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Review

Polychlorinated biphenyls in the environment

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ABSTRACT

This review surveys the problems arising from the release of PCBs into the environment from the point of view of the analytical chemist. These problems are very complex and interdependent and so it is essential to recognize their mutual links rather than to separate one problem from another (sources of contamination, fate in the environment, toxic properties and particular capabilities, limitations and purposes of analytical methods). Prominent attention should be paid in the future to congener-specific analyses of "toxic" congeners using high-resolution gas chromatography and to toxicity-assessing biological methods.

CONTENTS

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1. INTRODUCTION

Polychlorinated biphenyls (PCBs) are prominent environmental contaminants. They can be found in diverse areas of environment all over the world and their properties indicate that they are a probable threat to health.

Many analyses of PCBs have been reported and there is a vast literature, including several books [1–3]. This review is intended to survey the current status of various aspects of PCB analysis and related problems, their mutual links and trends. It is believed that for good experimental planning, proper analyses and effective interpretation of the data obtained, it is important to have an adequate knowledge both of suitable methods for PCB analyses and of the environmental and toxicological aspects of PCBs.

2. FUNDAMENTAL TERMS

PCBs are a group of compounds derived from biphenyl by substitution of one to ten atoms of hydrogen with atoms of chlorine. PCBs are also commonly called chlorinated byphenyls, chlorobiphenyls and polychlorobiphenyls.

The following terms are used in connection with PCBs. Homologues differ one from another in the numbers of chlorine atoms; there are ten PCB homologues. Isomers differ one from another in the substitution pattern of the chlorine atoms. Each homologue has a particular number of isomers: monochlorobiphenyl 3, di- 12, tri- 24, tetra- 42, penta- 46, hexa- 42, hepta- 24, octa- 12, nona- 3 and decachlorobiphenyl 1. Congener denotes each individual polychlorinated biphenyl, on other words, any isomer of any homologue. There are 209 different PCB congeners. However, sometimes the term "isomer" is used in the sense of congener in the literature.

In order to simplify the nomenclature of PCBs, systematic numbering has been introduced [4]. Each of the congeners has been designated with a number from 1 to 209. This systematic numbering is shown in Table 1.

3. PRODUCTION AND APPLICATION

PCBs were first synthesized in 1881 by Schmidt and Schulz [5] and their commercial production began in 1929 in the USA [6,7]. PCBs were produced by numerous manufacturers throughout the world. The prominent producers include Monsanto (St. Louis, MO, USA) (products sold under the tradename Aroclor), Kanegafuchi Chemical (Tokyo, Japan) (trademark Kanechlor) and Bayer (Leverkusen, Nordrhein-Westfalen, Germany) (tradename Clophen) [8]. In Czechoslovakia, Chemko (Strážske, East Slovakia) marketed products under the trade-name Delor and the PCBs with small amounts of additives as Delotherm and Hydelor [9]. The trademark is usually followed by a number that indicates an average degree of chlorination of the product. For example, Aroclor is followed by a four-digit number, the first two (12) indicating the type of compound (biphenyl) and the other two the average percentage of chlorine. The only exception is Aroclor 1016, which contains about 40% of chlorine and is similar to Aroclor 1242 [7]. Delors are designated with a three-digit number, the first two (10) indicating the type of the molecule and the third the average number of chlorine atoms in the molecule. Delors 103 and 106 were produced in the largest amounts [9].

Because of their properties (*e.g.*, chemical and thermal stability, low or no flammability, high permittivity, low vapour pressure at ambient temperature [8,10]), PCBs have extensive applications. They are utilized (alone or in mixtures) as heat-transfer fluids, dielectrics for capacitors and transformers, hydraulic fluids, lubricants, additives in plastics and dyes, etc. [8,10]. Since the hazardous properties and widespread environmental occurrence of PCBs have been discovered, their production has substantially decreased because many manufacturers have ceased production [8,11]. In 1984 the total cumulative world's production was estimated to $1.2 \cdot 10^9$ kg [11]. In Czechoslovakia $1.89 \cdot 10^7$ kg were produced and production ceased in 1984 [12].

4. CHARACTERIZATION OF PCB MIXTURES AND PCB-RELATED COMPOUNDS

Commercial products of PCBs are mixtures of a large number of congeners. During the manufacture of PCBs by reaction of gaseous chlorine with molten biphenyl under given conditions, the chlorination is controlled by the laws of thermodynam-

TABLE 1

SYSTEMATIC NUMBERING OF PCB CONGENERS INTRODUCED BY BALLSCHMITER AND ZELL [4] AND ACCEPTED BY IUPAC

No.	Substitution pattern	No.	Substitution pattern	No.	Substitution pattern	No.	Substitution pattern
1	2	53	2,2',5,6'	105	2,3,3',4,4'	157	2,3,3',4,4',5'
2	3	54	2,2',6,6'	106	2,3,3',4,5	158	2,3,3',4,4',6
3	4	55	2,3,3',4	107	2,3,3',4',5	159	2,3,3',4,5,5'
4	2,2'	56	2,3,3',4'	108	2,3,3',4,5'	160	2,3,3',4,5,6
5	2,3	57	2,3,3',5	109	2,3,3',4,6	161	2,3,3'.4.5'.6
6	2,3'	58	2,3,3',5'	110	2,3,3',4',6	162	2,3,3',4',5,5'
7	2,4	59	2,3,3',6	111	2,3,3',5,5'	163	2,3,3',4',5,6
8	2,4'	60	2,3,4,4'	112	2,3,3',5,6	164	2,3,3',4',5',6
9	2,5	61	2,3,4,5	113	2,3,3',5',6	165	2,3,3',5,5',6
10	2,6	62	2,3,4,6	114	2,3,4,4',5	166	2,3,4,4',5,6
11	3,3'	63	2,3,4',5	115	2,3,4,4',6	167	2,3',4,4',5,5'
12	3,4	64	2,3,4',6	116	2,3,4,5,6	168	2,3',4,4',5',6
13	3,4'	65	2,3,5,6	117	2,3,4',5,6	169	3,3',4,4',5,5'
14	3,5	66	2,3',4,4'	118	2,3',4,4',5	170	2,2',3,3',4,4',5
15	4,4'	67	2,3',4,5	119	2,3',4,4',6	171	2,2',3,3',4,4',6
16	2,2',3	68	2,3',4,5'	120	2,3',4,5,5'	172	2,2',3,3',4,5,5'
17	2,2',4	69	2,3',4,6	121	2,3',4,5',6	173	2,2',3,3',4,5,6
18	2,2',5	70	2,3',4',5	122	2',3,3',4,5	174	2,2',3,3',4,5,6'
19	2,2',6	71	2,3',4',6	123	2',3,4,4',5	175	2,2',3,3',4.5',6
20	2,3,3'	72	2,3',5,5'	124	2',3,4,5,5'	176	2,2',3,3',4,6,6'
21	2,3,4	73	2,3',5',6	125	2',3,4,5,6'	177	2,2',3,3',4',5,6
22	2,3,4'	74	2,4,4',5	126	3,3',4,4',5	178	2,2',3,3',5,5',6
23	2,3,5	75	2,4,4',6	127	3,3',4,5,5'	179	2,2',3,3',5,6,6'
24	2,3,6	76	2',3,4,5	128	2,2',3,3',4,4'	180	2,2',3,4,4',5,5'
25	2,3',4	77	3,3',4,4'	129	2,2',3,3',4,5	181	2,2',3,4,4',5,6
26	2,3',5	78	3,3',4,5	130	2,2',3,3',4,5'	182	2,2',3,4,4',5,6'
27	2,3',6	79	3,3',4,5'	131	2,2',3,3',4,6	183	2,2',3,4,4',5',6
28	2,4,4'	80	3,3',5,5'	132	2,2',3,3',4,6'	184	2,2',3,4,4',6,6'
29	2,4,5	81	3,4,4',5	133	2,2',3,3',5,5'	185	2,2',3,4,5,5',6
30	2,4,6	82	2,2',3,3',4	134	2,2',3,3',5,6	186	2,2',3,4,5,6,6'
31	2,4',5	83	2,2',3,3',5	135	2,2',3,3',5,6'	187	2,2',3,4',5,5',6
32	2,4',6	84	2,2',3,3',6	136	2,2',3,3',6,6'	188	2,2',3,4',5,6,6'
33	2',3,4	85	2,2',3,4,4'	137	2,2',3,4,4',5	189	2,3,3',4,4',5,5'
34	2',3,5	86	2,2',3,4,5	138	2,2',3,4,4',5'	190	2,3,3',4,4',5,6
35	3,3',4	87	2,2',3,4,5'	139	2,2',3,4,4',6	191	2,3,3',4,4',5',6
36	3,3',5	88	2,2',3,4,6	140	2,2',3,4,4',6'	192	2,3,3',4,5,5',6
37	3,4,4'	89	2,2',3,4,6'	141	2,2',3,4,5,5'	193	2,3,3',4',5,5',6
38	3,4,5	90	2,2',3,4',5	142	2,2',3,4,5,6	194	2,2',3,3',4,4',5,5'
39	3,4',5	91	2,2',3,4',6	143	2,2',3,4,5,6'	195	2,2',3,3',4.4'.5.6
40	2,2',3,3'	92	2,2',3,5,5'	144	2,2',3,4,5',6	196	2,2',3,3',4,4',5',6
41	2,2',3,4	93	2,2',3,5,6	145	2,2',3,4,6,6'	197	2,2',3,3',4,4',6,6'
42	2,2',3,4'	94	2,2',3,5,6'	146	2,2',3,4',5,5'	198	2,2',3,3',4,5,5',6
43	2,2',3,5	95	2,2',3,5',6	147	2,2',3,4',5,6	199	2,2',3,3',4,5,6,6'
44	2,2',3,5'	96	2,2',3,6,6'	148	2,2',3,4',5,6'	200	2,2',3,3',4,5',6,6'
45	2,2',3,6	97	2,2',3',4,5	149	2,2',3,4',5',6	201	2,2',3,3 ',4',5,5',6
46	2,2',3,6'	98	2,2',3',4,6	150	2,2',3,4',6,6'	202	2,2',3,3',5,5',6,6'
47	2,2',4,4'	99	2,2',4,4',5	151	2,2',3,5,5',6	203	2,2',3,4,4',5,5',6
48	2,2',4,5	100	2,2',4,4',6	152	2,2',3,5,6,6'	204	2,2',3,4,4',5,6,6'
49	2,2',4,5'	101	2,2',4,5,5'	153	2,2',4,4',5,5'	205	2,3,3',4,4',5,5',6
50	2,2',4,6	102	2,2',4,5,6'	154	2,2',4,4',5,6'	206	2,2',3,3',4,4',5,5',6
51	2,2',4,6'	103	2,2',4,5',6	155	2,2',4,4',6,6'	207	2,2',3,3',4,4',5,6,6'
52	2,2',5,5'	104	2,2',4,6,6'	156	2,3,3',4,4',5	208	2,2',3,3',4,5,5',6,6'
						209	2,2',3,3',4,4',5,5',6,6'

ics. As a result, certain substitution patterns are favoured and, consequently, some congeners are more abundant than the others [13]. Owing to the varied reaction conditions for different lots and variations in other technical operations (*e.g.*, distillation of raw products), the composition of the commercial products also varies [8,9]. Hence published data on compositions of commercial PCB mixtures [14–19], giving valuable information on the abundance of congeners, should not be considered as the exact composition of all lots of the same product marketed under the same name. Published results are also partly affected by more or less inaccurate determinations.

Congener compositions of samples from different areas of the environment and biological materials are influenced by the fact that each congener differs from the others in its properties such as water solubility [20–22], Henry's law constant [23], standard reduction potential [24], ability to undergo microbial transformation [25,26], partitioning between different compartments of the environment [27–29], accumulation, distribution and elimination by various organisms [30–38], including humans [39–41]. Consequently, the abundances of individual congeners in environmental and biological samples differ from those in the products from which the contamination originated [42,43]. Moreover, contamination may originate from more than one source and it has also been shown that a number of PCB congeners are produced unintentionally, *e.g.*, during combustion of chloride-containing wastes in incinerators [44] or during syntheses of azo dyes [45], phthalocyanine pigments, chlorinated paraffins, phenolic resins, etc. [46]. Some physico-chemical characteristics (melting point, water solubility and vapour pressure) and differences between them for different congeners can be seen in Table 2. The abundances of some congeners in two lots of Aroclor 1260 and in three biological samples (human milk and bird's eggs) are given in Table 3.

Environmental samples contaminated by PCBs and commercial PCB products may also contain other groups of chlorinated compounds which have

TABLE 2

MELTING POINTS, WATER SOLUBILITIES AND VAPOUR PRESSURES OF SOME PCB CONGENERS

Congener No.	Melting point (°C)[a]	Water solubility (μg/l)[a]	Vapour pressure at 25°C (mPa)[b]
1	34	6000	1120
2	25	2400	200
3	78	1200	612
4	61	1100	133
7	24	1100	239
15	149	56	2.53
18	44	410	12.0
29	78	130	43.9
30	63	200	117
40	121	29	9.71
47	83	92	11.4
52	87	29	4.92
66	124	4.6	6.12
77	180	9.2	0.306
86	100	21	0.0771
87	114	4.1	2.13
101	77	10	1.20
105	125[c]	2.1[c]	0.904
116	124	5.2	–
118	105	2.1[c]	1.20
126	125[c]	1.0[c]	–
128	150	0.57	0.346
138	79	1.8[c]	0.532
153	103	0.91	0.692
156	141[c]	0.36[c]	0.213
171	122	2.0	0.239
180	99[c]	0.63[c]	0.129
194	159	0.22	–
208	183	0.018	–
209	306	0.0013	–

[a] Adapted from ref. 21.
[b] Adapted from ref. 6.
[c] Estimates.

TABLE 3

ABUNDANCES OF SOME PCB CONGENERS IN TWO LOTS OF AROCLOR 126 IN HUMAN MILK AND IN EGGS OF TWO SPECIES OF BIRDS

Weight %, $\sum$PCBs = 100.

PCB No.	Aroclor 1260 [17]	Aroclor 1260 [47]	Human milk [17]	Eggs of black-winged stilt [48]	Eggs of gull-billed tern [48]
52	0.25	0.56	1.9	4.4	0.4
101	2.5	5.02	0.97	4.4	1.3
118	0.49	0.57	6.5	8.9	2.2
138	6.5	6.13	10.0	9.7	9.5
153	9.6	10.80	12.0	12.6	17.1
156	0.45	0.88	4.87	1.7	2.2
180	9.1	7.12	5.3	8.0	15.3

some properties in common with PCBs. Each group of these compounds may be a complex mixture containing many individual compounds cumulated by organisms, more or less persistent, having toxicological importance, some of them having the same mechanisms of action as the "toxic" PCBs congeners and some of them able to interfere in the determination of PCBs. The presence of these compounds may affect both chemical and biological assays of PCBs and thus lead to misinterpretation of the results. Basic information on such compounds is summarized in Table 4.

In addition to chlorinated compounds, there are also bromochlorinated and brominated compounds derived from PCBs, PCDFs and PCDDs [49,50]. All these groups of the compounds can be determined using gas chromatography.

5. PCB POLLUTION: A VIEW INTO THE FUTURE

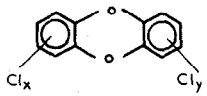
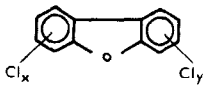
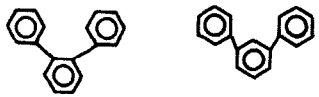
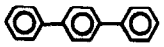
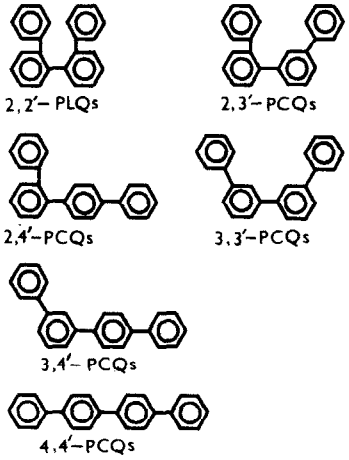
PCBs have been used for more than 60 years, but their presence in the environmental was not apparent until the electron-capture detector was extensively applied in gas chromatographic analyses [122]. In 1966 PCBs were first identified as pollutants [123] and subsequent investigations showed that they are ubiquitous [124]. Currently found concentrations are usually given in pg/m^3 in the atmosphere, ng/kg in surface waters, $\mu g/kg$ in sediments and soils and mg/kg in eggs of aquatic birds, fat of fishes, human adipose tissue and human milk fat. The amount of PCBs in the global environment has been estimated to be about $3.7 \cdot 10^8$ kg [11], and further $7.8 \cdot 10^8$ kg were estimated to be still available for utilization or deposited in different ways [11]. Hence it is of great importance for assessing the prospects for contamination to know what the fate of the non-utilized and deposited PCBs will be [11].

