4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Kinetic data on PCDFs have been reviewed (Olson, 1994).

In an individual exposed accidentally to PCDFs during a fire in Binghamton, NY, United States (see page 364), elimination half-lives of 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF and 1,2,3,4,6,7,8-HpCDF were found to be 4–7 years (Schecter *et al.*, 1990a). Flesch-Janys *et al.* (1996a) investigated 48 workers who had been exposed to PCDDs and PCDFs in a herbicide-producing plant and calculated median half-lives that ranged from 3.0 years for 1,2,3,4,5,6,7,8-HpCDF to 19.6 years for 2,3,4,7,8-PCDF. In *yucheng* patients who had ingested PCB-contaminated rice oil in 1979 (see pages 362–363), half-lives of 2–3 years were found for 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF and 1,2,3,4,6,7,8-HpCDF during the period 1–12 years after the incident. *Yusho* patients were contaminated in 1968 and were examined 15–25 years after the incident showed considerably longer half-lives of 8–13 years (Ryan *et al.*, 1993; Masuda, 1996). These data suggest an increase in half-lives at lower dose levels. This behaviour is reflected in the kinetic model of Carrier *et al.* (1995b).

Half-lives of 1.3 years for 2,3,7,8-TCDF and 6.3 years for 2,3,4,7,8-PeCDF were calculated (Schlatter, 1991) using a method that compares daily intakes and body burdens of PCDFs of the normal population with those of 2,3,7,8-TCDD.

Concentrations of 2,3,4,7,8-PeCDF and 1,2,3,4,7,8- and 1,2,3,6,7,8-HxCDFs were 3–5-fold higher in adipose tissue than in liver (wet weight basis) in deceased *yusho* patients but considerably lower in adipose tissue than in liver in *yucheng* patients (Olafsson *et al.*, 1988). In the normal population, the concentration ratios of liver : fat (on a wet weight basis) were 0.2 for 2,3,4,7,8-PeCDF, 0.5 for OCDF and 1.1 for 1,2,3,4,6,7,8-HpCDF (Thoma *et al.*, 1990; Wacker *et al.*, 1990).

During one year of breast-feeding, PCDD and PCDF levels in human milk fell by 50–70% and those in the milk of mothers nursing their second child were 20–30% lower than those in the milk of mothers nursing their first child (Fürst *et al.*, 1989). These results are concordant with predictions of kinetic models (Carrier *et al.*, 1995b; Van der Molen *et al.*, 1996).

4.1.2 *Experimental systems*

(*a*) *Absorption*

The efficiency of absorption of 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF has been studied in rats, hamsters and guinea-pigs after oral uptake using oily vehicles. For both compounds, 70–90% absorption from the gastrointestinal tract was observed (Birnbaum *et al.*, 1980; Yoshimura *et al.*, 1986; Brewster & Birnbaum, 1987; Kamimura *et al.*,

1988). In the guinea-pig, gastrointestinal uptake of 2,3,7,8-TCDF was more efficient than that of 2,3,7,8-TCDD and this was attributed to higher solubility of the former compound (Nolan *et al.*, 1979; Decad *et al.*, 1981a). As with PCDDs, gastrointestinal absorption of PCDFs depends on the vehicle, molecular size and solubility of the congener. The latter two properties appear to be the more significant in decreasing absorption of the hepta- and octa-CDFs (McLachlan *et al.*, 1990). As for 2,3,7,8-TCDD, it was shown that enterohepatic circulation was not significant for 1,2,3,7,8-PeCDF or its metabolites in rats (Brewster & Birnbaum, 1988).

Percutaneous absorption of 2,3,4,7,8-PeCDF in rats was age-dependent, with much more effective uptake in younger animals (Banks *et al.*, 1990). The dermal absorption of 2,3,7,8-TCDF and 1,2,3,7,8- and 2,3,4,7,8-PeCDFs in rats was also found to be dose- and structure-dependent, with 2,3,7,8-TCDF absorbed most efficiently (Brewster *et al.*, 1989). Compared with oral uptake, skin permeation is much slower. After dermal application of 1,2,3,7,8-PeCDF to the skin of a rhesus monkey, 99% of the dose was still present at the application site after 6 h (Brewster *et al.*, 1988). The uptake of seven compounds from a dermal application site showed a good inverse correlation with the octanol–water partition coefficients (Jackson *et al.*, 1993).

As with PCDDs, the adsorption of PCDFs on environmental matrices such as soil and combustion particles can strongly reduce the bioavailability of these compounds. The oral bioavailability factor which has been suggested for PCDDs (25–50% for Cl_4- and Cl_6-congeners) can also be considered applicable to PCDFs (Van den Berg *et al.*, 1994).

(*b*) *Distribution*

The PCDFs have a similar tissue distribution to that of the PCDDs in both rodents and non-human primates, the liver, adipose tissue and skin being the major storage sites. The 2,3,7,8-substituted PCDFs are the major congeners retained in most mammalian tissues and fluids. In this respect, the guinea-pig forms a distinct exception, as it also retains in the liver PCDFs with a 2,3,(4),6,7-chlorine substitution pattern, which apparently cannot be effectively metabolized by the cytochrome P450 system of the guinea-pig (Van den Berg *et al.*, 1986c; Ahlborg *et al.*, 1990). In other rodent species, the retention of these 'pseudolateral' PCDFs (for example, 2,3,4,6,7-PeCDF) is rarely observed (Van den Berg *et al.*, 1994). Some tissue retention of non-2,3,7,8-substituted PCDFs has been found in rats and marmosets (Abraham *et al.*, 1989; Neubert *et al.*, 1990a), but the concentrations observed are not considered to be toxicologically relevant when compared with the predominance of 2,3,7,8-substituted congeners. An increasing binding affinity to plasma proteins is found for the higher-chlorinated PCDFs and binding to plasma proteins and lipoproteins appears to be the major mode of transport in the blood (Patterson *et al.*, 1989; Schecter *et al.*, 1990e).

