Chapter 5

Toxicity of PCBs, PCDFs and Related Compounds

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At the outbreak of the Yusho incident, a special rice oil produced by the K company was suspected to be the causative agent for Yusho and a few months later, Kanechlor 400 (a commercial brand of PCBs) was identified as a contaminant in this oil. In the early toxicity studies on this specified oil and Kanechlor 400, various experimental animals such as chickens, mice, rats, guinea pigs, rabbits and monkeys were used to test the inductivity of lesions in the skin and liver, which had been seen in Yusho patients. Since then, the toxicological effects of possibly Yusho-related compounds other than Kanechlor 400 have been studied in other types of Kanechlor and other contaminants such as polychlorinated dibenzofurans, using various experimental animals, from toxicological, biochemical and histopathological aspects. Although some of the toxicity studies have been carried out using pure chemicals, the majority of the studies on polychlorinated biphenyls have been done with mixtures, commercial preparations of Kanechlor, especially in early period of Yusho study. Therefore, it is difficult to ascribe positive results with those biphenyl mixtures to specific isomer(s).

The purpose of this chapter is to attempt to delineate the specific aspects of toxicity for PCBs, PCDFs and other compounds related to Yusho, mainly in experimental animals.

5.1. Acute, Subacute and Chronic Toxicity

Although it is difficult to differentiate the toxicity of PCBs, PCDFs and other related compounds, which are contained in the Yusho inducing oil, in clinical cases, several major causal agents of Yusho have been suspected based on the results of chemical analysis of blood and tissue samples in the patients in various periods after the incident and in toxicological studies on PCBs and related compounds using experimental animals.

In the course studying the Yusho disease, it was found that commercial PCBs contained significant levels of PCDFs which may be more toxic than the parent compound, although no comprehensive toxicological studies have yet been fully conducted. Among the PCBs and related compounds, it has been recognized that the relative toxicity is dioxin > furan >> PCBs >> naphthalene, if a given animal species is exposed to the most toxic isomer of each class of compound (McConnel and Mckinney,1978). In the early experiments on Yusho, it was reported that the oral LD_{50} value of Kanechlor 400 was about 2.0 g/kg in male and female CF-1 mice (Tanaka et al., 1969). Moreover, the oral LD_{50} value of polychlorinated

Table 5.1. Lesions Produced by PCBs and Related Compounds

	Lesions	Acute	Chronic	Comments
1.	Thymus (atrophy)	+++	+++	in young animals
2.	Spleen (atrophy)	+	±	in lethal exposures
3.	Bone marrow (atrophy)	+++	++	in lethal exposures
4.	Liver			
	necrosis	±~+++	±~++	animal species related
	hyperplasia	±	+~++	animal species related
5.	Gall bladder (hyperplasia)	–	±~++	animal species related
6.	Stomach and colon (hyperplasia)	–~±	–~++	animal species related
7.	Kidney (hyperplasia)	–~++	–~++	animal species related
8.	Skin and sebaceous glands			
	(hyper- and metaplasia)	–	–~+++	animal species related
9.	Thyroid (hypertrophy)	–	+	animal species related
10.	Adrenal (atrophy)	++	+	in lethal exposures
11.	Testicle (atrophy)	±~++	±~++	in lethal exposures

Note: –: no change, ±: minimal, +: mild, ++: moderate, +++: severe.

dibenzofurans was approximately 200 mg/kg in male and 400 mg/kg in female CF-1 mice (Nishizumi, 1978).

Different animal species vary widely in their susceptibility to intoxication by these compounds. In general, female animals are more sensitive than males, and young animals are more sensitive than adults. Poultry, guinea pigs and nonhuman primates are the most susceptible animals. In fact, millions of chickens in southwestern Japan were affected by a "Dark Oil" incident which was induced by the use of a commercial chicken feed contaminated with organochlorine compounds in 1968 (Shoya, 1974; see Appendix 6). It is now considered that the causative agents for this incident are the same chemical compounds as those found in the Yusho incident, since these two incidents show a close correlation in both the manufacturer and in the time of contamination. This "Dark Oil" intoxication in chickens also coincided with an incident of so-called chick edema disease, which was first recognized in the southeastern United States in 1957 (Sanger et al., 1958).

The toxic lesions produced by these chemical compounds vary with the species of animals exposed, the duration and dose of exposure, and to some degree the age and sex of the animals exposed. However, if the parameters of exposure are the same, including the same relative toxic dose of the chemicals, the toxic lesions are usually comparable within the same species of animals (Table 5.1).

In acute lethal studies, weight loss becomes progressive until death. In the rat, guinea pig and the mouse, weight loss with general debilitation may be the only signs observed prior to death. In contrast, birds, particularly chickens, may actually show an increase in body weight just prior to death, as a result of an extracel-

