.2	2.7*	1.7*	1.5	2.7	1.7
2,5,2'4'5'	54/60	1.0	1.9	3.1	0.6	3.7	0.1
2,4,2'4'5'	54/60	1.2	2.9*	5.7*	14	45	6
2,3,2'4'5'	54/60	1.0	1.0	1.9	1.6	1.6	0.6
2,5,2'3'4'	54/60	0.4	0.9	1.1	8.0	2.9	1.8
2,4,2'3'4'	1254	1.6	1.5	1.6	2.6	4.4	1.3
2,3,2'3'4'	54/60	2.4	2.5	2.6	0.7‡	5.5‡	2.0
2,5,2'3'5'6'	54/60	0.6	0.5	1.9	0.3	0.7	0.4
2,3,2'3'5'6'	54/60	ND	ND	0.2	3.3	6.7	0.0
2,3,4,2'3'6'	54/60	0.2	0.7	0.9	1.3	2.5	0.9
3,4,3'4'	1254	0.8	0.7	0.7	3.5	28	2.0
2,3,6,2'3'4'6'	54/60	0.5	0.6	0.7	1.8	2.3	2.3
2,4,5,2'4'5'	54/60	ND	ND	ND	0.9	1.2	0.3
2,3,4,2'4'5'	54/60	2.8	1.9	0.4	2.3	2.2	1.0
3,4,2'3'4'6'	54/60	1.6	1.6	0.5	2.8	1.7	0.3
2,3,4,2'3'4'	54/60	1.7	1.4	1.0	1.0	0.8	0.04
2,3,6,2'3'4'5'6'	54/60	ND	ND	ND	120	85	50
3,4,2'3'4'5'	54/60	9.4	9.4	11	0.2	0.2	ND
2,3,4,5,2'3'5'6'	1260,	0.03	0.5	0.3	0.2	0.4	0.1
Total PCB		100	532	266	331	586	243

[1] ND <0.01 ng/l

* † ‡ Probability that sites identical <0.005

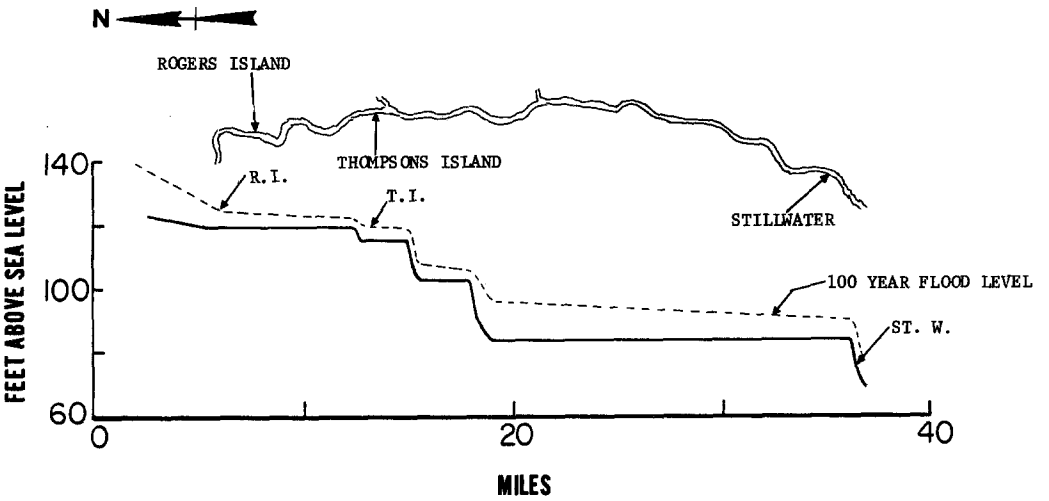

Figure 1. Macroinvertabrate sampling sites in the upper Hudson River.

