Bull. Environ. Contam. Toxicol. (1988) 41:523–530

Environmental Contamination and Toxicology

First Studies on the Bioconcentration and Immunotoxicity of Tetrachlorodiarylmethanes in the Rat

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In Italy too the PCBs have given rise to widespread phenomena of environmental contamination and are present in the surface waters, in animals, in foodstuffs and in human tissues (Leoni et al., Leoni and Biocca, 1982). Accordingly the use of PCBs in Italy was discontinued in 1984, while to replace them among other things tetrachlorodiarylmethanes (TCDAM, Fig.1) were proposed, to be used in electrical equipment mixed with trichlorobenzenes (TCBs).

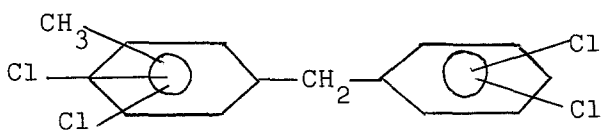

Figure 1 : Structural formula of tetrachlorodiaryl-methanes (TCDAMs).

TCDAMs are produced by the ATOCHEM Company (France) and marketed under the name of "UGILEC 141" . The isomers theoretically possible number about 70, but as yet separate individual isomers are not available. The acute toxicity of the TCDAM mixture is low (oral LD50 in rat 4600 mg/kg) and these products, compared to PCBs, would seem to be less dangerous to the environment (CI50 Daphnia 0.4 mg/l, TLm 48 h fishes 400 mg/l) and more biodegradable. The ATOCHEM Company also carried recently some oral subchronic toxicity

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This research was founded by the National Council of Research (CNR)-(contact No. 86.01838.56).
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studies on rats (30 and 90 days). We undertook an immunotoxicity study on TCDAMs , comparing them to one of the PCBs most widely used in Italy (Fenclor 54, containing 54% chlorine). A number of studies have been carried out on the immunotoxicity of PCBs both in animals (Street and Sherma, 1975, Koller, 1977, Thomas and Hinsdill, 1978, Talcott and Koller, 1983) and in man (accidental ingestion in a population group in Taiwan) where they have been shown to cause a decrease in the concentration of IgMs and IgAs, a decrease in the percentage of T-lymphocytes and T-helper lymphocytes, and a normal percentage of T-suppressor lymphocytes (Lu and Wu, 1985). These studies were recently reviewed by Decotes (1986). No relative reviews are available on TCDAMs. In our research we carried out a humoral immunotoxicity test by administrating tetanus toxoid to rats fed either with a standard diet or with diets containing TCDAMs, PCBs and hydrocortisone (HY) and evaluating the serum level of tetanus antitoxin using the passive hemoagglutination technique (PHA). The investigation was completed with a gas chromatographic assessment of TCDAM and PCB deposits in fatty tissue, in liver and the brain of a number of the experimental animals.

MATERIALS AND METHODS

Wistar albino rats five weeks old and weighing approximately 60-70 g were randomly assigned to 5 groups of 12 males and 12 females and then huosed in plastic and metal cages (mesuring 40 x 25 x 15 cm, with four animals per cage) at 20-25 C. Five types of diet were then prepared (A - E) : the basic diet (A) was the standard Hawk and Oser diet containing 10% hydrogenated cotton oil; while diet B contained 200 ppm of a PCB with 54% of chlorine (Fenclor 54, produced by the Caffaro Company, Italy) and diets C and D contained 200 and 1000 ppm respectively of TCDAM (produced by Atochem Company, France) and diet E contained 1000 ppm of Hydrocortisone 21-hemisuccinate (produced by the Lepetit Company, Italy). All the animals were initially fed the basic diet for one week, and thereafter the diets containing the PCBs and the TCDAMs for a period of ten weeks, while the control groups received the basic diet throughout. Group E (treated with hydrocortisone) alternatively received the treated diet and the basic diet, because the hydrocortisone diet

