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Porphyrinogenesis study in workers exposed to polychlorinated biphenyls (PCBs)

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Polychlorinated biphenyls (PCBs) show porphyrinogenic activity in experimental animals as well as in in vitro models (1). This paper reports on investigations on urinary elimination of porphyrins performed in two independent studies involving workers exposed to PCBs in the Washington D.C. area, and in northern Italy. The complete reports of these studies, which comprised many other investigations, have been published in details elsewhere (2, 3) and only data relevant to the porphyrin studies are excerpted and summarized here.

METHODS

American study: The workers exposed to PCBs consisted of 55 transformer repairmen who had been predominantly in contact with Aroclor 1260 and, to a smaller extent, with Aroclor 1254 and 1242. An industrial hygiene survey performed in their working environment had shown very low air concentrations of PCBs but a widespread PCB contamination of the workplace surfaces. 2,3,7,8-tetrachlorodibenzofuran was detected in some transformer oils sampled at the workplace (highest concentration: 31 ppb) (4).

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Some of the workers (n = 38) were currently exposed to PCBs at the time of the study, the others had been exposed to PCBs only in the past, the time elapsed from last exposure ranging from 6 months to 5 years. The average lenght of employment with PCB exposure was 7 years.
In order to compare the results obtained in the exposed workers, a reference group (n = 56) was selected among workers employed at the same employer with jobs not involving exposure to PCBs. The demographic characteristics chosen to match the comparison subjects were: sex, age, smoking habits, alcohol intake, place of residence, level of education and marital status. The resulting group did not differ significantly from the exposed group for any of the above parameters.
The internal levels of PCBs in the exposed and comparison subjects were assessed by measuring serum and adipose-tissue-lipid PCB concentrations by packed column GC/ECD (3).
Urinary elimination of porphyrins was determined on 24 hr urine samples by thin layer chromatography (5, 6).

Italian study: The workers exposed to PCBs consisted of capacitor manufacturers, who were exposed to Aroclor 1254 in the past and to Pyralene 3010 (equivalent to Aroclor 1242) at the time of the study. Also in this study, an industrial hygiene survey in the working environment had shown low level of air PCB contamination but very remarkable PCB pollution of the workplace surfaces (7).
The study group was selected so as to include only those subjects who were non-smokers or smoked less than 10 cigarettes per day and who did not consume alcoholic beverages in excess of 30 g alcohol per day. The resulting group consisted of 51 subjects (25 males and 26 females), with a mean age of 39 years and a mean lenght of employment on PCB exposure of 10 years (range: 1 to 30 years).
The comparison group was identified among electronic-component assembly workers and matched with the exposed workers for age, sex, and place of residence, after excluding the subjects who did not meet the smoking and alcohol criteria adopted for the selection of the exposed workers. The resulting comparison group consisted of 67 subjects (30 males and 37 females).
Blood PCB concentration measured by packed column GC/ECD was available for 28 of the exposed workers (14 males and 14 females) and for none of the comparison subjects (3).
Urinary porphyrin concentration was determined in morning spot urine specimens by thin layer chromatography, with

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quantification of all the porphyrinic homologues from 8 to 4 carboxylic groups (8).

RESULTS

Table 1 shows the internal levels of PCBs and the urinary excretion of aminolevulinic acid (ALA), coproporphyrin, and uroporphyrin, observed in the American workers. When tested with the analysis of variance, the PCB concentrations were remarkably different between the exposed and comparison workers ($p < 0.01$), although the mean values in the exposed group suggested a low level of exposure.

Table 1: Serum and adipose tissue PCB concentrations and urinary excretion of ALA porphyrins in the American workers.