Generally, PCBs are very persistent compounds. There are three natural processes of degradation of environmentally dispersed PCBs: combustion, photolysis and biodegradation [125]. Natural combustion is very rare and photolysis needs access of light for a sufficiently long period of time. Moreover, during PCB photolysis (and also combustion) PCDFs and other related toxic compounds can be formed from the PCBs [126,127]. The crucial role in the clearance of the environment is played by microbial biodegradation processes. These processes are highly congener dependent. There are two basic types of microbial degradation: aerobic degradation, affecting especially the lightly chlorinated PCBs (as a result the highly chlorinated PCBs become more abundant) [128–131], and anaerobic degradation, occurring in sediments and causing reductive dechlorination of highly chlorinated PCBs (as a result the lightly chlorinated PCBs become more abundant). It is of particular interest that anaerobic dechlorination selectively removes *meta*- and *para*-chlorines and thus affects the "toxic" congeners (see Section 6.3.). The less chlorinated congeners that arise by anaerobic degradation can be then subjected to aerobic degradation [128, 129, 132]. Hence it seems that nature may have the potential to remove PCBs from the environment. These processes, however, occur under specific conditions and anaerobic degradation also needs relatively high concentrations of PCBs (on decreasing the concentration of Aroclor 1242 from 140 to 14 ppm the dechlorination becomes unobservable) [133]. This is unfortunate because the environmental concentrations are usually much lower and the higher (and potentially dangerous) concentrations usually occur in higher animals and humans owing to bioaccumulation.

Assessment of time trends is complicated owing to the difficulty of obtaining true values for PCB concentrations, to changes in analytical methods over the years and also due to seasonal variations in PCB levels (*e.g.*, [134]). Moreover, the trends may differ for different compartments of the environment. For these reasons, mere comparisons of results from various time periods (and from various locations) may in some instances be misleading and the time trends can hardly be determined exactly. However, the results obtained so far indicate that after a significant drop in PCB levels in heavily polluted areas during the 1970s (mainly as a result of decreases in leakage and of diffusion to less contaminated areas), from the early 1980s the PCB levels have been fluctuating or declining much more slowly in the samples used for monitoring environmental pollution and in humans [107,135–139] (e.g., see Table 5 and Fig. 1). Hence it seems likely that the end of PCB pollution can hardly be expected in the near future.

TABLE 4

IMPORTANT PCB-RELATED COMPOUNDS

Compound	Structure	Sources	Comments
Polychlorinated dibenzo-*p*-dioxins (PCDDs) Polychlorinated dibenzoforans (PCDFs)	 PCDDS  PCDFs	Heating and especially combustion of PCBs and other chlorine-containing compounds [51–63], unwanted by-products in several chemicals (including in PCBs). PCDDs formed also in enzyme-mediated reactions [64,65]. Never produced intentionally	Extremely persistent and some congeners (2,3,7,8-TCDD) extremely toxic. Found in numerous environmental and biological samples all over the world [66–84]. PCDDs include 75 congeners and PCDFs include 135 congeners [67,68]. PCDDs and PCDFs may co-elute with PCBs when common GC stationary phases are used [85].
Polychlorinated terphenyls (PCTs)	Three types of skeletal structures:  *ortho* *meta*  *para*	Produced, marketed and used for similar purposes to PCBs, sometimes in mixtures with PCBs [8,86,87]	High concentrations of PCTs have been recently found in US water sediments and biota [88]. Little information is available on the distribution, fate and effects of the PCTs [88]. The number of congeners is enormous. PCTs, particularly the lower chlorinated ones, may co-elute with PCBs when common GC stationary phases are used [89]
Polychlorinated quaterphenyls (PCQs)	Six skeletal structures:  2,2′– PLQs 2,3′– PCQs 2,4′–PCQs 3,3′–PCQs 3,4′– PCQs 4,4′–PCQs	Dimerization of PCBs	All six skeletal structures of PCQs have been identified in human adipose tissue and blood [90,91]. The number of congeners is enormous (more than 100 000) [90]

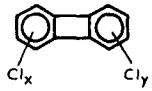
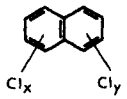
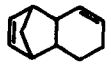
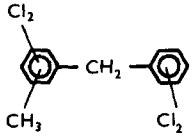
Polychlorinated biphenylenes (PCBPs)		Combustion of PCBs [92]	2,3,6,7-Tetrachlorobiphenylene was found to be equipotent with 2,3,7,8-TCDD in inducing microsomal enzymes [93]. There are 75 possible congeners
Polychlorinated naphthalenes (PCNs)		Produced commercially (under the trademark Halowax) and used mainly in the electrical industry and to impregnate wood, paper and textiles [94]	PCNs have been found in various parts of environment [95–97]. Some of them are potent AHH and EROD inducers [98]. There are 75 congeners [94]. PCNs may co-elute with PCBs when common GC stationary phases are used [99]
Polychlorinated camphenes (PCCs), also called polychlorinated bornanes (Toxaphene, Campheclor)	One of the skeletal structures of the main components is 	Produced commercially and used as pesticides [100]	Global pollutant [100–102]. Extremely complex mixture of more than 600 individual components, only a few of which have been identified [100,103]. PCCs may co-elute with PCBs when common GC stationary phases are used [100]
Technical chlordane (polychlorinated methanoindenes and indanes)	Basic tricyclic structure: 	Produced commercially and used as pesticides [104]	Global pollutant [105–108]. In addition to 48 congeners derived from the basic tricyclic chlordene structure, it also contains a number of other chlorinated compounds [109,110]. Technical chlordane may co-elute with PCBs when common GC stationary phases are used [105]
Tetrachlorobenzyltoluenes (TCBTs), also called monomethyl-substituted diphenylmethanes (MePCDMs)		Produced commercially and used in several cases as PCBs substitutes [111–114]	Found in fish and sediment samples [115,116]. There are 96 congeners (in this instance the same as isomers) [117]. TCBTs may co-elute with PCBs when common GC stationary phases are used [118]
PCB metabolites: Oxygenated PCBs (PCB-ols) Methylsulphonyl PCBs (MSF-PCBs) Hydroxymethylsulphonyl PCBs (HMS-PCBs)	Some chlorines are substituted with –OH Substituted with $-SO_2CH_3$ Substituted with $-SO_2CH_3$ and –OH	Biological modifications of PCBs	MSF-PCBs have been found in human tissues [119] and the same is true for HMS-PCBs [120]. PCB-ols have been found also in marine sediments and biota [121]. MSF-PCBs are very persistent and are suspected to cause some distress in PCBs-affected humans [119]

TABLE 5

TIME TREND OF PCB LEVELS IN THE LIZARD GOBY FISH (RHINOGOBIUS FLUMINEUS) FROM THE RIVER NAGARAGAWA, JAPAN (ACCORDING TO REF. 107)

Year	Total PCBs (mg/kg wet weight basis)	Year	Total PCBs (mg/kg wet weight basis)
1968	14	1979	0.37
1969	15	1980	0.20
1970	13	1981	0.21
1971	2.7	1982	0.12
1972	3.1	1983	0.10
1973	2.6	1984	0.11
1974	0.58	1985	0.16
1977	0.38	1986	0.15
1978	0.34		

6. BIOLOGICAL ASPECTS OF PCBs

6.1. Effects of high doses on laboratory animals

Effects on animals by high doses of PCBs include body weight loss and lesions and dysfunctions of the skin (chloracne), liver (hepatomegaly, haemorrhage, porphyria) bile duct, gall bladder, urinary tract, endocrine system and reproductive system and also teratogenesis and carcinogenesis. There are large differences in sensitivity and types of effects between species. These effects are preceded by induction of xenobiotic metabolizing enzymes [140–147]. On the other hand, there are also antagonistic relationships between PCBs and some other toxic chemicals (*e.g.*, inhibition of aflatoxin B_1-mediated carcinogenesis by PCBs) [146,148–152].

Acute toxicity testing of commercial mixtures has not revealed a dependence of toxicity merely on the degree of chlorination. Of the Aroclors, the most toxic in rats (oral application) were Aroclor 1254 (LD_{50} 1.295 g/kg) and Aroclor 1260 (LD_{50} 1.315 g/kg) and the least toxic were Aroclor 1248 (LD_{50} 11 g/kg), 1262 (LD_{50} 11.3 g/kg) and 1268 (LD_{50} 10.9 g/kg) [153]. As will be discussed in Section 6.3., however, there are vast differences in toxicity between individual congeners.

Mutagenicity testing by the most commonly applied Ames test has shown that none of the PCBs gives positive results, either with metabolic activation or without it [146].

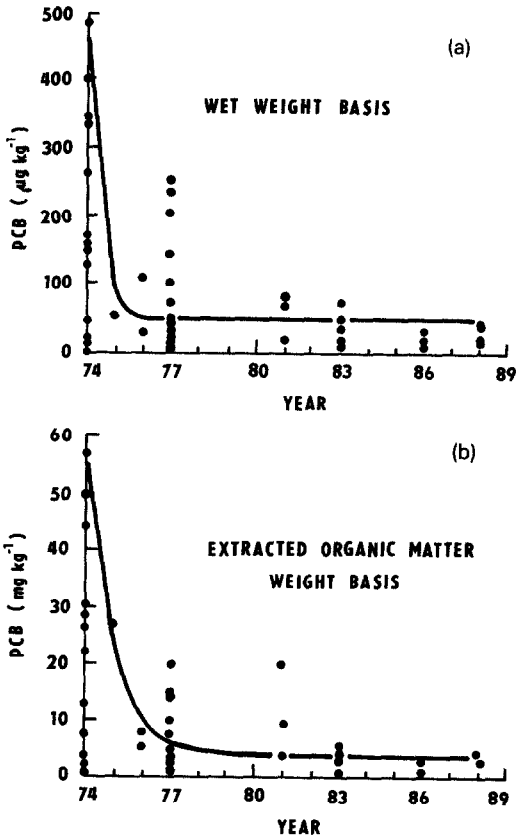


Fig. 1. Time trend of PCBs levels in mussels from the Rijeka Bay (Adriatic Sea), Croatia. Monitoring was carried out by a single analytical group (mostly by the same analyst) using uniform methodology for 14 years. Reprinted from ref. 138.

6.2. Effects of high doses on humans

The effects of high doses on humans can be investigated in individuals exposed to PCBs either occupationally or due to poisoning. There have been two accidents of mass PCB poisoning caused by ingestion of edible oil. The first, called "Yusho", happened in 1968 in Japan [154] and the second, called "Yucheng" happened in 1979 in Taiwan [39,155]. In the poisoned humans many effects were observed such as liver damage, dermal lesions, respiratory disorders, severe ocular signs, various neurological symptoms, damage to the endocrine system, immunodeficiency and reproduction disorders [154,156]. The most common symptom in occupationally exposed humans is chloracne. Liver injury and changes in liver-related serum analytes (bi-

lirubin, transferases, β-glucoronidase, etc.) have also been observed. Other responses are more rare [156–159].

More serious problem may be the effects of PCBs on children, even in cases of non-professional (environmental) exposure. They may be exposed to PCBs during foetal development and after birth they may be fed with breast milk which may contain significant levels of PCBs [160,161]. It has been found that infants from mothers with higher PCBs concentrations in the blood had significantly decreased weights and gestational ages [162] and women with miscarriages had higher average PCBs levels in the blood than control women [163]. It has been also proposed that late haemorrhagic disease, which is an important cause of morbidity and mortality in infants older than 1 month, is caused by PCBs and related compounds given to the infants in the breast milk [164,165]. It has been observed that children who had been breast fed with milk containing higher levels of PCBs for longer periods (1 year) were less active than the less exposed children, which suggests a possible vulnerability of certain behavioural systems to PCBs (and related compounds) [166].

Carcinogenity of PCBs in humans has not been proved reliably so far. However, significantly increased levels of some oncogene proteins [167] and chromosomal aberrations of human peripheral blood lymphocytes [168] have been found in workers exposed to PCBs.

Correct interpretations of the effects of PCBs on humans are difficult owing to the possible presence of PCB-related compounds together with PCBs (*e.g.*, the contaminated Yusho oil also contained PCDFs, PCDDs and PCQs [154,169] and it is probable that the largest part of toxicity in this case arose from PCDFs and PCDDs [170], and the Yucheng oil contained PCDFs, PCQs and PCTs [155,171]) and also because an important role is played by other factors such as exposure to various chemicals, way of life and genetic differences, which are specific to individuals and which are very difficult to assess.

6.3. Biochemical insight: cytochrome P-450, its induction by PCBs and links with toxicity

Most lipophilic foreign compounds (xenobiotics) undergo in organisms enzyme-mediated biotransformation reactions, the purpose of which is elimination of these xenobiotics. The detoxification has two phases. During the first phase the polarity of a xenobiotic increases and during the second phase conjugation occurs, which means that the metabolite is combined with a product of normal metabolism (endogenous product). Such a conjugate can usually be easily excreted. However, metabolism does not lead inevitably to detoxification of a xenobiotic, as conversion into more toxic product(s) that are not conjugated and thus not excreted may also occur.

A critical role in the metabolism of many xenobiotics (and also endogenous compounds) is played by a family of enzymes named cytochrome P-450 [172]. The enzymes belonging to such a family are generally called isozymes. P-450 is a constituent of enzymatic complexes called P-450-mediated monooxygenases or mixed function oxidases (MFO). The term "P-450" arises from the fact that the reduced form of cytochrome P-450 bound to carbon monoxide exhibits an absorption maximum at about 450 nm. Various isozymes of cytochrome P-450 exist in plant and in bacterial and animal organisms including man [173–177], and there are indications that cytochrome P-450 is ubiquitous in all living organisms [172]. It is not known how many isozymes of cytochrome P-450 exist, but the data imply that there may be up to 100 enzymes [173]. The main effort has been exerted to investigate P-450 in mammals (particularly in rats and mice and in humans) and in chick embryos.

The important feature of the P-450 is its inductibility, which means that its production by cells in the organism can be increased by various xenobiotics. These xenobiotics are called inducers. Each inducer exerts a characteristic impact both on the induction of the total amount of the cytochrome P-450 and on the levels of individual cytochrome P-450 isozymes [178,179]. The isozymes have their own, but overlapping, substrate specificities [180]. Higher amounts of these isozymes in organisms are manifested by changes in the metabolism of the corresponding xenobiotics. Such stimulated metabolical modifications have been observed both in various species of laboratory animals and in humans [180]. The following cytochrome P-450 isozymes appear to be important for investigation of the effects of PCBs: P-450IA1 (also called in rats P-450c,

in mice P_1-450 or P-448 and in humans P_1-450), P-450IA2 (also called in rats P-450d and in mice and humans P_3-450), P-450IIB1 (also called in rats P-450b) and P-450IIB2 (also called in rats P-450e) [181].

Inducers are classified into groups according to the cytochrome P-450 isozymes that they induce. For the study of PCB toxicology there are two important groups of inducers. A typical representative of the first group is phenobarbital (PB) and of the second group the polycyclic aromatic hydrocarbon 3-methylcholanthrene (3-MC). Accordingly, a chemical inducing a spectrum of cytochrome P-450 isozymes similar to that induced by PB (or 3-MC) is classified as a PB-type inducer (or a 3-MC-type inducer). PB-type inducers typically significantly increase the levels of P-450IIB1 and P-450IIB2 and 3-MC-type inducers the levels of P-450IA1 and P-450IA2 isozymes. Induction of a particular cytochrome results in increasing activity of enzymatic complexes in which the particular cytochrome is involved. Such enzymatic complexes of special interest are aryl hydrocarbon hydroxylase (AHH) responsible for the metabolism of polycyclic aromatic hydrocarbons and exthoxyresorufin-*o*-deethylase (EROD). The catalytic activities of both AHH and EROD are associated with 3-MC-type inducers, or strictly, with isozyme P-450IA1 [173,178,179,182, 183].

The influence of cytochrome P-450 as such on the toxicity of xenobiotics in organisms is complicated. The P-450 activities may lead, on the one hand, to inactivation and elimination of toxic xenobiotics or, on the other, to conversion of other (or even the same) foreign compounds to more toxic or carcinogenic intermediates, and as a result, the P-450 may prevent intoxication or cause intoxication, and may protect against chemical carcinogenesis or increase the risk of cancer. The results depends on many factors, such as the nature of the foreign compound, the route into the organism, specific tissue susceptibility, the isozymal spectrum of induced P-450, and the ratio between P-450 and other related enzymes and compounds [179,180,184]. The influence of these factors on the metabolism of a xenobiotic has been most extensively investigated in benzo[*a*]pyrene [185–191].

