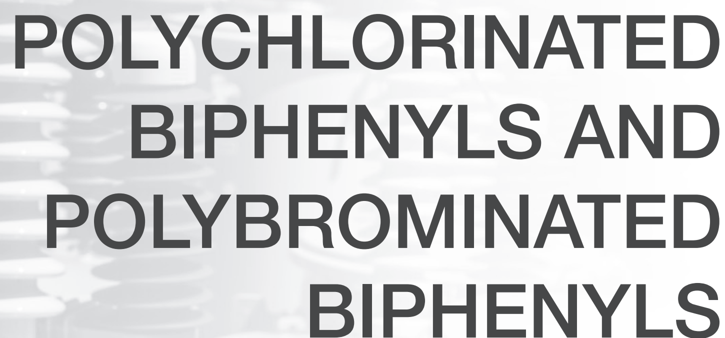
IARC MONOGRAPHS



POLYCHLORINATED BIPHENYLS AND POLYBROMINATED BIPHENYLS

VOLUME 107

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 12–19 February 2013

Lyon, France - 2016

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

4. MECHANISTIC AND OTHER RELEVANT DATA

4.1 Absorption, distribution, metabolism, and excretion

In this Section, the most recent Ballschmiter & Zell (BZ) nomenclature was used throughout (see Mills *et al.*, 2007). For the full corresponding IUPAC nomenclature, the reader is referred to Section 1.1, Tables 1.1–1.3. For the methyl sulfonyl metabolites, and wherever the nomenclature reported is unclear, the name of the metabolite is given as reported in the article, followed by, where appropriate, the abbreviation as well as the structural name (Maervoet *et al.*, 2004; Grimm *et al.*, 2015).

4.1.1 Absorption

(a) Oral exposure

(i) Humans

The absorption of polychlorinated biphenyls (PCBs) was studied in four breastfed infants in Sweden by Dahl *et al.* (1995). Absorption was measured by comparing the estimated total intake and the excretion in faeces for 48 hours, at 1, 2, and 3 months postpartum. The concentrations of 56 congeners in maternal milk were determined. For tetrachlorosubstituted to octachlorosubstituted congeners, absorption was found to be close to 100%, while absorption of trichlorinated congeners was 60–98%, probably due to the low levels at which they were present and ensuing analytical difficulties in detection. [Another possible explanation could be metabolism of the trichlorinated congener.]

The gastrointestinal absorption of 10 congeners from food was investigated using a mass balance approach in seven individuals aged 24–81 years with different contaminant body burdens (Schlummer *et al.*, 1998). The difference between ingested and excreted amounts of the chlorinated compounds was defined as net absorption. Nearly complete net absorption was observed for PCB-28, PCB-52, PCB-77, PCB-101, and PCB-126. Absorption of PCB-105, PCB-138, PCB-153, and PCB-180 was > 60% in most volunteers, but limited absorption was observed in the three older subjects. In all cases, absorption of PCB-202 was < 52%.

(ii) Experimental systems

Several reports have been published on the dietary absorption of PCBs, mostly individual congeners. Gastrointestinal absorption of congeners with between one and six chlorine atoms has been investigated by monitoring faecal excretion in rats fed individual congeners at doses ranging from 5 to 100 mg/kg bw. Absorption of the administered dose was > 90% for all 20 congeners tested (Albro & Fishbein, 1972). Metabolic studies in rodents given oral doses of various radiolabelled PCBs with three to six chlorine atoms (i.e. PCB-31, PCB-47, PCB-85, PCB-101, and PCB-153) indicated that gastrointestinal

absorption was highest for the trichlorobiphenyl congener (about 94% of the administered dose), and lowest for the hexachlorobiphenyl PCB-153 (28%) (Bergman *et al.*, 1982). In a study by Tanabe *et al.* (1981), absorption efficiency was 95% for dichlorobiphenyls, but only 75% for octachlorobiphenyls. These data suggested that, in rats, absorption of PCBs decreases as the number of chlorine atoms increases.

(b) Inhalation

(i) Humans

There is indirect evidence for absorption of PCBs via inhalation in humans; several congeners have been detected in body fluids of people exposed in occupational settings or frequenting contaminated buildings, such as schools, where air concentrations of PCBs have also been measured (Wolff, 1985; Wolff *et al.*, 1992; Schwenk *et al.*, 2002; Liebl *et al.*, 2004).

(ii) Experimental systems

Hu *et al.* (2010) used a nose-only exposure system to assess the time course of PCB vapour uptake from commercial products in animals. Rats (average weight, 188 g) were exposed to vapours of Aroclor 1242 (PCB concentration, 2.4 mg/m^3; total amount, 40 µg) for a total of 2 hours, with a 1-hour break, and killed at 0, 1, 3, 6, and 12 hours after exposure. Congeners detected in tissues included mostly PCBs with mono- or di-*ortho*-substitution, ranging from mono- to pentachlorobiphenyls, with the majority being tri- and tetrachlorobiphenyls. PCB-20 + PCB-28 co-elution was most abundant in every tissue. When compared with the air mixture, most of the material retained in the tissues had shifted from mono- and dichlorinated PCBs to tri- and tetra- or even more highly chlorinated biphenyls. The amount of PCBs measured in the five tissues collected (liver, lung, blood, adipose tissue, and brain) was 5 µg per rat. The measured body burden (i.e. the sum of PCBs loaded at the end of exposure) was 33 µg per rat, suggesting pulmonary absorption of close to 100%. Casey *et al.* (1999) found that uptake of PCBs was greater by inhalation than by ingestion in a comparison of rats exposed to Aroclor 1254 for 30 days via inhalation (0.9 µg/m^3) or in the diet (0.436 µg/g).

(c) Dermal exposure

(i) Humans

Studies on exposure of capacitor workers to PCBs suggested that these compounds are well absorbed by skin contact (Wolff, 1985). Skin samples collected from human cadavers and exposed in vitro to [^{14}C]-labelled Aroclor 1254 and Aroclor 1242 retained 43–44% of the administered dose over a 24-hour period when the mixtures were formulated in water (Wester *et al.*, 1990, 1993). A lower retention was observed when PCBs were formulated in mineral oil or adsorbed on contaminated soil.

(ii) Experimental systems

In rhesus monkeys, percutaneous absorption in vivo of [^{14}C]-labelled Aroclor 1242 and Aroclor 1254 formulated in mineral oil was 20.4 ± 8.5% and 20.8 ± 8.3% of the administered dose, respectively, as determined by urinary and faecal excretion of radiolabel for 30 days after topical application (Wester *et al.*, 1990).

In rats given selected mono-, di-, tetra- and hexachlorobiphenyls as a single dermal dose (0.4 mg/kg bw), dermal penetration varied inversely with the degree of chlorination Garner & Matthews (1998). At 48 hours, dermal penetration ranged from about 100% for the monochlorobiphenyl to about 30% for the hexachlorobiphenyl.

In rats given a topical dose of [^{14}C]-labelled PCB-77 or PCB-153, absorption at 24 hours after dosing ranged from 5% to 8% for both compounds (Hughes *et al.*, 1992). Skin retention was 3–31% for PCB-77 and 3–12% for PCB-153. Dermal absorption was similar for all application forms (solid, aqueous paste, aqueous suspension, dissolved in ethanol). For PCB-153, absorption

was significantly higher when PCB-153 was applied as a solid compared with in ethanol.

Male F344 rats were given single doses (0.4 mg/kg bw) of [^{14}C]-labelled mono-, di-, tetra- and hexachlorobiphenyls applied to 1 cm^2 areas of the dorsal skin (Garner *et al.*, 2006). The more highly chlorinated PCBs were slowly absorbed and accumulated in the adipose tissue and skin. Excretion of absorbed radiolabel varied with chlorine content, ranging from 27% to about 100% at 2 weeks after dosing (Garner *et al.*, 2006).

4.1.2 Distribution

The distribution of PCBs is dependent on the structure and the physicochemical characteristics of the individual congeners, and also on dose.

(a) Humans

No studies of quantitative distribution in humans after controlled exposure to PCBs were available to the Working Group. However, some information existed regarding the concentration of PCBs in human tissues and biological fluids after occupational or dietary exposure. PCBs distribute preferentially to adipose tissue and concentrate in human breast milk due to its high fat content. The pattern of congeners observed in tissues does not correspond with the profiles of commercial PCB mixtures.

The most commonly detected PCBs in plasma and in adipose tissue of occupationally exposed individuals are the hexa- and heptachlorobiphenyls. PCB congeners with chlorine atoms in the 4 and 4′ positions were generally found at relatively high concentrations, while PCBs with nonsubstituted 3,4-positions on at least one ring were present at lower concentrations (ATSDR, 2000).

In Greenlanders exposed through high consumption of fat from sea mammals, the most abundant PCB congeners found in adipose tissue, plasma, and liver were PCB-138, PCB-153, and PCB-180 (Dewailly *et al.*, 1999).

Some studies focused on transplacental transfer of PCBs, as determined by measurement of PCB concentrations and congener profiles in maternal blood, placenta and cord blood. Tsukimori *et al.* (2013) investigated concentrations of four non-*ortho* PCBs (PCB-77, PCB-81, PCB-126, PCB-169) in maternal blood, placenta, and cord blood in 19 pregnant women from Fukuoka City, Japan. Mean concentrations were 3.95, 0.87, and 1.08 pg toxic equivalency (TEQ)/g lipid in maternal blood, placenta, and cord blood, respectively. Among specific congeners, PCB-126 showed the highest ratio for cord blood to maternal blood (0.3). PCBs are able to cross the placental barrier in humans, with PCB concentration in cord blood being 25–50% of that in maternal blood.

A study of 360 second-grade schoolchildren (a subgroup of the cohort in Hesse, Germany) in 1995 (Karmaus *et al.*, 2001a, b) found a significant dose-dependent relationship between the duration of breastfeeding (0, 1–4 weeks, 5–8 weeks, 9–12 weeks, > 12 weeks) and blood concentrations of all organochlorine compounds, including PCBs. Breastfeeding for more than 12 weeks was associated with a doubling of concentrations of organochlorine compounds in the children's blood.

Scheele *et al.* (1992) measured the concentrations of PCB-138, PCB-153, and PCB-180 in 38 children with leukaemia and 15 children in a control group. The PCB concentrations in bone marrow were higher by two- to threefold than those in fat tissue; however, there was no significant difference between PCB concentrations in bone marrow of children with leukaemia and of children in the control group.

PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, and PCB-180 were analysed in six post-mortem samples of human lung (Rallis *et al.*, 2012). The limit of quantification (LOQ) varied from 1.7–4.5 ng/g tissue. PCB-153 (detected in two cases), PCB-138 and PCB-180 (detected in three cases) were found at highest concentrations, ranging from < LOQ to 6.3 ng/g.

In 107 post-mortem samples of human brain (Mitchell *et al.*, 2012), eight congeners (PCB-28, PCB-95, PCB-105, PCB-118, PCB-138, PCB-153, PCB-170, and PCB-180) were analysed. PCB-138, PCB-153, and PCB-180 were most frequently detected, at average concentrations of 5.5–8 ng/g lipid. PCB-95 was mainly detected in samples from individuals with neurodevelopmental disorders with a known genetic basis, compared with neurologically typical controls.

In addition to the parent PCBs, hydroxylated metabolites have been detected in human serum and adipose tissue (Fernandez *et al.*, 2008). The concentrations of hydroxylated PCBs (OH-PCBs; 14 congeners), methylsulfonyl PCBs ($MeSO_2$-PCBs; 24 congeners), and parent PCBs (17 congeners) in five paired samples of human liver and adipose tissue were reported by Guvenius *et al.* (2002). The sum of OH-PCB congeners was higher in liver (7–175 ng/g lipid) than in adipose tissue (0.3–9 ng/g lipid), with 3′-OH-PCB-138 and 4′-OH-PCB-130 as the predominant OH-PCB metabolites. The sum of $MeSO_2$-PCBs was of the same order of magnitude as OH-PCB congeners in the same samples: 12–358 ng/g lipid and 2–9 ng/g lipid in liver and adipose tissue, respectively. The concentrations of parent PCBs were similar in liver and adipose tissue, at 459–2085 ng/g lipid and 561–2343 ng/g lipid, respectively.

Concentrations and congener profiles of PCBs and OH-PCBs in placenta samples from a population in Madrid, Spain, were reported by Gómara *et al.* (2012). The sum of PCB concentrations in placenta samples ranged from 943–4331 pg/g fresh weight, and their hydroxylated metabolites showed a 20-times lower concentration (53–261 pg/g fresh weight). PCB-52 and PCB-101 accounted for more than 44% of the total amount of PCBs. The OH-PCB profiles were dominated by 4-OH-PCB-187 and 4-OH-PCB-146, representing > 50% of the sum concentration of OH-PCBs in the placenta samples.

The concentration of OH-PCBs may comprise 10–20% of total PCBs in human serum, and as many as 38 different OH-PCBs were structurally identified in human plasma, pooled from 10 randomly selected male donors. Only a few of these make up the major proportion of the OH-PCBs present in human blood (Hovander *et al.*, 2002).

$MeSO_2$ metabolites of PCBs were investigated in serum samples from pregnant women from Slovakia and in a selected number of paired samples of cord blood (Linderholm *et al.*, 2007). The major methylsulfone in most samples was a non-identified $MeSO_2$-hexachlorinatedbiphenyl, followed by 4′-$MeSO_2$-PCB-101, 4′-$MeSO_2$-PCB-87, and 4-$MeSO_2$-PCB-149. The concentrations of $MeSO_2$-PCBs in maternal serum were about 1.5 times higher than in the corresponding cord serum on a lipid-weight basis. In samples of human adipose tissue, 4-$MeSO_2$-PCB-49 [4-$MeSO_2$-2,2′,4′,5-tetraCB; 4′-$MeSO_2$-PCB-49], 4-$MeSO_2$-PCB-101 [4′-$MeSO_2$-PCB-101; 4-$MeSO_2$-2,2′,4′,5,5′-pentaCB], and 3-$MeSO_2$-PCB-110 [5-$MeSO_2$-PCB-110; 3-$MeSO_2$-2,3′,4′,5,6- pentaCB] were the predominant $MeSO_2$ metabolites (Karásek *et al.*, 2007).

(b) Experimental systems

(i) PCB mixtures

Adult rhesus monkeys were given Aroclor 1248 as a single dose at 1.5 or 3.0 g per kg bw by gastric intubation, and killed after 4 days (Allen *et al.*, 1974). At the lowest dose tested, average concentrations found in liver, kidney, and brain were 25, 12, and 17 µg/g tissue, respectively. In another study, two groups of eight adult rhesus monkeys were exposed to diets containing Aroclor 1248 at 2.5 ppm (Allen & Barsotti, 1976). After 6 months of exposure, the monkeys were successfully bred. After 2 months, milk samples after birth were obtained from four lactating mothers exposed at 2.5 ppm. Concentrations of PCBs ranged from 0.154 to 0.397 µg per g milk in

three samples of milk fat, and reached 16.44 µg per g in milk fat in the fourth sample.

PCBs were analysed in blood, adipose tissue, liver, kidney and brain from female rhesus monkeys fed Aroclor 1254 at a daily dose of 0, 5, 20, 40, or 80 µg/kg bw for approximately 6 years (16 animals per group) (Mes *et al.*, 1995a). Offspring were nursed for 22 weeks and fed no additional PCBs until necropsy at approximately 120 weeks after birth. PCB concentrations in all tissues of the adult monkeys (mothers and offspring) increased with increasing dose. Mes *et al.* (1994) reported that for groups exposed to higher doses (≥ 40 µg/kg bw), tissues of infants from dosed dams contained higher concentrations of PCBs than tissues of infants from control dams. The PCB distribution pattern in tissues from a dosed mother/infant pair differed considerably. A larger percentage of heptachlorobiphenyls was found in the infants than in their dams.

In rats given a single dose of Aroclor 1254 at 500 mg/kg bw by gavage, the highest PCB concentrations were found in adipose tissue (996 µg/g wet weight), liver (116 µg/g wet weight), and brain (40 µg/g wet weight), indicating that PCBs are able to cross the blood–brain barrier (Grant *et al.*, 1971). The relative amounts of PCBs in the brain, liver, spleen, blood, testes, heart, kidney, and adipose tissue of rats killed 3 weeks after treatment were 10%, 16%, 20%, 21%, 22%, 24%, 36%, and 67%, respectively, of those found in animals killed after 2 days. In a subsequent long-term study, Grant *et al.* (1974) fed rats with Aroclor 1254 at a dietary concentration of 0, 2, 20, or 100 mg/kg feed and found highest concentrations of PCBs after 246 days in adipose tissue, with concentrations reaching 26.1 ± 2.9 µg/g wet tissue at the lowest contamination tested (2 mg/kg feed). Levels of PCBs in all tissues analysed were dose-related, and generally, the tissue concentrations did not increase significantly after 64 days of exposure. The residues present in the adipose tissue, liver, and brain had decreased by 64%, 75%, and 10% respectively, 182 days after removal of Aroclor 1254 at 2 mg/kg from the diet. Part of the decrease observed in the adipose tissue and the liver resulted from a dilution effect due to weight increase in these tissues.

The analysis of individual congeners in tissues of rats fed diets containing Aroclor 1254 for 84 days demonstrated a limited accumulation of PCB congeners with a low level of chlorine substitution (tri- and tetrachlorobiphenyls) (Nims *et al.*, 1994). In these rats, time- and dose-dependent increases in the relative concentrations of PCB-138 and PCB-153 were detected in the liver and adipose tissue. Increases in PCB-99 concentrations in hepatic and adipose tissues, and in PCB-156 in adipose tissue, were also observed.

Aroclor 1254 was given to pregnant rats once daily on days 7–15 of gestation (Curley *et al.*, 1973). The concentrations of PCBs found in fetuses were higher by twofold in the group at 50 mg/kg bw compared with the group at 10 mg/kg bw. The mean concentrations of PCB-derived components found in brain, liver, and kidney in weanlings aged 21 days (27 days after the last dose was given to the mother in the group at 10 mg/kg bw) were approximately 2, 4, and 2 µg/g wet tissue, respectively. Concentrations in milk sampled from the same group were between 16 and 25 µg/g.

Samples of brain, adipose tissue, and liver from rat pups and dams exposed to Aroclor 1254 were analysed by Shain *et al.* (1986). In adipose tissue, most congeners were detected at concentrations close to the feed concentration, but the following congeners accumulated to tissue concentrations 10-fold those in the feed: PCB-176, PCB-146, PCB-138 + PCB-168 + PCB 178 (co-eluted), and PCB-177. In the liver and the brain, the congeners present at the highest concentrations were PCB-85 and PCB-179 + PCB-188 (co-eluted). Bioaccumulation of congeners in the milk closely resembled that observed in fat samples from the dams. The chromatographic pattern

of bioaccumulated congeners in pup liver was different from that observed in the dams. The congener found at the highest concentration in samples of newborn rat brain was PCB-85. Shain *et al.* (1986) estimated that the transfer of PCBs through the mammary gland and milk in rats may be 100 times higher than the transfer across the placenta, resulting in a higher accumulation during lactation than during pregnancy.

Kodavanti *et al.* (1998) investigated the congener-specific distribution of PCBs in blood, brain, liver, and adipose tissue of adult rats given repeated doses of Aroclor 1254 (30 mg/kg bw per day; once per day, 5 days per week for 4 weeks). Total PCB congeners in control rat brain were < 0.02 µg/g tissue. Mean concentrations of total PCBs in treated rats in the frontal cortex, cerebellum, and striatum were 15.1, 13.1, and 8.2 µg/g tissue, respectively; those in the blood, liver, and adipose tissue were 1.6, 38.3, and 552 µg/g tissue, respectively. In addition to differential total uptake between tissues, there was differential accumulation of PCBs with respect to number of chlorine substituents. In all tissues, heavily (hexa- to nona-) chlorinated congeners were present in higher proportions than in the parent mixture, Aroclor 1254, while less highly (tetra- and penta-) chlorinated congeners were present to a lesser degree than their respective proportions in Aroclor 1254. This shift towards accumulation of heavily chlorinated congeners appeared to be more pronounced in the brain than in liver and fat.

In rats exposed via inhalation to vapour-phase PCBs generated from Aroclor 1242 for 10 days, much higher amounts of PCBs (× 400) were found in liver and lung than in blood (Hu *et al.*, 2010). PCB-20 + PCB-28 (co-eluted), PCB-49 + PCB-69 (co-eluted), PCB-52, PCB-60, PCB-61 + PCB-70 + PCB-74 + PCB-76 (co-eluted), PCB-66, PCB-83 + PCB-99 + PCB-112 (co-eluted), PCB-85 + PCB-116 + PCB-117 (co-eluted), PCB-90 + PCB-101 + PCB-113 (co-eluted), PCB-105, and PCB-118 were the major congeners in these tissues.

The presence of $MeSO_2$-PCB atropisomers was determined in liver, lung, and adipose tissues of rats orally exposed to Clophen A50. In all tissues analysed, especially lung, *para*-$MeSO_2$ PCBs were more abundant than *meta*-derivatives. An excess of the atropism 2(A_2) of 4-$MeSO_2$-PCB-149 – (*R*)-3-$MeSO_2$-PCB-149 – in lung extracts was observed (Larsson *et al.*, 2002). The enantiomeric enrichment of PCB atropoisomers was reported in selected tissues from rats exposed to Aroclor 1254 (Kania-Korwel *et al.*, 2006). Both PCB-95 and PCB-149 were enantiomerically enriched to a significant extent in adipose tissue, liver, and skin.

A few studies on complex mixtures such as Aroclor 1254 mention substantial retention of certain congeners in lung of treated mice (Anderson *et al.*, 1993).

In mice exposed to contaminated soil (retrieved from a Superfund site before remediation) through their bedding for 4 weeks, total PCB residues in skin and fat declined about 80% during the 4-week recovery period. PCB residues were detected in the ear skin (total PCBs, 208 mg/kg of tissue), trunk skin (total PCBs, 129 mg/kg of tissue), and in body fat (total PCBs, 370 mg/kg), confirming these tissues as important PCB reservoirs (Imsilp & Hansen, 2005).

(ii) Individual congeners

Several experiments carried out in mammals, including non-human primates, confirm the data obtained with complex mixtures. The congeners investigated were unlabelled or labelled PCB-3, PCB-5, PCB-15, PCB-30, PCB-31, PCB-47, PCB-65, PCB-77, PCB-101, PCB-116, PCB-118, PCB-126, PCB-153, and PCB-196 (Goto *et al.*, 1974a, b; Matthews & Anderson, 1975a, b; Abdel-Hamid *et al.*, 1981; Beran *et al.*, 1983; Shimada & Sawabe, 1984; Koga *et al.*, 1990; van Birgelen *et al.*, 1996; Pereg *et al.*, 2001; NTP, 2006a, b, 2010). In some cases, mixtures of individual congeners were used (Öberg *et al.*, 2002; NTP, 2006c, d). Taken together, the data indicated that an oral

dose of PCBs results in an initially high concentration in liver and serum, followed by a decrease in concentrations in the liver, and a concomitant increase in adipose tissue and lipid-rich tissues. This redistribution generally occurred during the first week after dosing, and the differences between the congeners were mainly dependent on the number of chlorine atoms (ATSDR, 2000). In rodents, the hepatic retention/accumulation of non-*ortho*-substituted PCBs such as PCB-126 may occur to a higher extent than in adipose tissue, including after long-term exposure (NTP, 2006a). This was not the case for congeners with chlorine atoms in *ortho* positions, such as PCB-153 (van Birgelen *et al.*, 1996; NTP, 2006a, b).

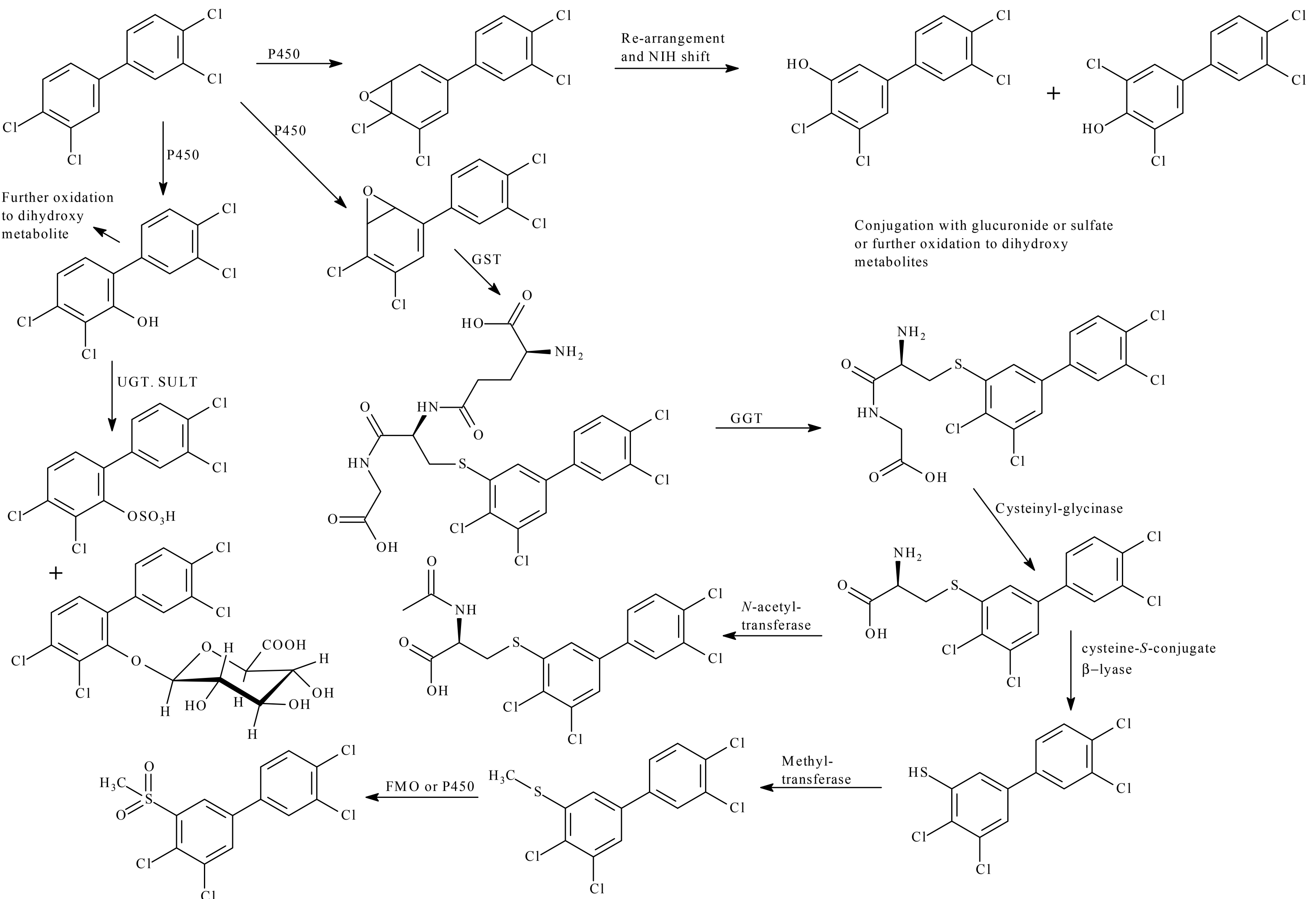
4.1.3 Metabolism

There is evidence that most known PCBs are subject to biotransformation (metabolism) in humans and other animals through enzymatic processes (Safe, 1993). Biotransformation is important for the eventual elimination of PCBs from the body, as most (but not all) of the metabolites are more water-soluble than the parent compound. As well as serving as substrates for biotransformation enzymes, some PCBs and PCB metabolites can interact with several drug-metabolizing enzymes as inducers or inhibitors, as discussed further below.

The first step in metabolism targets the biphenyl ring carbons, and is catalysed by cytochrome P450 (CYP) monooxygenase enzymes. Subsequent metabolism involves one or more of several other possible enzymatic pathways (James, 2001). Some of the major pathways of PCB metabolism are illustrated in Fig. 4.1, with PCB-77 as an example. Fig 4.2 shows structures of representative PCB metabolites. The rate and extent of biotransformation of a particular PCB congener depend upon its chlorination pattern, the number of chlorine substituents, the species, age, and sometimes sex of the animal, and in some cases whether or not the exposure is continuous or a single exposure. The number of chlorine substituents and substitution pattern determine how well a particular PCB congener binds to and can be metabolized by the biotransformation enzyme (Matthews & Dedrick, 1984). In general, congeners with more than four chlorine substituents are more slowly metabolized than those with four or fewer chlorines, and congeners with unsubstituted 3,4-positions in one or both rings are more readily metabolized than those without such substitution patterns (Hansen, 2001). Biotransformation enzymes with similar functions often differ between animal species in properties of substrate recognition and binding, which contributes to species differences in metabolism. Very young animals often have lower levels of several biotransformation enzymes than adults, resulting in age-related differences in metabolism (Hines, 2008). In rodents, sex affects the expression of several important biotransformation enzymes, particularly CYP, which can lead to sex-specific differences in PCB metabolism. The reason that continuous exposure to certain PCB congeners can affect rate and extent of metabolism is that such exposure can result in upregulation of expression of enzymes that biotransform PCBs, through receptor-mediated processes. PCBs that bind the aryl hydrocarbon receptor (AhR) (see Section 4.3.1) are known to induce CYP isoforms in the 1 family (CYP1A1, CYP1A2 and CYP1B1) as well as epoxide hydrolase, some isoforms of uridine diphosphate-glucuronosyltransferase (UGT) and glutathione *S*-transferase (GST) (Parkinson *et al.*, 1980; 1983). PCBs that bind the nuclears receptors, the pregnane-X receptor (PXR) and the constitutive androstane receptor (CAR) have been shown to induce CYP3A4 and CYP2B isoforms (Petersen *et al.*, 2007; Al-Salman & Plant, 2012).

In the context of carcinogenesis, biotransformation to electrophilic metabolites that are more chemically reactive than the parent PCB is likely to be an important component. Being more biotransformed, the metabolized congeners are more likely to undergo bioactivation.

Fig. 4.1 Metabolic pathways for polychlorinated biphenyls, showing PCB-77 (3,3′,4,4′-tetrachlorobiphenyl) as an example

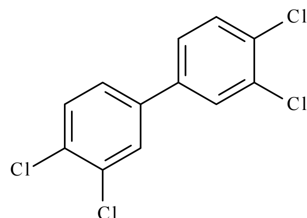


P450, cytochrome P450; UGT, UDP-glucuronosyltransferase; SULT, 3′-phosphoadenosine-5′-phosphosulfate-sulfotransferase; GST, glutathione-*S*-transferase; GGT, gamma-glutamyl transpeptidase; FMO, flavin monooxygenase

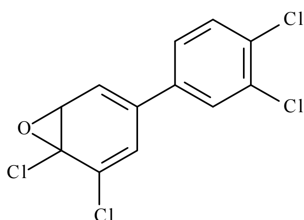
The NIH shift causes non-enzymatic migration of chlorine atoms to an adjacent carbon.

Compiled by the Working Group

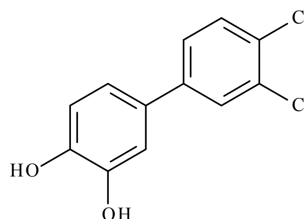
Fig. 4.2 Representative metabolites derived from PCB-77 (3,3′,4,4′-tetrachlorobiphenyl)



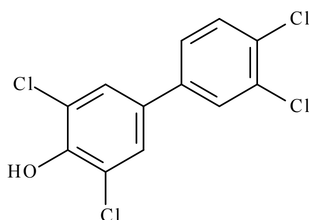
3,3',4,4'-tetrachlorobiphenyl (parent)



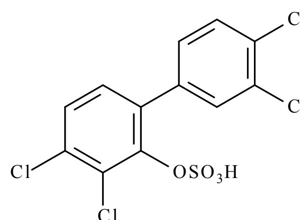
4,5-arene oxide of 3,3',4,4'-tetrachlorobiphenyl



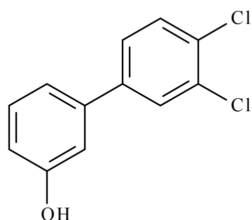
3',4'-dihydroxy-3,4-dichlorobiphenyl
(catechol metabolite)



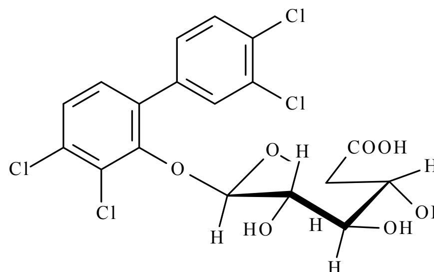
4'-hydroxy-,3,3',4,5'-tetrachlorobiphenyl



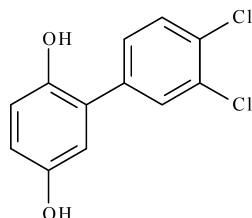
2'-hydroxy-3,3',4,4'-tetrachlorobiphenyl-2'-sulfate



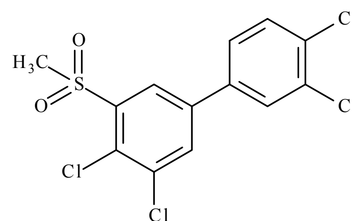
3'-hydroxy-3,4-dichlorobiphenyl



2'-hydroxy-3,3',4,4'-tetrachlorobiphenyl-2'-glucuronide



2',5'-dihydroxy-3,4-dichlorobiphenyl
(semiquinone metabolite)



5-methyl-sulfonyl-3,3',4,4'-tetrachlorobiphenyl
(methyl sulfone metabolite)

The following sections describe the different enzymatic pathways known to be involved in PCB metabolism.

(a) CYP

The first step in biotransformation of PCBs is introduction of oxygen, catalysed by one or more members of the CYP superfamily of monooxygenase enzymes (Guengerich, 2008). Two mechanisms are known, H• radical abstraction and recombination of the short-lived chlorobiphenyl radical with an OH• radical from the active site of CYP to give a hydroxylated (phenolic) metabolite, and formation of an arene oxide by addition of oxygen across an aromatic bond in the biphenyl ring. The arene oxide is an electrophilic metabolite that can rearrange non-enzymatically to form a phenolic metabolite. If one of the carbons that forms part of the arene oxide is substituted with chlorine then, during the non-enzymatic rearrangement, that chlorine can migrate to the adjacent non-chlorine-substituted carbon, while the phenolic hydroxy group attaches to the carbon previously substituted with chlorine, a mechanism known as the NIH shift (shown in Fig. 4.1). Alternatively, the arene oxide may undergo further metabolism by epoxide hydrolase or GST, or may bind with a nucleophilic site on DNA, such as the N7 of guanine, to form an adduct.

As noted above, the chlorine substitution pattern, number of chlorine substituents and presence or absence of unsubstituted 3,4-positions are important factors in determining how readily a particular congener is metabolized by CYP. There are more than 50 isoforms of CYP in humans, and a similar number in experimental animals (Guengerich, 2008). Studies to date have shown that several human isoforms can biotransform one or more PCB congeners; these include CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CY2C9, CYP2C19, and CYP3A4 (Ariyoshi *et al.*, 1995; McGraw & Waller 2006, 2009; Warner *et al.*, 2009; Yamazaki *et al.*, 2011). Related isoforms usually metabolize the same congeners in rat (Morse *et al.*, 1995; Warner *et al.*, 2009; Wu *et al.*, 2011), mouse (Curran *et al.*, 2011), and other species such as fish (Schlezinger *et al.*, 2000). Studies have suggested that congeners with one or no *ortho*-chlorine substitutent are more likely to be metabolized by CYP1 family isoforms. Although it has not been explicitly demonstrated, CYP1B1 metabolizes many of the same substrates as CYP1A1 and CYP1A2 (Shimada *et al.*, 1997) and may also metabolize congeners with one or no *ortho* chlorine. However, CYP1B1 protein is not constitutively expressed in liver, the major drug-metabolizing tissue, and is generally very low in normal tissues (Murray *et al.*, 2001). Congeners with two or more *ortho*-chlorine substituents are usually metabolized by CYP2A, CYP2B, CYP2C and CYP3A subfamily isoforms. It is not well understood which isoforms are involved in monooxygenation of each known PCB congener. This is partly because of difficulties in studying monooxygenation of some of the congeners in vitro with hepatic microsomes or expressed recombinant individual CYP isoforms. The less chlorinated congeners, which tend to be readily metabolized by CYP, are easily studied in vitro; however, until recently they attracted much less attention than the more highly chlorinated congeners (Espandiari *et al.*, 2004). The difficulty in studying highly chlorinated congeners is that they are very slowly metabolized, and conditions for incubation in vitro are difficult to set up to produce sufficient hydroxylated metabolite for analysis. With increasingly sensitive analytical techniques, this problem can be overcome (Yamazaki *et al.*, 2011). While some early publications claimed that certain congeners did not produce metabolites in particular species (Murk *et al.*, 1994), these congeners were later shown through studies in vivo to produce hydroxylated metabolites (Buckman *et al.*, 2007).

An important determinant of the activity of CYP is whether or not the isoform that metabolizes a particular congener is subject to induction, either through exposure to PCBs or through

exposure to other agents known to induce that form of CYP. For example, many congeners with no or one *ortho* chlorine are metabolized by CYP1A1 or CYP1A2 (Curran *et al.*, 2011), and these congeners, like dioxin, polycyclic aromatic hydrocarbons and some components of tobacco smoke, induce CYP1A1 and CYP1A2 by binding to and activating AhR (Parkinson *et al.*, 1983; Safe, 1993). CYP1B1 is also induced by compounds that bind and activate AhR (Murray *et al.*, 2001). A study in which wildtype and knockout mouse strains were exposed in utero and by lactation to a complex mixture of PCBs showed that mice with poor-affinity AhR and lacking CYP1A2 (*Cyp1a2*$^{(-/-)}$ knockout) had higher concentrations of congeners with no or one *ortho* chlorine in tissues than mice with high-affinity AhR and CYP1A2 (*Cyp1a2*$^{(+/+)}$ wild-type), consistent with low metabolism of these PCB congeners in the knockout mice (Curran *et al.*, 2011). PCBs with two or more *ortho* chlorines and at least one *para* chlorine interact with rat and human CAR and induce CYP2B family isoforms, including CYP2B1 and CYP2B6 in a similar manner to the classic inducer, phenobarbital (Parkinson *et al.*, 1980; Al-Salman & Plant, 2012). Some PCBs with two or more *ortho* chlorines have been shown to bind to the human and rat PXR and to human CAR, resulting in upregulation of CYP3A4 (Waller *et al.*, 1996; Petersen *et al.*, 2007; Al-Salman & Plant 2012). CYP3A4 converts PCB-101 and PCB-118 to hydroxylated metabolites (McGraw & Waller, 2009). Activation of CAR also results in upregulation of CYP2B isoforms, several of which have been shown to metabolize PCBs with two or more *ortho* chlorines. For example, human CYP2B6 and the related enzyme, canine CYP2B11, were shown to convert PCB-153 to the 3-hydroxylated metabolite, albeit very slowly (Ariyoshi *et al.*, 1995).

An interesting subgroup of PCBs comprises the 19 chiral PCB congeners, all of which have three or more *ortho* chlorines, which limit rotation around the biphenyl bond. There was evidence that these compounds are enantioselectively metabolized, resulting in depletion of one enantiomer through metabolism, while the form that is resistant to metabolism accumulates (Kania-Korwel *et al.*, 2008; Lehmler *et al.*, 2010). Forms of CYP identified as metabolizing chiral PCBs are rat CYP2B1 and human CYP2B6. For example, there was evidence that PCB-45, PCB-84, PCB-91, PCB-95, PCB-132 and PCB-136 were enantioselectively metabolized to hydroxylated metabolites in vitro by purified rat CYP2B1 and human CYP2B6, leading to alterations in the enantiomeric fractions of the parent congeners (Warner *et al.*, 2009). The positions of hydroxylation were not identified. In a separate study, rat liver microsomes preferentially metabolized (+)-2,2′,3,3′,6,6′-hexachlorobiphenyl (PCB-136) to 5-hydroxy-PCB-136 (5-OH-PCB-136), and treatment of rats with phenobarbital, which induces CYP2B1, further increased the formation of 5-OH-PCB-136 from (+)-PCB-136, compared with untreated rats, thereby leaving an excess of the less readily metabolized (–)-PCB-136 (enantiomeric enrichment) (Wu *et al.*, 2011). There was also a slight increase in 5-OH-PCB-136 formation in dexamethasone-treated rats, which have induced CYP3A, compared with controls. The minor metabolites, 4-OH-PCB-136 and 4,5-dihydroxy-PCB-136 were also formed preferentially by microsomes from phenobarbital-treated rats compared with controls. Since the ryanodine receptor is sensitized only by (–)-PCB-136, more rapid metabolism of (+)-PCB-136 means that the more toxic enantiomer is preferentially retained in the body.

Once formed, OH-PCBs are sometimes further hydroxylated by CYP and perhaps other oxygenases to dihydroxy-PCBs (McLean *et al.*, 1996a; Garner *et al.*, 1999; Wu *et al.*, 2011). If the two OH groups are *ortho* to each other, the metabolites are termed catechols (Garner *et al.*, 1999), and if the two OH groups are *para* to each other, the metabolites are termed hydroquinones (or semiquinones) (Fig. 4.2).

(b) Other oxidative enzymes

PCB catechols and hydroquinones can undergo oxidation to PCB quinones, which are electrophilic, potentially reactive, metabolites. One pathway for quinone formation is through the action of prostaglandin endoperoxide H synthase, an enzyme expressed in extrahepatic tissues, including prostate, ovary, and breast (Wangpradit *et al.*, 2009). Hydroquinones can also be converted to quinones by peroxidases such as horseradish peroxidase, myeloperoxidase, and lactoperoxidase (Srinivasan *et al.*, 2002).

(c) Epoxide hydrolase

If not quickly rearranged to form a phenolic metabolite, an arene oxide metabolite can be converted to a dihydrodiol by addition of water, in a reaction catalysed by epoxide hydrolase (Ota & Hammock, 1980). The dihydrodiol metabolite is generally non-toxic and readily eliminated as the dihydrodiol or as a glucuronide conjugate. It has been suggested that dihydrodiol metabolites of PCBs may be oxidized by dihydrodiol dehydrogenase to the corresponding catechol metabolite, thereby restoring aromaticity to the ring (Garner *et al.*, 1999). Furthermore, catechols could be converted to the *ortho* quinone, which is chemically reactive and can bind to protein and DNA (Zhao *et al.*, 2004).

(d) PCB oxygenation and formation of ROS

PCB biotransformation by CYP can sometimes give rise to formation of reactive oxygen species (ROS) of PCBs, through uncoupling of the CYP cycle. Formation of ROS during PCB monooxygenation by CYP most likely occurs if the congener binds to the CYP substrate-binding site in an orientation that is not favourable for rapid monooxygenation: this has been demonstrated for PCB-77 biotransformation by CYP1A from fish and other vertebrates (Schlezinger *et al.*, 1999, 2000). PCB-77 has been shown to inhibit ethoxyresorufin *O*-deethylase (EROD) activity at high concentrations, perhaps by competitive inhibition of CYP1A by PCB-77 (Hahn *et al.*, 1993). Formation of ROS through uncoupling of the CYP1A cycle has been demonstrated with two other non-*ortho*-substituted PCB congeners, PCB-126 and PCB-169 (Schlezinger *et al.*, 2006). PCB-126 and PCB-169 were also shown to uncouple human CYP1B1 and produce ROS (Green *et al.*, 2008). Since CYP1B1 is expressed and inducible in tissues that are frequent targets for cancer, including colon, breast, lung, endometrium, ovary, and prostate, formation of ROS in these tissues could result in genotoxicity.

PCB metabolism by peroxidases and prostaglandin H synthase (also called cyclooxygenase; COX) can also give rise to ROS (Gonçalves *et al.*, 2009). Another pathway leading to ROS production during PCB metabolism occurs when quinone metabolites are formed. Quinones undergo redox cycling through reaction with glutathione (GSH) to form adducts through Michael addition. The quinone-GSH-adduct can be converted back to the semi-quinone or catechol and recycled through this pathway (Amaro *et al.* 1996; Oakley *et al.*, 1996a). This cycling results in depletion of the important cellular antioxidant, GSH, which can cause oxidative stress to the cell and formation of ROS. Redox cycling of the 2′,5′-dihydroxy metabolite of PCB-12 has been shown to result in DNA adducts through formation of ROS (Oakley *et al.*, 1996a).