Studies with 2,3,7,8-TCDF and -TCDD have shown these compounds to have similar tissue distribution in the rat (Birnbaum *et al.*, 1980). A number of higher-chlorinated PCDFs, especially 2,3,4,7,8-PeCDF, have a much higher liver affinity in rodents than 2,3,7,8-TCDD. For these PCDFs, liver retention of 75–90% of the administered dose has been reported (Van den Berg *et al.*, 1994). Studies with mixtures of both PCDDs and PCDFs showed that tissue distribution in rats and hamsters was not significantly different

between the hepta- and octa-CDFs and -CDDs (Van den Berg *et al.*, 1986c, 1987). In rhesus monkeys, the liver retention of 2,3,4,7,8-PeCDF, which is unusually high in rodents, is lower and not much different from that of 2,3,7,8-TCDD in rhesus monkeys (Kuroki *et al.*, 1980; Brewster *et al.*, 1988). In contrast, marmosets more closely resemble rats in tissue distribution of PCDFs as well as PCDDs (Abraham *et al.*, 1989). As with 2,3,7,8-TCDD, dose-dependent hepatic retention of PCDFs has been observed in a number of rodent studies, but some other studies have not found this dose-dependence (Van den Berg *et al.*, 1994). With respect to the occurrence of inducible hepatic binding sites in rodent liver (Poland *et al.*, 1989a), it should be noted that 2,3,4,7,8-PeCDF is a strongly binding substrate as well as an inducer of CYP1A2 (Yoshimura *et al.*, 1984; Kuroki *et al.*, 1986; Yoshimura *et al.*, 1987).

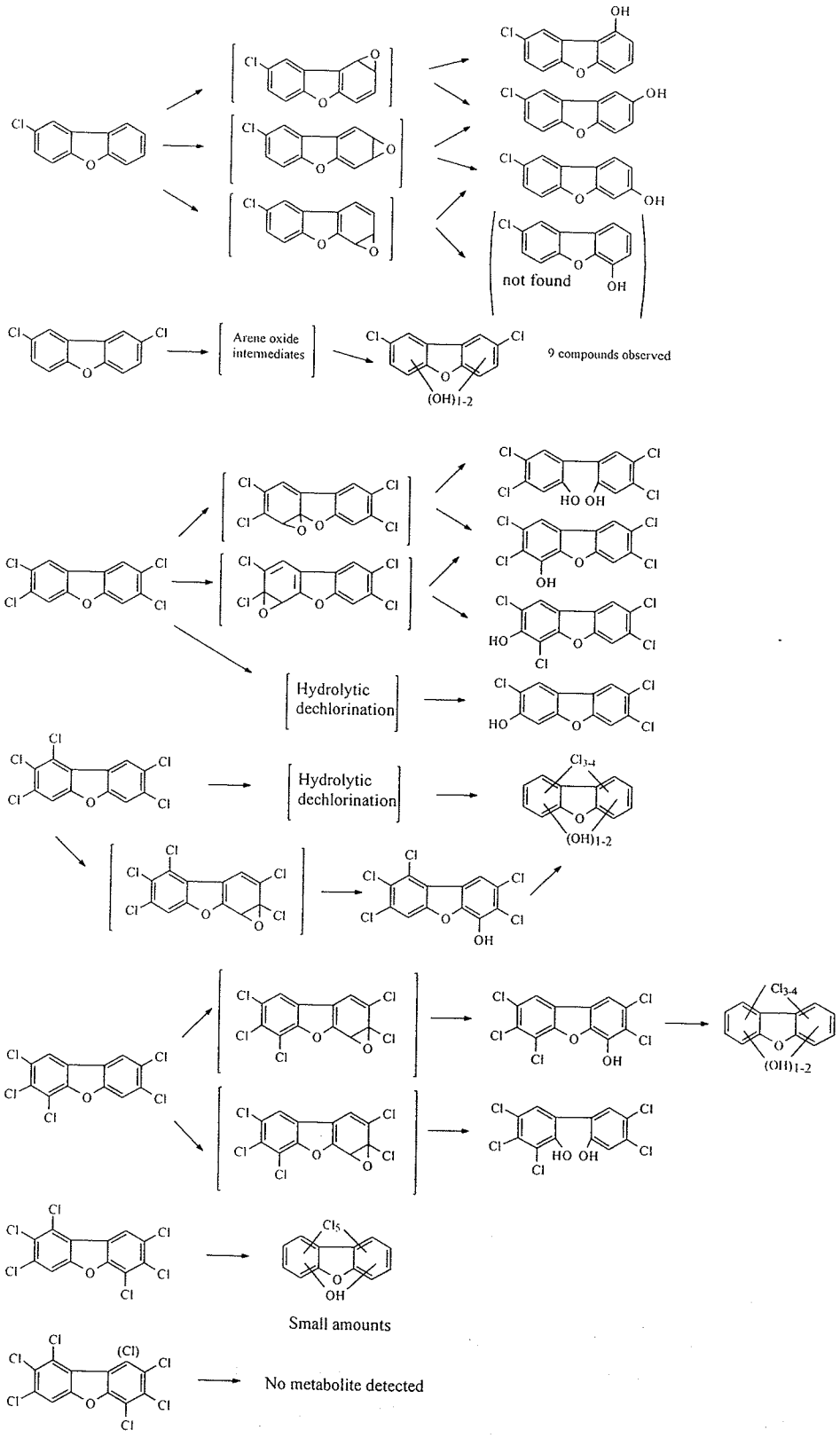
(*c*) *Metabolism*

As with the PCDDs, the oxidation of PCDFs occurs preferentially on the 2, 3, 7 or 8 positions, yielding a higher number of hydroxylated metabolites than with the PCDDs due to the asymmetric structure of the dibenzofuran molecule (Veerkamp *et al.*, 1981; Poiger *et al.*, 1989). Based on studies with rats and 2,3,7,8-TCDF, it appears that the preferred site of metabolism of 2,3,7,8-TCDF is near the furan oxygen, with oxygenation at C4 predominating over oxygenation at C3 (Burka *et al.*, 1990). In rats, the CYP1A1 protein is directly involved in phase I metabolism of 2,3,7,8-TCDF (Olson *et al.*, 1994) and not the CYP1A2 protein (Tai *et al.*, 1993). Sulfur-containing metabolites have also been observed as minor metabolites, with S substitution preferentially at the 4 position (Kuroki *et al.*, 1989, 1990). In contrast to the PCDDs, the 4-4a positions in the dibenzofuran molecule are more susceptible to metabolic mixed function oxidase attack (Plüss *et al.*, 1987; Burka *et al.*, 1990). As a result, the biotransformation of 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF is much more rapid than that of their dioxin analogues. If chlorine atoms on the 4 or 6 position are present in a 2,3,7,8-substituted PCDF, metabolism is strongly decreased, leading to an extremely low rate of elimination from the body (Brewster & Birnbaum, 1987, 1988; Van den Berg *et al.*, 1989a,b). Further chlorination of 2,3,7,8-substituted PCDFs results in a decrease in the number of metabolites and elimination rate (Veerkamp *et al.*, 1981; Poiger *et al.*, 1989). Virtually no information is available on differences between species in PCDF metabolism. In **Figure 1**, a generalized scheme for metabolic pathways of PCDFs is given, which is based on mammalian studies *in vivo* (Van den Berg *et al.*, 1994).

