3. Studies of Cancer in Experimental Animals

2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (2,3,7,8-TCDD)

Long-term carcinogenicity studies of 2,3,7,8-TCDD in experimental animals are summarized in **Table 40**.

3.1 Oral administration

3.1.1 *Mouse*

Groups of 45 male outbred Swiss/H/Riop mice, 10 weeks of age, were given gastric instillations of 0.007, 0.7 or 7.0 μg/kg bw 2,3,7,8-TCDD [purity unspecified] dissolved in sunflower oil once a week for one year. A control group received vehicle only. Animals were followed for the rest of their life span. Survival was significantly decreased in the high-dose animals (average life span, 424 days compared with 588 days in controls). It was reported that all organs [unspecified] were examined histologically. Treatment with 2,3,7,8-TCDD caused severe chronic, ulcerous skin lesions, followed by generalized lethal amyloidosis and was associated with an increased incidence of liver tumours (hepatocellular adenomas and carcinomas combined) [the two histological types were not reported separately]: control, 7/38; low-dose, 13/44; mid-dose, 21/44 ($p < 0.01$, χ^2 test) and high-dose, 13/43. No statistically significant increase in the incidence of lung tumours or lymphomas was observed (Toth *et al.*, 1979). [The Working Group noted that mortality-adjusted analysis was not performed and, therefore, the tumour incidence in the high-dose group may be underestimated.]

Groups of 50 male and 50 female B6C3F1 mice, six weeks of age, were given gastric instillations of 0.01, 0.05 or 0.5 μg/kg bw (males) and 0.04, 0.2 or 2.0 μg/kg bw (females) 2,3,7,8-TCDD (purity, 99.4%) in a vehicle of 9 : 1 corn oil–acetone twice a week for 104 weeks. One control group of 75 males and 75 females received vehicle alone and another group of 50 males and 50 females was untreated. Mean body weights of the treated groups were comparable with those of the vehicle-control group. Treatment did not affect survival: 30/50 untreated control, 38/74 vehicle-control, 30/50 low-dose, 31/50 mid-dose and 31/50 high-dose males were still alive at 105–107 weeks and 34/50 untreated control, 58/75 vehicle-control, 37/50 low-dose, 36/50 mid-dose and 32/50 high-dose females were still alive at 106–107 weeks. Treatment-related increases in

Table 40. Summary of long-term carcinogenicity studies on 2,3,7,8-TCDD in experimental animals

Species	Sex	Dose and route	Target organ	Tumour type	Lowest effective dose for significant increase in tumours	Reference
Mouse	M	0.007, 0.7, 7.0 µg/kg bw orally once a week for 1 year and observed for lifetime	Liver	Hepatocellular adenoma and carcinoma	0.7 µg/kg/bw	Toth *et al.* (1979)
	M	0.01, 0.05, 0.5 µg/kg bw orally twice a week, 104 weeks	Liver Lung	Hepatocellular carcinoma Alveolar/bronchiolar adenomas or carcinoma	Dose-related trend Dose-related trend	United States National Toxicology Program (1982a)
	F	0.04, 0.2, 2.0 µg/kg bw orally twice a week, 104 weeks	Liver Thyroid Haematopoietic system Skin	Hepatocellular carcinoma Follicle-cell adenoma Lymphoma Subcutaneous fibrosarcoma	Dose-related trend Dose-related trend Dose-related trend Dose-related trend	
	F	0.005 µg/animal, skin, 3 times per week for 104 weeks	Integumentary system	Fibrosarcoma	0.001 µg/animal	United States National Toxicology Program (1982b)
	M	2.5, 5.0 µg/kg bw orally once a week for 52 weeks, followed until 104 weeks of age	Liver	Hepatocellular carcinoma	2.5 µg/kg bw	Della Porta *et al.* (1987)
	F	2.5, 5.0 µg/kg bw orally once a week for 52 weeks, followed until 104 weeks of age	Liver	Hepatocellular carcinoma	2.5 µg/kg bw	

Table 40 (contd)

Species	Sex	Dose and route	Target organ	Tumour type	Lowest effective dose for significant increase in tumours	Reference
Mouse (immature)	M	1, 30, 60 µg/kg bw i.p. once a week for 5 weeks and observed until 78 weeks of age	Thymus Liver	Lymphoma Hepatocellular adenoma and carcinoma	Dose-related trend Dose-related trend	Della Porta *et al.* (1987) (contd)
	F	1, 30, 60 µg/kg bw i.p. once a week for 5 weeks and observed until 78 weeks of age	Thymus Liver	Lymphoma Hepatocellular adenoma and carcinoma	Dose-related trend Dose-related trend	
Rat	M	22, 210, 2200 ppt in diet, for 2 yrs (equiv. to 0.001, 0.01, 0.1 µg/kg bw)	Hard palate Nasal turbinates Tongue	Squamous-cell carcinoma Squamous-cell carcinoma Squamous-cell carcinoma	0.1 µg/kg bw 0.1 µg/kg bw 0.1 µg/kg bw	Kociba *et al.* (1978)
	F	22, 210, 2200 ppt in diet, for 2 yrs (equiv. to 0.001, 0.01, 0.1 µg/kg bw)	Liver Liver Hard palate Nasal turbinates Lung Tongue	Hyperplastic nodule Hepatocellular carcinoma Squamous-cell carcinoma Squamous-cell carcinoma Squamous-cell carcinoma Squamous-cell carcinoma	0.01 µg/kg bw 0.1 µg/kg bw 0.1 µg/kg bw 0.1 µg/kg bw 0.1 µg/kg bw 0.1 µg/kg bw	
	M	0.01, 0.05, 0.5 µg/kg bw orally, twice a week, 104 weeks	Thyroid Liver	Follicular-cell adenoma Neoplastic nodule	Dose-related trend Dose-related trend	United States National Toxicology Program (1982a)
	F	0.01, 0.05, 0.5 µg/kg bw orally, twice a week, 104 weeks	Thyroid Liver	Follicular-cell adenomas Neoplastic nodules	0.5 µg/kg bw 0.5 µg/kg bw	
Hamster	M	50, 100 µg/kg bw i.p. or s.c. 6 times at 4 wk intervals and observed for 1 year	Skin	Squamous-cell carcinomas	100 µg/kg bw	Rao *et al.* (1988)

i.p., intraperitoneally; s.c., subcutaneously

hepatotoxicity were found in high-dose animals of both sexes. The incidences of hepatocellular carcinoma were dose-related, with significantly higher incidence in the high-dose groups (males, $p = 0.002$; females, $p = 0.014$, Fisher's exact test) than in the vehicle-control groups (males: vehicle-control, 8/73; low-dose, 9/49; mid-dose, 8/49; and high-dose, 17/50; females: vehicle-control, 1/73; low-dose, 2/50; mid-dose, 2/48; and high-dose, 6/47; males, $p = 0.002$; females, $p = 0.008$ Cochran–Armitage test for trend). Dose-related increases in the incidence of follicular-cell adenomas of the thyroid were observed in female mice, with significantly higher incidence ($p = 0.009$) in the high-dose group than in the vehicle-control group (vehicle-control, 0/69; low-dose, 3/50; mid-dose, 1/47; and high-dose, 5/46; $p = 0.016$ for trend). In female mice, there was a significant increase in the incidence of lymphomas at the high dose (18/74 controls, 11/50 low-dose, 13/48 mid-dose and 20/47 high-dose; $p = 0.029$). There was also a significant increase in the incidence of subcutaneous fibrosarcomas in high-dose females (1/74 controls, 1/50 low-dose, 1/48 mid-dose and 5/47 high-dose; $p = 0.032$). A dose-related increase ($p = 0.04$, Cochran–Armitage test for trend) in lung tumours (alveolar/bronchiolar adenomas or carcinomas) was observed in male mice (10/71 controls, 2/48 low-dose, 4/48 mid-dose and 13/50 high-dose) (United States National Toxicology Program, 1982a).

Groups of 45–55 male and female (C57BL/6J × C3Hf)F1 mice, six weeks of age, were given gastric instillations of 0, 2.5 or 5.0 μg/kg bw 2,3,7,8-TCDD [laboratory grade; purity unspecified] in 0.01 mL/kg bw corn oil vehicle (containing 1.2% acetone) once a week for 52 weeks. At 31–39 weeks of age, 41 males and 32 females in the 2.5-μg/kg bw group were erroneously treated once with a dose of 25 μg/kg bw 2,3,7,8-TCDD. The treatment of these mice was interrupted for five weeks and then continued until week 57, as for the other treated mice. At the end of treatment, all groups were kept under observation until 110 weeks of age, when all survivors were killed. Histopathological examination was carried out on Harderian glands, pituitary, thyroid, tongue, oesophagus, trachea, lungs, liver, pancreas, mesenteric lymph nodes, small intestine, spleen, kidney, adrenal glands, testis or ovaries, uterus, urinary bladder and all other organs with apparent or suspected pathological alterations. Treatment with 2,3,7,8-TCDD at both dose levels caused a marked depression in mean body weight in both males and females. Survival was significantly reduced in male and female 2,3,7,8-TCDD-treated mice ($p < 0.001$). Long-term administration of 2,3,7,8-TCDD increased the incidence of some non-neoplastic lesions, including liver necrosis, amyloidosis of multiple tissues and nephrosclerosis. Dermatitis developed in most 2,3,7,8-TCDD-treated mice and regressed after cessation of treatment. Tumour incidence was evaluated statistically adjusting for intercurrent mortality. [The authors report that, due to the toxicity of the treatment, it was not possible to distinguish between fatal and incidental tumours and both statistical methods were used.] Hepatocellular adenomas occurred in 10/45 control, 11/51 low-dose and 10/50 high-dose males ($p > 0.05$, incidental tumour test). However, when fatal tumours were considered, there was a significant increase ($p < 0.001$, fatal tumour test). In females, the incidence of hepatocellular adenomas was 2/49 control, 4/42 low-dose and 11/48 high-dose animals ($p < 0.01$, fatal tumour test; $p < 0.001$, incidental tumour test). The incidences of hepatocellular carcinomas were: 5/43 control, 15/51 low-dose and 33/50 high-dose males ($p < 0.001$ for both fatal and incidental tumour tests); and

1/49 control, 12/42 low-dose and 9/48 high-dose females ($p < 0.01$, fatal tumour test; $p < 0.05$, incidental tumour test). The incidence of any other tumour type was not associated with treatment (Della Porta *et al.*, 1987).

