Review

Carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in experimental models

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The contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a prototype compound of a whole class of halogenated aromatic hydrocarbons termed 'dioxinlike' contaminants present in food, human tissue, mothers milk, and environmental samples. Among the various adverse effects caused by TCDD in animal experiments, its carcinogenic effects caused particular concern. In rodents, long-term TCDD treatment leads to the development of tumors of the liver, thyroid, lung, skin, oral cavity and other sites. The occurrence of liver tumors mainly observed in female rats has been used as a basis for quantitative cancer risk assessment for TCDD. TCDD does not behave like a 'complete carcinogen', *i.e.* no DNA binding of the parent compound or metabolites thereof could be detected. However, enhanced oxidative damage of hepatic DNA was observed, probably resulting from a dramatic induction of cytochrome P450 enzymes, which are under the regulatory, transcriptional control of the TCDD-activated aryl hydrocarbon receptor. The marked enhancement of TCDD-related oxidative liver DNA damage in rats by estrogens warrants further mechanistic investigation. Furthermore, TCDD acts as a tumor promoter, *i.e.* it facilitates the growth of putative preneoplastic hepatic lesions after initiation with a complete carcinogen. The mechanisms underlying this effect may be related to altered intracellular signaling involving pronounced changes in the phosphorylation pattern of proteins regulating growth and apoptosis. These effects are thought to result in an enhanced survival of preneoplastic cells, some of which can undergo further steps on the way to malignancy. In summary, a better understanding of the mechanisms of the carcinogenicity of TCDD is mandatory to provide a rational basis for a better inter-species extrapolation. The final aim of these efforts is a more reliable risk assessment for the carcinogenic potency of the class of dioxinlike contaminants in humans.

Keywords: Arylhydrocarbon receptor / Cancer / Dioxin / TCDD / Tumor promotion

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1 Tumor formation in experimental animals

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the prototype compound of a number of chlorinated aromatic compounds which are found as persistent contaminants in the environment, in the food chain and in human samples. The individual members of the TCDD or 'dioxinlike' chemicals belong to the larger groups of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls. In order to express their relative biological potency and toxicity in relation to TCDD, the most toxic congener, they have been attributed TCDD or toxicity equivalency factors (TEF) [1].

A major cause of concern related to exposure to dioxinlike compounds is their possible carcinogenicity in humans. Since carcinogenic effects were first reported for TCDD, and most experimental data are available on this compound, the carcinogenicity of TCDD in experimental models is reviewed here.

When administered by different routes and at low doses to rodents (rats, mice, hamsters), TCDD caused tumors at multiple sites. Tumors were observed in various tissues of both sexes, *e.g.* liver, lung, nasal turbinates, hard palate, thyroid and tongue. Tumor promotion experiments (prior

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Abbreviations: AhR, arylhydrocarbon receptor; **CYP**, cytochrome P450; **DEN**, diethyl-N-nitrosamine; **EGF**, epidermal growth factor; **ROS**, reactive oxygen species; **TCDD**, 2,3,7,8-tetrachlordibenzo-*p*-dioxin

Table 1. Long-term carcinogenicity studies on 2,3,7,8-TCDD in rodents ([37]; modified)[a)]

