HUMAN MONITORING OF PCB BY URINE ANALYSIS

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ABSTRACT

Experiments with Wistar derived albino rats and with Macaca nemestrina have shown that urinary PCB concentration is correlated with serum PCB concentration. Sufficient analytical sensitivity was achieved for the analysis of human urine and when a set of normal human samples was analyzed they could be discriminated according to race.

INTRODUCTION

Monitoring of PCB in humans, generally for the purpose of detecting suspected exposure to PCB, has been carried out by analyzing either serum or whole blood (1) also a limited amount of work has been done with human milk (2,3). The large majority of work was with packed column chromatography although a limited number of samples have been analyzed on capillary columns. Electron capture detection has been used exclusively for reasons of analytical sensitivity (1).

Use of serum for PCB monitoring has many disadvantages and has only really been used to date as a pis aller. Not only is invasive sampling frequently ethically unjustified in speculative research, particularly when small children are involved, but the pattern of PCB congeners found in blood does not resemble the pattern ingested by a mammal (4). The cause is ready liver metabolism of PCB congeners without 4,4'-substitution (5). Hence if a subject is exposed to a PCB mixture such as Aroclor 1016, only two congeners, 2,4,4'-trichlorobiphenyl and 2,4,2',4'-tetrachlorobiphenyl will be expected to be elevated and hence the amount of data available for epidemiological study is very limited.

During chronic feeding studies of rats and monkeys primarily for the investigation of neurological effects of PCB, the opportunity was taken to investigate the relative concentration of PCB in serum and urine and as a result of an apparent correlation between PCB levels in serum and urine, preliminary work with human urine has been initiated. Studies in the three species will be described here.

METHODS

Animals. Rats were fed chow contaminated with a mixture or Aroclor 1016 and Aroclor 1260 (0.5 and 1 mg/g) for eighteen days. They were housed in metabolism cages. Two were sacrificed on alternate days for brain catecholamine analysis and for congener specific PCB analysis. Monkeys were housed in suspended stainless steel cages and fed Aroclor 1016 or Aroclor 1260 once a day on a piece of bread (0.8, 1.6 and 3.2 mg(kg·day)). Urine was collected in a large pan beneath the cage on the same day as blood samples were drawn. Serum was prepared after coagulation by centrifugation. Urine and serum samples were stored at -40 C until analyzed.

Sample preparation. Serum was analyzed by the addition of methanol and subsequent extraction with diethyl ether/hexane 1:1 (6). Rat and monkey urine were analyzed by the same procedure but human urine required some method development due to the low levels present. Diethyl ether was eliminated from the procedure and hexane was redistilled in glass with a lagged six ball Snyder column, collecting at a 1:3 reflux ratio. Eventually, methanol was also eliminated and the sample (100 ml) was extracted three times with hexane after acidification with 1 N sulfuric acid (30 ml). Recovery efficiency was determined by spiking with an acetone solution containing a 1:1.1:1 mixture of Aroclors 1221, 1016, 1254 and 1260 (total PCB concentration 20ng/ml)

Gas Chromatography. A Hewlett-Packard 5840 gas chromatograph was used with autoinjector, electron capture detector, 5880 splitless capillary injector and 25 M crosslinked 5% phenylmethyl silicone fused silica column (Hewlett-Packard film thickness 0.11 micron, phase ratio 450). The microprocessor was calibrated with a 1:1:1:1 mixture of Aroclors 1221, 1016, 1254 and 1260 (200 ng/ml of each). 78 chromatographic domains using peak identities based on 56 synthetic PCB, and the work of Mullin et al and Schultz et al (7,8) were calibrated. The finished data table was transmitted to a personal computer via the ASCII interface board and logged using Kermit software. Data were manipulated using the Biomedical Statistics Package (BMDP) and Lotus with three dimensional addition (Intex Solutions Inc Wellesley, MA).

Gas chromatographic detection limits were determined by running the calibration standard seven times at 4, 20, 40 and 160 ng/ml total PCB, and determining the standard deviation of each peak as it approached detectability (the relative standard deviation approached 30%). The limit was defined as 3.14 times the standard deviation when the chance the peak is not there is <0.01 and the chance that it is there is >0.988.

RESULTS AND DISCUSSION

Detection limits were all less than 0.4 ng/mL except for two poorly resolved domains which were 1.4 and 0.55 ng/mL respectively (which comprise <2% of the total mass in the 1:1:1:1 standard). In the extreme case of human urine therefore, where 100mL of urine are reduced to 1.0 mL detection limits are 0.004 ng/mL per congener. Recovery from human urine at 10ng/mL total PCB ranged from 60 to 80% for different congeners.

