Effects of exposure to a human milk PCB-DDT-DDE mixture from day 1 to 20, or to TCDD on day 18 of age, in prepubertal females, and on the development of methylnitrosourea-induced mammary tumors in the adult rat.

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Introduction

Epidemiological studies remain controversial with respect to identifying a link between organochlorine (OC) exposure and breast cancer. In the rat, antitumorigenic activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; Holcomb & Safe, 1994) and methylated trichlorodibenzofurans (6- and 8-MCDF; McDougal et al., 1997) has been demonstrated using the chemically-induced mammary tumour rat model. In contrast, tumorigenic effects of prenatal TCDD (Brown et al., 1998), and 3,3',4,4'-tetrachlorobiphenyl (Nesaretnam et al., 1998) have been observed. Exposure to OCs, such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCB) during critical periods of development, is hypothesized to increase the risk of developing breast cancer (Trichopoulos, 1990; Dewailly et al., 1994; Davis et al., 1997). Thus, our objective was to determine if the most abundant and toxic OCs found in human milk, ingested during the neonatal period, modulate the development of mammary tumors in the rat.

Materials and methods

A mixture composed of *p,p'*-DDT, its major metabolite, dichlorodiphenyldichloroethene (*p,p'*-DDE), and 19 PCB congeners, was prepared according to their median concentrations found in the milk of Canadian women (Table 1). The proliferative effects of this mixture were tested *in vitro* using the estrogen dependent human breast cancer MCF7-E3 cells (Butler et al., 1986; Wiese et al., 1992), according to the method described by Desaulniers et al. (1998). *In vivo*, the toxic effects of this mixture were tested in a first experiment including nine treatment groups. Two groups, water and oil-dmso, tested the effects of the vehicle. The complete mix was tested at 10, 100 and 1000 times the typical daily intake of a human baby. Four other groups were treated with the 1000x level of the major components of the mixture, the non-ortho, mono-ortho, and di-ortho chlorinated PCBs, and a pesticide DDT-DDE group. At ages 1, 5, 10, 15, and 20 days, the female pups received the appropriate vehicle or mixture by gavage, each dose representing 5 days of ingestion. At 21 days of age, the rats were sacrificed and the blood and tissues collected to assess a number of endpoints including hepatic enzyme induction, mammary gland development and endocrine disturbance. In the second experiment, the neonates were gavaged with the complete mix, as described above, at the 10x, 100x and 1000x dose levels. At 18 days of age, one oil-control group received 2.5 μg TCDD/kg b.w, by gavage. On day 21 of age, all treatment groups, except for the control and a 1000x-mix group, received a single i.p. injection of the initiator of the carcinogenic process, methylnitrosourea (MNU, 30 mg/kg b.w.). Rats were palpated regularly for the presence of tumors and sacrificed when their tumors reached 1 cm in size, or at the end of the experiment. Mammary tumors, and the mammary glands, were prepared for histological investigation.

Table 1: Concentrations of PCB congeners and DDT isomers in human milk, culture medium, and gavaging solution.

Family of xenobiotics	Selected congeners [1]	[Milk fat] (ng/g)[2]	Concentration (ng/2 ml of culture medium)[3]		Amount Gavaged (ng/g b.w. per day)[4]	Relative % per	
			1 X	5000 X	1 X	Congener	family
PCBs[5]:							
Non-ortho	77	0.008	0.0006	3	0.00004	0.002	0.03
	126	0.080	0.0059	30	0.0004	0.018	
	169	0.033	0.0024	12	0.0001	0.007	
Mono-ortho	28	8	0.59	2960	0.04	1.800	10.80
	66	4	0.30	1480	0.02	0.900	
	74	15	1.11	5550	0.07	3.376	
	118	16	1.18	5920	0.07	3.601	
	156	5	0.37	1850	0.02	1.125	
Di-ortho	99	23	1.70	8510	0.10	5.176	28.81
	128	4	0.30	1480	0.02	0.900	
	138	27	2.00	9990	0.12	6.077	
	153	27	2.00	9990	0.12	6.077	
	170	9	0.67	3330	0.04	2.026	
	180	18	1.33	6660	0.08	4.051	
	183	3	0.22	1110	0.01	0.675	
	187	7	0.52	2590	0.03	1.575	
	194	3	0.22	1110	0.01	0.675	
	201	4	0.30	1480	0.02	0.900	
	203	3	0.22	1110	0.01	0.675	
DDT:	*p,p*-DDE	233	17	86358	1.04	52.530	60.36
	p,p-DDT	35	3	12876	0.15	7.832	
Total xenobiotics:		444	33	164399	1.97	100	100

[1]: Only the congeners present in more than 75% of Canadian women were included in the mixture (Mes et al., 1993a, 1993b). Non ortho-chlorinated PCBs (77, 126 and 169), although not detectable in 75% of Canadian women, were included in the mixture in the proportion measured in human milk because of their high toxicity. Numbering system according to Ballschmiter and Zell (1980).

[2]: Median concentration of each congener in human milk fat (Mes et al., 1993a, 1993b).

[3]: Concentrations in the culture medium reflect multiples of what is present in 2 mL of human milk containing 3.7% fat. 10x, 100x, 500x, and 1000x multiples were also tested.

[4]: Animals received 10X, 100X and 1000X multiples of this dose.

[5]: PCB congeners are separated according to the position of the chlorine substitution (non-ortho, mono-ortho and di-ortho substituted).