Species	Sex	Dose	Target organ	Tumor	Lowest effective dose	Reference
Rat	M	1, 5, 50, 500 ppt 1, 5, 50, 500, 1000 ppb for 78 weeks in the diet	Lung	Squamous cell carcinoma	5 ppb	2
			Liver	Neoplastic nodules	5 ppb	
				Cholangiocarcinoma	1 ppb	
	M	22, 210, 2200 ppt for 2 years in the diet	Hard palate	Squamous cell carcinoma	0.1 μg/kg bw	3
			Nasal turbinates	Squamous cell carcinoma	0.1 μg/kg bw	
			Tongue	Squamous cell carcinoma	0.1 μg/kg bw	
	F	22, 210, 2200 ppt for 2 years in the diet	Liver	Hyperplastic nodule	0.01 μg/kg bw	3
			Liver	Hepatocellular carcinoma	0.1 μg/kg bw	
			Hard palate	Squamous cell carcinoma	0.1 μg/kg bw	
			Nasal turbinates	Squamous cell carcinoma	0.1 μg/kg bw	
			Lung	Squamous cell carcinoma	0.1 μg/kg bw	
			Tongue	Squamous cell carcinoma	0.1 μg/kg bw	
	M	0.01, 0.05, 0.5 μg/kg bw for 104 weeks, orally	Thyroid	Follicular cell adenoma	Dose-related	4
			Liver	Neoplastic nodule	Dose-related	
	F	0.01, 0.05, 0.5 μg/kg bw for 104 weeks, orally	Thyroid	Follicular cell adenoma	0.5 μg/kg bw	4
			Liver	Neoplastic nodules	0.5 μg/kg bw	
	F	3, 10, 22, 46, 100 ng/kg bw 5days/week for 104 weeks	Liver	Cholangiocarcinoma	22 ng/kg bw	5
				Hepatocellular adenoma	100 ng/kg bw	
			Lung	Cystic keratinizing epithelioma	100 ng/kg bw	
			Oral mucosa	Gingvial squamous cell carcinoma	100 ng/kg bw	
			Uterus	Squamous cell carcinoma	46 ng/kg bw	
Mouse	M	0.007, 0.7, 7.0 μg/kg bw for 1 year, orally once a week; observed for lifetime	Liver	Hepatocellular adenoma and carcinoma	0.7 μg/kg bw	7
	M	0.01, 0.05, 0.5 μg/kg bw for 104 weeks, orally twice a week	Liver	Hepatocellular carcinoma	Dose related	5
			Lung	Alveolar/bronchiolar adenomas & carcinomas	Dose related	
	F	0.04, 0.2, 2.0 μg/kg bw for 104 weeks, orally twice a week	Liver	Hepatocellular carcinoma	Dose related	5
			Thyroid	Follicle cell adenoma	Dose related	
			Hematopoietic system	Lymphoma	Dose related	
			Skin	Subcutaneous fibrosarcoma	Dose related	
	F	0.005 μg per animal, skin 3 × per week for 104 weeks	Integumentary system	Fibrosarcoma	0.001 μg per animal	6
	M	2.5, 5.0 μg/kg bw for 52 weeks, orally once a week, followed until 104 weeks	Liver	Hepatocellular carcinoma	2.5 μg/kg bw	8
	F	2.5, 5.0 μg/kg bw for 52 weeks, orally once a week, followed until 104 weeks	Liver	Hepatocellular carcinoma	2.5 μg/kg bw	8
	M	1, 30, 60 μg/kg bw i.p. once a week for 5 weeks; observed until 78 weeks of age	Thymus	Lymphoma	Dose related	8
			Liver	Hepatocellular adenoma and carcinoma	Dose related	
	F	1, 30, 60 μg/kg bw i.p. once a week for 5 weeks; observed until 78 weeks of age	Thymus	Lymphoma	Dose related	8
			Liver	Hepatocellular adenoma and carcinoma	Dose related	
Hamster	M	50, 100 μg/kg bw i.p. or s.c. 6 × at 4 week intervals; observed for 1 year	Skin	Squamous cell carcinoma	100 μg/kg bw	9

a) Abbreviations: bw, body weight; F, female; M, male; s.c., subcutaneous.

exposure to a known carcinogen and subsequent exposure to TCDD) enhanced tumor incidence. Additionally, tumors were observed at earlier times.

Carcinogenicity studies of TCDD given by oral administration were performed in four experiments in rats, in three experiments in mice and in one experiment in hamsters. In one experiment, TCDD was administered dermally on the clipped back of mice (Table 1).

1.1 Rats

Van Miller *et al*. [2] administered 1, 5, 50, 500 ppt and 1, 5, 50, 500, 1000 ppb, respectively, of TCDD for 78 weeks in the diet to male Sprague-Dawley rats. All animals treated with the highest doses (50, 500 and 1000 ppb) died within 4 weeks. At the end of the experiment after 95 weeks, various types of neoplasms were observed, namely squamous cell tumors in the lung, neoplastic nodules and cholangiocarcinomas in the liver of the animals.

In a study by Kociba *et al*. [3] male and female Sprague-Dawley rats were fed with 22, 210 and 2200 ppt TCDD in the diet for 2 years. Non-neoplastic, treatment-related pathological changes were observed, especially in the liver as well as multiple hepatocellular necrosis and inflammatory changes in high- and mid-dosed animals. In female rats, the incidence of hepatocellular hyperplastic nodules and hepatocellular carcinomas was increased as well as squamous cell carcinomas of the hard palate, lung, tongue and nasal turbinates. In males, thyroid tumors, squamous cell carcinomas of the hard palate, nasal turbinates and the tongue were observed.

In a study by the United States National Toxicology Program [4], Osborne-Mendel rats of both sexes were given 0.01, 0.05 or 0.5 μg/kg body weight TCDD by gastric instillation twice a week for 104 weeks. Treatment-related increased incidences of thyroid follicular cell adenomas and neoplastic nodules of the liver were observed in males and females.