(*d*) *Excretion*

The elimination of PCDFs, like that of the PCDDs, depends strongly on the position of the chlorine atoms. Those congeners with a 2,3,7,8-chlorine substitution pattern exhibit the slowest elimination rates in all laboratory species studied. As PCDFs are stored primarily in the liver and adipose tissue, the whole-body half-life of these compounds is governed mainly by the elimination from these two body compartments. Although kinetic information for PCDFs is more limited, elimination rates and half-lives

Figure 1. A generalized scheme of pathways for the biotransformation of PCDFs based on the information from in-vivo mammalian studies



From Van den Berg *et al.* (1994)

appear to be similar to those of the PCDDs (Van den Berg *et al.*, 1994). Exceptions are seen with 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF, for which elimination in rodents is much faster than for the other 2,3,7,8-substituted PCDFs. This rapid elimination can be directly attributed to the higher susceptibility of the C-4 position to metabolic attack in the dibenzofuran molecule. The presence of a chlorine atom on the C-4 position dramatically decreases the rate of elimination (Birnbaum *et al.*, 1981; Brewster & Birnbaum, 1988; Brewster *et al.*, 1988; Van den Berg *et al.*, 1989a,b; Ahlborg *et al.*, 1990). As a result, the half-life in the liver of the rat increases from several days for 1,2,3,7,8-PeCDF to more than 100 days for 2,3,4,7,8-PeCDF (Brewster & Birnbaum, 1988; Van den Berg *et al.*, 1989b). This importance of chlorine substitution on the 4/6 position is also seen in the short half-life of 1,2,3,7,8,9-HxCDF of less than 10 days in rats, compared with those of 2,3,4,6,7,8-HxCDF, 1,2,3,6,7,8-HxCDF and 1,2,3,4,7,8-HxCDF (Ahlborg *et al.*, 1990). Guinea-pigs eliminate 2,3,7,8-TCDF less efficiently than mice, the half-life in guinea-pigs being 20 days, compared with 4 days in DBA/2J mice and just 2 days in C57BL/6J mice (Decad *et al.*, 1981a,b; Ioannou *et al.*, 1983). The fact that the acute toxicities of 2,3,7,8-TCDD and -TCDF in the guinea-pig are in the same range has been attributed to the limited ability of this species to metabolize and eliminate the latter congener (Van den Berg *et al.*, 1994).

As in rodents, the elimination rates of 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF in primates are faster than those of the other 2,3,7,8-substituted congeners. The half-lives for both compounds in rhesus monkeys and marmosets have been estimated to be approximately one week or less (Birnbaum *et al.*, 1981; Neubert *et al.*, 1990a,b). As for PCDDs, the elimination of PCDFs from most body compartments can be described as a one-compartment open model, but models with two- or three-phase eliminations for 2,3,7,8-TCDF in rodents and monkeys have been reported. In view of the non-linear distribution of PCDDs in many experimental systems, the use of physiologically based pharmacokinetic models has been successfully applied (Carrier *et al.*, 1995a,b).

4.2 Toxic effects

4.2.1 *Humans*

The results in this section are not lipid-based.

[The Working Group noted that in the *yusho* and *yucheng* incidents, exposures were to PCDFs and planar and non-planar PCBs and that one cannot unequivocally attribute the effects to one class of chemicals or the other.]

(*a*) *Non-cancer effects of ingestion of rice oil contaminated with polychlorinated dibenzofurans, quaterphenyls and biphenyls in Japan* (yusho) *and Taiwan* (yucheng)

In both groups, the most notable acute effects were dermatological and neurological signs and symptoms of fatigue, headaches and gastrointestinal distress (nausea, vomiting, abdominal pain) (Kuratsune, 1989; Rogan, 1989).

Yusho

The initial recognition of *yusho* occurred in 1968. About 2000 individuals were identified as part of the *yusho* population (Masuda *et al.*, 1985). Tissue concentrations of PCDFs in these people are given in **Table 18**.

Effects observed shortly after exposure included elevated triglyceride levels and effects on female reproductive hormones manifest as changes in menstrual and basal body temperature patterns and lowered excretion of oestrogens and pregnanediol in exposed women (Kuratsune, 1989). However, fertility and other measures of reproductive function were not evaluated. Evidence of chronic bronchitis and respiratory infections still remained 14 years after exposure ended (Nakanishi *et al.*, 1985). However, more than 10 years after exposure, PCB levels were not related to serum levels of triiodothyronine, thyroxine and thyroxine-binding globulin (Murai *et al.*, 1987). Although the liver is the suspected target organ for halogenated hydrocarbons and marked proliferation of the endoplasmic reticulum was observed in that organ, clinical evidence of liver damage, such as alterations in liver enzymes or liver disease, was not observed (Kuratsune, 1989).

Dermatological effects were the most evident signs, characterized by hyperpigmentation of the nails, gingivae and face and by nail deformities, horny plugs, comedones, acneform eruptions, cysts and other abnormal keratotic changes. Acneform eruptions were observed on the face, cheeks, auricles, retroauricular areas, inguinal regions and external genitalia (Urabe & Asahi, 1985). More than 80% of *yusho* cases experienced one or more dermatological effects (Kuratsune, 1989), which diminished in severity over time (Urabe & Asahi, 1985).

Ophthalmological effects were characterized by swelling and hypersecretion of the meibomian glands and pigmentary changes of the conjunctiva (Kuratsune *et al.*, 1972). More than 80% of *yusho* cases exhibited ocular changes, which, in some cases, appeared to persist 15 years after exposure ended (Kuratsune, 1989).