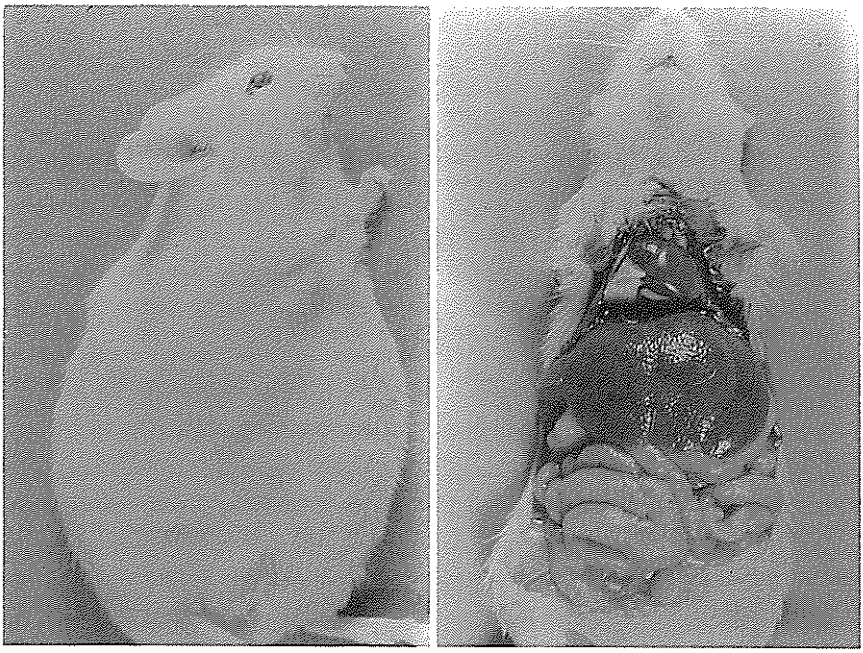

Fig. 5.1. A Mouse Exposed to PCDFs (0.5 g/kg p.o.). Left: a distended abdomen due to ascites and an enlarged liver. Right: the mouse has been opened to demonstrate a markedly enlarged liver and an absence of normal body fat with some fluid accumulation.

lular accumulation of body fluids (subcutaneous edema, ascites, hydrothorax and hydropericardium). One of the interesting aspects of acute poisoning with these compounds, especially with PCDFs and PCDDs, is the median time until death, which is generally 2–3 weeks after a single exposure for most small laboratory animals and even longer for larger animals, dogs and monkeys. In acute but sublethal PCBs toxicity studies in rodents, the initial signs of toxicity is weight loss or a depressed weight gain, which is only partially related to a decrease in food or water consumption. In chicks given a diet laced with toxic rice oil at the 5% level, labored respiration and a distended abdomen were observed on the 17th day. At necropsy, hydropericardium, ascites and pale edematous enlarged kidney were all evident. These same changes were also seen in chicks fed a diet laced with Kanechlor 400 at the 400 ppm level (Goto et al., 1969).

In subacute stages, a depression in the body weight increase, a decrease in the food intake, a loss of vigor and swelling of the eyelids were seen in mice and rats given approximately 0.1 g/kg of Kanechlor 400 for 1 to 3 months (Tanaka et al., 1969; Nishizumi et al., 1969). In monkeys, 0.5 mg Kanechlor 400/kg for 3 months induced a loss of body weight, a loss of hair, swelling of the eyelids and eye dis-

Table 5.2. Organ Weight Changes Produced by PCBs

Brain	↔	
Heart	↑	relative to body weight and in lethal exposures
Lung	↔	
Liver	↑↑	at lethal and sublethal exposures
Spleen	↑	at low doses
	↓	at high doses
Thymus	↓↓↓	
Kidney	↓	absolute weight and in lethal exposures
Adrenal	↑	relative to body weight and in lethal exposures
Testicle	↓	species related, at lethal exposures

Note: ↔ No change. ↑ Mild to ↑↑ moderate increase. ↓ Mild to ↓↓↓ marked decrease.

charge (Yoshihara et al., 1979, Yoshimura et al., 1981). In subacute toxicity studies, autopsy results show a reduction of the thymus weight (thymus atrophy) and an increase in the liver weight (liver enlargement) (Fig. 5.1). A decrease in the organ weight of the spleen and testicles are also sometimes shown in animals that are exposed to lethal levels of these chemicals (Table 5.2).

Since an enlargement of the liver is observed in all the animals tested, an increase in the weight of the liver may be a sensitive and useful indicator of toxicity from PCBs and related chemicals. The morphological expression for hepatomegaly is hepatocellular hypertrophy and possibly hyperplasia. The ultrastructural basis for hepatocyte enlargement is due to a large extent to the proliferation of endoplasmic reticulum (ER), especially the smooth endoplasmic reticulum (SER) type (Fig. 5.2), which is the morphological expression of an increase in the activity of hepatic microsomal enzymes (Nishizumi, 1970, Yoshimura et al., 1979). An increase in the lipid droplets and lysosomes in the hepatocytes is also observed. The use of long-wavelength (366 nm) ultraviolet on tissue specimens (particularly the liver) from intoxicated mice, rats and birds for the presence of red fluorescence as an indication of excess porphyrin accumulation is often useful in establishing the degree of intoxication.

A reduction of the size and weight of the thymus was markedly observed in immature animals. Microscopically, a diminution in size is reflected as a loss in the cortical lymphocytes (Fig. 5.3). These lesions are clinically expressed by changes in the immune system, which will be described later. Some reports show the reduction in the weight of the spleen in animals exposed to lethal levels of these chemicals. It was reported that PCB congeners, which are the 3-methylcholanthrene-type inducers of hepatic enzymes exhibited high acute toxicities, such as a depression of the body weight increase, liver enlargement and atrophy of the thymus and the spleen (Yoshimura et al., 1979).