Results

In vitro, at a concentration 5000 times higher than the human milk level, the mixture stimulated the proliferation of MCF7-E3 breast cancer cells. *In vivo*, the mixture had no uterotrophic effects. The highest dose induced hepatic microsomal enzymes (ethoxy- and benzyloxy- (CYP-1A1, -2B/3A, $p<0.05$) and pentoxy- resorufin-*o*-deethylase activities (CYP-2B, not significantly), and

increased liver weights. The non-ortho treatment also increased EROD activity and liver weights. Prolactin in pituitary gland homogenates was increased in the DDT-DDE and the 100X dose groups compared to controls ($p<0.05$). There were no indications of synergistic effects between different categories of OCs. In the second study, growth was significantly reduced by the TCDD or the MNU treatments. Day of vaginal opening was delayed ($p<0.05$) in the MNU-10 and MNU-100 dose groups compared to non-MNU treated control rats. The metabolism of tritiated estradiol by hepatic microsomes was more important in rats treated with the highest dose. Although there were no significant treatment effects on the conversion into 16-OH or 2-OH-estradiol, assessed by thin layer chromatography, 2-OH-estradiol conversion significantly decreased with age. The percentage of rats developing benign or malignant mammary tumors (Table 2) was similar among dose groups. The incidence curves (the delay between the time of MNU injection until the detection of a palpable tumor) obtained from the treatment groups were similar to that of the control group, however, the incidence curve for the TCDD group differed from the 1000X group (Figure 1. Survival curve analysis, Kaplan-Meir method, $p<0.05$).

Discussion

The estrogen-like effect detected by the MCF7-E3 breast cancer cells could not predict the absence of uterotrophic effects *in vivo*. The highest dose of the mixture of PCB-DDT-DDE had minimal toxic effects in the 21 day old female rats. The absence of significant differences in the number of rats developing mammary tumors suggest that TCDD, or the mixture, did not promote the initiation of the carcinogenic process by MNU. The delay in the development of palpable tumors in the 1000X group suggests that promotional factors could be reduced by the treatment. Given that mammary tumors are mostly estrogen dependent, perhaps the delay in tumor development could be associated with a reduced ovarian estradiol output in the 1000X dose group. Although the acute single dose treatment of TCDD had no significant effects, the elevated incidence curve (Figure 1) in this group provided us with the incentive to initiate a similar experiment testing the dose response effects of human milk dioxins and furans.

Table 2: Percentage of rats with abnormal mammary structures.

Type	OC/Mixture	0	1000	Methylnitrosourea treated groups				
				0	10	100	1000	TCDD
	N:	30	33	41	28	31	34	32
Benign	Fibroadenomas	0	0	29.3	46.4	19.4	26.5	31.3
	Papillomas	0	0	7.3	3.6	9.7	2.9	15.6
	Adenomas	0	0	12.2	14.3	12.9	23.5	18.8
	IDP's /Hyperplasia	0	3.0	39.0	32.1	32.3	41.2	46.9
Malignant	In situ carcinomas	0	3.0	12.2	17.9	25.8	20.6	12.5
	Adenocarcinomas	0	0	26.8	42.9	32.3	35.3	40.6
Other Mammary	Adenocystic Changes	0	0	22.0	28.6	19.4	32.4	28.1
	Fibrosis	3.3	0	58.5	75.0	58.1	55.9	56.3
	Cysts	36.7	39.4	70.7	64.3	61.3	64.7	53.1
	Fibromas	0	0	2.4	0	0	0	0
	Fibrohistiocytic tumor	0	0	0	0	0	0	3.1
	Lactational changes	6.7	0	51.2	60.7	54.8	52.9	34.4

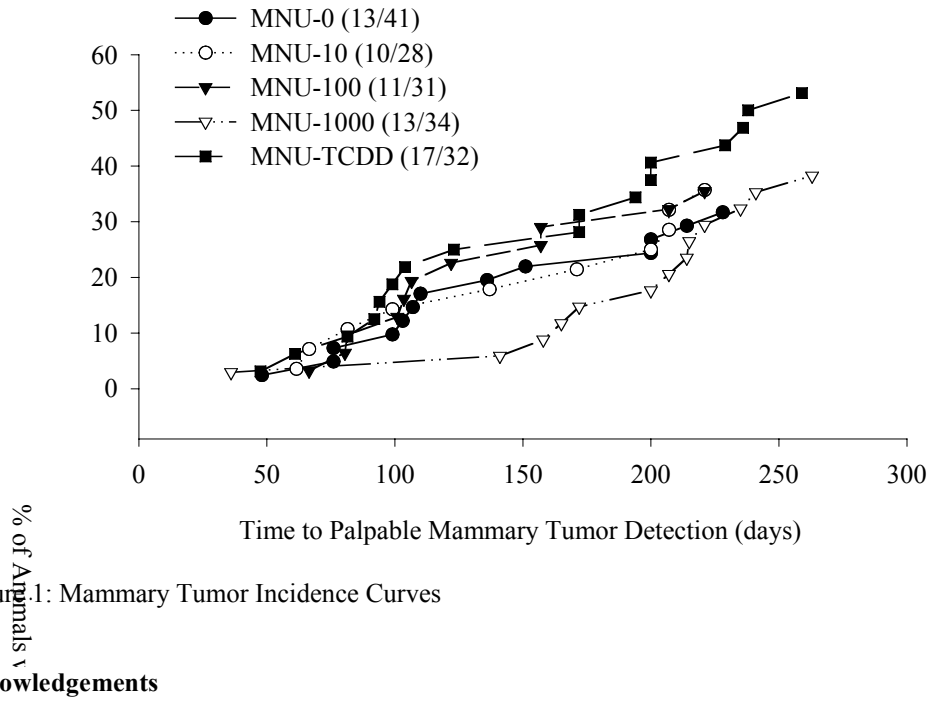

Figure 1: Mammary Tumor Incidence Curves

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