Thirty per cent of the cases reported having at least one symptom consistent with neurological involvement, such as limb paraesthesia and spasms, weakness, headaches and fatigue (Kuratsune, 1972). As summarized by Kuratsune (1989), Kuriowa *et al.* (1969) found mostly sensory deficits, identified through slowed nerve conduction velocities in 23 cases. Follow-up of these cases indicated that the neurological symptoms disappeared over time; however, conduction velocity measurements were not repeated.

A number of studies examined the immune status of *yusho* cases (Kuratsune, 1989). Significant decreases in mean IgA and IgM and increases in IgG were noted in 28 cases tested in 1970 ($p < 0.05$). Within two years, means levels of all three immunoglobulins returned to normal. Small increases in the percentage of $CD4^+$ cells, small decreases in the percentage of $CD8^+$ cells and enhanced lymphocyte stimulation were also noted in *yusho* cases (Nakanishi *et al.*, 1985).

Mortality in the *yusho* population was assessed among 1761 patients registered by the end of 1983. Among 887 men and 874 women, there were 79 and 41 deaths, respectively (Masuda, 1994). Mortality from chronic liver disease and cirrhosis was elevated in men only (6 deaths; SMR, 2.7).

Studies of offspring of *yusho* cases have been limited to descriptions of effects on newborns exposed *in utero*. An early description of 13 children born to exposed mothers noted two stillborn infants, one of whom was diffusely and deeply hyperpigmented (Rogan, 1982). Neonates described in other reports were darkly pigmented and had marked secretions of the conjunctival palpebra, gingival hyperplasia, hyperkeratosis, calcification of the skull, low birth weight and natal teeth (Yamashita & Hayashi, 1985). The abnormal pigmentation disappeared after 2–5 months. No other physical abnormalities (neurological, cardiovascular or malformations) were identified.

Yucheng

The initial recognition of *yucheng* occurred in 1979. As of 1983, approximately 2000 individuals were found to have been exposed to the contaminated rice oil. Serum concentrations of PCDFs in these people are given in **Table 19**.

The ophthalmological and dermatological changes observed in *yucheng* cases were very similar in character and anatomical distribution to those noted in *yusho* cases (Lü & Wu, 1985). In 89 cases followed for up to 17 months, dermatological conditions of 38% of the cases improved, 54% remained the same and 7% showed deterioration of their condition (Lü & Wong, 1984).

Like *yusho* cases, *yucheng* cases examined within two years of exposure for nerve function exhibited slowing of sensory nerve conduction. They also exhibited motor nerve slowing and mixed deficits (Chen *et al.*, 1981, 1983; Chia & Chu, 1984; Chen *et al.*, 1985a). Of a population of 27 individuals, 20% also had abnormal electroencephalograms (EEGs) (Chia & Chu, 1984). However, the authors suggested that any correlation between PCB exposure and the abnormal EEGs might be spurious due to low PCB levels in the cerebrospinal fluid (0.5–2.3 μg/kg, measured in four subjects), despite much higher blood PCB levels of 48–64 μg/kg. A sample of 28 individuals with peripheral neuropathy in 1980 was re-examined in 1982 and was found to have normal EEGs and some recovery of sensory and motor nerve conduction velocity (Chia & Chu, 1985).

In 1981, immunological function was assessed on several subsets of *yucheng* cases and summarized by Lü and Wong (1984). In 30 cases compared with unexposed controls, both IgA and IgM were significantly decreased, while IgG did not differ from controls. In this same group, percentages of active T cells and T cells (E-rosette lymphocytes) were significantly decreased ($p < 0.05$), while total lymphocyte count and percentage of B cells were unchanged. Significant decreases in helper T cells (T4) but not suppressor T cells (T8) were also observed. In another group of cases, response to lymphocyte-stimulating mitogens was mixed and the findings were unclear. In 143 cases, reaction to streptococci antigen appeared to be significantly ($p < 0.05$) depressed relative to controls.

Alterations in porphyrin levels and liver enzymes have been identified as acute reactions to exposure to halogenated polycyclic hydrocarbons, including PCBs. Porphyrin levels were measured in two exposed groups (Chang *et al.*, 1980; Gladen *et al.*, 1988). In 1980, statistically significant elevations in 24-h urinary excretion of uroporphyrin (exposed, 41.23 μg ± 24.56; unexposed, 13.57 μg ± 11.76; $p < 0.01$) and

α-aminolaevulinic acid (exposed, 1.002 mg ± 0.600; unexposed, 0.715 mg ± 0.337; $p < 0.05$) were noted among 69 poisoned and 20 normal subjects (Chang *et al.*, 1980). Coproporphyrin and porphobilinogen levels were increased (but not significantly) in the exposed group. The second study group was composed of 75 children born between June 1978 and March 1985 to mothers who ingested contaminated rice oil (Gladen *et al.*, 1988). Spot urines were collected in 1985. Mean total porphyrin (exposed, 95.2 µg/L; unexposed, 80.7 µg/L) and coproporphyrin (exposed, 72.4 µg/L; unexposed, 59.8 µg/L) excretion was elevated in the exposed, possibly due to extremely high levels (> 200 µg/L) in eight exposed children and two controls (Rogan *et al.*, 1988). However, no porphyria cutanea tarda, a severe form of porphyria, was observed in either group of children. Moderate, but statistically significant, increases were observed in aspartate transaminase and alanine transaminase levels in 23 cases tested one year after exposure (Lü & Wong, 1984). Lactate dehydrogenase and bilirubin levels were not significantly elevated. As in *yusho* cases, triglyceride levels were significantly increased to approximately twice the level in unexposed controls.

Effects observed in offspring of *yucheng* cases are described in Section 4.4.1.

4.2.2 *Experimental studies*

(*a*) *Species comparisons of toxic effects*

(i) *General effects*

Thirteen-week dietary studies of Sprague-Dawley rats given 1,2,3,4,8-PeCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF or 1,2,3,6,7,8-HxCDF revealed that both toxicity, including body weight loss and thymic atrophy, and depletion of hepatic vitamin A followed the rank order of the compounds to bind to the Ah receptor and to induce CYP1A activity (Plüss *et al.*, 1988a,b; Håkansson *et al.*, 1990). When these PCDFs were administered as a mixture, it was observed that the individual PCDF toxicity was additive (Plüss *et al.*, 1988b). Toxicity of 1,2,3,7,8-PeCDF was significantly lower than that of 2,3,4,7,8-PeCDF and this was attributed to rapid detoxification by biotransformation (see Section 4.1.2(*c*)) (Brewster & Birnbaum, 1988; Plüss *et al.*, 1988a).