In hematology, it was shown that the erythropoietic system is more sensitive to

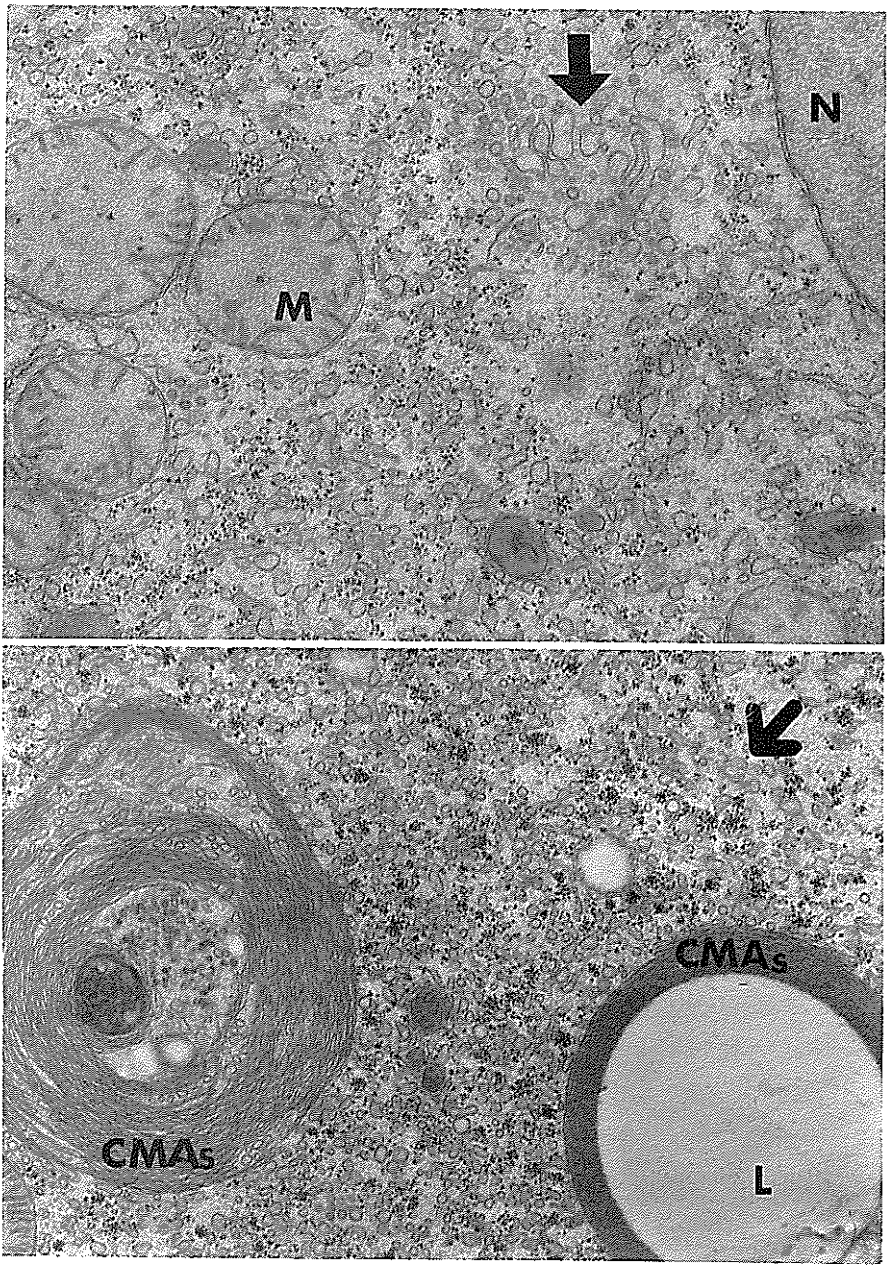

Fig. 5.2. Electron Micrographs from a Mouse Liver after Being Orally Administered Kanechlor 400 2.0 g/kg for 8 Weeks. Note the marked proliferation and dilatation of the smooth endoplasmic reticulum (arrows), and concentric membrane arrays (CMAs) either with or without lipid droplets (L). N = nucleus, M = mitochondria. Top: × 30,000; bottom: × 17,000.

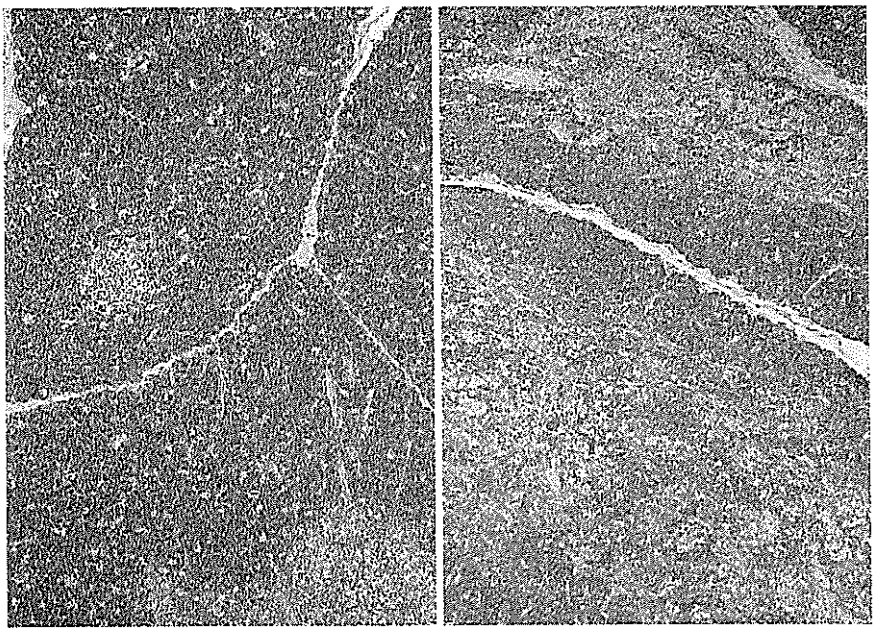

Fig. 5.3. Photomicrographs of Thymic Tissue from a Normal Rat (Left) and a Rat Exposed to Kanechlor 400 2.5 g/kg (Right). Atrophy of the thymus cortex is clearly evident in a PCB-exposed rat compared to the dark highly cellular cortex in a normal rat. H & E stain: × 65.

the toxic effects of these compounds than the white cell components of the blood, and that the effect to the red blood cell picture is both dose- and time-dependent (McConnell, 1989). In an experiment using rhesus monkeys, which were given either Kanechlor 400 (0.25 mg/kg/day) or PCDFs (0.625 μg/kg/day) for 175 days, mild anemia was clearly observed in both groups. A diffuse atrophy of the bone marrow has been reported in lethal animals exposed to these chemicals.