3.1.2 *Rat*

Groups of 10 male Sprague-Dawley rats, weighing approximately 60 g, were fed diets containing 1, 5, 50, 500 ng/kg diet (parts per trillion; ppt), 1, 5, 50, 500 or 1000 µg/kg diet (parts per billion; ppb) 2,3,7,8-TCDD [purity unspecified]. One control group was maintained on basal diet. Animals were maintained on the diets for 78 weeks, at which time the treated animals were changed to the basal diet. At 65 weeks of the experiment, laparotomies were performed on all surviving animals and at 95 weeks all surviving animals were killed. All animals treated with the three highest doses (50, 500 and 1000 ppb) died between the second and fourth week of the experiment. At the end of the experiment, four, eight, six, six and five, zero and zero animals were alive in the control, 1-, 5-, 50- and 500-ppt and 1- and 5-ppb groups, respectively. Various types of neoplasm were observed in the treated rats and none in the control rats. The numbers of animals with neoplasms were zero, zero, five, three, four, four and seven in the control, 1-, 5-, 50- and 500-ppt and 1- and 5-ppb groups, respectively. In the 5-ppb group, four squamous-cell tumours of the lung, four neoplastic nodules of the liver and two cholangiocarcinomas of the liver were observed. One cholangiocarcinoma was observed in the 1-ppb group (Van Miller *et al.*, 1977). [The Working Group noted the small number of animals per group, but that cholangiocarcinomas are relatively rare in rats and may be related to treatment.]

Groups of 50 male and 50 female Sprague-Dawley rats, six to seven weeks of age, were fed 22, 210 or 2200 ppt (ng/kg) 2,3,7,8-TCDD (purity, > 99%) in the diet, corresponding to 0.001, 0.01 or 0.1 µg/kg bw daily for two years. Control groups of 86 male and 86 female rats received a basal diet containing the vehicle (acetone) only. Terminal necropsy was performed at 105 weeks. Representative portions of most organs and any additional gross lesions were preserved in formalin fixative. Histological examination was conducted on an extensive list of tissues from control and high-dose rats. All rats in the low- and mid-dose groups were subjected to histological examination of tissues identified as possible target organs and all gross lesions. Reduced survival was observed in high-dose females and in mid- and high-dose males. Mean body weights of high-dose males and mid- and high-dose females were below those of the control animals throughout the major portion of the study [details not reported]. Non-neoplastic, treatment-related pathological changes were reported, especially in the liver. High- and mid-dose rats had multiple hepatocellular necrosis and inflammatory changes. In males, the incidences of hepatocellular hyperplastic nodules (6/85 controls, 0/50 low-dose, 3/50 mid-dose and 2/50 high-dose) and hepatocellular carcinomas (2/85 controls, 0/50 low-dose, 0/50 mid-dose and 1/50 high-dose) were not increased. In female rats, the incidence of hepatocellular hyperplastic nodules was 8/86 control, 3/50 low-dose, 18/50 mid-dose and 23/49 high-dose animals ($p < 0.05$, Fisher's exact test) and that of hepatocellular carcinomas was 1/86 control, 0/50 low-dose, 2/50 mid-dose and 11/49 high-dose animals

($p < 0.05$). The lipid concentrations of 2,3,7,8-TCDD in the low-, mid- and high-dose rats were 540, 1700 and 8100 ng/kg. Squamous-cell carcinomas of the hard palate or nasal turbinates occurred in 4/49 ($p < 0.05$) high-dose and 1/50 mid-dose females, squamous-cell carcinomas of the lung were observed in 7/49 ($p < 0.05$) high-dose females and squamous-cell carcinomas of the tongue occurred in 2/49 high-dose females; tumours at these sites were not observed in the other groups. In high-dose males, 4/50 rats developed squamous-cell carcinomas of the hard palate or nasal turbinates ($p < 0.05$), one developed a squamous-cell carcinoma of the lung and three squamous-cell carcinomas of the tongue ($p < 0.05$). In comparison with the high incidence of endocrine-related tumours (pituitary adenomas, phaeochromocytomas and pancreatic islet-cell tumours) in controls, reduced incidences were observed in the high-dose group (Kociba *et al.*, 1978).

A re-evaluation of the slides of liver specimens from the female animals studied by Kociba *et al.* (1978) was performed by a panel of pathologists (Keenan *et al.*, 1991). In contrast to the original findings, they found about two-thirds fewer tumours present in the livers of female Sprague-Dawley rats. On the basis of their results, they established a no-observed-adverse-effect level for hepatocellular carcinomas of 0.01 μg/kg bw per day 2,3,7,8-TCDD. [The Working Group noted that the results of this re-evaluation do not change substantially the positive findings of Kociba *et al.* (1978) on the liver carcinogenicity of 2,3,7,8-TCDD in female rats.]

Liver slides from the study by Kociba *et al.* (1978) were further reviewed by a pathology working group with the aim of evaluating proliferative lesions in the livers of female rats. The results of the working group substantially confirmed the previous results on dose-related increased tumour incidence (hepatocellular adenomas: 2/81 control, 1/50 low-dose, 9/50 mide-dose and 14/45 high-dose; hepatocellular carcinomas: 0/86, 0/50, 0/50 and 4/45). There was a dose-related increase in the incidence of hepatic eosinophilic foci (31/86 control, 23/50 low-dose, 37/50 mid-dose and 40/45 high-dose) (Goodman & Sauer, 1992).

Groups of 50 male and 50 female Osborne-Mendel rats, six weeks of age, were given gastric instillations of 0.01, 0.05 or 0.5 μg/kg bw 2,3,7,8-TCDD (purity, > 99.4%) in a vehicle of 9 : 1 corn oil–acetone twice a week for 104 weeks. One control group of 75 male and 75 female rats received vehicle alone and another control group of 50 males and 50 females was untreated. Mean body weights of the high-dose groups were lower than those of the corresponding controls after week 55 in males and after week 45 in females. Treatment did not affect survival: 23/50 untreated control, 29/75 vehicle-control, 17/50 low-dose, 20/50 mid-dose and 19/50 high-dose males were alive at the end of the experiment at 105–108 weeks; 29/50 untreated control, 39/75 vehicle-treated control, 29/50 low-dose, 34/50 mid-dose and 32/50 high-dose females were alive at the end of the experiment at 105–107 weeks. Treatment-related increased hepatotoxicity was observed in high-dose males and females. Treatment-related increased incidences of follicular-cell adenomas of the thyroid were seen in males and were significantly higher ($p = 0.001$; Fisher's exact test) in the high-dose group than in the vehicle controls (1/69 vehicle-control, 5/48 low-dose, 6/50 mid-dose and 10/50 high-dose; $p = 0.006$, Cochran–

Armitage test for trend) and in high-dose female rats (3/73 vehicle-control, 2/45 low-dose, 1/49 mid-dose and 6/47 high-dose; $p = 0.022$, Cochran–Armitage test for trend). The incidence of neoplastic nodules of the liver was significantly higher ($p = 0.006$) in high-dose females than in vehicle-control females (5/75 vehicle-control, 1/49 low-dose, 3/50 mid-dose and 12/49 high-dose). In males, a dose-related positive trend ($p = 0.005$) was seen (0/74, 0/50, 0/50 and 3/50, respectively) (United States National Toxicology Program, 1982a).

3.2 Administration to immature animals

Mouse: Groups of 89–186 male and female (C57BL/6J × C3Hf)F1 (B6C3) and (C57BL/6J × BALB/c)F1 (B6C) mice, 10 days old, were given five weekly intraperitoneal injections of 0, 1, 30 or 60 μg/kg bw 2,3,7,8-TCDD [laboratory grade; purity unspecified] in 0.01 mL/g bw corn oil vehicle (containing 1.2% acetone). Treatment-related mortality was high, especially with the mid and high doses. In both hybrids, 2–6% of vehicle-control and low-dose mice died between the end of treatment and week 13 of age, compared with 12–20% of mid-dose and 26–30% of high-dose mice. A mean body weight depression of 5% was observed throughout the experiment in mid- and high-dose B6C3 and B6C males compared with the control groups. Animals received no further treatment until 78 weeks of age, when all survivors (73–100% of males and 81–100% of females) were killed. Gross examination was performed on all animals that died or were killed. Histopathological examination was carried out on the liver, kidney and any other organ with apparent or suspected pathological alterations. The incidences of thymic lymphomas were: 0/45 control, 0/55 low-dose, 1/52 mid-dose and 2/43 high-dose B6C3 males; 0/42 control, 0/57 low-dose, 0/48 mid-dose and 5/57 high-dose B6C3 females; 0/32 control, 0/54 low-dose, 2/27 mid-dose and 2/30 high-dose B6C males; and 0/48 control, 0/57 low-dose, 1/39 mid-dose and 2/38 high-dose B6C females. Thymic lymphomas developed between 16 and 41 weeks of age and most of them (11/15) were seen within 26 weeks of age. The χ^2 test for trend was significant ($p < 0.05$) in all four groups (male and female B6C3 and B6C mice). The authors considered the development of thymic lymphomas to be treatment-related due to the rarity of this tumour type in untreated groups of B6C3 and B6C mice. The incidences of hepatocellular adenomas were: 6/45 control, 5/55 low-dose, 5/52 mid-dose and 11/43 high-dose B6C3 males ($p = 0.043$, χ^2 test for trend); and 0/42 control, 1/57 low-dose, 1/48 mid-dose and 5/57 high-dose B6C3 females ($p = 0.014$, χ^2 test for trend). The incidences of hepatocellular carcinomas were: 3/45 control, 1/55 low-dose, 9/52 mid-dose and 14/43 high-dose B6C3 males ($p < 0.001$, χ^2 test for trend; $p = 0.002$, Fisher's exact test, high-dose compared with control); and in 0/42 control, 0/57 low-dose, 1/48 mid-dose and 1/57 high-dose B6C3 females. Hepatocellular carcinomas were not observed in B6C mice of either sex (Della Porta *et al.*, 1987).