Table 2. Transport of PCB down the Hudson

		Flow m^3/min*	Kg Total PCB (Kg/month)#	Transport (2+2,2'+ 2,6) Kg/month#	Remainder Kg/month#
July	RI	3668	16	1	15
	TI	3218**	96	62	34
	ST	2769	39	18	21
August	RI	6163	42	13	29
	TI	6350**	104	30	74
	ST	6537	57	21	36
Mean Transport			50 ± 16#	24 ± 8#	34 ± 8#
Mean Transport Kg/ per annum			600 ± 200#	320 ± 120#	420 ± 90#

* From U.S. Geological Survey

±Standard error of the mean

** Mean of RI & ST

MATERIALS AND METHODS

Water samples from each site were collected 0.2 m from the surface in triplicate in n-hexane washed 2 liter reagent bottles. The bottles were filled and emptied three times to equilibrate the inner surfaces to the water -- since PCB's in solution rapidly sorb to glass surfaces. After being filled a fourth time they were placed on ice and transported to the laboratory where they were placed under refrigeration at 4°C. The following morning very little sedimentation had occurred as the samples were virtually free of sediments. Approximately 1300-1600 ml were carefully decanted into separatory funnels for extraction. Ten milliliters of n-hexane were added to each sample. The samples were shaken for 1 min, allowed to separate and then the extract was collected and dried with hexane washed sodium sulfate; the water being collected in a conical flask and its volume eventually measured accurately. This process was repeated 3 times for each sample. The collected extracts were then concentrated to 1 ml using Kuderna-Danish receivers and 4-ball Snyder columns in preparation for GC analysis. All PCB congeners were recovered at the 95% level by this procedure (unpublished results).

The caddisfly larvae were collected with a D-frame aquatic dip net or by picking organisms from rocks removed from the river bed. In the laboratory, organisms were identified to the lowest possible taxon, weighed on a tared pan with an electrobalance, freeze-dried and reweighed. The samples were then transferred to a 10 ml beaker and crushed with a glass rod. Hexane (2 ml) was added and the samples were homogenized with a Tekmar Tissuemizer until the solids were reduced to a fine powder. The contents after settling were transferred onto a layer (1 cm) of sodium sulfate on a 1-cm-diameter glass column containing 10 g of 2% deactivated Florisil. The column was eluted with hexane and the first 40 ml of the eluate was collected and concentrated to 1.0 ml in a Kuderna-Danish evaporator (Bush and Barnard 1982).

The extracts in hexane were analysed using a Hewlett-Packard 5840A gas chromatograph with a 5880 splitless glass capillary inlet and an ASCII interface board through which the calibrated data tables were transmitted to a VAX computer for analysis using the BMDP 81 statistics package (BUSH et al. 1983).

RESULTS AND DISCUSSION

The mean concentration in water of the major PCB congeners which together comprise more than 60% of the total PCB are shown in Table 1. Figure 2 shows the chromatogram of a water sample taken at Thompson's Island. The concentration of the lower chlorinated congeners are much enhanced in the water compared with the mixture of Aroclors 1221. 1016, 1254 and 1260, also shown in Fig. 2. The variability between triplicate samples was ±80% at Roger's Island, ±12% at Thompson's Island and ±10% at Stillwater. The high variance at Rogers Island is attributable to the turbulence of the riffle area at that site and possibly the heterogeneity and