(e) GST

Arene oxide metabolites of PCBs are potential substrates for GSTs, as shown in Fig. 4.1. After initial formation of a conjugate with GSH, the two terminal amino acids of the tripeptide are enzymatically removed, leaving a cysteine conjugate of the PCB. This metabolite may be converted to a mercapturic acid, which is readily excreted in urine or bile (Bakke *et al.*, 1982). Alternatively, the cysteine conjugate may be a substrate for cysteine conjugate β-lyase, which converts the cysteine conjugate to a thiol. The

thiol metabolite of the PCB can then be methylated by methyltransferase and oxidized by flavin monooxygenase or CYP to yield the $MeSO_2$-PCB (Mio & Sumino 1985). Depending on its structure, the $MeSO_2$-PCB metabolite may not be readily excreted and may accumulate in tissues, particularly liver, lung, and adipose tissue (Haraguchi *et al.*, 1997a, b; Guvenius *et al.*, 2002; Hovander *et al.*, 2006; Karásek *et al.*, 2007). Chiral PCBs were shown, by analysis of the $MeSO_2$-PCBs present in human adipose tissue, seal blubber and pelican muscle, to form $MeSO_2$-PCBs in an enantioselective manner (Karásek *et al.*, 2007). Tissue accumulation can occur in fatty tissues because the $MeSO_2$-PCBs are very lipid soluble, especially those that are highly chlorinated. Accumulation in lung appears to be due to specific binding of the $MeSO_2$-PCBs to an uteroglobin-like protein/PCB-binding protein, a protein that is synthesized in non-ciliated bronchiolar Clara cells of the lung epithelium (Nordlund-Möller *et al.*, 1990; Anderson *et al.*, 1993). Formation of $MeSO_2$-PCBs and their retention in tissues are of concern because several of these metabolites have been shown to interact with the glucocorticoid receptor (Johansson *et al.*, 1998), and to be antiestrogenic (Letcher *et al.*, 2002). Some $MeSO_2$-PCBs such as 3-$MeSO_2$-2,2′,4′,5-tetrachlorobiphenyl [3′-$MeSO_2$-PCB-49] and 3-$MeSO_2$-2,2′,4′,5,5′-pentachlorobiphenyl [3′-$MeSO_2$-PCB-101] were potent inducers of CYP2B1 and CYP2B2 in rats (Kato *et al.*, 1997).

(f) Glucuronosyltransferase and sulfotransferase

OH-PCBs may be expected to be conjugated with glucuronic acid or sulfate to form non-toxic, readily excreted metabolites, in reactions catalysed by uridine 5′-diphosphate-(UDP)-glucuronosyl transferase (UGT) or 3′-phosphoadenosine-5′-phosphosulfate (PAPS)-sulfotransferase (SULT). Glucuronide and sulfate conjugates of a hydroxylated metabolite of PCB-3 were identified in urine of rabbits given 1 g by gavage (Block & Cornish, 1959).

Studies of glucuronidation have not been conducted with human liver microsomes or UGTs; however, two studies demonstrated formation of glucuronide conjugates with several OH-PCB metabolites, using rat liver microsomes and expressed rat UGTs in yeast strain AH22 (Tampal *et al.*, 2002; Daidoji *et al.*, 2005). In a further study of OH-PCB glucuronidation, it was noted that rates of conjugation varied with the particular OH-PCB substitution pattern, in catfish as well as in rats (Sacco *et al.*, 2008). Those OH-PCBs with only one chlorine flanking a 4-OH group exhibited a higher V_{max} for glucuronidation than OH-PCBs with a 4-OH-3,5-dichloro substitution pattern. The more slowly glucuronidated 4-OH-3,5-dichloro-substituted OH-PCBs were shown to be more potent inhibitors of human estrogen sulfotransferase (human SULT1E1) than those lacking two flanking chlorine atoms (Kester *et al.*, 2000).

In addition to a study in rabbits, which were shown to excrete glucuronide and sulfate conjugates of OH-PCB-3 (Block & Cornish, 1959), further evidence that OH-PCBs are sulfonated in vivo was provided by a study of the fate of PCB-3 in male rats given a dose of 112 mg/kg bw by intraperitoneal injection (Dhakal *et al.*, 2012). The major metabolite was the 4′-sulfate of PCB-3, with little evidence for the glucuronide conjugate; 4′-OH-PCB-3 was converted to the 4′-sulfate conjugate by rat SULT1A1 (Liu *et al.*, 2009). The 4′-OH-PCB-3 was also a substrate for human hepatic cytosolic SULT1A1 (Wang *et al.*, 2006). Other OH-PCBs tested were very poor substrates for human SULT1A1 (Wang *et al.*, 2006), human SULT2A1 (Liu *et al.*, 2006; Ekuase *et al.*, 2011), rat liver SULT1A1, or rat liver SULT2A3 (Liu *et al.*, 2009).

As noted above, OH-PCBs with a 4-OH-3,5-dichloro- structural motif are potent inhibitors of human SULT1E1, with 17-β-estradiol as substrate (Kester *et al.*, 2000). Recent studies showed that some OH-PCBs inhibit sulfonation of dehydroepiandrosterone (DHEA), catalysed by human SULT2A1 or rat SULT2A3 (Liu

et al., 2006, 2009; Ekuase *et al.*, 2011). As well as inhibiting sulfonation of estradiol and DHEA, OH-PCBs inhibit sulfonation and glucuronidation of xenobiotic substrates. Several OH-PCBs were potent inhibitors (low µM values for IC_{50}, the concentration producing 50% inhibition) of sulfonation of 3-hydroxy-benzo[*a*]pyrene catalysed by human liver cytosol, human SULT1A1 and human SULT1E1, but were very weak inhibitors or did not inhibit SULT1A3 (Wang *et al.*, 2005).

(g) Sites of metabolism

For most xenobiotics, including PCBs, the liver is the major organ of metabolism, as most of the drug-metabolizing enzymes are expressed in liver in high concentrations. This is true for several isoforms of CYP, epoxide hydrolase, GST, glucuronosyltransferase, and sulfotransferase; however, the liver is not the only site where these enzymes are expressed. The intestine expresses many of the same enzymes as the liver. The liver is able to convert PCBs to reactive metabolites, and to respond to PCBs that interact with AhR, but the role of metabolism in other tissues is not always clear. Tissues where there are associations between PCB exposure and cancer include the liver, lung, oral mucosa, uterus, thyroid, pancreas, adrenal, breast, skin, blood and lymphatic system, and these effects in some instances may be due to tissue distribution of PCBs or metabolite. As noted above, CYP1B1 is inducible by AhR agonists and has been shown to be expressed in colon, breast, lung, endometrium, ovary and prostate (Schmidt & Bradfield, 1996; Green *et al.*, 2008). Prostaglandin endoperoxide H synthase, implicated in formation of quinone metabolites from OH-PCBs, is expressed in high concentrations in the prostate gland, and is also found in ovary and breast (Wangpradit *et al.*, 2009). The skin contains inducible CYP1A, as well as other drug-metabolizing enzymes (Costa *et al.*, 2010).

4.1.4 Excretion

(a) Humans

Two well designed studies (taking into account ongoing exposure and body weight changes, and not limited by small sample size or short sampling interval) showed that highly chlorinated congeners persist in the body, with half-lives averaging about 8–15 years, while less chlorinated PCBs clearly have shorter half-lives (Table 4.1; Grandjean *et al.*, 2008; Ritter *et al.*, 2011).

Few studies on the faecal (Schlummer *et al.*, 1998; Moser & McLachlan, 2001), or urinary excretion (Price *et al.*, 1972; ATSDR, 2000) of PCBs in humans have been published. A substantial part of absorbed or retained PCBs may be eliminated via breast milk (see Section 1.4 in this *Monograph*). Concentrations varying from 9 to 1915 ng/g lipid have been reported in the general population. Not only parent compounds, but also OH-PCBs were detected in breast milk. Traces of OH-PCBs (median of the sum of 12 congeners, 3 pg/g milk) were found in milk samples collected in 2000–2001 from 15 mothers living in Stockholm; the ratio of total PCBs to total OH-PCBs was approximately 1400, and the major metabolite was an unresolved mixture of 4-OH-CB-107 [4-OH-2,3,3′,4′,5-pentaCB; 4-OH-PCB-109] and 4′-OH-CB-108 [4′-OH-2,3,3′,4,5′-pentaCB; 4-OH-PCB-107] (Guvenius *et al.*, 2003). Adenugba *et al.* (2009) analysed 15 samples of human bile, collected endoscopically, for seven PCB congeners (PCB-28, PCB-52, PCB-101, PCB-118, PCB-153, PCB-138, and PCB-180). Total PCB concentrations in bile ranged from 6 to 49 ng/mL, and PCB-28 was the predominant congener.

(b) Experimental systems

Elimination half-lives have been estimated in different animal species. In rats, elimination half-lives vary from days (di- and trichlorobiphenyl) to more than 3 months (penta- and

Table 4.1 Estimated human elimination half-lives for nine PCB congeners at background concentrations

Age group	Elimination half-life (years)								
	PCB-28	PCB-52	PCB-105	PCB-118	PCB-138	PCB-153	PCB-170	PCB-180	PCB-187
Children[a]	NR	NR	5.4	5.7	3.7	8.4	7.6	9.1	8
Adults[b]	5.5	2.6	5.2	9.3	10.8	14.4	15.5	11.5	10.5

[a] Grandjean *et al.* (2008), *n* = 200
[b] Ritter *et al.* (2011), *n* = 229
NR, not reported
Adapted from Ritter *et al.* (2011)

hexachlorobiphenyl), while a half-life of approximately 10 months was estimated for Aroclor 1254 in weanling pigs (ATSDR, 2000). Half-lives of a group of congeners (PCB-105, PCB-118, PCB-128, PCB-138, PCB-153, PCB-156, PCB-157, PCB-180, PCB-183) were estimated in monkeys dosed with Aroclor 1254. On average, half-lives varied from 0.4 years (PCB-105) to 1.9 years (PCB-128); however, a wide range of estimates (0.42–7.58 years, depending on individuals) was reported for PCB-128 (Mes *et al.*, 1995b). [These data indicated that PCB half-lives vary according to species, and that PCB half-lives are longer in humans than in experimental animals, including monkeys.]

In rodents, PCBs administered by different routes are mainly excreted in the faeces, with urine usually representing a minor route of excretion.

PCB metabolites that have been identified in urine are mentioned in Section 4.1.3. In addition to OH-PCBs and dihydroxylated PCBs and corresponding glucuronides also observed in other studies, the elimination in urine of sulfated metabolites of PCB-3, PCB-3 2′-sulfate, PCB-3 3′-sulfate, and PCB-3 4′-sulfate after a single intraperitoneal dose of PCB-3 (112 mg/kg bw) was reported. In rats, approximately 3% of the administered dose was excreted in the urine as sulfates over 36 hours, with peak excretion occurring 10–20 hours after exposure (Dhakal *et al.*, 2012). Mercapturic acid of [^{14}C]-2,4′,5-trichlorobiphenyl (PCB-31) was isolated from the urine of rats treated with this congener (Bakke *et al.*, 1982). This metabolite represented 0.3% of the administered dose of 4 mg per rat. About 57% of the administered dose was excreted in the bile, and 30–35% was present as metabolites in the mercapturic acid pathway.

Lactation is also a major route of excretion of PCBs in animals. In monkeys exposed to different doses of Aroclor 1254 in long-term studies, approximately 4% of the intake was eliminated in milk (Mes *et al.*, 1994). The transfer of [^{14}C]-labelled congeners PCB-77, PCB-126, PCB-169, and PCB-105 to milk has been investigated in mice (Sinjari *et al.*, 1996). These compounds were administered intraperitoneally to lactating mice at a single dose of 2.0 µmol/kg bw each on postnatal day 11. Concentrations of PCB-126, PCB-169 and PCB-105 in milk 1 day after administration were higher (1450–2520 pmol/mL) than concentrations of PCB-77 (580 pmol/mL).

In addition to these routes of elimination, other minor pathways have been reported. Studies by Yoshimura & Yamamoto (1975) on PCB-66 in rats have suggested that excretion of unmetabolized PCB through the small intestinal wall may occur. In other experiments with rats, PCBs were excreted unchanged in hair and through the skin (Matthews *et al.*, 1976).

4.2 Genetic and related effects

Since the first *IARC Monograph* on PCBs (IARC, 1978), the genetic and related effects of PCBs have been studied in several experimental systems and in humans (for details and references, see Tables 4.2, 4.3, 4.4, 4.5, 4.6), and summarized in numerous reviews (Safe, 1989; Silberhorn *et al.*, 1990; ATSDR, 2000; Ludewig, 2001).

4.2.1 Exposed humans

Several studies have used cytogenetic effects (structural chromosome aberration, sister-chromatid exchange, and DNA adducts) in cells from body fluids (blood and semen) as biomarkers in humans occupationally or environmentally exposed to PCBs (see Table 4.2).

(a) Genotoxicity and cytogenicity from occupational exposure

Peripheral lymphocytes from 32 workers exposed occupationally to commercial PCB mixtures (DELOR 103 and 106) for up to 25 years were examined for cytogenetic changes. All workers with PCB exposure were smokers and moderate drinkers, and control groups were chosen accordingly: control group 1 consisted of 20 people working outside the PCB-production unit, and control group 2 consisted of 20 employees from administrative offices and the research department (Kalina *et al.*, 1991). Workers with PCB exposure were also exposed to formaldehyde and benzene, but at levels not exceeding national exposure limits. Occupational exposure to PCB mixtures led to an increase in PCB plasma concentrations of more than 100-fold (305–487 µg/L), when compared with the control groups (1.5–3 µg/L). A significant increase in the frequency of chromosomal aberration and sister-chromatid exchange was observed in workers exposed to PCBs for at least 11 years; however, no dose–response effect was observed between cytogenetic effects and PCB blood concentrations. [The Working Group was not able to determine how the PCB plasma concentrations were measured. No quantitative data were provided on the exposure of the workers to benzene and formaldehyde, or on whether all three groups were similarly exposed to benzene and formaldehyde. The choice of control group used for the *t*-test analysis was not clearly indicated].

An increase in structural chromosomal aberration in lymphocytes was also observed in workers occupationally exposed to PCBs when compared with a non-exposed control group; however, no information on PCB blood concentrations or confounders was available (Joksić & Marković, 1992).

Peripheral blood lymphocytes from male workers (n = 21) exposed occupationally to PCBs for 2–5 years at a factory decontaminating industrial transformers and capacitors and from workers in an industrial control group (87; 53 men and 34 women) were analysed for structural and numerical chromosomal aberrations. Significant increases of twofold in the frequency of structural chromosomal aberration and four- to sixfold in the frequency of premature centromere division in mitotic chromosomes were observed in the PCB-exposed group (Jakab *et al.*, 1995; Major *et al.*, 1999). [The Working Group noted that PCB concentrations in blood and/or air were not monitored, the industrial control group was not further specified, and no adjustment for confounders was made.]

Two studies of occupational exposure examined workers exposed to PCBs after a fire at an electric station (Elo *et al.*, 1985; Melino *et al.*, 1992). In one study, maximum blood PCB concentrations (median, 14 µg/L) were reached 3 days after exposure and declined over the course of 1 month to background levels (≤ 2 µg/L). No exposure-related increases in the frequency of structural chromosomal aberration and sister-chromatid exchange in 15 PCB-exposed workers were observed for

Table 4.2 Genetic effects and markers of oxidative DNA damage in humans exposed to PCBs

Target tissue	End-point	Result	Comments	Reference
Occupational exposure				
Peripheral blood lymphocytes	Chromosomal aberration Sister-chromatid exchange	– –	Exposed, 15; unexposed, not defined No details on individual numbers and statistical analysis	Elo *et al.* (1985)
Peripheral blood lymphocytes	Chromosomal aberration	–	Exposed, 45 (29 men, 12 women, 4 children) living within 2 km from capacitor-manufacturing plant (24 workers, 21 residents); unexposed; pre-employment test from workers Heavy smokers excluded; no statistical analysis; no correlation with PCB concentrations (11 congeners) in blood and adipose tissue [no details on PCB concentrations were given]	Tretjak *et al.* (1990)
Peripheral blood lymphocytes	Chromosomal aberration Sister-chromatid exchange	+ ($P < 0.01$) + ($P < 0.05$)	Exposed to technical PCB mixture, 32; unexposed group 1 (working outside production unit), 20; unexposed group 2 (administration and research), 20. Positive correlation with duration of exposure but not blood PCB levels	Kalina *et al.* (1991)
Peripheral blood lymphocytes	Chromosomal aberration Micronucleus formation Sister-chromatid exchange	? + +	Exposed, 48; unexposed, 15 No statistical analysis performed	Jokšić & Marković (1992)
Peripheral blood lymphocytes	Chromosomal aberration Sister-chromatid exchange	– –	Exposed, 12; unexposed, 19 No serum PCB concentrations; both groups contained moderate smokers; no confounder taken into account	Melino *et al.* (1992)
Peripheral blood lymphocytes	Chromosomal aberration Premature centromere division	+ ($P < 0.01$) + ($P < 0.01$)	Exposed, 21 (men); unexposed, 87 (53 men,34 women) Heavy smokers (> 20 cigarettes/day); heavy drinkers (> 100 g alcohol/day); donors with neoplasia	Jakab *et al.* (1995), Major *et al.* (1999)
Urine	Oxidative DNA damage (8-OHdG)	–	Study cohort: 64; pre- and post-shift workplace exposure	Wen *et al.* (2008)
Environmental exposure				
Peripheral blood lymphocytes	Chromosomal aberration	–	Exposed, 36 (Yucheng; 17 men, 19 women); unexposed 10 (5 men, 5 women) Sampling of exposed group occurred 3 years after exposure; chromosomal aberrations included breaks, exchanges, acentric fragments, and gaps.	Wuu & Wong (1985)
Peripheral blood lymphocytes	Chromosomal aberration Sister-chromatid exchange *After exposure of lymphocytes to α-naphthoflavone* in vitro*:* Chromosomal aberration Sister-chromatid exchange	– – – + ($P < 0.001$)	Exposed, 35 women (Yucheng victims); unexposed, 24 Blood samples of exposed individuals were taken in 1985 or 5 years after the exposure had occurred; unexposed women were from the same county; all participants were nonsmokers	Lundgren *et al.* (1988)

Table 4.2 (continued)

Target tissue	End-point	Result	Comments	Reference
Peripheral blood lymphocytes	Sister-chromatid exchange	–	Exposed, 16 Yusho patients; unexposed, 39	Nagayama *et al.* (2001)
	Sister-chromatid exchange after exposure of lymphocytes to α-naphthoflavone	–		
Peripheral blood lymphocytes	Micronucleus formation	+ ($P < 0.01$; PCB-118)	Study cohort: up to 1583; age 50–65 years; confounder: age, sex, smoking, lifestyle, body mass index	De Coster *et al.* (2008)
	DNA damage (comet assay)	+ ($P < 0.05$; PCB-118)		
Blood serum	Prostate specific antigen	–		
	Carcinoembryogenic antigen	–		
	TP53	+ ($P < 0.05$; sum of PCB-138, PCB-153, PCB-180)		
Leukocytes	DNA adduct	–	Study cohort: 103 Inuits, categorized into low (1.7–20 µg/L; $n = 54$), medium (21–40 µg/L; $n = 21$) and high (41–143 µg/L; $n = 28$) PCB exposure	Ravoori *et al.* (2008)
Leukocytes	DNA adduct	Negative correlation with PCB ($P < 0.0001$)	Study cohort: 83 Inuits: 56 women, 27 men Effect of age, sex, smoking status, PCB and selenium concentrations on DNA adduct accumulation taken into account	Ravoori *et al.* (2010)
	DNA adduct and 8-OHdG	Negative correlation in the high selenium/PCB ratio group ($P < 0.01$ and $P = 0.014$; respectively)		
Sperm	XY disomy	+ ($P < 0.001$)	Study cohort: 192 men from subfertile couples	McAuliffe *et al.* (2012)
	Total sex-chromosome disomy	+ ($P < 0.001$)		
	XX disomy	Negative correlation ($P < 0.001$)		
Sperm	Sperm chromatid structure	+ ($P < 0.01$)	Study cohort: 176 adult men (Swedish)	Rignell-Hydbom *et al.* (2005)
Sperm	Sperm chromatid structure (DNA fragmentation)	+	Study cohort: 707 adult men (193 Greenland Inuits, 178 Swedish, 141 Polish, and 195 Ukrainian) Statistically positive association for Ukranian and Swedish cohorts, and for European cohorts combined (Sweden, Poland, Ukraine)	Spanò *et al.* (2005)
Sperm	Sperm chromatid structure (DNA fragmentation)	+ ($P < 0.05$)	Study cohort: 652 adult men (200 Greenland Inuits; 166 Swedish, 134 Polish, and 152 Ukrainian) Significant association only for European cohorts combined (Sweden, Poland, Ukraine)	Stronati *et al.* (2006)
Urine	Oxidative DNA damage (8-OHdG)	–	Study cohort: up to 1583; age 50–65 years; confounder: age, sex, smoking, lifestyle, body mass index	De Coster *et al.* (2008)

8-OHdG, 8-hydroxy-2′-deoxyguanosine

Table 4.3 Genetic and related effects of commercial PCB mixtures in experimental systems in vitro

Agent	Test system	Results[a]		Dose[b] (LED or HID), µg/mL	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
Non-mammalian systems					
Aroclor 1221	*Salmonella typhimurium* TA1538, reverse mutation	–	(+)	200	Wyndham *et al.* (1976)
	Saccharomyces cerevisiae, strain RS112, interchromosomal recombination	+	+	10 000	Schiestl *et al.* (1997)
	Salmonella typhimurium TA98, TA1538, reverse mutation	–		5000 µg/plate	Shahin *et al.* (1979)
Aroclor 1254	*Salmonella typhimurium* C3076, D3052, G46, TA98, TA1000, TA1535, TA1537, TA1538, and *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	NR	Probst *et al.* (1981)
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, reverse mutation	–	–	500	Bruce & Heddle (1979) Schoeny *et al.* (1979)
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538, and *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	333	Dunkel *et al.* (1984)
	Salmonella typhimurium TA1538, reverse mutation	–	–	200	Wyndham *et al.* (1976)
	Salmonella typhimurium TA98, TA100, *Escherichia coli* WP2 *uvrA,* reverse mutation	–	–	200 µg/plate	Evandri *et al.* (2003)
	Saccharomyces cerevisae, heterozygous transgenic for human MS32 minisatellite, length mutation	+	NR	6000	Appelgren *et al.* (1999)
Aroclor 1260	*Saccharomyces cerevisiae*, strain RS112, interchromosomal recombination	+	+	15 000	Schiestl *et al.* (1997)
Aroclor 1268	*Salmonella typhimurium* TA1538, reverse mutation	–	–	200	Wyndham *et al.* (1976)
Kanechlor 300	*Salmonella typhimurium* TA1535, TA1536, TA1537, TA1538, TA98, TA100, reverse mutation	–	–	NR	Odashima (1976)
	Salmonella typhimurium TA98, TA100, *Escherichia coli* WP2, reverse mutation	–	–	NR	Sugimura *et al.* (1976)
Kanechlor 500	*Salmonella typhimurium* TA1535, TA1536, TA1537, TA1538, TA98, TA100, reverse mutation	–	–	NR	Odashima (1976)
	Salmonella typhimurium TA98, TA100, *Escherichia coli* WP2, reverse mutation	–	–	NR	Sugimura *et al.* (1976)
Clophen 30	*Drosophila melanogaster*, genetic crossing-over, sex-chromosome loss	–		250	Nilsson & Ramel (1974)
Clophen 50	*Drosophila melanogaster*, genetic crossing-over, sex-chromosome loss	–		200	Nilsson & Ramel (1974)
Mammalian cells in vitro					
Aroclor 1221	Intrachromosomal (non-homologous) recombination at *Hprt* locus, Chinese hamster lung Sp5/V79 cells	–		30	Helleday *et al.* (1998)
	Intrachromosomal (homologous) recombination *Hprt* locus, Chinese hamster lung SPD8/V79 cells	+		20	

Table 4.3 (continued)

Agent	Test system	Results[a]		Dose[b] (LED or HID), μg/mL	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
Aroclor 1221 (cont.)	Intrachromosomal recombination by deletion in *HPRT* locus, human lymphoblastoid GM6804 cells	+		5	Aubrecht *et al.* (1995)
Aroclor 1016	DNA adducts ^{32}P-postlabelling, primary human hepatocytes (three donors)	(+)		23	Borlak *et al.* (2003)
Aroclor 1242	Gene mutation (ouabain resistance), Chinese hamster fibroblast V79 cells	–		150	Hattula (1985)
	Chromosomal aberrations, chicken embryo (*Gallus domesticus*)	–		20	Blazak & Marcum (1975)
Aroclor 1254	DNA single-strand breaks, alkaline elution, rat hepatocytes	+		100	Sina *et al.* (1983)
	DNA strand breaks (comet assay), rat primary prostate cells	+		1	Cillo *et al.* (2007)
	Unscheduled DNA synthesis, primary rat hepatocytes	+		20 (MED)	Althaus *et al.* (1982)
	Unscheduled DNA synthesis, primary F344 rat hepatocytes	–		[16] 50 μM	Probst *et al.* (1981)
	DNA adducts ^{32}P-postlabelling, primary fetal rat hepatocytes		–	[16] 50 μM	Dubois *et al.* (1995)
	DNA adducts ^{32}P-postlabelling, human hepatocarcinoma HepG2 cells	–		[16] 50 μM	Dubois *et al.* (1995)
	DNA adducts ^{32}P-postlabelling, primary human hepatocytes (three donors)	(+)		[20] 60 μM	Borlak *et al.* (2003)
	Detection of repairable adducts by growth inhibition (DRAG) assay in wildtype and DNA repair-deficient Chinese hamster ovary cells	–		135/114, 127, 132[c]	Johansson *et al.* (2004)
	Micronucleus formation, human keratinocytes	–		3	van Pelt *et al.* (1991)
	Chromosomal aberrations, human lymphocytes (five donors)	+		0.1	Sargent *et al.* (1989)
	Cell transformation, Syrian hamster embryo cells	–		50	Pienta (1980)
Clophen A60	Gene mutation (ouabain resistance), Chinese hamster fibroblast V79 cells	–		150	Hattula (1985)
Kanechlor 500 + 600 (plus PCDD/PCDF/ PCB-77, PCB-126, PCB-169 as 0.5% wt)	Sister-chromatid exchange, human lymphocytes	+	+	[0.4 ng WHO-TEQ/g; 0.25 ng WHO-TEQ/g]	Nagayama *et al.* (1994)

[a] +, considered to be positive; (+), considered to be weakly positive in an inadequate study; –, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an inadequate study)

[b] Approximately minimal lethal dose not reported.

[c] Dose 135 μg/mL is the IC_{50} concentration inhibiting growth of wildtype CHO cells (AA8) by 50%; doses 114, 127 and 132 μg/mL are the IC_{50} for repair-deficient CHO cells EM9, UV4 and UV5, respectively.

HID, highest effective dose; LED, lowest effective dose; MED, maximum effective dose; PCDD/PCDF, polychlorinated dibenzodioxin/polychlorinated dibenzofuran; TEQ, toxic equivalency

Table 4.4 Genetic and related effects of commercial PCB mixtures in experimental animals in vivo

Agent	Test system	Results[a]	Dose[b] (LED or HID)	Reference
Aroclor 1221	Intrachromosomal recombination by DNA deletion, homozygous C57BL/6J p^{un}/p^{un} mouse	+	1000 ip × 1	Schiestl *et al.* (1997)
Aroclor 1242	DNA adducts ^{32}P-postlabelling, and 8-OHdG, HPLC/ECD-analysis, male Lewis rat liver, thymus, glandular stomach, spleen, testes, seminal vesicles and prostate gland	–	20 po × 1	Schilderman *et al.* (2000)
	Chromosomal aberrations (structural), male Osborne-Mendel rat bone-marrow and spermatogonial cells	–	5000 po × 1	Green *et al.* (1975a)
		–	500 po × 4	
	Dominant lethality, Osborne-Mendel rat	–	2500 po × 1	Green *et al.* (1975b)
		–	250 po × 5	
Aroclor 1254	DNA adducts (I-compounds only) ^{32}P-postlabelling, male Sprague-Dawley rat liver, kidney, lung	–	500 ip × 2	Nath *et al.* (1991)
	DNA adducts ^{32}P-postlabelling, male F344 rat liver	–	25 po × 35	Chadwick *et al.* (1993)
	Unscheduled DNA synthesis, Sprague-Dawley rat, primary hepatocytes	–	300 ip × 1	Kornbrust & Dietz (1985)
	Unscheduled DNA synthesis, rat, primary hepatocytes	–	500 ip × 1	Shaddock *et al.* (1989)
	Unscheduled DNA synthesis, male cynomolgus monkey, primary hepatocytes	–	50 ip × 1	Hamilton *et al.* (1997)
		–	50 ip × 2	
	Micronucleus formation, fish (*C. carpio*), erythrocytes	+	50 ip × 1	Al-Sabti (1986)
	Micronucleus formation, B6C3F$_1$ mouse, bone-marrow cells	–	15 000 ip × 5	Bruce & Heddle (1979)
	Chromosomal aberrations (structural), fish (*C. carpio; T. tinica; C. idella*), kidney cells	+	50 ip × 1	Al-Sabti (1985)
	Chromosomal aberrations (structural), Sprague-Dawley rat, spermatogonial cells	–	50 po × 7	Dikshith *et al.* (1975)
	Chromosomal aberrations (structural), male Osborne-Mendel rat, bone-marrow cells	–	300 po × 5	Green *et al.* (1975a)
	Chromosomal aberrations (structural), male Holtzman rat, bone-marrow and spermatogonial cells	–	500 ppm, 5 weeks	Garthoff *et al.* (1977)
	Sperm morphology, B6C3F$_1$ mice	–	7500 ip × 5	Bruce & Heddle (1979)
	Germline length mutation PC-1 minisatellite, male C57B1/6 mouse, liver	+	100 ip × 1	Hedenskog *et al.* (1997)
	Germline length mutation PC-2 minisatellite, male C57B1/6 mouse, liver	–	100 ip × 1	Hedenskog *et al.* (1997)
	Dominant lethal mutation, Osborne-Mendel rats	–	300 po × 5	Green *et al.* (1975b)
	Gene mutation, transgenic male BigBlue™ mice	(+)	100 ppm in diet, 7 weeks	Davies *et al.* (2000)
Aroclor 1260	Intrachromosomal recombination by DNA deletion, homozygous C57BL/6J p^{un}/p^{un} mouse	+	500 ip × 1	Schiestl *et al.* (1997)
	DNA adducts ^{32}P-postlabelling, male and female B6C3F$_1$ mouse, liver	–	50 po × 1	Whysner *et al.* (1998)
		–	200 ppm × 2 weeks	

Table 4.4 (continued)

Agent	Test system	Results[a]	Dose[b] (LED or HID)	Reference
Kaneclor 300	Chromosomal aberrations, mouse, bone-marrow cells	–	NR[c]	Odashima (1976)
	Chromosomal aberrations, rat, bone-marrow cells	–	NR[c]	Odashima (1976)
Kaneclor 500	Micronucleus formation, male ddY mice, bone-marrow cells	(+)	100 po × 6	Watanabe *et al.* (1982)
		–	100 sc × 6	
	Chromosomal aberrations, mouse, bone-marrow cells	+	NR	Odashima (1976)
	Chromosomal aberrations, rat, bone-marrow cells	–	NR	Odashima (1976)
Kanechlor [no further specification given]	DNA strand breaks (comet assay), ddY male mouse (stomach, colon, liver, kidney, urinary bladder, lung, brain, bone marrow)	–	1000 po × 1	Sasaki *et al.* (2000)
PCB_3[c]	Micronucleus formation, fish (*Misgurnus anguillicaudatus*), erythrocytes	+	0.5 mg/L × 7 d	Chu *et al.* (1996a)
		+	1 mg/L × 2 d	
		–	10 ppm × 12 mo	

[a] +, considered to be positive; (+), considered to be weakly positive in an inadequate study; –, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an inadequate study)
[b] In-vivo tests, mg/kg bw
[c] Commercial PCB mixture manufactured in China, the composition of which was similar to that of Aroclor 1242 (see Section 1.1, Table 1.8)
CB, chlorobiphenyl; d, day; HID, highest effective dose; HPLC/ECD, high-performance liquid chromatography electrochemical detection; ip, intraperitoneal; LED, lowest effective dose; mo, month; NR, not reported; 8-OHdG, 8-hydroxy-2’-deoxyguanosine; po, oral administration; TEQ, toxic equivalency

Table 4.5 Genetic and related effects of PCB congeners and their metabolites in experimental systems in vitro

PCB congener Structural name	BZ nomenclature[a]	Test system	Results[b]		Dose[c] (LED or HID), µg/mL	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Non-mammalian systems						
2-MonoCB	PCB-1	*Salmonella typhimurium* C3076, D3052, G46, TA98, TA1000, TA1535, TA1537, TA1538, reverse mutation	–	–	1000	McMahon et al. (1979)
4-MonoCB	PCB-3	*Salmonella typhimurium* C3076, D3052, G46, TA98, TA1000, TA1535, TA1537, TA1538, reverse mutation	–	–	1000	McMahon et al. (1979)
4-MonoCB	PCB-3	*Salmonella typhimurium* TA1538, reverse mutation	?	+	50 µg/plate	Wyndham et al. (1976)
4-MonoCB	PCB-3	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, reverse mutation	–	–	200	Schoeny (1982)
4,4-DiCB	PCB-15	*Salmonella typhimurium* TA98, TA100, reverse mutation	–	–	100	Butterworth et al. (1995)
4,4'-DiCB	PCB-15	*Drosophila melanogaster*, somatic mutation and recombination, eye mosaic test	+	+	223	Butterworth et al. (1995)
2,2',4,4'-TetraCB	PCB-47	*Salmonella typhimurium* TA98, TA100, reverse mutation	–	–	200	Schoeny (1982)
2,2',5,5'-TetraCB	PCB-52	*Salmonella typhimurium* TA1538, reverse mutation	–	–	200 µg/plate	Wyndham et al. (1976)
2,2',5,5'-TetraCB	PCB-52	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, reverse mutation	NT	–	200 µg/plate	Hsia et al. (1978)
4-OH-2,2',5,5'-TetraCB	4-OH-PCB-52	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, reverse mutation	NT	–	20 µg/plate	Hsia et al. (1978)
3,4-Epoxy-2,2',5,5'-tetraCB	3,4-Epoxy-PCB-52	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, reverse mutation	NT	–	200 µg/plate	Hsia et al. (1978)
3,3',4,4'-TetraCB	PCB-77	*Salmonella typhimurium* TA98, TA100, reverse mutation	–	–	200	Schoeny (1982)
2,2',4,4',6,6'-HexaCB	PCB-155	*Salmonella typhimurium* TA98, TA100, reverse mutation	–	–	200	Schoeny (1982)
2,2',3,3',4,4',5,5',6,6'-DecaCB	PCB-209	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	5000	Han et al. (2009)

Table 4.5 (continued)

PCB congener Structural name	BZ nomenclature[a]	Test system	Results[b]		Dose[c] (LED or HID), µg/mL	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Mammalian cells in vitro						
2',5'-HQ-2-MonoCB	Metabolite of PCB-1	Polyploidy, Chinese hamster lung V79 cells	–		4.4	Flor & Ludewig (2010)
2',5'-HQ-2-MonoCB	Metabolite of PCB-1	Sister-chromatid exchange, Chinese hamster lung V79 cells	–		4.4	Flor & Ludewig (2010)
2',5'-HQ-3-MonoCB	Metabolite of PCB-2	Polyploidy, Chinese hamster lung V79 cells	+		1.1	Flor & Ludewig (2010)
2',5'-HQ-3-MonoCB	Metabolite of PCB-2	Sister-chromatid exchange, Chinese hamster lung V79 cells	–		2.2	Flor & Ludewig (2010)
4-MonoCB	PCB-3	Binding (covalent) to DNA, RNA or protein, Chinese hamster ovary cells	+		2	Wong et al. (1979)
4-MonoCB	PCB-3	Unscheduled DNA synthesis, Chinese hamster ovary cells	(+)		2	Wong et al. (1979)
4-MonoCB	PCB-3	DNA adducts (^{32}P-postlabelling), primary human hepatocytes (three donors)	+		43	Borlak et al. (2003)
4-MonoCB	PCB-3	Gene mutation, Chinese hamster lung V79 cells, *Hprt* locus	–		56	Zettner et al. (2007)
2'-OH-4-MonoCB	Metabolite of PCB-3		–		20	
3'-OH-4-MonoCB	Metabolite of PCB-3		–		20	
4'-OH-4-MonoCB	Metabolite of PCB-3		–		20	
2',5'-HQ-4-MonoCB	Metabolite of PCB-3		–		1.7	
3',4'-HQ-4-MonoCB	Metabolite of PCB-3		–		5.5	
2',5'-Q-4-MonoCB	Metabolite of PCB-3		+		0.1	
3',4'-Q-4-MonoCB	Metabolite of PCB-3		+		0.1	
4-MonoCB	PCB-3	Micronucleus formation, Chinese hamster lung V79 cells	–		38	Zettner et al. (2007)
2'-OH-4-MonoCB	Metabolite of PCB-3		+		10	
3'-OH-4-MonoCB	Metabolite of PCB-3		+		20	
4'-OH-4-MonoCB	Metabolite of PCB-3		+		15	
2',5'-HQ-4-MonoCB	Metabolite of PCB-3		+		0.6	
3',4'-HQ-4-MonoCB	Metabolite of PCB-3		+		3.3	
2',5'-Q4-MonoCB	Metabolite of PCB-3		+		0.1	
3',4'-Q-4-MonoCB	Metabolite of PCB-3		+		0.5	

Table 4.5 (continued)

PCB congener Structural name	BZ nomenclature[a]	Test system	Results[b]		Dose[c] (LED or HID), µg/mL	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
4-MonoCB	PCB-3	Aneuploidy, Chinese hamster lung V79 cells	–		38	Zettner et al. (2007)
2'-OH-4-MonoCB	Metabolite of PCB-3		+		10	
3'-OH-4-MonoCB	Metabolite of PCB-3		+		20	
4'-OH-4-MonoCB	Metabolite of PCB-3		+		15	
2',5'-HQ-4-MonoCB	Metabolite of PCB-3		+		0.6	
3',4'-HQ-4-MonoCB	Metabolite of PCB-3		+		3.3	
2',5'-Q-4-MonoCB	Metabolite of PCB-3		+		0.5	
3',4'-Q-4-MonoCB	Metabolite of PCB-3		+		1.1	
2',5'-HQ-4-MonoCB	Metabolite of PCB-3	Polyploidy, Chinese hamster lung V79 cells	+		1.1	Flor & Ludewig (2010)
3',4'-HQ-4- MonoCB	Metabolite of PCB-3	Polyploidy, Chinese hamster lung V79 cells	–		2.2	Flor & Ludewig (2010)
2',5'-HQ-4-MonoCB	Metabolite of PCB-3	Sister-chromatid exchange, Chinese hamster lung V79 cells	–		2.2	Flor & Ludewig (2010)
3',4'-HQ-4-MonoCB	Metabolite of PCB-3	Sister-chromatid exchange, Chinese hamster lung V79 cells	+		1.1	Flor & Ludewig (2010)
2',5'-Q-4-MonoCB	Metabolite of PCB-3	Micronucleus formation, human breast epithelial MCF-10A cells	+		0.1	Venkatesha et al. (2008)
2',5'-Q-4-MonoCB	Metabolite of PCB-3	Micronucleus formation, Chinese hamster lung V79 cells	+		0.6	Jacobus et al. (2008)
2,2',5,5'-TetraCB	PCB-52	DNA strand breaks (alkaline sedimentation), mouse fibroblast L-929 cells	+		20	Stadnicki et al. (1979)
4-OH-/3-OH-2,2',5,5'-TetraCB (4 : 1)	Metabolites of PCB-52	DNA strand breaks (alkaline sedimentation), mouse fibroblast L-929 cells	+		20	Stadnicki et al. (1979)
3,4-Epoxy-2,2',5,5'-TetraCB	Metabolite of PCB-52	DNA strand breaks (alkaline sedimentation), mouse fibroblast L-929 cells	+		10	Stadnicki et al. (1979)
2,2',5,5'-TetraCB	PCB-52	DNA strand breaks (comet assay), human lymphocytes (six donors)	(+)		0.3	Sandal et al. (2008)
2,2',5,5'-TetraCB	PCB-52	Sister-chromatid exchange, human lymphocytes (four donors)	–		1	Sargent et al. (1989)
2,2',5,5'-TetraCB	PCB-52	Chromosomal aberrations, human lymphocytes (5–9 donors)	–		1	Sargent et al. (1989)

Table 4.5 (continued)

PCB congener Structural name	BZ nomenclature[a]	Test system	Results[b]		Dose[c] (LED or HID), μg/mL	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
2,2',5,5'-TetraCB and 3,3',4,4'-tetraCB	PCB-52 + PCB-77	Chromosomal aberrations, human lymphocytes (5–9 donors)	+		$1 + 10^{-5}$	Sargent et al. (1989)
2,2',5,5'-TetraCB and 3,3',4,4'-tetraCB	PCB-52 + PCB-77	Sister-chromatid exchange, human lymphocytes (four donors) in vitro	–		$1 + 10^{-5}$	Sargent et al. (1989)
3-$MeSO_2$-2',3',4,5-TetraCB	5-$MeSO_2$-PCB-56	Sister-chromatid exchange, human lymphocytes	–		7.1	Nagayama et al. (1999)
3-$MeSO_2$-2',3',4,5-TetraCB	5-$MeSO_2$-PCB-56	Micronucleus formation, human lymphocytes	–		7.1	Nagayama et al. (1995)
3,3',4,4'-TetraCB	PCB-77	DNA strand breaks (comet assay), human lymphocytes (three donors)	–		25	Belpaeme et al. (1996a)
3,3',4,4'-TetraCB	PCB-77	DNA strand breaks (comet assay), human lymphocytes (six donors)	(+)		3	Sandal et al. (2008)
3,3',4,4'-TetraCB	PCB-77	DNA adducts ^{32}P-postlabelling, human hepatocarcinoma HepG2 cells	+		15	Dubois et al. (1995)
3,3',4,4'-TetraCB	PCB-77	DNA adducts ^{32}P-postlabelling, primary fetal rat hepatocytes		+	15	Dubois et al. (1995)
3,3',4,4'-TetraCB	PCB-77	Sister-chromatid exchange, human lymphocytes (four donors) in vitro	–		0.1	Sargent et al. (1989)
2,2',5,5'-TetraCB and 3,3',4,4'-tetraCB	PCB-52 + PCB-77	Sister-chromatid exchange, human lymphocytes (four donors) in vitro	–		$1 + 10^{-5}$	Sargent et al. (1989)
3,3',4,4'-TetraCB	PCB-77	Micronucleus formation, human lymphocytes (two donors)	–		500	Belpaeme et al. (1996a)
3,3',4,4'-TetraCB	PCB-77	Chromosomal aberrations (structural), human lymphocytes (5–9 donors)	+		0.01	Sargent et al. (1989)
3-$MeSO_2$-3',4,4',5-TetraCB	5-$MeSO_2$-PCB-77	Sister-chromatid exchange, human lymphocytes	–		6.8	Nagayama et al. (1999)
3-$MeSO_2$-3',4,4',5-TetraCB	5-$MeSO_2$-PCB-77	Micronucleus formation, human lymphocytes	–		7.8	Nagayama et al. (1995)
4,4'-$(OH)_2$-3,3',5,5'-TetraCB	Metabolite of PCB-80	Detection of repairable adducts by growth inhibition (DRAG) assay in wildtype and DNA repair-deficient Chinese hamster ovary cells	(+)		140/102, 92, 91[d]	Johansson et al. (2004)
4-$MeSO_2$-2,2',3',4',5-PentaCB	4'-$MeSO_2$-PCB-87	Sister-chromatid exchange, human lymphocytes	+		5.8	Nagayama et al. (1999)

Table 4.5 (continued)

PCB congener Structural name	BZ nomenclature[a]	Test system	Results[b]		Dose[c] (LED or HID), μg/mL	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
4-MeSO$_2$-2,2',3',4',5-PentaCB	4'-MeSO$_2$-PCB-87	Micronucleus formation, human lymphocytes	–		5.8	Nagayama et al. (1995)
2,2',4,5,5'-PentaCB	PCB-101	DNA strand breaks (comet assay), fish fibroblast RTG-2 cells	+		16	Marabini et al. (2011)
2,2',4,5,5'-PentaCB	PCB-101	Micronucleus formation, fish fibroblast RTG-2 cells	+		16	Marabini et al. (2011)
3-MeSO$_2$-2,2',4',5,5'-PentaCB	3'-MeSO$_2$-PCB-101	Sister-chromatid exchange, human lymphocytes	+		5.2	Nagayama et al. (1999)
3-MeSO$_2$-2,2',4',5,5'-PentaCB	3'-MeSO$_2$-PCB-101	Micronucleus formation, human lymphocytes	–		5.2	Nagayama et al. (1995)
4-OH-2,3,3',4',5-PentaCB	Metabolite of PCB-109	Detection of repairable adducts by growth inhibition (DRAG) assay in wildtype and DNA repair-deficient Chinese hamster ovary cells	–			Johansson et al. (2004)
2,3',4,4',5-PentaCB	PCB-118	DNA strand breaks (comet assay), fish fibroblast RTG-2 cells	+		10	Marabini et al. (2011)
2,3',4,4',5-PentaCB	PCB-118	Micronucleus formation, fish fibroblast RTG-2 cells	+		10	Marabini et al. (2011)
3,3',4,4',5-PentaCB	PCB-126	Micronucleus formation, human hepatoma HepG2 cells in vitro	–		0.003	Wei et al. (2009b)
2,2',3,4,4',5'-HexaCB	PCB-138	DNA strand breaks (comet assay), fish fibroblast RTG-2 cells	+		25	Marabini et al. (2011)
2,2',3,4,4',5'-HexaCB	PCB-138	Micronucleus formation, fish fibroblast RTG-2 cells	–		25	Marabini et al. (2011)
4-MeSO$_2$-2,2',3',5,5',6'-HexaCB	4'-MeSO$_2$-PCB-151	Sister-chromatid exchange, human lymphocytes	–		9.6	Nagayama et al. (1999)
4-MeSO$_2$-2,2',3',5,5',6'-HexaCB	4'-MeSO$_2$-PCB-151	Micronucleus formation, human lymphocytes	–		9.6	Nagayama et al. (1995)
2,2',4,4'5,5'-HexaCB	PCB-153	Chromosomal aberrations (structural), human lymphocytes (5–9 donors)	+		1	Sargent et al. (1989)

Table 4.5 (continued)

PCB congener Structural name	BZ nomenclature[a]	Test system	Results[b]		Dose[c] (LED or HID), µg/mL	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
2,2',4,4',5,5'-HexaCB	PCB-153	Micronucleus formation, human breast epithelial MCF-10A cells	+		0.4	Venkatesha et al. (2008)
2,2',4,4',5,5'-HexaCB	PCB-153	Micronucleus formation, human hepatoma HepG2 cells	+		36	Wei et al. (2009a)
2,2',4,4',5,5'-HexaCB	PCB-153	DNA strand breaks (comet assay), fish fibroblast RTG-2 cells	+		11	Marabini et al. (2011)
2,2',4,4',5,5'-HexaCB	PCB-153	Micronucleus formation, fish fibroblast RTG-2 cells	+		11	Marabini et al. (2011)
4-OH-2,2',3,4',5,5',6-HeptaCB	Metabolite of PCB-187	Detection of repairable adducts by growth inhibition (DRAG) assay in wildtype and DNA repair-deficient Chinese hamster ovary cells	–		23	Johansson et al. (2004)
2,2',3,3',4,4',5,5',6,6'-DecaCB	PCB-209	Gene mutation, mouse lymphoma L5178Y cells, *Tk*$^{+/-}$ locus	–	–	150	Han et al. (2009)

[a] BZ nomenclature as listed in Table 1.1, Section 1

[b] +, considered to be positive; (+), considered to be weakly positive in an inadequate study; –, considered to be negative;?, considered to be inconclusive (variable responses in several experiments within an inadequate study); 0, not tested.