	CURRENTLY EXPOSED N = 38	PAST EXPOSED N = 17	COMPARISON N = 56
Serum PCBs (ppb)	12.2 (nd-300)	5.9 (nd-30)	4.6 (nd-15)
Adipose tissue lipid PCBs (ppm)	2.1 (nd-33)	0.8 (0.3-5.1)	0.6 (nd-3)
ALA (mg/24 hrs)	2.9 (0.9-4.6)	2.6 (1.1-4.7)	2.5 (0.2-5.0)
Coproporphyrin (µg/24 hrs)	79.5 (26.2-142.5)	70.0 (7.2-139.5)	92.6 (5.5-246.6)
Uroporphyrin (µg/24 hrs)	10.7 (1.7-29.4)	9.9 (3.9-24.7)	10.9 (2.0-28.9)

Values are geometric means for PCB concentrations and arithmetic means for ALA and porphyrins. Maximum and minimum in brackets.

The urinary excretion of ALA and porphyrins did not show significant differences between the groups.
Serum and adipose tissue PCB congeners detected in these subjects were mostly highly chlorinated, reflecting the PCB pattern of the Aroclor 1260 mixture.

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In the Italian workers exposed to PCBs, the total blood PCB concentrations ranged from 88 to 1359 ppb with a geometric mean of 308 ppb. When the blood PCB peaks were divided into low and high chlorinated PCBs, according to whether the peaks corresponded to an Aroclor 1242 or an Aroclor 1254 mixture respectively, the geometric means were 87 ppb for the lower chlorinated PCBs and 211 for the higher chlorinated PCBs. Blood PCB concentrations were not available for comparison subjects, but we assume that their concentrations were of the same order of magnitude as those observed in the comparison group in the American study (see Table 1). In fact, the subjects of the two comparison groups live in comparable areas and limited data on human PCB concentrations collected in northern Italy support this assumption.
Urinary porphyrin concentrations are shown in Table 2.

Table 2: Urinary porphyrin excretion (μg/l) in the PCB workers and the comparison group in the Italian study.

	PCB WORKERS (N = 51)		COMPARISON GROUP (N = 67)	
	Range	Geometric Mean	Range	Geometric Mean
Total Porph.	7.1-273.7	74.4	9.1-127.3	36.5
8-COOH Porph.	0.1-45.2	8.2	0.1-16.5	4.3
7-COOH Porph.	0.1-21.7	2.2	0.1-3.4	0.8
6-COOH Porph.	0.1-10.4	0.5	0.1-2.9	0.2
5-COOH Porph.	0.1-5.6	1.2	0.1-6.0	0.4
4-COOH Porph.	6.7-237.0	59.1	7.8-111.0	25.7

All the differences are significant at $p < 0.05$ when tested with the Student t test on log-converted values.

Workers exposed to PCBs showed an excretion of porphyrins about doubled as compared with the non-exposed workers. The enhanced total porphyrin elimination was the result of a generalized increase in every urinary fraction. The comparison

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of the porphyrin excretion values corrected for urine creatinine concentration between the groups (not shown in the Table) suggested that the observed difference was not related to an urine dilution effect.
Where blood PCB concentrations were available, correlation coefficients were calculated between respectively total PCB, low and high chlorinated PCB concentrations and the urinary excretion of total porphyrins and single porphyrinic homologues. None of the Pearson's correlation coefficients was higher than 0.2 or statistically significant.

DISCUSSION

Polychlorinated biphenyls are porphyrinogenic agents in animals and in in vitro experimental models (1). Experience in man is limited to the two poisoning episodes occurred in Japan in 1968 and in Taiwan in 1979, where thousands of subjects were intoxicated by cooking oils contaminated with PCBs. In the Yusho epidemics, no reports were made of clinical porphyria or increased urinary excretion of porphyrins in the poisoned patients (9). In the Yu-Cheng accident, porphyrinogenesis alterations were investigated in a limited cohort of patients and only slight increases in the mean excretion of ALA and uroporphyrin were found (10). These findings are markedly different from those observed in patients intoxicated with chlorinated hydrocarbons such as hexachlorobenzene or 2,3,7,8-tetrachlorodibenzodioxin, where marked biochemical modifications of porphyrin metabolism progressing up to clinically overt porphyria were documented in several cases.
In our studies, the porphyrinogenic effects found were very slight in the Italian group and totally absent in the American group.
The absence of correlation observed in the Italian workers between blood total PCB concentration and porphyrin excretion could indicate that a contaminant, rather than PCBs themselves, may have been responsible for the observed effects. An alternative explanation could be that only a few PCB congeners, not proportionally represented in the workers' blood PCBs, were the effective porphyrinogenic chemicals.
So far available evidence indicates that type of PCBs, internal concentration, presence of contaminants and, possibly, route of exposure are all determinant factors in human porphyrinogenesis by PCBs. Human porphyrinogenic effects by PCBs, on the whole, seem to be rather slight. This is not