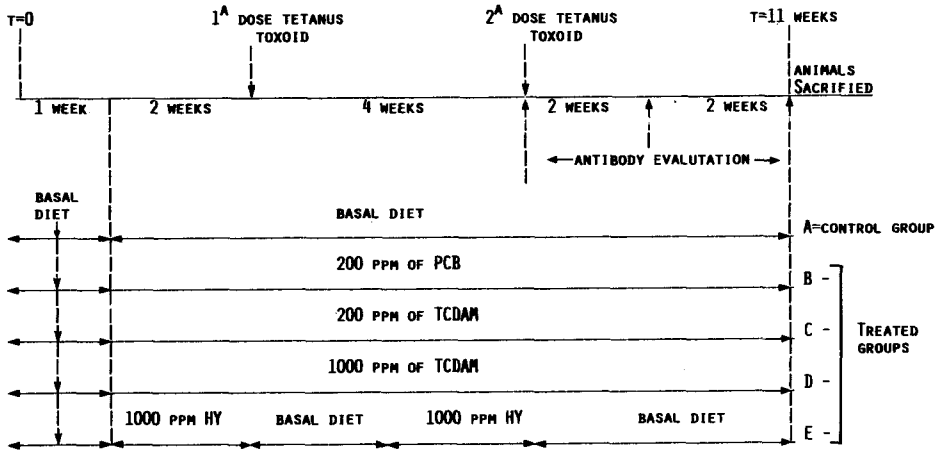

Figure 2 : Experimental pattern of treatment of rats with tetanus anatoxin, polychlorinated biphenyls (PCB), tetrachlorodiarylmethanes (TCDAMs) and hydrocortisone (HY).

produced high mortality. Two weeks after the treatment was initiated, all animals received a dose (1.5 Lf = 6 I.U.) of tetanus anatoxin (Tetatox Berne, Switzerland). A second dose of 1.5 Lf was administered four weeks after the first dose. To establish the level of antibodies in the serum, blood samples were taken from the tails of animals at six, eight and ten weeks after treatment and evaluated using the PHA technique employing the Tetan Test kit (of the Istituto Sieroterapico S. Belfanti, Milan, Italy). The results obtained were expressed in international units (I.U.). Fig. 2 shows the experimental results. At the time the first samples were taken, two male and five female rats from the control group died,due to a laboratory accident, while during the last four weeks of treatment one male of the group treated with PCB and seven males and five females of the group treated with hydrocortisone died. No deaths occurred in the group treated with TCDAMs. At the end of experiment, all the animals were sacrificed in sinly nitrogen and tissue from groups of three animals were chosen at random for analysis. The TCDAMs in brain, liver and abdominal fat were determined by gas chromatography. To accomplish this the tissue samples (0,7 - 3 g) were extracted in a blender with n-hexane and the extract diluted to 100 ml. A quantity of 10 ml of extract was treted with

0.5 ml of concentrated H2SO4, according to the standardised technique of Waliszewski and Szymczynski (1982) for chlorinated pesticides. The procedure gave excellent results for the TCDAMs and PCBs. A proportion of the treated extract, appropriately diluted, was assessed using a VARIAN 3700 gaschromatograph complete with electron capture detector (Ni 63) and 2 m x 3 mm i.d. column filled with 1.5% SP 2250 + 1.95% SP 2401 on 100 - 120 mesh Supelcoport (temperature : column 200 C, injector 220 C, and detector 300 C) and nitrogen carrier gas at 40 ml / minute. Quantitation was achieved on the basis of the total area of the gaschromatogram. Similar steps were taken to determine the PCBs in the tissues of a number of animals fed with the diet containing 200 ppm of Fenclor 54.

RESULTS AND DISCUSSION

Table 1 shows the geometric means of the levels of antibodies for tetanus toxin in the animals treated. The results relative to the PCBs do not indicate a reduction of humoral response in the rat for treatment of 200 ppm for ten weeks, while on the contrary the level of antibodies at the second and third sampling is usually significantly higher than in the controls. Various researches (Imanishi et al.,1980, Loose et al., 1978) have shown that treatment with PCB makes the animals (mice) highly susceptible to viral and bacterial agents; while the investigations of Talcott and Koller (1983) failed to demonstrate immunotoxic effects of PCBs in mice born of mothers treated with PCB. In man (Chang et al., 1981) an immunosuppressing effect of PCB on the B-cell system may be hypothesised. Our experiment, at the doses employed, did not confirm a reduction in the titrable antibodies in the serum; and this might perhaps be explained by the high deposits of PCB (Fenclor 54) in the fatty tissues in the rat, and a lower metabolic availability of the compound. As far as the rats treated with TCDAM are concerned, the kinetics of the antibody response indicates for the primary response a statistically significant reduction compared to the controls in the males treated with 200 ppm and in the males and females treated with 1000 ppm. On the contrary the secondary response is significantly higher in the treated animals than in the controls, but on the third sampling, the

Table 1: Geometric means (U.I./ml) of antibody for tetanic toxin in treated and control rats (12 male and 12 female/group).

