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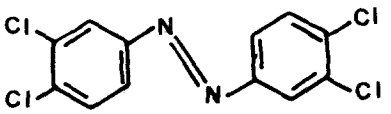
Effects of 3,3′,4,4′-Tetrachcloroazobenzene and 3,3′,4,4′-Tetrachloroazoxybenzene on Rat Liver Microsomes

G. Saint-Ruf[1], Do Phuoc Hien[1], and M. Mercier[2]

[1]*Centre Marcel Delépine, C.N.R.S., 45045 Orléans Cedex, France, and*
[2]*Laboratoire de Biotoxicologie, Tour Van Helmont, UCL-7369, Avenue Emmanuel Mounier, 73, 1200 Bruxelles, Belgique*

There have been recent reports (POLAND et al. 1976, TAYLOR et al. 1977) of several cases of chloracne in almost 90% of the workers in a factory engaged in the manufacture of 3,4-dichloroaniline-based herbicides. The physico-pathological manifestations of this syndrome are quite similar to those observed after exposure to 2,3,7,8-tetrachloro-p-dioxin (TCCD) (I.A.R.C. MONOGRAPHS 1977). These industrial accidents were attributed to 3,4,3',4'-tetrachloroazobenzene, or TCAB (1) and its oxidation product, 3,4,3',4'-tetrachloroazoxybenzene, or TOCAB (2), two by-products of 3,4-dichloroaniline manufacture (POLAND et al. 1976). They present, at least in their trans form, a certain structural analogy with dioxin (3). In addition, these molecules are found in soil as degradation products of 3,4-dichloroaniline and of various herbicides derived from this compound (BARTHA & PRAMER 1967 1970, MANSOUR & KORTE 1976). They may also form by the action of ultraviolet light on the derivatives (MANSOUR & KORTE 1976).



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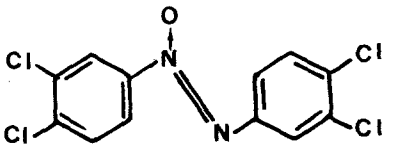

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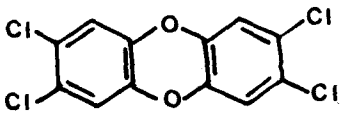

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It was also shown by POLAND et al. (1976) that TCAB and TOCAB induce aryl hydrocarbon hydroxylase (AHH) in chicken embryos to a comparable extent as does dioxin, the most potent inducer known and that there was a certain degree of competition between TCCD and TCAB or TOCAB in the hepatic cytosol, where the receptors for AHH induction are located.

In order to broaden the study of the biochemical effects of TCAB and TOCAB and to extend the comparison with the effects of TCCD, we sought possible effects of these compounds on three important biological systems: 1) the activity of a typical enzyme, zoxazolamine hydroxylase; 2) the properties of cytochrome P-450; 3) hepatic arginase levels.

MATERIALS AND METHODS

TCAB was prepared by oxidizing 3,4-dichloroaniline with active manganese dioxide, as described by WHELER & GONZALES (1964). TOCAB was obtained by the controlled reduction of 3,4-dichloronitrobenzene by $LiAlH_4$ in anhydrous ether (CORBETT & HOLT 1963).

Male Sprague-Dawley rats weighing 100 to 130 g and maintained on a vitamin-rich diet were used in the biological tests.

The effects on zoxazolamine hydroxylase activity were measured with the technique used for TCCD and TBrDD (BUU-HOI et al. 1971, SAINT-RUF et al. 1975). Various doses of TCAB and TOCAB were intraperitoneally injected in dimethylsulfoxide (DMSO) solution. Zoxazolamine was dissolved in 1 volume of 1 N HCl, 3 volumes of isotonic saline were added and 60 mg/kg were intraperitoneally injected.

Cytochrome P-450 was assayed with the technique of McLEAN & DAY (1974). The quantity of cytochrome P-450 in a liver homogenate was calculated as a function of the intensity of the absorption peak between 450 and 490 nm, using the millimolar absorption coefficient of 91 after the addition of dithionite.

Hepatic arginase was assayed according to NG-HUY et al. (1972) and the results were expressed in arginase units (1 U = hydrolysis of 1 nanomole of arginine/min) per mg of liver fresh weight.

RESULTS AND DISCUSSION

1. Effect on zoxazolamine hydroxylase biosynthesis.

The effect of various doses of TCAB and TOCAB were measured as a function of the percent reduction of the duration of paralysis caused by zoxazolamine in the treated animals (Table 1). The results show that both compounds have high zoxazolamine hydroxylase inducing activities which are still manifest (75%) at a dose of 0.05 mg/kg. At higher doses, the effect increases to reach a considerable maximum (95%) at the maximum effect does of 10 mg/kg, as we have shown for dioxin (BUU-HOI et al. 1971).