In a second study by the NTP [5], female Sprague-Dawley rats were given 3, 10, 22, 46 and 100 ng/kg body weight TCDD 5 days per week for 104 weeks. There was clear evidence for carcinogenic activity of TCDD in female Sprague-Dawley rats based on increased incidences of cholangiocarcinomas and hepatocellular adenomas of the liver, cystic keratinizing epitheliomas of the lung, and gingival squamous cell carcinomas of the oral mucosa. The increased incidence of squamous cell carcinomas of the uterus was also considered to be related to TCDD administration. The marginally increased incidences of pancreatic neoplasms and occurrences of hepatocholangiomas and cholangiomas of the liver may have been related to TCDD administration.

1.2 Mice

In a third study by the NTP [6], male and female Swiss-Webster mice received doses of 0.001 or 0.005 μg/animal TCDD on the clipped back on 3 days per week for 99 or 104 weeks. In females the incidence of fibrosarcomas of the integumentary system was significantly increased in TCDD treated animals, compared to vehicle control. In males, this effect was statistically not significant.

Male Swiss/H/Riop mice were given gastric instillations of 0.007, 0.7 or 7.0 μg/kg body weight TCDD once a week for 1 year in a study by Toth *et al*. [7]. The animals were observed for lifetime. Treatment with TCDD caused increased incidences of liver tumors (hepatocellular adenomas and carcinomas). No increased incidences of lung tumors or lymphomas were reported.

Male and female B6C3F1 mice were given gastric instillations of 0.01, 0.05 or 0.5 μg/kg body weight (males) and 0.04, 0.2 or 2.0 μg/kg body weight (females) TCDD twice a week for 104 weeks. The incidences of hepatocellular carcinomas were dose related, compared to vehicle controls. Incidences of follicular-cell adenomas of the thyroid were increased in a dose-related manner in female animals, as well as a significant increase in the incidence of lymphomas and subcutaneous fibrosarcomas. In male mice, a dose-related increase of lung tumors was observed [4].

In a study by Della Porta *et al*. [8] male and female C57BL/6 mice were given gastric instillations of 2.5 or 5.0 μg/kg body weight TCDD once a week for 52 weeks. The incidence of non-neoplastic lesions (liver necrosis, amyloidosis of multiple tissues and nephrosclerosis) was increased in treated animals compared to vehicle control. A significant increase in hepatocellular adenomas and carcinomas was observed in a dose-related manner, the incidence of any other tumor type was not associated with TCDD treatment.

In the same study, 10-days-old male and female C57BL/6 (B6C3 and B6C) mice were given five weekly intraperitoneal (i.p.) injections of 1, 30 or 60 μg/kg body weight TCDD. Animals did not receive further doses until 78 weeks of age when they were killed. Thymic lymphomas were observed in a dose-dependent manner in both male and female B6C3 and B6C animals. Hepatocellular carcinomas were only observed in B6C3 males.

1.3 Hamsters

In a study by Rao *et al*. [9], male Syrian golden hamsters received six i.p. or subcutaneous injections of 50 or 100 μg/kg body weight TCDD at 4-week intervals. A second group received only two i.p. injections of 100 μg/kg

body weight TCDD. Animals were observed until 13 months. Squamous-cell carcinomas of the facial skin were observed in the high-dosed animals. No animal of the second group or of the low-dosed group developed tumors at any site.

2 Tumor promotion studies

Administration of TCDD after initiation with known carcinogens enhanced the incidences of various tumor types, *e.g.* skin papillomas, lung and liver adenomas, or hepatoblastomas in mice. In several rat strains the incidences of hepatic lesions was increased after initiation with N-nitrosamines [10].

2.1 Rats

The lung was identified as the target organ in a study by Clark *et al*. [11]. Female Sprague-Dawley rats were ovariectomized or sham-operated and i.p. initiated with a single dose of 200 mg/kg body weight diethyl-N-nitrosamine (DEN). Ten days later they were given 1.4 μg/kg body weight TCDD every 2 weeks for 60 weeks. Adenocarcinomas and one squamous-cell carcinomas of the lung were found in ovariectomized rats.

In some of the studies, DEN was given after partial hepatectomy or ovariectomy as initiating agent, followed by administration of TCDD. Detailed information about dosage and administration is listed in Table 2. As a conclusion, all initiation-promotion experiments with a partial hepatectomy resulted in increased levels of putative preneoplastic hepatic foci. The studies selected different markers to detect hepatic foci, namely γ-glutamyl transpeptidase (γ-GT), adenosine triphosphatase (ATPase), glucose-6-phosphatase (G6Pase) or glutathione S-transferase P (GST) [12–18].

A second study design, which was likewise utilized in various experiments, also performs initiation with DEN followed by promotion with TCDD, foregoing partial hepatectomy. Detailed information about dosage and administration can be obtained from Table 2. Similar to experiments with partially hepatectomized animals, these initiation-promotion experiments also resulted in increased levels of altered hepatic foci [19–25].