Skin lesions appeared as an initial sign in Yusho after several months of exposure. In some animal species such as cattle, chronic cutaneous manifestations are distinctive feature which have diagnostic value (McConnell, 1989). Toxicity of the skin in experimental animals are described later.

Hypertrophic lesions mixed with ulceration and inflammation in the epithelial tissue of the stomach and colon were reported in monkeys chronically exposed to TCDD (Allen et al., 1974).

Some reports showed the toxicity of PCBs in respiratory organs which revealed necrosis of nonciliated bronchiolar epithelial cells (Clara cells) in mice, rats and beagle dogs. In addition, PCDFs induced more severe toxicity to bronchiolar Clara cells (Nagata et al., 1989)

Basic lesions in the kidney and urinary tract were reported to be a hyperplasia of

the transitional epithelium from the terminal positions of the collecting ducts of the renal medulla to the renal pelvis, ureter and urinary bladder. Although most kidney lesions were not apparent by light microscopy, a disruption and distortion of the mitochondria followed by necrosis and the appearance of SER were seen in the epithelial cells lining the proximal convoluted tubules in ultramicroscopy (unpublished observations).

Changes in the thyroid in rats exposed to PCBs included the presence of smaller thyroid follicles. In addition, an ultrastructural examination of the thyroid revealed some changes in the follicular epithelium (Collins et al., 1977).

Lesions in the testicle were reported to vary from almost complete atrophy of the seminiferous components to the development of abnormal (multinucleated) cell types (McConnell et al., 1978). It is not clear, however, whether this is a primary toxic response to these chemicals or a secondary phenomenon to body weight loss associated with toxicity.

In the neurological aspects of PCB toxicity, decreased motor conduction velocity and early features of segmental demyelination were noted in the peripheral nerves of rats (Ogawa, 1971). On the other hand, no remarkable changes have yet been observed in the central nervous function in animal experiments.

Many parameters, especially the serum enzymes in blood chemistries after exposure to these classes of compounds, tend to vary markedly in animals depending on the dose, the period of the time during and after exposure, and the state of the host at the time the blood samples were obtained. Among the changes in blood serum chemistries in "Yusho" disease, the most prominent findings were elevated serum triglyceride levels. This abnormal value was confirmed in both the rat and rabbit (Tanaka et al.,1969; Uzawa, H. et al., 1971). A decrease in the postheparin lipolytic activity and impaired plasma triglyceride removal are also suspected to play an important role as a mechanism of hyperglyceridemia. In many studies, triglycerides and free fatty acids have been reported to increase, while the findings of total cholesterol remained unclear. These changes seem to be a reflection of liver dysfunction and the debilitated condition of the animals. There are also marked species difference with regard to these parameters. Other abnormalities in the serum protein and transaminase were shown in lethal animals.

Regarding disturbances of the porphyrin metabolism due to PCBs, it has been reported that the oral administration of commercial PCBs to chickens caused hepatic-type porphyria (Vos and Koeman, 1970). Although PCBs and TCDD have been shown to be porphyrogenic in experimental animals and in men since then, only a few studies have been carried out related to the porphyrin metabolism in Yusho. This is partly due to the fact that no increase in urinary porphyrins was observed in Yusho patients who were examined approximately 10 years after the

incident (Ishimoto, 1983). In Yucheng cases, only a modest 3-fold increase in uroporphyrin excretion was seen in 69 human subjects immediately after the incident in 1979 (Lü and Wong, 1984). In rodents, the porphyria has been shown to be characterized by a delayed onset as well as the accumulation of uroporphyrins. An accumulation of hepatic porphyrin occurred in dd-K strain mice given Kanechlor 400 and its change was enhanced by 0.1% griseofulvin. However, no change in the hepatic delta aminolevulinic acid dehydratase (ALA-D) activity was observed, even though an elevation of ALA-D activity was seen in the erythrocytes (Nonaka et al., 1985). Based on these results, they concluded that an abnormality of the ALA-D activity in the PCB poisoning seemed to occur secondarily following the accumulation of hepatic porphyrin. Sano et al. (1985) studied the structural requirements of synthetic PCB for porphyrinogenic activities by using cultured chick embryo liver cells, examining the relationship between the induction of the δ-amino levulinic acid (ALA) synthetase and the inhibition of uroporphyrinogen decarboxylase. Only 3,4,3′,4′-tetrachlorobiphenyl and 3,4,5,3′,4′,5′-hexachlorobiphenyl produced a marked accumulation of uroporphyrin, and these two compounds strongly inhibited uroporphyrinogen decarboxylase directly at two stages, i.e. first in the formation of hexacarboxylic porphyrinogen III from heptacarboxylic porphyrinogen III and second in the formation of heptacarboxylic porphyrinogen III from uroporphyrinogen III. Thus, porphyrinogenic PCBs primarily inhibited uroporphyrinogen decarboxylase, leading to a depletion of heme, which may also be accelerated by induction of apocytochrome P-450 by PCB. As a result, the synthesis of ALA synthetase increased leading to an accumulation of uroporphyrin in the liver.