In male Sprague-Dawley rats fed 10 mg 2,3,7,8-TCDF per kg of diet, thymus, ventral prostate and seminal vesicle weights were significantly decreased (Oishi *et al.*, 1978), and haemoglobin and haematocrit values were reduced.

Guinea-pigs given a single oral dose of 1–15 µg/kg 2,3,7,8-TCDF or 2,3,4,7,8-PeCDF showed a reduction in body weight gain. All animals that received 10 µg/kg or 15 µg/kg 2,3,7,8-TCDF or 2,3,4,7,8-PeCDF died 9–20 days after dosing. In mice, 22 daily oral doses of 30–300 µg/kg bw/day 2,3,7,8-TCDF did not induce clinical signs of toxicity (Moore *et al.*, 1979). In contrast with other rodents, 2,3,7,8-TCDF was highly toxic to guinea-pigs, the acute toxicity being similar to that of 2,3,7,8-TCDD in this species (Ioannou *et al.*, 1983; Moore *et al.*, 1979). The high sensitivity of the guinea-pig was attributed to the low biotransformation rate in this species (see Section 4.1.2(*b*)) (Van den Berg *et al.*, 1994).

A single dose of 1500 µg/kg 2,3,7,8-TCDF was lethal to 2/2 rhesus monkeys (Moore *et al.*, 1979). Those animals which survived a dose of 1000 µg/kg (2/4) developed facial

oedema and loss of eyelashes, fingernails and toenails. Most animals accumulated a white, wax-like exudate in the ear canal and showed a dry, leathery texture of the skin. Blood analysis 28 days after dosing revealed mild anaemia, relative lymphopenia and marked relative and absolute neutrophilia. Serum cholesterol was decreased by 33–50%. Major microscopic lesions were hyperkeratosis of the epidermis, squamous metaplasia of the meibomian and ceruminous glands (eyelid and ear canal, respectively) and a dilatation of hair follicles filled with keratinaceous debris in the facial area. The thymus showed extensive reduction of the cortex and necrotic debris in the medulla. Furthermore, the bile duct mucosa was found to be extremely hyperplastic with cystic dilatation and inflammation. Mild skin lesions were also found in the two rhesus monkeys that had received 500 μg/kg.

Packed blood cell volume and serum triglyceride and bile acid concentrations were significantly increased in rhesus monkeys after a single intravenous dose of 34 μg/kg 2,3,4,7,8-PeCDF (Brewster *et al.*, 1988). Serum cholesterol, protein, albumin, triiodothyronine and thyroxine concentrations were decreased. After 28–58 days, the animals exhibited alopecia, hyperkeratinization of the toe- and finger-nails, facial chloracne-like lesions and loss of body weight. Two out of three animals subsequently died. Pathological findings indicated hyperplastic and metaplastic changes in the gastric mucosa, the meibomian glands of the eyelid and the ceruminous glands of the ear.

In cynomolgus monkeys (*Macaca fascicularis*), PCB mixtures similar to those ingested by *yusho* patients but without PCDFs caused immunosuppression and enlargement and histopathological changes of the liver (interstitial inflammation, proliferation of bile-duct epithelial cells). Treatment with a PCDF-containing PCB mixture, however, led to more pronounced decreases in body weight, immunosuppression, fatty liver and histopathological changes. In addition, the PCDF-containing mixture caused hair loss, acneform skin eruptions, oedema of the eyelid, congestions and abscesses of the meibomian gland and cornification of the skin (Hori *et al.*, 1982).

(ii) *Skin*

In haired and hairless newborn and adult mice, dermal application of 2,3,4,7,8-PeCDF or 1,2,3,4,7,8-HxCDF caused involution of sebaceous glands (Puhvel & Sakamoto, 1988). Epidermal hyperplasia and hyperkeratinization, however, were induced in the hairless mice only. The density of inflammatory cell infiltrates in the dermis was not reduced by topical treatment with anti-inflammatory agents. The distinct pattern of chloracne observable in hairless mice (Puhvel *et al.*, 1982) did not include hyperkeratinization of the sebaceous follicles typical of human chloracne. Histopathological changes observed with all acnegenic compounds were epidermal hyperkeratosis and hyperplasia, loss of sebaceous glands, keratinization of intradermal pilar cysts and diffuse lymphohistiocytic infiltration of the dermis. Atrophy or complete absence of the hair follicles were evident in severe lesions (Hébert *et al.*, 1990a). In these cases, the epidermis was hypoplastic with increased keratin on the surface. The data for dermal toxicity and changes in body weight and organ weights indicated that 2,3,4,7,8-PeCDF was 0.2–0.4 times, and 1,2,3,4,7,8- HxCDF 0.08–0.16 times, as toxic as 2,3,7,8-TCDD following repeated dermal exposure.

The ability of several PCDFs to induce a flat, cobblestone-like morphology in cell cultures was studied in a nonkeratinizing derivative (XBF) of the keratinizing XB mouse epithelial cell line cocultured with irradiated 3T3 feeder cells. The minimum concentrations required to produce these changes from the normal spindle-shape cells, over a 14-day exposure period, were > 2.38 μg/kg for 2,6-DCDF, 0.032 μg/kg for 2,3,7,8-TCDF, 0.378 μg/kg for 2,3,4,6,7,8-HxCDF and 4.48 μg/kg for OCDF (Gierthy & Crane, 1985).

Osborne and Greenlee (1985) reported that 2,3,7,8-TCDF decreased DNA synthesis, proliferation and epidermal growth factor (EGF) binding, and induced differentiation in several lines of human keratinocytes.

(iii) *Liver*

While no histopathological signs of liver damage were observed in guinea-pigs treated with 15 μg/kg 2,3,7,8-TCDF or 20 μg/kg 2,3,4,7,8-PeCDF, Sprague-Dawley rats showed liver cell vacuolization, necrosis of single hepatocytes and Kupffer cell hypoplasia after treatment with 1,2,3,6,7,8-HxCDF (200 μg/kg in the diet). These alterations were less pronounced with 1,2,3,7,8-PeCDF (200 μg/kg in the diet) and 1,2,3,6,7,8-HxCDF (20 μg/kg in the diet). No liver lesions were observed after administration of 1,2,3,4,8-PeCDF (6000 μg/kg in diet) (Plüss *et al.*, 1988a).