3.3 Intraperitoneal or subcutaneous administration

Hamster: Groups of 10–24 male Syrian golden hamsters, weighing 65–80 g [age unspecified] received six intraperitoneal or subcutaneous injections of 50 or 100 μg/kg

bw 2,3,7,8-TCDD [purity unspecified] in dioxane or dioxane alone at four-week intervals. A further group received only two intraperitoneal injections of 100 μg/kg bw 2,3,7,8-TCDD. Animals were observed until 12–13 months after the beginning of treatment. Complete necropsies were performed on all animals, and tissues were fixed in formalin and examined microscopically. Toxicity and early mortality were observed in the high-dose groups. Squamous-cell carcinomas of the facial skin developed in 4/18 intraperitoneally high-dosed animals and 3/14 subcutaneously high-dosed animals. No animal in the control groups, the low-dose groups or the groups receiving two intraperitoneal injections of the high dose developed tumours at any site (Rao *et al.*, 1988). [The Working Group noted the small number of animals per group.]

3.4 Skin application

Mouse: Groups of 30 male and 30 female Swiss-Webster mice (six weeks old) received 2,3,7,8-TCDD (purity, 99.4%) on the clipped back at doses of 0.001 μg/animal (males) and 0.005 μg/animal (females), suspended in 0.1 mL acetone, on three days per week for 99 (males) or 104 weeks (females). As vehicle controls, 45 mice of each sex received 0.1 mL acetone three times per week. Thirty animals of each sex served as untreated controls. Mean body weights were similar in the 2,3,7,8-TCDD- and vehicle-treated groups, but untreated controls of both sexes had slightly higher body weights. Survival in both sexes was decreased by 2,3,7,8-TCDD treatment (p = 0.005 and p = 0.031 in males and females, respectively, Cox's test). Among males, 27/30, 33/45 and 27/30 untreated control, vehicle-control and 2,3,7,8-TCDD-treated mice, respectively, were alive at week 52 of the study; 28/30, 43/45 and 29/30 untreated control, vehicle-control and 2,3,7,8-TCDD-treated females, respectively, were alive at week 52 of the study. In males, the incidence of fibrosarcomas of the integumentary system was 3/42 and 6/28 in the vehicle-control and 2,3,7,8-TCDD-treated mice, respectively (not a statistically significant difference). In female mice, the incidence of fibrosarcomas of the integumentary system was significantly higher in animals treated with 2,3,7,8-TCDD (8/27) compared with vehicle-controls (2/41; p = 0.007, Fisher's exact test) (United States National Toxicology Program, 1982b).

3.5 Exposure by immersion in water

Fish: Preliminary results of a study in progress reported in an abstract indicate that exposure of medaka fish [*Oryzias latipes*] to 2,3,7,8-TCDD producing a body concentration of 2 μg/kg was associated with tumours at several sites — gills, thyroid and swim bladder (Johnson *et al.*, 1992b).

3.6 Administration with known carcinogens and modifying factors

For an overview of studies on administration of 2,3,7,8-TCDD and related PCDDs with known carcinogens, see **Table 41**. [The Working Group noted that the experimental approach used in these studies is frequently termed a tumour promotion protocol. This terminology is used in Section 4.6.2 of the present monograph.]

Table 41. Studies on 2,3,7,8-TCDD and related PCDDs administered with known carcinogens and modifying factors

Tumour type Strain/species (sex)	Known carcinogen	Route of administration	Interval	Dose and frequency of PCDD (times per week/number of weeks)	Route of administration	Enhancement[a]	Reference
Skin							
CD1 mice (F)	200 nmol DMBA	Skin	1 week	0.1 µg 2,3,7,8-TCDD/2 per wk/30 wk	Skin	–	Berry *et al.* (1978)
HRS/J haired mice (*hr/+*) (F)	200 nmol DMBA	Skin	None	20 ng 2,3,7,8-TCDD/2 per wk/8 wk then 50 ng/17 wk	Skin	–	Poland *et al.* (1982)
HRS/J hairless mice (*hr/hr*) (F)	200 nmol DMBA	Skin	None	20 ng 2,3,7,8-TCDD/2 per wk/8 wk then 50 ng/17 wk	Skin	+	
HRS/J hairless mice (*hr/hr*) (F)	5 µmol MNNG	Skin	None	3.75 ng 2,3,7,8-TCDD/2 per wk/20 wk	Skin	+	
	5 µmol MNNG	Skin	None	7.5 ng 2,3,7,8-TCDD/2 per wk/20 wk	Skin	+	
	5 µmol MNNG	Skin	None	15 ng 2,3,7,8-TCDD/2 per wk/20 wk	Skin	+	
	5 µmol MNNG	Skin	None	30 ng 2,3,7,8-TCDD/2 per wk/20 wk	Skin	+	
	5 µmol MNNG	Skin	None	50 ng 2,3,7,8-TCDD/5 wk/ then 20 ng/15 wk	Skin	+	
	5 µmol MNNG	Skin	None	20 µg 2,7-DCDD/2 per wk/20 wk	Skin	–	
HRS/J hairless mice (*hr/hr*) (F)	5 µmol MNNG	Skin	7 days	2.5 ng 2,3,7,8-TCDD/2 per wk/20 wk	Skin	+	Hébert *et al.* (1990a)
	5 µmol MNNG	Skin	7 days	5 ng 2,3,7,8-TCDD/2 per wk/20 wk	Skin	+	
	5 µmol MNNG	Skin	7 days	10 ng 2,3,7,8-TCDD/2 per wk/20 wk	Skin	+	
Lung							
Swiss mice (M)	25 mg/kg bw NDMA	i.p.	3 weeks	1 × 1.6 µg/kg bw 2,3,7,8-TCDD	i.p.	+	Beebe *et al.* (1995a)
	25 mg/kg bw NDMA	i.p.	3 weeks	1 × 16 or 48 µg/kg bw 2,3,7,8-TCDD	i.p.	– (toxic)	
	25 mg/kg bw NDMA	i.p.	3 weeks	20 × 0.05 µg/kg bw 2,3,7,8-TCDD	i.p.	–	
C57BL/6 mice (M)	90 mg/kg bw NDEA	i.p.	3 weeks	0.05 µg/kg bw 2,3,7,8-TCDD/weekly/20 wk	i.p.	–	Beebe *et al.* (1995b)
B6D2F1 mice (M)	90 mg/kg bw NDEA	i.p.	3 weeks	0.05 µg/kg bw 2,3,7,8-TCDD/weekly/20 wk	i.p.	–	
DBA/2 mice (M)	90 mg/kg bw NDEA	i.p.	3 weeks	0.05 µg/kg bw 2,3,7,8-TCDD/weekly/20 wk	i.p.	–	
SD rats (F)	200 mg/kg bw NDEA	i.p.	10 days	1.4 µg/kg bw 2,3,7,8-TCDD/biweekly/60 wks	Oral	–	Clark *et al.* (1991)
SD rats (F) (ovariectomized)	200 mg/kg bw NDEA	i.p.	10 days	1.4 µg/kg bw 2,3,7,8-TCDD/biweekly/60 wks	Oral	+	

Table 41 (contd)

Strain/species (sex)	Known carcinogen	Route of administration	Interval	Dose and frequency of PCDD	Route of administration	Enhancement[a]	Reference
Liver							
C57BL/6 mouse	90 mg/kg bw NDEA	i.p.	3 weeks	0.05 µg/kg bw 2,3,7,8-TCDD/weekly/20 wk	i.p.	–	Beebe *et al.*
B6D2F1 mouse	90 mg/kg bw NDEA	i.p.	3 weeks	0.05 µg/kg bw 2,3,7,8-TCDD/weekly/20 wk	i.p.	+	(1995b)
DBA/2 mouse	90 mg/kg bw NDEA	i.p.	3 weeks	0.05 µg/kg bw 2,3,7,8-TCDD/weekly/20 wk	i.p.	–	
SD rats (F)	PH/10 mg/kg bw NDEA	oral	7 days	0.14 µg/kg bw 2,3,7,8-TCDD/biweekly/7 mo	i.m.	–	Pitot *et al.*
	PH/10 mg/kg bw NDEA	oral	7 days	1.4 µg/kg bw 2,3,7,8-TCDD/biweekly/7 mo	i.m.	+	(1980)
Fischer 344 rats (F)	PH/ 10 mg/kg bw NDEA	oral	14 days	0.0014 mg/kg bw 2,3,7,8-TCDD/biwekkly/6 mo	i.m.	–	Pitot *et al.*
	PH/ 10 mg/kg bw NDEA	oral	14 days	0.014 µg/kg bw 2,3,7,8-TCDD/biweekly/6 mo	i.m.	–	(1987)
	PH/ 10 mg/kg bw NDEA	oral	14 days	0.14 µg/kg bw 2,3,7,8-TCDD/biweekly/6 mo	i.m.	–	
	PH/ 10 mg/kg bw NDEA	oral	14 days	1.4 µg/kg bw 2,3,7,8-TCDD/biweekly/6 mo	i.m.	+	
SD rats (F)	PH/30 mg/kg bw NDEA	i.p.	7 days	0.7 µg/kg bw 2,3,7,8-TCDD/weekly/14 wk	s.c.	–	Flodström &
	PH/30 mg/kg bw NDEA	i.p.	7 days	0.7 µg/kg bw 2,3,7,8-TCDD/weekly/26 wk	s.c.	+	Ahlborg
	PH/30 mg/kg bw NDEA	i.p.	35 days	3.5 then 0.7 µg/kg bw 2,3,7,8-TCDD/weekly/9 wk	s.c.	+	(1989);
	PH/30 mg/kg bw NDEA	i.p.	35 days	3.5 then 0.7 µg/kg bw 2,3,7,8-TCDD/weekly/21 wk	s.c.	+	Flodström
	PH/30 mg/kg bw NDEA	i.p.	35 days	0.35 then 0.07 µg/kg bw 2,3,7,8-TCDD/weekly/15 wk (normal vitamin A)	s.c.	–	*et al.* (1991)
	PH/30 mg/kg bw NDEA	i.p.	35 days	0.35 then 0.07 µg/kg bw 2,3,7,8-TCDD/weekly/15 wk (marginal or low vitamin A)	s.c.	+	
	PH/30 mg/kg bw NDEA	i.p.	35 days	3.5 then 0.7 µg/kg bw 2,3,7,8-TCDD/weekly/15 wk (normal, marginal, low vitamin A)	s.c.	+	
Fischer 344 rats (F)	PH/10 mg/kg bw NDEA	oral	2 weeks	0.14 µg/kg bw 2,3,7,8-TCDD/biweekly/6 mo	s.c.	+	Dragan *et al.* (1991)
SD rats (F)	PH/30 mg/kg bw NDEA	i.p.	35 days	0.44 then 0.088 µg/kg bw 1,2,3,7,8-PeCDD/weekly/ 20 wk	s.c.	+	Waern *et al.*
	PH/30 mg/kg bw NDEA	i.p.	35 days	1.75 then 0.35 µg/kg bw 1,2,3,7,8-PeCDD/weekly/20 wk	s.c.	+	(1991)
	PH/30 mg/kg bw NDEA	i.p.	35 days	7 then 1.4 µg/kg bw 1,2,3,7,8-PeCDD/weekly/20 wk	s.c.	+	
	PH/30 mg/kg bw NDEA	i.p.	35 days	0.22 then 0.044 µg/kg bw 2,3,7,8-TCDD/weekly/20 wk	s.c.	+	
	PH/30 mg/kg bw NDEA	i.p.	35 days	0.88 then 0.175 µg/kg bw 2,3,7,8-TCDD/weekly/20 wk	s.c.	+	
	PH/30 mg/kg bw NDEA	i.p.	35 days	3.5 then 0.7 µg/kg bw 2,3,7,8-TCDD/weekly/20 wk	s.c.	+	