Some time ago, it was revealed that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), an extremely toxic and carcinogenic compound related to PCBs, is also an extremely potent inducer of AHH activity. It was also found that the biological activity is structure dependent and that the pattern of pathological changes caused by certain PCB congeners is identical with that caused by 2,3,7,8-TCDD [192–200].

After an effort to find the relationships between structure, AHH (or P-450IA1 or EROD or 3-MC type) induction activity and toxicity [201–207], a classification of PCBs into categories has been proposed [208]. The congeners having two *para*-chlorines and at least one *meta*-chlorine (according to similarity with 2,3,7,8-TCDD) have been considered. The first category, "coplanar" PCBs (having no *ortho*-chlorine), has two subcategories (the term "coplanar" is commonly applied to them because of the absence of *ortho*- and the presence of *meta*- and *para*-chlorine atoms make a coplanar conformation more probable [209]). The first subcategory contains congeners 77, 126 and 169. These are the most potent inducers of P-450IA1 and P-450IA2 (and hence AHH and EROD activity) both *in vivo* (male Wistar rats) and *in vitro* (rat hepatoma H-4-II E cells), they are "pure" 3-MC-type inducers and are the most toxic PCB congeners known. The second subcategory contains congeners 37 and 81. These are less potent AHH inducers than congeners in the first subcategory and they are mixed-type inducers, *i.e.*, they induce P-450 isozymes typical for both 3-MC and PB. PCB 37 is the least active congener in the first category and does not cause typical toxic responses. The second category comprises mono-*ortho* "coplanar" PCBs having one *ortho*-chlorine (congeners 105,114,118,123,156,157,167 and 189). They are also potent inducers of AHH activity, they are mixed-type inducers and in many of them toxic effects similar to those of 2,3,7,8-TCDD have been proved. The next category contains di-*ortho* "coplanar" PCBs (congeners 128, 137, 138, 153, 158, 166, 168, 170, 180, 190, 191, 194 and 205). Their AHH-inducing potency *in vivo* is significantly diminished (*in vitro* they are inactive) and another shift to a PB-type induction pattern is apparent [206–208,210,211].

Congeners belonging to groups other than those above (mono-*ortho* "coplanar" congeners without one *para*-chlorine or tri-*ortho* "coplanar" congeners) can also to some extent induce in high doses

(tens and hundreds of mg/kg) AHH and EROD activities *in vivo* (in mice) [212], but their inducing potencies are considerably lower in comparison with AHH-type inducers between PCBs or 2,3,7,8-TCDD.

It is noticeable that in fish only the non-*ortho* "coplanar" congeners induce EROD activity; other congeners did not cause such effects at the concentrations tested (1 and 5 mg/kg) [213].

Systematic tests in rats have revealed that there are very good correlations (in terms of orders of magnitude) between AHH- and EROD-inducing potencies *in vivo* and *in vitro* and between the inducing potencies and typical acute toxic effects (body weight loss, thymic atrophy). Such correlations have been demonstrated in selected PCB congeners [208,210,214,215] and in PCDD and PCDF congeners [216–218]. Certain PCB congeners, namely 77 and 105, however, have shown some deviations from these correlations, probably owing to their easier metabolizability *in vivo* [207]. The results also show that there are vast differences in inducing potencies and toxicities between various individual congeners. The above-mentioned correlations imply that a congener which is a potent inducer of P450IA1 is expected to also be considerably toxic (and *vice versa*). This is why the non-*ortho* and mono-*ortho* "coplanar" congeners have begun to be called "toxic" congeners. Consequently, there are thirteen or, when congener 37 is not included, twelve "toxic" congeners. The term "toxic" currently used for the twelve (or thirteen) PCB congeners does not mean that the other PCB congeners are harmless [212,219], but is reflects the very large differences in toxic potencies between the congeners.

In a search for the mechanism of P450IA1 induction and toxic effects, the ability of P450IA1-inducing chemicals to bind as ligands to an intracellular protein exhibiting typical properties of a receptor has been discovered [220–222]. This receptor is called Ah-receptor (aryl hydrocarbon or aromatic hydrocarbon receptor) or, sometimes, 2,3,7,8-TCDD receptor. There is evidence that the complex of the Ah-receptor with the ligand is the mediator of both the P-450IA1 induction and the typical toxic effects [208,221–224]. The Ah-receptors are widely distributed in many animal tissues and species and in humans [172,179,225–230] and their nature is still an object of investigation. It has recently been proposed that the toxic effects may be caused by certain products of the metabolism of arachidonic acid, namely epoxyeicosatrienoic acid and monohydroxyeicosatetranoic acid. The metabolism is P-450 dependent and highly inducible by 2,3,7,8-TCDD [231]. Nevertheless, it is probable that although Ah-receptor plays a critical role in the toxicity, it is not a mediator of all the toxic responses of P450IA1-inducing compounds [232].

It is characteristic for receptor-mediated biological process (because of limited binding sites per cell) that only certain maximum induced enzymatic activity can be reached (at doses associated with acute toxicity so-called "super-maximum" induction of hepatic EROD (but not AHH) activity has been also observed in mice [233,234], which is not considered in the following text). The dose or concentration of a congener that just induces half of the maximum value, ED_{50} (effective dose) or EC_{50} (effective concentration), is called the biological potency and serves as a measure of the biological activity of the congener. Owing to the similarity of toxic responses and mechanisms of the "toxic" PCB congeners and 2,3,7,8-TCDD, the biological potency is often expressed relative to that of 2,3,7,8-TCDD and is then termed relative biological potency [210,220] or toxic equivalent factor (TEF) [235]. The relative biological potencies of the "toxic" congeners for EROD and AHH induction are shown in Table 6 [210]. The corresponding TEFs are also available for toxic PCDDs and PCDFs [236].

Several more or less different systems of "composite" TEFs have been developed for PCDD and PCDF congeners. In addition to P450IA1 induction potency, the composite TEFs should also reflect various toxic effects including carcinogenity in different animals [235,237,238]. Recently, "international TEFs" have been introduced in order to replace the other composite TEFs systems used for PCDDs and PCDFs [239]. Composite TEFs have recently been proposed also for PCBs. These are 0.1 for congener 126, 0.05 for 169, 0.01 for 77, $1 \cdot 10^{-3}$ for mono-*ortho* "coplanar" PCBs and $2 \cdot 10^{-5}$ for di-*ortho* "coplanar" PCBs [49]. The application of TEFs enables the data on chemical composition for a sample containing PCBs (and PCB-like chemicals) to be converted into a number that indicates

TABLE 6

TOXIC EQUIVALENT FACTORS OF THE "TOXIC" PCB CONGENERS BASED ON INDUCTION OF AHH AND EROD ACTIVITY IN RAT HEPATOMA H-4-II E CELLS IN CULTURE

The values are given in the form of fraction $1/x$, where x is the molar concentration of the particular congener in solution that would cause the same biological response as a solution of 2,3,7,8-TCDD having unit concentration. TEF = EC_{50} (2,3,7,8-TCDD)/EC_{50}(PCB congener). EC_{50} (2,3,7,8-TCDD) for AHH induction is $9.6 \cdot 10^{-11}$ *M* and for EROD induction $8.02 \cdot 10^{-11}$ *M*. Modified from ref. 210.

PCB No.	AHH	EROD
77	1/370	1/1100
81	1/120 000	1/24 000
105	1/910	1/1500
114	1/41 000	1/14 000
118	1/120 000	1/110 000
123	1/10 000	1/7000
126	1/2.5	1/3
156	1/22 000	1/11 000
157	1/7400	1/16 000
167	1/140 000	1/112 000
169	1/630	1/300
189	1/120 000	1/98 000
(3-MC	1/13 000	1/7900)

how much 2,3,7,8-TCDD would cause the same biological effects as PCBs (and PCB-like compounds) contained in the sample. This value is called the 2,3,7,8-TCDD toxic equivalent [236] or toxic equivalent quantity (TEQ) [235], and it seems to be extremely useful for risk assessment. It can be obtained by congener-specific chemical analysis and calculation or, when the TEQs are derived only from enzyme induction, also by biological assay.

Vast differences between the toxicities of congeners can be illustrated by the results of a study of the mortality of chick embryos. The results have shown that a dose of 2 μg/kg of congener 126 in an egg caused mortality in 90% of the embryos 14 days after injection and 100 μg/kg congener 169 caused 80% mortality, whereas a dose of $5 \cdot 10^4$ μg/kg of congener 153 did not cause any mortality or even any abnormalities in the embryos. Corresponding results were also obtained when the inducing potencies of EROD in the chick embryos were tested [240]. It is of interest that in another similar test congener 70, belonging to the group of mono-*ortho* "coplanar" congeners without one *para*-chlorine atom (this group contains congeners 55, 56, 61, 63, 67, 68, 70, 76, 106, 107, 108, 120, 122, 124, 159 and 162) caused certain embryonic mortality, namely a dose of 5 mg/kg caused mortality of 40% of chick embryos. For the tested mono-*ortho* "coplanar" congeners with *para*-chlorines, the corresponding values were 5 mg/kg of congener 118, 45%; 5 mg/kg of congener 167, 0%; 2.5 mg/kg of congener 105, 85%; 2.5 mg/kg of congener 157, 90%; and 2.5 mg/kg of congener 156, 95% embryonic mortality [241].

An important question is whether there are any additive, synergistic or antagonistic effects both mutually between PCB congeners and between PCBs and other chemicals that may be present together in environmental and biological samples. Owing to the extreme complexity of such environmental mixtures, this question can hardly be answered definitively. The concept of toxicity equivalent factors requires additivity of the toxic effects. This seems to be generally fulfilled, but the results published so far are sometimes contradictory and they show that besides additive effects antagonisms also exist, *e.g.*, between 2,3,7,8-TCDD and technical mixtures and some congeners of PCBs, between 2,3,7,8-TCDD and some of its derivatives and between 2,3,7,8-TCDD and hexachlorobenzene [149–151,212,242–247]. A recent study, on the other hand, shows considerable synergistic effects of a combination of PCB 52 and 77 (*in vivo*) in rats [248]. The antagonistic and/or synergistic effects can be covered only by biological assays.

The considerations in this section can lead to the conclusion that there may be several PCB congeners which cause the toxic effects observed in living organisms and which represent the risk to humans of exposure to PCBs. The question is, which congeners contribute to the toxicity of PCB mixtures to such an extent that the possible effects of the other congeners in some sample (or environment) can be assumed to be negligible? It seems very likely that the congeners should have high potency of Ah-receptor-mediated induction of cytochrome P450IA1 and should bind to Ah-receptor preferentially to other binding sites that may occur in cells because of the generally very low concentrations of PCBs in cells.

The values of toxic equivalent quantities of commercial mixtures of PCBs obtained both by chemical analysis and calculation using TEFs based on

AHH and EROD induction potencies and by a biological assay using rat hepatoma H-4-II E cells in culture have been compared considering six PCB congeners (77, 105, 118, 126, 156 en 169) and eleven PCDFs congeners. The results were comparable. Interestingly, the PCDFs did not contribute significantly to the overall toxicity [249].

It is probable that the twelve "toxic" congeners contribute essentially to the overall PCBs toxicity in environmental and biological samples. Some other congeners, however, namely those belonging to the groups of di-*ortho* "coplanars" and also mono-*ortho* "coplanars" without one *para*-chlorine, could be of some importance. Steps towards a firmly based determination of toxic equivalent factors of the toxic congeners and assessement of toxicological properties of the congeners belonging to the suspected groups are of utmost importance. Results of toxicological research can guide analytical chemists in targetting their analyses effectively.

The limit concentrations of PCBs used for regulatory purposes are based either on the "total PCB" level or, more recently, on "standard" individual congeners (28, 52, 101, 138, 153 and 180) chosen in order to cover a wide range of chlorination (from 3 to 7 chlorine atoms) and taking into consideration their relatively high levels in samples. For example, the limits set by the US Food and Drug Administration (FDA) (in 1972) are for milk and milk products 1.5 mg "total PCBs"/kg milk fat, for poultry 3.0 mg/kg fat, fish (the limit was set in 1978) 5.0 mg/kg edible fraction and foodstuffs for infants 0.2 mg/kg [250]. The limits set in Germany in 1988 are for milk and milk products 40 and 50 μg/kg milk fat for congeners 28, 52, 101 and 180, and 138 and 153, respectively, and for edible animal fat 80 and 100 μg/kg for congeners 28, 52, 101 and 180, and 138 and 153, respectively [251].

7. ANALYTICAL CHEMISTRY OF PCBs

7.1. Analytical procedure in general

Considering the properties of PCBs and their distribution and concentrations in the environment (see also Table 10), the general analytical procedure is similar to that for the trace analysis of other lipophilic organic substances, *e.g.*, halogenated pesticides. The procedure in most instances consists in the following basic steps: (1) sampling; (2) extraction; (3) clean-up; and (4) determination and evaluation. Concentration steps usually follow steps 2 and 3. The particular execution of the procedure depends above all on the type of sample to be analysed and on the expected range of PCB levels.

The sample types can be divided as follows: (a) gaseous matrices (mostly air); (b) water and aqueous solutions; (c) solid matrices with no or negligible fat content (soils, water sediments); and (d) matrices containing fat (human and animal tissues, blood, milk and milk products, etc.).

A vast number of particular procedures and their various modifications (especially as for steps 2 and 3) have been described. The recoveries mentioned are mostly comparable. With respect to the differences that always exist between analytical laboratories (*e.g.*, differences between the same types of matrices, lots of chemicals used, individual styles of work of analytical chemists) it can be hardly decided which method will be the best for a specific laboratory with its own particular conditions.

7.2. Sampling and sample extraction

There is comprehensive specialized literature dealing with methods to take appropriate samples [252–254]; only sampling from air and water will be mentioned here.

For the sampling of PCBs in air, polyurethane foam is commonly used [255–259]. Chromosorb [260], Florisil [260], silica gel [108], Tenax GC [260–262], XAD-2 resin [260] and glass beads [263] have also been applied. Air particles are usually captured using glass-fibre filtration [256,261–263]. The amounts of the air pumped through the sorbent are commonly hundreds to thousand(s) of cubic metres [255,256,262]. PCBs are then extracted with various organic solvents, commonly with light petroleum [255,256] or hexane [261,262] using a Soxhlet extractor, or with dichloromethane [263] or a mixture of organic solvents, *e.g.*, acetone and hexane [258]. A recent approach is to use supercritical fluid extraction [257,264].

Water sampling is made difficult by the usually very low concentrations of PCBs in environmental waters. This makes effects such as adsorption a serious problem with a potentially substantial effect on the results [265]. Owing to adsorption, the use of polymers other than PTFE should generally be avoided during any PCB analysis, as demonstrated

by following adsorption experiment: 90 ml of a solution of 100 mg/l of Aroclor 1254 in deionized water (pH 6.9) with addition of 0.25% (w/v) of Triton X-100 (added because it increases the solubility of Aroclor) was mixed and shaken at 34°C with various polymers of approximately equal surface areas (216 cm^2). The amounts of the Aroclor 1254 adsorbed by the tested polymers were red vacuum rubber 99.0, latex 97.8, norprene 97.7, polypropylene 96.7, Tygon 96.2, polyethylene 95.7, Monosil (silicone) 93.6, phenoxyresin 33.0, nylon 22.9 and PTFE 3.4 mg/l [266].

Water samples are either extracted directly with water-immiscible organic solvents, commonly with hexane [267,268] or dichloromethane [256,263,269], or the PCBs in water are first captured on a solid sorbent (XAD-2 resin [270,271], Tenax [272] or polyurethane foam [258]) and then solvent extracted. The amounts of environmental waters taken for analysis are usually from a litre up to hundreds of litres [263,267,271,273,274].

The results of the analyses may also be substantially affected by pH. It has been shown that acidic or neutral extraction may lead to an underestimation of PCB concentrations in fresh water [275]. A promising method of sampling PCBs (and other lipophilic pollutants) in environmental waters is to use solvent-filled dialysis membranes. The sampling and extraction are carried out in one step and, what is important, the samples obtained normally do not need clean-up [276].

Samples of sediments are usually extracted (after homogenization and conditionally drying) with a mixture of acetone and a light aliphatic hydrocarbon [267,277–282] in a Soxhlet apparatus [267, 277,281], a separating funnel [279] or an ultrasonic bath [280]. Other media used are hexane [283], acetone [281] or ethyl acetate [284] applied individually or isopropyl alcohol and dichloromethane [256] or methanol and dichloromethane [285] used consecutively. Steam distillation [259,281] and supercritical fluid extraction [286] techniques have been also utilized. Dichloromethane [224,287] and ethyl acetate [288] have been applied to extract PCBs from soils.

Fat-containing samples are commonly extracted in such a way that the fat is obtained together with PCBs and removed in the following step.