5.2. Acnegenicity

As dermatological changes in Yusho patients, acneform eruptions with hyperpigmentation and hyperkeratosis were observed in the early period. Many experimental studies have been done to examine the induction of acne-like lesions by exposing various species of animals to PCBs and related compounds.

Chloracne-like lesions were not usually induced in rodents, although they were reported to be seen in the ears of rats exposed to PCDFs (Oishi et al., 1978). Eruptions with hyperkeratosis were observed in "hairless" (actually not hairless since remnants of follicles are present) mice after the ingestion of a diet containing the toxic rice oil at a 10% concentration for 10 weeks (Inagami et al., 1969). These skin eruptions started to appear after 7 weeks on the lower part of the abdominal wall, and thereafter expanded to other parts of the body. In many studies, a histological examination of the eyelids of mice and rats exposed to PCBs showed more

or less cystic dilatation and hyperkeratosis of the hair follicles.

Since the inner surface of the rabbit ear is sensitive to the effect of these compounds, the ear of the rabbit was used to test for the presence of and relative toxic potentials of mixtures containing PCBs and related compounds (Jones and Krizek, 1962). This test was used to detect skin lesions by 3,4,3′,4′-tetrachlorobiphenyl and also to compare the relative potency of PCBs, PCDFs and PCDDs to induce skin lesions. The application of pure crystalline 3,4,3′,4′-tetrachlorobiphenyl to the rabbit ear induced hyperkeratosis, dilatation of hair follicles and the formation of cysts filled with keratinaceous materials, which were almost identical to those induced by Kanechlor 400 (Komatsu and Kikuchi, 1972). PCDDs was also shown to be the most potent irritant to the rabbit ear compared with PCDFs and PCBs (Nishizumi et al., 1975). It should be noted that the cutaneous absorption of these compouds in rabbits is so efficient that generalized intoxication may develop before hyperkeratosis with folliculitis develops fully (Kimmig and Schultz, 1957). The skin lesions on the inner side of rabbit ear, consisting of hyperkeratosis, cystic hair follicles filled with karatinaceous debris and the formation of granuloma, were observed in the rabbit exposed to Kanechlor 400 (0.1% in diet) orally for 3 months (Uzawa et al., 1971).

As an animal susceptible to intoxication by PCBs, monkeys, nonhuman primates, have been used (Allen, 1975, Yoshihara et al., 1979; Yoshimura et al., 1981). Lesions of the eyelids, especially changes in the Meibomian (tarsal) glands, were the most sensitive morphological indication of intoxication in monkeys. A swelling of the eyelids and an enlargement of the Meibomian glands, which corresponds to the keratinizing cysts in the histological findings, were characteristic in the acute stages of PCB intoxication (Nishizumi et al., 1969; Yoshihara et al., 1979). At three years after the termination of PCB exposure, the Meibomian glands abnormalities still remained after the prominent clinical signs subsided, although the pathological changes were milder than those in the acute stages (Kohno and Ohnishi, 1987). The changes observed in the skin (chloracne), eyelids and ear canal in the monkeys had a common pathogenesis, which was related to the changes in the sebaceous glands and hair follicles. The major histological changes in the dental tissue of the monkeys exposed to Kanechlor 400 were the hyperkeratotic and proliferative invasion of the gingival epithelium and the formation of keratocysts in the lamina propria (Yoshihara et al., 1979). In a comparative study on the biological effects of PCBs, PCQs and PCDFs, monkeys treated with PCDFs showed Meibomian retention cysts, acneform eruptions on face, enlargement of hyperkeratinosis of pilar pores and the degeneration of hair root on the dorsal skin (Kunita et al., 1985). In contrast, the monkeys treated with PCBs or PCQs did not show such clinical symptoms.

The Meibomian glands of beagle dogs exposed to PenCB (0.05 mg/kg, twice) showed dilatation of the duct and squamous metaplasia of the alveolar cells at 50 days after the last exposure (Kohno and Ohnishi, 1989).

In animal models, it has been found that the chloracneogenic potential of the halogenated aromatic compounds examined corresponds with the relative affinity of these same compounds for the cytosolic TCDD receptor, which controls the coordinate expression of a number of inducible enzyme activities and in certain cell targets can alter normal programs of proliferation and differentiation (Greenlee et al., 1985).

5.3. Endocrine Effects

In Yusho patients, some deleterious effects regarding the poisoning on the function of the thyroid, adrenals and gonads were suspected and several clinical tests were also done, as described elsewhere (Watanabe et al., 1971; Nagai et al., 1971, Kusuda et al., 1975; Murai et al., 1985).

The action of PCBs on estrogen was also examined by measuring the uterine wet weight in ovariectomized rats. It was shown that PCBs themselves were not estrogenic but instead potentiate the uterotropic action of the estradiol (Komatsu, 1972). A decrease in the serum estradiol and progesterone was observed in rhesus monkeys exhibiting reproductive dysfunction due to TCDD exposure (Barsotti et al., 1979). On the effects of TCDD, it is considered that the decreases in the effects of exogenous estradiol probably reflect the decreases in the levels of the estrogen receptor and the resultant decreased responsiveness of the tissue estradiol.