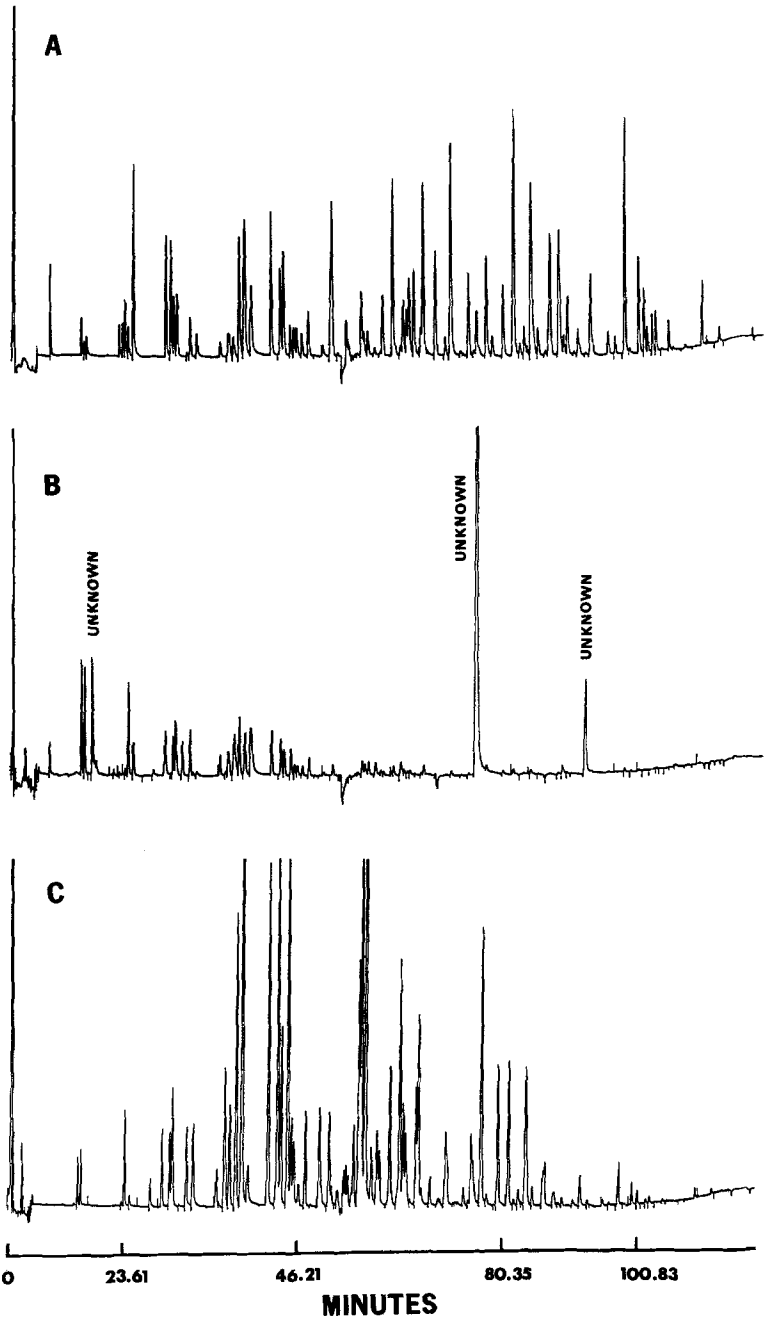

Figure 2. Chromatograms of (A), 200 ng/mL mixture of Aroclors 1221, 1016, 1254 and 1260 (1:1:1:1) in hexane; (B) Hudson River water from Thompson Island concentrated 1,500 times (collected 15 Aug 1983) (C) extract from caddisfly larvae (Pycnopsyche guttifer) collected from Hudson River at Stillwater (11 July 1983).

instability of remnant deposits containing PCB's directly upstream. The Thompson Island and Stillwater sites are located below impoundments in fast flowing riffles with rocky bottoms (Fig. 1). The water is relatively sediment free as the water in the navigable reaches above the impoundments is slow flowing allowing sedimentation of even small particles at the low flow periods of the year. Since the water was subsequently allowed to settle overnight and then carefully decanted; the analyses represent as nearly true estimates of dissolved PCB as is technically possible to attain in field work. The clarity of the water rendered centrifugation unnecessary, and the additional sample handling might reduce the reliability of the ultra-trace PCB analysis.

The concentrations shown in Fig. 2 are low when compared to drinking water standards (0.7 parts per billion compared with the N.Y. State recommended maximum drinking water concentration of 50 parts per billion). Structures of the observed peaks were confirmed by gas chromatography/mass spectrometry using the same Apiezon L column and an MS-25 spectrometer (Kratos Inc.).