Animal and human tissues are extracted, after homogenization and drying (frequently with anhydrous sodium sulphate), commonly with hexane [35,105,250,289–294] or light petroleum [137,277, 279]. Dichloromethane [284,295,296], cyclohexane [297], ethyl acetate [298] and benzene [299] have also been used. Often mixtures of solvents, are applied, *e.g.*, a small amount of diethyl ether in light petroleum [300–302] or mixtures of benzene and acetone [303,304], hexane and acetone [28,100, 267,305–308] and toluene and ethyl acetate [309]. Several extraction steps, each with a different extraction medium [102] and using more complicated solvent mixtures [310], have also been reported. A generally applied method is also to saponify the fat first and then use a light aliphatic hydrocarbon for extraction [33,169,311]. Comparison of different extraction methods has shown large differences between extraction efficiences of various solvents for different types of fish species and congeners and the necessity for a sufficiently long duration of Soxhlet extraction (at least 6 h) [312]. Plant tissues have been extracted with hexane [313,314].

Important fat-containing liquid matrices are blood and milk. PCBs in blood are determined either in whole blood or in serum. In both instances the usual organic solvents are used (*e.g.*, hexane [39,40,315,316], hexane–diethyl ether [35,317–320], acetone–hexane [321] and acetone–benzene [303]). For whole blood, saponification is often employed [39,40,316]. For milk, in addition to extraction methods similar to those used for blood [321–325], adsorption on a solid sorbent has been utilized. Milk was first mixed with fibrous cellulose and Florisil and, after evaporation of water, the mixture was extracted with hexane [326]; alternatively, the milk was mixed with Lipidex 5000 gel and the chemicals of interest (in addition PCBs also some pesticides and PCDFs and PCDDs) were successively eluted with a series of solvent mixtures [327] and then cleaned up.

To reduce the amount of extracts after extraction and to increase the concentration of PCBs prior to determination, evaporation steps are usually carried out. Common methods are to use a rotary vacuum evaporator for rough concentration and a Kuderna–Danish evaporator or a gentle stream of nitrogen for fine concentration.

7.3. Clean-up

The point of clean-up is to remove the substances that could interfere in a determination. For clean-up the methods commonly used are liquid–solid adsorption chromatography, gel permeation chromatography and chemical methods. Other techniques, such as liquid–liquid partitioning or removing fat by low-temperature precipitation, are much less frequent.

Clean-up procedures are often modifications of older procedures applied for the determination of organochlorine pesticides (*e.g.*, DDT) and they are empirical. In many instances PCBs are determined in one sample together with these pesticides.

For clean-up utilizing liquid–solid adsorption chromatography, the use of most common adsorbents and many organic solvents and their mixtures as eluents has been described. Both very different clean-up systems for nearly identical extracts and, on the other hand, identical clean-up systems for very different extracts have appeared in the literature, probably because in liquid–solid chromatography successful results can frequently be obtained under conditions which may be far from optimum for the system [328]. When fatty samples are cleaned up, fat is retained and PCBs are eluted.

The common adsorbents and eluents and their applications are as follows:

Florisil: elution with hexane [36,288,303,314,329, 330], hexane or light petroleum with small amounts of diethyl ether [7,300,331,332] or benzene [333] or stepwise elution to obtain fractions with various pesticides in addition to PCBs [279,323,334]. Florisil chromatography has been utilized for analyses of air [255], paper [335], sewage sludge [329], soils [288], sediments [7,279], various building materials [332], human and animal tissues [36,291,300,303, 304,333], plant tissues [314], milk [323,334] and blood [36,303].

Silica gel: elution with hexane [39,250,317,336, 337], benzene–hexane [338], stepwise elution using hexane and dichloromethane [269,339] or more complicated solvent systems [340]. Silica gel is sometimes impregnated with concentrated sulphuric acid [267,284] (and sodium hydroxide [341]) or oleum [45]. In these instances hexane [267,341] or small amounts of benzene in hexane [284] or cyclohexane [45] are applied. By means of silica gel extracts of water [267,269], sediments [267,284], pigments [45], wood [339], various oils [336], plant tissues [338], animal tissues [102,250,288,340,342], blood [39] and blood serum [317–319,337] have been cleaned up, in some instances after saponification [39,169,316].

Alumina: elution with light aliphatic hydrocarbons (light petroleum [255], pentane [288,322], hexane [33], isooctane [281]) or with a small amount of dichloromethane in hexane [321]. Extracts may be from air [255], sediments [281], animal tissues [33,288,308], milk [321,322] or blood [321], sometimes after saponification [33,288].

Activated carbon is special adsorbent for PCB clean-up because it is used in combination with other clean-up methods for the separation of coplanar PCBs and/or PCDDs and PCDFs. Elution is always stepwise and several eluents and their mixtures (hexane, dichloromethane, ethyl acetate, benzene, toluene) are applied successively [277,284,304, 311,342–346]. The possibility of separating PCBs according to planarity (and hence toxicity) is very useful and it can be expected that with increasing numbers of analyses for toxicity evaluation in the future, activated carbon might be widely applied. Figs. 2 and 3 show an example of the application of

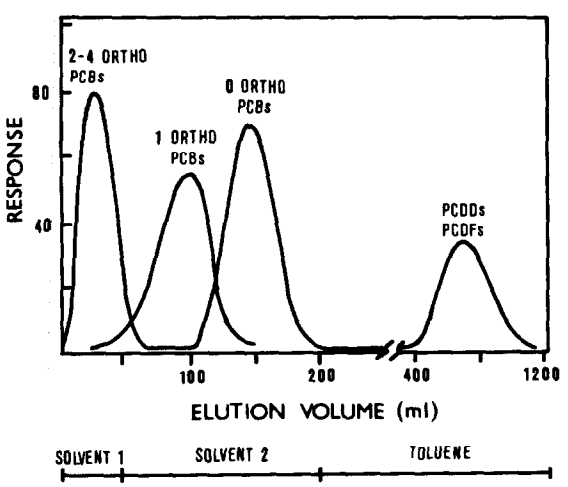


Fig. 2. Application of activated carbon for the separation of PCB congeners according to the number of *ortho*-chlorine atoms: elution profile of a PCB mixture. Sorbent, 750 g of a 1:12 mixture of activated carbon AX-21 (obtained from Anderson Development, Adrian, MI, USA) and LPS-2 silica gel (obtained from Whatman, Hillsboro, OR, USA), placed between two layers of silica gel; chromatographic column, 8 cm × 8 mm I.D.; solvent 1, dichloromethane–hexane (20:80); solvent 2, benzene–ethyl acetate (50:50). Reprinted from ref. 346.

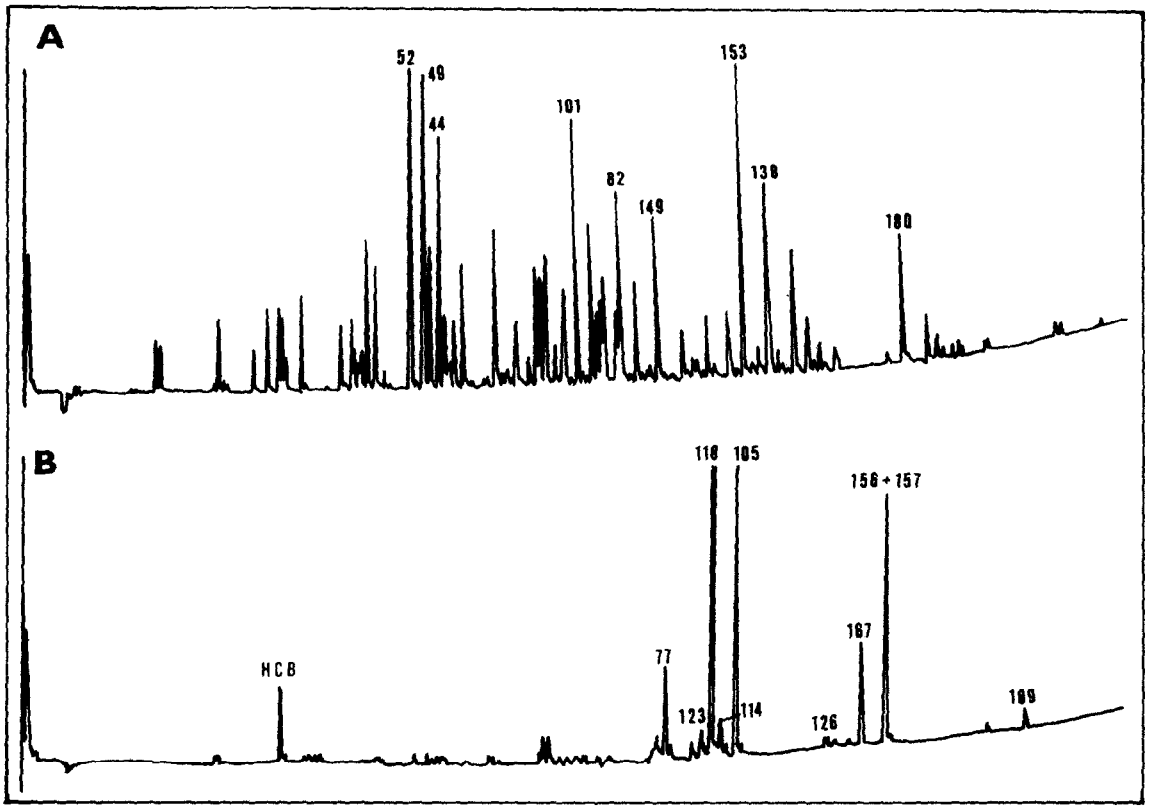


Fig. 3. Gas chromatograms of extracts from a Hudson River fish preseparated using activated carbon chromatography as shown in Fig. 2 into two fractions, the first fraction (A) containing di-to tetra-*ortho*-chlorine-substituted PCBs and the second fraction (B) containing mono- and non-*ortho*-chlorine substituted PCBs. Chromatographic conditions: 60-m glass capillary column coated with Apiezon L; temperature programme, 60°C for 2 min, increased at 10°C/min to 120°C, then at 1°C/min to 250°C; carrier gas, helium at 172 kPa (25 p.s.i.); make-up gas, argon–methane (5:95) at 40 ml/min; injector temperature, 250°C; electron-capture detector temperature 250°C. Reprinted from ref. 346.

active carbon to separate toxic congeners in fish samples [346].

Sometimes two sorbents are used for clean-up of one extract. Silica and alumina have been applied to clean up extracts from air [259], water [271], sediments [256,283], rice bran oil [155] and animal and human tissues [137,289]. Silica and Florisil have been employed to clean up extracts from sediments [249] and human and animal tissues [107,249,347]. For clean-up of extracts from fish, combinations of alumina and Florisil [100] and also alumina and silica gel have been used [267].

Another important clean-up technique is gel permeation chromatography (GPC), which is utilized for removing fat from extracts. The most widely applied gel is BioBeads S-X3 [298,301,302,344,348–350], but other gels, such as BioBeads S-X4 [297], S-X8 [297] and S-X12 [298], Sephadex LH-20 [351] and PLRP-S [326], have also been applied.

Eluents are mostly mixtures, *e.g.*, cyclohexane–ethyl acetate [298], cyclohexane–dichloromethane [302,306,348,349], toluene–ethyl acetate [301,309] and 2-propanol–heptane [326]. Because the fat removal is not so complete as when, for example, Florisil is used, it has been recommended [349] either to use GPC only when the PCB levels are high enough (more than 0.1 μg/kg) or to use GPC and then apply other clean-up technique, *e.g.*, Florisil [301,302] or silica gel [306,348] chromatography.

The point of clean-up by chemical methods is to eliminate possible interferences by means of chemical changes. Elimination of interfering substances,

particularly fat, with sulphuric acid is a commonly applied clean-up method. The sulphuric acid applied is either concentrated [95,102,258,259,267, 278,293,319,320,352–356] or fuming [169,311,357]. Sometimes the application of sulphuric acid is the only clean-up step [258,293,352–355,357,358] and sometimes it is used together with other clean-up steps [102,169,250,259,267,278,311,319,320,343, 356].

In some instances, particularly when determination is done by gas chromatography using packed columns, *p,p'*-DDE, which is often present particularly in extracts from biological samples, interferes with PCBs. This problem was solved by oxidizing the *p,p'*-DDE to 4,4'-dichlorobenzophenone using chromium trioxide or sodium dichromate [343]. The benzophenone was then removed using liquid–solid adsorption chromatography [290,343].

Saponification is also a chemical method of clean-up, but as in practice it precedes extraction, it has already been mentioned in that context.

Methods such as liquid–liquid partitioning (*e.g.*, between hexane and acetonitrile [292] or dimethylformamide and hexane [100]) and low-temperature precipitation [359], which are both utilized to remove mainly fatty substances, have relatively low efficiency and have to be used together with other clean-up methods.

When sediments or related matrices are analysed, it is often necessary to remove sulphur. In this instance either TBA–sulphite reagent (hexane-extracted tetrabutylammonium hydrogensulphate saturated with sodium sulphite) [283,352] or acid-activated fine copper [256,282,284,308,338] or mercury [28] can be applied.

It is very difficult to say in advance which particular clean-up method and technique would be the best or at least sufficient in a particular case.

Liquid–solid adsorption chromatographic (LSC) techniques are efficient and can be substantially modified. A serious problem may be reproducibility and hence standardization. The same sorbents made by different manufacturers or even from the same source but originating from different lots can show different properties. Hence the suitability of a particular adsorbent should be properly tested. Attention should be paid also to the capacity of the selected sorbent with regard to sample amount. It has been shown that the capacity of a 10 cm × 0.2 cm I.D. silica gel column to retain fat was 25 mg for elution with hexane, 20 mg for dichloromethane–hexane (5:95) and 2.5 mg for 2-propanol–hexane

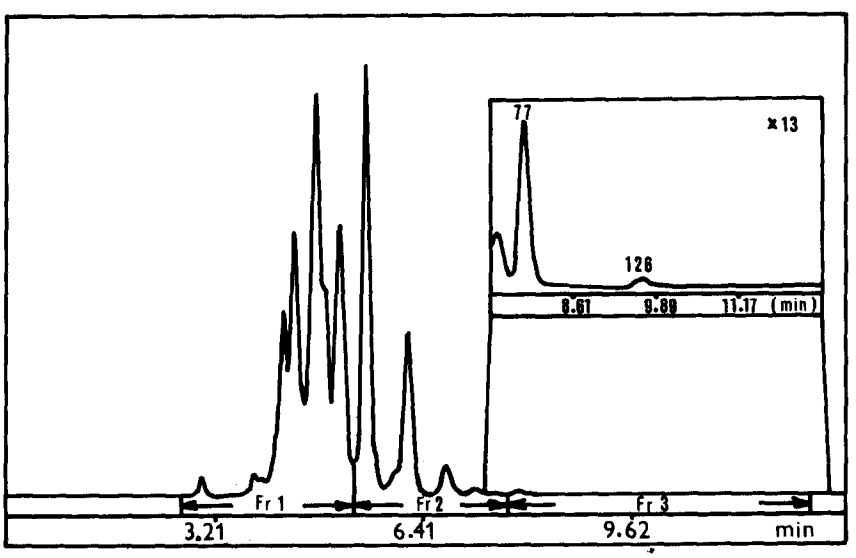


Fig. 4. Application of a 2-(1-pyrenyl)ethyldimethylsilylated silica gel (Cosmosil 5-PYE, Nacalai Tesque, Kyoto, Japan) column for the separation of PCB congeners according to number of *ortho*-chlorine atoms: HPLC of Clophen A 50. Column, 150 × 4.6 mm I.D., particle size 5μm; mobile phase, *n*-hexane; flow-rate, 0.7 ml/min; UV detection at 254 nm. Reprinted from ref. 361.