Table 41 (contd)

Strain/species (sex)	Known carcinogen	Route of administration	Interval	Dose and frequency of PCDD	Route of administration	Enhancement[a]	Reference
Liver (contd)							
SD rats (F)	200 mg/kg bw NDEA	i.p.	7 days	1.4 µg/kg bw 2,3,7,8-TCDD/biweekly/30 wk	Oral	+	Lucier *et al.* (1991)
SD rats (F) (overiectomized)	200 mg/kg bw NDEA	i.p.	7 days	1.4 µg/kg bw 2,3,7,8-TCDD/biweekly/30 wk	Oral	+	
SD rats (F)	PH/10 mg/kg bw NDEA	oral	7 days	0.14 µg/kg bw 2,3,7,8-TCDD/biweekly/1 mo	i.p.	+	Dragan *et al.* (1992)
	PH/10 mg/kg bw NDEA	oral	7 days	0.14 µg/kg bw 2,3,7,8-TCDD/biweekly/3 mo	i.p.	+	
	PH/10 mg/kg bw NDEA	oral	7 days	0.14 µg/kg bw 2,3,7,8-TCDD/biweekly/5 mo	i.p.	+	
SD rats (F)	175 mg/kg bw NDEA	i.p.	14 days	3.5 ng/kg bw 2,3,7,8-TCDD/biweekly/30 wk	Oral	–	Maronpot *et al.* (1993)
	175 mg/kg bw NDEA	i.p.	14 days	10.7 ng/kg bw 2,3,7,8-TCDD/biweekly/30 wk	Oral	–	
	175 mg/kg bw NDEA	i.p.	14 days	35.7 ng/kg bw 2,3,7,8-TCDD/biweekly/30 wk	Oral	–	
	175 mg/kg bw NDEA	i.p.	14 days	125 ng/kg bw 2,3,7,8-TCDD/biweekly/30 wk	Oral	+	
Wistar rats (F)	5 × 10 mg/kg bw NDEA	oral	14 days	1.4 µg/kg bw 2,3,7,8-TCDD/biweekly/9 wk	s.c.	+	Buchmann *et al.* (1994)
	5 × 10 mg/kg bw NDEA	oral	14 days	1.4 µg/kg bw 2,3,7,8-TCDD/biweekly/13 wk	s.c.	+	
	5 × 10 mg/kg bw NDEA	oral	14 days	1.4 µg/kg bw 2,3,7,8-TCDD/biweekly/17 wk	s.c.	+	
	5 × 10 mg/kg bw NDEA	oral	14 days	70 µg/kg bw HpCDD/biweekly/9 wk	s.c.	+	
	5 × 10 mg/kg bw NDEA	oral	14 days	70 µg/kg bw HpCDD/biweekly/13 wk	s.c.	+	
	5 × 10 mg/kg bw NDEA	oral	14 days	70 µg/kg bw HpCDD/biweekly/17 wk	s.c.	+	
Wistar rats (F)	80 mg/L *N*-nitroso-morpholine in drinking-water for 25 days	oral	14 days	2 ng/kg bw 2,3,7,8-TCDD/daily/13 wk	s.c.	+	Schrenk *et al.* (1994a)
		oral	14 days	20 ng/kg bw 2,3,7,8-TCDD/daily/13 wk	s.c.	+	
		oral	14 days	200 ng/kg bw 2,3,7,8-TCDD/daily/13 wk	s.c.	+	
		oral	14 days	100 ng/kg bw HpCDD/daily/13 wk	s.c.	–	
		oral	14 days	1000 ng/kg bw HpCDD/daily/13 wk	s.c.	–	
		oral	14 days	10 000 ng/kg bw HpCDD/daily/13 wk	s.c.	+	
		oral	14 days	200 ng/kg bw PCDD mixture/daily/13 wk	s.c.	+	
		oral	14 days	2000 ng/kg bw PCDD mixture/daily/13 wk	s.c.	+	
		oral	14 days	20 000 ng/kg bw PCDD mixture/daily/13 wk	s.c.	+	
SD rats (F)	10 mg/kg bw NDEA	i.p.	30 days	0.007 µg/kg bw 2,3,7,8-TCDD/day (150 ppt in diet) until day 450	Oral	+	Sills *et al.* (1994)
	10 mg/kg bw NDEA	i.p.	170 days	0.007 µg/kg bw 2,3,7,8-TCDD/day (150 ppt in diet) until day 450	Oral	+	
	10 mg/kg bw NDEA	i.p.	240 days	0.007 µg/kg bw 2,3,7,8-TCDD/day (150 ppt in diet) until day 450	Oral	+	

Table 41 (contd)

Strain/species (sex)	Known carcinogen	Route of administration	Interval	Dose and frequency of PCDD	Route of administration	Enhancement[a]	Reference
Liver (contd)							
SD rats (F)	PH/30 mg/kg bw NDEA	i.p.	35 days	0.5 then 0.1 μg/kg bw 2,3,7,8-TCDD/weekly/20 wk	s.c.	–	Hemming
	PH/30 mg/kg bw NDEA	i.p.	35 days	1.58 then 0.316 μg/kg bw 2,3,7,8-TCDD/weekly/20 wk	s.c.	–	*et al.* (1995)
	PH/30 mg/kg bw NDEA	i.p.	35 days	5 then 1 μg/kg bw 2,3,7,8-TCDD/weekly/20 wk	s.c.	+	
Wistar rats (F)	10 × 10 mg/kg bw NDEA	oral	56 days	1.4 μg/kg bw 2,3,7,8-TCDD once	s.c.	–	Stinchcombe
	10 × 10 mg/kg bw NDEA	oral	56 days	1.4 μg/kg bw 2,3,7,8-TCDD/biweekly/16 wk	s.c.	+	*et al.* (1995)
SD rats (F)	175 mg/kg bw NDEA	i.p.	14 days	1.75 μg/kg bw 2,3,7,8-TCDD/biweekly/30 wk	Oral	+	Tritscher *et al.* (1995)

DMBA, 7,12-dimethylbenz[*a*]anthracene; MNNG, *N*-methyl-*N* -nitro-*N*-nitrosoguanidine; NDMA, *N*-nitrosodimethylamine; NDEA, *N*-nitrosodiethylamine; SD, Sprague-Dawley; PH, partial hepatectomy; F, female; M, male; i.p., intraperitoneal injection; s.c., subcutaneous injection; i.m., intamuscular injection

[a]Enhancement corresponds to promotion as used in Section 4.6.2.

3.6.1 *Skin*

Mouse: Groups of 30 female CD1 mice, seven to nine weeks of age, received skin applications of 2 μg 2,3,7,8-TCDD or 2.56 μg 7,12-dimethylbenz[*a*]anthracene (DMBA) per animal or both chemicals together in 0.2 mL acetone solvent. Starting one week later, the mice received thrice weekly applications of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (5 μg/animal) in 0.2 mL acetone for 32 weeks. The 2,3,7,8-TCDD/TPA-treated mice had 0.1 papillomas/mouse (14% incidence), the 2,3,7,8-TCDD + DMBA/TPA-treated mice had 2.2 papillomas/mouse (63% incidence) and the DMBA/TPA-treated mice had approximately 1.8 papillomas/mouse (40% incidence) (DiGiovanni *et al.*, 1977). [The Working Group noted that adequate control groups were not available, precluding an evaluation.]

Three groups of 30 female CD1 mice, six to eight weeks of age, received single skin applications of 0.2 μmol/animal (60 μg) DMBA in 0.2 mL acetone or solvent alone. Starting one week later, mice were treated with an acetone solution of 0.1 μg 2,3,7,8-TCDD or 2 μg TPA per animal (positive control) or acetone solvent alone twice weekly for 30 weeks, at which time the experiment was terminated. The percentages of mice with skin papillomas were DMBA/2,3,7,8-TCDD, 0%; DMBA/TPA, 92% and acetone/-TPA, 0% (Berry *et al.*, 1978).

In groups of female HRS/J haired (*hr/+*) mice given single skin applications of DMBA (0.2 μmol in acetone) followed by twice weekly applications of 20 ng/animal 2,3,7,8-TCDD for eight weeks then 50 ng/animal for 17 weeks, no skin tumours were found. In contrast, in HRS/J hairless (*hr/hr*) mice treated with 0.2 μmol/animal DMBA and the same regimen of 2,3,7,8-TCDD (25 weeks), 15/19 surviving mice developed skin tumours (1.4 tumours per mouse). In hairless animals treated with DMBA alone, one skin papilloma was found and, in the absence of DMBA, 2,3,7,8-TCDD produced no skin tumour (Poland *et al.*, 1982).