Reductions in the blood levels of thyroxine in rats were induced by exposure to TCDD (Bastomsky, 1977a) and PCBs (Collins et al., 1977), but the serum T_3 levels were elevated (Bastomsky, 1977a). An ultrastructural examination of the thyroid follicular epithelium of the PCBs-exposed rats revealed a projection of the cytoplasm into the adjacent colloid, which was interpreted as compensatory hypertrophy in response to the lowered thyroxine levels. Aroclor 1254 and 2,3,7,8-TCDD increased the biliary excretion of thyroxine glucuronide. 2,3,7,8-TCDD and PCBs also decreased the serum thyroxine (T_4) levels (by approximately 50%), increased the serum TSH levels approximately 4-fold, increased the ^{131}I uptake by the thyroid (approximately 2-fold), and also increased the thyroid weights significantly (Bastomsky, 1977a, b).

The exposure of rats to Aroclor 1221 at a concentration of 250 ppm in water for 10 weeks induced an increase of the corticosterone plasma level which complied with the morphological feature of hyperfunction of the adrenal zona fasciculata, while a diet containing Kanechlor 400 induced a decrease in the ability of corticoid

hormone synthesis in rats. 2,3,7,8-TCDD appeared to alter the circadian rhythm of serum corticosterone levels, suppressing the peak of the serum corticosterone levels, and it also altered the circadian rhythm of the serum levels of prolactin, which is thought to play a role in the regulation of corticosterone secretion in response to ACTH (Jones et al., 1987).

5.4. Immunosuppressive Effects

The first indication that PCBs might affect the immune system was based on the observed weight decrease and histological changes of lymphoid organs. Atrophy of lymphoid tissues such as a small spleen, a significant reduction in the relative thymus weight and atrophy of the cortex of the thymus, as induced by PCBs ingestion were all observed in chickens, mice, rats, guinea pigs, rabbits and monkeys (Vos and Luster, 1989). The changes in the thymus induced by 2,3,7,8-TCDD and 2,3,7,8-TCDF were also similar in structure and pathology including severe thymic atrophy to those induced by PCBs.

The suppressive effects of PCB exposure on humoral immunity have been well documented in many studies using Aroclor and Clophen (Vos and Luster, 1989). Referring to Yusho, the biological activities including Anti-Sheep Red Blood Cell (SRBC) antibody responses of PCBs, PCQs and PCDFs contained in the rice oil causing Yusho and Yucheng disease have also been reported (Kunita et al., 1985). Male Sprague-Dawley rats received 22 daily oral doses of 1 mg PCBs, 1 mg PCQs, 10 μg PCDFs or a mixture of these compounds. Female cynomolgus monkeys received daily doses of 5 mg PCBs for 20 weeks, 5 mg PCQs or a mixture containing 5 mg PCBs + 20 μg PCDFs. Treatment with PCBs but not with PCQs suppressed the antibody responses to SRBC in rats and monkeys. Treatment with PCDFs and the mixture caused the most severe immunosuppression. Weight loss and thymic atrophy were also caused by these treatments.

PCB exposure studies in utero have been reported. Among them, the suppression of T helper cell activity was observed in the offspring of C3H mice treated orally with Kanechlor 500 (Takagi et al., 1987). Female mice were orally treated with 50 mg Kanechlor 500/kg body weight, twice a week for 3 weeks, before mating. Cross-fostering procedures were employed prior to nursing in order to separate prenatal from postnatal influences. The T helper cell activity was reduced in the prenatally exposed groups to about 20% of the control value at 4 weeks after birth, and to 50% of the control value in the postnatal exposure group. However, the B cell activity in the offspring, assessed at 4, 7, 11 and 15 weeks after birth by determining the plaque-forming cell response to the T cell-independent antigen DNP-dextran, was not suppressed.

In general, PCDFs lead to severe toxicity of the thymus and induce a selective suppression of helper T cells. In C3H female mice intraperitoneally given 5 μg PCDF, an analysis of the T-cell subsets of mice blood at 4 weeks showed a lower level of anti Thy-1,2 positive cells (pan T cells) and a low Thy 1/2 ratio (helper/suppressor ratio) as compared with that of PCBs (Nakanishi et al., 1985). This depression thereafter gradually recovered to the control level.

The effect on host resistance of Kanechlor 500 feeding against viral infection was studied by Imanishi et al. (1980). The mice exposed to 100, 200 or 400 mg of Kanechlor 500/kg diet for 3 weeks were more susceptible to Herpes simplex infection, while mortality from an ectromelia virus infection was increased at the 200 and 400 mg/kg dietary level.

The immune alterations in victims of the Yusho and Yucheng incident (Shigematsu et al., 1978; Lü and Wu, 1985), including frequent respiratory infections, suggested suppressed host defense mechanisms, a depression of serum immunoglobulin level (both of serum Ig A and Ig M), elevation of Ig G and a depression of delayed-type hypersensitivity, which were all consistent with those observed in animal studies.

Regarding the mechanisms of immunotoxicity following PCB exposure, the suppression of the antibody synthesis is considered to be mediated by the aromatic hydrocarbon (Ah) cytosolic receptor. The immunotoxicity induced by TCDD, TCDF and the planar PCBs appears to also be associated with stereospecific binding to the Ah or TCDD receptor, originally described by Poland et al. (1976) in hepatic cytosol, and recently identified in a variety of tissues and cells.

5.5. Carcinogenicity

The presence of carcinogenicity in PCBs and PCDFs is one of the greatest concerns of Yusho patients, because of the high persistence of these chemicals in the human body.