TABLE 1

Induction of Zoxazolamine Hydroxylase in Rats by TCAB and TOCAB

Dose (mg/kg)	Length of paralysis (min)† Controls	TCAB	TOCAB	Reduction of length (%)†† TCAB	TOCAB
10	200±68(6)	12±1(6)	11±1(6)	94	95
5	"	23±3(6)	22±7(6)	89	89
2	"	23±4(6)	23±4(6)	89	89
1	"	25±5(6)	27±7(6)	88	87
0.5	168±54(6)	27±9(6)	28±3(6)	84	83
0.25	"	33±7(6)	31±6(6)	80	81
0.10	"	39±8(6)	38±8(6)	77	77
0.05	"	44±9(6)	39±8(6)	74	77

† The number of rats used is indicated in parentheses. The first figure is the mean, the second the standard deviation of the mean. In all cases, $p<0.01$ (Student's test).

†† Calculated in relation to controls

2. Effect on cytochrome P-450.

It is known that cytochrome P-450 plays an important role in drug metabolism and it is admitted that a large number of carcinogens owe their effects to an activation by the mixed function oxidase centered on the P-450 system. Thus, it was shown that the alterations of the properties of rat liver microsomal P-450 caused by TCCD are similar to those caused by 20-methylcholanthrene, one of the most powerful carcinogens knwon.

The effects of TCAB and TOCAB on rat liver microsomal cytochrome P-450 are shown in Table 2. In particular, it can be seen that both compounds lead to a doubling of control values. Here again, there is a good correlation with previous observations obtained for dioxin (GREIG & MATTEIS 1973).

3. Effect on hepatic arginase levels.

The tests were performed on 36 male rats divided into 4 groups of 3 lots each. Each group contained a lot of 3 controls, 3 TCAB-treated animals and 3 TOCAB-treated animals. Both substances were dissolved in DMSO and intraperitoneally injected at a dose of 10 mg/kg. Injections were repeated at 5 day intervals; the first group received 1 injection, the second 2, the third 3 and the fourth 4. Controls received 2.5 ml of DMSO/kg.

The results in Table 3 show that although both compounds lead to slight increases in liver weight, they apparently have no significant effect on arginase activity, even after the fourth in-

jection. In addition, we noted no unusual variations of body weight in treated animals, which exhibited the same growth curves as controls. These results differ from those we previously obtained under almost identical conditions, but with TCCD and TBrDD (BUU-HOI et al. 1971, SAINT-RUF et al. 1975): we observed not only decreased growth curves, but also a gradual and ultimately severe decrease in arginase levels, similar to that obtained with liver-homing carcinogenic chemicals. This apparent dicrepancy between the pronounced effects on the hydroxylase system (zoxazolamine hydroxylase and cytochrome P-450) and the negligible action on hepatic arginase could be explained, as suggested by POLAND et al. (1976), by the fact that TCAB and TOCAB are metabolized more rapidly than TCCD.

TABLE 2

Hepatic Cytochrome P-450 Content after Treatment of Animals with 10 mg of TCAB or TOCAB per kg.

	Cytochrome P-450 (nmoles/g fresh weight)		
Assay	Control	TCAB	TOCAB
1	26.6	54.9	56.25
2	24.4	53.68	51.44
3	29.76	56.45	59.31
4	27.00	61.07	62.18
5	23.36	59.75	59.87
6	26.59	62.55	63.78
Mean ± S.E.M.	26.28±2.23	58.06±3.57	57.64±4.98

TABLE 3

Effects of TCAB and TOCAB on Liver Arginase Levels.

Sacrifice: days after first injection	No. of injections	Mean liver weight (g)			Mean arginase activity (units/g of liver)		
		Controls	TCAB	TOCAB	Controls	TCAB	TOCAB
1	1	5.40	7.10	6.90	470	466	470
7	2	7.10	9.12	11.20	410	385	385
14	3	8.35	10.50	12.10	474	454	396
21	4	9.55	12.25	13.10	510	463	385

In addition, it has been reported that TCAB is an anti-adenocarcinomic agent in mice (CHILD et al. 1972). It is thus possible that there is a relationship between this property and the probable absence of an effect on the hepatic arginase levels. Whatever the reality of the situation, the acnegenic properties of TCAB and TOCAB and their effects on the enzymatic system of the liver cell lead to the classification of these compounds as dangerous substances, since they are potential agents of environmental pollution.

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