[c] Approximately minimal lethal dose not reported.

[d] Dose 140 µg/mL is the IC_{50} concentration inhibiting growth of wildtype CHO cells (AA8) by 50%; 102, 92 and 91 are the IC_{50} for repair-deficient CHO cells EM9, UV4 and UV5, respectively.

CB, chlorobiphenyl; HID, highest effective dose; HQ, hydroquinone; LED, lowest effective dose; MED, maximum effective dose; $MeSO_2$, methyl sulfonyl; OH, hydroxyl

For the nomenclature of PCB metabolites, the reader is referred to the review by Grimm *et al.* (2015).

Table 4.6 Genetic and related effects of PCB congeners and their metabolites in experimental animals in vivo

PCB congener Structural name	BZ nomenclature[a]	Test system	Results[b]	Dose[c] (LED or HID)	Reference
4-MonoCB 4′-OH-4-MonoCB	PCB-3 Metabolite of PCB-3	Gene mutation, transgenic male BigBlue® rat, liver	+ –	113 ip × 4 82 ip × 4	Lehmann *et al.* (2007)
4-MonoCB 4′-OH-4-MonoCB	PCB-3 Metabolite of PCB-3	Gene mutation, transgenic male BigBlue® rat, lung	(+) (+)	113 ip × 4 (1/week) 82 ip × 4 (1/week)	Maddox *et al.* (2008)
4-MonoCB 4′-OH-4-MonoCB	PCB-3 Metabolite of PCB-3	Gene mutation, transgenic female BigBlue® rat, liver	– –	113 ip × 4 82 ip × 4	Jacobus *et al.* (2010)
2,2′,5,5′-TetraCB	PCB-52	Chromosomal aberrations (numerical and structural), female Sprague-Dawley rat, 70% hepatectomy, bone-marrow cells	–	10 ppm, 1 year	Meisner *et al.* (1992)
2,2′,5,5′-TetraCB	PCB-52	Chromosomal aberrations (numerical), female Sprague-Dawley rat, liver cells after 70% hepatectomy	– –	10 ppm × 7 mo 10 ppm × 12 mo	Sargent *et al.* (1992)
3,3′,4,4′-TetraCB	PCB-77	Chromosomal aberrations (numerical & structural), female Sprague-Dawley rat, 70% hepatectomy, bone-marrow cells	–	0.1 ppm, 1 year	Meisner *et al.* (1992)
3,3′,4,4′-TetraCB	PCB-77	Chromosomal aberrations (numerical), female Sprague-Dawley rat liver cells after 70% hepatectomy	 –	0.1 ppm × 7 mo 0.1 ppm × 12 mo	Sargent *et al.* (1992)
3,3′,4,4′-TetraCB	PCB-77	DNA strand breaks (comet assay) and micronucleus formation, fish (*Salmo trutta fario*) erythrocytes	–	0.9 µg/mL	Belpaeme *et al.* (1996b)
3,3′,4,4′-TetraCB and 2,2′,5,5′-tetraCB	PCB-77 + PCB-52	Chromosomal aberrations (numerical & structural), female Sprague-Dawley rat, 70% hepatectomy, bone marrow cells	+	0.1 + 10 for 1 year	Meisner *et al.* (1992)
3,3′,4,4′ -TetraCB + 2,2′,5,5′-tetraCB	PCB-77 + PCB-52	Chromosomal aberrations (numerical), female Sprague-Dawley rat liver cells after 70% hepatectomy	–	0.1 + 10 ppm for 7 mo	Sargent *et al.* (1992)
3,3′,4,4′,5-PentaCB	PCB-126	Gene mutation, transgenic Muta™Mouse fetus, day 18 of gestation, after exposure on day 10, in utero	–	0.5 po × 1	Inomata *et al.* (2009)
3,3′,4,4′,5-PentaCB	PCB-126	DNA adducts, M_1dG secondary oxidative DNA lesion, LC-MS/MS female Sprague-Dawley rat, liver	+	0.001 po × 5 per week for 53 weeks	Jeong *et al.* (2008)
2,2′,4,4′,5,5′-HexaCB	PCB-153	DNA adducts, M_1dG secondary oxidative DNA lesion, LC-MS/MS female Sprague-Dawley rat, liver	–	1 po × 5 per week for 53 weeks	Jeong *et al.* (2008)
2,2′,4,4′,5,5′-HexaCB	PCB-153	DNA adducts, M_1dG secondary oxidative DNA lesion, LC-MS/MS female Sprague-Dawley rat, brain	–	1 po × 5/week for 53 weeks	Jeong *et al.* (2008)

Table 4.6 (continued)

PCB congener Structural name	BZ nomenclature[a]	Test system	Results[b]	Dose[c] (LED or HID)	Reference
3,3′,4,4′,5-PentaCB and 2,2′,4,4′,5,5′-hexaCB	PCB-126 + PCB-153	DNA adducts, M_1dG secondary oxidative DNA lesion, LC-MS/MS female Sprague-Dawley rat, liver	+	0.0003 + 3 po × 5/week for 53 weeks	Jeong *et al.* (2008)
3,3′,4,4′,5-pentaCB and 2,2′,4,4′,5,5′-hexaCB	PCB-126 + PCB-153	DNA adducts, M_1dG secondary oxidative DNA lesion, LC-MS/MS female Sprague-Dawley rat, brain	–	0.001 + 1 po × 5/week for 53 weeks	Jeong *et al.* (2008)
2,2′,3,3′,4,4′,5,5′,6,6′-DecaCB	PCB-209	Micronucleus formation, male and female Crl:CD1 mice bone-marrow cells	–	2000 po × 1	Han *et al.* (2009)
1 : 2 : 3 : 2 Mixture of 2,3′,4,4′,5-pentaCB, 2,2′,3,4,4′,5′-hexaCB, 2,2′,4,4′,5,5′-hexaCB, and 2,2′,3,4,4′,5,5′-heptaCB	PCB-118, PCB-138, PCB-153, PCB-180	DNA adducts, M_1dG secondary oxidative DNA lesion, LC-MS/MS female C57BL/6J mouse, liver	–	10 ng TEQ/kg bw ip × 1	Jeong *et al.* (2008)

[a] BZ nomenclature as listed in Table 1.1, Section 1.

[b] +, considered to be positive; (+), considered to be weakly positive in an inadequate study; –, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an inadequate study)

[c] In-vivo tests, mg/kg bw

CB, chlorobiphenyl; HID, highest effective dose; ip, intraperitoneal; mo, month; LED, lowest effective dose; po, oral administration; TEQ, toxic equivalency; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; HPLC/ECD, high-performance liquid chromatography electrochemical detection; I-compounds, take from Table 4.3 or Table 4.4

7 months (Elo *et al.*, 1985). [The Working Group noted that the control group was not defined. The data on observed chromosomal aberration and sister chromatid exchange, and the statistical method used, were not provided.] The other study reported a non-significant increase by fourfold in the frequency of chromosomal aberration, but no increase in the frequency of sister-chromatid exchange in a group of 12 workers (Melino *et al.*, 1992).

In another report, the study group consisted of 45 randomly selected people (workers, residents, or children) living within 2 km of a capacitor-producing factory known to cause occupational and environmental exposure to PCBs, in Semic, Slovenia, and was compared to workers that had pre-employment tests. An abnormally high frequency of structural chromosome aberration (55%) was observed in peripheral lymphocytes from workers and residents when compared with the control group (Tretjak *et al.*, 1990). However, these findings were not correlated to environmental or blood PCB concentrations. [The Working Group noted that no PCB concentrations in blood were reported. No matched control group was available and no statistical analysis was performed. Heavy smokers and people who had had recent X-ray examinations were excluded from the study].

Men working in Chinese electrical and electronic equipment waste-dismantling factories were shown to be exposed occupationally to PCBs, tetrachlorodibenzo-*p*-dioxins and dibenzofurans (TCDD/Fs) and polybrominated diphenyl ethers (PBDEs). Urine concentrations of 8-hydroxydeoxyguanosine (8-OHdG), a product of oxidative DNA damage, were significantly increased in workers after their working shift when compared with levels before the working shift. However, no correlation could be drawn between the observed increase in urinary 8-OHdG concentrations and occupational exposure to any of the organochlorine compounds (Wen *et al.*, 2008).

(b) Genotoxicity and cytogenicity from non-occupational exposure

Three years after accidental contamination of cooking oil with PCBs in Taiwan, China (see Section 1.4.8), blood samples were taken from 36 patients with Yucheng ("oil disease"); lymphocytes were analysed for chromosomal aberrations and compared with lymphocytes from age- and sex-matched laboratory staff (n = 10). Blood PCB concentrations ranged from 6.4 to 101.8 μg/L. A high frequency of chromosomal aberration was observed in 19 out of 36 (53%) PCB-exposed patients, while none was seen in the control group. The findings could not be correlated with the blood PCB levels (Wuu & Wong, 1985). [The Working Group noted that no details on the statistical evaluation or adjustment for confounders were given.]

The frequencies of chromosomal aberration and sister-chromatid exchange in peripheral lymphocytes from 35 nonsmoking women from Taiwan, China, exposed to PCBs through contaminated rice oil ("Yucheng") were similar to those from matched controls. However, when peripheral blood lymphocytes were treated with α-naphthoflavone in vitro [to increase sensitivity], a small (20%) but significant increase in frequency of sister-chromatid exchange, but not chromosomal aberration, was observed (Lundgren *et al.*, 1987, 1988).

Similarly, 27 years after exposure to high concentrations of PCBs, the frequency of sister-chromatid exchange in lymphocytes of 16 victims of the "Yusho" food poisoning incident (see Section 1.4.8 in this *Monograph*) were not significantly different from those of a non-exposed control group, despite persistently elevated blood PCB concentrations in these patients (281 pg/g fat versus 41 pg/g fat in the control group). Addition of α-naphthoflavone did not increase the frequency of sister-chromatid exchange (Nagayama *et al.*, 2001).

Blood concentrations of cadmium, lead, *p,p′*-dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene, PCBs (PCB-99, PCB-118, PCB-170, PCB-138, PCB-153, PCB-180), and dioxin-like activity (Calux assay) were analysed in 1583 residents in nine different industrialized regions in Belgium (De Coster *et al.*, 2008). Also analysed as effect biomarkers were the percentage of cells with micronucleus formation, DNA damage (comet assay) in peripheral blood cells, and 8-OHdG in urine. Overall significant differences between the different regions were found for micronucleus formation, DNA damage, and urinary 8-OHdG concentrations. Among these, positive correlations were reported between PCB-118 concentrations and both micronucleus formation and DNA damage.

In a group of 103 Inuit people from Northern Canada exposed to high dietary concentrations of PCBs and selenium, plasma PCB concentrations and DNA adduct profiles in leukocytes were determined (Ravoori *et al.*, 2008). The ^{32}P-postlabelling technique used allowed for differentiation between polar and lipophilic adducts. Plasma PCB concentrations were significantly correlated with increasing age [$P < 0.01$]. The most abundant PCB congeners in the plasma were PCB-138, PCB-153, and PCB-180. The most abundant adduct was 8-OHdG, which accounted for 51–57% of the total adduct burden. No correlation between adduct levels and specific PCB congeners, smoking status, or sex were observed.

In a follow-up study in 83 subjects, Ravoori *et al.* (2010) reported 30–800-fold interindividual variability in levels of unidentified polar DNA adducts (indicative of oxidative stress) in leukocytes. Negative associations were observed between total DNA adduct levels and selenium, and PCB concentrations, the latter being significant. After grouping the individuals according to selenium/PCB ratio as high-ratio (ratio, > 33; mean, 75.5; $n = 41$), or low-ratio (ratio, ≤ 33; mean, 18; $n = 42$), levels of 8-OHdG and total DNA adducts were significantly negatively correlated with the high-ratio group ($P = 0.014$ and $P < 0.01$, respectively), while there was no correlation with the low-ratio group, indicating a mitigating effect of selenium on the toxicity of PCBs.

(c) Sperm DNA damage

Sex-chromosome disomy in sperm nuclei was determined in 192 men from subfertile couples. A positive association with YY, XY, and total sex-chromosome disomy and an inverse association with XX disomy were observed with higher serum concentrations of four PCBs (PCB-118, PCB-138, PCB-153, and PCB-180) (McAuliffe *et al.*, 2012). Other environmental organochlorine pollutants may also have contributed to sex-chromosome aneuploidy, since plasma DDE concentrations were positively associated with increased rates of XX, XY, and total sex-chromosome disomy.

In a group of 176 Swedish fishermen with low or high consumption of fatty fish, the DNA fragmentation index in sperm was compared with serum PCB concentrations (Rignell-Hydbom *et al.*, 2005). Plasma concentration of PCB-153 was statistically significantly associated with an increase in DNA fragmentation ($P < 0.001$); however, when adjusted for age, which was strongly associated with percentage DNA fragmentation index, this association was no longer significant ($P = 0.28$). When PCB-153 concentrations were categorized into quintiles, the lowest-exposure quintile had significantly lower levels of DNA fragmentation than the other quintiles ($P < 0.001$), even after adjustment for age ($P = 0.006$). The association between DNA fragmentation and DDE concentrations was not significant (Rignell-Hydbom *et al.*, 2005).

In sperm samples from 707 adult men (193 Inuits from Greenland, 178 Swedish fishermen, 141 men from Poland, and 195 men from Ukraine), DNA fragmentation was correlated with serum PCB-153 concentrations (Spanò *et al.*, 2005). After adjustment for age, period of sexual abstinence, and serum PCB-153 concentration,

levels of DNA fragmentation betwen men in the three European groups did not differ considerably, but were significantly higher than those found in Inuit men. While DNA fragmentation in sperm was unrelated to PCB-153 concentration among the Inuits (very high PCB concentrations) and Polish men (very low PCB concentrations), increasing serum PCB-153 concentrations were significantly associated with increased DNA fragmentation in the Swedish ($P = 0.001$), and Ukranian cohorts ($P = 0.027$), and in the three European groups combined ($P < 0.0001$). No correlation between DNA fragmentation index and serum DDE concentrations was seen.

Similar results were observed in a subsequent study with a largely overlapping study population (Stronati *et al.*, 2006).

(d) Gene mutation

A possible correlation between PCB exposure and cancer of the pancreas has been discussed earlier (see Section 2.3.5). An analysis of blood organochlorine concentrations and *KRAS* mutations in tissue from pancreatic cancer found a significant correlation between tumours harbouring *KRAS* mutations and PCB-138, and PCB-153, and between the two most common mutations in *KRAS* and PCB-138 concentrations (Porta *et al.*, 2009). The dose–response pattern was approximately linear only for PCB-138.

Another study analysed post-mortem samples of brain from patients with neurodevelopmental disorders with a known genetic basis ($n = 32$), autism of unknown etiology ($n = 32$), and controls ($n = 43$) for eight PCBs (PCB-28, PCB-95, PCB-105, PCB-118, PCB-138, PCB-153, PCB-170, and PCB-180) (Mitchell *et al.*, 2012). The concentration of PCB-95 was significantly higher in the group with genetic neurodevelopmental diseases. In fact, PCB-95 was detected nearly exclusively in the brain of patients from mothers with a specific duplication in the long arm of chromosome 15 (dup15q11–q13) or deletions in the same chromosome 15q11–q13 in patients with Prader-Willie syndrome. Five out of six patients with dup15q11–q13, which is related to autism spectrum disorder, were born after 1976.

(e) Epigenetic effects

In the study by Mitchell *et al.* (2012) cited above, samples of brain showing dup15q also showed a lower level of methylation in regions of repetitive DNA, suggesting that PCBs may have caused hypomethylation in these regions, resulting in chromosome instability and a higher risk of duplication.

Rusiecki and coworkers used pyrosequencing to estimate global DNA methylation via repetitive elements *Alu* and (long interspersed nucleotide element) LINE-1 assays of bisulfite-treated DNA in 70 samples from Inuit people in Greenland to examine epigenetic effects of high PCB contamination (Rusiecki *et al.*, 2008). They observed significant inverse correlations between percentages of methylcytosine and plasma concentrations of DDT, DDE, β-hexachlorocyclohexane, oxychlordane, α-chlordane, mirex, sum of PCBs, and sum of all persistent organic pollutants, after adjusting for age and cigarette smoking.

(f) Changes in gene expression

In samples taken in 2007 from 139 daughters of members of a cohort of fish-consumers in Michigan, there was no correlation between serum concentrations of PCB, PBDE, or DDE, and expression of four genes encoding 17-α-hydroxylase (CYP17A1), aromatase (CYP19A1), and estrogen receptor α and β (ESR1 and ESR2) (Karmaus *et al.*, 2011). In contrast, maternal concentrations of serum PCB (prenatal PCB concentration), measured in 1973–1991, were highly significantly associated with decreased expression of the steroid synthesis genes *CYP17* and *CYP19* in blood lymphocytes. Other persistent organic pollutants were not correlated.

4.2.2 Experimental systems

(a) Commercial PCB mixtures

Table 4.3 and Table 4.4 summarize data with commercial PCB mixtures in in-vitro and in-vivo studies respectively. For each category of test (non-mammalian systems, mammalian cells in vitro, and in-vivo assays), the data are presented by commercial PCB mixture in increasing order of chlorination, and for each commercial mixture, by end-point.

(i) Non-mammalian systems

All PCB mixtures tested for their ability to induce gene mutation in bacteria, i.e. PCB mixtures with chlorination levels ranging from ~20% (e.g. Aroclor 1221) to ~70% (e.g. Aroclor 1268) were not mutagenic in different strains of *Salmonella typhimurium* and *Escherichia coli* in the absence or presence of an exogenous metabolic activation system comprising induced and non-induced liver microsomes (Table 4.3). However, only Aroclor 1254 was tested up to the recommended limit dose for hazard assessment of 5000 μg/plate (Shahin *et al.*, 1979), not all strains typically used in the Ames test battery (*S. typhimurium* TA98, TA100, TA1535, TA1537) or *E. coli* WP2 *uvrA* were tested, and an exogenous metabolic system was not always included.

In contrast, Aroclor 1221 and Aroclor 1260 did induce intrachromosomal recombination in *Saccharomyces cerevisiae* cells in the absence and presence of exogenous metabolic activation. Since Aroclor 1221 was effective at lower concentrations than Aroclor 1260, chlorination level seemed to be inversely correlated to mutagenicity of PCBs in this test system (Schiestl *et al.*, 1997).

Additionally, Aroclor 1254 induced mutations in the number of tandem repeats in *S. cerevisiae* transgenic for the human MS32 minisatellite (Appelgren *et al.*, 1999).

Clophen mixtures did not induce somatic mutation in the fruit fly *Drosophila melanogaster* (Nilsson & Ramel, 1974).

(ii) Mammalian cells in vitro

Aroclor 1254 caused DNA strand breaks (detected by alkaline filter elution) in primary rat hepatocytes (Sina *et al.*, 1983) and in primary rat prostate cells (comet assay; Cillo *et al.*, 2007), while evidence for induction of unscheduled DNA synthesis in primary rat hepatocytes was equivocal (Probst *et al.*, 1981; Althaus *et al.*, 1982). An increase in the frequency of DNA adducts (detected by ^{32}P-postlabelling) was observed in primary human hepatocytes from three different donors (Borlak *et al.*, 2003), but not in cultured human hepatocarcinoma HepG2 cells or dexamethasone-treated primary rat fetal hepatocytes (Dubois *et al.*, 1995). A dose-dependent increase in structural chromosomal aberration starting at concentrations of less than 1 μg/mL was seen in cultured human lymphocytes (Sargent *et al.*, 1989).

Aroclor 1221 caused intrachromosomal recombination at the *Hprt* locus in a mutant Chinese hamster V79 cell line (Helleday *et al.*, 1998), and in human lymphoblastoid cells (Aubrecht *et al.*, 1995). Aroclor 1016 enhanced DNA-adduct formation in primary human lymphocytes (Borlak *et al.*, 2003); no increase in the frequency of chromosomal aberration was seen in chicken embryos and ouabain-resistant colonies in Chinese hamster V79 cells treated with Aroclor 1242 (Blazak & Marcum, 1975; Hattula, 1985).

(iii) In-vivo assays

Repeated doses of Aroclor 1254 did not alter hepatic levels of DNA adducts (as measured by ^{32}P-postlabelling) in male Sprague-Dawley (given two intraperitoneal doses of 500 mg/kg bw) or male Fischer 344 rats (given 35 oral doses of 25 mg/kg bw) compared with controls (Nath *et al.*, 1991; Chadwick *et al.*, 1993).

When used for hepatic enzyme induction, a single intraperitoneal application of Aroclor 1254 of up to 500 mg/kg bw in rats (Kornbrust & Dietz, 1985; Shaddock *et al.*, 1989) and 50 mg/kg bw

in cynomolgus monkeys (Hamilton *et al.*, 1997) did not enhance unscheduled DNA synthesis in isolated primary hepatocytes.

Dietary exposure of male C57BL/6 (Big Blue®) mice transgenic for bacterial *lacI* to Aroclor 1254 at 100 ppm (0.01%) for 7 weeks caused a significant, but less than twofold, increase in the frequency of mutation in the liver (Davies *et al.*, 2000).

No increase in the frequency of structural chromosomal aberration in bone marrow and spermatogonial cells was observed in rats given repeated doses of Aroclor 1254 by gavage (300 mg/kg bw for five consecutive days or 50 mg/kg bw for seven consecutive days) or in the diet (500 ppm for 5 weeks) (Dikshith *et al.*, 1975; Green *et al.*, 1975a; Garthoff *et al.*, 1977). Aroclor 1254 did not increase the frequency of micronucleus formation in bone marrow of $B6C3F_1$ mice given Aroclor 1254 as intraperitoneal injections of 15 000 mg/kg bw on five consecutive days (Bruce & Heddle, 1979).

In contrast to the observations in rodents, a single intraperitoneal injection of Aroclor 1254 induced a dose-dependent increase in the frequency of micronucleus formation in fish (*Cyprinus carpio*) erythrocytes (Al-Sabti, 1986), and aberrant metaphases and structural chromosomal aberration in fish kidney cells (*Cyprinus carpio*, *Tinca tinica*, *Ctenopharyngodon idella*), from the starting dose of 50 mg/kg bw (Al-Sabti, 1985). In addition, Aroclor 1254 induced germline length mutation in the PC-1 but not PC-2 minisatellite region in male C57B1/6 mice given a single intraperitoneal dose of Aroclor 1254 at 100 mg/kg bw (Hedenskog *et al.*, 1997).

Kanechlor 500 (which has a similar level of chlorination as Arochlor 1254) caused a weak (less than twofold) increase in the frequency of micronucleus formation in bone-marrow cells in male ddY mice given an oral dose at 100 mg/kg bw for 6 days, but not when applied subcutaneously at the same dose (Watanabe *et al.*, 1982).

A single dose of Aroclor 1242 did not enhance levels of DNA adducts (as measured by ^{32}P-postlabelling) or 8-OHdG formation (as measured by high-performance liquid chromatography/electrochemical detection) in liver, glandular stomach, spleen, thymus, prostate, testes, and seminal vesicles of male Lewis rats, nor did Aroclor 1242 induce structural chromosomal aberrations in bone marrow and spermatogonial cells of Osborne-Mendel rats given a single oral dose of 5000 mg/kg bw, or repeated doses of 500 mg/kg bw for 4 days (Green *et al.*, 1975a; Schilderman *et al.*, 2000).

Aroclor 1242, like Aroclor 1254, did not reduce the number of mitotic spermatogonial cells in Osborne-Mendel rats at the highest doses tested (Green *et al.*, 1975a), and had no effect on the number of dominant lethals (Green *et al.*, 1975b).

A study by Desaulniers *et al.* (2009) examined the effects of PCB and organochlorine pesticide mixtures on DNA methylation in the liver of exposed rats. The PCB mixture, but not the organochlorine pesticide mixture, reduced the mRNA abundance of DNA methyltransferase-1, -3a, and -3b, reduced the abundance of the methyl donor *S*-adenosylmethionine, and decreased the methylation of CpG sites in the promoter region of the tumour suppressor gene $p16^{INK4a}$.

Another group analysed histone post-translational modifications in chromatids from liver of rats exposed to PCBs in early life (Casati *et al.*, 2012). There was a decrease in levels of histone H4K16Ac and histone H3K4me3, and an increase in the expression of *SirtT1* and *Jarid1b*, genes encoding two chromatid-modifying enzymes (histone demethylases). A decrease in the abundance of mRNA of androgen receptor, a histone enzyme coregulator, was also reported.

Ghosh *et al.* (2011) applied the tools of global gene expression and Ingenuity biological functions analysis to peripheral blood mononuclear cells (PBMC) exposed in vitro to PCB-138 (0.87 ng/mL) or PCB-153 (1.42 ng/mL) for 48

hours. The expression of several biologically significant genes was highly modulated in vitro, in general by downregulation, and differential gene expression was specific to the PCB used. Exposure to PCB-153 identified genes involved in three Ingenuity Pathway Analysis (IPA) networks involved in cellular movement, development and function of the haematological system, immune cell trafficking, molecular transport, and cancer. Exposure to PCB-138 resulted in significant expression of several genes including tumour necrosis factor-associated protein 1 (*TRAP1*), contactin 5 (human neuronal NB-2 gene) (*CNTN5*), glial cell line-derived neurotrophic factor family receptor α-1 (*GFRA1*), von Willebrand factor D and EGF domains (*VWDE*), and *CYP1A2*. Notable among these are the upregulated genes *TRAP1*, *CNTN5*, *GFRA1*, which are important in the activation of *TRAP-1*.

Using the same genomic methods, Hochstenbach *et al.* (2010) reported alterations indicative of exposure to immunotoxicants in whole genome gene-expression profiles (transcriptomic changes) in human PBMC from two healthy donors exposed in vitro to a range of immunotoxic chemicals including PCB-153.

Wens *et al.* (2013) studied gene-expression profiles in PBMC exposed in vitro to a dioxin-like polychlorinated biphenyl, PCB-126 (1 µM), or a non dioxin-like polychlorinated biphenyl, PCB-153 (10 µM). Hierarchal cluster analysis created distinct clustered gene groups for samples exposed to PCB-126 or PCB-153. The number of differentially expressed genes varied with the compound used and ranged from 60 to 192. As expected, exposure to PCB-126 caused induction of the AhR signalling pathway. Exposure to PCB-153, which is known to disrupt thyroid metabolism, resulted in expression of the nuclear estrogen receptor *ESR2*.

(b) Individual congeners and their metabolites

In this section, the data in the text are presented first for non-mammalian systems and then combined for cell culture tests and in-vivo assays, by PCB congener and corresponding metabolite(s) (Table 4.5 and Table 4.6). Data in the table are presented first for non-mammalian systems and cell culture tests (Table 4.5), and then for in-vitro assays (Table 4.6).

(i) Non-mammalian systems

In tests for gene mutation in bacteria, the PCB congeners PCB-1, PCB-3, PCB-15, PCB-47, PCB-52, PCB-77, PCB-155, and PCB-209 were not mutagenic in various strains of *Salmonella typhimurium* and *Escherichia coli* in the absence or presence of exogenous metabolic activation (induced and non-induced liver microsomes), except in one study with PCB-3 in *S. typhimurium* TA1538 in the presence of rabbit liver microsomes (Wyndham *et al.*, 1976). Only PCB-209 was tested up to the recommended limit dose of 5000 µg/plate and in all strains typically used in the Ames test battery, i.e. *S. typhimurium* TA98, TA100, TA1535, TA1537, and in *E. coli* WP2 *uvrA* (Han *et al.*, 2009).

The less chlorinated congener PCB-15 was reported to induce somatic mutation in *Drosophila melanogaster* (Butterworth *et al.*, 1995).

(ii) Cell culture tests and in-vivo assays

Several studies have shown in vitro or in non-humans in vivo that PCB congeners with one to four chlorine atoms are bioactivated to DNA- and protein-binding intermediates in vitro and in vivo. Each congener produced multiple different DNA adducts, particularly with guanine. The most prominent ultimate DNA-binding intermediates were quinone metabolites, but some binding of epoxide intermediates was suggested. Rodent and human liver microsomes produced similar or different adduct patterns depending on the PCB congener used, indicating that

species differences exist. Reactive intermediates can bind to cellular macromolecules, including DNA and DNA-maintenance proteins, and such adducts can be detected in multiple organs (Morales & Matthews, 1979; Amaro *et al.*, 1996; McLean *et al.*, 1996b; Oakley *et al.*, 1996a, 1996b; Lin *et al.*, 2000; Pereg *et al.*, 2001, 2002; Srinivasan *et al.*, 2002; Arif *et al.*, 2003; Zhao *et al.*, 2004; Bender *et al.*, 2006; Bender & Osheroff, 2007).

PCB-1, PCB-2, PCB-3 and metabolites

Without exogenous metabolic activation, tritium-labelled PCB-3 was reported to bind to DNA, RNA, and cellular proteins in cultured Chinese hamster ovary cells (Wong *et al.*, 1979). PCB-3 also enhanced unscheduled DNA synthesis by 1.6-fold in the same cell line (Wong *et al.*, 1979), and increased DNA-adduct formation dose-dependently in primary human hepatocytes, as determined by ^{32}P-postlabelling (Borlak *et al.*, 2003). Maximum adduct levels were observed 24 hours after exposure and declined to control levels within 48 hours (Borlak *et al.*, 2003).

The mutagenicity of PCB-3, its mono- and dihydroxylated metabolites, and its 3′,4′- and 2′,5′-quinones was investigated in cultured Chinese hamster V79 cells (Zettner *et al.*, 2007). Induction of gene mutations at the *Hprt* locus was determined by 6-thioguanine resistance. Induction of chromosomal and genomic mutation was assessed by micronucleus formation and immunochemical differentiation of micronuclei containing whole chromosomes (kinetochore-positive) or DNA fragments (kinetochore-negative). Both quinones, but not the PCB-3 itself or its mono- or dihydroxylated metabolites, caused a dose-dependent increase in the frequency of 6-thioguanine-resistant colonies at non-cytotoxic concentrations, and an increase in chromosomal and genomic mutation was observed at higher, cytotoxic concentrations.

In addition, the 2′,5′-dihydroxylated metabolites of PCB-3 and PCB-2, but not of PCB-1, or the 3′,4′-dihydroxy-PCB-3 induced polyploidy in V79 cells; of these dihydroxylated metabolites, only 3′,4′-dihydroxy-PCB-3 increased the levels of sister-chromatid exchange (Flor & Ludewig, 2010).

As in V79 cells, PCB-3–2′,5′-quinone caused a dose-dependent increase in the frequency of micronucleus formation in human breast epithelial MCF-10A cells (Venkatesha *et al.*, 2008). At the concentrations tested, electron paramagnetic resonance showed an increase in steady-state levels of ROS, and detected the presence of a semiquinone radical, suggesting redox cycling of the 2′,5′-quinone. Furthermore, the increase in number of micronucleated cells observed with PCB-3–2′,5′-quinone and also with PCB-153 was consistent with an increase in levels of phosphorylated histone protein γ-H2AX (Venkatesha *et al.*, 2008). The 2′,5′-quinone of PCB-3 also caused significant and dose-dependent shortening of the telomeres in human keratinocyte HaCaT cells after 11 weeks of exposure, and an increase in frequency of micronucleus formation in V79 cells (Jacobus *et al.*, 2008).

Induction of gene mutation in vivo by PCB-3 and its monohydroxylated metabolite 4′-OH-PCB-3 was investigated in male and female transgenic Fischer 344 (Big Blue®) rats given four intraperitoneal injections of PCB-3 at 113 mg/kg bw and 4′-OH-PCB-3 at 82 mg/kg bw over 4 weeks. Seventeen days after the last injection, the frequency and spectrum of mutation in the *lacI* gene were determined in the liver (Lehmann *et al.*, 2007) and lung (Maddox *et al.*, 2008) of males, and in the liver of females (Jacobus *et al.*, 2010). Both PCB-3 and its 4′-OH-metabolite caused a similar, more than twofold, increase in mutation frequency in the liver of male rats; however, only the increase observed with PCB-3 was statistically significant. Although the mutation spectrum induced by PCB-3 was different from that in control rats, and similar to that induced by the positive control, 3-methylcholanthrene, only the proportion of transitions was statistically different from that in control

rats. In contrast, the mutation spectrum for 4′-OH-PCB-3 differed only slightly from that in the control group (Lehmann *et al.*, 2007). In the liver of female rats treated with PCB-3 and its 4′-OH-metabolite, mutation frequencies and mutation spectra were not significantly different from those observed in control rats (Jacobus *et al.*, 2010). PCB-3 and its 4′-OH-metabolite caused a twofold, but not statistically significant, increase in mutation frequency in the lungs of treated males. However, a shift in the mutation spectra, especially with PCB-3, and an increase in the frequency of mutation outside of the hotspot region for spontaneous mutation of *lacI* (base pairs 1–400) were observed (Maddox *et al.*, 2008). The genotoxicity profile of metabolites of PCB-3 is summarized in Table 4.7.

PCB-28, PCB-52, PCB-77

PCB-52 enhanced the frequency of DNA strand breaks in human lymphocytes (comet assay) and mouse fibroblast L-929 cells (alkaline sedimentation), but had no effect on the level of sister-chromatid exchange and structural chromosomal aberration in human lymphocytes (Stadnicki & Allen, 1979; Stadnicki *et al.*, 1979; Sargent *et al.*, 1989; Sandal *et al.*, 2008). However, the addition of PCB-77 at non-genotoxic concentrations led to a threefold increase in the frequency of chromatid breaks compared with that in control cells (Sargent *et al.*, 1989).

PCB-28, PCB-52, and a synthetic mixture of PCBs similar to that present in air in Chicago, USA, at equimolar concentrations all caused a 30–40% reduction in telomerase activity in human skin HaCaT keratinocytes, but the effect on telomere length differed, with shortening effects caused by PCB-28, PCB-52, and the Chicago air mixture of about 10%, 40%, and 5%, respectively, compared with controls after 6 weeks of exposure (Senthilkumar *et al.*, 2011).

PCB-77 caused DNA-adduct formation in human hepatocarcinoma HepG2 cells and in dexamethasone-treated primary rat fetal hepatocytes. In human lymphocytes, PCB-77 induced structural chromosomal aberration, but no increase in the frequency of micronucleated cells and sister-chromatid exchange was observed (Sargent *et al.*, 1989; Dubois *et al.*, 1995; Belpaeme *et al.*, 1996b).

Long-term dietary exposure of female hepatectomized Sprague-Dawley rats to PCB-52 at 10 ppm for 7 months, or PCB-77 at 0.1 ppm for 1 year, did not enhance the frequency of structural or numerical chromosomal aberration in liver and bone-marrow cells (Meisner *et al.*, 1992). However, coexposure to PCB-52 and PCB-77 at the doses given above for 1 year increased the frequency of polyploidy and structural chromosome aberration in bone-marrow cells. Although the frequency of numerical and structural chromosomal aberration in primary hepatocytes remained unaffected after coexposure to PCB-52 and PCB-77 for 7 months, the liver became more susceptible to diethylnitrosamine-induced genotoxicity (Sargent *et al.*, 1992).

PCB-101, PCB-118, PCB-138

PCB-101, PCB-118, and PCB-138 were able to induce DNA strand breaks (comet assay) and micronucleus formation (except PCB-138) in fish fibroblast RTG-2 cells [usually not used for genotoxicity testing], in a single dose experiment. However, the time course of markers for oxidative stress (carboxy-dichlorofluorescein oxidation, intracellular GSH, lipid peroxidation, and superoxide dismutase activity) did not correspond with the observed genotoxicity (Marabini *et al.*, 2011).

PCB-126

PCB-126 did not increase the frequency of micronucleus formation in human hepatoma HepG2 cells, but did cause a significant, but not dose-dependent, increase in levels of the DNA repair protein XPA (Western blot), whereas XPC protein levels were unaffected (Wei *et al.*, 2009b).

Table 4.7 Genotoxicity profile of metabolites of PCB-3

Compound	Lowest effective dose (μM)						
	Gene mutation (thioguanine resistance)[a]	**Micronucleus (clastogenic effect)[a]**	**Micronucleus (aneuploidy: chromosomal loss)[a]**	**SCE[b]**	**Polyploidy[b]**	**DNA damage (comet assay)[c]**	**ROS[c]**
PCB-3	-	-	-	-	-	-	-
2-OH-PCB-3	-	-	50	-	-	-	-
3-OH-PCB-3	-	-	100	-	-	-	-
4-OH-PCB-3	-	75	75	-	-	-	-
3,4-dihydroxy-PCB-3	-	25	15	5	-	-	-
3,4-*ortho*-quinone	0.6	15	5	-	-	-	-
2,5-hydroquinone	-	5	2.5	-	7.5	10 (at 37°C, not 6°C, in HL-60 cells; not in Jurkat cells at 37°C)	5 (ROS increased in HL-60 cells at 37°C, not at 6°C; no effect on ROS in Jurkat cells)
2,5-*para*-quinone	0.5	1	2.5	-	-	5 (at 37°C or 6°C in HL-60 cells; at 37°C in Jurkat cells)	2.5 (ROS increased in HL-60 cells and in Jurkat cells)

[a] From Zettner *et al.* (2007)
[b] From Flor & Ludewig (2010)
[c] From Xie *et al.* (2010)
PCB, polychlorinated biphenyl; ROS, reactive oxygen species; SCE, sister-chromatid exchange
Adapted from Robertson & Ludewig (2011)

PCB-126 did not increase the frequency of mutation in fetuses of the transgenic Muta™Mouse on day 18 of gestation after a single maternal oral dose of 0.5 mg/kg bw on day 10 of gestation (Inomata *et al.*, 2009).

PCB-126 and PCB-153

The role of oxidative DNA damage in carcinogenesis caused by PCB-126, PCB-153, and a combination thereof, was investigated by measuring in treated animals the accumulation of a DNA adduct, namely 3-(2′-deoxy-β-D-erythro-pentafuranosyl)-pyrimido[1,2-α]-purin-10-one (M1dG) (the pyrimidopurinone of deoxyguanosine) (Dedon *et al.*, 1998), which can be formed by reaction of lipid-peroxidation derived malonedialdehyde or by oxidation of deoxyribose-derived DNA base propenal and deoxyguanosine. Accumulation of M1dG adducts was assessed in the liver of female C57BL/6J mice given a single dose and in Sprague-Dawley rats exposed for 1 year. A single dose of a mixture consisting of four dioxin-like compounds (including PCB-126), or a mixture consisting of four non-dioxin-like PCBs (PCB 118, 138, 153, 180), did not increase M1dG accumulation in the mouse liver. In female Sprague-Dawley rats exposed to PCB-126, PCB-153, or a combination of both for 1 year (see Section 3; NTP, 2006a, b, c), an increase in hepatic levels of M1dG was observed in rats treated with PCB-126, and in rats treated with a combination of PCB-126 + PCB-153. In female rats coexposed to PCB-126 + PCB-153, the observed levels of M1dG adducts correlated with the observed incidence of liver tumours (Jeong *et al.*, 2008).

PCB-153

PCB-153 induced structural chromosomal aberration in human lymphocytes (Sargent *et al.*, 1989) and a statistically significant dose-dependent increase in the frequency of micronucleus formation in human breast epithelial MCF-10A cells (Venkatesha *et al.*, 2008). PCB-153 also induced a significant and dose-dependent twofold increase in the frequency of micronucleation in human hepatocarcinoma HepG2 cells. Coexposure to PCB-153 and benzo[*a*]pyrene significantly and dose-dependently increased the frequency of micronucleus formation by 60%. When α-naphthoflavone (an inhibitor of CYP1A1) was added to cultures exposed to PCB-153 and PCB-153 + benzo[*a*]pyrene, the frequency of micronucleation decreased almost to control levels (Wei *et al.*, 2009a).

PCB-153 was able to induce DNA strand breaks and micronucleus formation in fish fibroblast RTG-2 cells (Marabini *et al.*, 2011; see above for comments).

Treatment of immortal human skin HaCaT keratinocytes with PCB-153 at a single concentration resulted in a decrease in telomerase activity (~20% after 1 week to ~40% after 7 weeks of exposure) and telomeres were shortened by about 40% (Senthilkumar *et al.*, 2012). Shortening of telomeres was also observed in normal human foreskin keratinocytes exposed to PCB-153 in culture, but the difference compared with the control cells was not statistically significant on any of the days analysed.

PCB-209

PCB-209 did not induce mutation at the thymidine kinase locus in mouse lymphoma L5178Y/$T^{+/-}$ cells, and did not cause an increase in micronucleus formation in bone-marrow cells of male and female Crl:CD1 mice given a single oral dose at 2000 mg/kg bw (Han *et al.*, 2009).

$MeSO_2$-PCB metabolites

$MeSO_2$-PCBs did not induce micronucleus formation in cultured human lymphocytes, but some, namely 3-$MeSO_2$-2,5,2′,4′,5′-pentaCB [3′-$MeSO_2$-PCB-101;3-$MeSO_2$-2,2′,4′,5,5′- pentaCB] and 4-$MeSO_2$-2,5,2′,3′,4′-pentaCB [4’-$MeSO_2$-PCB-87; 4-$MeSO_2$-2,2′,3′,4′,5- pentaCB], enhanced levels of sister-chromatid exchange (Nagayama *et al.*, 1995, 1999).

(c) Summary

Numerous cell-based test systems, and animal models, have been used to investigate the genotoxic potential of commercial PCB mixtures. However, only 13 individual congeners have been examined so far in studies of genotoxicity and related effects. Seven congeners (PCB-3, PCB-52, PCB-77, PCB-118, PCB-138, PCB-153, PCB-209) have been investigated in both cellular systems and animals. An additional four congeners (PCB-15, PCB-47, PCB-101, and PCB-155) were tested only in cellular systems, and two congeners (PCB-126 and PCB-180) have been tested only in cellular systems or animals, respectively.

Studies on induction of gene mutation in bacteria exposed to PCB mixtures, or to the few individual congeners tested, gave negative results. However, these data were of limited value for assessing this end-point because the doses applied were usually < 1000 μg/plate and/or where this was not the case, testing with an exogenous metabolic system was omitted. Studies with PCB-209 were not subject to the aforementioned limitations.

When high concentrations of commercial PCB mixtures were tested in *Saccharomyces cerevisiae*, genotoxicity was observed with Arochlor 1254, Aroclor 1221, and Aroclor 1260. In mammalian cells in vitro, Aroclor 1254 was reported to produce DNA adducts, unscheduled DNA synthesis, DNA strand breaks and, to some extent, chromosomal aberration. Although these end-points were negative when tested in rodents in vivo, Aroclor 1254 did increase chromosomal aberration and micronucleation in fish, and mutation frequency in the liver of transgenic Big Blue® mice. Aroclor 1254 induced cell transformation in cultured Syrian hamster embryo cells.

As for the individual congeners, the most comprehensive data on genetic effects were available for PCB-3 and its metabolites. PCB-3 did not induce gene mutation in bacteria at doses up to 1000 μg/plate in the presence or absence of an exogenous metabolic system, except for one study in strain TA1538 in the presence of rabbit liver microsomes (see Table 4.4). However, PCB-3 was reported to bind to DNA and to cause an increase in levels of DNA adducts in primary human hepatocytes.

The cell lines commonly used for mutagenicity testing (Chinese hamster lung fibroblast V79, Chinese hamster ovary fibroblast, and mouse lymphoma L5178Y cells) have no or only very limited biotransformation capability, a problem for test compounds that require metabolic activation. Using instead a series of synthetic PCB-3 metabolites in the V79 gene mutation assay, the *ortho* (3,4-) and *para* (2,5-) quinones were shown to efficiently induce mutation at the *Hprt* locus at non-cytotoxic concentrations, while none of the tested mono- or dihydroxylated metabolites or PCB-3 itself induced mutation (see Table 4.4). In addition, an increase in chromosomal and genomic mutation was observed for all tested PCB-3 metabolites at higher, cytotoxic concentrations. Also, the 2′,5′-dihydroxylated metabolites of PCB-3 and PCB-2, but not metabolites of PCB-1 or the 3′,4′-dihydroxylated PCB-3, induced polyploidy in V79 cells, indicating strict structure–activity requirements for this type of DNA damage. The 2′,5′-quinone of PCB-3 induced an increase in levels of ROS via a semiquinone radical at concentrations inducing micronucleation, suggesting redox cycling of the 2′,5′-quinone. PCB-3–2′,5′-quinone caused telomere shortening in cultured HaCaT cells exposed for 11 weeks, an effect that may have been caused by oxidative stress.