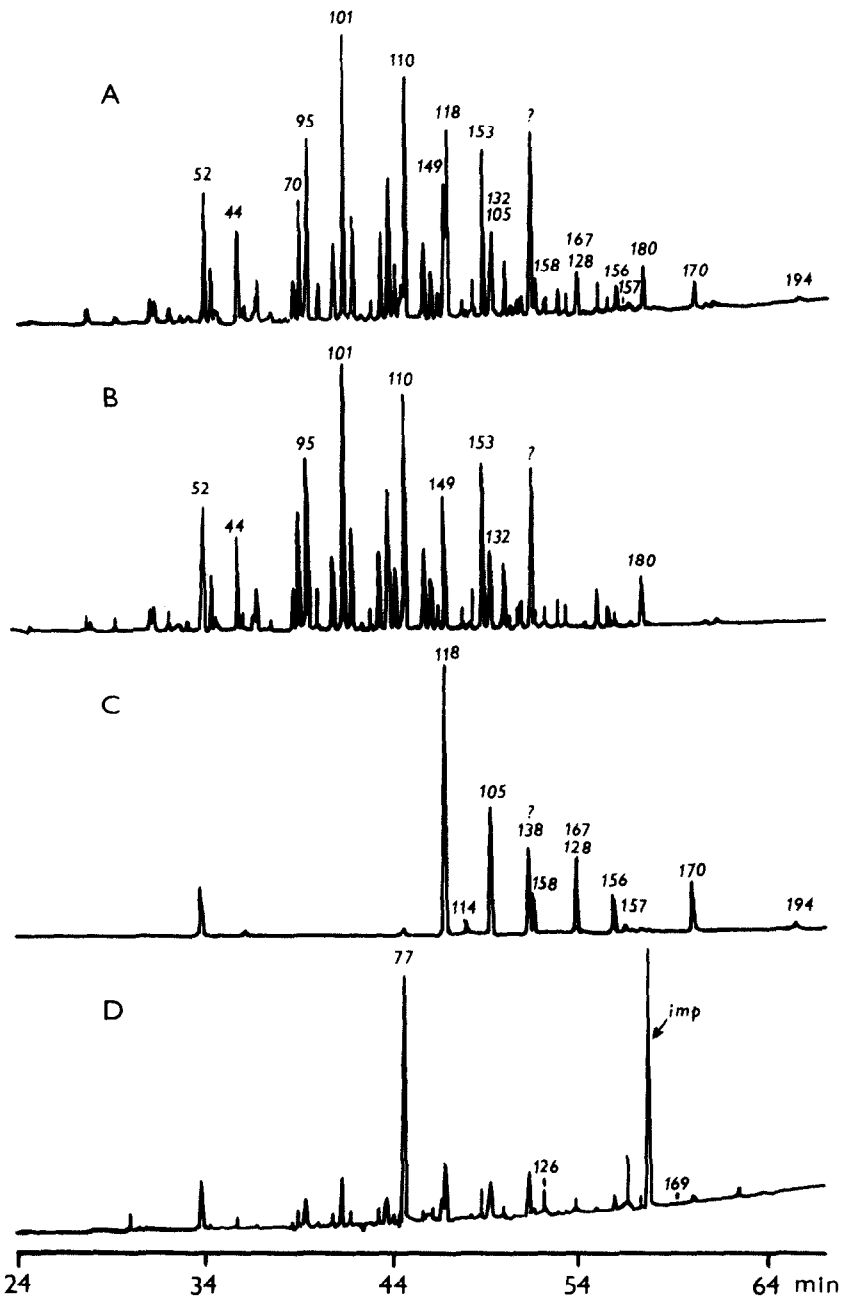


Fig. 5. Gas chromatograms of (A) Clophen A 50 and (B, C and D) the three fractions 1, 2 and 3 of Clophen A 50 obtained by HPLC as shown in Fig. 4. Chromatographic conditions: fused-silica capillary column, 50 m × 0.2 mm I.D.; stationary phase, 5% polymethylsiloxane (Ultra 2, Hewlett-Packard); film thickness, 0.33 μm; temperature programme, 70°C for 4 min, increased at 30°C/min to 180°C, held at 180°C for 2 min, then increased at 2°C/min to 300°C, and held at 300°C for 10 min; carrier gas, helium; flow-rate, 0.3 ml/min; splitless injection (90 s); electron-capture detector. The peak labelled "?" is suspected to contain two different PCB congeners; "imp" means impurity. Reprinted from ref. 361.

(0.5:99.5). Nevertheless, all the capacity cannot be fully exploited. It has also been shown that the more fat is loaded on a column, the lower are the retention volumes of eluted (separated) compounds [360].

Perhaps the most progressive modification of LSC is high-performance liquid chromatography (HPLC), *e.g.*, Nucleosil 100-5 (Machery–Nagel) stationary phase and pentane–dichloromethane as mobile phase [305], especially coupled on-line with gas chromatography [283]. However, another clean-up step is usually necessary, at least when the matrices contain fat or sulphur.

HPLC with a special stationary phase [2-(1-pyrenyl)ethyldimethylsilylated silica gel Cosmosil 5-PYE column, particle size 5 μm] and hexane as mobile phase has been shown very useful for the preseparation of PCBs according to planarity. The column separates PCBs in almost the same way as activated carbon, but this HPLC method seems to have several advantages. A high efficiency (*ca.* 45 000 theoretical plates/m) resulting in sharp peaks with good symmetry permits the accurate isolation of the mono-*ortho* "coplanar" congeners and the use of only one solvent (hexane) and small volumes of fractions, about 2–3 ml (with activated carbon stepwise elution is necessary and the volumes of fractions are commonly hundreds of ml) make reduction of the sample volume before gas chromatographic analysis very easy [361]. Fig. 4 shows the HPLC preseparation of Clophen A 50 into three fractions and Fig. 5 the gas chromatographic separation of Clophen A 50 (A) and its three fractions (B–D) [361].

GPC techniques are not as efficient as LSC and they are limited to the elimination of large molecules. However, it may be automated and made easy to operate and in comparison with LSC there is a significantly lower consumption of clean-up medium.

Sulphuric acid is an efficient clean-up agent. Problems may be caused by the fact that sulphuric acid may affect other compounds (halogenated pesticides) that are sometimes determined together with PCBs in one extract. There is also significantly slower reaction rate with decrease in the concentrations of the removed substances. Moreover, it is not desirable to handle concentrated or fuming sulphuric acid, the amounts of which can be relatively high.

Application of alkali digestion (saponification), which is limited to fat, facilitates another clean-up carried out mostly by LSC and may also increase the efficiency of extraction [312].

7.4. Determination and data evaluation

The dominant position is held by gas chromatography (GC) and other chromatographic techniques are rarely used. The development of biochemical and especially biological methods is in progress. There are also special methods that are used in particular cases, *e.g.*, determination of PCBs as total organic chlorine in transformer oils.

7.4.1. Gas chromatography. GC has several advantages over other techniques, *e.g.*, very sensitive detectors and the possibility of coupling the gas chromatograph with a device permitting identification (*e.g.*, a mass spectrometer), very efficient separation and good reproducibility. GC is therefore extremely useful for the determination of PCBs and related compounds.

Each GC process includes injection, separation and detection. Injection is not a special problem when packed columns are used. When capillary columns are used, it is mostly necessary (owing to the trace amounts present) to use either splitless injection or on-column injection. Splitless injection may cause significant discrimination [362] owing to the wide range of boiling points of PCB mixtures. The presence of dirt may substantially increase this discrimination. On-column injection yields better results [362], but it is much more sensitive to dirt than splitless injection. This can be improved by means of a retention gap. A retention gap also enables relatively large sample volumes to be injected.

The most frequently used detection system for PCBs is electron-capture detection (ECD). This is the most sensitive detection method available for GC in routine use and it is selective towards halogenated compounds. Its extreme sensitivity, on the other hand, makes ECD vulnerable to dirt and overloading. The ECD response is variable and it varies from one detector to another; in one detector it also varies with particular conditions such as detector temperature, quality of gas passing through the detector, flow-rate of the gas and cleanliness of the detector, etc. Despite the selectivity, many non-halogenated compounds may substantially interfere (*e.g.*, fatty substances, phthalate esters, elemental

sulphur). Hence careful clean-up is necessary. The linearity of an electron-capture detector working in the pulse modulated mode is approximately four orders of magnitude.

The response of a chlorinated compound depends significantly on both the number of chlorine atoms and their positions in the molecule and, consequently, there is a wide range of response factors

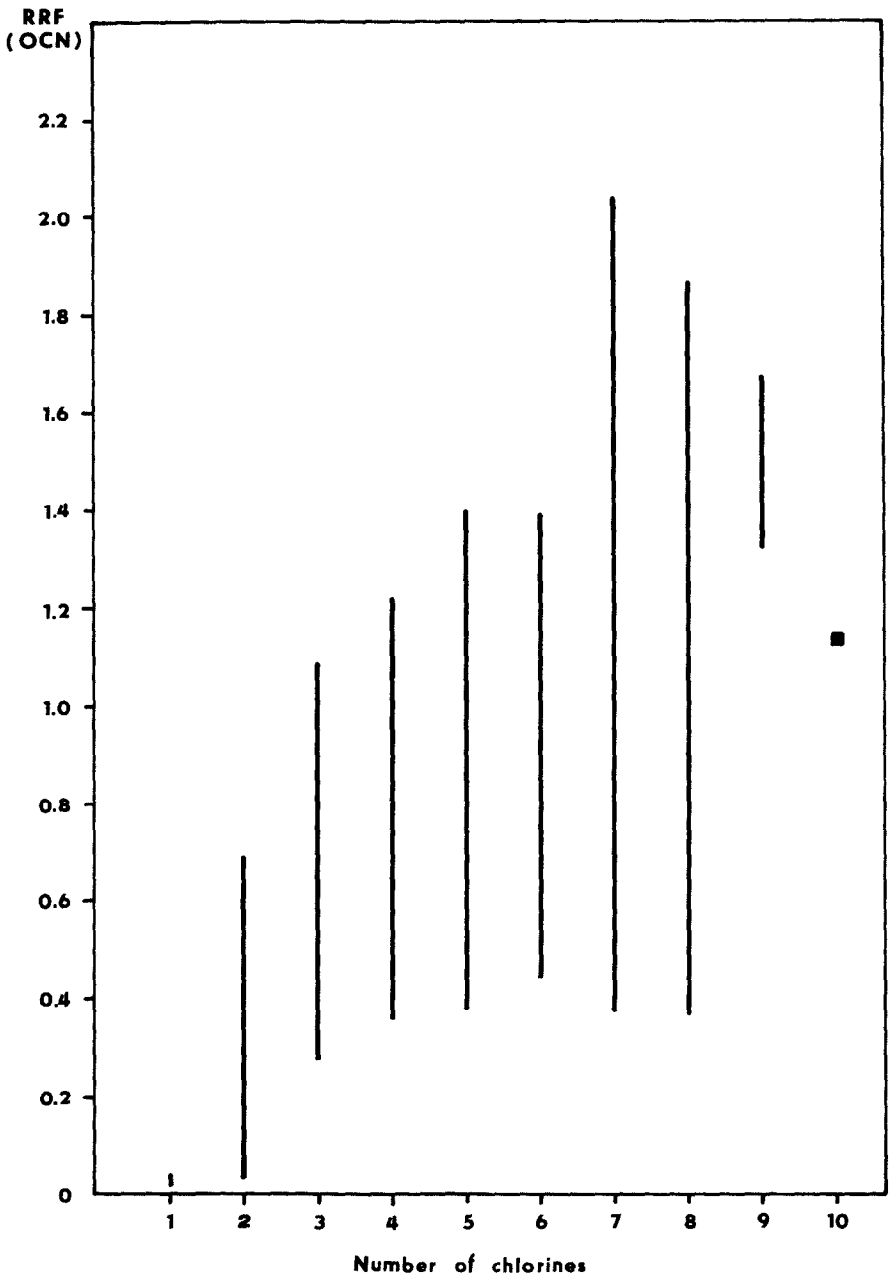


Fig. 6. Relative response factors of PCBs (relative to octachloronaphthalene) for an electron-capture detector. Vertical bars show the ranges of the RRFs within isomer groups. Horizontally can be seen differences between various isomer groups (homologues). According to ref. 363.

of PCB congeners. The relative response factors (RRFs) (relative to octachloronaphthalene) of all the PCB congeners were first reported by Mullin *et al.* [363]. Fig. 6 shows the ranges of RRFs within the isomer groups and differences between the isomer groups. The RRFs vary from one detector to another and are also dependent on the particular conditions, hence the tabulated values cannot be applied generally. These variations can be seen in RRFs published by different workers [363–365]. These significant differences in responses make determinations ambiguous unless all the congeners determined are separated and compared with the appropriate individual standards. Methods for simplifying the wide range of RRFs have been suggested, *e.g.*, grouping of congeners according to their RRFs into 31 groups. Each group is then represented by one surrogate standard [366].

The second most frequently used detection system is mass spectrometry (MS). The system provides another data dimension from which information on structure and/or molecular masses of analyte substances can be derived. It permits confirmation of identification and also the use of labelled compounds as recovery surrogates. The mass spectrometer is also very useful when the matrices contain large amounts of chlorinated organic compounds, as when determining by-product PCBs in certain commercial products (*e.g.*, in azo dyes [45], phthalocyanine pigments or chlorinated paraffins [46]) and in wastes. There are two modes in which the mass spectrometer is employed to detect PCBs: electron impact (EI) ionization and chemical ionization with negative-ion detection (NICI).

EI mass spectra are fairly reproducible, they show relatively intense molecular ions and the natural isotopic distribution of chlorine gives rise to typical clusters, which are easily recognizable (see Table 7) [367]. Nevertheless, except for the "*ortho*-effect", which allows one to distinguish several congeners having three *ortho*-chlorines and congener 52, it is impossible to distinguish between the spectra of isomers [368]. A major disadvantage of this mode is the relatively high minimum detectability, which is 2–3 orders of magnitude higher than that of ECD [369]. In order to improve the minimum detectability, limited mass scanning (LMS) over a certain mass range of interest or selected ion monitoring (SIM) are employed. On the other hand, these techniques increase the uncertainty of identification [368]. For quantification a suitable ion(s) in each isomer group is (are) chosen and the masses of these ions are monitored [326,370–372]. Ion(s) other than those used for quantification must be monitored in order to prevent misidentification and thus incorrect determination, *e.g.*, because of the tendency of PCBs to lose two chlorines [46,326,373]. Such an ion detection programme is shown in Table 8. The response factors in EI-MS differ between isomers by no more than about twofold [365], which is much less than in ECD. It is therefore possible to use one surrogate standard within each isomer group [371,374]. The surrogate standards should be those congeners whose response factors are nearest to the average response factors of corresponding isomer groups [46,371].

The response mechanism of NICI-MS is in some respects similar to that of ECD [364]. The major advantage of NICI in comparison with EI is the lower minimum detectability, particularly for highly chlorinated species [375]. Similarly to ECD, there are large differences in response factors between congeners [364]. The NICI mass spectra are considerably dependent on particular conditions such as pressure and composition of the reagent gas and source temperature [364,375]. The spectra also give less information on the structure of compounds [364]. Lightly chlorinated PCBs have a tendency to exhibit fragments (*e.g.*, chlorine atoms) and only the higher chlorinated PCBs exhibit pronounced molecular ions [364,368]. The high dependence on the conditions make it possible, however, that the optimum NICI-MS conditions have not yet been found. NICI is not currently routinely used in PCB analyses.

The impossibility of distinguishing between PCB congeners is a challenge. Distinguishing between five hexachlorobyphenyl isomers by ion–molecule reactions using ammonia in a triple-quadrupole instrument has recently been reported [376].

Other detector systems are not frequently employed for PCB analyses. The hall electrolytic conductivity detector working in the reductive mode is selective to halogens and has been employed to determine the chlorine content in peaks representing parts of Aroclor mixtures [377]. Although generally its response depends only on the chlorine content, a dependence on structure for some chlorinated com-

TABLE 7

MOLECULAR ION CLUSTER OF PCB HOMOLOGUES (ACCORDING TO REF. 367)

m/z	Relative abundance (%)	*m/z*	Relative abundance (%)	*m/z*	Relative abundance (%)
Monochlorobiphenyls		*Hexachlorobiphenyls*		*Nonachlorobiphenyls*	
188	100.00	358	51.12	460	25.89
189	13.53	359	6.83	461	3.48
190	33.86	360	100.00	462	75.55
191	4.34	361	13.32	463	10.01
Dichlorobiphenyls		362	80.95	464	100.00
222	100.00	363	10.35	465	13.27
223	13.28	364	35.61	466	72.07
224	64.84	365	4.65	467	9.69
225	8.59	366	8.71	468	35.93
226	10.54	367	1.21	469	4.95
227	1.95	368	1.17	470	11.59
Trichlorobiphenyls		369	0.15	471	1.42
256	100.00	*Heptachlorobiphenyls*		472	2.43
257	13.45	392	43.78	473	0.25
258	92.61	393	5.90	474	0.33
259	12.13	394	100.00	*Decachlorobiphenyl*	
260	29.81	395	13.02	494	25.59
261	3.43	396	96.09	495	3.34
262	3.16	397	13.08	496	78.55
263	0.26	398	53.02	497	9.84
Tetrachlorobiphenyls		399	6.51	498	100.00
290	77.14	400	16.51	499	14.28
291	10.44	401	2.12	500	92.42
292	100.00	402	3.18	501	11.64
293	13.67	403	0.45	502	51.69
294	48.84	404	0.30	503	6.86
295	6.82	*Octachlorobiphenyls*		504	20.28
296	10.84	426	34.36	505	2.71
297	1.50	427	4.56	506	5.42
298	0.96	428	88.10	507	0.75
Pentachlorobiphenyls		429	11.53	508	1.04
324	61.84	430	100.00	509	0.14
325	7.85	431	13.43		
326	100.00	432	65.33		
327	12.96	433	8.73		
328	66.33	434	26.11		
329	8.72	435	3.39		
330	21.94	436	6.99		
331	2.99	437	0.87		
332	3.86	438	1.16		
333	0.37	439	0.11		
334	0.24	440	0.11		

pounds has been mentioned [378]. The minimum detectability is too high and the detector is also difficult to operate. The Coulson conductivity detector is an older modification of the Hall electrolytic conductivity detector [379].