2.2 Mice

Tumor promotion experiments on skin were performed in mice only. Berry *et al*. [26] initiated with a single skin application of 60 μg per animal 7,12-dimethylbenz[a]anthracene (DMBA) in female CD1 mice, followed by 0.1 μg TCDD twice weekly for 30 weeks. No skin papillomas were observed.

In a study by Poland *et al*. [27], female haired and hairless HRS/J mice were used in the experiments using DMBA and N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) as initiating agents. Detailed information about dosage and administration can be obtained from Table 2. Tumors (skin papillomas) were only observed in hairless mice.

Hebert *et al*. [28] also initiated with skin applications of 5 μmole MNNG per animal in female HRS/J mice. Then doses of 2.5, 5 or 10 ng per animal TCDD were applied twice weekly for 20 weeks. Papillomas, carcinomas and hyperproliferative nodules of the skin were observed.

Tumor promotion in the lung was investigated in two studies by Beebe and co-workers. Male Swiss mice were given a single dose of dimethyl-N-nitrosamine (DMN) followed by either a single i.p. injection of 1.6, 16 or 48 μg/kg TCDD or weekly doses of 0.05 μg/kg body weight for 20 weeks. Alveolar-cell adenomas and carcinomas were found in all mice in all treatment groups. Additionally, tumor multiplicity was significantly increased [29].

In the second study, male C57BL/6, DBA/2 and B6D2F1 mice were initiated with a single dose of 90 mg/kg body weight DEN. Weekly i.p. injections of 0.05 μg/kg body weight TCDD for 20 weeks (followed by observation up to 52 weeks) did not increase lung tumor incidence, because a high incidence of lung tumors induced by DEN alone precluded the detection of a TCDD-mediated effect. However, TCDD increased liver tumor incidence (all types) in B6D2F1 mice, compared to DEN alone. No significant increase in liver tumors was observed in the C57BL/6 and DBA/2 strain [30].

3 Mechanistic aspects

3.1 Conflicting results on genotoxicity

In the Ames test either in the presence or in absence of an exogenous metabolic system, TCDD failed to induce mutations [31]. Furthermore, unscheduled DNA synthesis was not induced in normal human mammary epithelial cells [32], while increased formation of sister chromatid exchange was observed in human lymphocytes *in vitro* [33]. An increase in DNA single-strand breaks was found in rat liver [34], and in rat peritoneal lavage cells [35]. A mixture of TCDD and other congeners caused increased DNA single-strand breaks in liver and brain of treated rats [36]. There is no evidence for the formation of TCDD-derived DNA adducts [37].

Table 2. Studies on 2,3,7,8-TCDD administered with known tumor promoters in rodents ([37]; modified)[a)]