Table 2 gives the river flow at the times of sampling. The river flow is controlled by sluices impounding an enormous flood prevention reservoir (Great Sacandaga Lake). The low flow at Stillwater in July is accounted for by a surge caused by recent sluice opening 10 miles upstream of Roger's Island, which had not yet reached Stillwater. In spite of these sporadic variations it was considered useful to estimate the monthly transport of dissolved PCB down the river, to gain an impression of the order of magnitude of the transport represented by the data in Table 1: it approaches metric tones per annum. The most surprising feature is that one half of that which is transported is comprised of only three low chlorinated PCB congeners. Of course this estimate is rough, and during high flow seasons the major transport will be via suspended sediment, which will be expected to carry PCBs without the enhancement in concentration of mono- and dichlorobiphenyls seen with aqueous dissolution here.

Analysis of variance (ANOVA) was used to discover discernible differences between sites, for each congener analysed. Statistically discernible differences ($p < 0.005$) are marked in Table 1. Notice that no discernible differences in total PCB were apparent in the August sampling, whereas individual congeners did differ, emphasizing the value of congener-by-congener analysis. The next most noticeable feature of the table is that differences are attributable only to congeners which are components of Aroclors 1221 and 1242. All congeners of Aroclor 1221 diminished between Thompson's Island and Stillwater in July, whereas the majority of the congeners of Aroclors 1221 and 1242 increased between Roger's Island and Thompson's Island, most noticeably 2-chlorobiphenyl, 2,2'-dichlorobiphenyl and 2,6-dichlorobiphenyl and account for 65% of the mass of PCB transported down the river in July at Thompson's Island. In August when the flow was higher, the Aroclor 1221 differences disappeared but the Aroclor 1242