The recently developed helium discharge detector

TABLE 8

ION DETECTION PROGRAMME FOR EI-MS DESIGNED IN ORDER TO INCREASE THE LIMIT OF DETECTION AND TO DECREASE THE UNCERTAINTY

This programme is (with various slight modifications) currently used for EI-MS of PCBs. (According to ref. 373).

PCBs	Mass range scanned	Ion for identification	Ions for quantification
Monochloro-	186 – 190	188, 190	188, 190
Dichloro-	220 – 226	222, 224, 226	222, 224
Trichloro-	254 – 260	256, 258, 260	258
Tetrachloro-	288 – 294	290, 292, 294	292, 294
Pentachloro-	322 – 328	324, 326, 328	326, 328
Hexachloro-	356 – 364	358, 360, 362	360, 364
Heptachloro-	386 – 400	394, 396, 398	394, 398
Octachloro-	426 – 434	428, 430, 432	430, 432
Nonachloro-	460 – 468	462, 464, 466, 468	464, 468
Decachloro-	494 – 504	496, 498, 500, 502	498

has been employed to determine PCBs in model samples. The response of the detector applied to the element-selective detection of chlorine emission is based solely on the number of chlorine atoms present. Its minimum detectability (the reported limit of detection is 10–35 pg) may limit its application in some instances [380].

The flame ionization detector is not selective and has too high a minimum detectability to be used for trace environmental analyses of PCBs. It has been employed to characterize the composition of commercial PCB products [381].

Fourier transform infrared (FT-IR) spectrometry is a unique detection technique available for GC. It provides, like mass spectrometry, another data dimension but these data (infrared spectra) are, unlike mass spectrometric data, intrinsic to a particular compound. The results obtained so far indicate that each of PCB congeners has its own specific infrared spectrum [382]. It would therefore be possible to identify unambiguously any congener without a standard by comparison with a library of the spectra of the PCB congeners. The main drawback is very high minimum detectability of FT-IR detectors. When a cryogenic matrix isolation sample collector was utilized as an interface between a gas chromatograph and an IR spectrometer, the limit of detection for PCB congeners ranged from 1 to 10 ng [383]. This is why (apart from high price of the instrumentation) GC–FT-IR spectrometry, although from many points of view an extremely powerful tool, could be employed only in very limited cases.

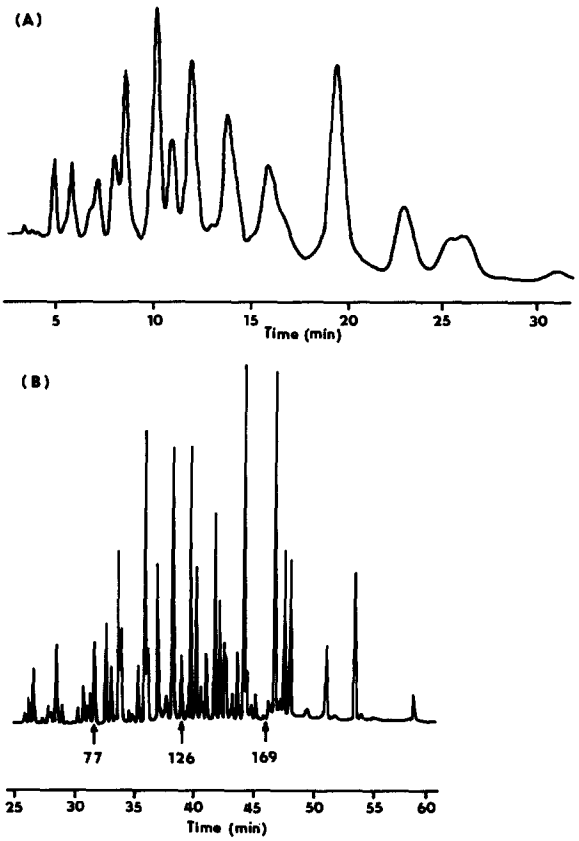


Fig. 7. Chromatograms of hazardous waste extract using (A) packed and (B) capillary columns. (A) Column, 6 ft. (183 cm) × 1/8 in. (3.2 mm) I.D. with 3% SP-2100 liquid stationary phase on 100–120-mesh Supelcoport; carrier gas, nitrogen; flow-rate, 30 ml/min; temperatures, injector 225°C, electron-capture detector 300°C, column 190°C. (B) Column, 30 m × 0.25 mm I.D. fused silica with DB-5 liquid stationary phase (J&W Scientific); carrier gas, helium; constant head pressure, 21 p.s.i. (145 kPa); 27 cm/s at 235°C; injector purge, 12 ml/min; make-up gas, nitrogen at 60 ml/min; temperatures, on-column injector at room temperature, electron-capture detector 280°C, column temperature programmed from 100 to 150°C at 10°C/min and from 150 to 235°C at 2°C/min. Reprinted from ref. 355.

For separation, either packed columns or, for high-resolution GC, capillary columns are employed. Stationary phases are commonly non-polar or slightly polar.

When packed columns are used, the chromato-

gram of a commercial product or of a mixture of PCBs in an environmental sample shows a few peaks (about 10–15, see Fig. 7A). Each of the peaks usually represents several congeners.

There are several methods of quantification using packed column gas chromatography:

"Method of selected peaks" ("method of peak comparison"): one, a few or all resolved peaks are selected to represent the total mixture. Commercial products (*e.g.*, Aroclors) are employed as standards. Areas (or heights) of the corresponding selected peaks in chromatograms of samples and standards are compared [7,292,309,329]. The results are reported as, *e.g.*, "Aroclor 1254" [292] or "total Aroclor" [329].

Webb–McCall method: in 1973, Webb and McCall [384] worked out a method of quantification using packed column GC. They chromatographed (under the same conditions) Aroclors using a Coulson conductivity detector, an electron-capture detector and a mass spectrometer. From the findings they determined the weight percent which each resolved peak of Aroclors 1221, 1232, 1242, 1248, 1254 and 1260 represents in the chromatogram and summarized the results in tables. Use of these tables and the standards enables response factors to be calculated for other electron-capture detectors and to be employed to quantify the samples. The method should give better results than the method of selected peaks but it is necessary to use the same chromatographic conditions and exactly the same standards, which the authors did. A similar paper was published in 1978 by Sawyer [377].

The congeneric compositions of PCBs in real samples are more or less different from those of commercial products employed as standards, which is why the results obtained using the above-mentioned methods should be considered only as estimates because the methods are based on conventions [288].

Perchlorination: another possibility is to convert all the PCB congeners in cleaned-up extracts to decachlorobiphenyl using a chlorination agent (*e.g.*, antimony pentachloride) and to determine the decachlorobiphenyl [385–387]. The method represents a real quantification from a chromatographic point of view because the determined compound and the standard are unambiguously defined chemical individuals. Another advantage is lower limit of detection in comparison with above-mentioned methods.

The method nevertheless has several considerable disadvantages: perchlorination causes loss of information about the congeneric composition of PCBs in the sample and the yield of the reaction may be significantly affected by small changes in the reaction conditions, especially temperature [387]. The major drawback is that several other groups of compounds may be converted into decachlorobiphenyl and cause gross errors [388]. The results obtained using perchlorination may be up to several ten times higher than those using the method of selected peaks. This is especially true for sewage sludges, paper and paper board. Although biological samples generally do not give such erroneous results, in some instances (*e.g.*, brains and livers of herons) the errors are extremely large. It has been shown that the most important interfering role is played by hydrogenated terphenyls (having one of three rings hydrogenated) which yield decachlorobiphenyl during perchlorination of PCBs. Biphenyl, hydrogenated biphenyls, polybrominated biphenyls and to a small extent polychlorinated naphthalenes may also be converted to some extent into decachlorobiphenyl and to contribute to erroneous results, but in much less extent than hydrogenated terphenyls [388].

Dechlorination: dechlorination involves the conversion of all PCB congeners to an unambiguously defined compound, biphenyl. The reducing agent may be, *e.g.*, hydrogen in the presence of palladium catalyst [389] or solution of lithium aluminium hydride ($LiAlH_4$) in diethyl ether [390]. Interfering compounds may be polybrominated biphenyls, hydroxylated PCBs and biphenyl, which in practice do not cause such large errors as hydrogenated terphenyls in the case of perchlorination [390]. Determination is carried out by GC with flame ionization detection [389]. Application of HPLC with UV detection has also been suggested for determining both decachlorobiphenyl and biphenyl [388,390,391] (see Section 7.4.2.).

When PCBs are chromatographed using a high-resolution capillary column, a mixture is separated into much more components than when using packed columns (see Fig. 7B). Nevertheless, a single capillary column is not able to separate all the 209 PCB congeners [392]. Consequently, another separation or a simplification has to be applied. The

TABLE 9

RELATIVE RETENTION TIMES (WITH RESPECT TO OCTACHLORONAPHTHALENE) FOR ALL PCB CONGENERS

Conditions: fused-silica capillary column (50 m × 0.2 mm I.D.), SE-54, programmed from 100 to 240°C at 1.0°C/min; carrier gas, hydrogen, constant pressure 221 kPa (2.25 kg/cm^2), linear velocity 45 cm/s at 100°C; injector temperature, 270°C; detector temperature, 330°C; splitting ratio, 1:10. Retention time of octachloronaphthalene was 124.9 min. (According to ref. 363).

PCB No.	Relative retention time	PCB No.	Relative retention time	PCB No.	Relative retention time	PCB No.	Relative retention time	PCB No.	Relative retention time	PCB No.	Relative retention time
1	0.1544	35	0.4738	70	0.5407	105	0.7049	140	0.6707	175	0.7611
2	0.1937	36	0.4375	71	0.4989	106	0.668	141	0.7203	176	0.7305
3	0.1975	37	0.4858	72	0.4984	107	0.6628	142	0.6848	177	0.8031
4	0.2245	38	0.4593	73	0.4554	108	0.6626	143	0.6789	178	0.7537
5	0.2785	39	0.4488	74	0.5341	109	0.6016	144	0.6563	179	0.7205
6	0.2709	40	0.5102	75	0.4643	110	0.6314	145	0.6149	180	0.8362
7	0.2566	41	0.499	76	0.5408	111	0.6183	146	0.6955	181	0.7968
8	0.2783	42	0.487	77	0.6295	112	0.5986	147	0.6608	182	0.7653
9	0.257	43	0.4587	78	0.6024	113	0.5862	148	0.6243	183	0.772
10	0.2243	44	0.4832	79	0.5894	114	0.6828	149	0.6672	184	0.7016
11	0.3238	45	0.4334	80	0.5464	115	0.6171	150	0.5969	185	0.7848
12	0.3298	46	0.445	81	0.6149	116	0.6132	151	0.6499	186	0.7416
13	0.3315	47	0.4639	82	0.6453	117	0.615	152	0.6062	187	0.7654
14	0.2973	48	0.4651	83	0.6029	118	0.6693	153	0.7036	188	0.692
15	0.3387	49	0.461	84	0.5744	119	0.5968	154	0.6349	189	0.9142
16	0.3625	50	0.4007	85	0.6224	120	0.6256	155	0.5666	190	0.874
17	0.3398	51	0.4242	86	0.6105	121	0.5518	156	0.8105	191	0.8447
18	0.3378	52	0.4557	87	0.6175	122	0.6871	157	0.8184	192	0.8269
19	0.3045	53	0.4187	88	0.5486	123	0.6658	158	0.7429	193	0.8397
20	0.417	54	0.38	89	0.5779	124	0.6584	159	0.7655	194	0.962
21	0.4135	55	0.5562	90	0.5814	125	0.6142	160	0.7396	195	0.9321
22	0.4267	56	0.5676	91	0.5549	126	0.7512	161	0.6968	196	0.8938
23	0.377	57	0.5155	92	0.5742	127	0.7078	162	0.7737	197	0.8293
24	0.3508	58	0.5267	93	0.5437	128	0.7761	163	0.7396	198	0.8845
25	0.3937	59	0.486	94	0.5331	129	0.7501	164	0.7399	199	0.8494
26	0.3911	60	0.5676	95	0.5464	130	0.7284	165	0.692	200	0.8197
27	0.3521	61	0.5331	96	0.5057	131	0.6853	166	0.7572	201	0.8875
28	0.4031	62	0.4685	97	0.61	132	0.7035	167	0.7814	202	0.8089
29	0.382	63	0.529	98	0.5415	133	0.6871	168	0.7068	203	0.8938
30	0.3165	64	0.4999	99	0.588	134	0.6796	169	0.8625	204	0.8217
31	0.4024	65	0.4671	100	0.5212	135	0.6563	170	0.874	205	0.9678
32	0.3636	66	0.5447	101	0.5816	136	0.6257	171	0.8089	206	1.0103
33	0.4163	67	0.5214	102	0.5431	137	0.7329	172	0.8278	207	0.9423
34	0.3782	68	0.504	103	0.5142	138	0.7403	173	0.8152	208	0.932
		69	0.451	104	0.4757	139	0.6707	174	0.7965	209	1.0496

rigorous determination of all congeners in a sample requires their complete separation and also the application of pure standards of all the congeners. The complete separation can be achieved using a multidimensional gas chromatograph with two capillary columns with different stationary phases. This system enables the parts of a sample which were not resolved in the first column to be transferred to the other column where their separation can be completed [18,47,392–394]. The system allows the complete and rigorous characterization of PCB mixtures.

Unfortunately, the rigorous determination of all components is extremely expensive and time consuming and, therefore, exceptional. A single capillary column is usually used, some congeners are left

TABLE 10

REPORTED CONCENTRATIONS OF "TOXIC" (T) AND "STANDARD" (S) PCB CONGENERS IN VARIOUS MATRICES AND COUNTRIES

PCB No.	Source[a]																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
28 S	–	–	–	–	–	–	–	–	–	0.79	0.13	3.70	–	–	–	–	–	–	–	2.39	0.65	–	6.3	13.6
52 S	62	ND[b]	1.7	ND	0.44	0.75	84.3	1.6	79.1	0.60	0.17	4.80	15	2.4	62	–	–	–	–	1.60	1.05	–	3.2	20.7
77 T	–	–	–	–	–	–	–	–	–	–	–	9.10	–	–	–	18	22.5	<5	<0.5	–	0.0165	–	–	–
81 T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	90	–	17	<0.5	–	–	–	–	–
101 S	49	14	0.97	2.3	0.84	0.39	76.4	0.9	50.0	0.46	0.16	5.03	19	3.8	270	–	–	–	–	1.16	–	–	0.3	3.6
105 T	–	–	–	–	–	–	–	–	–	–	–	–	12	0.8	–	181	486	427	5.8	–	1.55	–	162.3	65.7
114 T	–	–	–	–	–	–	–	–	–	–	–	14.10	–	–	–	–		57	1.0	–	–	–	–	–
118 T	11	10	0.3	ND	0.63	0.15	17.7	0.6	26.7	0.17	0.08	71.70	21	1.7	250	443	–	1350	11	–	3.05	40.61	30.9	5.4
123 T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	109	1.4	–	–	–	–	–
126 T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	19	6.96	<5	<0.5	–	0.072	–	–	–
138 S	8.1	3.6	0.14	0.67	0.25	0.23	31.0	2.0	94.5	0.55	0.26	146.00	15	1.2	260	–	–	826	6.2	2.72	14.75	107.31	5.4	3.6
153 S	–	–	–	–	–	–	33.7	1.5	70.8	0.34	0.15	129.70	23	2.2	430	723	–	–	–	2.47	19.9	150.36	36.0	33.6
156 T	–	–	–	–	–	–	–	–	–	–	–	18.20	2.3	0.2	34	78	–	79	1.7	–	1.95	–	12.4	4.3
157 T	–	–	–	–	–	–	–	–	–	–	–	4.08	–	–	–	–	–	76	<0.5	–	–	–	–	–
167 T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	77	<0.5	–	–	–	–	–
169 T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	ND	0.62	<5	<0.5	–	0.049	–	–	–
180 S	ND	ND	0.16	0.82	0.23	ND	4.5	0.5	21.7	0.30	0.13	67.20	13	0.8	200	–	–	–	–	1.74	15.6	77.13	0.7	4.5
189 T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	29	<0.5	–	–	–	–	–

[a] Sources:

1 = Air vapour, Siskiwit Lake, Isle Royale, Lake Superior, MI, USA, summer concentrations, average of 13 samples (pg/m^3).
2 = Air vapour, Siskiwit Lake, Isle Royale, Lake Superior, MI, USA, winter concentrations, average of 7 samples (pg/m^3).
3 = Air particles, Siskiwit Lake, Isle Royale, Lake Superior, MI, USA, summer concentrations, average of 13 samples (pg/m^3).
4 = Air particles, Siskiwit Lake, Isle Royale, Lake Superior, MI, USA, winter concentrations, average of 7 samples, (pg/m^3).
5 = Rain, Siskiwit Lake, Isle Royale, Lake Superior, MI, USA, average of 12 samples (ng/l).
6 = Snow, Siskiwit Lake, Isle Royale, Lake Superior, MI, USA, one sample (ng/l).
7 = Air vapour, city of Kiel, Schleswig-Holstein, Germany, one sample (pg/m^3).
8 = Air particles, city of Kiel, Schleswig-Holstein, Germany, one sample (pg/m^3).
9 = Rain, city of Kiel, Schleswig-Holstein, Germany, one sample (pg/l).
10 = Water in the North Atlantic, depth 10 m, June 1987, one sample (pg/l).
11 = Water in the North Atlantic, depth 4000 m, June 1986, one sample (pg/l).
12 = Adipose tissue of birds (female razorbills), Isle of May, the Firth of Forth, Scotland, 6 samples obtained between 1978 and 1984 (mg/kg wet weight adipose tissue).
13 = Suspended sediments, Lake Ontario, average of 10 samples (μg/kg dry weight).
14 = Plankton, Lake Ontario, average of 3 samples (μg/kg wet weight).
15 = Fish, Lake Ontario, average of 60 samples (μg/kg wet weight).
16 = Fish (lake trout), Lake Ontario, composite sample of 3 fish (μg/kg whole fish).
17 = Fish (lake trout), Lake Ontario, composite sample of 50 fish (μg/kg fish lipid).
18 = Fish (whole carp), Saginaw Bay, Lake Huron, MI, USA (μg/kg).
19 = Sediment, Green Bay, Lake Michigan, USA (μg/kg).
20 = Cow milk, Bayern, Germany, average of 556 samples (μg/kg fat).
21 = Human milk, Stockholm, Sweden, average of two analyses of one sample (μg/kg fat).
22 = Human milk, city of Certaldo, Tuscany, Italy, average of 31 samples (μg/kg dry weight).
23 = Human adipose tissue, North America, one sample (μg/kg fat).
24 = Human liver, North America, one sample (the same man as in 23) (μg/kg fat).

References: 1–6 [256], 7–9 [263], 10,11 [270], 12 [405], 13–15 [28], 16 [235], 17 [406], 18,19 [345], 20 [407], 21 [408], 22 [409], 23,24 [299].

[b] ND = Not detected.

unresolved and only certain congeners are determined [48,319,324,356,362,395,396]. The columns commonly employed are 25–60 m long with I.D. 0.2–0.32 mm. The temperature is programmed, often with an extremely slow programming rate. Quantification is sometimes also done using secondary standards instead of individual congeners, which are PCB mixtures (often commercial) of known composition. Computer assistance is utilized [28,296] and retention data have to be employed. Very useful is a list of the retention data of all the 209 congeners relative to octachloronaphthalene, measured by Mullin *et al.* [363] using a column coated with SE-54 stationary phase (Table 9). There are also papers dealing with predictions of retention times from experimental data for several congeners and descriptors derived from molecular structures [44,397,398]. Metals (metal columns, metal GC–MS interface) may cause catalytic dechlorination and/or redistribution of the chlorine atoms [399,400] and, therefore, any contact of PCBs with metal should be avoided in the gas chromatograph.

Determination of only a few particularly selected congeners facilitates the use of individual congeners as standards and makes identification more certain and quantification more accurate. There is a problem as to which congeners should be selected for determination so that the analyses will provide the most useful information. There are two main approaches: either to determine the congeners that are usually present in relatively high concentrations and considering their amounts to be related to the total amount of PCBs present, or to determine the congeners most important from the toxicological point of view.

In the former instance, congeners 28, 52, 101, 138, 153 and 180 (and sometimes also 118) are most commonly determined. These "standard" congeners (PCB 118 belongs to the "toxic" congeners) cover a wide range of chlorine numbers and some of them are usually present in most samples. Determination of these congeners is now widely performed in many laboratories. Nevertheless, recent publications demonstrate that some other congeners (*e.g.*, 84, 90 and 163) may also interfere [394,401–404].

It is assumed that the principal toxic effects of PCBs are connected with interaction with Ah-receptors, it may be considered that it would be possible to determine only the congeners showing significant activity in this respect and to utilize the toxicity equivalent factors and in such a way obtain results showing the toxic potency of PCBs in a sample. This means determining the "toxic" congeners (see Section 6.3.).

The chief problems with their analyses arise from the usually very low levels of the toxic congeners in samples (reported samples and countries are shown in Table 10). It follows that very good separation is necessary (otherwise the small peaks belonging to the toxic congeners could easily be missed, see Fig. 7B), detection systems are limited to ECD and MS and effects such as adsorption become much more significant. The difficulty of such analyses can be documented, *e.g.*, by comparison of results obtained using ECD and MS detection. The median concentrations of PCBs 77, 126 and 169 was 50, 177 and 28 ng/kg, respectively, using ECD and 10, 55 and 7.7 ng/kg, respectively, using MS detection for the same samples (ten samples of horse fat) [350].

The above-mentioned carbon liquid chromatographic preseparation or similar preseparations are very useful in this instance and satisfactory separations can be achieved using multi-dimensional GC [18]. Methods for the determination of the three [311] or more [232,284,293,346] most toxic PCB congeners in environmental samples (sediments [284], fish [284,346], blubber of a marine mammal [311], eggs of snapping turtle [293], of fish and of birds [232]) have been developed and described and the problems of the analysis of the "coplanar" and mono- and di-*ortho* "coplanar" PCB congeners have recently been reviewed [410].

Special stationary phases can facilitate the determination of the "toxic" congeners. For example, *n*-octyl-(50%)–methylpolysiloxane stationary phase (SB Octyl 50; Lee Scientific) is normally used in supercritical fluid chromatography, in which the order of elution depends considerably on the degree of coplanarity of the congeners [411,412]. The application of liquid crystals as stationary phases is very interesting; the retention is highly dependent on the geometry of the molecules. Retention data for some PCB congeners on SB Octyl 50 [411] and the nematic liquid crystal N,N′-bis(*p*-methoxybenzylidene)-α,α′-bi-*p*-toluidine [413] are given in Table 11. The results show that the application of liquid crystalline stationary phases for the separation of "toxic" congeners (and not only those of PCBs) is

TABLE 11

RELATIVE RETENTION TIMES (RRT) (RELATIVE TO PCB 1) OF SELECTED PCB CONGENERS ON SPECIAL STATIONARY PHASES: SB OCTYL 50 AND BMBT LIQUID CRYSTAL

Conditions: (A) stationary phase SB-Octyl 50 (Lee Scientific), capillary column 50 m × 0.2 mm I.D., film thickness 0.25 μm, carrier gas helium, column head pressure 207 kPa (30 psi), temperature programme 100°C for 3 min, increased at 20°C/min to 160°C, then at 1.5°C/min to 268°C, 15 min isothermal, injector temperature 250°C, splitless injection (adapted from ref. 411); (B) stationary phase N,N′-bis(*p*-methoxybenzylidene)α,α′-bi-*p*-toluidine (BMBT), glass packed column 183 cm (6 ft.) × 2 mm I.D., 1.5% BMBT on 100–120-mesh Chromosorb W HP, carrier gas helium, flow-rate 30 ml/min, column temperature (isothermal) 150°C (according to ref. 413).

Congener No.	Conditions (A): RRT SB-Octyl-50	Conditions (B): corrected RRT BMBT	Congener No.	Conditions (A): RRT SB-Octyl-50	Conditions (B): corrected RRT BMBT
4	1.23	1.28	112	2.98	43.68
8	1.50	3.32	114	3.44	–
15	1.80	19.64	115	3.11	30.54
21	2.10	8.36	116	3.08	20.08
28	2.08	–	118	3.38	–
30	1.71	2.72	121	2.80	8.16
50	2.02	3.64	123	3.64	–
52	2.26	4.60	126	–	–
54	1.84	3.00	128	3.81	88.86
69	2.28	5.76	153	3.56	4.30
70	2.72	12.34	156	4.08	10.52
77	3.19	55.54	157	4.09	–
81	3.11	–	167	3.96	–
93	2.66	8.16	169	4.34	–
101	2.90	9.62	171	4.00	58.54
104	2.36	4.26	180	4.21	–
105	3.50	–	183	3.87	31.52
			209	–	126.84

very promising [414,415]. The purpose of the application of such phases is to introduce another type of separation mechanism and thus another type of elution order than with commonly used stationary phases, to improve the separation (also of multidimensional GC) and also to facilitate the identification of the "toxic" congeners by MS because they often elute between congeners having a different (smaller) number of chlorines. Chromatograms of a mixture of 51 PCB congeners on three stationary phases, low-polarity CP-Sil 8 (95% methyl–5% phenyl siloxane), moderately polar CP-Sil 19 (85% methyl–7% methyl–7% cyanopropyl–1% vinyl polysiloxane) and liquid crystalline polysiloxane SB-Smectic (Lee Scientific), are shown in Figs. 8–10. The liquid crystalline stationary phases, however, suffer from thermal and chemical instability, *e.g.*, with SB-Smectic some compounds such as octachloronaphthalene are affected, hydrogen cannot be used as the carrier gas because of its adverse effect on the phase and at higher temperatures regular significant bleeding occurs [414].

7.4.2. Other methods. In addition to the widely used GC, other methods can be employed to determine PCBs. Among these, methods utilizing biochemical and biological processes seem to be the most progressive.

HPLC. HPLC with UV detection has been used to detemine PCBs, especially in order to characterize commercial mixtures [416–418] and to determine decachlorobiphenyl (after perchlorination of PCBs) [391] or biphenyl (after dechlorination of PCBs) [390].

The retention times of PCBs decrease with increasing number of chlorine atoms in the molecules (generally opposite to the GC retention order) when a column packed with 5-μm LiChrosorb Si 60 silica gel (Merck) as stationary phase and *n*-hexane as mobile phase is used [416,417]. The "normal" elution order gives a reversed-phase system with microparticulate silica (μBondapak C_{18}; Waters Assoc.) as stationary phase and water–acetonitrile as mobile phase [418]. The system silica gel–*n*-hexane has also been employed for the determination of both decachlorobiphenyl and biphenyl [390,391]. Decachlorobiphenyl showed maximum absorbance

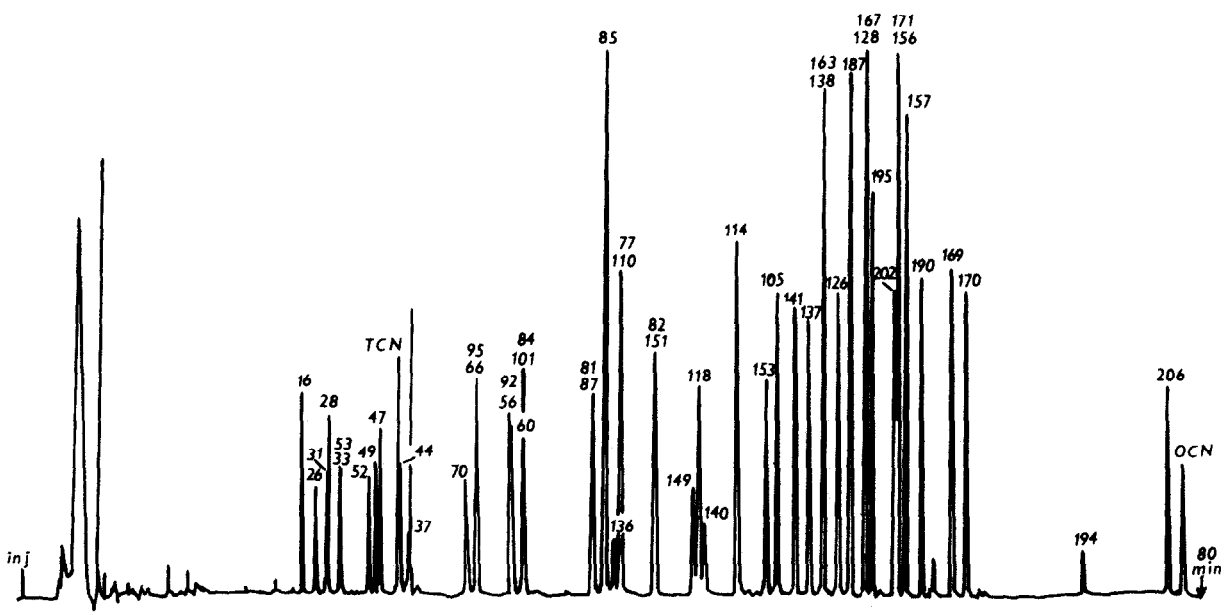


Fig. 8. Gas chromatogram of a mixture of 51 PCB congeners. Chromatographic conditions: splitless injection (2 min); injector temperature, 270°C; electron-capture detector temperature, 360°C; purge gas, nitrogen at 60 ml/min. Columns: WCOT CP-Sil 8 CB fused-silica, 95% methyl–5% phenyl polysiloxane (Chrompack, Middelburg, Netherlands), 50 m × 0.15 mm I.D.; film thickness, 0.30 μm; carrier gas, hydrogen at 310 kPa (0.31 ml/min, 27 cm/s); temperature programme, 90°C for 3 min, increased at 30°C/min to 215°C, held at 215°C for 40 min then increased at 5°C/min to 270°C and held at 270°C for 22 min. Reprinted from ref. 414.

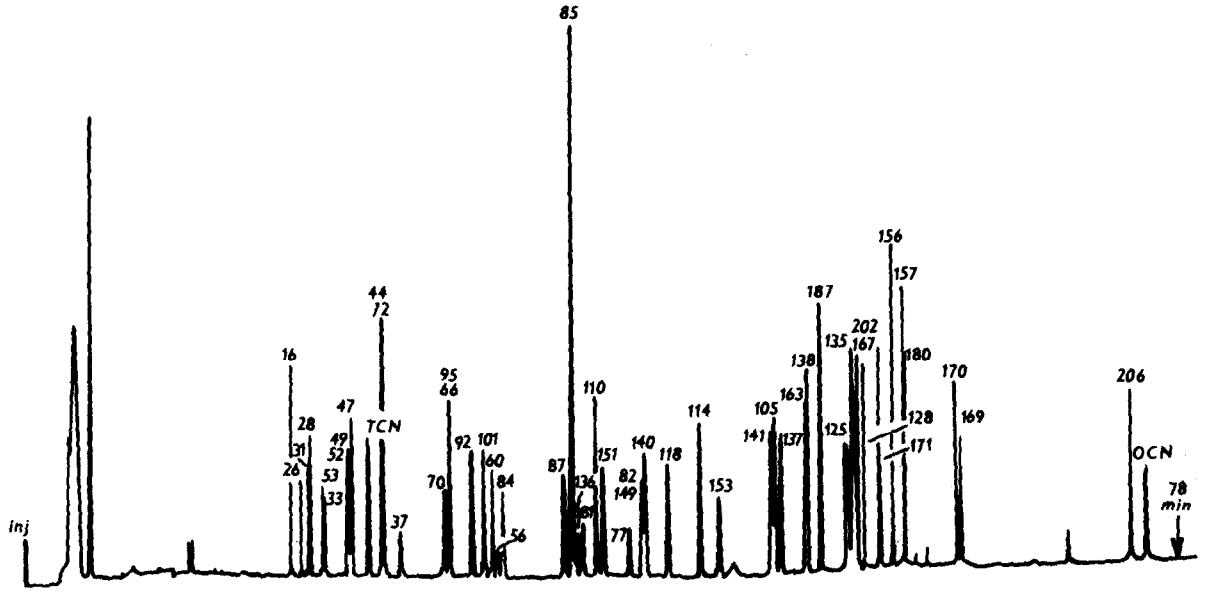


Fig. 9. As Fig. 8 except for column: WCOT CP-Sil 19 CB fused-silica, 85% methyl–7% phenyl–7% cyanopropyl–1% vinyl polysiloxane (Chrompack, Middelburg, Netherlands), 60 m × 0.15 mm I.D.; film thickness, 0.20 μm; carrier gas, hydrogen at 345 kPa (0.32 ml/min, 30 cm/s); temperature programme, 90°C for 3 min, increased at 30°C/min to 215°C, held at 215°C for 40 min, then increased at 5°C/min to 270°C and held at 270°C for 20 min. Reprinted from ref. 414.