Table 1 shows the pattern difference between rat feed, and the resultant serum and urinary levels. Notice how much more closely the urine pattern resembles the food intake pattern than the serum pattern. From this it became clear that if urinary concentrations could be measured precisely at environmental levels, this fluid might give a better indication of current exposure to PCB than does serum analysis. Using the BMDP scatter plot program, correlation coefficients between urine and serum for congeners which comprised >1% of the residue in serum were significantly different from 0 ($p<0.05$) indicating that urine was a predictor for serum so that it may be used as an indicator of PCB status at least as well as serum.

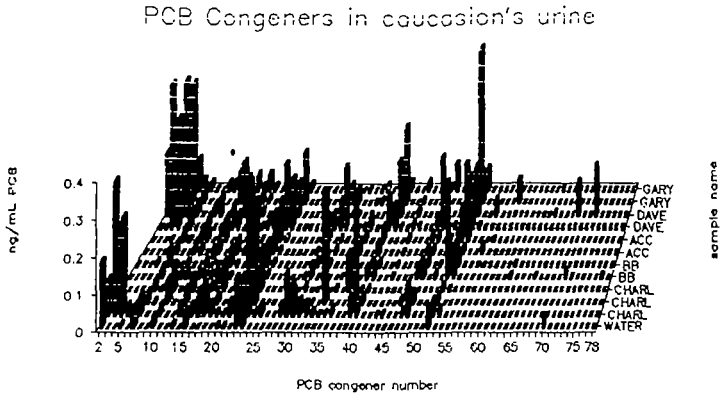
TABLE 1. Congeners comprising >1% of total PCB in rat experiment

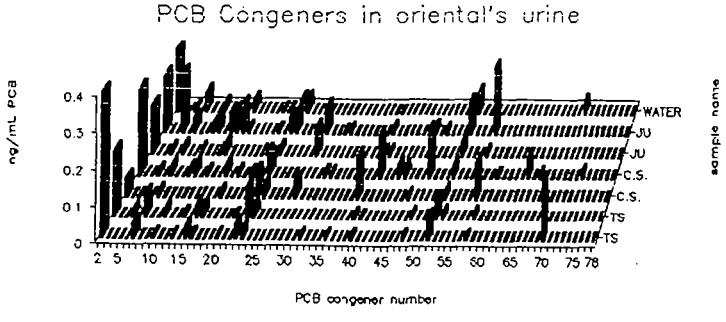
Congener	Food	Percent of Total Serum	Urine
2,5,2'	5	<	5
2,4,2'	2	<	2
2,5,3'	1	<	1
2,4,3'	4	<	1
2,4,4	6	1	3
2,3,4'	3	<	1
2,5,2',5'	3	<	13
2,4,2',5'	3	<	4
2,3,2',5'	3	<	3
2,4,2',4'	1	<	4
2,3,6,3'	2	<	1
2,3,6,4'	3	<	1
2,4,5,2',5'	2	<	2
2,3,5,2',4',5'	1	2	3
2,4,5,2',4',5'	6	14	9
2,3,4,2',4',5'	4	10	7
2,3,4,6,3',4'	1	1	1
2,3,4,5,6,3',4'	2	8	3
14 Additional congeners	>1% in urine and in serum		
Total Contents	1mg/g	2.8ug/g	0.4ug/g

In the monkeys exposed to Aroclor 1016, the urine pattern resembled the serum pattern more closely than in the rat, which doubtless indicates the difference in liver metabolic power between species.

A series of human volunteers was surveyed using morning urine samples. 25 ml were processed using the same sample analysis as for serum except redistilled hexane was employed. Several differences between the participants in the survey were apparent, the most pronounced was between races, Orientals were plainly less contaminated than caucasians (Fig 1). One oriental subject has resided in the USA for 15 years the other two entered the USA from mainland China within the last few months. The Caucasians have lived near Albany NY for at least 15 years. The data illustrated in Fig 1 could not have been obtained using a more complicated or invasive sampling system. They also indicate the utility of PCB urine analysis, although, of course no epidemiological conclusions can be drawn from such a small sample. The method of presentation which allows for the display of 78 PCB analyses per sample and comparison between samples at a glance is also an important innovation in PCB analytical technology.

Fig 1 displays the water and reagent blank which is clearly unsatisfactory. The data reported here make it clear that urine analysis for PCB is worth pursuing and method improvement is therefore being undertaken. Reduction of pH with sulfuric acid and extraction of 100 ml of urine with hexane produced a large reduction in background. Recovery and analytical precision are being determined.





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