C57BL/6fh(J67) mice receiving 22 daily oral doses of 300 μg/kg 2,3,7,8-TCDF showed a 17% increase in liver weight and a 25% increase in liver/body weight ratio; fluorescence indicative of porphyria was not observed. Guinea-pigs receiving single oral doses of up to 15 μg/kg 2,3,7,8-TCDF did not develop liver pathology. In rhesus monkeys, single oral doses of 2,3,7,8-TCDF up to 1500 μg/kg resulted in inconsistently increased liver weight but no histopathological liver lesion (Moore *et al.*, 1979). Brewster *et al.* (1988) did not report histopathological liver changes except for deposits of haemosiderin in Kupffer cells after administration of a single intravenous dose of 34 μg/kg 2,3,4,7,8-PeCDF to rhesus monkeys.

(*b*) *Immunological responses*

Only five out of 135 PCDF congeners have been studied for their effects on the mammalian immune system (Holsapple, 1995).

Kerkvliet *et al.* (1985) studied the humoral immunosuppressive effect of a single oral dose of 1,2,3,4,6,7,8-HpCDF in C57BL/6 mice, two days before sheep red blood cell (SRBC) challenge. Splenic IgM antibody response was measured five days later ('HAIR-assay'). The 50% immunosuppressive dose (ID_{50}) was calculated as 208 μg/kg, while the ID_{50} for 1,2,3,4,5,6,7,8-HpCDD was 85 μg/kg. [For comparison, the ID_{50} for 2,3,7,8-TCDD was 0.74 μg/kg (Kerkvliet & Brauner, 1990)].

Davis and Safe (1991) compared the effects of a series of congeners with respect to their suppression of the in-vitro plaque-forming anti-SRBC response using cells from either C57BL/6 or DBA/2 mice. The immunosuppressive potencies of 2,3,4,7,8-PeCDF, 2,3,7,8-TCDF, 1,2,3,7,9-PeCDF and 1,3,6,8-TCDF in this in-vitro assay were similar to each other and to that of 2,3,7,8-TCDD, using spleen cell cultures from both mouse

strains, although *in vivo* their immunotoxic potentials differ by up to 14 900-fold in C57BL/6 mice (Davis & Safe, 1988).

Harper *et al.* (1993) studied the effects of a single intraperitoneal injection of 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, 1,2,3,7,9-PeCDF or 1,3,6,8-TCDF on the splenic plaque-forming cell (PFC) response to the T-cell-independent antigen TNP-LPS in C57BL/6 and DBA/2 mice. The effective doses (μg/kg) required to decrease by 50% (ED_{50}) the end-point 'PFCs/10^6 viable cells' were:

Congener	C57BL/6 mice	DBA/2 mice
2,3,7,8-TCDD	1.5	9.7
2,3,4,7,8-PeCDF	2.0	2.6
1,2,3,7,9-PeCDF	391	4 690
1,3,6,8-TCDF	1 484	17 167

Similarly designed experiments were performed with B6C3F1 mice. The effects induced by the same four congeners after intraperitoneal injection were compared with those observed after in-vitro exposure of mouse splenocytes. The ED_{50} of the PFC response to 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDF and 1,3,6,8-TCDF was 14.1, 5.5, 1695 and 34 800 nmol/kg, respectively. Corresponding values derived from in-vitro studies were 7.0, 10.6, 149 and 2325 nM, respectively (Harper *et al.*, 1995).

Vecchi *et al.* (1983) studied the suppressive effects of a single intraperitoneal injection of 180 μg/kg bw 2,3,7,8-TCDF on antibody production in C57BL/6 mice and in DBA/2 mice. A pronounced decrease in the number of PFC as a response to the injection of SRBCs was observed in C57BL/6 mice only.

A single dose of 20 ng/kg 2,3,4,7,8-PeCDF had no effect on the proportions of sub-populations of lymphocytes in peripheral blood of marmosets, studied by flow cytometry. In contrast, 10 μg/kg bw 2,3,7,8-TCDD induced a decrease in the number of $CD20^+$ cells and the number of $CD4^+$ $CDW29^+$ cells (Neubert *et al.*, 1993b).

(c) *Biochemical responses*

There appear to have been few studies of the biochemical responses attributable to PCDF exposure, other than those on induction of CYP1A1 and CYP1A2 expression (see Section 4.3).

EGF receptor autophosphorylation was decreased in placenta after in-utero exposure to PCBs and PCDFs ingested from contaminated rice oil in the *yucheng* incident (Sunahara *et al.*, 1987). In contrast, EGF receptor expression was increased in mouse embryonic palatal medial epithelial cells (Abbott & Birnbaum, 1989b). Support for the role of the Ah receptor in mediating downregulation of the EGF receptor was supported by structure–activity studies in mice (Ryan *et al.*, 1989b) and the differential responsiveness of congenic mice differing only at the Ah locus (Lin *et al.*, 1991a).

4.3 Interaction with Ah receptor and its early molecular consequences and other biochemical responses

Laterally substituted PCDF congeners bind to the Ah receptor and produce the same biological and toxic effects as the PCDDs. Among these PCDFs, the congeners with the 2,3,7,8-chlorine substitution pattern are the most potent ones (Poland & Knutson, 1982; Safe, 1990). The binding affinities of 2,3,7,8-TCDF, 1,2,3,7,8- and 2,3,4,7,8-PeCDFs to the Ah receptor are of the same order of magnitude as that of 2,3,7,8-TCDD (see Section 4.3 in the monograph on PCCDs in this volume). The Ah receptor binding affinity for the class of congeners is dependent upon the extent and pattern of chlorination (Whitlock, 1986; Okey, 1990; Safe, 1990).

In general, induction of *CYP1A1* gene expression by PCDFs tends to follow the same rank order of potency as receptor binding *in vitro*. Like many planar aromatic substances, including 2,3,7,8-TCDD, the 2,3,7,8-substituted PCDFs also induce CYP1A2 and bind strongly to this protein (Yoshimura *et al.*, 1984; Kuroki *et al.*, 1986; Poland *et al.*, 1989b). Ah receptor-regulated genes encoding phase-two metabolizing enzymes (e.g., UDP-glucuronosyl transferase and DT diaphorase) are also induced following PCDF exposure, but information is limited (Safe, 1990; Van den Berg *et al.*, 1994).