In a further experiment, groups of 20 female HRS/J hairless (*hr/hr*) mice, seven weeks of age, were given single skin applications of 5 μmol (0.75 mg)/animal *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) in 50 μL acetone followed by twice weekly doses of 0 or 3.75–30 ng/animal 2,3,7,8-TCDD or 1 μg or 3 μg/animal TPA as positive controls. The numbers of mice with skin tumours at 20 weeks are shown in **Table 42** (Poland *et al.*, 1982).

Groups of 26 female HRS/J hairless (*hr/hr*) mice, eight weeks of age, were given single skin applications of 0 or 5 μmol/animal MNNG in 50 μL acetone followed by 50 ng/animal 2,3,7,8-TCDD for five weeks then 20 ng/animal for 15 weeks, both in 50 μL acetone twice weekly during the 20-week period. Skin tumours developed in 16/19 (1.6 tumours per mouse) mice treated with MNNG plus 2,3,7,8-TCDD compared with 0/18 in mice treated with 2,3,7,8-TCDD alone (Poland *et al.*, 1982).

Three groups of 20 female HRS/J hairless (*hr/hr*) mice, five to eight weeks of age, were given single skin applications of 5 μmol/animal MNNG in 50 μL acetone. Seven days later, the mice were treated with 2.5, 5 or 10 ng/animal 2,3,7,8-TCDD in 25 μL acetone twice weekly for 20 weeks. A control group of 20 mice received acetone

followed by 10 ng/animal 2,3,7,8-TCDD. The numbers of surviving mice with papillomas, carcinomas or hyperproliferative nodules of the skin were 8/20, 8/19 and 7/18 after treatment with MNNG and 2.5, 5 or 10 ng/animal 2,3,7,8-TCDD compared with 0/18 mice treated with 2,3,7,8-TCDD alone (Hébert *et al.*, 1990a).

Table 42. Induction of skin tumours in female HRS/J hairless mice

Initiation	Promotion		Tumour incidence (surviving mice with tumours/ surviving mice)	Tumour multiplicity (average no. of papillomas/ surviving mice)
MNNG	Acetone		1/19	0.05
MNNG	TCDD	3.75 ng	11/20	0.7
MNNG	TCDD	7.5 ng	13/17	1.5
MNNG	TCDD	15 ng	10/10	4.0
MNNG	TCDD	30 ng	15/19	1.6
Acetone	TCDD	30 ng	0/19	0
MNNG	TPA	1 μg	5/19	0.4
MNNG	TPA	3 μg	13/18	1.6

From Poland *et al.* (1982)

In two experiments with female Sencar mice, it was shown that prior skin application of 2,3,7,8-TCDD reduced tumorigenesis of DMBA (or monofluoro derivatives of DMBA) and benzo[*a*]pyrene (Cohen *et al.*, 1979; DiGiovanni *et al.*, 1983).

3.6.2 *Lung*

(*a*) *Mouse*

Groups of male Swiss mice [initial numbers unspecified], five weeks of age, were given a single intraperitoneal injection of 25 mg/kg bw *N*-nitrosodimethylamine (NDMA) in saline. Three weeks later, the mice were given either single intraperitoneal injections of 1.6, 16 or 48 μg/kg bw 2,3,7,8-TCDD in olive oil, weekly doses of 0.05 μg/kg bw 2,3,7,8-TCDD weekly for 20 weeks or olive oil alone. All mice were killed after 52 weeks. Alveolar-cell adenomas and carcinomas were found in 100% of mice in all treatment groups (NDMA/olive oil, 24/24; NDMA/2,3,7,8-TCDD (repeated 0.05 μg/kg bw/week), 30/30; NDMA/2,3,7,8-TCDD (1.6 μg/kg bw), 15/15; NDMA/-2,3,7,8-TCDD (16 μg/kg bw), 19/19; NDMA/2,3,7,8-TCDD (48 μg/kg bw), 11/11). However, tumour multiplicity was significantly increased in mice receiving weekly injections of 0.05 μg/kg bw 2,3,7,8-TCDD (18 ± 1.7 versus 12 ± 1.5; $p = 0.031$) and in mice given a single dose of 1.6 μg/kg bw 2,3,7,8-TCDD (20 ± 2.6 versus 12 ± 1.5; $p = 0.016$) compared with those given NDMA only (Beebe *et al.*, 1995a).

In another experiment with male C57BL/6, DBA/2 and B6D2F1 mice, lung tumour incidence following a single intraperitoneal injection of 90 mg/kg bw *N*-nitrosodiethylamine (NDEA) was not increased by weekly intraperitoneal injections of 0.05 μg/kg bw

2,3,7,8-TCDD given three weeks later for 20 weeks followed by observation up to 52 weeks (C57BL/6: NDEA alone, 20/26; NDEA + 2,3,7,8-TCDD, 25/31; DBA/2: NDEA alone, 24/28; NDEA + 2,3,7,8-TCDD, 22/26; B6D2F1: NDEA alone, 33/34; NDEA + 2,3,7,8-TCDD, 33/33) (Beebe *et al.*, 1995b). [The Working Group noted that the high incidence of lung tumours induced by NDEA alone precluded the detection of an effect of 2,3,7,8-TCDD.]

(*b*) *Rat*

Groups of 45 female Sprague-Dawley rats were ovariectomized or sham-operated at 56 days of age. At 70 days of age, they were given a single intraperitoneal injection of 200 mg/kg bw NDEA in saline followed 10 days later by oral administration of 0 or 1.4 μg/kg bw 2,3,7,8-TCDD in olive oil every two weeks for 60 weeks. Lung carcinomas were found in 0/37 sham-operated and 4/39 (3 adenocarcinomas and 1 squamous-cell carcinoma) ovariectomized rats treated with NDEA and 2,3,7,8-TCDD (Clark *et al.*, 1991a). [The Working Group noted that no information was reported on whether NDEA alone caused lung tumours in sham-operated or ovariectomized rats.]

3.6.3 *Liver*

(*a*) *Mouse*

Groups of 18–30 male C57BL/6, DBA/2 and B6D2F1 mice were given a single intraperitoneal injection of 90 mg/kg bw NDEA or the solvent tricaprilyn at five weeks of age. Starting three weeks later, 0.05 μg/kg bw 2,3,7,8-TCDD was given weekly for 20 weeks, and animals were observed until the terminal killing at 52 weeks. No significant increase was observed in liver tumours (all types) due to co-administration of NDEA and 2,3,7,8-TCDD in C57BL/6 (NDEA alone, 4/28; NDEA + 2,3,7,8-TCDD, 6/32) or DBA/2 (NDEA alone, 6/28; NDEA + 2,3,7,8-TCDD, 10/39) mice. However, 2,3,7,8-TCDD did increase the incidence of liver tumours (all types) as compared to NDEA alone in B6D2F1 mice (NDEA alone, 7/33; NDEA + 2,3,7,8-TCDD, 17/33). This increase was particularly due to an increase in hepatoblastomas (NDEA alone, 1/33; NDEA + 2,3,7,8-TCDD, 8/33) (Beebe *et al.*, 1995b). [The Working Group noted that only one dose level of 2,3,7,8-TCDD was used in this study.]

(*b*) *Rat*

Groups of 4–7 female Sprague-Dawley rats, weighing 200–250 g, were subjected to 70% partial hepatectomy and 24 h later treated once with saline or 10 mg/kg bw NDEA in saline by gastric instillation. Starting seven days later, the rats were given 0, 0.14 or 1.4 μg/kg bw 2,3,7,8-TCDD by subcutaneous injection every two weeks for 28 weeks at which time all surviving rats were killed. There was an increase in the number and size of focal and nodular hepatic lesions with 2,3,7,8-TCDD in the low- and high-dose groups. Focal and nodular hepatic lesions were identified by γ-glutamyl transpeptidase (GGT), canalicular adenosine triphosphatase (ATPase) and glucose-6-phosphatase (G6Pase). The number of focal lesions was 309 ± 98 (NDEA alone), 34 ± 17 (low-dose 2,3,7,8-TCDD), 25 ± 7 (high-dose 2,3,7,8-TCDD), 1068 ± 166 (NDEA followed by low-

dose 2,3,7,8-TCDD) and 871 ± 66 (NDEA followed by high-dose 2,3,7,8-TCDD). The corresponding volume fractions of liver occupied by these lesions were 0.7, 0.2, 0.1, 9.0 and 43.0% (Pitot *et al.*, 1980).

Female Fischer 344 rats (150–220 g) [number not stated] were subjected to a 70% partial hepatectomy and given 10 mg/kg bw NDEA orally 24 h after the surgery. Starting two weeks after surgery, the rats were given intramuscular injections of 0, 0.0014, 0.014, 0.14 or 1.4 μg/kg bw 2,3,7,8-TCDD in corn oil every two weeks for six months. Treatment with 2,3,7,8-TCDD alone had virtually no effect on the number or volume fraction of altered hepatic foci. No effect on the number or volume fraction of focal hepatic lesions was observed at the three lowest doses. Only the highest dose (0.1 μg/kg bw per day) significantly increased the number and volume fraction of hepatic foci (detected by GGT, ATPase and G6Pase) (approximately 10 000 foci/liver with NDEA + 2,3,7,8-TCDD compared with 4000 foci/liver with NDEA alone; volume fraction approximately 2.8% with NDEA + 2,3,7,8-TCDD compared with 0.8% with NDEA alone) (Pitot *et al.*, 1987).

Groups of 10 female Sprague-Dawley rats (140–160 g) were subjected to a 70% partial hepatectomy and 24 h later were given a single intraperitoneal injection of 30 mg/kg bw NDEA. Starting one week later, the rats were given weekly subcutaneous injections of the corn oil solvent or 2,3,7,8-TCDD (0.7 μg/kg bw) for 14 or 26 weeks. Alternatively, some groups of rats were given a single loading dose (3.5 μg/kg bw) of 2,3,7,8-TCDD at five weeks after NDEA treatment or solvent in order to attain the same total dose. These latter groups were then given a weekly maintenance dose of 0.7 μg/kg bw 2,3,7,8-TCDD for nine or 21 weeks until killing. Focal hepatic lesions (detected by GGT) were significantly increased with weekly administration of 0.7 μg/kg bw 2,3,7,8-TCDD for 26 weeks. In the group receiving a loading dose of 2,3,7,8-TCDD, the percentage of liver occupied by hepatic foci was even greater than in the group treated with the same cumulative dose (Flodström & Ahlborg, 1989). Using the same experimental design, the influence of vitamin A deficiency upon the 2,3,7,8-TCDD response was investigated. Vitamin A deficiency increased the mean volume fraction of foci following NDEA + 2,3,7,8-TCDD treatment (Flodström *et al.*, 1991).