Until now, PCBs have been shown to produce neoplastic hepatic nodules or hepatocellular carcinoma in rodents. Prolonged feeding of PCB mixtures induced hepatic tumors in rats (Kimbrough et al., 1972; Kimura and Baba, 1973; Ito et al., 1974; Kimbrough et al., 1975) and mice (Nagasaki et al., 1972; Ito et al., 1973; Kimbrough and Linder, 1974). Adenofibrosis, a lesion consisting of a mixture of proliferating fibrous connective tissue and bile ducts, has also been observed, concomitantly with hepatocellular carcinoma (Kimbrough et al., 1972, Kimbrough and Linder, 1974). On the carcinogenic potency of Kanechlor, higher chlorinated mixtures showed a somewhat higher rate in the appearance of liver tumors (Nagasaki et al., 1972, Ito et al., 1974)

A mixture of PCBs also effectively promoted the induction of hepatic tumors by benzene hexachloride (Ito et al., 1973). Kanechlor 400 (400 μg/g diet given for 6 months) after 3′-methyl-4-dimethylaminoazobenzene administration increased the incidence of hepatocarcinoma over that for the initiator alone in female Donryu rats in the 800 day study (Kimura et al., 1976). Kanechlor 500 (0.01 ml given twice weekly by gastric intubation for 12 weeks) resulted in more liver tumors at 40 and 52 weeks in male Wistar rats given diethylnitrosamine (Nishizumi, 1979). Kanechlor 500 (500 or 1,000 μg/g diet) caused the development of hepatic neoplastic nodules in male F344 rats when given for 8 weeks after a non-tumorigenic dose of N-2-fluorenyl-acetamide (2-FAA) (Tatematsu et al., 1979). Aroclor 1254 was also shown to have a promoting effect on hepatocarcinogenesis (Preston et al., 1981; Pereira et al., 1982). Tumor promotion was still evident when impurities such as dibenzofurans were removed from the PCB mixture (Preston et al., 1981). In addition to the lengthy carcinogenicity test, the method counting and measuring preneoplastic enzyme-altered foci in the liver, which are considered to be a precursor lesion of hepatocellular carcinoma, has also been used as an indicator of the tumor promoting ability of chemicals such as PCBs. By using this procedure, PCBs were shown to have greater promoting effects in the livers from female rats when compared to the effects in males, while weanling rats were much more susceptible to this promoting effect of PCBs than adult rats (Oesterle and Deml, 1983). The tumor-promoting action of PCBs was also revealed in the tumor initiated by N-nitrosodimethylamine in Swiss male mice (Anderson et al., 1986).

Regarding hepatocarcinogenesis, it is difficult to distinguish a weak initiator from a strong promoter. The identification of trans-dihydrodiol in the rat (Norback et al., 1976) supports the supposition that PCBs are metabolized via arene oxides of a PCB analog, which is an electrophilic intermediate metabolite capable of forming an DNA-adduct. On the other hand, reports that the PCB mixture was not mutagenic in a Salmonella assay in the absence or presence of rat liver homogenate (Shahin et al., 1979) provides evidence against the ability of PCBs to act as an initiator.

Interestingly, the inhibitory effects of PCBs on hepatocarcinogenesis were shown, when PCBs are given to rodents before the administration of hepatocarcinogens such as 3′-methyl-4-dimethylaminoazobenzene, 2-FAA, or diethylnitrosamine (Makiura et al., 1974; Kimura et al., 1976). This inhibitory action of PCBs on hepatocarcinogenesis was observed in the offspring pre-exposed to PCBs in utero and via the mothers milk, when they were given diethylnitrosamine after birth (Nishizumi, 1980). The suppression of 20-methylcholanthrene(MC)-induced skin carcinogenesis by PCBs was also observed. The inhibitory effect of Kanechlor 400 to MC-induced skin carcinogenesis has also been reported (Hori et

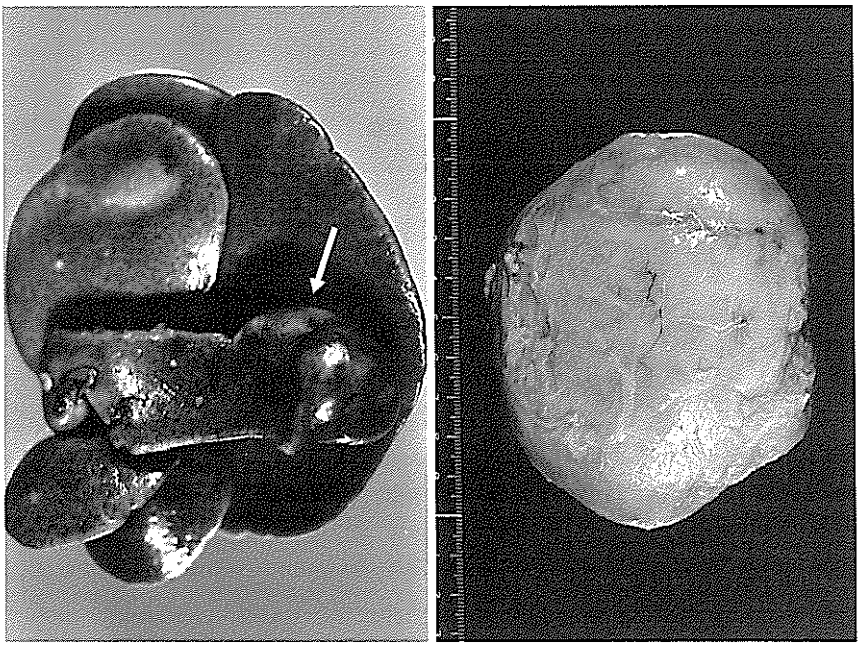

Fig. 5.4. Tumors Produced by PCDFs. Left: the liver from a rat exposed to 2,3,4,7,8-PenCDF, showing a tan tumor (arrow) in the right lobe. Right: fibrosarcoma from a rat exposed to 1,2,3,4,7,8-HCDF, obtained from the subcutaneous tissue.

al., 1985). It is one of the possible explanations for these results that these chemicals are potent inducers of hepatic microsomal enzymes and then enzymes can metabolize many carcinogens to less potent metabolites. The carcinogenic potential of PCBs for other organs has not yet been demonstrated.