Although the binding of some 2,3,7,8-substituted PCDFs to the Ah receptor and associated *CYP1A1* induction *in vitro* is similar to that of 2,3,7,8-TCDD, the general toxicity of some of these congeners is significantly lower due to faster biotransformation in several rodent species. This is especially the case for 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF (Van den Berg *et al.*, 1994).

Enzyme induction has been observed in both pre- and postnatally exposed rats; the endocrine implications of these effects are unclear (Waalkens-Berendsen *et al.*, 1996).

Numerous PCDF congeners have been shown to produce Ah receptor-mediated responses such as thymic atrophy, immunotoxicity and teratogenicity in many mammalian species (reviewed by Safe, 1990).

Like the 2,3,7,8-substituted PCDDs (see Section 4.3 in the monograph on PCDDs), PCDFs negatively modulate some 17β-oestradiol-induced biological responses in certain target tissues. The above effects can be of the same order of magnitude as those produced by 2,3,7,8-TCDD (Safe *et al.*, 1991).

The binding of these compounds to the Ah receptor and associated biological responses depend on the cell type, species, sex, age and assay used (Poland & Knutson, 1982; Safe, 1986; Whitlock, 1986; Okey, 1990; Safe, 1990).

Four PCDFs have been shown to increase levels of both hepatic and urinary porphyrins following subchronic exposure in mice, as observed with 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD (van Birgelen *et al.*, 1996b).

4.4 Reproductive and developmental effects

4.4.1 *Humans*

Fetal PCB syndrome, as described among babies in Japan born to mothers who consumed contaminated oil, is characterized at birth by brown pigmentation ('cola-coloured babies') on the skin and the mucous membrane, gingival hyperplasia, very early postnatal eruption of the teeth or natal teeth, calcification of the skull and low birth weight (Yamashita & Hayashi, 1985). In addition, among *yucheng* babies born between 1979 and 1983, a high perinatal mortality rate was observed (eight of 39) (Hsu *et al.*, 1985). Retrospective ascertainment of neonatal dermatological findings among 128 children exposed transplacentally and born in Taiwan between 1979 and 1985, indicated increased rates of hyperpigmentation, eyelid swelling and discharge, deformed nails, acne, natal teeth and swollen gums, compared with 115 control children (Rogan *et al.*, 1988; Gladen *et al.*, 1990). In neither Japan nor Taiwan was there a clear relationship between symptoms or fetopathy and PCB dose (Yu *et al.*, 1991).

Many follow-up studies were initiated among *yucheng* children to assess metabolic impairment or anomalies in physical or cognitive development. In 1985, a cohort was constructed to include all children born between June 1978 and March 1985 who had been exposed prenatally. The exposed cohort consisted of 132 children, living in 1985, from 159 pregnancies occurring among 74 women (Rogan *et al.*, 1988). In April 1985, 117 exposed children aged one month to six years (average, 32 months) and 108 control children (average age, 31 months) were examined. Exposed children were smaller (93% of control weight and 97% of control height) than controls of the same age and sex. Medical histories since birth indicated a higher rate of bronchitis among exposed children. Clinical examination showed a higher frequency of hyperpigmentation and nail deformities, differences in eyebrow flare, hypertelorism and clinodactyly, and an increased prevalence of clinically detectable developmental delay (10% exposed versus 3% controls).

One hundred and fifteen exposed children from the original cohort and 115 highly matched controls were tested for cognitive development annually from 1985 through 1990. The exposed children scored approximately five points lower on age-appropriate tests of intelligence from the age of two to the age of seven. Children born later were as affected as children born shortly after the outbreak (Yu *et al.*, 1991; Chen *et al.*, 1992; Lai *et al.*, 1994).

A behavioural survey was performed on the same groups (Chen *et al.*, 1994). At each year of follow-up and at each age, exposed children scored higher on tests for hyperactivity and conduct disorders.

At school-age, there was evidence of higher prevalence of congenital lack of permanent teeth among some exposed *yucheng* children (five of 18) compared with controls (one of 44) matched for sex, age, father's occupation, family economic status and area of residence (Lan *et al.*, 1989). [Selection of exposed children is not clearly described, and control children had a low participation rate of 61%.]

In a series of 55 *yucheng* children (out of 132 identified during the same period of 1978–85) and 55 controls matched for age and sex, there was evidence in 1991 of decreased height and decreased muscular development (as indicated by total lean mass) among exposed children (Guo *et al.*, 1994).

Seven to nine years after the poisoning, there was no difference in any immunological or haematological parameters investigated between 19 exposed children and 32 matched controls (Lan *et al.*, 1990).

In an examination conducted in 1993 of 104 exposed children, *yucheng* girls were significantly shorter (2.5 cm) than controls, and the penile length of *yucheng* boys, aged 11–14 years, was shorter than that of controls. Neither effect was related to sexual development by the Tanner scale (Guo *et al.*, 1993). In a separate examination, 22% of tympanic membranes in 110 *yucheng* children were abnormal versus 17% of controls ($p < 0.01$) (Chao & Hsu, 1994).

Analysis of physical and cognitive development began in October 1991 of 104 children whose mothers were exposed and 109 children whose fathers but not mothers were exposed and of three matched controls born after 1985 (Guo *et al.*, 1993; Chen *et al.*, 1992). Like children born before 1985, the later-born children were shorter in stature and lower in weight than controls, although the differences were no longer statistically significant. *Yucheng* children were reported to have higher activity levels but no physical temperament, habit or other behavioural problems. Overall, scores on all tests among paternally exposed children were similar to those of the controls. However, maternally exposed children scored lower on the Stanford-Binet IQ test Wechsler and on all subscales of the Wechsler Intelligence Scale for Children. In a follow-up study based on a random sample of the above children, the exposed children had significantly lower verbal and full-scale IQs and auditory event related potential. No neurophysiological changes were observed, including pattern visual evoked potentials and short-latency somatosensory evoked potentials.

In summary, there is evidence that babies born after the *yusho* incident or after the *yucheng* incident (for which more data are available) present signs of intra-uterine growth retardation and congenital anomalies at birth. Some authors have proposed that these findings were consistent with a generalized disorder of ectodermal tissue (Rogan *et al.*, 1988). Sunahara *et al.* (1987) showed that *yucheng* babies with low birth-weight had depressed autophosphorylation capacity of the EGF receptor in the placenta, induced by exposure to PCBs and PCDFs during gestation. There is evidence of deficits on cognitive development scores among *yucheng* children up to seven years of age.