Table 3. PCB congener concentrations (µg/g wet weight) in caddisfly larvae

	Roger's Island				Thompson's Island						Stillwater	
	H. leo.		Chemo(g)		H. leo.		P. gut.		Chemo(g)		P. gut.	
Congener	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2	.08	.05	.07	.04	.6	.2	0.3	0.1	.3	.04	.004	.002
2,2'	.03	.01	.05	.01	3	.8	.2	.09	.8	.04	.1	.01
2,3'	.03	.01	.06	.01	.06	.02	.01	.008	.07	.002	.07	.002
2,4'	.04	.008	.06	.01	.5	.2	.05	.02	.3	.01	.01	.002
2,2',5'	.02	.01	.5	.1	1.2	.2	.1	.05	.6	.03	.08	.004
2,2',4'	.06	.03	.1	.03	.7	.1	.05	.02	.3	.07	.03	.001
2,2',3' + 3,2',6'	.04	.02	.1	.03	0	0	0	0	0	0	0	0
4,2',6'	.1	.06	.4	.1	2.0	.4	.1	.04	.9	.02	.08	.004
4,4'	.06	.03	.09	.03	.3	.07	.2	.02	.1	.05	.3	.009
2,2',4',6'	.1	.04	.1	.06	1.1	.2	.06	.02	.4	.03	.03	.004
3,2',5'	.06	.02	.1	.02	1.0	1.0	0	0	0	0	.05	.05
3,2',4'	.1	.1	0	0	1.6	.07	.4	6.0	.8	.03	.7	.2
3,2',3' + 4,2',4'	.2	.1	.8	.1	.7	.03	.09	.05	.8	.1	.06	.03
4,2',3'	.4	.2	.7	.2	2.6	.4	.2	.08	1.3	.05	.1	.03
2,5,2',5'	1.2	.5	2.3	.5	7.4	.9	.7	.1	3.1	.1	.5	.04
2,4,2',5'	.5	.2	1.0	.2	1.8	.2	.2	.04	.8	.06	.1	.01
2,3,2',5'	1.1	.5	2.3	.5	12.	1.6	1.7	.07	3.5	.1	1.2	.2
2,4,2',4'	.3	.1	.7	.1	1.6	.2	.1	.02	.6	.03	.1	.01
CL4C	.5	.2	1.4	.3	2.5	.4	.1	.05	1.2	.03	.08	.01
CL4D	.6	.2	1.8	.4	3.7	.6	.3	.06	2.0	.06	.2	.06
2,3,2',3',6'	.07	.03	.2	.04	.5	.02	.05	.02	.4	.03	.02	.003
2,5,3',4'	.8	.3	1.6	.3	2.6	.1	.4	.03	1.2	.03	.2	.02
2,4,3',4'	.5	.2	1.1	.2	1.6	.2	.5	.05	.5	.01	.1	.02
2,5,2',4',5'	.2	.06	.3	.07	.9	.2	.1	.01	.6	.005	.07	.01
2,4,2',4',5'	.2	.06	.3	.06	.5	.2	.2	.02	.5	.006	.08	.04
2,3,2',4',5'	.1	.06	.3	.06	.6	.09	.1	.005	.4	.009	.07	.01
2,5,2',3',4'	.3	.1	.6	.1	1.3	.3	.1	.02	.5	.1	.08	.02
2,4,2',3',4'	.2	.8	.4	.9	1.4	.2	.2	.02	.7	.004	.2	.03
2,3,2',3',4'	.3	.1	.7	.1	1.8	.3	.2	.005	1.3	.02	.1	.01
2,5,2',3',5',6'	.01	.005	.02	.008	.3	.05	.02	.003	.2	.01	.01	.003
2,3,2',3',5',6'	.003	.003	.01	.004	.03	.004	0	0	.1	.1	.004	.002
2,3,4,2',3',6'	.05	.008	.1	.03	.4	.1	.06	.01	.4	.003	.04	.01
3,4,3',4'	0	0	.004	.002	.06	.06	0	0	.002	.002	.01	.006
2,3,6,2',3',4',6'	.2	.07	.4	.08	1.0	0.1	.2	.03	.7	.1	.2	.02
2,4,5,2',4',5'	0	0	0	0	.3	.3	0	0	0	0	.3	.3
2,3,4,2',4',5'	.2	.05	.3	.06	.6	.2	.1	.02	.5	.05	.1	.006
3,4,2',3',4',6'	.03	.006	.04	.008	.1	.03	.03	.006	.2	0.1	.02	.002
2,3,4,2',3',4'	.03	.02	.02	.003	.2	.01	.04	.005	0.3	.04	.03	.008
3,4,2',3',4',5'	.02	.01	.05	.007	.1	.03	.004	.002	0.1	.001	.002	.002
2,3,4,5,2',3',5',6'	.02	.007	.02	.002	.09	.006	.01	.006	0.1	0.1	.008	.00002
TOTAL	9.6	4	22	5	66	12	8	.7	31	1	6.0	3

difference persists (2-chlorobiphenyl is almost absent from Aroclor 1242). No differences were discernible for the more chlorinated congeners nor for total PCB in August at the 0.005 probability level.

The majority of the PCB in the river was contained by an earth dam near Roger's Island until that dam was taken out in 1973. Large quantities of the retained sediment were then shifted down into the reach ending at the dam just above the riffle area at Thompson's Island due to the 100 year flood (level shown in Fig. 1) in 1974. Aroclors 1221 and 1242 are the major constituents of this sediment (Horn et al. 1979, Sloan et al. 1983). The topography of the river between sites is shown in Fig. 1 which if drawn strictly to scale would show an extremely thin layer of water, moving very slowly between PCB contaminated sediment and the atmosphere. It is postulated that the lower chlorine homologues diffuse into the water phase and are dissolved preferentially according to their water solubility and affinity for the sediment, and hence they change most between Roger's Island and Thompson's Island as the water slowly flows over the contaminated river bed. Downstream of Thompson's Island, where the contamination is lower, the volatile congeners may be lost by evaporation into the atmosphere, the loss being discernible at low flow rate in July for the Aroclor 1221 congeners. Ultra-violet degradation may also be a route of degradation.

It appears that the congeners of the more chlorinated Aroclors do not vary greatly from site to site and may be a steady constituent of the water even above Roger's Island, whereas the congeners of Aroclors 1221 and 1242 vary between sites and hence are more dynamic in their input and escape from the river water.