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necessarily in contrast with experimental evidence.
In the American study, serum and adipose tissue samples were also analyzed by capillary column GC/ECD in order to identify and quantitate individual PCB congeners (11). The analysis identified more than 80 different PCB congeners both in serum and adipose tissue. Six major chromatographic peaks accounted for more than 50% of the total PCB concentration. Table 3 illustrates the congeners identified in each peak and the experimental evidence of porphyrinogenic activity available for them. As indicated in the Table, only two congeners have been tested and only one is frankly positive as a porphyrinogenic substance.

Table 3 Major PCB congeners in serum and adipose tissue determined in workers exposed to Aroclor 1260

PEAK RANK (1)	CONGENERS (2)	EXPERIMENTAL PORPHYRINOGENIC ACTION (3)
1°	2,3,5,6,3',4',5' / 2,3,4,5,2',4',5' } HEPTA-CB	not tested
2°	2,3,4,2',4',5'-HEXA-CB	+ liver of rodents and birds
3°	2,4,6,3',4',5' / 2,4,5,2',4',5' / 2,3,4,5,2',5' } HEXA-CB	- rodents/+chick embryo
4°	2,3,4,5,2',3',4'-HEPTA-CB	not tested
5°	2,3,4,5,2',3',5',6' / 2,3,4,5,6,2',3',5' } OCTA-CB	not tested
6°	2,4,5,3',4' / 3,4,5,2',3' } PENTA-CB	not tested

(1) In order of decreasing concentration
(2) Some chromatographic peaks are not resoluble in a single PCB congener
(3) See Reference 1.

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In conclusion, porphyrinogenic activity of industrial PCB mixtures in man appears to be rather weak and not uniform for different PCBs.
Further toxicological studies are needed on the porphyrinogenic power of the single PCB congeners more represented in human tissue.

ACKNOWLEDGEMENTS

The experimental work has been in part supported with the contracts n. 83.02646.56, 84.02332.56 and 85.000581.56 from the National Research Council of Italy (C.N.R.) - Target Project "Preventive Medicine and Rehabilitation"; Subproject "Toxicological Risk", and with a grant of Ministry of Education.

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DSW 476038.1130

STLCOPCB4043291

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ELLIS HORWOOD LIMITED
Publishers.Chichester

Halsted Press: a divison of
JOHN WILEY & SONS
New York.Chichester.Brisbane.Toronto

DSW 476038.1131

STLCOPCB4043292

First published in 1987 by
ELLIS HORWOOD LIMITED
Market Cross House, Cooper Street,
Chichester, West Sussex, PO19 1EB, England

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Baffins Lane, Chichester, West Sussex, England

North and South America and the rest of the world:
Halsted Press: a division of
JOHN WILEY & SONS
605 Third Avenue, New York, NY 10158, USA



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IUPAC Secretariat:
Bank Court Chambers, 2-3 Pound Way, Cowley Centre, Oxford OX4 3YF, UK

British Library Cataloguing in Publication Data
OCCUPATIONAL AND ENVIRONMENTAL CHEMICAL HAZARDS:
Cellular and Biochemical Indices for Monitoring Toxicity. –
(Ellis Horwood series in chemical science)
1. Toxicity testing
I. Foà, Vito
II. International Union of Pure and Applied Chemistry
615.9'07 RA1199

ISBN 0–7458–0088–2 (Ellis Horwood Limited)
ISBN 0–470–20802–3 (Halsted Press)

LIBRARY OF CONGRESS Card No. 86–27801

Printed in Great Britain by Unwin Bros. of Woking

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DSW 476038.1132

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