Female Fischer 344 rats (130–220 g) were subjected to a 70% partial hepatectomy and 24 h later were given a single gastric instillation of 10 mg/kg bw NDEA. Starting two weeks later, nine rats were administered oil and four rats were given 0.14 μg/kg bw 2,3,7,8-TCDD by subcutaneous injection every two weeks for six months. The number and volume fraction of the liver occupied by focal hepatic lesions (detected by glutathione *S*-transferase P (GSTP), GGT, ATPase, G6Pase) in the 2,3,7,8-TCDD-treated group was significantly increased compared with controls. The numbers of focal hepatic lesions per liver were 90 ± 30 (NDEA alone) and 14 220 ± 1340 (NDEA followed by 2,3,7,8-TCDD), while the volume fractions of liver were 0.14 ± 0.05% (NDEA alone) and 1.23 ± 0.11% (NDEA followed by 2,3,7,8-TCDD) (Dragan *et al.*, 1991).

Groups of 10–20 female Sprague-Dawley rats were subjected to a 70% partial hepatectomy followed by a single intraperitoneal injection of 30 mg/kg bw NDEA. Starting five weeks later, rats were given a loading dose of 0.22, 0.88 or 3.5 μg/kg bw 2,3,7,8-

TCDD by subcutaneous injection followed by a weekly maintenance dose of one fifth of the loading dose of 2,3,7,8-TCDD. One group received no 2,3,7,8-TCDD. The rats were killed after 20 weeks of treatment. A significant increase in the percentage of liver occupied by GGT-positive focal hepatic lesions was observed for all doses compared with NDEA controls (approximately 0.15% in controls, 0.6% in the low-dose, 0.59% in the mid-dose and 1.15% in the high-dose rats). A significant increase was seen in the number of foci at all doses (approximately: low-dose, 5000; mid-dose, 4250, high-dose, 7500) as compared to NDEA alone (2000) (Waern *et al.*, 1991).

Groups of nine female Sprague-Dawley rats, 70 days of age, were ovariectomized or sham-operated and were given an intraperitoneal injection of saline or 200 mg/kg bw NDEA. One week later, the rats were administered 2,3,7,8-TCDD in corn oil by intragastric instillation biweekly to provide a daily dose of approximately 100 ng/kg bw per day. The rats were killed after 30 weeks of 2,3,7,8-TCDD administration and GSTP- and GGT-positive foci were examined. For the GGT-positive foci, the percentages of the liver occupied by foci were 0.01 ± 0.01 (control), 0.01 ± 0.01 (2,3,7,8-TCDD alone), 0.3 ± 0.1 (NDEA alone) and 0.87 ± 0.06 (NDEA followed by 2,3,7,8-TCDD) in the intact animals. In the ovariectomized animals, the corresponding GGT-positive volume fractions were 0, 0, 0.03 ± 0.01 and 0.08 ± 0.04. The percentages of liver occupied by GSTP-positive foci were 0.04 ± 0.02 (control), 0.02 ± 0.01 (2,3,7,8-TCDD alone), 0.35 ± 0.11 (NDEA alone) and 1.17 ± 0.26 (NDEA followed by 2,3,7,8-TCDD) in the intact animals and 0.01 ± 0.01, 0.03 ± 0.01, 0.16 ± 0.05 and 0.57 ± 0.1 in the corresponding groups of ovariectomized animals. There was a reduction in the volume fraction of liver occupied by GSTP lesions in ovariectomized rats treated with NDEA alone and NDEA followed by 2,3,7,8-TCDD (Lucier *et al.*, 1991).

Groups of 5–10 female Sprague-Dawley rats were subjected to a 70% partial hepatectomy and administered oil vehicle or 10 mg/kg bw NDEA 24 h later by gastric instillation. Starting one week after the surgery, the rats were given biweekly subcutaneous injections of 0.14 μg/kg bw 2,3,7,8-TCDD for one, three or five months and either killed or maintained for a further six months before killing. The percentages of liver occupied by focal hepatic lesions (as identified by GSTP, GGT, ATPase and G6Pase) were 0.17 ± 0.03 (NDEA alone) and 1.47 ± 0.19 (NDEA and 2,3,7,8-TCDD) at the end of the first five months. The corresponding values in the group observed for an additional six months were 2.00 ± 0.27 (NDEA followed by 2,3,7,8-TCDD) and 1.35 ± 0.16 (NDEA alone, killed at the 11-month time point) (Dragan *et al.*, 1992).

Groups of 8–10 female Sprague-Dawley rats (70 days of age) were given a single intraperitoneal dose of saline or 175 mg/kg bw NDEA. Starting two weeks later, these rats were then administered an average of 0, 3.5, 10.7, 35.7 and 125 ng/kg bw 2,3,7,8-TCDD biweekly in corn oil by gastric instillation for 30 weeks and the number and volume fraction of GSTP-positive foci were determined. Dose-related increases in the number of foci per liver and the volume percentage of liver occupied by foci (as identified by GSTP) were found (see **Table 43**) (Maronpot *et al.*, 1993).

Groups of 20 female Wistar rats (100 g) were administered doses of 10 mg/kg bw NDEA in water by gastric instillation on five consecutive days. Starting two weeks after

NDEA administration, rats were treated with either corn oil or 1.4 μg/kg bw 2,3,7,8-TCDD biweekly by subcutaneous injection. Focal hepatic lesions (detected by ATPase deficiency) were quantitated at 9, 13 and 17 weeks for 4–8 rats per treatment group. In the control rats, the percentage of the liver occupied by foci was zero at all time points. In the group treated with NDEA alone, the percentages of liver occupied by foci were 0.025 ± 0.007 (at 9 weeks), 0.045 ± 0.017 (at 13 weeks) and 0.048 ± 0.018 (at 17 weeks). In the rats treated with 2,3,7,8-TCDD alone, the percentages of the liver occupied by ATPase-deficient hepatic foci were 0.030 ± 0.021 (at 9 weeks), 0.020 ± 0.011 (at 13 weeks) and 0.283 ± 0.211 (at 17 weeks). In the NDEA/2,3,7,8-TCDD-treated rats, the percentages of liver occupied by foci were 0.043 ± 0.02 (at 9 weeks), 0.211 ± 0.065 (at 13 weeks) and 0.313 ± 0.215 (at 17 weeks) (Buchmann *et al.*, 1994).

Table 43. Induction of focal hepatic lesions in rats treated with 2,3,7,8-TCDD and/or NDEA

	2,3,7,8-TCDD only		+ NDEA (175 mg/kg bw)	
2,3,7,8-TCDD dose (ng/kg bw)	No. of foci	Vol. %	No. of foci	Vol. %
0	327 ± 418	0.01 ± 0.01	5748 ± 3 923	0.57 ± 0.44
3.5	457 ± 1 122	0.02 ± 0.05	10 552 ± 7 941	0.87 ± 0.40
10.7	447 ± 614	0.02 ± 0.03	11 482 ± 8 879	1.00 ± 0.16
35.7	1 533 ± 1 794	0.06 ± 0.11	7 157 ± 3952	0.93 ± 0.56
125	693 ± 921	0.04 ± 0.07	11 989 ± 6 798	2.23 ± 1.47

From Maronpot *et al.* (1993)

Groups of five female Wistar rats (190–210 g) were given *N*-nitrosomorpholine (80 mg/L) in the drinking-water for 25 days. Starting two weeks later, the rats were given biweekly subcutaneous injections of corn oil or 2,3,7,8-TCDD for 13 weeks. The calculated average daily dose was 2, 20 or 200 ng/kg bw. The number and volume fraction of hepatic focal lesions (ATPase-deficient) were increased following 13 weeks of 2,3,7,8-TCDD administration at all doses. The numbers of hepatic focal lesions were approximately 125/cm^3 (control), 250/cm^3 (low-dose 2,3,7,8-TCDD), 250/cm^3 (mid-dose 2,3,7,8-TCDD) and 500/cm^3 (high-dose 2,3,7,8-TCDD), while the corresponding volume fractions were approximately 0.125% (control), 0.10% (low-dose 2,3,7,8-TCDD), 0.125% (mid-dose 2,3,7,8-TCDD) and 0.6% (high-dose 2,3,7,8-TCDD) (Schrenk *et al.*, 1994a).

Groups of 6–15 female Sprague-Dawley rats, 21 days of age, were treated with 10 mg/kg bw NDEA by intraperitoneal injection. After 30 days, rats were given either a basal diet or the basal diet supplemented with 2,3,7,8-TCDD (150 ppt [ng/kg diet], equivalent to a daily dose of about 0.007 μg/kg bw). Hepatic lesions were scored as ATPase-deficient foci. A time-dependent increase was noted in the volume fraction of hepatic foci in NDEA + 2,3,7,8-TCDD-treated rats (170 days after NDEA, 2%; 240 days, 4%;

450 days, 17%) as compared to NDEA alone (170 days, 0.6%; 240 days, 1.0%; 450 days, 4.0%). No hepatotoxicity was detected in any of the groups. Other rats were treated with NDEA (as above) followed by phenobarbital (PB) in the diet (500 ppm) for 30–170 days, at which time the rats were either returned to the basal diet or exposed to dietary 2,3,7,8-TCDD (150 ppt; 0.007 μg/kg bw per day). There was a time-dependent increase in the volume fraction of focal hepatic lesions, which was enhanced in the NDEA + PB + 2,3,7,8-TCDD group (240 days, 2%; 450 days, 13%) as compared to NDEA + PB (240 days, 1%; 450 days, 4%). However, when comparing the groups given NDEA + PB + 2,3,7,8-TCDD and NDEA + 2,3,7,8-TCDD (240 days, 4%; 450 days, 17%), no difference was seen. This suggests that phenobarbital does not enhance the ATPase-negative foci caused by NDEA + 2,3,7,8-TCDD. [The Working Group noted that loss of ATPase does not identify all possible preneoplastic lesions.] Hepatic tumours were examined at 450 days after NDEA treatment. They were primarily eosinophilic and expressed *ras*. The number of combined hepatocellular adenomas and carcinomas was 1/12 in the NDEA group, 5/15 in the NDEA + 2,3,7,8-TCDD group, 5/15 in the NDEA + PB group and 6/15 in the NDEA + PB + 2,3,7,8-TCDD group (Sills *et al.*, 1994).