The mutagenic activity of PCB-3 was also tested in an assay in transgenic rats in vivo. In the liver of male rats exposed to PCB-3, the mutation frequency was significantly increased and the mutation spectrum changed from predominantly transitions in the controls to predominantly G:C → T:A transversions in the rats exposed to PCB-3. 4′-OH-PCB-3 caused a similar, but not statistically significant, increase

in mutation frequency and a minor shift in the mutation spectrum compared with rats in the control group. A sex-specific and organ-specific difference was noted, since the response was less pronounced in livers of female Big Blue® rats and lungs of males, in which the observed increases in mutation frequency were below the level of statistical significance.

The non-dioxin-like PCB-52 was not tested for gene mutation in bacteria and cultured mammalian cells. Data on chromosomal aberration in cultured mammalian cells were ambiguous, but also of limited value since PCB-52 was never tested in the presence of a metabolic activation system. There were, however, indications of DNA damage caused by PCB-52 metabolites in studies in vitro and in vivo in rats coexposed to PCB-52 and dioxin-like PCB-77 for 1 year. Negative outcomes in other studies of chromosomal aberration in vivo may be attributed to the low doses tested.

The dioxin-like PCB-77 increased the level of DNA adducts in cultured mammalian cells. The lack of data on mutagenicity testing of PCB-77 did not allow for an interpretation of these findings with regard to gene mutation. Data on structural/numerical chromosomal aberrations, including micronucleus formation, were inconclusive in vitro, and negative for chromosomal aberration in female rats after long-term dietary exposure.

The limited data available on PCB-126 suggested no genotoxic potential in vitro or in vivo. However, increased levels of DNA adduct (M_1dG) indicative of the formation of ROS and/or lipid peroxidation were seen in female rats exposed to PCB-126 and PCB-126 + PCB-153 for 1 year (Jeong *et al.*, 2008).

Non-dioxin like PCB-153 gave positive results when tested for micronucleus formation in two cultured mammalian cell lines and one fish cell line. Also, reduction in telomerase activity corresponding to shortened telomeres was reported in cultured human cells. [Since no in-vivo data were available, the significance of these in-vitro results could not be assessed by the Working Group.]

For the decachlorinated PCB-209, a series of standard assays for genotoxicity that followed internationally accepted testing guidelines for regulatory purposes were performed under good laboratory practice (GLP) conditions, and showed no mutagenic and/or genotoxic potential.

4.3 Biochemical and cellular effects

4.3.1 AhR binding and activation

(a) AhR activity

AhR is a cytosolic, ligand-activated transcription factor that mediates many toxic and carcinogenic effects in vertebrates. TCDD has extremely high affinity to the AhR and is the reference AhR agonist and toxicant. AhR-mediated toxic responses are consequences of deregulated physiological functions, and sustained (chronic) AhR activation by persistent "dioxin-like" compounds is the key process in dioxin-like toxicity (Bock & Köhle, 2006). Toxicological evaluation of dioxin-like-PCBs (DL-PCBs) is based on various end-points associated with activation of the AhR and AhR-mediated physiological and toxic responses (Haws *et al.*, 2006). The major advantages of this concept are that most (if not all) effects of dioxin-like compounds are mediated via AhR activation, and that various effects of TCDD reported in many in-vivo and in-vitro models associated with carcinogenesis and tumour promotion may be extrapolated for DL-PCBs (IARC, 2012).

Effects of AhR-mediated changes in gene expression include the control of xenobiotic-metabolizing enzymes, modulations in cell cycle progression and cell proliferation, suppression of apoptosis, and perturbation of various developmental signalling pathways involved in carcinogenic processes (Vezina *et al.*, 2004; Sartor *et al.*, 2009; Faust *et al.*, 2013). In addition, AhR interacts with other signalling and transcription pathways,

including estrogen, thyroid and retinoic acid receptors, mitogen-activated protein kinases (MAPKs), NF-κB, retinoblastoma protein, and hypoxia-inducible factor-1 α (Tian *et al.*, 2002; Beischlag *et al.*, 2004; Murphy *et al.*, 2007; Puga *et al.*, 2009). Several molecular mechanisms that are related to AhR and that may contribute to carcinogenesis have been proposed:

- Induction of CYP1 enzymes linked to toxicity and cancer initiation (DNA-adduct formation and oxidative DNA damage);
- Sustained AhR-dependent expression of genes directly or indirectly controlling the cell cycle, proliferation and apoptosis, and cross-talk between genes in the AhR and growth-regulatory pathways;
- AhR-mediated cytoskeletal remodelling, reduced cell–cell contacts, modulation of developmental/differentiation pathways, cell plasticity and invasiveness affecting tumour progression;
- Upregulation of proinflammatory genes (Gasiewicz *et al.*, 2008).

Correlations between the immunosuppressive effects of PCBs and activation of the AhR pathway have been also reported (see Section 4.3.4).

(b) Concepts of TEF and TEQ

The concept of toxic equivalency (TEQ) is based on a common mechanism of action (mediated through AhR activation) of persistent organic pollutants (including polyhalogenated dibenzo-*p*-dioxins, dibenzofurans and biphenyls). It uses relative effective potencies (REP) of individual compounds to activate the AhR, and AhR-dependent toxic or biological effects relative to the reference toxicant TCDD; toxic equivalency factors (TEFs) for individual compounds were established/extrapolated from the database of many in-vivo studies. Since the 1980s, the TEF concept has been developed and refined (Safe *et al.*, 1985; Safe, 1990; Ahlborg *et al.*, 1994; Van den Berg *et al.*, 1998). Current TEF values were reevaluated recently using a refined TEF database (Haws *et al.*, 2006; Van den Berg *et al.*, 2006).

TEQ is defined by the sum of concentrations of dioxin-like compounds multiplied by their TEF values. A limitation of the concept is the additivity model being used, but its major advantage is the transformation of data on chemical concentration of complex mixtures into a single TCDD-like activity of the mixture. Many experimental studies with complex mixtures have confirmed that the TEQ approach is consistent with an additive model, although some deviations from additivity are observed. Another disadvantage is that the potential toxic and carcinogenic effects of non dioxin-like-PCBs (NDL-PCBs) are not included in this concept; high levels of NDL-PCBs may even suppress AhR-mediated toxicity, and thus act as antagonists.

Importantly, studies of carcinogenic and tumour-promoting activity were accounted for in the refined TEF database. Based on the TEF approach, carcinogenic hazard in humans may only be identified for DL-PCBs. The current TEF values for the PCB congeners included in the TEF concept are presented in Section 1, Table 1.4.

(c) Validation in experimental systems

AhR activation by DL-PCBs has been reported in many studies in vitro and in vivo, including comparative toxicogenomic analyses in primary human, monkey, and rodent hepatocytes (Silkworth *et al.*, 2005; Westerink *et al.* 2008). In a comparative in-vitro study in primary cultures of human and rat hepatocytes exposed to TCDD or PCB-126 at various concentrations for 48 hours, dose–responses and relative effective potencies (REP-values) were calculated for induction of CYP1A1 and other AhR-responsive genes (Carlson *et al.*, 2009). Previously, Silkworth *et al.* (2005) found that human cells are about 10–1000 times less sensitive to TCDD, PCB-126, and Aroclor 1254 than are rat and monkey cells.

Importantly, the newly calculated rat–human interspecies relative potency factors for PCB-126 were more than 100 times lower than the current rodent-derived value (Silkworth *et al.*, 2005).

These and other studies showed a relative insensitivity of the human AhR and human cells to PCB-126. In addition to a lesser potency of TCDD in human models (Haws *et al.*, 2006), lower potencies of PCB-126 might be due to species differences in relative intrinsic efficacy and/or species-specific differences in recruitment of transcriptional co-activators (Carlson *et al.*, 2009). In spite of the discrepancies between relative potencies of PCB-126 and TCDD in rodent and human liver cells, REP estimates based on induction of CYP1A1 or other AhR target genes might be relevant to evaluate the carcinogenic and hepatotoxic potential of TCDD and PCB-126 in humans.

The TEF approach and additivity concept were evaluated in 2-year cancer bioassays in groups of 53–55 female Harlan Sprague-Dawley rats receiving TCDD at a dose of 3–100 ng/kg bw per day, PCB-126 at a dose of 30–1000 ng/kg bw per day, 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) at a dose of 6–200 ng/kg bw per day, or a mixture of the three toxicants. Dose–response curves for hepatic, pulmonary, and oral mucosal neoplasms showed that carcinogenic effects could be predicted from the WHO TEF values (Walker *et al.*, 2005).

In a short-term study, female Harlan Sprague-Dawley rats were exposed for 13 weeks to toxicologically equivalent doses of four polychlorinated aromatic hydrocarbons based on their TEF: TCDD (100 ng/kg bw per day), PeCDF (200 ng/kg bw per day), PCB-126 (1000 ng/kg bw per day), or PCB-153 (1000 µg/kg bw per day) (Vezina *et al.*, 2004). The AhR agonists (TCDD, PeCDF, and PCB126) produced very similar global gene-expression profiles, while PCB-153 showed a different, non-AhR-mediated response. All four compounds induced significant liver hypertrophy. TCDD and PCB-126 were more effective in activating AhR-dependent gene expression and inducing hepatic hypertrophy than was PeCDF, although the administered doses of each compound were based on equal TEQ values. These data fitted perfectly with the TEF value for PCB-126 in rats. Nevertheless, the gene-expression data might not bear a direct relevance to carcinogenicity of the studied compounds (Vezina *et al.*, 2004).

Global gene expression was investigated in vitro in the contact-inhibited rat liver progenitor WB-F344 cells exposed to PCB-126 at a concentration of 100 nM, or TCDD at 1 nM, for 6, 24, and 72 hours (Faust *et al.*, 2013). AhR dependency was validated using both chemical inhibition of AhR and knockdown of the AhR or the aryl hydrocarbon receptor nuclear translocator (ARNT) using small interfering RNA (siRNA). Gene ontology analysis revealed that, apart from deregulation of drug and lipid metabolism, genes participating in regulation of the cell cycle and growth control, developmental and cancer pathways, cell–cell communication and adhesion were significantly affected. Importantly, transcriptional regulation mediated by PCB-126 was very similar to that induced by TCDD in rat liver in vivo (Vezina *et al.*, 2004), and in rat liver progenitor WB-F344 cells. [Nevertheless, the relevance of these data to human carcinogenesis remained limited due to the species-specific pattern of AhR-dependent gene expression (Dere *et al.*, 2011).]

4.3.2 Cell death and proliferation

(a) Apoptosis, cell proliferation, and cell cycle control

(i) Apoptosis

DL-PCBs and NDL-PCBs have been shown to suppress DNA damage-induced apoptosis in vitro (Knerr & Schrenk, 2006; Al-Anati *et al.*, 2010).

PCB-28, PCB-101, and PCB-187 inhibited ultraviolet irradiation-induced apoptosis in hepatocytes from male Wistar rats pre-exposed to ultraviolet radiation before being treated with PCBs for 12 hours. A statistically significant suppression of apoptosis was found after the treatment with PCB-28 at 1 nM, PCB-101 at 10 nM, or PCB-187 at 1 μM (Bohnenberger *et al.*, 2001; Schrenk *et al.*, 2004).

PCB-126, and several NDL-PCBs (concentration range, 0.01–10 μM), attenuated the TP53-mediated apoptotic response via phosphorylation of the regulatory protein MDM2 in human hepatoma HepG2 cells (Al-Anati *et al.*, 2009). PCB-28, PCB-101, and PCB-153 reduced benzo[*a*]pyrene-induced phosphorylation of MDM2, and amplified the benzo[*a*]pyrene-induced TP53-dependent apoptotic response; however, benzo[*a*]pyrene-induced apoptosis was inhibited. Reduced levels of phosphorylated forkhead family transcription factor FOXO3a [FOXO3] were also reported after treatment with NDL-PCBs (Al-Anati *et al.*, 2010). FOXO3a probably functions as a trigger for apoptosis through expression of genes necessary for cell death. Thus NDL-PCBs may also inhibit benzo[*a*]pyrene-induced apoptosis by preventing phosphorylation of FOXO3a (Al-Anati *et al.*, 2010).

(ii) Cell proliferation

Cell proliferation can be caused either by cytotoxicity/injury and regenerative proliferation, or by a sustained increase in proliferation. It is mediated via several signal-transduction pathways leading to pro-proliferative changes in gene expression (controlled by specific transcription factors, such as AhR, CAR, NF-κB or AP-1). These events may drive genotoxic and nongenotoxic processes associated with tumour promotion and progression. PCBs have been reported to induce such proliferative events in a series of experimental in-vitro and in-vivo models (Tharappel *et al.*, 2002; Marlowe & Puga, 2005; Puga *et al.*, 2009).

CAR is known to control the hepatic expression of detoxification enzymes and to induce sustained cell proliferation in the liver. *Ortho*-substituted PCBs induce expression of CYP isoenzymes (see Section 4.1.3) via CAR (Muangmoonchai *et al.*, 2001). The activation of CAR-dependent gene expression by NDL-PCBs in vivo has been observed, e.g. in rat liver after 28-day exposure to PCB-180 (Roos *et al.*, 2011), or in the liver of immature, ovariectomized C57BL/6 mice treated with PCB-153 (Kopec *et al.*, 2010). Using a range of genetically engineered human cell models derived from liver, lung, and colon tissues, it has been shown that several NDL-PCBs, such as PCB-99, PCB-138, PCB-153, PCB-180 or PCB-194, may activate CAR-controlled reporter vectors, as well as PXR reporters, in a tissue-specific manner (Al-Salman and Plant, 2012). [The Working Group was aware that the relevance to human risk of CAR-driven hepatocarcinogenic effects seen in rodents has been questioned (Holsapple *et al.*, 2006).]

In the 13-week study by Vezina *et al.* (2004), modulation of global gene expression was analysed in liver of female rats given PCB-153 at a dose of 1000 μg/kg bw per day. In addition to CYP2B1 and CYP2B2, PCB-153 also modulated the expression of anti-apoptotic genes (*Bcl2* and *Wee1* were downregulated), and other genes associated with liver injury. PCB-153 selectively enhanced expression of the cAMP response element modulator (CREM), which is a signature response to liver regeneration after hepatocyte injury.

In an initiation–promotion study in female Sprague-Dawley rats, an increase in the frequency of several preneoplastic foci, and increased NF-κB and AP-1 binding activities were observed in the liver of rats given PCBs (Tharappel *et al.*, 2002). Although cell proliferation was not affected by PCB-153, apoptotic indexes were decreased in focal hepatocytes by PCB-153. The induction of altered hepatic foci appeared to be related to compensatory cell proliferation in rats treated

with PCB-77, while inhibition of apoptosis appeared to be important for rats treated with PCB-153 (Tharappel *et al.*, 2002). In a subsequent study, a single dose of PCB-153 (at 150 or 300 μmol/kg bw), but not PCB-77, induced hepatocyte proliferation and hepatic NF-κB activation in male Sprague Dawley rats (Lu *et al.*, 2003). Comparison of the effects of PCB-153 in wild-type mice and in mice deficient in the NF-κB p50 subunit suggested possible involvement of NF-κB in PCB-153-modulated cell proliferation and apoptotic changes (Lu *et al.*, 2004). Absence of the NF-kB p50 subunit inhibited the promoting activity of PCB-153, as illustrated by the NF-κB knockout study in mice treated with diethylnitrosamine/PCB-153. Taken together these data implicate a possible role for oxidative stress-mediated activation of specific transcription factors, such as NF-κB, as a possible mode of action for NDL-PCBs (Glauert *et al.*, 2008).

Brown *et al.* (2007) have reported a correlation between incidence of tumours of the liver and increased activity of mixed function oxidases and increased expression of proliferating cell nuclear antigen (the indicator of cell proliferation) in Sprague-Dawley rats exposed to repeated doses of Aroclor mixtures for 24 months. [From these data, it was not clear to which class of PCB congeners (DL- or NDL-PCBs) the effects could be attributed.]

In nontumorigenic human mammary epithelial MCF-10A cells, PCB-153 at a concentration of 1–15 μM, Aroclor 1254 and 2-(4-chlorophenyl) benzo-1,4-quinone increased levels of reactive oxygen species, and caused cell-cycle delay and growth inhibition by suppressing levels of cyclin D1 (Venkatesha *et al.*, 2008, 2010; Chaudhuri *et al.*, 2010).

Further studies also examined the role of AhR in PCB-mediated deregulation of cell proliferation. Activation of AhR is known to cause a delay in cell-cycle progression in several cancer cell lines, models of differentiated cells (e.g. rodent hepatoma cells), and in primary rodent hepatocytes (Elferink, 2003; Marlowe & Puga, 2005). However, AhR ligands have been found to elicit opposite effects in liver progenitor cells: induction of cell proliferation in contact-inhibited rat liver progenitor cells in vitro by DL-PCBs was reported to be an AhR-dependent process (Vondráček *et al.*, 2005). Like TCDD, PCB-126 at 100 pM, 4′-OH-PCB-79 (a metabolite of coplanar PCB-77) at 1 μM, or PCB-105 (mono-*ortho*-chlorinated congener) at 10 μM increased the percentage of cells in S-phase and the total number of cells. In contrast, the NDL-PCBs and their metabolites had no effect on cell proliferation at concentrations up to 10 μM. Only PCB-126 (AhR-activating), and not PCB-153 (not AhR-activating), upregulated levels of cyclin A and D2 protein (Vondráček *et al.*, 2005). The proliferative effects of PCB-126 were further potentiated by tumour necrosis factor-α (Umannová *et al.*, 2007).

(iii) DNA synthesis

The rate of DNA synthesis in altered hepatic foci and in tumours in PCB-treated rats and mice was studied by Tharappel *et al.* (2002), who gave rats DEN at a dietary concentration of 150 mg/kg followed by four biweekly intraperitoneal injections of PCB-77 or PCB-153 at a dose of 100 or 300 μmol/kg bw. Rats were given bromodeoxyuridine (BrdU) in Alzet osmotic pumps for the measurement of DNA synthesis in focal and nonfocal hepatocytes. PCB-77 increased the BrdU labelling indexes in GSTP-positive foci and in normal hepatocytes, but PCB-153 did not. Similarly, PCB-153 did not influence the BrdU labelling index in DEN-initiated hepatic tumours in mice (Glauert *et al.*, 2008). Haag-Grönlund *et al.* (2000) found that weekly subcutaneous injections of PCB-118 at doses of 10–10 000 μg/kg bw did not increase BrdU labelling in focal hepatocytes after 20 weeks, but that PCB-118 at a dose of 10 000 μg/kg bw increased the BrdU labelling index after 52 weeks.

(b) Cell–cell communication

Several studies have demonstrated that PCBs can inhibit gap-junctional intercellular communication (GJIC) both in vivo (Krutovskikh *et al.*, 1995; Bager *et al.*, 1997) and in vitro in rat liver epithelial cells, mouse and rat hepatocytes, human keratinocytes, and normal human breast epithelial cells (Ruch & Klaunig, 1986; Swierenga *et al.*, 1990; Hemming *et al.*, 1991; Kang *et al.*, 1996). The *ortho*-substituted PCBs were potent inhibitors of GJIC at low micromolar concentrations, while the coplanar PCBs did not inhibit GJIC after a single dose (Machala *et al.*, 2003). The assay for GJIC inhibition showed good predictability for tumour promotion of *ortho*-substituted PCBs. Recently, inhibition of GJIC has been confirmed using single doses of ultrapure NDL-PCB congeners (Hamers *et al.*, 2011).

Different cell- and connexin-specific mechanisms of action probably account for the inhibitory effects of PCBs on GJIC. Of the NDL-PCBs, PCB-153 decreased the number of gap-junction plaques, and decreased levels of connexin 43 (constitutive protein of gap junctions) in liver epithelial cells. PCB-153 enhanced proteasomal and lysosomal degradation of connexin 43 and inhibited trafficking of connexin 43 to the plasma membrane (Šimečková *et al.*, 2009a). In contrast, inhibition of GJIC by AhR ligands (i.e. DL-PCBs such as PCB-126) seems to proceed mainly through downregulation of mRNA of connexin 32 in hepatocyte-derived models (Herrmann *et al.*, 2002).

(c) Other cellular mechanisms relevant to PCB-induced carcinogenesis

NDL-PCBs have been shown to elicit additional nongenomic effects on membrane-associated proteins, which are closely related to tumour promotion and progression.

PCB-153 was found to increase the incidence of glutamine synthetase-positive tumours of the liver in male B6129sf2/J mice, and almost 90% (34 out of 38) of all tumours from mice treated with PCB-153 contained mutations in the β-catenin gene (*Catnb*), compared with ~45% (17 out of 37) of tumours in the control group. Tumours containing mutations of Ha-*ras* [*Hras*] and B-*raf* [*Braf*] were rare and not significantly different between treatment groups. Exposure to PCB-153 appeared to strongly select for *Catnb*-mutated, glutamine synthetase-positive tumours of the liver in mice (Strathmann *et al.*, 2006).

In the rat liver progenitor WB-F344 cell line, PCB-153 was found to decrease levels of several proteins at adherens junctions involved in cell–cell communication and intracellular signalling, including E-cadherin, β-catenin, and plakoglobin (Šimečková *et al.*, 2009b). Such mechanisms may be involved in the effects of NDL-PCBs, contributing to promotion of tumours.

Oral administration of dioxin-like PCB-126, mono-*ortho*-substituted PCB-118, and non-dioxin-like PCB-153 differentially altered expression of the tight junction proteins claudin 5, occludin, and ZO-1 in brain capillaries in C57/B16 mice. These alterations were associated with increased permeability of the blood–brain barrier. Most importantly, exposure to individual PCB congeners enhanced the rate of formation and progression of brain metastases by luciferase-tagged melanoma cells (Seelbach *et al.*, 2010).

As vascular endothelial cells create a selective barrier to the passage of cancer cells, it is of interest to note that non-dioxin-like PCB-104 induced endothelial hyperpermeability of human microvascular endothelial cells HMEC-1 and trans-endothelial migration of human breast cancer cells MDA-MB-231; these effects were associated with overexpression of vascular endothelial growth factor (Eum *et al.*, 2004).

Structurally different PCBs may induce proinflammatory mediators, which further contribute to metastasis. PCB-77, PCB-104 and PCB-153 induced expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and monocyte

chemoattractant protein-1 (MCP-1) in the liver, lung, and brain of male C57Bl/6 mice. PCB-77 and PCB-104 also increased levels of matrix metalloproteinase-7 (MMP-7) mRNA in the liver and brain (Sipka *et al.*, 2008).

The mixture of seven NDL-PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180, and PCB-209) increased cell motility of human non-metastatic MCF-7 cells and human metastatic breast cancer MDA-MB-231 cells in vitro via production of reactive oxygen species, and activation of the Rho-associated kinase (ROCK). In a follow-up study in vivo, the PCB mixture enhanced the capability of metastatic breast cancer cells to metastasize to bone, lung, and liver (Liu *et al.*, 2010).

To explore the possible effects of PCBs on telomeres and telomerase, human skin keratinocytes were exposed to a synthetic mixture of volatile PCBs, or the prominent airborne PCB congeners, PCB-28 or PCB-52, for up to 48 days (see also Section 4.2.2b). The PCB mixture and the two congeners significantly inhibited telomerase activity from day 18, while telomere length was reduced by PCB-52 from day 18, and by PCB-28 and by the mixture from day 30 onwards (Senthilkumar *et al.*, 2011).

New bioanalytical tools (e.g. transcriptomics) applied in human, animal, and in-vitro studies might improve the ability to predict the potential carcinogenicity of chemicals by elucidation of similar mechanisms (Guyton *et al.*, 2009). Several analyses of global gene expression in rodent models included identification of the effects of DL-PCBs, especially PCB-126, on pathways related to carcinogenicity.

4.3.3 Endocrine disruption

Extensive data indicate an association between exposure to PCBs and endocrine disruption. The effects include primarily interference with the function of sex hormones, i.e. estrogens and androgens, and their receptors (reviewed by Bonefeld-Jørgensen, 2010; Bonefeld-Jørgensen *et al.*, 2011; Crinnion, 2011; Fucic *et al.*, 2012). In addition, PCBs are able to bind to thyroxine transport protein (TTR), human thyroxine-binding globulin, and thyroid-hormone receptors (reviewed by Cheek *et al.*, 1999; Kawano *et al.*, 2005; Grimm *et al.*, 2013); disruption of the thyroid-hormone system was observed up to 30 years after exposure (Masuda, 2001). Furthermore, PCBs affect hormone-metabolizing enzymes, e.g. of the CYP1, CYP2, CYP3A subfamilies, and uridine-diphosphate-glucuronyl transferase, iodothyronine deiodinase, and sulfotransferase (Brouwer *et al.*, 1998).

OH-PCB and PCB-catechol and PCB-quinone metabolites formed by CYP and other oxidative enzymes have been implicated as direct or indirect endocrine-disrupting agents. The interactions found depended upon the position of hydroxylation, as well as the proximity of chlorine substitutents and the substitution pattern. Some OH-PCBs are retained in blood because they bind to transthyretin (TTR) (Lans *et al.*, 1993). Several OH-PCBs, PCB-catechols and PCB-quinones interact with estrogen receptors and other cellular receptors as agonists or antagonists (Garner *et al.*, 1999). Other OH-PCBs inhibit human estrogen sulfotransferase, thyroid hormone sulfotransferase and phenol sulfotransferases, with inhibitory potencies (IC_{50}) ranging from less than nM to low µM (Schuur *et al.*, 1998a). Species differences in the protein structures of these sulfotransferases are such that there are differences in potency of inhibition of the corresponding sulfotransferases from other species such as fish (Wang & James, 2007). The human sulfotransferase enzymes are more potently inhibited by OH-PCB than those of other species (see details below).

(a) Humans

(i) Effects on sex hormones and their receptors

Serum samples were collected from male residents of an area in eastern Slovakia with extensive environmental contamination from a former PCB-production site, as well as from a neighbouring non-industrial region. The highest quartile of PCB concentrations was significantly associated with reduced estrogen receptor-mediated activity, and a negative correlation was observed between total estrogenic activity and dioxin-like activity. No correlation was found between E_2 [17beta-estradiol] concentrations and total PCB concentrations (R_s = 0.078). E_2 was largely responsible for the estrogenic activity identified in total serum extracts (Plísková *et al.*, 2005).

PCB-induced endocrine dysfunction related to the hypothalamic–pituitary–gonadal axis was evaluated in a birth-cohort study in Germany, initiated in 2000. Healthy mother–infant pairs were recruited in the industrialized city of Duisburg. Dioxins, DL-PCBs, and six indicator PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180) were measured in maternal blood during pregnancy and in breast milk. Concentrations of testosterone and estradiol were measured in maternal and cord serum of 104 mother–infant pairs. Linear-regression analysis was used to describe the association of PCBs in maternal blood or milk with the serum concentrations of the sex steroids, after adjustment for confounding. Median concentrations for the sum of indicator PCBs were 149 ng/g in maternal blood fat and 177 ng/g in milk fat. Typically, reduction in testosterone concentrations was more pronounced in the cord serum of female babies. In contrast, male babies showed a stronger reduction in estradiol concentrations. The only statistically significant reduction associated with the six indicator PCBs was for testosterone in girls (means ratio, 0.76; 95% CI, 0.61–0.96) (Cao *et al.*, 2008).

Serum concentrations of testosterone in relation to concentrations of PCBs were investigated in an adult Native American (Mohawk) population. Fasting serum samples were collected from 257 men and 436 women, and analysed for the presence of 101 PCB congeners, and for testosterone, cholesterol, and triglycerides. The associations between testosterone and tertiles of PCB concentrations in serum (both adjusted for wet weight and lipid) were assessed by use of a logistic regression model, controlled for age, body mass index (BMI), and other factors. The lowest tertile was taken as the reference level. Testosterone concentrations in men were inversely correlated with total PCB concentration in serum, and with concentrations of the congeners PCB-74, PCB-99, PCB-153, and PCB-206, but not PCB-52, PCB-105, PCB-118, PCB-138, PCB-170, PCB-180, PCB-201, or PCB-203. Testosterone concentrations in women were much lower than in men, and not significantly correlated with serum concentrations of PCBs (Goncharov *et al.*, 2009).

A possible correlation between exposure to PCBs and testosterone concentrations was studied in 834 men from Eastern Slovakia (age, 21–78 years; median age, 48 years), of whom 432 were from a highly polluted area, and 402 were from an area with background pollution. Serum concentrations of 15 PCB congeners were measured by gas chromatography/mass spectrometry, and total testosterone was determined immunochemically (electrochemiluminescence). Correlation coefficients for each PCB congener and for the total of 15 PCBs (Σ15PCBs) with testosterone were determined. The full cohort of 834 men (median concentration of Σ15PCBs, 885 ng/g lipid) showed a highly statistically significant negative correlation between testosterone concentration and age (r = 0.303; $P < 0.0001$). A significant negative correlation ($P < 0.05$) with testosterone concentration was seen only for two mono-*ortho*-congeners, i.e. PCB-105 and PCB-118. No significant correlations were found in the subcohort of 444 men

in a narrower age range (41–55 years), in which there was no effect of age on testosterone concentrations (Langer *et al.*, 2010).

A follow-up study by the same authors included 429 men (age, 41–55 years) from a highly polluted area in Eastern Slovakia. For all subjects, the serum concentrations of 15 PCB congeners and several other chemicals were measured by gas chromatography/mass spectrometry, and total testosterone in serum was determined by electrochemiluminescence immunoassay. Similarly to the previous analysis, there was no statistically significant correlation between Σ15PCBs and testosterone (Langer *et al.*, 2012).

The association of PCBs with sex-hormone concentrations in serum was assessed in 341 men from an infertility clinic in the USA, whose exposure levels to PCBs were comparable to those observed in the general population. In crude regression models, inverse correlations were found between serum concentrations of PCBs and steroid hormone-binding globulin (SHBG) and total and free testosterone. However, after adjustment for lipids, age, and body-mass index, nearly all the significant associations disappeared: an inverse correlation remained between PCB-118 and SHBG ($P < 0.01$), while those between DL-PCBs and SHBG and total testosterone, and between PCB-118 and total testosterone, were suggestive but not statistically significant (Ferguson *et al.*, 2012).

A few studies explored the relationship between levels of steroid hormones in consumers of contaminated fatty fish from the Great Lakes (Persky *et al.*, 2001; Turyk *et al.*, 2006; see below).

(ii) Effects on the thyroid-hormone system

In a study of more than 600 children in Germany, blood samples collected from 320 children showed a significant positive correlation between serum concentrations of PCBs and increased levels of thyroid-stimulating hormone (TSH), and a significant inverse correlation with free total thyroxine (T4), as was to be expected when TSH increases (Osius *et al.*, 1999).

Hagmar *et al.* (2001a) studied the relationship between the amounts of various organohalogenic compounds in fatty fish from the Baltic Sea and hormone levels in adult men who consumed these fish. Plasma samples from 110 men (43 from south-eastern Sweden, 67 from Latvia; age range, 23–79 years) who consumed up to 32 fish-meals per month were analysed for the presence of 18 PCB congeners, five OH-PCBs, and various other chemicals. In addition, plasma concentrations of follicle-stimulating hormone, luteinizing hormone, prolactin, plasma thyrotropin, free and total triiodothyronine (T3), free and total T4, and free testosterone were measured. After adjustment for age, no significant associations were found between any of these markers and any of the PCBs or OH-PCBs. However, a study among 182 fishermen’s wives (age range, 23–46 years) from the east coast of Sweden, who had a median consumption of contaminated fatty fish from the Baltic Sea of two meals per month (range, 0–12 meals), found a significant inverse correlation between PCB-153 concentrations (range, 16–776 ng/g lipid) and total T3 levels in plasma, also after adjustment for age ($P < 0.001$) (Hagmar *et al.*, 2001b). An inverse correlation was also observed with total T4, which was borderline significant ($P = 0.07$).

Parallel to a larger investigation of consumption of contaminated fatty fish from the Great Lakes and effects on reproductive function, the association between PCB intake via consumption of fish and effects on thyroid and steroid hormones was studied in 178 men, and on thyroid hormones in 51 women (Persky *et al.*, 2001). Serum concentrations of PCBs and fish consumption were associated with significantly lower levels of T4 and a significantly lower free T4 index in women. Fish consumption, but not serum PCB concentration, was associated with a higher uptake of T3 in men. Results for TSH were inconsistent. Among men, there were significant

inverse associations for serum PCB concentration and fish consumption with SHBG-bound testosterone, but no association with SHBG itself, or with free testosterone. There were no significant overall associations for serum PCB concentration or fish consumption with estrone sulfate, follicle-stimulating hormone, luteinizing hormone, or dehydroepiandrosterone sulfate.

The relationsip between levels of steroid and thyroid hormones and total NDL-PCBs was investigated in 56 men who were frequent or infrequent consumers of fish from the Great Lakes (Turyk *et al.*, 2006). The men had consumed fish meals for 15–57 years. Significant inverse associations with serum PCB concentrations were found for T3, T4, TSH, and SHBG-bound testosterone, after adjustment for age, body-mass index, and use of medication. Follicle-stimulating hormone, luteinizing hormone, free testosterone, and SHBG were not associated with PCB concentrations in serum.

To assess the relationship between exposure to organochlorine compounds and thyroid function and neurodevelopment, a population-based birth-cohort study was conducted on the Faroe Islands (Denmark), where the regular consumption of PCB-contaminated fish is an important source of exposure (see Section 1.4.1). The study included 182 newborns who were followed up until age 54 months. PCB levels (calculated as the sum of congeners PCB-138, PCB-153, and PCB-180, multiplied by two) were measured in breast milk and maternal serum, and maternal blood and cord blood were collected for measurement of thyroid parameters. After covariate adjustments, consistent inverse and monotonic associations were observed between total PCB exposure and the resin T3 uptake ratio, a proxy measure of the binding capacity of T4-binding globulin sites that are not saturated with T4. The resin T3 uptake ratio is high in hyperthyroidism and low in hypothyroidism. No associations with other thyroid parameters (TSH, free T3, free T4) were observed (Julvez *et al.*, 2011).

In a study in 39 healthy pregnant women in the metropolitan area of Tokyo, Japan, associations were studied between in-utero exposure to PCBs or OH-PCBs and free T4 or TSH status in newborns. The concentration of total OH-PCBs and of OH-metabolites of PCB-187 in umbilical cord tissue was significantly correlated with higher levels of free T4 in heel-prick blood samples obtained from neonates aged 4–6 days. On the other hand, the concentration of total PCBs and of the congeners PCB-118, PCB-138, PCB-153, and PCB-180 showed no relationship with free T4 and TSH levels (Otake *et al.*, 2007).

In a study in 232 healthy mother–infant pairs recruited between 2000 and 2002 in the industrialized city of Duisburg, Germany, TSH, total T4, T3, free T4 and free T3 were measured in serum of the pregnant women and in cord serum (Wilhelm *et al.*, 2008). Blood levels (n = 182) of WHO 2005 TEQ (which includes PCDD/PCDF + PCBs) were in the range of 3.8–58.4 pg/g lipid (median, 19.3 pg/g lipid). The corresponding value for human milk (n = 149) was 2.6–52.4 pg/g lipid (median, 19.7 pg/g lipid). Multiple regression analyses did not detect any effects on thyroid hormones related to WHO 2005 TEQs in blood or milk of mothers and their newborns.

In a study among Inuit women and their infants, a positive correlation was found between concentrations of OH-PCBs and total T3 in plasma of 120 women at delivery ($\beta = 0.57$; $P = 0.02$). In umbilical cord plasma of 95 newborns, PCB-153 concentrations were negatively correlated with T4-binding globulin concentrations ($\beta = -0.26$; $P = 0.01$). No associations were observed between organochlorine contaminants and thyroid hormones in blood plasma collected from infants aged 7 months (Dallaire *et al.*, 2009).

(b) Experimental systems

(i) Effects on sex hormones and their receptors

Experimental animals in vivo

Groups of pregnant Wistar WU rats received a daily oral dose of 4-OH-2,3,3′,4′,5-pentachlorobiphenyl [4-OH-PCB-109] at 0.5 or 5.0 mg/kg bw, or Aroclor 1254 at 25 mg/kg bw, on days 10–16 of gestation. The diestrous stage of the estrous cycle was significantly prolonged in 75% and 82% of female offspring exposed to 4-OH-PCB-109 at the lower and higher dose, respectively, compared with 64% of Aroclor-exposed offspring. This effect resembled a state of pseudopregnancy. Plasma estradiol concentrations in female offspring were significantly increased (50%) in the proestrous stage after exposure to 4-OH-PCB-109 at the higher dose, while no effects on estradiol were seen in rats treated with Aroclor 1254 (Meerts *et al.*, 2004).

In the offspring (age, 17 weeks) of Sprague-Dawley dams treated intragastrically with PCB-77 at a dose of 250 ng/kg bw on days 13–19 post-conception, the concentrations of follicle-stimulating hormone, luteinizing hormone, and testosterone were similar to those in the controls (Wakui *et al.*, 2012).

In-vitro assays

In an in-vitro estrogen-reporter assay with T47 human breast-cancer cells, the less chlorinated congeners (PCB-28, PCB-52, PCB-66, and PCB-74) were estrogenic, while the more highly chlorinated congeners (PCB-138, PCB-153, PCB-170, PCB-180, PCB-187, PCB-194, PCB-199, and PCB-203) acted as anti-estrogens. Co-planar PCBs had no effect on estrogen-receptor activation in this assay (Plísková *et al.*, 2005).

Less chlorinated, *ortho*-substituted, non-coplanar PCBs were weakly estrogenic in some in-vitro assays. Results in MCF-7 human breast-cancer cells were generally consistent with, but not absolute in, the requirement for *ortho*-chlorine substitution and *para*-hydroxylation for estrogenic potency (Gierthy *et al.*, 1997).

In MCF-7 human breast-cancer epithelial cells, three abundant PCBs, i.e. PCB-138, PCB-153 and PCB-180, showed pleiotropic effects on the estrogen and androgen receptors. Slightly increased cell proliferation was observed at low PCB concentrations (1–10 nM) in cells co-treated with E_2 at 0.01 nM, while the PCBs significantly inhibited cell growth at higher concentrations (1 and 10 µM). In a reporter assay (ERE-*tk*-CAT analysis), the three congeners induced a significant decrease of ER-E_2-mediated CAT activity. PCB-138 had a dose-dependent antagonistic effect on androgen-receptor activity in transiently co-transfected Chinese hamster ovary cells, with an IC_{50} of 6.2 µM. Thus the three PCBs compete with the binding of two natural hormone-receptor ligands (Bonefeld-Jørgensen *et al.*, 2001). In reporter-based assay with LNCaP human prostate-cancer cells, the congeners PCB-42, PCB-128, PCB-138 and the Aroclor mixtures 1242, 1248, 1254, and 1260, showed antagonizing effects on androgen-receptor activity (Portigal *et al.*, 2002).

The effects of PCB-77, PCB-118, PCB-126, and PCB-153 (at 0.01–20 µg/mL) on the human prostatic carcinoma cell-line LNCaP were investigated in vitro. PCB-77 and PCB-126 reduced androgen-dependent prostate-specific antigen (PSA) secretion and LNCaP cell proliferation, and inhibited 5-α-reductase activity. PCB-118 and PCB-153 had no effect on 5-α-reductase, but showed a biphasic effect on LNCaP cell proliferation, with low concentrations (0.1–1 µg/mL) causing an increase, and higher concentrations (10–20 µg/mL) a significant reduction. Likewise, PCB-118 and PCB-153 enhanced PSA secretion at low concentrations and reduced it at higher concentrations. Since induction of ethoxyresorufin-O-deethylase (EROD) and inhibition of 5-α-reductase activity were not observed, these PCBs act through an AhR- and androgen-receptor-independent mechanism. The anti-androgenic effects of the *meta*- and *para*-substituted PCB-77 and PCB-126 are more pronounced than

those of *ortho*-substituted PCB-118 and PCB-153 (Endo *et al.*, 2003).

The estrogenicity of binary mixtures of the OH-PCBs 2,4,6-trichloro-4′-biphenylol (4′-OH-PCB-30) and 2,3,4,5-tetrachloro-4′-biphenylol (4′-OH-PCB-61), was examined in the MCF-7 focus assay and a competitive estrogen-receptor binding assay. Although the individual OH-PCBs were estrogenic in both assays, there was no synergy when they were combined at various concentrations as equimolar mixtures (Arcaro *et al.*, 1998). Likewise, the estrogenic activities of these two OH-PCBs were additive when tested as equimolar mixture in several systems (MCF-7 cells, MDA-MB-231 human breast-cancer cells, mouse uterus) at high and low levels of estrogen-receptor expression, confirming the lack of a synergistic effect (Ramamoorthy *et al.*, 1997).

PCB-138, PCB-153, and PCB-180, as well as other non-*ortho*- and di-*ortho*-substituted PCBs, were shown to interfere with the function of the androgen and estrogen receptors in vitro (Schrader & Cooke, 2003; Hjelmborg *et al.*, 2006). Similarly, some OH-PCBs showed estrogenic and/or anti-estrogenic effects (Jansen *et al.*, 1993; Rasmussen *et al.*, 2003).

PCB-54 was chosen as a prototypical *ortho*-substituted PCB to test the hypothesis that *ortho* substitution in the absence of *para*- or *meta*-substituted chlorines may lead to enhanced estrogenic activity. The results indicated that PCB-54 is estrogenic both in vitro in the MCF-7 cell-focus test, and in vivo in the rat uterotropic assay (Arcaro *et al.*, 1999). The estrogenic activity of PCB-54 in MCF-7 cultures was inhibited by the estrogen-receptor antagonist LY156758. Competitive binding assays with recombinant human (rh) estrogen receptor indicated that PCB-54 does not bind to rhERalpha or rhERbeta, but the 4-hydroxylated metabolite of PCB-54 does. This metabolite was also 10-fold more estrogenic than PCB-54 in the MCF-7 focus assay, but was not detected in the medium of MCF-7 cultures exposed to PCB-54. These results suggested that the estrogenicity observed in the human breast-cancer cells and the rat uterus may be due to (i) binding of an undetected metabolite of PCB-54 to the estrogen receptor; (ii) direct binding of PCB-54 to a novel form of the estrogen receptor; or (iii) an unknown mechanism involving the estrogen receptor (Arcaro *et al.*, 1999).

Evidence that PCB-77 can act as an estrogen – with effects mediated by the estrogen receptor– was based on results from a variety of assays, including those assessing binding to the receptor in a competitive binding assay (where PCB-77 at 700-fold molar excess inhibited [^{3}H]-estradiol binding to the estrogen receptor by 50%); regulation of gene expression from a transfected exogenous (ERE-*tk*-CAT) or endogenous (*pS2*) estrogen-regulated gene; regulation of cell growth in the estrogen-dependent human breast-cancer cell lines MCF-7 and ZR-75–1; and activity in the immature mouse uterine-weight bioassay in vivo. These data demonstrated that PCB-77 mimics estrogenic action at concentrations in the nanomolar range (292 ng/L), which is comparable to concentrations of PCBs found in human tissues (Nesaretnam *et al.*, 1996).

The estrogenic effects of PCBs may be mediated in part by their hydroxylated metabolites. Both the parent compound and the OH-metabolite show low affinities for both the α- and β-isoform of the estrogen receptor, which suggests that they have only weak activity as estrogen-receptor agonist or antagonist. However, PCBs and OH-PCBs may be indirectly estrogenic by inhibiting human estrogen sulfotransferase (hEST). When 31 OH-PCBs were tested for their inhibitory effect on hEST, hydroxylation of one of the phenyl rings appeared to increase the inhibitory effect in the order *para*-OH > *meta*-OH > *ortho*-OH. Indeed, various environmentally relevant OH-PCBs (e.g. 4-OH-2,3,3′,4′,5-pentachlorobiphenyl, 4-OH-PCB-109; and 4,4′-dihydroxy-3,3′,5,5′-tetrachlorobiphenyl, 4,4′-$(OH)_2$PCB-80) are very potent inhibitors of hEST. Since sulfation by this enzyme is an

important pathway for E_2 inactivation, inhibition of this metabolic step would lead to increased bioavailability of estradiol. This would explain the indirect estrogenicity of hEST inhibitors (Kester *et al.*, 2000).

A series of twelve PCBs were investigated for their ability to bind to the uterine estrogen-receptor protein, by use of a competitive equilibrium-binding assay with enriched cytosol-receptor preparations (0–40% ammonium sulfate fraction) from uteri of ovariectomized mice. PCBs that showed strong affinities generally possessed either single or multiple *ortho*-chlorine substituents. For OH-metabolites, *ortho*-chlorine substitution on the phenolic ring seemed less effective than on the nonphenolic ring. Thus 4′-OH-2,4,6-trichlorobiphenyl (4′-OH-PCB-30), which has two *ortho* chlorines and a *para* substituent, had the strongest binding affinity. For PCBs without *ortho* chlorines, the binding activity decreased 10–100-fold. PCBs that demonstrated appreciable receptor-binding activity were also active in vivo in stimulating an increase in uterine weight, while weak binders were inactive in this respect. The *ortho*-chlorine substitution appears essential in determining receptor-binding activity, probably because of decreased conformational flexibility due to restricted rotation about the inter-ring bond (Korach *et al.*, 1988).