Species	Strain & sex	Initiation dose	Administration	Dose & frequency of TCDD	Administration	Promotion	Reference
Skin							
Mouse	F; CD1	200 nM DMBA	Skin; 1 week interval	0.1 µg TCDD; 2 × per week; 30 weeks	Skin	–	26
	F; HRS/J haired	200 nM DMBA	Skin; no interval	20 ng TCDD; 2 × per week; 8 weeks, then 50 ng; 17 weeks	Skin	–	27
	F; HRS/J hairless	200 nM DMBA	Skin; no interval	20 ng TCDD; 2 × per week; 8 weeks, then 50 ng; 17 weeks	Skin	+	27
	F; HRS/J hairless	5 µmol MNNG	Skin; no interval	3.75, 7.5, 15, 30 ng TCDD; 2x per week; 20 weeks	Skin	+	27
	F; HRS/J hairless	5 µmol MNNG	Skin; no interval	50 ng TCDD; 5 weeks; then 20 ng; 15 weeks	Skin	+	27
	F; HRS/J hairless	5 µmol MNNG	Skin; 7 days interval	2.5, 5, 10 ng TCDD; 2x per week; 20 weeks	Skin	+	28
Lung							
Mouse	M; Swiss	25 mg/kg bw DMN	i.p.; 3 weeks interval	1.6 µg/kg bw TCDD	i.p.	+	29
				16, 48 µg/kg bw TCDD		– (toxic)	
				20 × 0.05 µg/kg bw TCDD		–	
	M; C57/B6, B6D2F1, DBA/2	90 mg/kg bw DEN	i.p.; 3 weeks interval	0.05 µg/kg bw TCDD; weekly; 20 weeks	i.p.	–	30
Rat	F; Sprague-Dawley	200 mg/kg bw DEN	i.p.; 10 days interval	1.4 µg/kg bw TCDD; biweekly; 60 weeks	oral	–	11
						+ (ovariecto-mized)	
Liver							
Mouse	C57/B6	90 mg/kg bw DEN	i.p.; 3 weeks interval	0.05 µg/kg bw TCDD; weekly; 20 weeks	i.p.	–	30
	B6D2F1					+	
	DBA/2					–	
Rat	F; Sprague-Dawley	PH; 10 mg/kg bw DEN	oral; 7 days interval	0.14 µg/kg bw TCDD; biweekly; 7 months	i.m.	–	12
				1.4 µg/kg bw TCDD; biweekly; 7 months	+		
	F; Fisher F344	PH; 10 mg/kg bw DEN	oral; 14 days interval	0.0014, 0.014, 0.14 µg/kg bw TCDD; biweekly; 6 months	i.m.	–	13
				1.4 µg/kg bw TCDD; biweekly; 6 months	+		
	F; Sprague-Dawley	PH; 30 mg/kg bw DEN	i.p.; 7 days interval	0.7 µg/kg bw TCDD; weekly; 14 weeks	s.c.	–	109
				0.7 µg/kg bw TCDD; weekly; 26 weeks	+		
	F; Sprague-Dawley	PH; 30 mg/kg bw DEN	i.p.; 35 days interval	3.5 then 0.7 µg/kg bw TCDD; weekly; 9 weeks	s.c.	+	14
				3.5 then 0.7 µg/kg bw TCDD; weekly; 21 weeks	+		
				0.35 then 0.07 µg/kg bw TCDD; weekly; 15 weeks (normal Vit A)	–		
				0.35 then 0.07 µg/kg bw TCDD; weekly; 15 weeks (marginal or low Vit A)	+		
				3.5 then 0.07 µg/kg bw TCDD; weekly; 15 weeks (normal, marginal, low Vit A)	+		
	F; Fisher F344	PH; 10 mg/kg bw DEN	oral; 2 weeks interval	0.14 µg/kg bw TCDD; biweekly; 6 months	s.c.	+	15
	F; Sprague-Dawley	PH; 30 mg/kg bw DEN	i.p.; 35 days interval	0.22 then 0.044 µg/kg bw TCDD; weekly; 20 weeks	s.c.	+	16
				0.88 then 0.175 µg/kg bw TCDD; weekly; 20 weeks			
				3.5 then 0.7 µg/kg bw TCDD; weekly; 20 weeks			
	F; Sprague-Dawley	200 mg/kg bw DEN	i.p.; 7 days interval	1.4 µg/kg bw TCDD; biweekly; 30 weeks	oral	+	
						+ (ovariecto-mized)	

Table 2. Continued

Species	Strain & sex	Initiation dose	Administration	Dose & frequency of TCDD	Administration	Promotion	Reference
	F; Sprague-Dawley	PH; 10 mg/kg bw DEN	oral; 7 days interval	0.14 µg/kg bw TCDD; biweekly; 1 month	i.p.	+	18
				0.14 µg/kg bw TCDD; biweekly; 3 month			
				0.14 µg/kg bw TCDD; biweekly; 5 month			
	F; Sprague-Dawley	175 mg/kg bw DEN	i.p.; 14 days interval	3.5 ng/kg bw TCDD; biweekly; 30 weeks	oral	–	19
				10.7 ng/kg bw TCDD; biweekly; 30 weeks	–		
				35.7 ng/kg bw TCDD; biweekly; 30 weeks	–		
				125 ng/kg bw TCDD; biweekly; 30 weeks	+		
	F; Wistar	5 × 10 mg/kg bw DEN	oral; 14 days interval	1.4 µg/kg bw TCDD; biweekly; 9 weeks	s.c.	+	20
				1.4 µg/kg bw TCDD; biweekly; 13 weeks			
				1.4 µg/kg bw TCDD; biweekly; 17 weeks			
	F; Wistar	80 mg/l n-nitro-somorpholine in drinking water for 25 days	oral; 14 days interval	2, 20, 200 ng/kg bw TCDD; daily; 13 weeks	s.c.	+	21
	F; Sprague-Dawley	10 mg/kg bw DEN	i.p.; 30 days interval	0.007 µg/kg bw TCDD/day (150 ppt in diet) until day 450	oral	+	22
			i.p.; 170 days interval	0.007 µg/kg bw TCDD/day (150 ppt in diet) until day 450	+		
			i.p.; 240 days interval	0.007 µg/kg bw TCDD/day (150 ppt in diet) until day 450	+		
	F; Sprague-Dawley	PH; 30 mg/kg bw DEN	i.p.; 35 days interval	0.5 then 0.1 µg/kg bw TCDD; weekly; 20 weeks	s.c.	–	23
				1.58 then 0.316 µg/kg bw TCDD; weekly; 20 weeks	–		
				5 then 1 µg/kg bw TCDD; weekly; 20 weeks	+		
	F; Wistar	10x10 mg/kg bw DEN	oral; 56 days interval	1.4 µg/kg bw TCDD once	s.c.	–	24
				1.4 µg/kg bw TCDD biweekly; 16 weeks	+		
	F; Sprague-Dawley	175 mg/kg bw DEN	i.p.; 14 days interval	1.75 µg/kg bw TCDD; biweekly; 30 weeks	oral	+	25