The single and combined effects of 2,3,7,8-TCDD and 3,4,5,3′,4′-pentachlorobiphenyl (PCB 126) were examined in groups of 10–15 female Sprague-Dawley rats. Female rats (120–140 g) were subjected to a 70% partial hepatectomy and 24 h later received an intraperitoneal injection of corn oil or 30 mg/kg bw NDEA. Five weeks later, the rats were administered either PCB 126, 2,3,7,8-TCDD or a combination of the two, as an initial loading dose (equivalent to five single doses) followed by weekly injections for 19 weeks, at which time the rats were killed. Weekly doses of PCB 126 were 0, 0.316, 1, 3.16 or 10 μg/kg bw, while those of 2,3,7,8-TCDD were 0.1, 0.316 or 1 μg/kg bw. The doses of the combinations were 1 μg/kg bw PCB 126 plus 0.1 μg/kg bw 2,3,7,8-TCDD, 3.16 μg/kg bw PCB 126 plus 0.316 μg/kg bw 2,3,7,8-TCDD and 10 μg/kg bw PCB 126 plus 1 μg/kg bw 2,3,7,8-TCDD. The number and volume fraction of the liver occupied by altered hepatic foci expressing GGT were increased with the highest dose of PCB 126 (10 μg/kg bw per week), with the highest dose of 2,3,7,8-TCDD (1 μg/kg bw per week), and with two of the combinations of PCB 126 with 2,3,7,8-TCDD (3.16 + 0.316 and 10 + 1 μg/kg bw per week of PCB 126 and 2,3,7,8-TCDD, respectively) (Hemming *et al.*, 1995).

Groups of four female Wistar rats (120 g) were given gastric instillations of 10 mg/kg bw NDEA in water daily for 10 days and were then allowed to recover for eight weeks. One group of rats was given a single subcutaneous injection of 1.4 μg/kg bw 2,3,7,8-TCDD ('acute'), while a second group received biweekly subcutaneous injections of 1.4 μg/kg bw 2,3,7,8-TCDD for 115 days ('chronic'). All rats were killed 26 weeks after the start of NDEA treatment. The volume fraction of altered hepatic foci, identified as GSTP-positive, was significantly increased in the 'chronic' 2,3,7,8-TCDD + NDEA group (3.4%) as compared to NDEA alone (1.2%). There was no difference between the 'acute' 2,3,7,8-TCDD + NDEA group (1.7%) and the NDEA-alone group (1.2%) (Stinchcombe *et al.*, 1995).

Groups of 7–11 female Sprague-Dawley rats given 0 or 175 mg/kg bw NDEA in saline by intraperitoneal injection followed by biweekly treatment with 1.75 μg/kg bw 2,3,7,8-TCDD in corn oil by gastric instillation for 30 weeks. The percentages of the liver occupied by focal hepatic lesions (detected by GSTP) were 0.01 ± 0.01 (non-NDEA-treated controls at 30 weeks), 2.23 ± 1.47 (NDEA followed by 2,3,7,8-TCDD at 30 weeks), 0.32 ± 0.97 (non-NDEA-treated controls at 62 weeks) and 6.05 ± 4.3 (NDEA followed by 2,3,7,8-TCDD for 30 weeks and a 32-week observation period). [The Working Group noted that this study did not compare the NDEA/2,3,7,8-TCDD group to an NDEA group not given 2,3,7,8-TCDD.] The incidence of liver neoplasms was increased in the NDEA/2,3,7,8-TCDD group relative to the controls (5/7 and 0/11) (Tritscher *et al.*, 1995).

Dibenzo-*para*-dioxin

Oral administration

Mouse

Groups of 50 male and 50 female B6C3F1 mice, five weeks of age, were administered 0 (control), 5000 (low-dose) or 10 000 (high-dose) mg/kg of diet (ppm) dibenzo-*para*-dioxin (purity, 99.5%) for 87 (high-dose males) or 90 (low-dose males and females, high-dose females) weeks. All surviving male mice were killed at 92–97 weeks, and all surviving female mice at 91–93 weeks. Mean body weights of the treated male and female mice were slightly lower than those of the corresponding controls. Survival of high-dose females was lower than that of the control and low-dose groups ($p < 0.001$, Tarone test). At week 90 of the study, 48/50 (96%), 50/50 (100%) and 46/50 (92%) control, low-dose and high-dose males, respectively, were still alive; and 44/50 (88%), 44/50 (88%) and 27/50 (54%) control, low-dose and high-dose females, respectively, were still alive. Tumours were not induced in mice of either sex at significantly higher incidence in the treated groups than in the corresponding control groups (United States National Toxicology Program, 1979a).

Rat

Groups of 35 male and 35 female Osborne-Mendel rats, five weeks of age, were administered 0 (control), 5000 (low-dose) or 10 000 (high-dose) mg/kg of diet (ppm) dibenzo-*para*-dioxin (purity 99.5%) in the diet for 110 weeks. Mean body weights of the treated male and female rats were lower that those of the corresponding controls. In male rats, survival in the control group was lower than in the treated groups ($p = 0.011$, Tarone test). Survival of high-dose female rats was lower than that of the control and low-dose groups ($p = 0.007$, Tarone test). At week 90 of the study, 24/35 control and 29/35 rats in each treated group of males were still alive, and 32/35, 31/35 and 20/35 control, low-dose and high-dose female rats, respectively, were still alive. Tumours were not induced in rats of either sex at significantly higher incidence in the treated groups than in the corresponding control groups (United States National Toxicology Program, 1979a).

2,7-Dichlorodibenzo-*para*-dioxin (2,7-DCDD)

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F1 mice, five weeks of age, were given 0 (control), 5000 (low-dose) or 10 000 (high-dose) mg/kg of diet (ppm) 2,7-DCDD for 90 weeks. Three impurities with peak areas 3–6% of the major peak were detected by gas chromatography. One was identified as trichlorodibenzo-*para*-dioxin. No 2,3,7,8-TCDD was detected. All surviving male mice were killed at 92–101 weeks and all surviving female mice at 91–98 weeks. Mean body weights of the male mice were unaffected by administration of the test chemical, whereas those of female treated mice were slightly lower than those of the corresponding controls. Survival in male mice was unaffected by treatment. Survival in high-dose female mice was significantly lower than that in control and low-dose groups ($p < 0.001$, Tarone test). At the end of the study, 48/50, 36/50 and 38/50 control, low-dose and high-dose males, respectively, were still alive; and 45/50, 46/50 and 28/50 control, low-dose and high-dose females, respectively, were still alive. Treated male and female mice showed an increased incidence of focal necrosis of the liver. The incidence of hepatocellular carcinomas in male mice was unaffected by treatment: 4/49, 5/50 and 5/42 control, low-dose and high-dose mice, respectively. However, the incidence of hepatocellular adenomas was 4/49, 15/50 and 12/42 in control, low-dose and high-dose males, respectively. The incidence of hepatocellular adenomas and carcinomas combined (8/49, 20/50 and 17/42) was significantly higher in the treated groups than in the control group ($p = 0.008$, Cochran–Armitage test for trend). The incidences in the low- and high-dose groups were both significantly higher than that in the control group ($p = 0.008$ and $p = 0.010$, respectively, Fisher's exact test). In male mice, the incidence of lymphoma or leukaemia was higher in the low-dose (6/50) than in the control group (0/50) ($p = 0.006$, Fisher's exact test). However, the incidence in the high-dose group (3/50) was not statistically different from that of controls. In the female mice, no tumours were observed at significantly higher incidence in the dosed groups than in the corresponding controls (United States National Toxicology Program, 1979b). [The Working Group noted that the presence of three impurities makes it impossible to ascribe the observed carcinogenic effect specifically to the 2,7-dichloro congener.]

3.1.2 *Rat*

Groups of 35 male and 35 female Osborne-Mendel rats, five weeks of age, were given 0 (control), 5000 (low-dose) or 10 000 (high-dose) ppm 2,7-DCDD in the diet for 110 weeks. Three impurities with peak areas 3–6% of the major peak were detected by gas chromatography. All surviving male rats were killed at 110–112 weeks, and all surviving female rats at 110–117 weeks. Mean body weights of the dosed groups were lower that those in the corresponding controls. Survival was not significantly affected by administration of 2,7-DCDD. At week 78 of the study, 30/35, 26/35 and 29/35 control, low-dose and high-dose males, respectively, were still alive; and 33/35, 30/35 and 28/35 control, low-dose and high-dose females, respectively, were still alive. Toxic hepatic

lesions characterized by centrilobular fatty metamorphosis (33–48%) and/or necrosis (6–20%) were observed in both low- and high-dose rats. Tumours were not induced in rats of either sex at significantly higher incidence in the dosed groups than in the corresponding control groups (United States National Toxicology Program, 1979b)

3.2 Administration with known carcinogens

Skin

Mouse: Groups of 20 female HRS/J hairless (*hr/hr*) mice, five to eight weeks of age, were treated with single skin applications of 0 or 5 μmol (0.75 mg) per animal MNNG in 50 μL acetone followed by 20 μg/animal 2,7-DCDD in 50 μL acetone twice weekly for 20 weeks. A control group of 20 mice was given acetone followed by 10 ng/animal 2,3,7,8-TCDD. Skin tumours developed in 0/19 mice treated with MNNG plus 2,7-DCDD and in 0/20 mice treated with 2,7-DCDD alone (Poland *et al.*, 1982).

