3. Studies of Cancer in Experimental Animals

3.1 Administration with known carcinogens

Studies on PCDFs in combination with known carcinogens are summarized in **Table 26**.

3.1.1 *Mouse skin*

2,3,7,8-Tetrachlorodibenzofuran

Groups of 20 female HRS/J hairless (*hr/hr*) mice, eight weeks of age, were given skin applications of 0 or 5 μmol/animal *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) in 50 μL acetone followed by 1 μg/animal 2,3,7,8-TCDF in 50 μL acetone twice weekly for 20 weeks. Skin papillomas developed in 19/19 mice (4.9 tumours/mouse) in mice treated with MNNG plus 2,3,7,8-TCDF compared with 1/20 (0.05 tumours/mouse) in mice treated with 2,3,7,8-TCDF alone and 0/23 with MNNG alone (Poland *et al.*, 1982).

2,3,4,7,8-Pentachlorodibenzofuran

Three groups of 20 female HRS/J hairless (*hr/hr*) mice, five to eight weeks of age, were treated with single skin applications of 5 μmol/animal MNNG in 50 μL acetone. Starting seven days later, the mice were treated with 25, 50 or 100 ng/animal 2,3,4,7,8-PeCDF in 25 μL acetone twice weekly for 20 weeks. A control group of 20 mice received acetone followed by 100 ng/animal 2,3,4,7,8-PeCDF. The numbers of surviving mice with papillomas of the skin were 9/19, 11/18 and 8/18 in mice treated with MNNG and 25, 50 or 100 ng/animal 2,3,4,7,8-PeCDF compared with 0/20 in mice treated with 2,3,4,7,8-PeCDF alone and 1/19 in mice treated with MNNG alone. Skin carcinomas were found in 1/19 mice treated with MNNG + 25 ng 2,3,4,7,8-PeCDF, 1/19 mice treated with MNNG + 100 ng 2,3,4,7,8-PeCDF and in 1/19 mice treated with MNNG alone (Hébert *et al.*, 1990a).

1,2,3,4,7,8-Hexachlorodibenzofuran

Three groups of 20 female HRS/J hairless (*hr/hr*) mice, five to eight weeks of age, were treated with single skin applications of 5 μmol/animal MNNG in 50 μL acetone. Starting seven days later, the mice were treated with 250, 500 or 1000 ng/animal 1,2,3,4,7,8-HxCDF in 25 μL acetone twice weekly for 20 weeks. A control group of 20 mice received acetone followed by 1000 ng/animal 1,2,3,4,7,8-HxCDF. The numbers of surviving mice with papillomas of the skin were 15/19, 7/14 and 3/17 in mice treated with MNNG and 250, 500 or 1000 ng/animal 1,2,3,4,7,8-HxCDF compared with 1/17 in mice treated with 1,2,3,4,7,8-HxCDF alone. Skin carcinomas occurred in 1/19 mice treated with MNNG + 250 ng 1,2,3,4,7,8-HxCDF, 2/17 mice treated with MNNG + 1000 ng 1,2,3,4,7,8-HxCDF and 1/19 mice treated with MNNG alone (Hébert *et al.*, 1990a).

Table 26. Enhancement of tumorigenesis in animals by administration of PCDFs in combination with known carcinogens

Strain/species (sex)	Known carcinogen	Route of administration	Interval	Dose and frequency	Route of administration	Enhancement	Reference
Skin							
HRS/J hairless mice (*hr/hr*) (F)	5 µmol MNNG	Skin		1 µg 2,3,7,8-TCDF/2 per wk/20 wk	Skin	+	Poland *et al.* (1982)
HRS/J hairless mice (*hr/hr*) (F)	5 µmol MNNG	Skin	7 days	25 ng 2,3,4,7,8-PeCDF/2 per wk/20 wk	Skin	+	Hébert *et al.* (1990a)
		Skin	7 days	50 ng 2,3,4,7,8-PeCDF/2 per wk/20 wk	Skin	+	
		Skin	7 days	100 ng 2,3,4,7,8-PeCDF/2 per wk/20 wk	Skin	+	
		Skin	7 days	250 ng 1,2,3,4,7,8-HxCDF/2 per wk/20 wk	Skin	+	
		Skin	7 days	500 ng 1,2,3,4,7,8-HxCDF/2 per wk/20 wk	Skin	+	
		Skin	7 days	1000 ng 1,2,3,4,7,8-HxCDF/2 per wk/20 wk	Skin	+	
Liver							
Wistar rats (M)	50 mg/L NDEA in drinking-water for 4 weeks	Oral	None	10 µg/kg bw 2,3,4,7,8-PeCDF per wk/16, 20 wk	s.c.	–	Nishizumi & Masuda (1986)
		Oral	None	10 µg/kg bw 2,3,4,7,8-PeCDF per wk/24 wk	s.c.	+	
		Oral	None	100 µg/kg bw 2,3,4,7,8-PeCDF per wk/16, 20, 24 wk	s.c.	+	
		Oral	None	10 µg/kg bw 1,2,3,4,7,8-HxCDF per wk/ 24 wk	s.c.	–	
		Oral	None	100 µg/kg bw 1,2,3,4,7,8-HxCDF per wk/ 24 wk	s.c.	+	
SD rat (F)	PH/30 mg/kg bw NDEA	i.p.	35 days	0.8 then 0.16 µg/kg bw 2,3,4,7,8-PeCDF per wk/20 wk	s.c.	–	Waern *et al.* (1991)
		i.p.	35 days	3.2 then 0.64 µg/kg bw 2,3,4,7,8-PeCDF per wk/20 wk	s.c.	+	
		i.p.	35 days	13 then 2.6 µg/kg bw 2,3,4,7,8-PeCDF per wk/20 wk	s.c.	+	

MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; NDEA, *N*-nitrosodiethylnitrosamine; i.p., intraperitoneal injection; PH, partial hepatectomy; F; female; M, male

3.1.2 *Rat liver*

2,3,4,7,8-Pentachlorodibenzofuran

Groups of 12 male Wistar rats, five weeks of age, were given 50 mg/L (ppm) *N*-nitrosodiethylamine (NDEA) in the drinking-water for four weeks. The rats were then given weekly subcutaneous injections of olive oil or 10 or 100 μg/kg bw 2,3,4,7,8-PeCDF for 16, 20 or 24 weeks. At the end of treatment, the animals were killed (four per treatment per time point) and the number and size of liver tumours (hepatocellular carcinomas and hyperplastic nodules) were assessed. The numbers of liver tumours per rat were significantly greater in the 2,3,4,7,8-PeCDF-NDEA animals (at 24 weeks, 10 μg 2,3,4,7,8-PeCDF + NDEA, 17/rat; 100 μg 2,3,4,7,8-PeCDF + NDEA, 24.3/rat) than in the rats treated with NDEA alone (at 24 weeks, 3/rat). The number of hepatocellular neoplasms was increased at the 16-week observation period in the 100 μg/kg 2,3,4,7,8-PeCDF/NDEA rats (3.3/rat) compared with those treated with NDEA alone (0.3/rat). The lesions were also larger in animals receiving the 2,3,4,7,8-PeCDF/NDEA combination treatment than in those treated with NDEA alone (Nishizumi & Masuda, 1986). [The Working Group noted that the number of animals with tumours was not given.]

Groups of 10 female Sprague-Dawley rats [age unspecified] were subjected to a 70% partial hepatectomy followed by administration of 30 mg/kg bw NDEA by intraperitoneal injection and treatment with corn oil vehicle or 2,3,4,7,8-PeCDF by subcutaneous injection starting five weeks later for 14 or 20 weeks. The 2,3,4,7,8-PeCDF was given as an initial loading dose (5 × maintenance dose) followed by weekly maintenance doses of 0.16, 0.64 and 2.6 μg/kg bw 2,3,4,7,8-PeCDF for 19 weeks. The rats were killed after 20 weeks of treatment with vehicle or 2,3,4,7,8-PeCDF and analysed for the presence of γ-glutamyltransferase-positive focal hepatic lesions. A significant increase in the percentage of liver occupied by these lesions was observed for all doses (approximately: low-dose, 0.25%; mid-dose, 0.5%; high-dose, 0.5%) compared with NDEA alone (0.15%). A significant increase in the number of foci per liver was also seen at the two highest doses (approximately: low-dose, 2500; mid-dose, 3500; high-dose, 4500) as compared to NDEA alone (2000) (Waern *et al.*, 1991).

1,2,3,4,7,8-Hexachlorodibenzofuran

Groups of 12 male Wistar rats, five weeks of age, were given 50 mg/L (ppm) NDEA in the drinking-water for four weeks. The rats were then given weekly subcutaneous injections of olive oil or 10 or 100 μg/kg bw 1,2,3,4,7,8-HxCDF for 16, 20 or 24 weeks. At the end of treatment, the animals were killed (four per treatment per time point) and the number and size of hepatocellular carcinomas and hyperplastic nodules were assessed. At 24 weeks, the highest dose of 1,2,3,4,7,8-HxCDF increased the number of liver tumours per rat (12/rat) as compared to NDEA alone (3/rat). No effect was seen at the low dose (2.3 tumours/rat) (Nishizumi & Masuda, 1986). [The Working Group noted that the number of animals with tumours was not given.]