a) Abbreviations: bw, body weight; DEN, diethyl-N-nitrosamine; DMBA, 7,12-dimethylbenz[a]anthracene; DMN, dimethyl-N-nitrosamine; F, female; i.m, intramuscular; M, male; MNNG, N-methyl-N′-nitro-N-nitrosoguanidine; PH, partial hepatectomy; s.c., subcutaneous.

3.2 The aryl hydrocarbon receptor

Since early investigations had revealed that the acute toxic and biochemical effects of TCDD were mainly if not exclusively dependent on its binding to the cytoplasmic aryl hydrocarbon receptor (AhR), it appeared likely that the AhR also plays a central role in the carcinogenicity of TCDD. The AhR is a member of the basic helix-loop-helix (bHLH) family of proteins [38, 39]. The bHLH motif is active in dimerization of the AhR with other proteins, and in DNA binding. The AhR functions as a ligand-activated nuclear transcription factor. Upon binding of agonists such as TCDD it forms a heterodimer with a structurally related protein called AhR nuclear translocator (ARNT). The dimeric complex binds to characteristic sequences in the promotor region of responsive genes. Binding to core DNA-sequences called xenobiotic-responsive elements (XRE) is needed for enhanced transcription of those genes *via* effective transactivation domains in the C-terminal portion of both the AhR and ARNT [40–42]. Many of the AhR/ARNT-regulated genes encode drug-metabolizing enzymes such as cytochrome P450 (CYP) 1A1, 1A2, 1B1 and a variety of phase II conjugating enzymes. Among the polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls, only those congeners bearing lateral chlorine substituents exhibit a signifi-

cant affinity to the AhR [43]. Among the polychlorinated biphenyls, chlorine substituents at one or more *ortho* positions lead to a decrease or complete loss of AhR affinity, probably because of a disturbance of the planar structure of the polychlorinated biphenyl molecule. The AhR-dependent phase I enzymes, most notably CYP1A1 also prefer planar substrates such as polycyclic aromatic hydrocarbons (PAH). Thus, it is tempting to speculate that induction of drug metabolism is aimed, at least in part, at the enhanced metabolic conversion and elimination of (the) AhR agonist(s) and/or its intermediate metabolites.

A variety of studies have dealt with the effects of AhR polymorphisms or variants in either spontaneous or TCDD-mediated carcinogenicity. In a transgenic mouse line expressing a constitutively active AhR (CA-AhR) spontaneous development of stomach tumors was reported [44]. Treatment of CA-AhR mice with DEN resulted in a pronounced increase in liver tumors when compared to DEN-treated wild-type mice [45]. Han/Wistar rats being relatively resistant to the acute toxicity of TCDD, bearing an AhR with an altered transactivation domain, were exceptionally resistant to liver tumor promotion by TCDD when compared to the more sensitive Long-Evans strain [46].

3.3 The role of drug metabolism

While AhR-regulated CYP are well known to play a central role in the metabolic activation of carcinogenic polycyclic hydrocarbons, it is unclear which role is played by the induction of drug metabolism in the carcinogenicity of TCDD and related potent AhR agonists. Park *et al*. [47] reported an enhanced release of 8-oxo-guanine from TCDD-treated Hepa1c1c7 cells. The authors postulated that induced CYP1A1 is the source for reactive oxygen species (ROS), leading to oxidative DNA modification. Treatment of rats with TCDD resulted in a marked increase in 8-oxo-deoxyguanosine (8-oxo-dG), a hallmark of oxidative DNA damage, in liver DNA. Since this effect was much more pronounced in the livers of female rats, which develop significantly more liver tumors at lower doses of TCDD, a correlation between 8-oxo-DG formation and TCDD-induced rat liver cancer was postulated [48]. In fact, the development of preneoplastic hepatic foci was significantly enhanced when male rats received TCDD together with 17β-estradiol [49]. Since ovariectomy led to a strong reduction in 8-oxo-dG formation in female rats [48], it could be speculated that 4-hydroxyestradiol, an estradiol metabolite formed by AhR-regulated CYP, may undergo redox-cycling *via* the intermediate formation of the corresponding catechol/semiquinone [50] and may thus act as a source for ROS. Alternatively, CYP induced by TCDD treatment may release ROS directly and may thus lead to oxidative DNA damage [47, 51]. The latter scenario does not explain, however, why female rats are much more sensitive to the hepatocarcinogenic action of TCDD. Possibly, ROS defense mechanisms including repair of oxidative DNA lesions are also affected by the presence of estrogens. In addition, estradiol can be activated via CYP-catalyzed formation of 4-hydroxy-estradiol. The latter is a precursor of the electrophilic estradiol-3,4-quinone that reacts with DNA *in vitro* [52].