Up to now, there have only been a few reports concerning the carcinogenicity of PCDFs. In the rats orally given 200 mg of 2,3,4,7,8-penCDF in total, a cholangiohepatoma and an osteosarcoma with hepatic nodules were induced among 8 rats that survived for 104 weeks after the start of the experiment. In the rats treated with 1,2,3,4,7,8-HexaCDF in a similar manner, two rats bearing hepatic nodules were observed out of 9 rats that survived (Nishizumi, 1991). Tumors in the liver or subcutaneous tissue were observed in 4 out of 13 rats subcutaneously injected 2,3,4,7,8-PenCDF, and in 5 out of 15 rats similarly treated with 1,2,3,4,7,8-HexaCDF (Nishizumi, 1989) (Fig. 5.4). The promoting action of 2,3,4,7,8-PenCDF on skin carcinogenesis induced by 20-methylcholanthrene in mice was recognized at a concentration of 0.5 ppm (Hirose, 1989).

Dioxins, specifically TCDD have been reported to be carcinogenic for liver in rats and mice (Kociba et al., 1978; NTP, 1982). Neoplasms have also been ob-

served in the thyroid gland, lungs and nasal epithelia in rats. Moreover, TCDD has been shown to be a potent promoter of liver tumor induced diethylnitrosamine (Pitot et al., 1980).

In humans, a preliminary cohort study on the mortality of Yusho patients showed significantly elevated standardized mortality ratios for cancer of the liver and of the lung in males but not in females (Ikeda et al., 1986).

5.6. Genetic Toxicity

The majority of studies of the genetic toxicity of PCBs have used commercial preparations of either Aroclor or Kanechlor (IARC, 1986). The mutagenicity test for Salmonella typhimurium of PCBs, mainly Aroclor 1254, unanimously resulted in nonmutagenicity. Likewise, Kanechlor 300 and Kanechlor 500 were tested by this assay (Kawachi et al., 1980; Oda et al., 1985), showing negative results. However, Miyauchi et al. (1983) reported that a number of mono-, di-, and trichlorinated nitrobiphenyl ethers, and their corresponding nitroso- and amino-derivatives, with the exception of 2,4,6-trichloro-4-nitrobiphenyl ether, were mutagenic in Salmonella.

Kanechlor 500 was positive in a test which measures the induction of the *umu* operon in Salmonella (Oda et al., 1985). Kanechlor 300 was reported to be positive but Kanechlor 500 was negative in the Bacillus subtilis rec assay (Kawachi et al., 1980).

There was no induction of micronuclei in the bone marrow of mice injected with Kanechlor 500 in ethanol, but a slight increase was obtained with Kanechlor 500 in corn oil administered by gavage (Watanabe et al., 1982).

Neither Kanechlor 300 nor Kanechlor 500 induced any mutations in silk worms (Kawachi et al., 1980).

The mutagenicity of the PCDFs in a number of tester strains of Salmonella was studied by Schoeny (1982) using S-9 from uninduced rats and rats that had been treated with various cytochrome P-448 inducers, including a PCB mixture and 2,3,7,8-tetrachlorodibenzofuran. As a result, 2,8- and 3,6-dichlorodibenzofuran, 2,3,7,8-tetrachlorodibenzofuran and octachlorodibenzofuran were all found to be nonmutagenic. The effect of PCDFs on pUC18 plasmid DNA was studied, and it was also suggested that PCDFs did react with pUC18 (Hori et al., 1991).

Although the positive Salmonella results were reported in the initial report on the mutagenicity of TCDD, subsequent Salmonella assays of TCDD have shown no mutagenic activity. In contrast to the polychlorinated dioxins, the nitrososubstituted dibenzo-p-dioxins were clearly mutagenic for Salmonella (Zeiger, 1989).

Using cultured human lymphocytes, the effects of 2,3,4,7,8-PenCDF,

3,4,5,3′,4′-PenCB and 2,3,7,8-TCDD on the induction of micronuclei were examined. All of these compounds significantly increased the frequency of micronuclei in almost the same dose-dependent manner based on an equivalent toxic concentration of TCDD toxic equivalent (Nagayama and Nagayama, 1993).

The mixtures of PCBs and related compounds, which resembled those remaining in the tissue specimens of healthy people, were examined for the induction of sister chromatid exchange (SCE) in a human whole blood culture system in order to clarify their genotoxicity by adding 7,8-benzoflavone. The tests showed a fairly good dose-response relationship between the concentration of the mixtures and the induction of SCEs/cell (Nagayama et al., 1991).

In conclusion, various animal toxicity studies on PCBs, PCDFs and related compounds were undertaken in order to determine the cause of Yusho, and the findings have resulted in the clarification of a wide variety of toxic responses. However, the basic mechanisms of their toxic actions are still not fully understood.

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