The effects of structure and substituent position on the estrogenic and anti-estrogenic activities of various OH-PCBs were investigated in a series of assays. The presence of an *ortho* or *meta* substitution in the phenolic ring had minimal effects on estrogenic activity, while the 2,4,6-trichloro- and 2,3,4,6-tetrachloro configuration in the non-phenolic ring were required for this response. Substitution in the phenolic ring had no effect on anti-estrogenic activity (Connor *et al.*, 1997).

In-vitro toxicity profiles were determined for 24 NDL-PCBs with respect to 10 different mechanisms of action. All NDL-PCBs antagonized androgen-receptor activation; none were androgenic. Less chlorinated NDL-PCBs (PCB-19, PCB-28, PCB-47, PCB-51, PCB-53, PCB-100, PCB-104, PCB-136) were weak estrogen-receptor agonists. More highly chlorinated NDL-PCBs (PCB-138, PCB-153, PCB-170, PCB-180, PCB-190) were weak estrogen-receptor antagonists; several inhibited estradiol-sulfotransferase activity by > 50% (PCB-28, PCB-47, PCB-51, PCB-53, PCB-100). On the basis of hierarchical analysis of the toxicity profiles, three separate clusters of NDL-PCBs and a fourth cluster of reference DL-PCBs could be distinguished. The indicators PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180 contributed most to the anti-androgenic, anti-estrogenic, anti-thyroidal, tumour-promoting, and neurotoxic potencies calculated for PCB mixtures reported in human samples, while the most potent AhR-activating DL-PCB, PCB-126, contributed at most 0.2% to any of these calculated potencies. It was suggested that PCB-168 should be added to the list of indicator congeners, given its relatively high abundance and its anti-androgenic and TTR-binding properties (Hamers *et al.*, 2011).

(ii) *Effects on the thyroid-hormone system*

Experimental animals in vivo

Marmoset monkeys were treated with oral doses of PCB-77 at 0.1, 1, or 3 mg/kg bw, twice per week, for 18–23 weeks. Histological examination of the thyroid gland showed dose-dependent hyperplasia of follicular cells, which was associated with various changes in thyroid function. The average serum concentrations of T4 during the treatment period were reduced by 35% in monkeys at 0.1 mg/kg bw, 81% at 1 mg/kg bw, and > 99% at 3 mg/kg bw. A reduction in serum concentrations of T_4 was observed from 2 weeks and throughout the entire treatment period (18–23 weeks), and was reflected in a decrease in the free T4 index in the groups at 1 and 3 mg/kg bw. Serum T3 concentrations were reduced in the group at 3 mg/kg bw within 2

weeks. Concentrations of TSH were increased in the group at the highest dose as a feedback response to the strongly reduced serum T4 concentrations (van den Berg *et al.*, 1988).

Pregnant Wistar WU rats were given Aroclor 1254 as daily oral dose at 5 or 25 mg/kg bw on days 10–16 of gestation to determine effects on thyroid-hormone concentrations in plasma and brain, on peripheral thyroid-hormone concentrations, and on peripheral thyroid-hormone metabolism in fetal and weanling rats. Maternal exposure to Aroclor 1254 significantly reduced fetal (day 20 of gestation) and neonatal (postnatal day 4) plasma concentrations of total T4 and free T4. These effects were less pronounced in offspring at age 21 days and absent at 90 days. T3 concentrations in brain tissue in the exposed fetuses were significantly decreased relative to controls, but only in the group at the lower dose. On postnatal day 21, T4 concentrations had significantly decreased in the forebrain of female weanling rats from the group at the higher dose, but no reductions were seen in male or female neonates. The deiodination of T4 to T3 was significantly increased in fetal forebrain homogenates at both doses. No alterations in thyroid-hormone metabolism were seen in forebrain homogenates from adult offspring exposed pre- and postnatally to Aroclor 1254. Accumulation of the PCB metabolite 2,3,3′,4′,5-pentachloro-4-biphenylol [4-OH-PCB-109] was observed in fetal plasma and forebrain tissue on day 20 of gestation, and in neonatal and weanling plasma on postnatal days 4, 21, and 90 (Morse *et al.*, 1996).

In groups of Sprague-Dawley rats given two or five weekly intraperitoneal injections of PCB-126 (0.2 mg/kg bw) or PCB-114 (20 mg/kg bw), total T4 concentrations in serum were lower than those in the controls. The expression of TTR was significantly higher in the PCB-treated group than in the control group (Han *et al.*, 2010).

Reduced thyroid-hormone levels were found in serum of Sprague-Dawley rats treated with $MeSO_2$ metabolites of the following PCB congeners: 3-$MeSO_2$-2,2′,3′,4′,5,6-hexachlorobiphenyl [5′-$MeSO_2$-PCB-132]; 3-$MeSO_2$-2,2′,3′,4′,5,5′-hexachlorobiphenyl [3′-$MeSO_2$-PCB-141]; 3-$MeSO_2$-2,2′,4′,5,5′,6-hexachlorobiphenyl [5-$MeSO_2$-PCB-149] and 4-$MeSO_2$-2,2′,4′,5,5′,6-hexachlorobiphenyl [4-$MeSO_2$-PCB-149]. These $MeSO_2$-PCBs are found in human milk, liver, and adipose tissue. All four metabolites (20 µmol/kg bw, intraperitoneal injection, once per day, for 4 days) reduced the serum concentration of total T4 by 22–44%, on days 2, 3, 4 and 7 after the last dose. Concentrations of total T3 were reduced by 37% on day 7 after treatment with 4-$MeSO_2$-PCB-149. A 30% increase in thyroid weight was seen after treatment with 3′-$MeSO_2$-PCB-141. These data suggest that these 3- and 4-$MeSO_2$ metabolites act as endocrine disrupters, but probably through different mechanisms (Kato *et al.*, 1998). A similar study was conducted with the *meta*-$MeSO_2$ metabolites of tetra- and pentachlorinated biphenyls: 3-$MeSO_2$-2,2′,4′,5-tetraCB [3′-$MeSO_2$-PCB-49], 3-$MeSO_2$-2,3′,4′,5-tetraCB [3-$MeSO_2$-PCB-70], 3-$MeSO_2$-2,2′,3′,4′,5-pentaCB [3′-$MeSO_2$-PCB-87], 3-$MeSO_2$-2,2′,4′,5,5′-pentaCB [3′-$MeSO_2$-PCB-101], and the *para*-$MeSO_2$-metabolite 4-$MeSO_2$-2,2′,4′,5,5′-pentaCB [4′-$MeSO_2$-PCB-101]. The data showed that all five $MeSO_2$-PCBs influence thyroid-hormone metabolism (Kato *et al.*, 1999). A further study by this group demonstrated that the *meta*-$MeSO_2$ metabolites of PCB-49, PCB-70, PCB-87, PCB-101, PCB-132, PCB-141, PCB-149 [3′-$MeSO_2$-PCB-49, 3-$MeSO_2$-PCB-70, 3′-$MeSO_2$-PCB-87, 3′-$MeSO_2$-PCB-101, 5′-$MeSO_2$-PCB-132, 3′-$MeSO_2$-PCB-141, 5-$MeSO_2$-PCB-149] and the *para*-$MeSO_2$ metabolite of PCB-101 [4′-$MeSO_2$-PCB-101] induced hepatic microsomal UDP-glucuronosyl transferase (UDP-GT) in male Sprague-Dawley rats. The increase in hepatic glucuronidation of T4 after the administration of the eight test compounds was the probable cause of the reduced serum concentration of T4 (Kato *et al.*, 2000).

Thyroid hormone status and metabolism were studied in groups of pregnant Wistar WU rats given oral doses of 4-OH-2,3,3′,4′,5-pentachlorobiphenyl [4-OH-PCB-109] ([^{14}C]-labelled or unlabelled) at 5 mg/kg bw on days 10–16 of gestation. Fetuses were studied at days 17 and 20 of gestation. The test compound accumulated in the fetal compartment, with fetal/maternal ratios of 11.0, 2.6, and 1.2 in liver, cerebellum, and plasma, respectively, at day 20. Radiolabel was bound to plasma TTR in dams and fetuses. Fetal plasma concentrations of total T4 and free T4 were significantly decreased at days 17 and 20 of gestation (89% and 41%, respectively, at day 20), while fetal concentrations of TSH were increased more than twofold at day 20 of gestation. No effects were seen on T3 concentrations in fetal brain (Meerts *et al.*, 2002).

In a study to investigate the effects of PCBs on thyroid-hormone status, female Sprague-Dawley rats were given Aroclor 1254 at a dose of 4 mg/kg bw per day by gastric intubation for 14 days. To test underlying mechanisms, microsomal enzyme activities (CYP isozymes and UDP-GT, indicating metabolic activation and/or biliary clearance), ex-vivo binding of [^{125}I]-T_4 to plasma proteins (suggesting effects on peripheral thyroid-hormone transport), and light microscope morphology of the thyroid gland were studied. The extent of thyroid-hormone reduction (free T4 to 30% and total T4 to 60% of control) observed after exposure to Aroclor 1254 corresponded with a decrease in the ex-vivo binding of [^{125}I]-T_4 to plasma TTR, and with induction of the microsomal phase-I enzymes (ethoxy- and methoxy-resorufin dealkylase, EROD and MROD). The phase-II enzyme UDP-GT was moderately elevated. The thyroid morphology showed activation of the epithelium, but no degenerative alterations correlated with exposure to Aroclor 1254. The results suggested that the decrease in T4 is mainly due to disturbed serum transport, as a result of binding of Aroclor 1254 metabolites to TTR (Hallgren & Darnerud, 2002).

Miller *et al.* (2012) studied the effects of exposure to PCBs and PBDEs on T4 levels in rat offspring from day 6 of gestation until postnatal day 21. In male rat offspring, exposure to PCBs or PBDEs at a dose of 1.7, 5, 10, 20, 40, or 60 µmol/kg bw per day induced equivalent and dose-dependent reductions in T4 from postnatal days 7 to 21. Exposure to equimolar mixtures of PCBs and PBDEs at a dose of 3.4, 10, 20, 40, or 80 µmol/kg bw per day additively reduced T4 levels during the exposure period. The effects on T4 levels were similar in males and females.

In-vivo and ex-vivo systems

The OH-PCB metabolites 4-OH-PCB-69, 4-OH-PCB-106, and 4-OH-PCB-121 were tested for capacity to disrupt the thyroid-hormone system via proliferation of thyroid hormone-dependent rat-pituitary GH3 cells. Growth of GH3 cells was stimulated by all three 4-OH-PCBs (Ghisari & Bonefeld-Jørgensen, 2005). These OH-PCBs were previously reported to bind to the thyroid receptor and to thyroid-hormone transport proteins (Cheek *et al.*, 1999).

PCBs are the most concentrated class of pollutant found in polar bears (*Ursus maritimus*). In plasma samples collected from polar bears, no binding of [^{125}I]-T4 to TTR was observed. Incubation of these plasma samples with [^{14}C]-2,3,3′,4′,5-pentachloro-4-biphenylol [[^{14}C]-4-OH-PCB-109], a PCB metabolite with a higher binding affinity to TTR than the endogenous ligand T4 itself, resulted in competitive binding. Incubation of plasma with T4 at up to 1 mM (a concentration that is not physiologically relevant) did not result in any detectable competition. These results suggested that the binding sites on TTR for T4 in wild polar bears are completely saturated (Gutleb *et al.*, 2010).

Disruption of thyroid-hormone transport may be an important mechanism by which PCBs can alter thyroid-hormone homeostasis. In a systematic in-vitro study of PCB-binding to TTR, the role of *ortho* substitution was investigated in

more detail. PCBs that have only *ortho* substitution show significant binding activity. The congeners most closely resembling the diiodophenolic ring of T4, i.e. di-*meta*-substitution in one or both rings, showed the highest binding activity to TTR. Multiple *ortho* substituents decreased the binding activity of such congeners. PCBs with a single *meta* substitution in one or both rings resemble more closely the monoiodophenolic ring of T3, and showed significantly lower binding activity to TTR. This was consistent with the relatively low binding activity of T3 and the smaller size of chlorine compared with iodine. The addition of *ortho* substituents gave variable results, depending on their position (Chauhan *et al.*, 2000).

In in-vitro studies that assessed the effect of OH-PCBs on thyroid-hormone sulfation, the inhibition of sulfotransferase activity towards 3,3′-diiodo-thyronine (T2) appeared to be similar to that towards T3. Hydroxylated metabolites of PCBs strongly inhibited T2 sulfotransferase activity, the most potent inhibitor being 3-OH-2,3′,4,4′,5-pentachlorobiphenyl (3-OH-PCB-118). An important structural requirement for inhibition of T2 sulfotransferase by OH-PCBs is the presence of a hydroxyl group in the *para* or *meta* position, with *ortho*-OH-PCBs being much weaker inhibitors (Schuur *et al.*, 1998a, b).

4.3.4 Effects on the immune system

The effects of PCBs on several parameters related to the immune system have been reported for humans, and more extensively for experimental animals (reviewed by Tryphonas & Feeley, 2001).

(a) Adults

Immunomodulatory effects of PCBs have been reported in workers occupationally exposed to these chemicals, in humans following consumption of contaminated fish, and in populations accidentally exposed to PCBs and their heat-degradation products, PCDFs, and polychlorinated quarterphenyls (PCQ) via consumption of contaminated rice oil (the Yusho and Yucheng poisoning incidents). In addition, PCB exposure during prenatal and early life has been associated with incidence of infectious and allergic diseases in children, and alterations in immune-system development.

Lawton *et al.* (1985) tested 194 workers exposed occupationally (152 men, 42 women) to one or more of the Aroclors 1254, 1242, and 1016 in a capacitor plant factory for an average duration of 17 years. The results taken in 1976 were compared with those from the same workers taken in 1979, two years after discontinuation of all PCB use in 1977. Significantly increased levels of leukocytes, with a concomitant increase in levels of lymphocytes, monocytes and eosinophils, were observed when these workers were tested in 1976. Interestingly, the levels of circulating polymorphonuclear cells were reduced in the same workers. Similar, but not statistically significant, shifts in leukocyte levels were noted when testing was repeated in 1979. A positive association was observed between serum PCB concentrations and blood monocytes, and was reported to persist even 2 years after discontinuation of PCB use. [The Working Group noted that the extent to which PCB exposure compromises the immune system could not be estimated on the basis of immune-cell alterations, since measurement of functional immune parameters was not part of the study protocol.]

In contrast, a study by Emmett *et al.* (1988a, b) of 55 transformer repairmen working in a factory and exposed to Aroclors 1260 and 1242 did not report any significant exposure-related effects on the immune system. The percentage of workers with positive skin responses (delayed-type hypersensitivity) to mumps and trichophyton antigens was similar to that of 56 nonexposed workers.

Follow-up studies of the Yusho and Yucheng populations indicated that several immune-related parameters were disrupted in exposed

adults. These included a statistically significant decrease in serum levels of immunoglobulins A and M, reduced T-helper (Th) and increased T-suppressor cells (Ts) resulting in reduced Th:Ts cell ratio, persistent respiratory distress caused by Gram-negative bacilli-infected airways, and increased in-vitro lymphoproliferative responses of peripheral blood leukocytes to phytohaemaglutinin, concanavalin A, and pokeweed mitogens at 1 and 3 years after exposure. Furthermore, a reduced number of patients with positive skin-test reactivity to streptokinase/streptodornase antigens was observed at 1 year after exposure, and to tuberculin antigens at up to 4 years after exposure (Lü & Wu, 1985; Nakanishi *et al.*, 1985), while some other immunological effects persisted up to 30 years after exposure (Masuda, 2001).

Consumption of contaminated fish has been associated with some effects on the immune system. High consumption of fatty fish from the Baltic Sea correlated positively with B-cell numbers, but negatively with the percentage of cytotoxic ($CD8^+$) T-cells in 68 fishermen in Latvia (Hagmar *et al.*, 1995). [The significance of these observations was not clear, since no functional immune parameters were examined.]

Svensson *et al.* (1994) studied levels of leukocytes in a group of 23 men in Sweden who consumed high levels of fatty fish species from the Baltic Sea and compared results with 20 men who ate practically no fish. No effects were reported on leukocyte counts, the number of total lymphocytes or their subsets, or serum immunoglobulin levels. A marginal reduction in natural killer (NK) cell activity was reported for the fish-eating population. This was in agreement with the weakly negative correlation observed between NK cell numbers and blood concentrations of PCB-126 and PCB-118 in some of the same subjects tested 3 years previously.

(b) Children

Weisglas-Kuperus *et al.* (1995) studied children residing in the Netherlands and who were exposed, in utero and via breastfeeding, to ambient concentrations of PCBs. The study group consisted of 207 healthy mother–infant pairs. Prenatal exposure to PCBs was estimated by the sum of PCB-118, PCB-138, PCB-153, and PCB-180 (ΣPCB) in maternal and cord plasma, and in breastfed infants by the TEQ levels (based on 17 dioxins and 8 dioxin-like PCBs) in human milk. A higher prenatal PCB/dioxin exposure was associated with increased numbers of T lymphocytes bearing T-cell receptors of the gamma/delta type, increased cytotoxic T-cells at age 18 months in breastfed infants; higher prenatal and postnatal concentrations of PCB/dioxin was associated with reduced monocytes and granulocytes at age 3 months. In follow-up studies, statistically significant associations were observed between prenatal PCB exposure and increased number of lymphocytes, T-cells, and cytotoxic ($CD3^+CD8^+$) cells, memory ($CD4^+$ $CD45RO^+$) cells, T-cell receptor (TcR) $\alpha\beta^+$, and activated T-cell ($CD3^+$ HLA-DR^+) numbers in the toddlers.

Horváthová *et al.* (2011a, b) collected blood specimens from newborns, and infants aged 6 and 16 months, from two districts in Slovakia, Michalovce and Svidnik/Stropkov, that had respectively high and low environmental PCB contamination, and correlated blood PCB concentrations with lymphocyte-receptor expression. The percentages of lymphoid dendritic cells and naive/resting T lymphocytes were significantly increased at 6 months in the Michalovce area compared with those in cord blood samples ($P < 0.001$). Overall there was a positive correlation of terminally differentiated effector memory T-lymphocyte population with age, and a negative linear correlation for myeloid dendritic cells from birth to 6 months in both regions. The Michalovce samples indicated

significantly higher expression of memory T lymphocytes (at birth, 6, and 16 months), terminally differentiated effector memory T lymphocytes (at birth and at 6 months), and lymphoid dendritic cells (at 6 months) than in samples from Svidnik/Stropkov.

Jusko *et al.* (2012) investigated the effect of several PCB congeners on thymus volume in 1134 mother–infant pairs residing in eastern Slovakia. Samples of maternal and infant (age 6 and 16 months) blood were collected and analysed for 15 PCB congeners. Higher maternal concentrations of PCBs were associated with reduced thymus volume at birth, while maternal PCB concentration was not predictive of thymus volume in the infants aged 6 and 16 months.

In a subgroup of 331 children aged 7–10 years from the Hesse, Germany cohort, mean concentrations of PCBs were 0.50 µg/L, and this value was significantly associated with increased levels of serum immunoglobulin M (IgM) (Karmaus *et al.*, 2005).

Similar immune-related sensitivities in adolescence were reported by Van Den Heuvel *et al.* (2002) for a study in Flanders, Belgium. In this study, serum concentrations of PCB-138, PCB-153 and PCB-180, and combined serum dioxin-like activity as determined by AhR-mediated expression of a reporter gene luciferase, were measured in samples from boys and girls (aged 17–18 years) with certain immune-related respiratory complaints. A significantly negative correlation between the percentage of eosinophils and NK cells in peripheral blood and TEQ in serum ($P = 0.009$ and $P = 0.05$, respectively) was observed. Similarly, significant negative correlations were calculated between serum TEQs and levels of specific IgE antibodies to allergens (cat dander, house dust mite, and grass pollen), and the incidence of reported allergies of the upper airways. A significant positive correlation was observed between increased serum TEQs and increased serum IgA levels ($P = 0.05$).

(c) Non-human primates

Unlike all other experimental animal models in which exposure levels were high, the available studies in non-human primates used PCB doses that were relatively low (< 1 mg). Such studies have shown that non-human primates are more sensitive to the immune-related effects of PCBs than any other experimental animal tested. Alterations in the immune system and immunotoxicity were also reported after PCB exposure during prenatal or early life.

Thomas & Hinsdill (1978) investigated immunological parameters in groups of eight rhesus monkeys fed diets containing Aroclor 1248 at a dose of 0.1 or 0.2 mg/kg bw per day for 11 months. The reported immune-related effects were seen only at 0.2 mg/kg bw and included significantly reduced titres of antibodies to sheep red blood cells (SRBC) at weeks 1 and 12 after primary immunization, and decreased percentage of gamma-globulin after 20 weeks, compared with a control group of five monkeys. The response to tetanus toxoid was not affected by treatment. Reduced titres to SRBC were also reported in the single female cynomolgus monkey *(Macaca fascicularis)* treated with a PCB mixture with constituents similar to those ingested by Yusho patients, and containing predominantly penta- and hexachlorobiphenyls and no PCDFs, prepared from Kanechlor 400 and administered at 5 mg per day for 20 weeks (Hori *et al.*, 1982).

Differences in PCB-induced toxicity were investigated in cynomolgus *(Macaca fascicularis)* and rhesus *(Macaca mulatta)* monkeys (Tryphonas *et al.*, 1986; Arnold *et al.*, 1990). In these studies, groups of four cynomolgus and four rhesus monkeys ingested Aroclor 1254 in apple juice-gelatin-corn oil emulsion at doses of 0.00 (control) or 280 µg/kg bw per day for 12–13 months (cynomolgus monkeys) and 27–28 months (rhesus monkeys) respectively. The total serum IgM levels and titres to anti-SRBC (primary response) antigens were significantly

reduced in both species. Based on clinical and pathological findings, the rhesus monkeys were more sensitive to PCB-induced toxicities than the cynomolgus monkeys, although effects on the immune system were similar in both species.

A long-term study with Aroclor 1254 (Tryphonas *et al.*, 1989, 1991a, b; Arnold *et al.*, 1993, 1995) was of particular significance since it was the only long-term study in which low doses (range, 5–80 µg/kg bw per day) of commercial PCB mixtures were used. Immunological effects were reported after 23–25 months (phase I) (Tryphonas *et al.*, 1989), during which time a blood PCB pharmacokinetic equilibrium was established, and after 55 months (phase II) (Tryphonas *et al.*, 1991a, b). Testing at phase I detected significant shifts in Th and Ts lymphocyte subsets (decreased Th, increased Ts and decreased Th:Ts cell ratio) at 80 µg/kg bw per day, and significantly reduced titres in response to SRBC antigens (Tryphonas *et al.*,1989). The response to SRBC antigens was significantly reduced even at a dose of 5 µg/kg bw per day. These effects in monkeys were comparable to those reported for the Yucheng population at 1 and 3 years after exposure (Lü & Wu, 1985). Several significant immune-related parameters were affected in monkeys exposed continuously to Aroclor 1254 for 55 months (phase II). Effects included: a dose-related decrease in the anamnestic (IgM and IgG) response to SRBC antigens; a dose-related decrease in the lymphoproliferative response of leukocytes to the mitogens concanavalin A and phytohaemaglutinin, but not to pokeweed mitogen (mostly B-cell dependent); reduced monocyte activity (peak chemiluminescence after phorbol myristate acetate activation); significantly higher levels of serum complement (CH_{50}) activity across all treated groups compared with controls; a dose-related significant increase in thymosin α1 ($T\alpha_1$) levels in treated groups compared with controls; a significant but not dose-related increase in levels of interferon at the 20 and 80 µg/kg bw per day, with a significantly reduced interferon level at 40 µg/kg bw per day. Tumour necrosis factor (TNF) levels were not affected significantly by treatment (Tryphonas *et al.*, 1991a, b).

Hand-reared infant rhesus (*Macaca mulatta*) monkeys (age, 66 weeks) were treated with a mixture of PCB congeners at a dose of 7.5 µg/kg bw per day, which represents the approximate daily intake of a nursing infant whose mother's breast milk contained PCBs at a concentration of 50 ppb. The PCB congeners used for treatment were those commonly found in human breast milk in Canada. Treatment continued until the monkeys reached age 20 weeks. Significant treatment-related effects characterized by reduced antibody responses to SRBC antigens, and reduced levels of the HLA-DR cell surface marker were observed (Arnold *et al.*, 1999).

Groups of eight adult female rhesus monkeys were fed diets containing Aroclor 1248 at a concentration of 2.5 or 5.0 ppm for approximately 1.5 years (Allen & Barsotti, 1976). Six of the eight monkeys treated with Aroclor 1248 at 5.0 ppm, and all monkeys at 2.5 ppm were successfully bred after 6 months of exposure. There was one live infant born among monkeys at 5.0 ppm, and five infants born to monkeys at 2.5 ppm. Infants were permitted to nurse with their mothers. Three infants died within 8 months, after 44, 112 and 239 days, respectively. At necropsy, histopathological observations of the infant tissues included a near complete absence of thymocytes in the cortical and medullary areas of the thymus, extremely small lymph nodules of the spleen with inapparent germinal centres, and hypocellularity of the bone marrow.

(d) Rodents and rabbits

(i) Effects on the thymus

Commercial PCB mixtures

Thymic atrophy was detected in female White New Zealand rabbits fed diets containing Aroclor 1260 at a dose of 118 mg/kg bw per

day for 38 days, or Aroclor 1260 at a dose of 120 mg/kg bw per day for 28 days (Vos &Beems, 1971; Vos & Notenboom-Ram, 1972); in male White New Zealand rabbits fed Aroclor 1254 at a dietary concentration of 20, 45.8, or 170 ppm [0.92, 2.10 or 6.54 mg/kg bw per day] for 56 days (Street & Sharma, 1975); in male Fischer 344 rats given Aroclor 1254 at a dose of 10 or 25 mg/kg bw per day by gavage for 15 weeks (Smialowicz *et al.*, 1989); in female guinea-pigs fed Clophen A60 at a dietary concentration of 50 ppm for 49 days (Vos & van Driel-Grootenhuis, 1972) and in male Sprague-Dawley rats fed Aroclor 1262, 1254, or 1248 at 1% of the diet for 6 weeks. The severity of thymic atrophy was Aroclor 1254 = Aroclor 1248 > Aroclor 1262 (Allen & Abrahamson, 1973).

Thymic atrophy was not detected upon exposure to Aroclor 1248 when fed to female outbred albino mice (50, 100, 500 or 1000 ppm) for 3 to 5 weeks (Thomas & Hinsdill, 1978), or to Aroclor 1242 (167 ppm) fed to Balb/c mice (Loose *et al.*, 1979).

PCB congeners

Thymic atrophy characterized by reductions in cortical and medullary volume was also reported in weanling male and female Sprague-Dawley rats treated with feed containing individual PCB congeners for 13 weeks at the following concentrations: PCB-126, 0.1–100 ppb (0.01–7.4 μg/kg bw per day) (Chu *et al.*, 1994); PCB-153, 0.05–50 ppm (3.6–3534 μg/kg bw per day) (Chu *et al.*, 1996b); PCB-28, 0.05–50 ppm (2.8–3783 μg/kg bw per day) (Chu *et al.*, 1996c); and PCB-105, 0.05–50 ppm (3.9–4327 μg/kg bw per day) (Chu *et al.*, 1998). In contrast, PCB-77, PCB-118, and PCB-128 did not have any significant effects on the thymus when fed to weanling male and female Sprague-Dawley rats for 13 weeks at the following concentrations: PCB-77: 0.01–10 ppm (0.73–768 μg/kg bw per day) in males; 0.01–10 ppm (0.92–892 μg/kg bw per day) in females (Chu *et al.*, 1995); PCB-118: 0.01–10 ppm (0.66–683 μg/kg bw per day) in males; 0.002–2 ppm (0.17–170 μg/kg bw per day) in females; PCB-128: 0.05–50 ppm (4.5–4397 μg/kg bw per day) (Lecavalier *et al.*, 1997).

In male C57BL/6 (Ah+) and DBA/2 (Ah-) mice given intraperitoneal doses of PCB-77 (DL-PCB) or PCB-52 (NDL-PCB) at 0, 10, or 100 mg/kg bw per day, thymic atrophy was observed only in C57BL/6 mice treated with PCB-77 (Silkworth & Grabstein, 1982). The results suggested that PCB immunotoxicity in mice is mediated through the AhR, present only in the C57BL/6 mice.

(ii) Effects on humoral immunity

Commercial PCB mixtures

Several studies reported effects of PCBs on humoral immune reactivity. A significant reduction in production of antibodies to tetanus toxoid was noted in guinea-pigs fed Clophen A60 (Vos & van Driel-Grootenhuis, 1972), to keyhole limpet haemocyanin (KLH) in rats fed Aroclor 1254 (Exon *et al.*, 1985), and to SRBC using the plaque-forming cell assay in mice given Aroclor 1254 intraperitoneally (Wierda *et al.*, 1981; Loose *et al.*, 1979). Mice genetically engineered to be either aryl hydrocarbon-responsive (Ah^b/Ah^b) or non-responsive (Ah^d/Ah^d) did not exhibit the same sensitivity to PCB-induced suppression in the plaque-forming cell assay. For example, C57BL/6N (Ah^b/Ah^b) mice injected intraperitoneally with Aroclor 1254 at a dose of 250–750 mg/kg bw exhibited significant reductions in plaque-forming cell numbers after 5 days, compared with controls, while DBA/2N (Ah^d/Ah^d) mice failed to demonstrate any significant PCB-induced effects on plaque-forming cell numbers, compared with controls (Lubet *et al.*, 1986).

PCB congeners

Cotreatment of C57BL/6 B6 mice with PCB-153 and TCDD showed that PCB-153 partially antagonized TCDD-mediated immunotoxicity in various assays (Biegel *et al.*, 1989).

Individual congeners were also assessed for their immunotoxicity in AhR-responsive or AhR-non-responsive mouse models. Bandiera *et al.* (1982) reported that PCB-77 binds AhR with high affinity and causes severe suppression of the humoral antibody response in C57BL/6 B6 (Ah^b/Ah^b) mice. In comparison, PCB-77 exhibited lower binding affinity for AhR in DBA/2N (Ah^d/Ah^d) mice and did not cause any immune-related effects (Silkworth & Grabstein, 1982). In contrast, the di-*ortho*-substituted PCB-52 had weak AhR binding affinity and was not immunosuppressive in either mouse strain (Silkworth & Grabstein, 1982).

(iii) Effects on cellular immunity

The effects of PCBs were less pronounced on cellular immune responses than on humoral immune reactivity. Reduced skin reactivity to tuberculin was detected in female guinea-pigs fed Clophen A60 at 50 or 250 ppm for 49 days (Vos & van Driel-Grootenhuis, 1972; Vos & Van Genderen, 1973). In contrast, no effects were detected when dinitrochlorobenzene was used as the skin sensitizer in female Swiss-Webster mice fed Aroclor 1254 at 10, 100, or 250 ppm [1.17, 116, 292 mg/kg bw per week] for 12 weeks (Talcott & Koller, 1983). Similarly, White New Zealand male rabbits fed Aroclor 1254 at 170 ppm [6.54 mg/kg bw per day] for 56 days did not show any effects on skin reactivity to tuberculin sensitization (Street & Sharma, 1975).

Studies on the mitogen-induced proliferative activity of splenic mononuclear leukocytes and the mixed lymphocyte response, both in-vitro correlates of cellular immune responses, also gave conflicting results and suggested that PCBs may affect a specific subpopulation of T lymphocytes. A few studies reported that phytohaemagglutinin-induced leukocyte blastogenic activity was increased upon exposure to Aroclors, while no effect was noted when concanavalin A, *S. typhimurium*, or pokeweed mitogens were used (Bonnyns & Bastomsky, 1976; Wierda *et al.*, 1981; Smialowicz *et al.*, 1989). The mixed lymphocyte response was not affected by treatment with the less chlorinated Aroclor 1242 (Carter & Clancy, 1980; reviewed by Silkworth & Loose, 1981).

Nakanishi *et al.* (1995) treated female Sprague-Dawley rats (age, 8 weeks) intraperitoneally with 5 mg of Kanechlor 400 in 2 mL of corn oil, and effects on the immune system were examined at termination of the study 4 weeks later. The percentage of T lymphocytes, and T-helper and T-suppressor cells, was significantly decreased in the treated groups compared with the controls. In contrast, the percentage of T lymphocytes in the bronchoalveolar lavage fluid was not significantly increased after treatment with Kanechlor 400. The percentage of T-suppressor cells increased significantly, while the percentage of T-helper cells was not affected by treatment. Release of O_2^- by alveolar macrophages, stimulated with either wheat germ lectin or phorbol myristate acetate, increased significantly compared with the controls (Martin *et al.*, 1981). In addition, there was mild inflammation of the alveoli after administration of PCBs. In support of this observation, Kikuchi *et al.* (1971) reported that lung autopsies for two Yusho patients showed the presence of pulmonary haemorrhage and pulmonary oedema. [It is conceivable that failure to remove O_2^- produced by macrophages might be responsible for the observed pathogenesis of interstitial changes of the lung after treatment with PCBs].

(iv) Effects on innate (non-specific) immunity

The cellular components of innate immunity, including phagocytic cells (neutrophils, macrophages) and NK cells, are targets of PCB-induced immunotoxicity. Functional impairment of these cells is characterized by reduced phagocytic activity and consequently diminished ability to eliminate pathogenic infections in PCB-exposed experimental animals, as well as compromised immunosurveillance mechanisms.

Male ICR mice fed diets containing Kanechlor 500 at 400, 200, or 100 µg per gram feed showed increased susceptibility to herpes simplex virus compared with control mice (Imanishi *et al.*, 1980). Likewise, Koller (1977) demonstrated that Balb/c male mice fed diets containing Aroclor 1242 at 375 ppm for 6 months showed a significant increase in susceptibility to Moloney leukaemia virus; this effect was not seen with Aroclor 1221. In Balb/c male mice given feed containing Aroclor 1242 at 167 ppm for 6 weeks, there was significantly increased susceptibility to *S. typhosa* endotoxin and to malaria parasite *Plasmodium berghei* (Loose *et al.*, 1979). Reduced clearance of *Listeria monocytogenes* was observed in adult and neonate male and female ICR mice given Aroclor 1254 at 75 mg/kg bw per day by gavage for 14 days (Smith *et al.*, 1978). Thomas & Hinsdill (1980) reported increased sensitivity to endotoxin challenge in outbred, female albino mice fed Aroclor 1248 at 100 ppm, but no effect on resistance to *S. typhimurium* in mice fed Aroclor 1248 at 1000 ppm.

NK-cell activity was reported to be decreased in male Fischer 344 rats exposed daily to Aroclor 1254 at 10 or 25 mg/kg bw by gastric intubation for up to 15 weeks (Smialowicz *et al.*, 1989), and in male Sprague-Dawley rats fed Aroclor 1254 at 50 or 500 ppm for 10 weeks (Talcott *et al.*, 1985; Exon *et al.*, 1985).

Paradoxically, despite the evidence that PCB-induced immunosuppression impairs immune surveillance, Aroclor 1254 protected mice and rats against certain kinds of experimentally induced tumours, such as Ehrlich's tumour ascites (Keck, 1981) and primary Walker 256 tumour (Kerkvliet & Kimeldorf, 1977).

(e) Fish and marine mammals

As top predators, marine mammals and large fish bioaccumulate PCBs at high concentrations in fat. Several studies have reported on the immunotoxic effects of PCBs on fish and marine mammals in contaminated environments (Mahy *et al.*, 1988; Osterhaus & Vedder, 1988; Cleland *et al.*, 1989; Dietz *et al.*, 1989; Visser *et al.*, 1993; De Swart *et al.*, 1994; Ross *et al.*, 1995, 1996; Hammond *et al.*, 2005; Iwanowicz *et al.*, 2009; Frouin *et al.*, 2010; Duffy-Whritenour *et al.*, 2010).

4.3.5 Effects on inflammatory response

Several in-vivo and in-vitro studies have investigated the role of PCBs in the development of inflammatory responses, and are reviewed in the following section. Pertinent to this review are the following questions: (i) is the observed inflammation in PCB-treated animals directly related to PCB exposure, or is it a secondary development following PCB-induced toxicity in target organs; and (ii) does inflammation play an active role in the development of cancer after PCB exposure?

(a) Humans

No studies defining an association between exposure to PCBs and the development of inflammation in relation to cancer in humans were available to the Working Group.

(b) Experimental animals in vivo

(i) Commercial PCB mixtures

Tryphonas *et al.* (1984) reported significant changes indicative of an ongoing inflammatory response in the liver of cynomolgus monkeys *(Macaca fascicularis)* treated with Aroclor 1254 or Aroclor 1248. These changes included "ground glass" appearance of the cytoplasm and pyknosis of the nuclei with or without neutrophil infiltration, eosinophilic necrosis of single or clusters of hepatocytes often with neutrophilic infiltration or collapse of the connective tissue framework, and moderate, diffuse sinusoidal fibrosis and hypercellularity, and were associated with PCB-induced necrosis of the liver.

Interstitial inflammation of the liver was also observed in cynomolgus monkeys fed with

P-KC-400 (Kanechlor 400 from which PCDFs had been removed, largely containing tri- and tetrachlorobiphenyls), or PY-PCB (a PCB mixture with constituents similar to those ingested by Yusho patients, and largely containing penta- and hexachlorobiphenyls and no PCDF) at 5 mg per day, for 20 weeks (Hori *et al.*, 1982).

(ii) PCB congeners

Inflammatory responses, presumably secondary to PCB-induced toxic effects, have been reported in long-term studies of carcinogenicity in rats treated with PCB-126 (NTP, 2006a), PCB-153 (NTP, 2006b), PCB-126 + PCB-153 (NTP, 2006c, varying ratios study), PCB-126 + PCB-118 (NTP, 2006d), and PCB-118 (NTP, 2010). The incidence and severity of inflammation in the treated groups varied according to the congener administered. For PCB-118 and PCB-126, the incidence of inflammation and degree of severity were significantly increased in core groups receiving the three higher doses than in the controls, while for PCB-153, the incidence in the core groups was only slightly increased compared with the controls and was not dose-dependent. In addition to the core groups, inflammation, albeit of low incidence and intensity, was also observed in the control groups in the studies with PCB-118 and PCB-126, and in the uterus of rats in the PCB-153 control group, but not in the ovary of rats in the same group.

Sipka *et al.* (2008) investigated the potential for various PCB congeners to induce inflammation in mice. Mice were given a single gavage dose (150 µmol/kg bw) of PCB-77, PCB-104, or PCB-153. The levels of specific inflammatory mediators including intercellular adhesion molecules (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) mRNA and monocyte chemoattractant protein-1 mRNA (MCP-1) were determined in the liver, lung, and brain. All three PCB congeners activated inflammatory mediators, and the organs affected varied according to the congener used. PCB-77 and PCB-104 caused induction of all three inflammatory mediators in the liver and lungs, but not in the brain. In contrast, the effects of PCB-153 varied across mediators and were predominantly seen in the lung and brain. Concentrations of PCB-153 were higher in the lung and brain than in the liver, and PCB-153 was the only PCB to be detected in the brain (Sipka *et al.*, 2008). These observations suggested that the observed differences in target organ for the effects on inflammatory mediators were due to differences in PCB-congener accumulation in the organs affected.

In another study, a single dose of PCB-77 resulted in increased expression of VCAM-1 only in the wildtype (AhR-positive) mice, and not in mice lacking the AhR gene (Hennig *et al.*, 2002b).

Sipos *et al.* (2012) suggested that exposure to environmental toxicants including PCBs may cause vascular inflammation that facilitates the development of brain metastases. The crucial event in metastasis is adhesion of blood-borne tumour cells to the vascular endothelium, followed by transcapillary migration. In wild-type or ICAM-1-deficient mice injected with Lewis lung carcinoma cells via the carotid artery, oral pretreatment with PCB-118 enhanced development of brain metastases by inducing overexpression of ICAM-1 (also designated as CD54) and VCAM-1 in the brain endothelium (Sipos *et al.*, 2012).

(c) In-vitro studies

In-vitro studies by Narayanan *et al.* (1998) indicated that Aroclor 1242 and PCB-47 (a major constituent of Aroclor 1242) impaired the oxidative burst (respiratory burst) in human neutrophils by inhibiting the antioxidant enzyme superoxide dismutase, which converts O_2^- to H_2O_2. Pre-incubation of neutrophils with Aroclor 1242 or PCB-47 before stimulation with phorbol 12-myristate 13-acetate, elevated the respiratory burst, and resulted in a significant increase in intracellular O_2^- production and a significant

decrease in H_2O_2 compared with that in unexposed but agonist-stimulated neutrophils.

Additional in-vitro studies indicated that non-coplanar PCBs stimulate neutrophil production of superoxide anions (O_2^-) by a mechanism that is structure-specific and dependent on the chlorine substitution pattern of the biphenyl rings. On the contrary, coplanar congeners with high affinity for AhR do not activate neutrophils to produce superoxide anions and may inhibit this response (Brown *et al.*, 1998). In these studies, neutrophils were isolated from male Sprague-Dawley rats and exposed to specific PCB congeners at 0 (vehicle), 10, or 50 μM for 30 minutes at 37 °C, before stimulation with phorbol 12-myristate 13-acetate at 0 or 20 ng/mL. PCB-4, PCB-8, or PCB-11 (50 μM) stimulated neutrophils to produce O_2^-. Incubation of neutrophils with PCB-15, PCB-126, PCB-127, or PCB-128 did not result in generation of O_2^-. Of the various congeners tested, PCB-8 elicited the highest production of superoxide anions.

Exposure to PCB-4, PCB-8, PCB-11, or PCB-128 before addition of phorbol myristate acetate caused a significant increase in the amount of O_2^- produced that was greater than that seen with either compound alone. Phorbol myristate acetate-stimulated production of O_2^- was unaffected by prior exposure to PCB-15, PCB-126, or PCB-127. In separate experiments, PCB-126 inhibited the amount of O_2^- produced in response to activation with either PCB-4 or PCB-11. From these results it appeared that non-coplanar congeners are capable of stimulating neutrophil production of O_2^-. Coplanar congeners with a high affinity for AhR do not activate neutrophils to produce O_2^- and may inhibit this response.

Kwon *et al.* (2002) investigated the effects of PCB-153 on the expression of cyclooxygenase-2 (COX-2) and pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α in a human leukaemic mast cell line. The expression of TNF-α and IL-1β mRNA was not dependent on PCB-153, while the expression of COX-2 and IL-6 mRNA was highly induced by PCB-153. Pre-treatment with pyrrolidine dithiocarbamate, an NF-κB-pathway inhibitor, suppressed induction of COX-2, TNF-α and IL-1β, and reduced the induction of IL-6 mRNA by PCB-153.

The effects of PCBs on the activation of human granulocytes were investigated by Voie *et al.* (1998). Respiratory burst activity was measured as luminol-amplified chemoluminescence in human granulocytes. *Ortho*-substituted PCB congeners (PCB-47 and PCB-4) stimulated chemoluminescence in a concentration-dependent manner (ED_{50}, approximately 10 μM), while *meta*- and *para*-substituted congeners had no significant effect. Furthermore, using several enzyme-specific inhibitors, it was shown that PCB-activated chemiluminescence was dependent on Ca^{++}-dependent phospholipase D or phospholipase C, phosphatidylinositol 3-kinase, and protein kinase C activation before activation of the NADPH oxidase.

In an early experiment, porcine pulmonary artery-derived endothelial cells were incubated for up to 24 hours with PCB-77, PCB-114, or PCB-153, which were selected for their varying binding avidities to AhR and different capacities to induce CYP (Toborek *et al.*, 1995). PCB-77 and PCB-114 significantly disrupted endothelial barrier function in a dose-dependent manner by allowing an increase in albumin transfer across endothelial monolayers. PCB-77 and PCB-114 also enhanced oxidative stress (increasing levels of 2,7-dichlorofluorescein fluorescence, lipid hydroperoxides, and intracellular calcium) and caused increased activity and level of CYP 1A, and decreased levels of vitamin E in the culture medium. In contrast, incubation of endothelial cells with the non-dioxin-like PCB-153 did not have any effect on cellular oxidation, intracellular calcium levels, or on endothelial barrier function.

Additional in-vitro experiments (Hennig *et al.*, 1999; 2002a, 2002b) further suggested

that PCBs are atherogenic, exerting their effect by disrupting normal cellular functions of the vascular endothelium, and confirmed that oxidative stress and activation of the CYP1A subfamily may play a role in the events that lead to atherogenicity.

Treatment of porcine endothelial cells with the DL-PCBs PCB-77, PCB-126, or PCB-169 resulted in increases in expression of the *CYP1A1* gene, oxidative stress, and the DNA-binding activity of NF-κB in a concentration-dependent manner. PCB-126 elicited a maximal response at the lowest concentration (0.5 μM) tested. In addition, all three coplanar PCBs increased endothelial production of IL-6. The expression of adhesion molecule VCAM-1 by endothelial cells was highest at 3.4 μM PCB-77 or PCB-169 (Hennig *et al.*, 2002b).

When human umbilical vein endothelial cells (HUVEC) were treated with PCB-104, a non-coplanar congener, PCB-104 increased the oxidative stress and markedly upregulated the expression of monocyte chemoattractant protein-1 (MCP-1), and the adhesion molecules E-selectin, and ICAM-1, at both the mRNA and protein levels, in a time and concentration-dependent manner. Furthermore, PCB-104 stimulated the adhesion of THP-1 cells (a human acute monocytic leukaemia cell line) to endothelial cell monolayers (Choi *et al.*, 2003).