A direct influence of TCDD on double strand-break repair was found by Chan *et al*. [53] in Chinese hamster ovary cells. By microarray analysis, Fletcher *et al*. [54] found that in rats relatively low doses of TCDD induced the hepatic expression of genes indicative for cellular stress or DNA damage. Treatment of mice with TCDD led to elevated hepatic levels of p53 and p21Waf1 [55], possibly indicating DNA damage.

Induction of drug-metabolizing enzymes is a common feature of many liver tumor promoters. Evidence was provided for a correlation between the tumor-promoting potency in rat liver and the efficacy of induction [56]. The mechanistic link between both effects is unclear, however.

Another link between altered drug metabolism and carcinogenicity of TCDD may exist with respect to the thyroid. Decreased serum thyroid hormone levels have been observed in laboratory animals after TCDD treatment [57 – 60]. This effect may be related to enhanced thyroid hormone glucuronidation resulting from induction of an AhR-regulated UDP-glucuronosyltransferase (UGT). Enhanced glucuronidation of thyroid hormones [61, 62] and/or induced biliary excretion of glucuronides [63] are thought to play a major role in reduction of thyroid hormone levels in blood. In response to this decrease the feedback suppression of the pituitary is released and thyroid-stimulating hormone (TSH) secretion is stimulated. Sustained growth stimulation of the thyroid resulting from enhanced TSH levels may facilitate the development (promotion) of thyroid tumors, as observed after phenobarbital treatment in two-stage rat carcinogenesis models [64, 65].

Another effect related to enhanced drug metabolism, is alteration in retinoid homeostasis. TCDD markedly decreases hepatic retinyl ester concentration in a variety of laboratory animal species [66 – 72]. In vitamin-storing hepatic stellate cells, TCDD treatment of rats resulted in a marked loss of retinol and retinyl palmitate. Nishimura *et al*. [73] reported that TCDD had no effects on retinoid levels in the liver of AhR-deficient mice. It was speculated that a loss of retinoids might facilitate the development of malignant tumors [74].

3.4 Altered regulation of growth and apoptosis

The interference of TCDD and related compounds with growth signaling was investigated in various cell types and tissues. It is noteworthy that a number of effects were reported to occur in a particular cell line but not in others. This fact highlights the need for detailed analysis of alterations in growth regulation in each individual experimental model.

In a number of cell types, TCDD decreased the binding of epidermal growth factor (EGF) and the auto-phosphorylation of the EGF receptor (EGFR) [75, 76]. It appears likely that the down-regulation of the EGFR is mediated by the AhR [77]. Interestingly, decreased EGF receptor binding was not observed in ovariectomized TCDD-treated rats, suggesting that growth-related signaling is altered depending upon ovarian hormones [78]. In fact, 10^{-12} M TCDD enhanced DNA synthesis stimulated by EGF and estradiol in cultured rat hepatocytes [79], while 10^{-9} M TCDD was ineffective or even inhibited DNA synthesis. The inhibitory action of TCDD on EGF-stimulated DNA synthesis was substantiated later in mouse hepatocytes in primary culture [80]. An interaction of TCDD and EGF was also observed in human epidermal cells, where TCDD prevented the EGF-dependent stimulation of telomerase activity in spontaneously immortalized but not in normal keratinocytes [81]. In confluent WB-F344 cells TCDD enhanced the otherwise low level of EGFR phosphorylation [82].

The expression and/or levels of other growth-related factors can be influenced by TCDD treatment in the case of transforming growth factor α, transforming growth factor β, and interleukin 1β [83–88]. In most cases, the effects were only seen in certain species, tissues or cell types, but not in others.