Group	Diet	Sex	Blood test I	Blood test II	Blood test III	Animal Surviving
A	Standard	M	0,9 (0,4-1,6)	1,0 (0,4-1,6)	7,0 (3,2-25,6)	11
		F	1,0 (0,8-1,6)	1,5 (0,8-1,6)	7,1 (3,2-12,8)	7
B	PCB 200 ppm	M	1,0 (0,4-3,2)	4,3* (1,6-12,8)	5,1 (1,6-12,8)	12
		F	1,0 (0,2-3,2)	10,8* (6,4-12,8)	13,6** (6,4-25,6)	11
C	TCDAM 200 ppm	M	0,3* (0,1-0,8)	8,1* (3,2-12,8)	10,8** (3,2-25,6)	12
		F	0,7 (0,4-1,6)	10,1* (6,4-12,8)	12,8 (6,4-25,6)	12
D	TCDAM 1000 ppm	M	0,2* (0,05-0,4)	8,5* (1,6-25,6)	8,1 (3,2-12,8)	12
		F	0,1* (0,05-0,2)	9,6* (6,4-25,6)	9,0 (6,4-25,6)	12
E	HY 1000 ppm	M	0,1* (0,0-0,4)	0,3*** (0,1-1,6)	0,8* (0,4- 3,2)	5
		F	0,2* (0,05-0,8)	3,5 (1,6-6,4)	3,2** (1,6-6,4)	7

* $p<0,001$; ** $p<0,05$; *** $p<0,01$: in relation to corresponding withdrawal of A group.

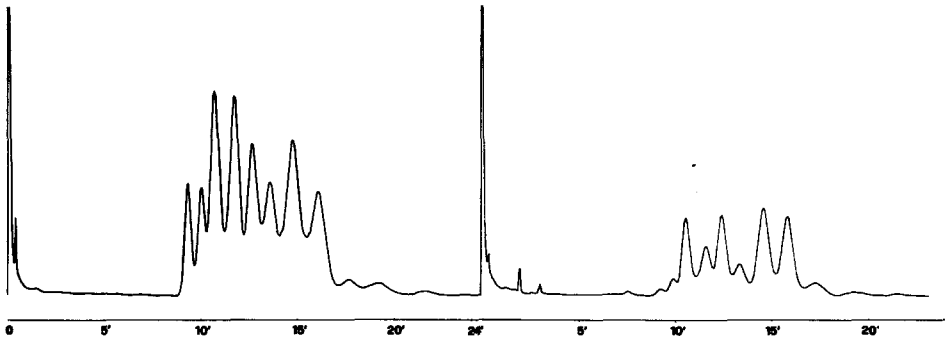

Figure 3 : Gaschromatograms of TCDAMs standard (on the left) and of extract of abdominal fat of rat fed with 1000 ppm of TCDAMs for 10 weeks (right side)

effect was statistically significant only for the animals treated with 200 ppm of TCDAM. The animals treated with hydrocortisone showed a high mortality and significant reduction in their antibody response througout the experiment.
Table 2 shows the deposits of TCDAMs in the tissues of rats fed 200 and 1000 ppm in their diets. These concentrations are referred to fresh tissue and calculated on the basis of the total area of the gaschromatogram; however Fig. 3 shows that important modifications occur during the metabolism of the TCDAMs, and that the deposit in fat is substantially determined by only of some isomers which we have yet to identify.
The amount of the deposits in the fatty tissues is clearly correlated to the doses in the diets. In comparison with the data obtained for the PCBs (but referred to a single animal per group) and on the basis of the same dose in the diet, the TCDAMs shows bioconcentration factors 25 - 30 times lower for the brain; 65 - 123 times lower for the liver; and 21 - 33 times lower for the fatty tissue. The TCDAMs in the rat are undoubtedly more metabolized than the PCBs, but these toxicological properties still have to be investigated in detail.

Acknowledgment : We would like to thank Mr. Giuseppe Fabiani for his invaluable technical collaboration.

Table 2: Concentrations (ppm) of TCDAM and PCB in fresh tissues of rats after 10 weeks of alimentation.

Diet	Brain		Liver		Addominal fat		Note
	M	F	M	F	M	F	
PCB 200 ppm	4,76	5,00	12,37	18,20	1069,0	750,0	One animal per group
TCDAM 200 ppm	0,15 (0,10-0,25)	0,20 (0,14- 0,25)	0,10 (0,08-0,12)	0,28 (0,18-0,36)	32,37 (22,69-42,54)	35,04 (29,67-40,35)	Three animals per group
TCDAM 1000 ppm	1,79 (1,14-2,91)	0,53 (0,42-0,74)	1,07 (0,42-2,36)	0,12 (0,09-0,15)	158,54 (126,62-191,38)	123,70 (75,82-183,95)	

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Received February 22, 1988; accepted May 19, 1988.