4.3.6 Quantitative structure–activity relationships (QSAR)

Based on their structure–activity characteristics, PCB congeners are generally grouped as dioxin-like and non-dioxin-like (see Section 1.1.1):

- DL-PCBs are *meta-/para*-chloro-substituted PCBs and include PCB-77, PCB-126, PCB-169 and their mono-*ortho*-chlorinated derivatives. These congeners can adopt a coplanar structure and display avid binding to AhR (avidity to AhR diminishes with *ortho*-chloro-substitution). AhR activation leads to a multitude of biological and toxic manifestations, referred to as "dioxin-like activity".
- NDL-PCBs are *ortho/para*-substituted PCBs. *Ortho/para*-substitution (at least two chlorines in *ortho* positions) is associated with the capacity to induce CAR/PXR-dependent gene expression (e.g. CYP2B, CYP3A isoenzymes). CAR agonists have substitutions in *ortho*, *para* with or without *meta* substitution, while PXR agonists and ryanodine agonists have multiple *ortho* positions substituted with chlorines.
- Some PCB congeners do not elicit activation of AhR, CAR, or PXR.

PCB congeners can also be grouped as lower- and higher-chlorinated congeners. The number of chlorine substituents is linked to persistency and bioaccumulation in animals and humans; less chlorinated congeners are typically volatile and metabolically active, and may produce ROS and genotoxic insults (see Section 4.2).

Additionally, a specific configuration may show activity in a specific bioassay, e.g. for endocrine effects (especially modulation of steroid and thyroid nuclear receptors), neurotoxic activities (release of a neurotransmitter, calcium homeostasis), and/or events associated with tumour promotion (e.g. inhibition of GJIC) (see Section 4.3.2).

The TEQ concept used for risk assessement of PCBs is based on AhR-mediated toxicity of DL-PCBs (see Section 4.3.1). In contrast, the toxicity profiles of NDL-PCBs are insufficiently characterized.

Defining key structural toxicity determinants of individual congeners modulating CAR-, PXR-, androgen receptor-, estrogen receptor-, and other receptor-dependent gene expression is not easy; with the exception of AhR, androgen receptor, and estrogen receptor, there were no systematic studies comparing a large series of PCB congeners in a receptor-based bioassay.

Only a few specific QSAR studies addressing carcinogenicity of PCBs have been published. Ruiz *et al.* (2008) attempted to predict mutagenicity and carcinogenicity of all 209 PCB congeners and some oxidative metabolites using experimental data on DNA-adduct formation, on GJIC-inhibition potency, and National Toxicology Program (NTP) rodent carcinogenicity bioassays. Interestingly, a positive mutagenicity activity was predicted for the less chlorinated PCBs and their hydroxy- and benzoquinone metabolites. Carcinogenicity of many di- to hexachlorinated PCBs was predicted by the QSAR based on NTP carcinogenicity studies in mice, while no carcinogenicity was predicted for tested congeners in the analysis for rats. [A significant drawback was that carcinogenicity predictions were not applicable for the highly abundant, higher-chlorinated congeners PCB-153, PCB-170 and PCB-180 (predicted values were outside the optimum prediction space). Therefore QSAR analyses of carcinogenicity of PCB congeners were inadequate, especially when regarding possible extrapolation to hazards in humans.]

An alternative and more complex approach was reported recently by Stenberg *et al.* (2011). Multivariate toxicity profiles and QSAR modelling of NDL-PCBs were used, based on a variety of molecular descriptors. The toxicity profiles of 24 selected PCBs were identified by in-vitro screening; the different mechanisms of action, which were mostly related to endocrine disruption and neurotoxicity, also included tumour promotion. NDL-PCBs were highly purified, to exclude any contaminating dioxin-like compounds before testing (Hamers *et al.*, 2011). QSAR analysis included also several parameters relevant to carcinogenicity, such as ROS production and inhibition of GJIC. Principal component analysis was used to derive general toxicity profiles from experimental in-vitro data, and individual QSAR models were calculated for each in-vitro response using a set of 67 chemical descriptors. It was shown that PCBs could be divided into at least three major clusters; the DL-PCBs, and two separate NDL-PCB clusters with similar toxicity profiles. The first NDL-PCB cluster included mainly less-chlorinated, *ortho*-substituted congeners with generally higher biological activities (e.g. PCB-28, PCB-95, PCB-101, PCB-136); this subset of congeners was also the most active in the study of GJIC inhibition. The second cluster of NDL-PCBs included congeners with a narrow effective concentration and lower biological activities, with the exception of three assays related to endocrine activity (e.g. PCB-118, PCB-138, PCB-153, PCB-170, PCB-180) (Stenberg *et al.*, 2011).

QSAR approaches might become a useful tool for evaluation and prediction of toxicity of PCBs related to carcinogenesis; however, currently their use is hampered by the lack of data on specific mechanisms of action for larger congener sets.

4.4 Organ toxicity relevant to carcinogenicity

The reader is referred to Section 3.1.2 and Table 3.1 for study design and additional results of the experiments described below.

4.4.1 Hepatic preneoplastic lesions

(a) Promotion of preneoplastic lesions

(i) Commercial PCB mixtures

PCB mixtures, including Aroclor 1254, Clophen A 30, Clophen A 50, and Phenoclor DP6, have shown promoting activity in liver carcinogenesis (Glauert *et al.*, 2001). Several initiating agents were used, including diethylnitrosamine (DEN), aflatoxin B_1, and benzo[*a*]pyrene. The following markers of altered hepatic foci were used in these studies: gamma-glutamyl transferase (GGT), ATPase, and glycogen. The promoting activity of PCBs was observed in males and females. In one study, the promoting activity

of Clophen A 50 was much higher in female rats than in males (Deml & Oesterle, 1982); a similar observation was made for phenobarbital (Xu *et al.*, 1990). In mice, males are more susceptible than females to hepatocarcinogenesis; higher production of IL-6 by Kupffer cells in males may be responsible for this sex-specific difference (Naugler *et al.*, 2007). In a dose–response study with Clophen A 50, a threshold dose (1 mg/kg bw, three times per week, for 11 weeks) was identified (Deml & Oesterle, 1987).

(ii) Individual congeners

Many studies have examined the ability of individual PCB congeners to promote altered hepatic foci in rat liver (Glauert *et al.*, 2001). Most of the studies used DEN as the initiating agent, whether as a single necrogenic dose, as a low dose in conjunction with partial hepatectomy, as a low dose in newborn animals, or in the drinking-water for 10–12 days. The following markers of altered hepatic foci were used in these studies: GGT, GSTπ, ATPase, and/or glucose-6-phosphatase. PCB congeners that had promoting activity included non-*ortho* PCBs (PCB-77 and PCB-126), which activated AhR; di-*ortho*-substituted PCBs (PCB-47, PCB-49, and PCB-153), which activated CAR; and mono-*ortho*-substituted PCBs (PCB-105, PCB-114, PCB-118, and PCB-156), which activated both receptors. Non-*ortho*-PCBs were the most efficacious (Glauert *et al.*, 2001). PCBs that did not induce (PCB-3 and PCB-15) or that weakly induced (PCB-28 and PCB-101) either receptor had poor promoting activity (Oesterle & Deml, 1981; Deml *et al.*, 1985; Buchmann *et al.*, 1991; Kunz *et al.*, 2006). [These differences could be due to pharmacokinetics as well as pharmacodynamics.]

(iii) Combinations of individual congeners

Several studies have investigated the effects of administering combinations of two or more PCB congeners. Most of these studies found that the co-administration of non-*ortho* and di-*ortho* PCBs produced less than additive effects, while administration of two non-*ortho* PCBs produced additive effects. These studies used DEN as the initiating agent, either as a low dose in combination with partial hepatectomy, or as a hepatotoxic dose.

In the earliest study, Sargent *et al.* (1991) examined the separate and combined effects of dietary administration of (di-*ortho*) PCB-52 at 10 ppm, and (non-*ortho*) PCB-77 at 0.1 ppm for 1 year in rats. When administered separately, PCB-77 did not increase the number or volume of altered hepatic foci, but PCB-52 increased the volume fraction but not the number of altered hepatic foci. Coadministration of PCB-52 and PCB-77, however, increased both the number and volume fraction of altered hepatic foci in a more than additive manner. In a study examining the interactive effects of a non-*ortho*-substituted PCB (PCB-126), a mono-*ortho*-substituted PCB (PCB-105), and a di-*ortho*-substituted PCB (PCB-153), no more than additive effects were observed. An additive effect was observed with PCB-105 + PCB-153, while less than additive effects were observed for PCB-126 + PCB-153, and for PCB-126 + PCB-105 (Haag-Grönlund *et al.*, 1998). In another study, PCB-77 and PCB-153 were administered every 2 weeks separately at 300 μmol/kg bw, or in combination at 150 μmol/kg bw (total PCB dose, 300 μmol/kg per injection) for four injections (Berberian *et al.*, 1995). Numbers and volume of foci induced by PCB-77 were decreased by the coadministration of PCB-153. In a study using a similar experimental design, rats were injected four times with PCB-77 or PCB-153 (100 or 300 μmol/kg bw), or PCB-77 + PCB-153 (100 μmol/kg bw each) biweekly. Both PCB-77 and PCB-153 separately increased the number and volume of GSTP-positive foci, but coadministration of PCB-153 inhibited the number and volume of foci induced by PCB-77 (Tharappel *et al.*, 2002). When PCB-126 (non-*ortho*) and PCB-153 (di-*ortho*) were coadministered using 14 combinations of doses, a less

than additive effect was observed (Dean *et al.*, 2002). Finally, the tumour-promoting activity of a polyhalogenated aromatic hydrocarbon mixture (TCDD; 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin; 2,3,4,7,8-pentachlorodibenzofuran; PCB-126; PCB-118; and PCB-156) with or without PCB-153 (di-*ortho*) was compared with that of TCDD alone, the mixture and TCDD having the same total TEF (van der Plas *et al.*, 1999). The mixture produced a lower mean volume of foci and volume fraction of foci in the liver than did TCDD alone. The addition of PCB-153 slightly increased the mean volume of foci and volume fraction of foci in the liver, but still not above that produced by TCDD alone. TCDD and PCB-126 (non-*ortho*) were found to have an additive effect in another study (Hemming *et al.*, 1995).

(b) Initiation of preneoplastic lesions

Studies examining the effect of PCBs as initiating agents fell into two categories: those that examined the effect of PCB treatment with no subsequent chemical treatment, and those in which PCB treatment was followed with protocols designed to shorten the latency period and increase the number and size of lesions, such as the Solt-Farber selection protocol (Solt *et al.*, 1977; Tsuda *et al.*, 1980; Semple-Roberts *et al.*, 1987). Groups of animals treated with PCBs only were often control groups in initiation–promotion studies, e.g. PCB-only groups being used to compare initiator + PCB groups.

Several studies have observed a small increase in the number of altered hepatic foci after treatment with PCBs only. These PCBs included Clophen A 50, PCB-49, PCB-77, and PCB-114 (reviewed in Glauert *et al.*, 2001). There are two possible explanations for this phenomenon: first, these PCBs have initiating activity; or second, these PCBs are very efficient at promoting cells that have initiated spontaneously (e.g. from errors in DNA replication, exposure to background chemicals or radiation, etc.). Other studies, however, have observed that certain PCBs, including Aroclor 1254, Clophen A 50, PCB-52, PCB-77, and PCB-153, do not produce any increase in the number of altered hepatic foci after treatment with the PCB congener only (reviewed in Glauert *et al.*, 2001). [Possible reasons for obtaining different results for the same PCBs included use of different doses, use of different proliferative stimuli, and different latency periods.]

Three studies have used PCBs as initiating agents in the Solt-Farber protocol to determine whether altered hepatic foci would develop. This protocol involves treatment with an initiating agent (either known or to be tested) in conjunction with a proliferative stimulus. After a recovery period (usually 2 weeks), rats are treated with 2-acetylaminofluorene (2-AAF; to inhibit cell proliferation), given either in the diet or by gavage, for 2 weeks, with a proliferative stimulus (usually an oral dose of carbon tetrachloride or partial hepatectomy) after the first week.

Hayes *et al.* (1985) assessed Aroclor 1254, a reconstituted human breast milk mixture of PCB congeners, PCB-47, PCB-52, and PCB-153, and found that none of them had initiating activity. Espandiari *et al.* (2003) examined less chlorinated PCBs, and observed that some (PCB-3, PCB-15, PCB-52, and PCB-77) increased the number of GGT-positive foci, while others did not (PCB-12 and PCB-38). A subsequent study showed that the PCB-3 metabolites 4-OH-PCB-3 and the *ortho* 3,4-quinone of PCB-3 acted as the proximate and ultimate carcinogens (Espandiari *et al.*, 2004). [Negative results obtained after the administration of PCBs could indicate lack of initiating activity, likely due to low metabolic activation, or could be caused by alteration of other components of the protocol, such as acetylaminofluorene metabolism and effects.]

4.4.2 Liver

Liver toxicity is commonly observed in long-term studies in rats and mice exposed to PCBs, with dose- and duration-dependent increases in the incidence, severity, and breadth of spectrum of lesions observed (Kimbrough & Linder 1974; Mayes *et al.*, 1998; NTP, 2006a, c, d, 2010).

For PCB-126, PCB-118, and binary mixtures of PCB-126 with PCB-153 or PCB-118, hepatic toxicity increased with increasing dose and duration of exposure, and was characterized by increases in the incidence and severity of hepatocyte hypertrophy (most likely due to alterations in PCB-induced CYP expression), diffuse fatty changes, multinucleated hepatocytes, pigmentation (likely due to haemosiderin accumulation), inflammation, altered hepatic foci, necrosis, oval cell hyperplasia, cholangiofibrosis, bile-duct hyperplasia, bile-duct cysts, and nodular hyperplasia (NTP, 2006a, c, d, 2010).

With PCB-153, hepatocyte hypertrophy was seen after 14, 31, and 53 weeks in female rats treated with doses of up to 3 mg/kg bw by gavage; at 2 years, there were also increases in the incidence of fatty change, bile-duct hyperplasia, oval-cell hyperplasia and pigmentation (NTP, 2006b).

While none of these hepatic responses are specifically preneoplastic, cholangiofibrosis and cholangiocarcinoma represent different diagnoses along the same continuum of pathogenesis. Cholangiofibrosis was seen in the above-mentioned NTP studies of female rats treated with specific PCB congeners by gavage, in female rats fed with Aroclor 1260 (Kimbrough *et al.*, 1975), and in female rats treated with other dioxin-like compounds by gavage (NTP, 2006e, f, g). In general, the higher the dose and duration of exposure, the higher the incidence, severity, and breadth of spectrum of responses observed. The observations of biliary and hepatocellular lesions are characteristic of an initial insult and the response of the liver to repair the injury and regenerate, leading to a hepatic stem-cell response and a bifurcating lineage of subsequent pathologies of both bile-duct cells and hepatocytes.

4.4.3 Lung

In the long-term NTP studies in female Harlan Sprague-Dawley rats treated with PCB-126, PCB-118, PCB-118 + 126 and PCB-126 + 153 by gavage, there were clear increases in the incidence of cystic keratinizing epithelium of the lung and of squamous cell carcinoma (NTP, 2006a, c, d, 2010). The two common effects seen in PCB-treated rats were an increased incidence of alveolar epithelial bronchiolar metaplasia and of squamous metaplasia of the lung (reviewed in Sells *et al.*, 2007). Squamous metaplasia was characterized by the transition of alveolar epithelial cells to squamous metaplastic cells with distortion of the normal architecture. Keratin formation was evident and inflammation was sometimes observed. The more expansive lesions formed keratinizing cysts, which consisted of a cystic structure with a thin uniform wall composed of mature squamous cells that contained various amounts of keratin. The term "cystic keratinizing epithelioma" was used for a benign neoplasm in this family of lesions, and "squamous cell carcinoma" was used as a diagnosis for the malignant form of the lesion (Sells *et al.*, 2007). Alveolar epithelial bronchiolar metaplasia was characterized by metaplasia of alveolar epithelium to respiratory type primarily at the junction of the terminal bronchioles and along alveolar ducts. Alveolar epithelial bronchiolar metaplasia did not appear to be associated with progression to neoplasia, but may have been characteristic of increased metabolic activity in the metaplastic area (Brix *et al.*, 2004).

No pulmonary toxicity was observed in a long-term NTP study with PCB-153 in female rats (NTP, 2006b). Pulmonary toxicity was not reported in long-term bioassays with Aroclors 1016, 1242, 1254, and 1260 in CD Sprague-Dawley

rats (Mayes *et al.*, 1998). [Differences between the studies included strain of rat used (Harlan Sprague-Dawley versus Charles River Sprague-Dawley), route of exposure (feed for Aroclors versus gavage for the PCB congeners), and use of complex mixtures (Aroclors) versus individual or binary mixtures of single PCB congeners.]

4.4.4 Thyroid

In long-term NTP studies of female Sprague-Dawley rats treated with PCB-126, PCB-118, PCB-126 + 118, and PCB-126 + 153 by gavage, there were increased incidences of follicular cell hypertrophy of the thyroid in the exposed groups at 14, 31, 53 weeks, and 2 years (NTP, 2006a, c, d, 2010; Yoshizawa *et al.*, 2010). Increased incidence of follicular cell hypertrophy was also seen with PCB-153 only at 53 weeks and 2 years (NTP, 2006b).

The observation of thyroid follicular cell hypertrophy in treated rats was attributed to alterations in the expression of UDP-GT in the liver, leading to a decrease in circulating T4, disruption of thyroid-hormone homeostasis, and compensatory hypertrophy (Hill *et al.*, 1989). [The Working Group noted that other mechanisms may be operational.] A persistent increase in the incidence of follicular cell hypertrophy has often been linked to increased incidences of follicular cell tumours of the thyroid in studies in experimental animals (see Section 3). No neoplasms were observed in treated females. Increased incidence of thyroid follicular cell tumours was seen in male CD SD rats exposed to Aroclors 1242, 1254, or 1260, although without significant increase in the incidence of thyroid follicular cell hypertrophy (Mayes *et al.*, 1998). The morphological appearance of the thyroid tumours was characteristic of those developed as a secondary response to chronic overstimulation of TSH. [This phenomenon is more common in males than females rats due to higher circulating levels of TSH in males.]

4.4.5 Adrenal gland

In the long-term NTP study in female Harlan Sprague-Dawley rats treated with PCB-126 by gavage, increased incidences of adrenal atrophy and cytoplasmic vacuolization were observed in those groups in which elevated incidences of adrenal adenoma were seen (NTP, 2006a). In long-term NTP studies in female rats treated with PCB-118, increases in the incidence of adrenal atrophy and cytoplasmic vacuolization, but not adrenal adenoma, were observed (NTP, 2010). Treatment with PCB-153 or Aroclors had no effect on the adrenal gland in long-term studies in female rats (Mayes *et al.*, 1998; NTP, 2006b).

4.4.6 Pancreas

A common occurrence in long-term studies with PCBs with dioxin-like activity (PCB-126, PCB-118, PCB-126 + PCB-118, and PCB-126 + PCB-153) in female rats (males were not studied) was toxicity in the pancreas (NTP, 2006a, c, d, 2010). In the NTP studies with PCB-126 and PCB-118, pancreatic acinar cytoplasmic vacuolization, atrophy, and chronic active inflammation were observed. No effect on the pancreas was seen in female rats exposed to PCB-153 at doses of up to 3 mg/kg bw per day for 2 years (NTP, 2006b). Increased incidence of acinar adenoma was observed in a long-term NTP study of PCB-126/153 in female rats, and sporadic incidences of acinar adenoma were observed in a long-term NTP study of PCB-118 in female rats, although it was uncertain whether this was a treatment-related effect (NTP, 2006c, 2010).

4.4.7 Female reproductive system

In the long-term NTP study of PCB-118 and PCB-153 in female Harlan Sprague-Dawley rats, there was no increase in the incidence of cystic endometrial hyperplasia of the uterus and squamous metaplasia of the uterus; the incidences of squamous metaplasia and cystic endometrial

hyperplasia in the core study groups were significantly less than the incidence in the vehicle-control group. In the PCB-118 stop-exposure group, in which exposure (to 4600 µg/kg bw) was for only 30 weeks followed by vehicle only (corn oil) for up to 2 years, the incidences of these two lesions were significantly increased compared with those in the core-study group exposed continually at 4600 µg/kg bw per day (NTP, 2006b, 2010). Accordingly, there was a significant increase in the incidence of uterine carcinoma in the stop-exposure group in which exposure was for only 30 weeks followed by vehicle only (corn oil) for up to 2 years, but not in the long-term exposure group (see Section 3.1.1). While the mechanism was not known, it was speculated that exposure to PCB-118 for the first 30 weeks led to the early development of responsive uterine carcinoma, and that the subsequent cessation of exposure reestablished a normal estrogenic milieu that promoted the development of these uterine neoplasms, which would otherwise have been suppressed if exposure had been continued for the full 2 years (Yoshizawa *et al.*, 2009).

In the 2-year NTP study with PCB-153 in female Harlan Sprague-Dawley rats, there was a significant increase in the incidence of chronic active inflammation of the ovary; however, there was no increase in the incidence of ovarian tumours (NTP, 2006b).

4.4.8 Skin

Chloracne and other dermal alterations are well known effects of long-term exposure to PCBs and related compounds (ATSDR, 2000). These effects have been reported in workers exposed occupationally to PCBs, and also in individuals exposed by accidental ingestion of rice oil contaminated with high concentrations of PCBs (Yusho and Yucheng), and in rhesus monkeys fed a diet containing Aroclor 1248. Chloracne is probably caused by interference of PCBs with the metabolism of vitamin A in the skin, resulting in disturbances of the epithelial tissues of the pilo-sebaceous duct (Coenraads *et al.*, 1994).

(a) Human exposure

(i) Occupational exposure

Chloracne is the most easily recognized effect of exposure to PCBs and structurally related chlorinated organic chemicals (Rice & Cohen, 1996). Chloracne first develops on the face, under the eyes and behind the ears, but severe chloracne can cover the entire body. Histologically, the lesions consist of keratinous cysts caused by squamous metaplasia of sebaceous glands. The acute stage is followed by vermiculite skin atrophy. Mild to moderate chloracne was observed in 7 out of 14 workers exposed to Aroclors (formulation not specified) at 0.1 mg/m^3 for an average duration of 14.3 months (Meigs *et al.*, 1954). [Because PCBs were used as a heat-exchange material, it is possible that these workers were exposed to pyrolysis products.] Three cases of chloracne occurred among autoclave operators (number not specified) exposed to Aroclor 1254 at 5.2–6.8 mg/m^3 for 4–7 months (Bertazzi *et al.*, 1987). [The presence of pyrolysis products may have been a confounding factor.] In 1977, four more cases of chloracne were diagnosed among 67 workers from the same plant who were engaged in impregnating capacitors with Pyralene 3010 (0.048–0.275 mg/m^3) and had skin contact confirmed as a major exposure route. An increased incidence of non-adolescent acneiform eruptions was reported in workers exposed to various Aroclors at mean concentrations of 0.007–11 mg/m^3 for > 5 years; 40% of the workers had been exposed for > 20 years (Fischbein *et al.* 1979, 1982). Maroni *et al.* (1981a, b) reported ten cases of acne and/or folliculitis and five cases of dermatitis among 80 capacitor-manufacturing workers in Italy. All the workers with chloracne were employed in jobs with high exposure. Their blood PCB concentrations ranged from 300 to 500 µg/L. No definite association was found

between chloracne and blood PCB concentrations. Other dermal effects reported in workers included skin rashes, pigmentation, disturbances of skin and nails, erythema and thickening of the skin, and burning sensations (Ouw *et al.*, 1976; Fischbein *et al.*, 1979, 1982; Smith *et al.*, 1982). In these studies, the workers were exposed to various Aroclors at concentrations as low as 0.003 mg/m^3 for > 5 years. In those studies that looked at PCB profile of exposure, statistically significant associations between dermatological effects and plasma concentrations of more highly chlorinated PCB congeners were reported (Fischbein *et al.*, 1979, 1982; Smith *et al.*, 1982), while no relationships were found between the incidence of skin rash or dermatitis, and plasma concentrations of less chlorinated PCBs (Smith *et al.*, 1982).

(ii) Accidental exposure

Skin effects were widely reported among victims of the Yusho and Yucheng poisoning episodes (Lü & Wu, 1985; Kuratsune, 1989; Rogan, 1989; Guo *et al.*, 1999). However, these effects could not be attributed solely to exposure to PCBs, since the victims were also exposed to PCDFs and other chlorinated chemicals (ATSDR, 1994). Characteristic skin changes included marked enlargement, elevation and keratotic plugging of follicular orifices, comedo formation, acneform eruptions, hyperpigmentation, hyperkeratosis, and deformed nails. Dark-coloured pigmentation frequently occurred in the gingival and buccal mucosa, lips, and nails, and improved only gradually in most patients (Kuratsune *et al.*, 1971; Fu, 1984; Lü & Wu, 1985; Kuratsune, 1989; Rogan, 1989). At 14 years after the Yucheng incident, exposed men and women had a higher lifetime prevalence of chloracne, abnormal nails, hyperkeratosis, and gum pigmentation (Guo *et al.*, 1999). Skin lesions were commonly observed in children born to mothers exposed during the Yusho or Yucheng incidents (Gladen *et al.*, 1990).

(b) Experimental systems

(i) Animal studies in vivo

Female rhesus monkeys fed diets containing Aroclor 1248 at concentrations of 2.5 and 5.0 ppm developed facial oedema, swollen eyelids, erythema, loss of hair, and acne, within 2 months. After 6 months, the monkeys were bred with control males. In the seven offspring carried to term, and exposed for 4 months to PCBs via the lactating mother, focal areas of hyperpigmentation and acneiform lesions of the face developed within 2 months, and were accompanied by increased skin PCB concentrations (Allen & Norback, 1976).

Developing *Xenopus laevis* tadpoles were exposed to Aroclor 1254 at concentrations of 0 to 100 µg/mL from day 5 to day 9 after fertilization. Exposure at the higher concentrations (10, 50, and 100 µg/mL) caused statistically significant reductions in survival and body size, and resulted in histological abnormalities, including aberrant tail-tips, and aberrant myotomal and melanocyte morphologies; tadpoles treated with Aroclor 1254 were devoid of dendritic arborizations, resulting in decrease in total melanocyte area (Fisher *et al.*, 2003).

(ii) Human cells in vitro

Only two studies were available on the molecular effects of PCBs in human skin cells. Exposure of normal human melanocytes to TCDD resulted in activation of the AhR signalling pathway, AhR-dependent induction of tyrosinase, and consequently, elevated total melanin content. These effects were due to the induction of tyrosinase and tyrosinase-related protein 2-gene expression. Thus AhR is able to modulate melanogenesis by controlling the expression of melanogenic genes (Luecke *et al.*, 2010).

Exposure of human skin keratinocytes to a synthetic mixture of volatile PCBs, or the common airborne congeners PCB-28 or PCB-52

led to significant inhibition of telomerase activity and reduced telomere length. All PCBs decreased cell proliferation, and PCB-52 produced a small increase in the fraction of cells arrested in G0/G1 of the cell cycle. Changes in telomere length and telomerase activity are hallmarks of ageing and carcinogenesis; these effects suggested a potential mechanism by which exposure to PCBs could lead to skin cancer (Senthilkumar *et al.*, 2011).

4.5 Susceptibility

4.5.1 Genetic polymorphisms

Single nucleotide polymorphisms in the genes for metabolizing enzymes or receptors can potentially affect expression or inducibility (if these polymorphisms were in the promoter region of the gene), and stability or function of the protein (if they were in the coding region). The individual response to carcinogens may be influenced by polymorphisms in genes for metabolizing enzymes, including xenobiotic- and steroid-metabolizing CYP, GST, catechol *O*-methyltransferase (COMT), and others (Singh *et al.*, 2008); receptors that control expression of metabolizing enzymes such as AhR (Ng *et al.*, 2010) and the AhR repressor (Hung *et al.*, 2013); and receptors that interact with endogenous molecules such as steroid hormones.

(a) Metabolizing genes

As discussed in Section 4.1.3, CYP plays an important role in PCB metabolism. Knowledge of the particular CYP isoform most likely to bind and/or metabolize a PCB congener is important in evaluating risk from exposure to this congener. Many human CYP isoforms exhibit pharmacogenetic polymorphisms, which can affect expression levels, catalytic activity per unit enzyme with particular substrates, or both parameters (Ingelman-Sundberg *et al.*, 2007). Variations in activity due to polymorphism could lead to inter-individual differences in the capacity to metabolize particular congeners. If metabolism of the congener produced genotoxic metabolites, such as arene oxides, quinones, or reactive oxygen species through the action of CYP, this could mean that greater amounts of these potential carcinogens would be formed in some individuals with increased metabolic activity. Alternatively, people with a lower metabolic activity for some PCBs could accumulate greater amounts of those PCBs, if continually exposed. Both scenarios could lead to increased risk of cancer, through several mechanisms.

(i) Cancer of the breast

Epidemiological studies have provided evidence for increased risk of cancer of the breast in women with a particular genetic polymorphism in the *CYP1A1* gene and high serum PCB concentrations (Moysich *et al.*, 1999; Laden *et al.*, 2002; Charlier *et al.*, 2004; Zhang *et al.*, 2004; Li *et al.*, 2005). In the variant form, *CYP1A1*2C*, also called the m2 variant, has valine substituted for isoleucine at position 462 near the *C* terminus of the protein (Persson *et al.*, 1997). This variant is found in 10–15% of the white population and in a larger proportion of African-Americans (reviewed in Brody *et al.*, 2007). Persson *et al.* (1997) reported that the activity per unit enzyme of this variant, measured in vitro, was similar to that of wildtype *CYP1A1*. Polymorphisms in AhR, or its repressor, that influence the expression of *CYP1A1* may be more important than *CYP1A1* genotype in determining the in-vivo activity of *CYP1A1* (Smart & Daly, 2000; Hung *et al.*, 2013).

Among postmenopausal patients with cancer of the breast in western New York state, USA, the incidence of cancer of the breast was higher in women with total PCB concentrations (73 congeners) of 3.73–19.04 ng/g of serum and the *CYP1A1*2C* polymorphism than in women with lower PCB concentrations or wildtype *CYP1A1* (Moysich *et al.*, 1999). In a study of Caucasian women in Connecticut, USA, in which serum

concentrations of PCB-74, PCB-118, PCB-138, PCB-153, PCB-156, PCB-170, PCB-180, PCB-183, and PCB-187 were measured, cancer of the breast was more prevalent in postmenopausal women with lipid-adjusted serum concentrations of 611–2600 ng/g and the *CYP1A1*2C* polymorphism than in controls (Zhang *et al.*, 2004). If the *CYP1A1* polymorphism was absent (homozygous wildtype alleles), there was no effect of serum PCB concentration on incidence of cancer of the breast. An epidemiological study of African-American and white women in North Carolina, USA, examined lipid-adjusted total plasma PCB concentrations, *CYP1A1* polymorphism, and risk of cancer of the breast (Li *et al.*, 2005). Although results were not conclusive due to small sample size, premenopausal white women with cancer of the breast were more likely to have total PCB concentration > 0.35 ng/mL serum and the *CYP1A1*2C* polymorphism than were controls, while there was no relationship between cancer of the breast in women with total PCB concentration < 0.35 ng/mL serum or lacking this polymorphism. In the African-American women, total PCB concentrations were somewhat higher (≥ 0.430 ng/mL), and the *CYP1A1*3* polymorphism was more prevalent in pre- and postmenopausal patients with cancer of the breast (Li *et al.*, 2005).

Another study found a non-significantly elevated risk of cancer of the breast among women with the CYP1A1-m1 variant and high serum PCB concentrations (McCready *et al.*, 2004).

(ii) Cancer of the testis

Data from 568 cases of testicular cancer and 698 controls enrolled in the United States Servicemen's Testicular Tumor Environmental and Endocrine Determinants Study were used to examine associations between testicular germ cell tumours (TGCT) and exposure to PCBs, as affected by polymorphisms in several hormone-metabolizing genes, i.e. *CYP17A1*, *CYP1A1*, *HSD17B1*, *HSD17B4* and androgen receptor. Among these, the polymorphism rs384346 in *HSD17B4* modified the association of TGCT risk with PCB-118 and PCB-138 concentrations. Among men who were homozygous for the major allele genotype, there was a statistically significant dose-dependent reduction in risk (P for trend < 0.001) with higher exposure to PCB-118 and PCB-138. Men in the highest quartile of PCB-118 exposure had an almost 50% reduction in risk of TGCT (OR, 0.46, 95% CI, 0.31–0.70) compared with men in the lowest quartile; similar results were seen for PCB-138. For any minor allele of this *HSD17B4* polymorphism, there were no associations between PCB-118 and PCB-138 concentrations and risk of TGCT. No interactions between other PCB congeners of interest (PCB-153, PCB-156, PCB-163, PCB-170, PCB-180, and PCB-187) and enzyme polymorphism were observed (Chia *et al.*, 2010).

(b) Polymorphisms in other genes

Among highly exposed Yucheng patients, combined *CYP1A1-Msp1* mutant genotype and *GSTM1-null* genotype were associated with an increased risk of chloracne (OR, 2.8; 95% CI, 1.1–7.6). Among intermediately exposed individuals, the *GSTM1-null* genotype was associated with skin allergy (Tsai *et al.*, 2006).

Patients with non-Hodgkin lymphoma and PCB-118 concentrations in the highest quartile (> 12.85–202.13 μg/L plasma) were more likely to have a polymorphic variant of AhR (IVS + 4640 null; G/G genotype) than controls, although the effect was not strong and was also related to highest levels of oxychlordane and *trans*-nonachlor (Ng *et al.*, 2010).

Among women with cancer of the breast who carried a variant of the tumour-suppressor gene *TP53*, total PCB exposure in the highest quartile was associated with an increased risk of cancer of the breast, but this was not statistically significant (OR, 3.0; 95% CI, 0.66–13.62) (Høyer *et al.*, 2002).

4.5.2 Exposure in utero, postnatally, and of children

PCBs can pass through the placenta during embryonic development and accumulate in breast milk. In addition, compared with adults, children have a lower barrier to absorption through the skin, gastrointestinal tract and lungs, and lower levels of detoxifying enzymes (Lindström *et al.*, 1995). A combination of all these factors leads to a higher accumulation of PCBs in children.

(a) Toxicokinetics and distribution in tissues

(i) Children

Grandjean *et al.* (2008) studied the elimination kinetics of PCBs in two groups of children with elevated PCB concentrations due to breastfeeding. Children were followed from age 4.5 to 7.3 years (99 subjects) and 7 to 14 years (101 subjects). Subjects with exposures above the median and in the highest quartile showed half-lives of about 3–4 years for PCB-138; 4.5–5.5 years for PCB-105 and PCB-118; 6.5–7.5 years for PCBs 156, 170 and 187; and 7–9 years for PCBs 153 and 180. The longest half-lives correspond to elimination of the parent PCB solely with a daily fat excretion rate of 1–2 g, while shorter half-lives assume metabolic break-down.

Scheele *et al.* (1992) measured the concentrations of PCB-138, PCB-153, and PCB-180 in bone marrow (collected during routine bone-marrow aspiration) of 38 children with leukaemia and 15 control children (nine had idiopathic thrombocytopenia and six were bone-marrow donors). Most of the samples were pooled to ensure sufficient volume for analysis. Total PCB concentrations were determined on the basis of congeners PCB-138 + PCB-153 + PCB-180 and multiplied by 1.7 (Deutsche Forschungsgemeinschaft, 1988). The mean and median concentrations of total PCBs in bone marrow of children were 3.6 mg/kg fat basis and 2.9 mg/kg, respectively. PCB concentrations in bone marrow were two- to threefold those in fat tissue. [The reason for the high affinity of bone marrow for PCBs was not clear. It is possible that genetic factors may play a role.] There were no significant differences in PCB concentrations between the group of children with leukaemia and the control group. [The Working Group noted that the authors did not report whether parental smoking, an important confounding factor, was accounted for in their statistical analysis.]

A study in 360 schoolchildren (a subgroup of the Hesse, Germany cohort) in 1995 (Karmaus *et al.*, 2001a, b) focused on the potential of early childhood factors such as breastfeeding, parity, and parental smoking to contribute to the variety of effects observed with exposure to organochlorine compounds including PCBs, at approximately age 7 years. Concentrations of PCBs (sum of congeners PCB-101, PCB-118, PCB-138, PCB-153, PCB-170, PCB-180, PCB-183, and PCB-187) were determined in whole blood. A significant dose–dependent relationship ($P < 0.0001$) existed between the duration of breastfeeding (none, 1–4 weeks, 5–8 weeks, 9–12 weeks, > 12 weeks) and the sum of PCB concentrations. Of all the potential factors analysed, breastfeeding accounted for most of the variance in PCB concentrations. Exclusive breastfeeding beyond 12 weeks was associated with a doubling of PCB concentrations in whole blood compared with bottle-fed children (sum of PCBs, 0.25 μg/L versus 0.55 μg/L).

(ii) Experimental animals

Sixteen (eight/group) adult female rhesus monkeys were exposed to diets containing Aroclor 1248 at 2.5 or 5.0 ppm for approximately 1.5 years (Allen & Barsotti, 1976). Six out of the eight monkeys treated with Aroclor 1248 at 5.0 ppm, and eight out of the eight monkeys at 2.5 ppm were successfully bred after 6 months of exposure. One live infant was born to dams exposed at 5.0 ppm, and five infants were born to monkeys at 2.5 ppm. Infants were permitted to nurse with their mothers. All six surviving infants had PCBs

in their tissues at birth: PCB concentrations in skin biopsies (epidermis, dermis and the attached underlying subcutaneous tissue) ranged from 1.0 to 4.8 µg per g of tissue. By the third month, skin PCB concentrations ranged from 86.4 to 136.8 µg per g of tissue. The infant that died after 239 days had PCB concentrations of more than 20 µg per g in seven organs (adrenal gland, cerebrum, kidney, muscle, pancreas, testes, thymus). In the two infants that survived for shorter periods, this PCB concentration was exceeded only in three tissues (bone marrow, lung, thymus) in one infant and two tissues (bone marrow, pancreas) in the other.

Female rhesus monkeys were fed a daily dose of Aroclor 1254 (0, 5, 20, 40 or 80 µg/kg bw) for approximately 6 years (Arnold *et al.*, 1993, 1995; Mes *et al.*, 1994, 1995a). Blood and adipose tissue from offsprings exposed in utero/during lactation who had nursed for 22 weeks were analysed for PCB content at 120 weeks after birth. PCB concentrations in the adult monkeys increased with their dosage. Tissues of live infants of dosed dams contained more PCBs than those of infants of control dams, and less PCBs than those of still-born infants. Also, offspring with higher PCB concentrations showed a marked shift from tetra- and hexachlorobiphenyls to penta- and hepta-chlorobiphenyls. The PCB distribution pattern in tissues from a dosed mother–infant pair differed considerably. A larger percentage of heptachloro-biphenyls was found in the infant than in its dam (Mes *et al.*, 1995a). Depletion studies revealed that PCB concentrations in the blood of exposed infants declined rapidly after weaning due to growth dilution and approached maternal levels within 40–50 weeks. Approximately 100 weeks after weaning, PCB concentrations in adipose tissue of infants from treated dams reached levels of those in the control group (Mes *et al.*, 1994).

Male Swiss mice aged 8 days were given a single intraperitoneal injection of a mixture of PCB-99, PCB-105, PCB-118, PCB-128, PCB-138, PCB-153, PCB-156, PCB-170, and PCB-180 at 500 mg/kg bw (Anderson *et al.*, 1993). Groups of 25 mice were killed at 1 and 7 days, and at 8, 12, and 16 weeks after treatment. Congeners in group 1 (PCB-99, PCB-105, PCB-118, PCB-128) were eliminated from the body more rapidly than congeners in group 2 (PCB-138, PCB-153, PCB-156, PCB-170, PCB-180). PCB concentration in the carcass (adipose compartment) was the most predictable finding, since the congeners behaved similarly within each group. In contrast, in lung, after a rapid loss during the first week, all congeners except PCB-153 were retained and decreased in amount only as a function of dilution due to growth. Congeners PCB-105 and PCB-138 were present at higher proportions in the lung than in the carcass. In the liver, retention of all congeners was observed during the prepubertal growth phase, with specific enrichment of PCB-105, followed by more rapid depletion of certain congeners (Anderson *et al.*, 1993).

(b) *Effect on gene expression*

Dutta *et al.* (2012) used microarray-based differential gene expression analysis of a group of children (mean age, 46.1 months) of central European descent (Slovak Republic) to study the impact of PCBs on different cellular pathways and to explain their possible mode of action. The subset of children having high blood PCB concentrations (> 75th percentile) was compared with their low PCB counterparts (< 25th percentile), with mean lipid-adjusted PCB concentrations of 3.02 ± 1.3 and 0.06 ± 0.03 ng/mg of serum lipid, respectively. A set of 162 genes with statistically significant differential expression ($P < 10^{-5}$) between groups with high and low PCB concentration was identified. Analyses using the IPA tool indicated that cell–cell signalling and interactions, cellular movement, cell signalling, molecular transport, and vitamin and mineral metabolism were the major molecular and cellular functions associated with the genes differentially expressed in children with high PCB concentrations. Furthermore, the

differential gene expression appeared to play a pivotal role in the development of probable diseases and disorders, including cardiovascular disease and cancer. The analyses also pointed out possible organ-specific effects, e.g. cardiotoxicity, hepatotoxicity and nephrotoxicity in the children exposed to high concentrations of PCBs. Expression levels of *BCL2*, paraoxonase 1 (*PON1*), interleukin *IL1F7*, *IL23A* and integrin β 1 (*ITGB1*) were significantly altered in these children; more specifically, *BCL2* and *ITGB1* were downregulated, while *IL1F7*, *PON1*, and *IL23A* were upregulated.

(c) Enzymatic effects in feto-placental unit, and fetal and neonatal liver

Alvares & Kappas (1975) investigated the induction of aryl hydrocarbon hydroxylase (Ahh) by PCBs in the feto-placental unit, fetal livers and neonatal livers during lactation. For the in-utero exposure protocol, pregnant Sprague-Dawley rats were injected intraperitoneally with Aroclor 1254 (25 mg/kg bw per day) for 6 days, and killed 24 hours later on day 20 of gestation. For the lactation experiments, untreated mothers were injected intraperitoneally with Aroclor 1254 (25 mg/kg bw per day) for 6 days starting on day 2 postpartum; the offspring of these dams were killed on day 8 postpartum.

PCBs caused a 10-fold induction in Ahh activity in the placenta, but only a threefold induction in the fetal livers. Ahh activity in placentas of untreated rats was markedly lower than that observed in the fetal liver of the same rats. In the liver of neonates whose mothers were treated with Aroclor 1254 postpartum (infants exposed through lactation), there was an 18-fold increase in Ahh activity, a threefold increase in CYP content, and a twofold increase in *N*-demethylase activity. Thus Aroclor 1254 was a more potent inducer of Ahh activity in placenta and liver when exposure occurred through lactation than through in-utero exposure when administered to pregnant rats.

4.6 Mechanistic considerations

The group of PCBs comprises 209 individual congeners with widely different physical and chemical properties. The number of chlorine atoms on the two phenyl rings and their relative positions determine the biological and toxicological attributes of each congener. Some PCBs are susceptible to metabolic conversion, which may give rise to a series of metabolites, each with its own profile of biological and toxicological activities. In this section, various mechanisms of carcinogenesis will be identified and summarized for specific subgroups of PCBs and their metabolites.

4.6.1 Metabolic activation and genotoxicity of PCBs and their metabolites

(a) Metabolism leading to formation of electrophiles

The 209 PCB congeners vary greatly in their susceptibility to metabolic attack, with less chlorinated biphenyls being much more susceptible. The first metabolic step is mono-oxygenation, which leads to the formation of hydroxylated metabolites, a reaction that is mediated by enzymes of the CYP super-family. There are 837 possible mono-hydroxylated products (Rayne & Forest, 2010; Grimm *et al.*, 2015). Depending on the number of chlorines present, the arene oxide may emerge as a highly reactive, electrophilic species: the lower the number of chlorines, the more reactive the arene oxide.

Mono-hydroxylated PCBs may undergo a second hydroxylation, producing a dihydroxylated PCB derivative, either as catechol (hydroxyl groups in the *ortho* configuration) or as hydroquinone (hydroxyl groups in the *para* configuration) (McLean *et al.*, 1996a). The formation of dihydroxylated PCBs is catalysed primarily by CYP enzymes. PCB catechols and hydroquinones may then be oxidized by peroxidases, prostaglandin synthase, and probably other enzymes,

giving rise to the formation of highly reactive electrophilic PCB quinones (Amaro *et al.*, 1996; Oakley *et al.*, 1996a; Wangpradit *et al.*, 2009).

The oxygenated PCB intermediates and metabolites, i.e. the arene oxides and the quinones, are probably the most relevant to PCB-induced carcinogenesis, but many other metabolites may also be formed. For example, OH-PCBs are substrates for glucuronidation (Tampal *et al.*, 2002) and sulfation (Liu *et al.*, 2006, 2009; Ekuase *et al.*, 2011). All electrophilic PCB metabolites with elevated chemical reactivity, however, should be regarded as probable cancer initiators.