4.4.2 *Experimental systems*

PCDFs are teratogenic in mice, causing the same spectrum of birth defects and developmental toxicity as 2,3,7,8-TCDD (Birnbaum, 1991). Whether administered orally to the dam as a single dose during the middle of organogenesis or in divided doses on gestation days 10–13, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF cause cleft palate and hydronephrosis at doses which are not maternally or fetally toxic (Weber & Birnbaum, 1985; Birnbaum *et al.*, 1987a,b). The dose–response

curves for these four PCDFs are parallel to each other and to that of 2,3,7,8-TCDD. The ED_{50} values and the relative potency values for induction of cleft palate are as follows: 2,3,7,8-TCDD 3.4 μg/kg, 1.0; 2,3,7,8-TCDF 70.1 μg/kg, 0.05; 1,2,3,7,8-PeCDF 132.9 μg/kg, 0.025; 2,3,4,7,8-PeCDF 35.9 μg/kg, 0.1; and 1,2,3,4,7,8-HxCDF 344.8 μg/kg, 0.01. The ED_{50} for hydronephrosis was about five times lower than that for cleft palate. Mixtures of these chemicals demonstrate strict additivity for induction of cleft palate.

Prenatal exposure of mice to 2,3,4,7,8-PeCDF results in haemorrhage of embryonic blood into the maternal circulation because of rupture of the embryo–maternal vascular barrier (Khera, 1992). Exposure of pregnant rats on gestation day 1 to 43 nmol/kg (15 μg/kg) 2,3,4,7,8-PeCDF resulted in a decrease in sperm count in the male offspring and a delay or lack of vaginal opening in the females (Waalkens-Berensen *et al.*, 1996).

Oral treatment of adult mice with 100 μg/kg 2,3,4,7,8-PeCDF five times over a 16-week period led to an increase in the growth of surgically induced endometriosis (Johnson *et al.*, 1996).

4.5 Genetic and related effects (see also Appendix 3 and **Table 27**)

4.5.1 *Humans*

Peripheral lymphocytes from 35 Taiwanese women exposed in the *yucheng* incident (Lundgren *et al.*, 1988) that occurred in 1979 and those from 24 matched controls were assessed for the levels of sister chromatid exchange in the presence or absence of α-naphthoflavone and for chromosomal aberrations in 1985. Serum levels of PCBs were measured for 32 individuals and those of PCDFs were measured for only 12 exposed women. Blood concentrations of total PCBs in the exposed population and in controls averaged approximately 15 and 0.34 μg/kg, respectively. PCDFs detected were primarily 1,2,3,4,7,8-HxCDF (10.8 ng/kg) and 2,3,4,7,8-PeCDF (2.7 ng/kg). Sister chromatid exchange frequencies in the absence of α-naphthoflavone and chromosomal aberrations were similar in control and exposed populations. Differences in the level of α-naphthoflavone-induced sister chromatid exchange between the two groups were highly significant. These findings indicate that exposure to PCBs or PCDFs *in vivo* results in an enhanced sensitivity of lymphocytes to the sister chromatid exchange-inducing effects of α-naphthoflavone.

Placentas from nonsmoking Taiwanese women (38–35 years of age) from the *yucheng* cohort were obtained in 1983 and 1984. The formation of DNA adducts in placental DNA was investigated using ^{32}P-postlabelling, but none was detected (Gallagher *et al.*, 1994).

4.5.2 *Experimental systems* (see **Table 27**)

3-Chlorodibenzofuran induced reverse mutation in *Salmonella typhimurium*. Elevated frequencies of sister chromatid exchange and micronucleus formation were induced by 2,3,4,7,8-PeCDF in human lymphocytes *in vitro* in the presence or absence of α-naphthoflavone.

Table 27. Genetic and related effects of PCDFs

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
3-Chlorodibenzofuran				
SAO, *Salmonella typhimurium* TA100, reverse mutation	+	+	100	Matsumoto & Ando (1991)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	40	Matsumoto & Ando (1991)
2,3,4,7,8-Pentachlorodibenzofuran				
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.0008	Nagayama *et al.* (1995a)
MIH, Micronucleus test, human lymphocytes *in vitro*	+	NT	0.005	Nagayama *et al.* (1993)
BVD, Binding (covalent) to DNA, rat liver *in vivo* (^{32}P-postlabelling)	–		0.1 p.o. × 4	Randerath *et al.* (1993)
1,2,3,7,8-Pentachlorodibenzofuran				
BVD, Binding (covalent) to DNA, rat liver *in vivo* (^{32}P-postlabelling)	–		0.1 p.o. × 4	Randerath *et al.* (1993)
1,2,4,7,8-Pentachlorodibenzofuran				
BVD, Binding (covalent) to DNA, rat liver *in vivo* (^{32}P-postlabelling)	–		0.1 p.o. × 4	Randerath *et al.* (1993)
2,3,4,6,7,8-Hexachlorodibenzofuran				
BVD, Binding (covalent) to DNA, rat liver *in vivo* (^{32}P-postlabelling)	–		0.1 p.o. × 4	Randerath *et al.* (1993)
Mixed PCDFs and PCBs				
BVD, Binding (covalent) to DNA, human placenta *in vivo* (^{32}P-postlabelling)	–		NG	Gallagher *et al.* (1994)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; p.o., oral; NG, not given

Changes in DNA I (indigenous)-compound formation were studied in female Sprague-Dawley rats treated by gastric instillation with 1,2,3,7,8-PeCDF, 1,2,4,7,8-PeCDF, 2,3,4,7,8-PeCDF or 2,3,4,6,7,8-HxCDF (100 μg/kg bw in corn oil per week for four weeks). No test compound–DNA adducts were detected, but there were significant, structure-dependent reductions in hepatic I-compound formation. Potencies increased in the order: control (100%, 122 modifications in 10^9 DNA nucleotides) = 1,2,4,7,8-PeCDF (104%) < 1,2,3,7,8-PeCDF (80%) < 2,3,4,7,8-PeCDF (61%) = 2,3,4,6,7,8-HxCDF. These activities parallel the reported Ah receptor-binding activities (Randerath *et al.*, 1993).