The expression of plasminogen activator inhibitor-2 (PAI-2) was induced in a human keratinocyte cell line [85] and in human hepatocytes in primary culture [89]. In *in vitro* and/or *in vivo* experimental models, TCDD treatment resulted in increased expression of *ras*, *erbA* [90], *c-fos* and *c-jun* [91]. Modulation of protein phosphorylation is frequently linked to effects on growth and/or apoptosis. TCDD enhanced protein kinase C activity in many cell types [92, 93], and induces tyrosine phosphorylation [94–96]. Furthermore, cyclin D2/cdk2 activity was increased in WB-F344 cells [97]. An AhR-dependent mechanism was suggested for the activation/hyperphosphorylation of p38–MAP kinase [98]. In confluent WB-F344 cells, TCDD treatment led to *c-Src* activation [99], and to transloaction of *c-Src* tyrosine kinase activity from the cytoplasm to the membrane fraction [82]. Among the target proteins showing increased phosphorylation in certain cell types are cyclin-dependent kinases [100], p53 [101], retinoblastoma protein [102], and EGFR [99] but not of ERK. In MCF-7 cells, Wang *et al*. [103] found attenuated responses of several estradiol-induced cell proteins/activities when estradiol was combined with TCDD treatment. A direct interaction of the AhR with the cell cycle regulator retinoblastoma protein was found by Puga *et al*. [104] in MCF-7 cells. Induction of N-myristoyltransferase 2 activity found in TCDD-treated 5L rat hepatoma cells, may affect myristoylation of signaling proteins involved in carcinogenesis [105].

Effects of TCDD on apoptosis were found both *in vivo* and *in vitro*. In preneoplastic rat liver, TCDD treatment led to a decrease in the incidence of apoptosis, usually enhanced in preneoplastic *vs*. surrounding cells [24]. This effect was suggested to contribute to the tumor-promoting potency of TCDD in rat liver, as has been demonstrated for other tumor promoters [106]. In rat hepatocytes in primary culture, TCDD led to a suppression of UV-induced apoptosis and to a concomitant inhibition of the increase in the tumor suppressor p53 usually seen after UV irradiation [107]. Similar results were obtained by Park and Matsumura [108] in human MCF10A cells. The authors suggest that TCDD may act by mimicking the anti-apoptotic action of EGF through activation of the *c-Src*/ERK signaling pathway.

In TCDD-treated rats, Paajarvi *et al*. [109] found an attenuation of the hepatic p53 response to DNA-damaging agents and a concomitant decrease in apoptosis in an AhR-dependent manner. Furthermore, TCDD induced the p53 antagonist Mdm2 accompanied by enhanced Mdm2 phosphorylation at Ser166.

Another frequently observed property of tumor promoters is their suppressive action on intracellular gap junctional intercellular communication (GJIC) in target cells. This was also reported for TCDD, which inhibits GJIC in rat hepatocytes in primary culture [110]. The effect was suggested to be mediated by an AhR-dependent decrease in connexin 32 mRNA.

4 Concluding remarks

A variety of studies demonstrated the carcinogenicity of TCDD in rodents. Major target organs are the liver and thyroid, oral cavity and lung in rats, and the liver, thymus, and skin in mice. Interestingly, in rats a reduced incidence of mammary tumors was found.

The striking sex-difference in liver carcinogenicity of TCDD in Sprague-Dawley rats, mainly observed in females, led to the suggestion that ovarian hormones play an important role in this effect. Enhanced formation of ROS probably originating from massive induction of CYP may play a role as DNA-damaging, initiating event. Since CYP

induction by TCDD in rats is not clearly sex dependent, undefined, estrogen-dependent modulating factors must be involved. The better understanding of these mechanisms is crucial for the issue of species extrapolation of the liver carcinogenicity of TCDD. More work is also needed to elucidate the possible role of oxidative DNA damage in the other types and locations of tumors found in TCDD-treated rodents. The carcinogenic effect of TCDD on the thyroid may be explained by a relative lack of thyroid hormone, resulting from induced glucuronidation and elimination of thyroid hormones in TCDD-treated rats. Therefore, permanently enhanced TSH aimed at counter-balancing the relative deficiency in thyroid hormones, may act as a co-carcinogenic stimulator of thyroid growth. Further mechanistic research is required, to allow a scientifically based species comparison of this mechanism.

Furthermore, TCDD acts as a liver tumor promoter in rodents pre-treated with genotoxic hepatocarcinogens. For this effect, a variety of mechanisms have been suggested as crucial, including inhibition of apoptosis of preneoplastic hepatocytes, suppression of gap junctional intercellular communication, and release from intercellular/paracrine growth control. The molecular mechanisms responsible for these effects may have a common denominator, *e.g.* enhanced phosphorylation of signaling proteins crucial for growth regulation and apoptosis. It remains open, however, which kinase(s) are relevant for these effects and if the changes in phosphorylation are due to direct stimulation by the AhR and/or how they are related to the TCDD-mediated oxidative or cell stress accused to result, *e.g.* in DNA damage.

5 References

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