(b) Binding to DNA and protein

Covalent binding to cellular macromolecules (adduct formation) has been observed in mice treated with radiolabelled PCB-153 and PCB-136, the binding of the latter decreasing in the order RNA > protein > DNA (Morales & Matthews, 1979). Formation of protein and DNA adducts was observed in vitro with PCB-3 and the tetrachlorinated congeners PCB-47, PCB-49, PCB-52, and PCB-77 (Wyndham *et al.*, 1976; Shimada & Sawabe, 1984). DNA-adduct formation was also observed with a series of 15 mono- and dichlorinated PCBs, with but not without activation by microsomes, horseradish peroxidase and hydrogen peroxide (McLean *et al.*, 1996b). This suggested that quinones were the ultimate genotoxic agents. Indeed, tests with synthetic quinones of less chlorinated PCBs confirmed the extensive DNA-adduct formation, particularly with deoxyguanosine (Oakley *et al.*, 1996a; Zhao *et al.*, 2004).

These experiments indicated that PCBs require CYP-mediated metabolic activation, that a lower degree of chlorination favours bioactivation, that an arene oxide intermediate and/or possibly a semiquinone or quinone is the ultimate DNA-binding species, and that guanine is the major target site in DNA. Apart from binding to DNA, PCB quinones also bind cellular proteins, preferably, but not exclusively, to cysteine (Amaro *et al.*, 1996; Srinivasan *et al.*, 2002; Bender *et al.*, 2006).

(c) Indirect genotoxicity: metabolism-associated generation of ROS

The arene oxides and quinones are probably the metabolites with most relevance to the cancer-initiating activity of PCBs, since they can be regarded as direct-acting genotoxic intermediates. In addition, dihydroxylated PCBs and their corresponding PCB quinones may undergo redox cycling, thereby generating ROS, which are considered to be active in the initiation, promotion, and progression of cancer. For example, ROS formed during auto-oxidation of a PCB hydroquinone may give rise to oxidative DNA damage, e.g. 8-OHdG. Mutations induced by these lesions may lead to activation of oncogenes or inhibition of tumour-suppressor genes, thus contributing to the carcinogenic potential of PCBs (Amaro *et al.*, 1996; Oakley *et al.*, 1996a). Formation of ROS may also induce DNA strand breaks (Srinivasan *et al.*, 2001).

(d) Mutagenic effects

PCB-3, 4-OH-PCB-3, and two hydroquinones of PCB-3 were tested for mutagenicity in Big Blue® rats and in Chinese hamster V79 cells. These results demonstrated that monochlorinated PCBs are mutagenic in vivo in the target organ, the liver, and studies in vitro suggested that metabolic activation to electrophilic and mutagenic species plays a crucial role. Although the ultimate mutagenic metabolite (*ortho*- or *para*-quinone, or epoxide or other metabolite) could not be deduced with certainty, the evidence pointed towards adduct formation by a quinone, or quinone-induced redox cycling as the mode of action.

Apart from gene mutations, other forms of genotoxicity observed after exposure to PCBs included the induction of DNA strand breaks, and anomalous segregation of chromosomes.

Elevated concentrations of mono- and dihydroxylated metabolites of PCB-3 were shown to induce these types of lesions in vitro (Zettner *et al.*, 2007; Flor & Ludewig, 2010).

With regard to the PCB congeners considered to act primarily through *trans*-activation of nuclear receptors, the available data provided little evidence regarding genotoxicity (see Section 4.2).

(e) Cancer initiation and promotion

The ability of commercial PCB mixtures and individual PCB congeners to initiate and/or promote neoplastic lesions has been studied in rodent two-stage models of liver carcinogenesis. Aroclor 1254, which contains mainly tetra- and pentachlorobiphenyls, acted as a weak tumour initiator in the mouse two-stage model of skin carcinogenesis (DiGiovanni *et al.*, 1977). In contrast, when tested using the Solt-Farber protocol, Aroclor 1254 and the PCB-153, PCB-52, and PCB-47 did not produce a positive response in male F344 rats (Hayes *et al.*, 1985). No nodules were apparent in animals receiving PCB-12 (dichloro-) or PCB-138 (trichloro-) as initiator, while PCB-3 (mono-chlorinated) induced clearly visible nodules in 50% of the exposed rats (Espandiari *et al.*, 2003). Thus less chlorinated PCBs seem to be able to initiate hepatocarcinogenesis in the rat, but in view of the small number of congeners tested, a clear structure–activity relationship could not be established.

A series of synthetic oxygenated metabolites of PCB-3 were studied with respect to focus formation in rat liver. Test compounds included the 2-OH-, 3-OH-, 4-OH-, 2,3-dihydroxyl-, 3,4-dihydroxyl-, 2,5-dihydroxyl-, 2,3-quinone, 3,4-quinone, and 2,5-quinone metabolites of PCB-3. The 4-OH- and 3,4-quinone metabolites significantly increased focus number and focus volume, while none of the other metabolites had a significant effect on either parameter (Espandiari *et al.*, 2004, 2005). The 3,4-*ortho*-quinone of PCB-3 was the initiating metabolite, and that PCB-3 is metabolized in rat liver in vivo to yield this ultimate carcinogenic species.

(f) Direct and indirect endocrine disruption

After the liver, the thyroid gland is the second major target of the toxicity of PCBs. In rats, exposure to PCBs produced an increase in the mass and/or volume of the thyroid gland, and in the number of thyroid neoplasms (Mayes *et al.*, 1998). Both these changes may be linked to the PCB-driven reduction in serum T4 concentrations, a commonly measured effect of PCBs (Knerr & Schrenk, 2006; Pearce & Braverman, 2009). Suggested mechanisms include: (a) PCB-induced alterations in the structure and function of the thyroid gland; (b) PCB-induced alterations in thyroid-hormone metabolism, biliary excretion of T4-glucuronide (Martin *et al.*, 2012), and effects on de-iodonase activity; and (c) interference with the transport of T4. OH-PCBs are competitors for the T4-binding site in the transport protein TTR (Brouwer *et al.*, 1998; Gutleb *et al.*, 2010), with binding affinities up to an order of magnitude stronger than that of the natural ligand, T4 (Chauhan *et al.*, 2000). The sulfate conjugates of OH-PCBs also bind to TTR, with affinities similar to that of T4 (Grimm *et al.*, 2012).

Circulating steroid and thyroid hormones are sulfated by sulfotransferases, which is an important feature of their homeostatic control. Since OH-PCBs are both substrates and inhibitors of these enzymes, they may directly influence the circulating levels of steroids and thyroid hormones by affecting the rates of sulfation (Schuur *et al.*, 1998b, c; Kester *et al.*, 2000; Liu *et al.*, 2009; Ekuase *et al.*, 2011).

OH-PCBs have both estrogenic and anti-estrogenic properties (see Section 4.3.3).

4.6.2 Receptor-driven effects of PCBs and their metabolites

PCBs and their metabolites may bind to and activate a wide range of cellular receptors, as illustrated in Table 4.8. Activation of AhR, CAR, and other receptors results in extensive modulation of expression of genes involved in cell-cycle control, cell proliferation, apoptosis, cell–cell communication, cell adhesion and migration, the pro-inflammatory response, and endogenous metabolism. Deregulation of those processes is directly associated with carcinogenesis, i.e. tumour promotion and progression (see Sections 4.3.1 and 4.3.2). The most significant events include modulation of cell proliferation, suppression of apoptosis (i.e. survival of initiated cells), impaired plasma-membrane function and plasma membrane-mediated signal transduction (i.e. modulation of cell plasticity, cell–cell communication, adhesion and migration) and induction of proinflammatory mediators. In part, induction of cell proliferation may be a consequence of cytotoxicity and tissue injury – after biotransformation processes, oxidative stress, etc. – and is considered regenerative cell proliferation (see Section 4.3.2).

In addition, disruption of endocrine function, due to interaction of PCBs or their metabolites with steroid and thyroid hormone receptors and serum proteins, or as a result of changes in biosynthesis and catabolism of steroids, may be linked to cancer development in hormone target tissues (see Section 4.3.3). Receptor-mediated gene expression is also linked to induction of proinflammatory processes and immunotoxic effects (see Sections 4.3.4 and 4.3.5).

(a) Induction of xenobiotic metabolism

Many highly chlorinated PCB congeners are potent inducers of enzymes involved in the metabolism of xenobiotics (Parkinson *et al.*, 1983) via binding to AhR (Bandiera *et al.*, 1982). Efficient induction has been reported of a wide spectrum of enzymes, notably certain CYP-dependent mono-oxygenases of the CYP1A subfamily, as well as CYP2Bs and microsomal epoxide hydrolase (Parkinson *et al.*, 1983), glutathione transferases, and UDP-glucuronosyl transferases (for a review, see Parkinson *et al.*, 1980).

Individual PCB congeners that showed the strongest binding to the AhR were identified as those in which the chlorines are in the *meta* and *para* positions of the phenyl rings in the absence of *ortho* chlorines (see Section 1.1.1). These PCBs are referred to as "coplanar" or "dioxin-like," typical examples being PCB-77, PCB-126, and PCB-169. Other PCBs, characterized by substitution in the *ortho* and *para* positions of the phenyl rings (e.g. PCB-153), activate CAR. PCBs in this group induce CYP2B1/2 and other enzymes, and as such resemble the drug phenobarbital (Parkinson *et al.*, 1983). Many PCBs that activate CAR also activate the pregnane X receptor (Holsapple *et al.*, 2006). PCBs that have one chlorine in the *ortho* position may be mixed-type inducers of CYPs, for example PCB-118, which induces members of the CYP1A and the CYP2B subfamilies.

Exposure to PCBs may alter the metabolic status in the liver, which will change the metabolism of endogenous or other exogenous compounds. For example, PCBs induce CYPs in the liver, which may redirect the metabolism of endogenous estrogen to more harmful estrogen catechols (Ho *et al.*, 2008), or generate ROS that produce estrogen quinones (Brown *et al.*, 2007).

(b) Immunomodulation

The biochemical events leading to the observed PCB-induced immunomodulation have not been completely elucidated. Studies on structure–activity relationships, and structure–toxicity relationships have demonstrated that some of the PCBs share a common mechanism of action with other structurally related halogenated aromatic hydrocarbons such as dioxins and dibenzofurans (Safe, 1990). These studies

Table 4.8 PCBs and metabolites as ligands for cellular and nuclear receptors

Receptor		Ligands	Gene or function affected	References
AhR	Aryl hydrocarbon	Coplanar, ***meta*-**, ***para*-**PCBs	CYP1A activation	Bandiera *et al.* (1982)
CAR	Constitutive androstane receptor	*Ortho*-, *para*-PCBs	CYP2B activation	Denomme *et al.* (1983), Al-Salman & Plant (2012)
PXR	Pregnane X receptor	Multi-*ortho*-PCBs, PCB-47, PCB-184; PCB-138, PCB-153, PCB-180, PCB-194	CYP3A activation	Schuetz *et al.* (1998), Al-Salman & Plant (2012)
PPAR	Peroxisome proliferator receptor	Coplanar, *meta*-, *para*-PCBs	CYP4A, repression	Hennig *et al.* (2005), Robertson *et al.* (2007)
RyR	Ryanodine receptor	Non-dioxin-like-PCBs (optimal configuration, multi-*ortho*, *para*-PCBs), OH-PCBs, catechols, $MeSO_2$-PCBs	Ca^{2+}-channel	Pessah *et al.* (2006)
ER	Estrogen receptor	Multi-*ortho*-PCBs, OH-PCBs	Agonism and antagonism	Connor *et al.* (1997), Arcaro *et al.* (1999); Bonefeld-Jørgensen *et al.* (2001), Plísková *et al.* (2005), Hamers *et al.* (2011)
AR	Androgen receptor	Multi-*ortho*-PCBs	Antagonism	Portigal *et al.* (2002), Fang *et al.* (2003); Schrader & Cooke (2003), Hamers *et al.* (2011)
PR	Progesterone receptor	OH-PCBs	Antagonism	Connor *et al.* (1997)
TH	Thyroid hormone	PCBs, OH-PCBs	Disruption of thyroid receptor-dependent gene expression	Gauger *et al.* (2004), Miyazaki *et al.* (2004)
DAT or VMAT	Dopamine active transporter or vesicular monoamine transporters	Coplanar and multi-*ortho*-PCBs	Decrease or increase in dopamine levels	Bemis & Seegal (2004), Richardson & Miller (2004), Seegal *et al.* (2005)
GR	Glucocorticoid receptor	$MeSO_2$-PCBs, OH-PCBs, PCB-28, PCB-153, PCB-118	Competitive antagonism	Johansson *et al.* (1998), Bovee *et al.* (2011), Antunes-Fernandes *et al.* (2011)

$MeSO_2$-PCB, methyl sulfonyl PCB; OH-PCB, hydroxylated PCB; PCB, polychlorinated biphenyl
Adapted from Ludewig *et al.* (2007)

indicated that certain immunotoxic effects seen with dioxin-like PCB congeners depend on the presence of AhR, which regulates the synthesis of a variety of proteins (Safe, 1990). AhR is present in several tissues and cells of the immune system as shown in rodents (e.g. Mason & Okey, 1982), in non-human primates (Van Der Burght *et al.*, 1998) and in humans (Hakkola *et al.*, 1997).

AhR is present in several tissues and cells of the immune system in animals and in humans. Binding of PCBs to AhR is a prerequisite for some of the immunotoxic effects of the DL-PCBs (reviewed in Silkworth *et al.*, 1984; Safe, 1990). TEFs were calculated for individual PCB congeners and several commercial PCB products, based on the suppression of the response in a challenge test against sheep erythrocytes (SRBC) – a parameter predictive of effects on humoral immunity (Davis & Safe, 1989, 1990). Highly chlorinated commercial PCB products, including Aroclors 1260, 1254, and 1248 have higher TEFs, while lower TEFs were calculated for the less chlorinated Aroclors 1242, 1016, and 1232.

Clearly some PCBs produce their immunotoxic effects by binding to AhR present in tissues and cells of the immune system, while others may follow different pathways and produce similar effects. Furthermore, individual congeners in commercial PCB products may antagonize each other's effects by mechanisms that have not been fully elucidated (see Section 4.3.4).

Overproduction of IL-6 has been shown to be responsible for the pathogenesis of inflammation-associated colorectal cancer (Waldner *et al.*, 2012). Furthermore, activation of NF-κB, a hallmark of inflammatory responses, plays a fundamental role in the formation and development of malignant tissue changes caused by inflammation, and is thought to function as a tumour promoter in inflammation-associated cancer (Pikarsky *et al.*, 2004; Karin, 2006).

(c) Interference with endogenous transport by PCBs and their metabolites

Endogenous substances such as vitamins, metals, steroids, and hormones are transported throughout the body by virtue of their binding to serum proteins. Substances that interfere with these processes can severely impair their tissue availability. Notable examples are the ability of PCB metabolites to interfere with vitamin A homeostasis and T4 transport (Grimm *et al.*, 2012), and steroid metabolism (see Section 4.3.3).

Overall, PCBs can induce formation of ROS, genotoxic effects, immune suppression, inflammatory responses, and endocrine effects to various extents and through different pathways. DL-PCBs exert their effects mainly through activation of AhR and the downstream cascade of related events; less chlorinated PCBs act more readily through metabolic activation and the ensuing effects involving their metabolites.

References

Abdel-Hamid FM, Moore JA, Matthews HB (1981). Comparative study of 3,4,3′,4′-tetrachlorobiphenyl in male and female rats and female monkeys. *J Toxicol Environ Health*, 7(2):181–91. doi:10.1080/15287398109529971 PMID:6785443

Adenugba A, Khan SA, Taylor-Robinson SD, Cox IJ, Toledano MB, Thillainayagam AV *et al.* (2009). Polychlorinated biphenyls in bile of patients with biliary tract cancer. *Chemosphere*, 76(6):841–6. doi:10.1016/j.chemosphere.2009.04.003 PMID:19419750

Ahlborg UG, Becking GC, Birnbaum LS, Brouwer A, Derks HJGM, Feeley M *et al.* (1994). Toxic equivalency factors for dioxin-like PCBs: Report on WHO-ECEH and IPCS consultation. *Chemosphere*, 28(6):1049–67. doi:10.1016/0045-6535(94)90324-7

Al-Anati L, Högberg J, Stenius U (2009). Non-dioxin-like-PCBs phosphorylate Mdm2 at Ser166 and attenuate the p53 response in HepG2 cells. *Chem Biol Interact*, 182(2–3):191–8. doi:10.1016/j.cbi.2009.09.004 PMID:19751709

Al-Anati L, Högberg J, Stenius U (2010). Non-dioxin-like PCBs interact with benzo[*a*]pyrene-induced p53-responses and inhibit apoptosis. *Toxicol Appl Pharmacol*, 249(2):166–77. doi:10.1016/j.taap.2010.09.004 PMID:20840854

Al-Sabti K (1985). Carcinogenic-mutagenic chemicals induced chromosomal aberrations in the kidney cells of three cyprinids. *Comp Biochem Physiol C*, 82(2):489–93. doi:10.1016/0742-8413(85)90198-7 PMID:2866926

Al-Sabti K (1986). Clastogenic effects of five carcinogenic-mutagenic chemicals on the cells of the common carp, Cyprinus carpio L. *Comp Biochem Physiol C*, 85(1):5–9. doi:10.1016/0742-8413(86)90043-5 PMID:2877805

Al-Salman F, Plant N (2012). Non-coplanar polychlorinated biphenyls (PCBs) are direct agonists for the human pregnane-X receptor and constitutive androstane receptor, and activate target gene expression in a tissue-specific manner. *Toxicol Appl Pharmacol*, 263(1):7–13. doi:10.1016/j.taap.2012.05.016 PMID:22664347

Albro PW, Fishbein L (1972). Intestinal absorption of polychlorinated biphenyls in rats. *Bull Environ Contam Toxicol*, 8(1):26–31. doi:10.1007/BF01684500 PMID:4630020

Allen JR, Abrahamson LJ (1973). Morphological and biochemical changes in the liver of rats fed polychlorinated biphenyls. *Arch Environ Contam Toxicol*, 1(3):265–80. doi:10.1007/BF01985749 PMID:4130176

Allen JR, Barsotti DA (1976). The effects of transplacental and mammary movement of PCBs on infant rhesus monkeys. *Toxicology*, 6(3):331–40. doi:10.1016/0300-483X(76)90037-8 PMID:825993

Allen JR, Norback DH (1976). Pathobiological responses of primates to polychlorinated biphenyl exposure. In: Proceedings of the National Conference on Polychlorinated Biphenyls, Chicago, 1975. EPA-560/6–75–004. Washington (DC): Environmental Protection Agency, pp. 43–49

Allen JR, Norback DH, Hsu IC (1974). Tissue modifications in monkeys as related to absorption, distribution, and excretion of polychlorinated biphenyls. *Arch Environ Contam Toxicol*, 2(1):86–95. doi:10.1007/BF01985803 PMID:4208176

Althaus FR, Lawrence SD, Sattler GL, Longfellow DG, Pitot HC (1982). Chemical quantification of unscheduled DNA synthesis in cultured hepatocytes as an assay for the rapid screening of potential chemical carcinogens. *Cancer Res*, 42(8):3010–5. PMID:7093950

Alvares AP, Kappas A (1975). Induction of aryl hydrocarbon hydroxylase by polychlorinated biphenyls in the foeto-placental unit and neonatal livers during lactation. *FEBS Lett*, 50(2):172–4. doi:10.1016/0014-5793(75)80482-0 PMID:803459

Amaro AR, Oakley GG, Bauer U, Spielmann HP, Robertson LW (1996). Metabolic activation of PCBs to quinones: reactivity toward nitrogen and sulfur nucleophiles and influence of superoxide dismutase. *Chem Res Toxicol*, 9(3):623–9. doi:10.1021/tx950117e PMID:8728508

Anderson LM, Fox SD, Riggs CW, Issaq HJ (1993). Selective retention of polychlorinated biphenyl congeners in lung and liver after single-dose exposure of infant mice to Aroclor 1254. *J Environ Pathol Toxicol Oncol*, 12(1):3–16. PMID:8459365

Antunes-Fernandes EC, Bovee TF, Daamen FE, Helsdingen RJ, van den Berg M, van Duursen MB (2011). Some OH-PCBs are more potent inhibitors of aromatase activity and (anti-) glucocorticoids than non-dioxin like (NDL)-PCBs and $MeSO_2$-PCBs. *Toxicol Lett*, 206(2):158–65. doi:10.1016/j.toxlet.2011.07.008 PMID:21782008

Appelgren H, Hedenskog M, Sandström C, Cederberg H, Rannug U (1999). Polychlorinated biphenyls induce meiotic length mutations at the human minisatellite MS32 in yeast. *Environ Mol Mutagen*, 34(4):285–90. doi:10.1002/(SICI)1098-2280(1999)34:4<285::AID-EM9>3.0.CO;2-5 PMID:10618177

Arcaro KF, Vakharia DD, Yang Y, Gierthy JF (1998). Lack of synergy by mixtures of weakly estrogenic hydroxylated polychlorinated biphenyls and pesticides. *Environ Health Perspect*, 106:Suppl 4: 1041–6. doi:10.1289/ehp.98106s41041 PMID:9703490

Arcaro KF, Yi L, Seegal RF, Vakharia DD, Yang Y, Spink DC *et al.* (1999). 2,2′,6,6′-Tetrachlorobiphenyl is estrogenic in vitro and in vivo. *J Cell Biochem*, 72(1):94–102. doi:10.1002/(SICI)1097-4644(19990101)72:1<94::AID-JCB10>3.0.CO;2-Y PMID:10025670

Arif JM, Lehmler HJ, Robertson LW, Gupta RC (2003). Interaction of benzoquinone- and hydroquinone-derivatives of lower chlorinated biphenyls with DNA and nucleotides in vitro. *Chem Biol Interact*, 142(3):307–16. doi:10.1016/S0009-2797(02)00141-2 PMID:12453668

Ariyoshi N, Oguri K, Koga N, Yoshimura H, Funae Y (1995). Metabolism of highly persistent PCB congener, 2,4,5,2′,4′,5′-hexachlorobiphenyl, by human CYP2B6. *Biochem Biophys Res Commun*, 212(2):455–60. doi:10.1006/bbrc.1995.1991 PMID:7626059

Arnold DL, Bryce F, Karpinski K, Mes J, Fernie S, Tryphonas H *et al.* (1993). Toxicological consequences of Aroclor 1254 ingestion by female rhesus (Macaca mulatta) monkeys. Part 1B. Prebreeding phase: clinical and analytical laboratory findings. *Food Chem Toxicol*, 31(11):811–24. doi:10.1016/0278-6915(93)90219-O PMID:8258410

Arnold DL, Bryce F, McGuire PF, Stapley R, Tanner JR, Wrenshall E *et al.* (1995). Toxicological consequences of Aroclor 1254 ingestion by female rhesus (Macaca mulatta) monkeys. Part 2. Reproduction and infant findings. *Food Chem Toxicol*, 33(6):457–74. doi:10.1016/0278-6915(95)00018-W PMID:7797173

Arnold DL, Bryce F, Mes J, Tryphonas H, Hayward S, Malcolm S (1999). Toxicological consequences of feeding PCB congeners to infant rhesus (Macaca mulatta) and cynomolgus (Macaca fascicularis) monkeys. *Food Chem Toxicol*, 37(2–3):153–67. doi:10.1016/S0278-6915(98)00120-3 PMID:10227739

Arnold DL, Mes J, Bryce F, Karpinski K, Bickis MG, Zawidzka ZZ *et al.* (1990). A pilot study on the effects of Aroclor 1254 ingestion by rhesus and cynomolgus monkeys as a model for human ingestion of PCBs. *Food Chem Toxicol*, 28(12):847–57. doi:10.1016/0278-6915(90)90058-U PMID:2125970

ATSDR (1994). *Toxicological profile for chlorodibenzofurans*. Atlanta (GA): U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

ATSDR (2000). Toxicological profile for polychlorinated biphenyls (update). Atlanta (GA): Agency for Toxic Substances and Disease Registry. Available from: http://www.atsdr.cdc.gov/ToxProfiles/tp17.pdf, accessed 6 May 2015

Aubrecht J, Rugo R, Schiestl RH (1995). Carcinogens induce intrachromosomal recombination in human cells. *Carcinogenesis*, 16(11):2841–6. doi:10.1093/carcin/16.11.2841 PMID:7586207

Bager Y, Kato Y, Kenne K, Wärngård L (1997). The ability to alter the gap junction protein expression outside GST-P positive foci in liver of rats was associated to the tumour promotion potency of different polychlorinated biphenyls. *Chem Biol Interact*, 103(3):199–212. doi:10.1016/S0009-2797(97)03759-9 PMID:9134010

Bakke JE, Bergman AL, Larsen GL (1982). Metabolism of 2,4′,5-trichlorobiphenyl by the mercapturic acid pathway. *Science*, 217(4560):645–7. doi:10.1126/science.6806905 PMID:6806905

Bandiera S, Safe S, Okey AB (1982). Binding of polychlorinated biphenyls classified as either phenobarbitone-, 3-methylcholanthrene- or mixed-type inducers to cytosolic Ah receptor. *Chem Biol Interact*, 39(3):259–77. doi:10.1016/0009-2797(82)90045-X PMID:6804100

Beischlag TV, Taylor RT, Rose DW, Yoon D, Chen Y, Lee WH *et al.* (2004). Recruitment of thyroid hormone receptor/retinoblastoma-interacting protein 230 by the aryl hydrocarbon receptor nuclear translocator is required for the transcriptional response to both dioxin and hypoxia. *J Biol Chem*, 279(52):54620–8. doi:10.1074/jbc.M410456200 PMID:15485806

Belpaeme K, Delbeke K, Zhu L, Kirsch-Volders M (1996a). Cytogenetic studies of PCB77 on brown trout (Salmo trutta fario) using the micronucleus test and the alkaline comet assay. *Mutagenesis*, 11(5):485–92. doi:10.1093/mutage/11.5.485 PMID:8921510

Belpaeme K, Delbeke K, Zhu L, Kirsch-Volders M (1996b). PCBs do not induce DNA breakage in vitro in human lymphocytes. *Mutagenesis*, 11(4):383–9. doi:10.1093/mutage/11.4.383 PMID:8671762

Bemis JC, Seegal RF (2004). PCB-induced inhibition of the vesicular monoamine transporter predicts reductions in synaptosomal dopamine content. *Toxicol Sci*, 80(2):288–95. doi:10.1093/toxsci/kfh153 PMID:15115888

Bender RP, Lehmler HJ, Robertson LW, Ludewig G, Osheroff N (2006). Polychlorinated biphenyl quinone metabolites poison human topoisomerase IIalpha: altering enzyme function by blocking the N-terminal protein gate. *Biochemistry*, 45(33):10140–52. doi:10.1021/bi0524666 PMID:16906772

Bender RP, Osheroff N (2007). Mutation of cysteine residue 455 to alanine in human topoisomerase IIalpha confers hypersensitivity to quinones: enhancing DNA scission by closing the N-terminal protein gate. *Chem Res Toxicol*, 20(6):975–81. doi:10.1021/tx700062t PMID:17516663

Beran M, Brandt I, Slanina P (1983). Distribution and effect of some polychlorinated biphenyls in the hemopoietic tissues. *J Toxicol Environ Health*, 12(4–6):521–32. doi:10.1080/15287398309530446 PMID:6422048

Berberian I, Chen LC, Robinson FR, Glauert HP, Chow CK, Robertson LW (1995). Effect of dietary retinyl palmitate on the promotion of altered hepatic foci by 3,3′,4,4′-tetrachlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl in rats initiated with diethylnitrosamine. *Carcinogenesis*, 16(2):393–8. doi:10.1093/carcin/16.2.393 PMID:7859372

Bergman Å, Larsen GL, Bakke JE (1982). Biliary secretion, retention and excretion of five 14C-labelled polychlorinated biphenyls in the rat. *Chemosphere*, 11(3):249–53. doi:10.1016/0045-6535(82)90148-5

Bertazzi PA, Riboldi L, Pesatori A, Radice L, Zocchetti C (1987). Cancer mortality of capacitor manufacturing workers. *Am J Ind Med*, 11(2):165–76. doi:10.1002/ajim.4700110206 PMID:3103429

Biegel L, Harris M, Davis D, Rosengren R, Safe L, Safe S (1989). 2,2′,4,4′,5,5′-hexachlorobiphenyl as a 2,3,7,8-tetrachlorodibenzo-p-dioxin antagonist in C57BL/6J mice. *Toxicol Appl Pharmacol*, 97(3):561–71. doi:10.1016/0041-008X(89)90261-5 PMID:2558429

Blazak WF, Marcum JB (1975). Attempts to induce chromosomal breakage in chicken embryos with Aroclor 1242. *Poult Sci*, 54(1):310–2. doi:10.3382/ps.0540310 PMID:806068

Block WD, Cornish HH (1959). Metabolism of biphenyl and 4-chlorobiphenyl in the rabbit. *J Biol Chem*, 234:3301–3302.

Bock KW, Köhle C (2006). Ah receptor: dioxin-mediated toxic responses as hints to deregulated physiologic functions. *Biochem Pharmacol*, 72(4):393–404. doi:10.1016/j.bcp.2006.01.017 PMID:16545780

Bohnenberger S, Wagner B, Schmitz HJ, Schrenk D (2001). Inhibition of apoptosis in rat hepatocytes treated with 'non-dioxin-like' polychlorinated biphenyls. *Carcinogenesis*, 22(10):1601–6. doi:10.1093/carcin/22.10.1601 PMID:11576998

Bonefeld-Jørgensen EC (2010). Biomonitoring in Greenland: human biomarkers of exposure and effects - a short review. *Rural Remote Health*, 10(2):1362 PMID:20572746

Bonefeld-Jørgensen EC, Andersen HR, Rasmussen TH, Vinggaard AM (2001). Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. *Toxicology*, 158(3):141–53. doi:10.1016/S0300-483X(00)00368-1 PMID:11275356

Bonefeld-Jørgensen EC, Long M, Bossi R, Ayotte P, Asmund G, Krüger T *et al.* (2011). Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: a case control study. *Environ Health*, 10(1):88 doi:10.1186/1476-069X-10-88 PMID:21978366

Bonnyns M, Bastomsky CH (1976). Polychlorinated biphenyl-induced modification of lymphocyte response to plant mitogens in rats. *Experientia*, 32(4):522–3. doi:10.1007/BF01920835 PMID:817932

Borlak J, Hock A, Hansen T, Richter E (2003). DNA adducts in cultures of polychlorinated biphenyl-treated human hepatocytes. *Toxicol Appl Pharmacol*, 188(2):81–91. doi:10.1016/S0041-008X(02)00075-3 PMID:12691726

Bovee TFH, Helsdingen RJR, Hamers ARM, Brouwer BA, Nielen MW (2011). Recombinant cell bioassays for the detection of (gluco)corticosteroids and endocrine-disrupting potencies of several environmental PCB contaminants. *Anal Bioanal Chem*, 401(3):873–82. doi:10.1007/s00216-011-5162-5 PMID:21681646

Brix AE, Jokinen MP, Walker NJ, Sells DM, Nyska A (2004). Characterization of bronchiolar metaplasia of the alveolar epithelium in female Sprague-Dawley rats exposed to 3,3′,4,4′,5-pentachlorobiphenyl (PCB126). *Toxicol Pathol*, 32(3):333–7. doi:10.1080/01926230490431817 PMID:15204975

Brody JG, Moysich KB, Humblet O, Attfield KR, Beehler GP, Rudel RA (2007). Environmental pollutants and breast cancer: epidemiologic studies. *Cancer*, 109(12):Suppl: 2667–711. doi:10.1002/cncr.22655 PMID:17503436

Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E *et al.* (1998). Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicol Ind Health*, 14(1–2):59–84. doi:10.1177/074823379801400107 PMID:9460170

Brown AP, Olivero-Verbel J, Holdan WL, Ganey PE (1998). Neutrophil activation by polychlorinated biphenyls: structure-activity relationship. *Toxicol Sci*, 46(2):308–16. PMID:10048134

Brown JF Jr, Mayes BA, Silkworth JB, Hamilton SB (2007). Polychlorinated biphenyls modulated tumorigenesis in Sprague Dawley rats: correlation with mixed function oxidase activities and superoxide (O2*) formation potentials and implied mode of action. *Toxicol Sci*, 98(2):375–94. doi:10.1093/toxsci/kfm122 PMID:17510085

Bruce WR, Heddle JA (1979). The mutagenic activity of 61 agents as determined by the micronucleus, Salmonella, and sperm abnormality assays. *Can J Genet Cytol*, 21(3):319–34. PMID:393369

Buchmann A, Ziegler S, Wolf A, Robertson LW, Durham SK, Schwarz M (1991). Effects of polychlorinated biphenyls in rat liver: correlation between primary subcellular effects and promoting activity. *Toxicol Appl Pharmacol*, 111(3):454–68. doi:10.1016/0041-008X(91)90250-I PMID:1684070

Buckman AH, Brown SB, Small J, Muir DC, Parrott J, Solomon KR *et al.* (2007). Role of temperature and enzyme induction in the biotransformation of polychlorinated biphenyls and bioformation of hydroxylated polychlorinated biphenyls by rainbow trout (Oncorhynchus mykiss). *Environ Sci Technol*, 41(11):3856–63. doi:10.1021/es062437y PMID:17612160

Butterworth FM, Pandey P, McGowen RM, Ali-Sadat S, Walia S (1995). Genotoxicity of polychlorinated biphenyls (PCBs): recombinogenesis by biotransformation products. *Mutat Res*, 342(1–2):61–9. doi:10.1016/0165-1218(95)90090-X PMID:7885394

Cao Y, Winneke G, Wilhelm M, Wittsiepe J, Lemm F, Fürst P *et al.* (2008). Environmental exposure to dioxins and polychlorinated biphenyls reduce levels of gonadal hormones in newborns: results from the Duisburg cohort study. *Int J Hyg Environ Health*, 211(1–2):30–9. doi:10.1016/j.ijheh.2007.04.005 PMID:17660003

Carlson EA, McCulloch C, Koganti A, Goodwin SB, Sutter TR, Silkworth JB (2009). Divergent transcriptomic responses to aryl hydrocarbon receptor agonists between rat and human primary hepatocytes. *Toxicol Sci*, 112(1):257–72. doi:10.1093/toxsci/kfp200 PMID:19692669

Carter JW, Clancy J Jr (1980). Acutely administered polychlorinated biphenyls (PCBs) decrease splenic cellularity but increase its ability to cause graft-versus-host reactions in BALB/c mice. *Immunopharmacology*, 2(4):341–7. doi:10.1016/0162-3109(80)90018-1 PMID:6780487

Casati L, Sendra R, Colciago A, Negri-Cesi P, Berdasco M, Esteller M *et al.* (2012). Polychlorinated biphenyls affect histone modification pattern in early development of rats: a role for androgen receptor-dependent modulation? *Epigenomics*, 4(1):101–12. doi:10.2217/epi.11.110 PMID:22332662

Casey AC, Berger DF, Lombardo JP, Hunt A, Quimby F (1999). Aroclor 1242 inhalation and ingestion by Sprague-Dawley rats. *J Toxicol Environ Health A*, 56(5):311–42. doi:10.1080/009841099158033 PMID:10094245

Chadwick RW, George SE, Kohan MJ, Williams RW, Allison JC, Hayes YO *et al.* (1993). Potentiation of 2,6-dinitrotoluene genotoxicity in Fischer-344 rats by pretreatment with Aroclor 1254. *Toxicology*, 80(2–3):153–71. doi:10.1016/0300-483X(93)90178-U PMID:8327998

Charlier CJ, Albert AI, Zhang L, Dubois NG, Plomteux GJ (2004). Polychlorinated biphenyls contamination in women with breast cancer. *Clin Chim Acta*,

347(1–2):177–81. doi:10.1016/j.cccn.2004.04.025 PMID:15313156

Chaudhuri L, Sarsour EH, Kalen AL, Aykin-Burns N, Spitz DR, Goswami PC (2010). Polychlorinated biphenyl induced ROS signalling delays the entry of quiescent human breast epithelial cells into the proliferative cycle. *Free Radic Biol Med*, 49(1):40–9. doi:10.1016/j.freeradbiomed.2010.03.012 PMID:20307652

Chauhan KR, Kodavanti PR, McKinney JD (2000). Assessing the role of ortho-substitution on polychlorinated biphenyl binding to transthyretin, a thyroxine transport protein. *Toxicol Appl Pharmacol*, 162(1):10–21. doi:10.1006/taap.1999.8826 PMID:10631123

Cheek AO, Kow K, Chen J, McLachlan JA (1999). Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. *Environ Health Perspect*, 107(4):273–8. doi:10.1289/ehp.99107273 PMID:10090705

Chia VM, Li Y, Quraishi SM, Graubard BI, Figueroa JD, Weber JP *et al.* (2010). Effect modification of endocrine disruptors and testicular germ cell tumour risk by hormone-metabolizing genes. *Int J Androl*, 33(4):588–96. PMID:19627379

Choi W, Eum SY, Lee YW, Hennig B, Robertson LW, Toborek M (2003). PCB 104-induced proinflammatory reactions in human vascular endothelial cells: relationship to cancer metastasis and atherogenesis. *Toxicol Sci*, 75(1):47–56. doi:10.1093/toxsci/kfg149 PMID:12805654

Chu I, Poon R, Yagminas A, Lecavalier P, Håkansson H, Valli VE *et al.* (1998). Subchronic toxicity of PCB 105 (2,3,3′,4,4′-pentachlorobiphenyl) in rats. *J Appl Toxicol*, 18(4):285–92. doi:10.1002/(SICI)1099-1263(199807/08)18:4<285::AID-JAT510>3.0.CO;2-9 PMID:9719429

Chu I, Villeneuve DC, Yagminas A, Lecavalier P, Håkansson H, Ahlborg UG *et al.* (1995). Toxicity of PCB 77 (3,3′,4,4′-tetrachlorobiphenyl) and PCB 118 (2,3′,4,4’5-pentachlorobiphenyl) in the rat following subchronic dietary exposure. *Fundam Appl Toxicol*, 26(2):282–92. doi:10.1006/faat.1995.1099 PMID:7589917

Chu I, Villeneuve DC, Yagminas A, LeCavalier P, Poon R, Feeley M *et al.* (1994). Subchronic toxicity of 3,3′,4,4′,5-pentachlorobiphenyl in the rat. I. Clinical, biochemical, hematological, and histopathological changes. *Fundam Appl Toxicol*, 22(3):457–68. doi:10.1006/faat.1994.1051 PMID:8050640

Chu I, Villeneuve DC, Yagminas A, Lecavalier P, Poon R, Feeley M *et al.* (1996b). Toxicity of 2,2′,4,4′,5,5′-hexachlorobiphenyl in rats: effects following 90-day oral exposure. *J Appl Toxicol*, 16(2):121–8. doi:10.1002/(SICI)1099-1263(199603)16:2<121::AID-JAT320>3.0.CO;2-G PMID:8935785

Chu I, Villeneuve DC, Yagminas A, Lecavalier P, Poon R, Håkansson H *et al.* (1996c). Toxicity of 2,4,4′-trichlorobiphenyl in rats following 90-day dietary exposure. *J Toxicol Environ Health*, 49(3):301–18. PMID:8876656

Chu S, Xi Z, Xu X, Zhang Y, Xu Y (1996a). Induction of micronuclei in peripheral erythrocytes of Misgurnus anguillicaudatus by polychlorinated biphenyls. *Bull Environ Contam Toxicol*, 57(2):179–82. doi:10.1007/s001289900172 PMID:8661895

Cillo F, de Eguileor M, Gandolfi F, Brevini TA (2007). Aroclor-1254 affects mRNA polyadenylation, translational activation, cell morphology, and DNA integrity of rat primary prostate cells. *Endocr Relat Cancer*, 14(2):257–66. doi:10.1677/ERC-06-0081 PMID:17639042

Cleland GB, McElroy PJ, Sonstegard RA (1989). Immunomodulation in C57Bl/6 mice following consumption of halogenated aromatic hydrocarbon-contaminated coho salmon (Oncorhynchus kisutch) from Lake Ontario. *J Toxicol Environ Health*, 27(4):477–86. doi:10.1080/15287398909531317 PMID:2527307

Coenraads PJ, Brouwer A, Olie K, Tang N (1994). Chloracne. Some recent issues. *Dermatol Clin*, 12(3):569–76. PMID:7923954

Connor K, Ramamoorthy K, Moore M, Mustain M, Chen I, Safe S *et al.* (1997). Hydroxylated polychlorinated biphenyls (PCBs) as estrogens and antiestrogens: structure-activity relationships. *Toxicol Appl Pharmacol*, 145(1):111–23. doi:10.1006/taap.1997.8169 PMID:9221830

Costa C, Catania S, De Pasquale R, Stancanelli R, Scribano GM, Melchini A (2010). Exposure of human skin to benzo[a]pyrene: role of CYP1A1 and aryl hydrocarbon receptor in oxidative stress generation. *Toxicology*, 271(3):83–6. doi:10.1016/j.tox.2010.02.014 PMID:20307623

Crinnion WJ (2011). Polychlorinated biphenyls: persistent pollutants with immunological, neurological, and endocrinological consequences. *Altern Med Rev*, 16(1):5–13. PMID:21438643

Curley A, Burse VW, Grim ME (1973). Polychlorinated biphenyls: evidence of transplacental passage in the Sherman rat. *Food Cosmet Toxicol*, 11(3):471–6. doi:10.1016/0015-6264(73)90013-8 PMID:4199498

Curran CP, Vorhees CV, Williams MT, Genter MB, Miller ML, Nebert DW (2011). In utero and lactational exposure to a complex mixture of polychlorinated biphenyls: toxicity in pups dependent on the Cyp1a2 and Ahr genotypes. *Toxicol Sci*, 119(1):189–208. doi:10.1093/toxsci/kfq314 PMID:20961953

Dahl P, Lindström G, Wiberg K, Rappe C (1995). Absorption of polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans by breast-fed infants. *Chemosphere*, 30(12):2297–306. doi:10.1016/0045-6535(95)00102-E PMID:7620852

Daidoji T, Gozu K, Iwano H, Inoue H, Yokota H (2005). UDP-glucuronosyltransferase isoforms catalyzing glucuronidation of hydroxy-polychlorinated biphenyls in rat. *Drug Metab Dispos*, 33(10):1466–76. doi:10.1124/dmd.105.004416 PMID:16006569

Dallaire R, Muckle G, Dewailly E, Jacobson SW, Jacobson JL, Sandanger TM *et al.* (2009). Thyroid hormone levels of pregnant inuit women and their infants exposed to environmental contaminants. *Environ Health Perspect*, 117(6):1014–20. doi:10.1289/ehp.0800219 PMID:19590699

Davies R, Clothier B, Smith AG (2000). Mutation frequency in the lacI gene of liver DNA from lambda/lacI transgenic mice following the interaction of PCBs with iron causing hepatic cancer and porphyria. *Mutagenesis*, 15(5):379–83. doi:10.1093/mutage/15.5.379 PMID:10970442

Davis D, Safe S (1989). Dose-response immunotoxicities of commercial polychlorinated biphenyls (PCBs) and their interaction with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Lett*, 48(1):35–43. doi:10.1016/0378-4274(89)90183-5 PMID:2501913

Davis D, Safe S (1990). Interactions of 2,3,7,8-TCDD and PCB mixtures/congeners immunotoxicity studies. *Chemosphere*, 20(7–9):1141–6. doi:10.1016/0045-6535(90)90234-K

De Coster S, Koppen G, Bracke M, Schroijen C, Den Hond E, Nelen V *et al.* (2008). Pollutant effects on genotoxic parameters and tumor-associated protein levels in adults: a cross sectional study. *Environ Health*, 7(1):26 doi:10.1186/1476-069X-7-26 PMID:18522717

De Swart RL, Ross PS, Vedder LJ *et al.* (1994). Impairment of immune function in harbor seals (*Phoca vitulina*) feeding on fish from polluted waters. *Ambio*, 23:155–159.