1,2,3,6,7,8-Hexachlorodibenzo-*para*-dioxin and 1,2,3,7,8,9-hexachlorodibenzo-*para*-dioxin (mixture) (HxCDD)

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F1 mice, six weeks of age, were administered HxCDD (purity, 98.6%; 31% 1,2,3,6,7,8-HxCDD and 67% 1,2,3,7,8,9-HxCDD of the total HxCDD content), suspended in a vehicle of 9 : 1 corn oil–acetone, by gastric instillation twice a week for 104 weeks at doses of 1.25 (low), 2.5 (mid) or 5 (high) μg/kg per week for male mice and 2.5 (low), 5 (mid) or 10 (high) μg/kg per week for female mice. Groups of 75 mice of each sex served as vehicle controls and 50 mice of each sex as untreated controls. All surviving animals were killed at 105–108 weeks. The mean body weights and survival in the treated groups were similar to those of the vehicle-control groups. At the end of the study, 32/50, 38/75, 29/50, 26/50 and 23/50 untreated control, vehicle-control, low-, mid- and high-dose male mice, respectively, were still alive; for females, the corresponding numbers were 36/50, 58/75, 31/50, 33/50 and 36/50 animals. In males, hepatocellular adenomas occurred at increased incidence in the high-dose group (7/73, 5/50, 9/49 and 15/48 vehicle-control, low-, mid- and high-dose mice, respectively; $p = 0.003$, Fisher's exact test). There was no significant increase in the incidence of hepatocellular carcinomas (8/73, 9/50, 5/49 and 9/48). In females, hepatocellular adenomas occurred at incidences that were dose-related (2/73, 4/48, 4/47 and 9/47 in the vehicle-control, low-, mid- and high-dose mice, respectively; $p = 0.002$, Cochran–Armitage test for trend); the incidence in the high-dose group was significantly higher ($p = 0.003$, Fisher's exact test) than that in the corresponding vehicle-control group. Hepatocellular carcinomas were found in 1/73, 0/48, 2/47 and 2/47 females, respectively (United States National Toxicology Program, 1980a). [The Working Group noted that it is unlikely that the impurities consisting of various polyhalogenated dibenzo-*para*-dioxins were responsible for the observed carcinogenic effects.]

3.1.2 *Rat*

Groups of 50 male and 50 female Osborne-Mendel rats, six weeks of age, were administered HxCDD (purity, 98.6%; 31% 1,2,3,6,7,8-HxCDD and 67% 1,2,3,7,8,9-HxCDD of the total HxCDD content), suspended in a vehicle of 9 : 1 corn oil–acetone, by gastric instillation twice a week for 104 weeks at doses of 1.25 (low), 2.5 (mid) or 5 (high) μg/kg per week. Groups of 75 rats of each sex served as vehicle controls and 50 rats of each sex as untreated controls. All surviving animals were killed at 105–108 weeks. A dose-related depression in mean body weight was seen in both sexes. At the end of the study, 24/50, 29/75, 18/50, 19/50 and 19/50 untreated control, vehicle control, low-, mid- and high-dose rats, respectively, were still alive; for females, the corresponding numbers were 33/50, 39/75, 36/50, 36/50 and 37/50 animals. In male rats, hepatic neoplastic nodules occurred at incidences that were dose-related (0/74, 0/49, 1/50 and 4/48 in the vehicle-control, low-, mid- and high-dose groups, respectively ($p = 0.003$, Cochran–Armitage test for trend). No hepatocellular carcinomas occurred in males. In female rats, hepatic neoplastic nodules occurred at incidences that were dose-related (5/75, 10/50, 12/50 and 30/50 in the vehicle-control, low-, mid- and high-dose groups, respectively; $p < 0.001$, Cochran–Armitage trend test); the incidences in the mid- and high-dose groups were significantly higher ($p = 0.006$ and $p < 0.001$, respectively, Fisher's exact test) than that in the corresponding vehicle-control group. In addition, four high-dose females had hepatocellular carcinomas (United States National Toxicology Program, 1980a). [The Working Group noted that it is unlikely that the impurities consisting of various polyhalogenated dibenzo-*para*-dioxins were responsible for the observed carcinogenic effects.]

3.2 Skin application

Mouse: Groups of 30 male and 30 female Swiss-Webster mice, six weeks of age, received skin applications on the clipped back of 0.01 μg/animal HxCDD (purity, 98.6%; 31% 1,2,3,6,7,8-HxCDD and 67% 1,2,3,7,8,9-HxCDD of the total HxCDD content) suspended in 0.1 mL acetone on three days per week for 104 weeks. During the first 16 weeks, doses were 0.005 μg/animal per application. As vehicle controls, 45 mice of each sex received 0.1 mL of acetone three times per week. Thirty animals of each sex served as untreated controls. HxCDD treatment did not affect mean body weights or survival in either sex. At week 60 of the study, 23/30, 30/45 and 25/30 untreated control, vehicle-control and HxCDD-treated males, respectively, were still alive; and 28/30, 43/45 and 24/30 untreated control, vehicle-control and HxCDD-treated females, respectively, were still alive. In male mice, the incidence of alveolar/bronchiolar carcinomas in the group administered only HxCDD was significantly higher (5/30; $p = 0.045$, Fisher's exact test) than that in the vehicle controls (1/41), but similar to that in untreated controls (4/28). In male mice, the incidence of lymphomas or leukaemias was significantly lower (0/30; $p = 0.011$) than that in untreated controls (6/29). In female mice, the incidence of fibrosarcomas of the integumentary system was significantly higher in animals administered HxCDD (4/27; $p = 0.044$, Fisher's exact test) than that in untreated controls (0/30).

However, the incidences were not significantly elevated compared with those of the vehicle controls (2/41) (United States National Toxicology Program, 1980b).

1,2,3,7,8-Pentachlorodibenzo-*para*-dioxin (1,2,3,7,8-PeCDD)

Administration with known carcinogens

Liver

Rat: Waern *et al.* (1991) analysed the GGT-positive foci in groups of 10–20 female Sprague-Dawley rats subjected to a 70% partial hepatectomy followed by single intraperitoneal injection of 30 mg/kg bw NDEA and 1,2,3,7,8-PeCDD. Five weeks after NDEA administration, a loading dose of 1,2,3,7,8-PeCDD consisting of five times the weekly maintenance dose in each treatment group was given by subcutaneous injection. Weekly maintenance doses of 0.088, 0.35 and 1.4 μg/kg bw 1,2,3,7,8-PeCDD per week were then given for 20 weeks. The rats were killed at the end of the treatment. A significant increase in the percentage of liver occupied by GGT-positive focal hepatic lesions was observed for all doses of 1,2,3,7,8-PeCDD (approximately: low-dose, 0.35%; mid-dose, 0.7%; high-dose, 1.6%) compared with NDEA alone (0.15%). A significant increase in the number of foci per liver was also seen in the two highest-dose groups (approximately: low-dose, 2750; mid-dose, 5000; high-dose, 10 250) compared with NDEA alone (2000).

1,2,3,4,6,7,8-Heptachlorodibenzo-*para*-dioxin (1,2,3,4,6,7,8-HpCDD)

Administration with known carcinogens

Liver

Rat: Groups of 4–8 female Wistar rats (100 g) were given five doses of 10 mg/kg bw NDEA in water by gastric instillation. Starting two weeks later, rats were treated with either corn oil solvent or 70 μg/kg bw 1,2,3,4,6,7,8-HpCDD biweekly by subcutaneous injection. The development of focal hepatic lesions was determined at 9, 13 and 17 weeks. ATPase-deficient lesions were quantitated and the GSTP-positive focal population was used to determine focal proliferation rates from pulse labelling with bromodeoxyuridine (BrdU) before sacrifice. The numbers of ATPase-deficient lesions were 619 ± 113 (at 9 weeks), 551 ± 158 (at 13 weeks) and 1691 ± 652 (at 17 weeks) per cm^3 in the NDEA-treated rats given 1,2,3,4,6,7,8-HpCDD. The corresponding numbers in the groups given 1,2,3,4,6,7,8-HpCDD but no NDEA were 63 ± 35 (at 9 weeks), 87 ± 16 (at 13 weeks) and 120 ± 30 (at 17 weeks) per cm^3. The percentage volume fractions of ATPase-deficient lesions were 0.093 ± 0.38 (at 9 weeks), 0.091 ± 0.037 (at 13 weeks) and 0.76 ± 0.40 (at 17 weeks) (Buchmann *et al.*, 1994).

Groups of five female Wistar rats (190–210 g) were given *N*-nitrosomorpholine (80 mg/L) in the drinking-water for 25 days. Starting two weeks later, the rats were given biweekly subcutaneous injections of corn oil or 1,2,3,4,6,7,8-HpCDD for 13 weeks. The calculated average daily doses were 100, 1000 or 10 000 ng/kg bw. The number and volume fraction of ATPase deficient lesions were assessed following 13 weeks of admi-

nistration. The numbers of ATPase-deficient hepatic foci were approximately 125/cm^3 (controls), 450/cm^3 (low-dose), 250/cm^3 (mid-dose) and 500/cm^3 (high-dose), while the corresponding percentage volume fractions were approximately 0.125 (control), 0.25 (low-dose), 0.125 (mid-dose) and 0.9 (high-dose) (Schrenk *et al.*, 1994a).

Defined mixture of 49 polychlorinated dibenzo-*para*-dioxins (PCDDs)

Administration with known carcinogens

Liver

Rat: Groups of five female Wistar rats (190–210 g) were given *N*-nitrosomorpholine (80 mg/L) in the drinking-water for 25 days. Starting two weeks later, the rats were given biweekly subcutaneous injections of corn oil or a defined mixture of 49 PCDDs for 13 weeks at 200 ng, 2000 ng or 20 000 ng PCDD/kg. The number and volume fraction of ATPase-deficient lesions were assessed following 13 weeks of administration. The numbers of ATPase-deficient hepatic foci were approximately 125/cm^3 (controls), 125/cm^3 (200 ng/kg), 300/cm^3 (2000 ng/kg) and 800/cm^3 (20 000 ng/kg), while the corresponding percentage volume fractions were 0.125 (control), 0.2 (200 ng/kg), 0.25 (2000 ng/kg) and 0.75 (20 000 ng/kg) (Schrenk *et al.*, 1994a).