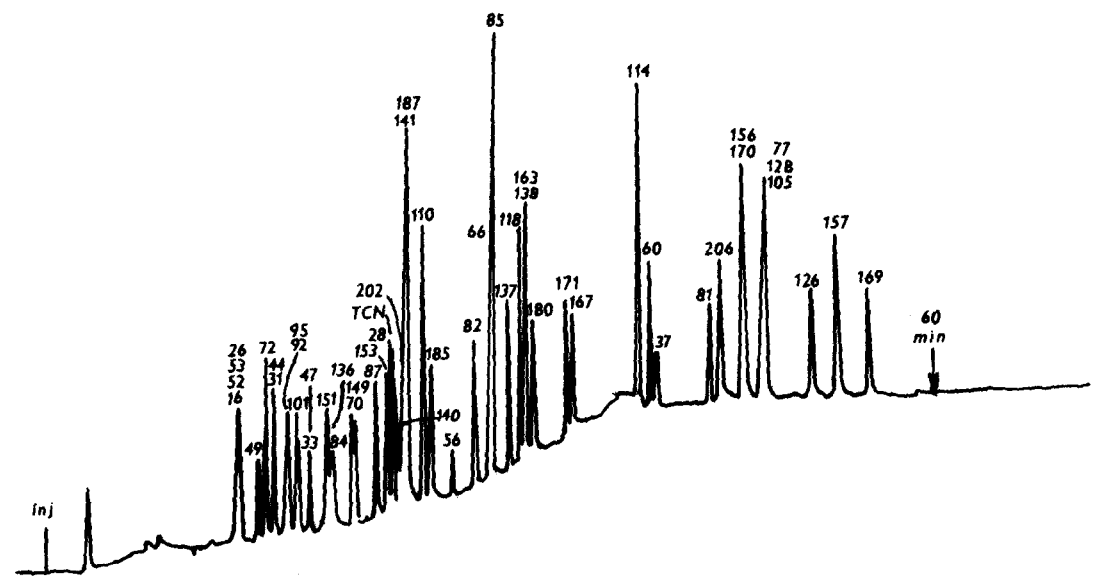


Fig. 10. As Fig. 8 except for column: SB-Smectic fused-silica liquid crystalline polysiloxane (Lee Scientific, Salt Lake City, UT, USA), 50 m × 0.20 mm I.D.; film thickness, 0.15 μm; carrier gas, nitrogen at 290 kPa (0.33 ml/min, 28 cm/s); temperature programme, 90°C for 3 min, increased at 30°C/min to 170°C, held at 170°C for 1 min, then increased at 1.5°C/min to 215°C and held at 215°C for 17 min. Reprinted from ref. 414.

at 215 nm [391] and biphenyl showed two maxima at 205 and 248 nm [390].

The decisive drawback of HPLC systems is the high limit of detection. The reported limit of detection for biphenyl is *ca.* 0.5 ng [390] and is correspondingly higher for a mixture of many congeners. For example, in a study of the characterization of commercial PCB mixtures, it has been reported that the amounts of Aroclors for each analysis could be reduced to 3 μg without markedly reducing the accuracy of the analysis [418]. Also, the resolution is lower than in high-resolution GC. HPLC may be very useful for the clean-up or preseparation of extracts before GC analysis.

Radioimmunoassay (RIA). The basis of RIA is the competition between an analyte compounds (which acts as a ligand) and a radioligand for binding sites in antiserum, which is a substance particularly prepared for the purpose employing the responses of immunity systems of animals, mostly rabbits. Sample preparation is the same as or similar to that in GC, *i.e.*, extraction and clean-up. The solvent is then evaporated to dryness and the residue is dissolved in dimethyl sulphoxide. Known amounts of antiserum and radioligand are added. After incubation, the bound and unbound ligands are separated. The amount of unbound radioligands is determined by measuring radioactivity and the amount of the ligands (*i.e.*, PCBs) in the sample is calculated. The results, expressed as "total" PCBs, are comparable to those obtained by using packed column GC. The binding affinity of PCBs to antiserum is fairly selective, but not specific, and this makes congener-specific analyses using RIA impracticable (the binding affinity is not related to toxicity). However, RIA is a relatively rapid method applicable to wide range of samples [321,419].

H-4-II E rat hepatoma cell bioassay. As discussed in Section 6.3, some PCB congeners, and also some other compounds, can significantly induce particular enzymatic systems (AHH, EROD) in animal cells. The induction potency of a congener *in vitro* generally correlates with the potency to cause toxic effects in living organisms. It is therefore possible to utilize cultured cells for the assessment of the overall Ah-receptor-related toxic potency of compounds in extracts from environmental samples. AHH and EROD induction in cultured H-4-II E rat hepatoma cells is very suitable for such an assay [236,420,421].

The sample extract dissolved in an appropriate solvent (dimethyl sulphoxide, isooctane) is added to the cells cultured in a Petri dish. After incubation (usually for 72 h [210,421,422]) the cells are harvested and the rate of conversion of benzo[*a*]pyrene to 3-hydroxybenzo[*a*]pyrene (AHH activity) [423] and/or the rate of conversion of ethoxyresorufin to resorufin (EROD activity) [424] is assessed using spectrofluorimetry. The rate of these conversions is a measure of the enzymatic complexes and hence a measure of inducing potency of the sample tested. The data obtained are compared with those obtained using a set of 2,3,7,8-TCDD standards of appropriate concentrations. The results are expressed as 2,3,7,8-TCDD toxicity equivalents. The limit of detection is 10 pg of 2,3,7,8-TCDD equivalent per plate [421]. The results include influences of all the AHH- and/or EROD-inducing compounds present, and also synergistic, antagonistic and additive effects between them. The assay does not, of course, provide information about which compounds are responsible for the induction. The assay has been successfully utilized to analyse extracts from environmental samples [421,422,425]. Although this method has not so far been used routinely, it seems that it has the potential to become (particularly together with high-resolution GC) a widely used tool in environmental studies and monitoring.

Competitive binding assay for ligands of the Ah-receptor. This method allows the calculation of the binding affinity of components in a sample to the Ah-receptor. The assay is based on competition between radioligand (2-[^{125}I]iodo-7,8-dibromodibenzo-*p*-dioxin) and components in the sample extract for binding to Ah-receptor prepared from livers of mice. The minimum detectable concentration is 0.8 pg of 2,3,7,8-TCDD in 0.25 ml [426,427]. This bioassay, which was described in 1988 and has certain features in common with H-4-II E rat hepatoma cell bioassay, is still awaiting application in environmental analyses.

In addition to the above-mentioned methods, there are several others that can be utilized in particular cases, *e.g.*, determination of chlorine in transformer oils using neutron activation and γ-ray spectrometry [428]. Conversion of chlorines in PCBs into chloride ions by metallic sodium [429,430] and their determination either using spec-

trophotometry [429] or by means of a chloride ion-selective electrode [430] have been also described. This method is limited to relatively high concentrations of PCBs and does not provide any qualitative information on the PCBs present. Its advantage is simplicity.

7.5. Interlaboratory tests

The results of interlaboratory tests provide information on the comparability of the results produced by different laboratories. The objective of such tests is especially to evaluate a proposed analytical method. The results are usually compared using relative standard deviations (R.S.D.). It is difficult to evaluate the accuracy in addition to the precision, because the determination methods are sometimes (and with packed columns always) conventional and the PCBs added may behave differently to PCBs naturally integrated into matrices. Moreover, these studies cannot give any information on problems of sampling, which may substantially affect the results of analyses. The tests indicate the present state of the routine analytical chemistry of the compounds in question.

On the basis of more than 150 interlaboratory studies, an equation has been proposed that relates R.S.D. and concentration:

$$\text{R.S.D.} = 2^{(1-0.5\log c)}$$

where c is the concentration expressed as the weight of the analyte in a sample divided by the weight of the sample [431]. It can be seen that according to this equation, for a concentration of, *e.g.*, 1 ppm the R.S.D. should be 16% and for 1 ppb the R.S.D. should be 45.3%. On the basis of theoretical considerations, another equation providing very similar results has been proposed [432]:

$$\text{R.S.D.} = c^{-0.15}/50$$

It is possible to consider that the lower the concentrations are, the more phenomena are capable of affecting the results (*e.g.*, adsorption, electronic noise, interferences) and the greater is the effort required to overcome these problems. These aspects

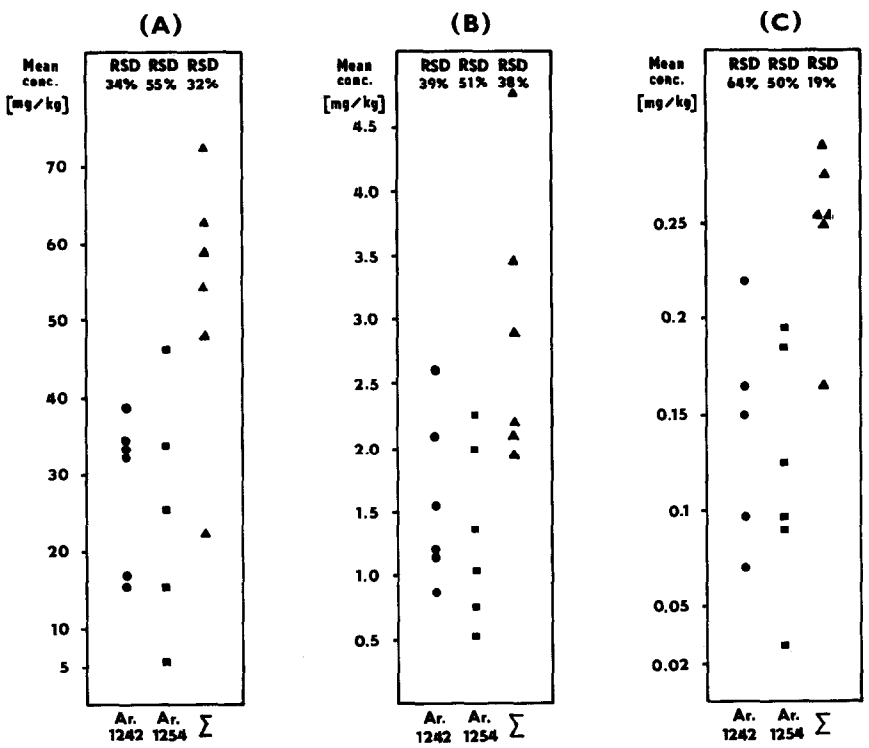


Fig. 11. Reported mean results for sediment sample analyses in an interlaboratory study [7]. For details, see text.

are very difficult to assess and hence the results obtained using the above equations should be treated with caution. The values can, however, indicate what results can be achieved in interlaboratory studies.

To illustrate the situation in the analysis of PCBs, some results from several recent interlaboratory tests using GC are presented below.

(1) Analyses of three series of contaminated marine sediment samples. Dried samples were Soxhlet extracted for 16 h with hexane–acetone (1:1), the extracts were concentrated in a Kuderna–Danish apparatus and then cleaned up by Florisil column chromatography and eluted with diethyl ether–hexane (6:94). Sulphur was removed with a tetrabutylammonium reagent. GC determinations were performed using a packed column containing methylsilicone stationary phase (SE-30, OV-1) with EC. The quantifications were made using the Webb–McCall procedure [384] and Aroclors 1242 and 1254 as standards. The real PCB concentrations were not known. From the measured data it can be seen that the concentrations in the three series of samples differed by about one and two orders of magnitude, respectively [7]. The mean measured PCB concentrations obtained by six participating laboratories are shown in Fig. 11. Interestingly, there was no apparent relation between concentration and RSD.

(2) Analyses of two series of water samples spiked with a mixture of Aroclors 1221, 1242, 1254, 1260 and 1268 so that the concentrations were 37 and 148 μg/l. The samples were extracted with dichloromethane in a separating funnel. GC determinations were performed using a 30 m × 0.25 or 0.32 mm I.D. fused-silica capillary column coated with dimethyldiphenylsiloxane (SE-54 or DB-5) with EI-MS detection. The response factors for each isomer group (except nonachlorobiphenyls) were determined using congeners 1, 5, 29, 50, 87, 154, 187, 200 and 209 and chrysene was used as an internal standard. Raw data were processed using special software. The results as mean percentage recovery reported by five participating laboratories are shown in Fig. 12 [371].

(3) Analysis of herring oil spiked with individual congeners 52 (82 μg/kg), 86 (77 μg/kg), 101 (63 μg/kg) and 153 (85 μg/kg). Clean-up was effected using chromatography on Florisil. Each of thirty participating laboratories used its own GC conditions. The results, expressed as percentage recoveries, were in the ranges 30–127%, 28–178%, 44–283% and 41–294% for PCBs 52, 86, 101 and 153, respectively. In addition to the common clean-up method, each laboratory also used its own method. The results did not show that the common clean-up gave more precise results than the laboratories' own methods [433].

The results from these three interlaboratory studies show that when current techniques and methods are employed, the differences between results obtained by various laboratories may be nearly one order of magnitude and R.S.D.s are higher than would be expected. If it is taken into consideration that the precision of routine analyses may be significantly worse than that of interlaboratory studies, it seems necessary to be cautious when comparing data on PCB pollution in various locations at various times and in various matrices. The only confirmed way to achieve R.S.D.s comparable to expected values is to introduce a learning programme covering

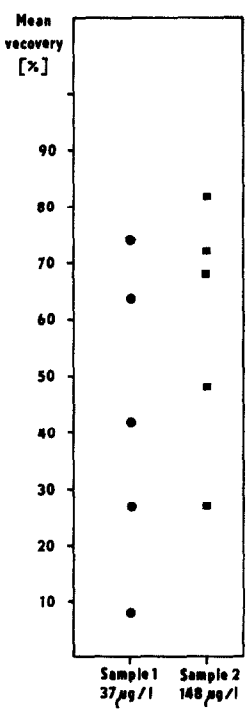


Fig. 12. Reported mean recoveries for two fortified water samples in an interlaboratory study [371]. For details, see text.

thoroughly all steps of the analytical procedure for all participants before a multi-laboratory test is carried out [434].

8. CONCLUSIONS

PCBs are amongst the most prominent environmental contaminants spread all over the world (Northern Europe [435,436], Southern Europe [437–440], Western Europe [274,441–443], Central Europe [444–446], Russia including Siberia [446], Vietnam [446], Hong Kong [447,448], Japan [449], Israel [450], Indonesia [273], North America [451–457] including Arctic regions [296], South America [458,459], Antarctica [271], oceans [270,460,461], etc.). The impact of their low-level presence in the environment, foodstuffs, humans, etc., is very difficult to assess. As discussed in Section 6, many studies show significant adverse effects of PCBs and PCBs-like compounds on living organisms; these effects, however, should be assessed realistically with respect to other environmental risks [462–464]. Interestingly, it has been shown that taking into consideration the environmental concentrations and toxicity equivalent factors, the overall toxicity of PCBs might be, owing to the "toxic" congeners, significantly greater than that of PCDDs and PCDFs together in environmental samples [235,345,465]. Moreover, it seems that an end to PCB environmental pollution cannot be expected in the near future. Therefore, the analytical chemistry of PCBs will be expected to provide not only other data, but also more valuable information.

The choice of analytical methods depends on the particular conditions and the purpose of the analysis. Conventional methods using packed column GC may be suitable for rapid screening and also for searching for sources of contamination. These methods are also used for regulatory purposes (*e.g.*, in the USA).

The perchlorination method is also relatively simple. Because the compound determined is an individual, the possibility of interference in minimized and confirmation is facilitated. There is, nevertheless, the above-mentioned possibility of gross errors [388]. The method is also included in US EPA methods.

Nevertheless, it seems obvious that in order to obtain real (not conventional) values of PCB concentrations, which could be readily comparable between laboratories, it is necessary to employ congener-specific analyses using individual standards.

Determination of the several (six or seven) "standard" congeners occurring in relatively high concentrations is now used for regulatory purposes in Western Europe (Germany, Netherlands).

Determination of the "toxic" congeners is the most progressive approach. The methods are, nevertheless, far from routine at present and represent a field for future research and development.

To assess a sample from the hygienic point of view, the best way would be, in general, to perform a simple biological test and in such a way to establish whether or not the sample may be dangerous. When this test gives positive results, then chemical analysis using high-resolution GC should be carried out to find the toxic compounds responsible. Such a biological test for PCBs and related compounds may be the H-4-II E rat hepatoma cell bioassay. However, it seems necessary to continue to work on the method in order to establish it firmly.

In general, for the future, there is a need to improve the methods for congener-specific analyses and short-term toxicity testing. These analytical approaches together with more extensive clinical and experimental data on the toxicity of PCBs will permit a real assessment of the risk that PCBs represent both in particular samples and in the global environment.

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