Eighteen species of macroinvertebrates were collected from the three sites. The collecting process depended entirely upon the presence and abundance of each species at each site. Table 3 shows the concentration of the predominant congeners in species which occurred at more than one site and which yielded enough sample mass to allow three analyses to be performed. Table 4 shows the apparent bioaccumulation factor ($x10^{-3}$) obtained by dividing water concentration into tissue concentration, for selected PCB congeners in species which were collected at several sites in July and August. Figure 2 shows representative chromatograms.

The bioaccumulation factors demonstrate selective uptake of different congeners by the various species. Although a more detailed consideration of these effects will be published elsewhere, at this time it is clear that Hydropsyche leonardi is an excellent representative species for PCB monitoring because its bioaccumulation factor was constant at each site at both sampling times. Non-homogeniety of PCB in the water flow at Roger's Island may account for the higher variability there. Unlike H. leonardi, Cheumatopsyche (green phase) is consistent neither in bioaccumulation of total PCB nor of

Table 4

Bioaccumulation factors* of macroinvertebrate species in the Hudson River

	July								August			
	Roger's Island		Thompson's Island			Stillwater			Roger's Island	Thompson's Island		Stillwater
Species[1] Congener	H. leo.	Chemo(g)	H. leo.	P. gut.	Chemo(g)	Chemo(b)	P. gut.	Macron.	H. leo.	H. leo.	Macron.	Macron.
2	175	151	4	2	2	1	7	14	0	3	2	3
2,2'	11	19	19	2	5	2	7	1	5	1	15	10
2,2',4'	64	136	114	9	54	20	8	49	64	143	72	130
2,2',4',6'	164	348	273	14	97	40	12	105	101	73	216	281
3,2',3'+4,2',4'	70	255	92	11	101	47	10	52	22	34	126	55
2,5,2',5'	509	992	719	64	307	133	72	276	167	582	328	425
2,3,2',5'	515	1100	2600	382	761	368	382	1030	142	678	401	515
2,3,2',3',6'	139	370	538	56	398	188	33	187	ND	54	77	286
2,5,2',4',5'	167	336	484	54	325	173	22	85	153	168	92	1290
2,3,2',4',5'	135	282	617	109	378	219	38	63	47	302	193	332
2,3,5,2',4',5'	150	313	1000	85	903	531	25	157	ND	ND	ND	ND
2,4,5,2',3',5',6'	3	5	18	2	152	63	10	26	50	ND	ND	449
2,4,5,2',3',4',5',6'	103	24	363	83	316	201	9	39	23	55	53	63
3,4,5,2',3',4',5'	ND	ND	178	8	221	92	2	26	2	117	67	6
Total	92	209	98	11	46	23	17	52	34	92	72	162
Standard Error	39	48	17	1	2	4	1	16	2	---	---	15
N	3	3	3	3	3	3	3	3	4	1	1	5

* Concentration in macroinvertebrates/mean concentration in water x 10^{-3}

[1] H. leo. = Hydropsyche leonardi; Chemo(g) = Cheumatopsyche green phase; P. gut. = Pycnopsyche guttifer; Chemo(b) = Cheumatopsyche brown phase; Macron. = Macronema carolina

individual congeners suggesting that it is not an appropriate taxon for PCB monitoring. These results support the need for species level ideniifications in this type of work, since the designation ''Cheumatopsyche (green phase)'' embraces several species of caddisflies which cannot be distinguished in the larval stages. P. guttifer accumulates less PCB than the other species in the same habitat.

Monitoring PCB by collecting and analysing macroinvertebrate species in a stream is a viable and relatively simple process. Clearly, different species bioaccumulate PCBs to different extents and hence, if the water concentration at a particular point integrated over recent time is to be determined, the bioaccumulation factors of each species to be utilized must be determined. This study indicates that such an approach to stream monitoring is practicable. More detailed analysis of these data, which will consider the known biology of the species, may yield further indications of the most useful species to be employed for such studies. Further studies of the rates of uptake and depuration in the laboratory will allow optimim integration times for different species to be determined for water quality surveillance in the field.

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Received March 2, 1984; Accepted April 5, 1984