Dean CE Jr, Benjamin SA, Chubb LS, Tessari JD, Keefe TJ (2002). Nonadditive hepatic tumor promoting effects by a mixture of two structurally different polychlorinated biphenyls in female rat livers. *Toxicol Sci*, 66(1):54–61. doi:10.1093/toxsci/66.1.54 PMID:11861972

Dedon PC, Plastaras JP, Rouzer CA, Marnett LJ (1998). Indirect mutagenesis by oxidative DNA damage: formation of the pyrimidopurinone adduct of deoxyguanosine by base propenal. *Proc Natl Acad Sci USA*, 95(19):11113–6. doi:10.1073/pnas.95.19.11113 PMID:9736698

Deml E, Oesterle D (1982). Sex-dependent promoting effect of polychlorinated biphenyls on enzyme-altered islands induced by diethylnitrosamine in rat liver. *Carcinogenesis*, 3(12):1449–52. doi:10.1093/carcin/3.12.1449 PMID:6217918

Deml E, Oesterle D (1987). Dose-response of promotion by polychlorinated biphenyls and chloroform in rat liver foci bioassay. *Arch Toxicol*, 60(1–3):209–11. doi:10.1007/BF00296982 PMID:2887150

Deml E, Oesterle D, Wiebel FJ, Wolff T (1985). Correlation between promotion of enzyme-deficient islands and induction of monooxygenase activities by halogenated hydrocarbons in rat liver. *Food Chem Toxicol*, 23(9):880 doi:10.1016/0278-6915(85)90327-8

Denomme MA, Bandiera S, Lambert I, Copp L, Safe L, Safe S (1983). Polychlorinated biphenyls as phenobarbitone-type inducers of microsomal enzymes. Structure-activity relationships for a series of 2,4-dichloro-substituted congeners. *Biochem Pharmacol*, 32(19):2955–63. doi:10.1016/0006-2952(83)90402-1 PMID:6414484

Dere E, Lee AW, Burgoon LD, Zacharewski TR (2011). Differences in TCDD-elicited gene expression profiles in human HepG2, mouse Hepa1c1c7 and rat H4IIE hepatoma cells. *BMC Genomics*, 12(1):193 doi:10.1186/1471-2164-12-193 PMID:21496263

Desaulniers D, Xiao GH, Lian H, Feng YL, Zhu J, Nakai J *et al.* (2009). Effects of mixtures of polychlorinated biphenyls, methylmercury, and organochlorine pesticides on hepatic DNA methylation in prepubertal female Sprague-Dawley rats. *Int J Toxicol*, 28(4):294–307. doi:10.1177/1091581809337918 PMID:19636072

Deutsche Forschungsgemeinschaft (1988). [Testing of residues in food. Notification XIII.] Verlag Chemic, Weinheim, page 24. [In German]

Dewailly E, Mulvad G, Pedersen HS, Ayotte P, Demers A, Weber JP *et al.* (1999). Concentration of organochlorines in human brain, liver, and adipose tissue autopsy samples from Greenland. *Environ Health Perspect*, 107(10):823–8. doi:10.1289/ehp.99107823 PMID:10504150

Dhakal K, He X, Lehmler HJ, Teesch LM, Duffel MW, Robertson LW (2012). Identification of sulfated metabolites of 4-chlorobiphenyl (PCB3) in the serum and urine of male rats. *Chem Res Toxicol*, 25(12):2796–804. doi:10.1021/tx300416v PMID:23137097

Dietz R, Heide-Jrgensen MP, Harkonen T (1989). Mass deaths of harbor seals (*Phoca vitulina*) in Europe. *Ambio*, 18:258–264.

DiGiovanni J, Viaje A, Berry DL, Slaga TJ, Juchau MR (1977). Tumor-initiating ability of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and Arochlor 1254 in the two-stage system of mouse skin carcinogenesis. *Bull Environ Contam Toxicol*, 18(5):552–7. doi:10.1007/BF01684000 PMID:412534

Dikshith TS, Rockwood W, Abraham R, Coulston F (1975). Effects of a polychlorinated biphenyl (Aroclor 1254) on rat testis. *Exp Mol Pathol*, 22(3):376–85. doi:10.1016/0014-4800(75)90082-9 PMID:805060

Dubois M, Pfohl-Leszkowicz A, Grosse Y, Kremers P (1995). DNA adducts and P450 induction in human, rat and avian liver cells after exposure to polychlorobiphenyls. *Mutat Res*, 345(3–4):181–90. doi:10.1016/0165-1218(95)90053-5 PMID:8552139

Duffy-Whritenour JE, Kurtzman RZ, Kennedy S, Zelikoff JT (2010). Non-coplanar polychlorinated biphenyl (PCB)-induced immunotoxicity is coincident with

alterations in the serotonergic system. *J Immunotoxicol*, 7(4):318–26. doi:10.3109/1547691X.2010.512277 PMID:20843273

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K *et al.* (1984). Reproducibility of microbial mutagenicity assays: I. Tests with Salmonella typhimurium and Escherichia coli using a standardized protocol. *Environ Mutagen*, 6(S2):Suppl 2: 1–251. doi:10.1002/em.2860060702 PMID:6394312

Dutta SK, Mitra PS, Ghosh S, Zang S, Sonneborn D, Hertz-Picciotto I *et al.* (2012). Differential gene expression and a functional analysis of PCB-exposed children: understanding disease and disorder development. *Environ Int*, 40:143–54. doi:10.1016/j.envint.2011.07.008 PMID:21855147

Ekuase EJ, Liu Y, Lehmler HJ, Robertson LW, Duffel MW (2011). Structure-activity relationships for hydroxylated polychlorinated biphenyls as inhibitors of the sulfation of dehydroepiandrosterone catalyzed by human hydroxysteroid sulfotransferase SULT2A1. *Chem Res Toxicol*, 24(10):1720–8. doi:10.1021/tx200260h PMID:21913674

Elferink CJ (2003). Aryl hydrocarbon receptor-mediated cell cycle control. *Prog Cell Cycle Res*, 5:261–7. PMID:14593720

Elo O, Vuojolahti P, Janhunen H, Rantanen J (1985). Recent PCB accidents in Finland. *Environ Health Perspect*, 60:315–9. doi:10.1289/ehp.8560315 PMID:3928359

Emmett EA, Maroni M, Jefferys J, Schmith J, Levin BK, Alvares A (1988b). Studies of transformer repair workers exposed to PCBs: II. Results of clinical laboratory investigations. *Am J Ind Med*, 14(1):47–62. doi:10.1002/ajim.4700140107 PMID:3136647

Emmett EA, Maroni M, Schmith JM, Levin BK, Jefferys J (1988a). Studies of transformer repair workers exposed to PCBs: I. Study design, PCB concentrations, questionnaire, and clinical examination results. *Am J Ind Med*, 13(4):415–27. doi:10.1002/ajim.4700130402 PMID:3129934

Endo F, Monsees TK, Akaza H, Schill WB, Pflieger-Bruss S (2003). Effects of single non-ortho, mono-ortho, and di-ortho chlorinated biphenyls on cell functions and proliferation of the human prostatic carcinoma cell line, LNCaP. *Reprod Toxicol*, 17(2):229–36. doi:10.1016/S0890-6238(02)00126-0 PMID:12642156

Espandiari P, Glauert HP, Lehmler HJ, Lee EY, Srinivasan C, Robertson LW (2003). Polychlorinated biphenyls as initiators in liver carcinogenesis: resistant hepatocyte model. *Toxicol Appl Pharmacol*, 186(1):55–62. doi:10.1016/S0041-008X(02)00018-2 PMID:12583993

Espandiari P, Glauert HP, Lehmler HJ, Lee EY, Srinivasan C, Robertson LW (2004). Initiating activity of 4-chlorobiphenyl metabolites in the resistant hepatocyte model. *Toxicol Sci*, 79(1):41–6. doi:10.1093/toxsci/kfh097 PMID:14976334

Espandiari P, Robertson LW, Srinivasan C, Glauert HP (2005). Comparison of different initiation protocols in the resistant hepatocyte model. *Toxicology*, 206(3):373–81. doi:10.1016/j.tox.2004.07.014 PMID:15588927

Eum SY, Lee YW, Hennig B, Toborek M (2004). VEGF regulates PCB 104-mediated stimulation of permeability and transmigration of breast cancer cells in human microvascular endothelial cells. *Exp Cell Res*, 296(2):231–44. doi:10.1016/j.yexcr.2004.01.030 PMID:15149853

Evandri MG, Mastrangelo S, Costa LG, Bolle P (2003). In vitro assessment of mutagenicity and clastogenicity of BDE-99, a pentabrominated diphenyl ether flame retardant. *Environ Mol Mutagen*, 42(2):85–90. doi:10.1002/em.10178 PMID:12929120

Exon JH, Talcott PA, Koller LD (1985). Effect of lead, polychlorinated biphenyls, and cyclophosphamide on rat natural killer cells, interleukin 2, and antibody synthesis. *Fundam Appl Toxicol*, 5(1):158–64. doi:10.1016/0272-0590(85)90060-0 PMID:3921421

Fang H, Tong W, Branham WS, Moland CL, Dial SL, Hong H *et al.* (2003). Study of 202 natural, synthetic, and environmental chemicals for binding to the androgen receptor. *Chem Res Toxicol*, 16(10):1338–58. doi:10.1021/tx030011g PMID:14565775

Faust D, Vondráček J, Krčmář P, Smerdová L, Procházková J, Hrubá E *et al.* (2013). AhR-mediated changes in global gene expression in rat liver progenitor cells. *Arch Toxicol*, 87(4):681–98. doi:10.1007/s00204-012-0979-z PMID:23196670

Ferguson KK, Hauser R, Altshul L, Meeker JD (2012). Serum concentrations of p, p'-DDE, HCB, PCBs and reproductive hormones among men of reproductive age. *Reprod Toxicol*, 34(3):429–35. doi:10.1016/j.reprotox.2012.04.006 PMID:22564984

Fernandez MF, Kiviranta H, Molina-Molina JM, Laine O, Lopez-Espinosa MJ, Vartiainen T *et al.* (2008). Polychlorinated biphenyls (PCBs) and hydroxy-PCBs in adipose tissue of women in Southeast Spain. *Chemosphere*, 71(6):1196–205. doi:10.1016/j.chemosphere.2007.09.064 PMID:18045642

Fischbein A, Thornton J, Wolff MS, Bernstein J, Selifoff IJ (1982). Dermatological findings in capacitor manufacturing workers exposed to dielectric fluids containing polychlorinated biphenyls (PCBs). *Arch Environ Health*, 37(2):69–74. doi:10.1080/00039896.1982.10667538 PMID:6462115

Fischbein A, Wolff MS, Lilis R, Thornton J, Selikoff IJ (1979). Clinical findings among PCB-exposed capacitor manufacturing workers. *Ann N Y Acad Sci*, 320:1 Health Effect: 703–15. doi:10.1111/j.1749-6632.1979.tb56645.x PMID:110206

Fisher MA, Jelaso AM, Predenkiewicz A, Schuster L, Means J, Ide CF (2003). Exposure to the polychlorinated biphenyl mixture Aroclor 1254 alters melanocyte and tail muscle morphology in developing Xenopus

laevis tadpoles. *Environ Toxicol Chem*, 22(2):321–8. doi:10.1002/etc.5620220212 PMID:12558163

Flor S, Ludewig G (2010). Polyploidy-induction by dihydroxylated monochlorobiphenyls: structure-activity-relationships. *Environ Int*, 36(8):962–9. doi:10.1016/j.envint.2010.03.012 PMID:20471090

Frouin H, Menard L, Measures L, Brousseau P, Fournier M (2010). T lymphocyte-proliferative responses of a grey seal (*Halichoerus grypus*) exposed to heavy metals and PCBs in vitro. *Aquat Mamm*, 36(4):365–71. doi:10.1578/AM.36.4.2010.365

Fu YA (1984). Ocular manifestation of polychlorinated biphenyls intoxication. *Prog Clin Biol Res*, 137:127–32. PMID:6425847

Fucic A, Gamulin M, Ferencic Z, Katic J, Krayer von Krauss M, Bartonova A *et al.* (2012). Environmental exposure to xenoestrogens and oestrogen related cancers: reproductive system, breast, lung, kidney, pancreas, and brain. *Environ Health*, 11:Suppl 1: S8 doi:10.1186/1476-069X-11-S1-S8 PMID:22759508

Garner CE, Demeter J, Matthews HB (2006). The effect of chlorine substitution on the disposition of polychlorinated biphenyls following dermal administration. *Toxicol Appl Pharmacol*, 216:157–167. doi:10.1016/j.taap.2006.04.013 PMID:16784763

Garner CE, Jefferson WN, Burka LT, Matthews HB, Newbold RR (1999). In vitro estrogenicity of the catechol metabolites of selected polychlorinated biphenyls. *Toxicol Appl Pharmacol*, 154(2):188–97. doi:10.1006/taap.1998.8560 PMID:9925803

Garner CE, Matthews HB (1998). The effect of chlorine substitution on the dermal absorption of polychlorinated biphenyls. *Toxicol Appl Pharmacol*, 149(2):150–8. doi:10.1006/taap.1998.8370 PMID:9571983

Garthoff LH, Friedman L, Farber TM, Locke KK, Sobotka TJ, Green S *et al.* (1977). Biochemical and cytogenetic effects in rats caused by short-term ingestion of Aroclor 1254 or Firemaster BP6. *J Toxicol Environ Health*, 3(4):769–96. doi:10.1080/15287397709529612 PMID:201769

Gasiewicz TA, Henry EC, Collins LL (2008). Expression and activity of aryl hydrocarbon receptors in development and cancer. *Crit Rev Eukaryot Gene Expr*, 18(4):279–321. doi:10.1615/CritRevEukarGeneExpr.v18.i4.10 PMID:18652561

Gauger KJ, Kato Y, Haraguchi K, Lehmler HJ, Robertson LW, Bansal R *et al.* (2004). Polychlorinated biphenyls (PCBs) exert thyroid hormone-like effects in the fetal rat brain but do not bind to thyroid hormone receptors. *Environ Health Perspect*, 112(5):516–23. doi:10.1289/ehp.6672 PMID:15064154

Ghisari M, Bonefeld-Jørgensen EC (2005). Impact of environmental chemicals on the thyroid hormone function in pituitary rat GH3 cells. *Mol Cell Endocrinol*, 244(1–2):31–41. doi:10.1016/j.mce.2005.01.013 PMID:16221524

Ghosh S, Zang S, Mitra PS, Ghimbovschi S, Hoffman EP, Dutta SK (2011). Global gene expression and Ingenuity biological functions analysis on PCBs 153 and 138 induced human PBMC in vitro reveals differential mode(s) of action in developing toxicities. *Environ Int*, 37(5):838–57. doi:10.1016/j.envint.2011.02.010 PMID:21470681

Gierthy JF, Arcaro KF, Floyd M (1997). Assessment of PCB estrogenicity in a human breast cancer cell line. *Chemosphere*, 34(5–7):1495–505. doi:10.1016/S0045-6535(97)00446-3 PMID:9134682

Gladen BC, Taylor JS, Wu YC, Ragan NB, Rogan WJ, Hsu CC (1990). Dermatological findings in children exposed transplacentally to heat-degraded polychlorinated biphenyls in Taiwan. *Br J Dermatol*, 122(6):799–808. doi:10.1111/j.1365-2133.1990.tb06269.x PMID:2142435

Glauert HP, Robertson LW, Silberhorn EM (2001). PCBs and tumor promotion . In: Robertson LW, Hansen LG editors. *PCBs: Recent Advances in Environmental Toxicology and Health Effects*. Lexington (KY): University Press of Kentucky, pp. 355–371.

Glauert HP, Tharappel JC, Banerjee S, Chan NL, Kania-Korwel I, Lehmler HJ *et al.* (2008). Inhibition of the promotion of hepatocarcinogenesis by 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153) by the deletion of the p50 subunit of NF-κ B in mice. *Toxicol Appl Pharmacol*, 232(2):302–8. doi:10.1016/j.taap.2008.06.013 PMID:18644402

Gómara B, Athanasiadou M, Quintanilla-López JE, González MJ, Bergman A (2012). Polychlorinated biphenyls and their hydroxylated metabolites in placenta from Madrid mothers. *Environ Sci Pollut Res Int*, 19(1):139–47. doi:10.1007/s11356-011-0545-x PMID:21698361

Gonçalves LL, Ramkissoon A, Wells PG (2009). Prostaglandin H synthase-1-catalyzed bioactivation of neurotransmitters, their precursors, and metabolites: oxidative DNA damage and electron spin resonance spectroscopy studies. *Chem Res Toxicol*, 22(5):842–52. doi:10.1021/tx800423s PMID:19374330

Goncharov A, Rej R, Negoita S, Schymura M, Santiago-Rivera A, Morse G *et al.* ; Akwesasne Task Force on the Environment(2009). Lower serum testosterone associated with elevated polychlorinated biphenyl concentrations in Native American men. *Environ Health Perspect*, 117(9):1454–60. doi:10.1289/ehp.0800134 PMID:19750113

Goto M, Sugiura K, Hattori M, Miyagawa T, Okamura M (1974a). Metabolism of 2,3-dichlorobiphenyl-^{14}C and 2,4,6-trichlorobiphenyl-^{14}C in the rat. *Chemosphere*, 3(5):227–32. doi:10.1016/0045-6535(74)90010-1

Goto M, Sugiura K, Hattori M, Miyagawa T, Okamura M (1974b). Metabolism of 2,3,5,6-tetrachlorobiphenyl-^{14}C and 2,3,4,5,6-pentachlorobiphenyl-^{14}C in the rat. *Chemosphere*, 3(5):233–8. doi:10.1016/0045-6535(74)90011-3

Grandjean P, Budtz-Jørgensen E, Barr DB, Needham LL, Weihe P, Heinzow B (2008). Elimination half-lives of polychlorinated biphenyl congeners in children. *Environ Sci Technol*, 42(18):6991–6. doi:10.1021/es800778q PMID:18853821

Grant DL, Moodie CA, Phillips WEJ (1974). Toxicodynamics of Aroclor 1254 in the male rat. *Environ Physiol Biochem*, 4(5):214–25. PMID:4218811

Grant DL, Phillips WEJ, Villeneuve DC (1971). Metabolism of a polychlorinated biphenyl (Aroclor 1254) mixture in the rat. *Bull Environ Contam Toxicol*, 6(2):102–12. doi:10.1007/BF01540090 PMID:5005159

Green RM, Hodges NJ, Chipman JK, O'Donovan MR, Graham M (2008). Reactive oxygen species from the uncoupling of human cytochrome P450 1B1 may contribute to the carcinogenicity of dioxin-like polychlorinated biphenyls. *Mutagenesis*, 23(6):457–63. doi:10.1093/mutage/gen035 PMID:18583386

Green S, Carr JV, Palmer KA, Oswald EJ (1975a). Lack of cytogenetic effects in bone marrow and spermatagonial cells in rats treated with polychlorinated biphenyls (Aroclors 1242 and 1254). *Bull Environ Contam Toxicol*, 13(1):14–22. doi:10.1007/BF01684858 PMID:805607

Green S, Sauro FM, Friedman L (1975b). Lack of dominant lethality in rats treated with polychlorinated biphenyls (Aroclors 1242 and 1254). *Food Cosmet Toxicol*, 13(5):507–10. doi:10.1016/0015-6264(75)90003-6 PMID:811517

Grimm FA, Hu D, Kania-Korwel I, Lehmler HJ, Ludewig G, Hornbuckle KC *et al.* (2015). Metabolism and metabolites of polychlorinated biphenyls. *Crit Rev Toxicol*, 45(3):245–72. doi:10.3109/10408444.2014.999365 PMID:25629923

Grimm FA, Lehmler HJ, He X, Robertson LW, Duffel MW (2013). Sulfated metabolites of polychlorinated biphenyls are high-affinity ligands for the thyroid hormone transport protein transthyretin. *Environ Health Perspect*, 121(6):657–62. doi:10.1289/ehp.1206198 PMID:23584369

Grimm FA, Lehmler H-J, Robertson LW, Duffel MW (2012). Sulfated metabolites of polychlorinated biphenyls bind with high affinity to the thyroid hormone transporter transthyretin. Society of Toxicology Annual Meeting. March 11–15, 2012. San Francisco, CA.

Guengerich FP (2008). Cytochrome P450 and chemical toxicology. *Chem Res Toxicol*, 21(1):70–83. doi:10.1021/tx700079z PMID:18052394

Guo YL, Yu ML, Hsu CC, Rogan WJ (1999). Chloracne, goiter, arthritis, and anemia after polychlorinated biphenyl poisoning: 14-year follow-Up of the Taiwan Yucheng cohort. *Environ Health Perspect*, 107(9):715–9. doi:10.1289/ehp.99107715 PMID:10464071

Gutleb AC, Cenijn P, Velzen M, Lie E, Ropstad E, Skaare JU *et al.* (2010). In vitro assay shows that PCB metabolites completely saturate thyroid hormone transport capacity in blood of wild polar bears (Ursus maritimus). *Environ Sci Technol*, 44(8):3149–54. doi:10.1021/es903029j PMID:20345174

Guvenius DM, Aronsson A, Ekman-Ordeberg G, Bergman A, Norén K (2003). Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorobiphenylols, and pentachlorophenol. *Environ Health Perspect*, 111(9):1235–41. doi:10.1289/ehp.5946 PMID:12842779

Guvenius DM, Hassanzadeh P, Bergman A, Norén K (2002). Metabolites of polychlorinated biphenyls in human liver and adipose tissue. *Environ Toxicol Chem*, 21(11):2264–9. doi:10.1002/etc.5620211102 PMID:12389902

Guyton KZ, Kyle AD, Aubrecht J, Cogliano VJ, Eastmond DA, Jackson M *et al.* (2009). Improving prediction of chemical carcinogenicity by considering multiple mechanisms and applying toxicogenomic approaches. *Mutat Res*, 681(2–3):230–40. doi:10.1016/j.mrrev.2008.10.001 PMID:19010444

Haag-Grönlund M, Conolly R, Scheu G, Wärngård L, Fransson-Steen R (2000). Analysis of rat liver foci growth with a quantitative two-cell model after treatment with 2,4,5,3′,4′-pentachlorobiphenyl. *Toxicol Sci*, 57(1):32–42. doi:10.1093/toxsci/57.1.32 PMID:10966509

Haag-Grönlund M, Johansson N, Fransson-Steen R, Håkansson H, Scheu G, Wärngård L (1998). Interactive effects of three structurally different polychlorinated biphenyls in a rat liver tumor promotion bioassay. *Toxicol Appl Pharmacol*, 152(1):153–65. doi:10.1006/taap.1998.8480 PMID:9772211

Hagmar L, Björk J, Sjödin A, Bergman A, Erfurth EM (2001a). Plasma levels of persistent organohalogens and hormone levels in adult male humans. *Arch Environ Health*, 56(2):138–43. doi:10.1080/00039890109604065 PMID:11339677

Hagmar L, Hallberg T, Leja M, Nilsson A, Schütz A (1995). High consumption of fatty fish from the Baltic Sea is associated with changes in human lymphocyte subset levels. *Toxicol Lett*, 77(1–3):335–42. doi:10.1016/0378-4274(95)03315-7 PMID:7618159

Hagmar L, Rylander L, Dyremark E, Klasson-Wehler E, Erfurth EM (2001b). Plasma concentrations of persistent organochlorines in relation to thyrotropin and thyroid hormone levels in women. *Int Arch Occup Environ Health*, 74(3):184–8. doi:10.1007/s004200000213 PMID:11355292

Hahn ME, Lamb TM, Schultz ME, Smolowitz RM, Stegeman JJ (1993). Cytochrome P4501A1 induction and inhibition by 3,3′,4,4′-tetrachlorobiphenyl in an Ah receptor-containing fish hepatoma cell line (PLHC-1). *Aquat Toxicol*, 26(3–4):185–208. doi:10.1016/0166-445X(93)90030-5

Hakkola J, Pasanen M, Pelkonen O, Hukkanen J, Evisalmi S, Anttila S *et al.* (1997). Expression of CYP1B1 in human adult and fetal tissues and differential inducibility of

CYP1B1 and CYP1A1 by Ah receptor ligands in human placenta and cultured cells. *Carcinogenesis*, 18(2):391–7. doi:10.1093/carcin/18.2.391 PMID:9054634

Hallgren S, Darnerud PO (2002). Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats-testing interactions and mechanisms for thyroid hormone effects. *Toxicology*, 177(2–3):227–43. doi:10.1016/S0300-483X(02)00222-6 PMID:12135626

Hamers T, Kamstra JH, Cenijn PH, Pencikova K, Palkova L, Simeckova P *et al.* (2011). In vitro toxicity profiling of ultrapure non-dioxin-like polychlorinated biphenyl congeners and their relative toxic contribution to PCB mixtures in humans. *Toxicol Sci*, 121(1):88–100. doi:10.1093/toxsci/kfr043 PMID:21357386

Hamilton CM, Dabbs JE, Cunningham GD, Vernetti LA, Mirsalis JC, Snyder RD (1997). Evaluation of positive controls for the in vitro unscheduled DNA synthesis assay using hepatocytes from induced (Aroclor 1254) and uninduced male cynomolgus monkey. *Environ Mol Mutagen*, 30(3):354–8. doi:10.1002/(SICI)1098-2280(1997)30:3<354::AID-EM15>3.0.CO;2-C PMID:9366915

Hammond JA, Hall AJ, Dyrynda EA (2005). Comparison of polychlorinated biphenyl (PCB) induced effects on innate immune functions in harbour and grey seals. *Aquat Toxicol*, 74(2):126–38. doi:10.1016/j.aquatox.2005.05.006 PMID:15982755

Han DY, Kang SR, Park OS, Cho JH, Won CK, Park HS *et al.* (2010). Hypothyroidism induced by polychlorinated biphenyls and up-regulation of transthyretin. *Bull Environ Contam Toxicol*, 84(1):66–70. doi:10.1007/s00128-009-9890-6 PMID:19806282

Han X, O'Connor JC, Donner EM, Nabb DL, Mingoia RT, Snajdr SI *et al.* (2009). Non-coplanar 2,2′,3,3′,4,4′,5,5′,6,6′-decachlorobiphenyl (PCB 209) did not induce cytochrome P450 enzyme activities in primary cultured rat hepatocytes, was not genotoxic, and did not exhibit endocrine-modulating activities. *Toxicology*, 255(3):177–86. doi:10.1016/j.tox.2008.10.013 PMID:19022331

Hansen LG (2001). Identification of Steady State and Episodic PCB Congeners from Multiple Pathway Exposures . In: Robertson LW, Hansen LG editors. *PCBs: Recent Advances in Environmental Toxicology and Health Effects*. Lexington (KY): The University Press of Kentucky, pp. 47–56.

Haraguchi K, Kato Y, Kimura R, Masuda Y (1997a). Comparative study on formation of hydroxy and sulfur-containing metabolites from different chlorinated biphenyls with 2,5-substitution in rats. *Drug Metab Dispos*, 25(7):845–52. PMID:9224779

Haraguchi K, Kato Y, Masuda Y, Kimura R (1997b). Metabolism of 3,3′,4,4′-tetrachlorobiphenyl via sulphur-containing pathway in rat: liver-specific retention of methylsulphonyl metabolite. *Xenobiotica*, 27(8):831–42. doi:10.1080/004982597240190 PMID:9293619

Hattula ML (1985). Mutagenicity of PCBs and their pyrosynthetic derivatives in cell-mediated assay. *Environ Health Perspect*, 60:255–7. doi:10.1289/ehp.8560255 PMID:3928351

Haws LC, Su SH, Harris M, Devito MJ, Walker NJ, Farland WH *et al.* (2006). Development of a refined database of mammalian relative potency estimates for dioxin-like compounds. *Toxicol Sci*, 89(1):4–30. doi:10.1093/toxsci/kfi294 PMID:16120753

Hayes MA, Safe SH, Armstrong D, Cameron RG (1985). Influence of cell proliferation on initiating activity of pure polychlorinated biphenyls and complex mixtures in resistant hepatocyte in vivo assays for carcinogenicity. *J Natl Cancer Inst*, 74(5):1037–41. PMID:2860266

Hedenskog M, Sjögren M, Cederberg H, Rannug U (1997). Induction of germline-length mutations at the minisatellites PC-1 and PC-2 in male mice exposed to polychlorinated biphenyls and diesel exhaust emissions. *Environ Mol Mutagen*, 30(3):254–9. doi:10.1002/(SICI)1098-2280(1997)30:3<254::AID-EM2>3.0.CO;2-L PMID:9366902

Helleday T, Arnaudeau C, Jenssen D (1998). Effects of carcinogenic agents upon different mechanisms for intragenic recombination in mammalian cells. *Carcinogenesis*, 19(6):973–8. doi:10.1093/carcin/19.6.973 PMID:9667733

Hemming H, Bager Y, Flodström S, Nordgren I, Kronevi T, Ahlborg UG *et al.* (1995). Liver tumour promoting activity of 3,4,5,3′,4′-pentachlorobiphenyl and its interaction with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Eur J Pharmacol*, 292(3–4):241–9. PMID:7796862

Hemming H, Wärngård L, Ahlborg UG (1991). Inhibition of dye transfer in rat liver WB cell culture by polychlorinated biphenyls. *Pharmacol Toxicol*, 69(6):416–20. doi:10.1111/j.1600-0773.1991.tb01323.x PMID:1766916

Hennig B, Hammock BD, Slim R, Toborek M, Saraswathi V, Robertson LW (2002a). PCB-induced oxidative stress in endothelial cells: modulation by nutrients. *Int J Hyg Environ Health*, 205(1–2):95–102. doi:10.1078/1438-4639-00134 PMID:12018021

Hennig B, Meerarani P, Slim R, Toborek M, Daugherty A, Silverstone AE *et al.* (2002b). Proinflammatory properties of coplanar PCBs: in vitro and in vivo evidence. *Toxicol Appl Pharmacol*, 181(3):174–83. doi:10.1006/taap.2002.9408 PMID:12079426

Hennig B, Reiterer G, Majkova Z, Oesterling E, Meerarani P, Toborek M (2005). Modification of environmental toxicity by nutrients: implications in atherosclerosis. *Cardiovasc Toxicol*, 5(2):153–60. doi:10.1385/CT:5:2:153 PMID:16046791

Hennig B, Slim R, Toborek M, Robertson LW (1999). Linoleic acid amplifies polychlorinated biphenyl-mediated dysfunction of endothelial cells. *J Biochem Mol Toxicol*, 13(2):83–91. doi:10.1002/

(SICI)1099-0461(1999)13:2<83::AID-JBT4>3.0.CO;2-7 PMID:9890193

Herrmann S, Seidelin M, Bisgaard HC, Vang O (2002). Indolo[3,2-b]carbazole inhibits gap junctional intercellular communication in rat primary hepatocytes and acts as a potential tumor promoter. *Carcinogenesis*, 23(11):1861–8. doi:10.1093/carcin/23.11.1861 PMID:12419834

Hill RN, Erdreich LS, Paynter OE, Roberts PA, Rosenthal SL, Wilkinson CF (1989). Thyroid follicular cell carcinogenesis. *Fundam Appl Toxicol*, 12(4):629–97. doi:10.1016/0272-0590(89)90001-8 PMID:2663577

Hines RN (2008). The ontogeny of drug metabolism enzymes and implications for adverse drug events *Pharmacol Ther*, 118:250–267.

Hjelmborg PS, Ghisari M, Bonefeld-Jørgensen EC (2006). SPE-HPLC purification of endocrine-disrupting compounds from human serum for assessment of xenoestrogenic activity. *Anal Bioanal Chem*, 385(5):875–87. doi:10.1007/s00216-006-0463-9 PMID:16791568

Ho PW, Garner CE, Ho JW, Leung KC, Chu AC, Kwok KH *et al.* (2008). Estrogenic phenol and catechol metabolites of PCBs modulate catechol-O-methyltransferase expression via the estrogen receptor: potential contribution to cancer risk. *Curr Drug Metab*, 9(4):304–9. doi:10.2174/138920008784220600 PMID:18473748

Hochstenbach K, van Leeuwen DM, Gmuender H, Stølevik SB, Nygaard UC, Løvik M *et al.* (2010). Transcriptomic profile indicative of immunotoxic exposure: in vitro studies in peripheral blood mononuclear cells. *Toxicol Sci*, 118(1):19–30. doi:10.1093/toxsci/kfq239 PMID:20702593

Holsapple MP, Pitot HC, Cohen SM, Boobis AR, Klaunig JE, Pastoor T *et al.* (2006). Mode of action in relevance of rodent liver tumors to human cancer risk. *Toxicol Sci*, 89(1):51–6. doi:10.1093/toxsci/kfj001 PMID:16221960

Hori S, Obana H, Kashimoto T, Otake T, Nishimura H, Ikegami N *et al.* (1982). Effect of polychlorinated biphenyls and polychlorinated quaterphenyls in Cynomolgus monkey (Macaca fascicularis). *Toxicology*, 24(2):123–39. doi:10.1016/0300-483X(82)90051-8 PMID:6814017

Horváthová M, Jahnová E, Palkovičová L, Trnovec T, Hertz-Picciotto I (2011a). The kinetics of cell surface receptor expression in children perinatally exposed to polychlorinated biphenyls. *J Immunotoxicol*, 8(4):367–80. doi:10.3109/1547691X.2011.620037 PMID:22047017

Horváthová M, Jahnová E, Palkovičová L, Trnovec T, Hertz-Picciotto I (2011b). Dynamics of lymphocyte subsets in children living in an area polluted by polychlorinated biphenyls. *J Immunotoxicol*, 8(4):333–45. doi:10.3109/1547691X.2011.615767 PMID:22013978

Hovander L, Linderholm L, Athanasiadou M, Athanassiadis I, Bignert A, Fängström B *et al.* (2006). Levels of PCBs and their metabolites in the serum of residents of a highly contaminated area in eastern Slovakia. *Environ Sci Technol*, 40(12):3696–703. doi:10.1021/es0525657 PMID:16830529

Hovander L, Malmberg T, Athanasiadou M, Athanassiadis I, Rahm S, Bergman A *et al.* (2002). Identification of hydroxylated PCB metabolites and other phenolic halogenated pollutants in human blood plasma. *Arch Environ Contam Toxicol*, 42(1):105–17. doi:10.1007/s002440010298 PMID:11706375

Høyer AP, Gerdes AM, Jørgensen T, Rank F, Hartvig HB (2002). Organochlorines, p53 mutations in relation to breast cancer risk and survival. A Danish cohort-nested case-controls study. *Breast Cancer Res Treat*, 71(1):59–65. doi:10.1023/A:1013340327099 PMID:11859874

Hsia MT, Lin FS, Allen JR (1978). Comparative mutagenicity and toxic effects of 2,5,2′,5′-tetrachlorobiphenyl and its metabolites in bacterial and mammalian test systems. *Res Commun Chem Pathol Pharmacol*, 21(3):485–96. PMID:151904

Hu X, Adamcakova-Dodd A, Lehmler HJ, Hu D, Kania-Korwel I, Hornbuckle KC *et al.* (2010). Time course of congener uptake and elimination in rats after short-term inhalation exposure to an airborne polychlorinated biphenyl (PCB) mixture. *Environ Sci Technol*, 44(17):6893–900. doi:10.1021/es101274b PMID:20698547

Hughes MF, Shrivastava SP, Sumler MR, Edwards BC, Goodwin JH, Shah PV *et al.* (1992). Dermal absorption of chemicals: effect of application of chemicals as a solid, aqueous paste, suspension, or in volatile vehicle. *J Toxicol Environ Health*, 37(1):57–71. doi:10.1080/15287399209531657 PMID:1522614

Hung WT, Lambert GH, Huang P-W, Patterson DG Jr, Guo YL (2013). Genetic susceptibility to dioxin-like chemicals' induction of cytochrome P4501A2 in the human adult linked to specific AhRR polymorphism. *Chemosphere*, 90(9):2358–64. doi:10.1016/j.chemosphere.2012.10.026 PMID:23168330

IARC (1978). Polychlorinated biphenyls and polybrominated biphenyls. *IARC Monogr Eval Carcinog Risk Chem Hum*, 18:1–124. PMID:215509

IARC (2012). Chemical agents and related occupations. *IARC Monogr Eval Carcinog Risks Hum*, 100F:1–599. PMID:23189753

Imanishi J, Nomura H, Matsubara M, Kita M, Won SJ, Mizutani T *et al.* (1980). Effect of polychlorinated biphenyl on viral infections in mice. *Infect Immun*, 29(1):275–7. PMID:6156912

Imsilp K, Hansen L (2005). PCB profiles in mouse skin biopsies and fat from an environmental mixture. *Environ Toxicol Pharmacol*, 19:71–84. doi:10.1016/j.etap.2004.04.007 PMID:21783463

Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C (2007). Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects.

Pharmacol Ther, 116(3):496–526. doi:10.1016/j.pharmthera.2007.09.004 PMID:18001838

Inomata T, Sekiguchi M, Hirayama S, Akahori F, Shirai M, Kashiwazaki N *et al.* (2009). An assessment of mutagenic effect of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) in muta mouse fetuses *J Vet Med Sci*, 71(4):529–33. doi:10.1292/jvms.71.529 PMID:19420863

Iwanowicz LR, Blazer VS, McCormick SD, Vanveld PA, Ottinger CA (2009). Aroclor 1248 exposure leads to immunomodulation, decreased disease resistance and endocrine disruption in the brown bullhead, Ameiurus nebulosus. *Aquat Toxicol*, 93(1):70–82. doi:10.1016/j.aquatox.2009.03.008 PMID:19406486

Jacobus JA, Flor S, Klingelhutz A, Robertson LW, Ludewig G (2008). 2-(4′-chlorophenyl)-1,4-benzoquinone increases the frequency of micronuclei and shortens telomeres. *Environ Toxicol Pharmacol*, 25(2):267–72. doi:10.1016/j.etap.2007.10.022 PMID:18438462

Jacobus JA, Wang B, Maddox C, Esch H, Lehmann L, Robertson LW *et al.* (2010). 3-Methylcholanthrene (3-MC) and 4-chlorobiphenyl (PCB3) genotoxicity is gender-related in Fischer 344 transgenic rats. *Environ Int*, 36(8):970–9. doi:10.1016/j.envint.2010.07.006 PMID:20739065

Jakab M, Major J, Tompa A (1995). Follow up cytogenetic analysis of peripheral blood lymphocytes in workers occupationally exposed to polychlorinated biphenyls. *Central Eur J Occup Environ Med*, 1:206–216.

James MO (2001). PCB: metabolism and metabolites . In: Robertson LW, Hansen LG editors. *Recent advances in the Environmental Toxicology and Health Effects of PCB*. Lexington (KY): The University Press of Kentucky, pp. 35–46.

Jansen HT, Cooke PS, Porcelli J, Liu TC, Hansen LG (1993). Estrogenic and antiestrogenic actions of PCBs in the female rat: in vitro and in vivo studies. *Reprod Toxicol*, 7(3):237–48. doi:10.1016/0890-6238(93)90230-5 PMID:8318755

Jeong YC, Walker NJ, Burgin DE, Kissling G, Gupta M, Kupper L *et al.* (2008). Accumulation of M_1dG DNA adducts after chronic exposure to PCBs, but not from acute exposure to polychlorinated aromatic hydrocarbons. *Free Radic Biol Med*, 45(5):585–91. doi:10.1016/j.freeradbiomed.2008.04.043 PMID:18534201

Johansson F, Allkvist A, Erixon K, Malmvärn A, Nilsson R, Bergman A *et al.* (2004). Screening for genotoxicity using the DRAG assay: investigation of halogenated environmental contaminants. *Mutat Res*, 563(1):35–47. doi:10.1016/j.mrgentox.2004.05.017 PMID:15324747

Johansson M, Nilsson S, Lund BO (1998). Interactions between methylsulfonyl PCBs and the glucocorticoid receptor. *Environ Health Perspect*, 106(12):769–72. doi:10.1289/ehp.98106769 PMID:9831536

Joksić G, Marković B (1992). [Cytogenetic changes in persons exposed to polychlorinated biphenyls] *Arh Hig Rada Toksikol*, 43(1):29–35.Croatian PMID:1510614

Julvez J, Debes F, Weihe P, Choi AL, Grandjean P (2011). Thyroid dysfunction as a mediator of organochlorine neurotoxicity in preschool children. *Environ Health Perspect*, 119(10):1429–35. doi:10.1289/ehp.1003172 PMID:21719373

Jusko TA, Sonneborn D, Palkovicova L, Kocan A, Drobna B, Trnovec T *et al.* (2012). Pre- and postnatal polychlorinated biphenyl concentrations and longitudinal measures of thymus volume in infants. *Environ Health Perspect*, 120(4):595–600. doi:10.1289/ehp.1104229 PMID:22275729

Kalina I, Srám RJ, Konecná H, Ondrusseková A (1991). Cytogenetic analysis of peripheral blood lymphocytes in workers occupationally exposed to polychlorinated biphenyls. *Teratog Carcinog Mutagen*, 11(2):77–82. doi:10.1002/tcm.1770110203 PMID:1686676

Kang K-S, Wilson MR, Hayashi T, Chang CC, Trosko JE (1996). Inhibition of gap junctional intercellular communication in normal human breast epithelial cells after treatment with pesticides, PCBs, and PBBs, alone or in mixtures. *Environ Health Perspect*, 104(2):192–200. PMID:8820588

Kania-Korwel I, Garrison AW, Avants JK, Hornbuckle KC, Robertson LW, Sulkowski WW *et al.* (2006). Distribution of chiral PCBs in selected tissues in the laboratory rat. *Environ Sci Technol*, 40(12):3704–10. doi:10.1021/es0602086 PMID:16830530

Kania-Korwel I, Hrycay EG, Bandiera SM, Lehmler H-J (2008). 2,2′,3,3′,6,6′-Hexachlorobiphenyl (PCB 136) atropisomers interact enantioselectively with hepatic microsomal cytochrome P450 enzymes. *Chem Res Toxicol*, 21(6):1295–303. doi:10.1021/tx800059j PMID:18494506

Karásek L, Hajslová J, Rosmus J, Hühnerfuss H (2007). Methylsulfonyl PCB and DDE metabolites and their enantioselective gas chromatographic separation in human adipose tissues, seal blubber and pelican muscle. *Chemosphere*, 67(9):S22–7. doi:10.1016/j.chemosphere.2006.05.081 PMID:17215020

Karin M (2006). Nuclear factor-kappaB in cancer development and progression. *Nature*, 441(7092):431–6. doi:10.1038/nature04870 PMID:16724054

Karmaus W, Brooks KR, Nebe T, Witten J, Obi-Osius N, Kruse H (2005). Immune function biomarkers in children exposed to lead and organochlorine compounds: a cross-sectional study. *Environ Health*, 4(1):5 doi:10.1186/1476-069X-4-5 PMID:15831097

Karmaus W, DeKoning EP, Kruse H, Witten J, Osius N (2001a). Early childhood determinants of organochlorine concentrations in school-aged children. *Pediatr Res*, 50(3):331–6. doi:10.1203/00006450-200109000-00007 PMID:11518819

Karmaus W, Kuehr J, Kruse H (2001b). Infections and atopic disorders in childhood and organochlorine exposure. *Arch Environ Health*, 56(6):485–92. doi:10.1080/00039890109602896 PMID:11958547

Karmaus W, Osuch JR, Landgraf J, Taffe B, Mikucki D, Haan P (2011). Prenatal and concurrent exposure to halogenated organic compounds and gene expression of CYP17A1, CYP19A1, and oestrogen receptor alpha and beta genes. *Occup Environ Med*, 68(6):430–7. doi:10.1136/oem.2009.053249 PMID:20924025

Kato Y, Haraguchi K, Shibahara T, Masuda Y, Kimura R (1998). Reduction of thyroid hormone levels by methylsulfonyl metabolites of polychlorinated biphenyl congeners in rats. *Arch Toxicol*, 72(8):541–4. doi:10.1007/s002040050540 PMID:9765071

Kato Y, Haraguchi K, Shibahara T, Shinmura Y, Masuda Y, Kimura R (2000). The induction of hepatic microsomal UDP-glucuronosyltransferase by the methylsulfonyl metabolites of polychlorinated biphenyl congeners in rats. *Chem Biol Interact*, 125(2):107–15. doi:10.1016/S0009-2797(99)00168-4 PMID:10699571

Kato Y, Haraguchi K, Shibahara T, Yumoto S, Masuda Y, Kimura R (1999). Reduction of thyroid hormone levels by methylsulfonyl metabolites of tetra- and pentachlorinated biphenyls in male Sprague-Dawley rats. *Toxicol Sci*, 48(1):51–4. doi:10.1093/toxsci/48.1.51 PMID:10330683

Kato Y, Haraguchi K, Tomiyasu K, Hiroyuki Saito , Isogai M, Masuda Y *et al.* (1997). Structure-dependent induction of CYP2B1/2 by 3-methylsulfonyl metabolites of polychlorinated biphenyl congeners in rats. *Environ Toxicol Pharmacol*, 3(2):137–44. doi:10.1016/S1382-6689(97)00150-6 PMID:21781771

Kawano M, Hasegawa J, Enomoto T, Onishi H, Nishio Y, Matsuda M *et al.* (2005). Hydroxylated polychlorinated biphenyls (OH-PCBs): recent advances in wildlife contamination study. *Environ Sci*, 12(6):315–24. PMID:16609671

Keck G (1981). [Effects of the contamination by polychlorobiphenyls (PCBs) on the growth of the Ehrlich tumor in mice (author's transl)] *Toxicol Eur Res*, 3(5):229–36. PMID:6803399

Kerkvliet NI, Kimeldorf DJ (1977). Antitumor activity of a polychlorinated biphenyl mixture, Aroclor 1254, in rats inoculated with Walker 256 carcinosarcoma cells. *J Natl Cancer Inst*, 59(3):951–5. PMID:408506

Kester MH, Bulduk S, Tibboel D, Meinl W, Glatt H, Falany CN *et al.* (2000). Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. *Endocrinology*, 141(5):1897–900. doi:10.1210/endo.141.5.7530 PMID:10803601

Kikuchi M, Mikagi Y, Hashimoto M, Kojima T (1971). Two autopsy cases of chronic chlorobiphenyls poisoning. *Fukuoka Acta Med.*, 62:89–103.

Kimbrough RD, Linder RE (1974). Induction of adenofibrosis and hepatomas of the liver in BALB-cJ mice by polychlorinated biphenyls (Aroclor 1254). *J Natl Cancer Inst*, 53(2):547–52. PMID:4367249

Kimbrough RD, Squire RA, Linder RE, Strandberg JD, Montalli RJ, Burse VW (1975). Induction of liver tumor in Sherman strain female rats by polychlorinated biphenyl Aroclor 1260. *J Natl Cancer Inst*, 55(6):1453–9. PMID:173869

Knerr S, Schrenk D (2006). Carcinogenicity of "non-dioxinlike" polychlorinated biphenyls. *Crit Rev Toxicol*, 36(9):663–94. doi:10.1080/10408440600845304 PMID:17050081

Kodavanti PRS, Ward TR, Derr-Yellin EC, Mundy WR, Casey AC, Bush B *et al.* (1998). Congener-specific distribution of polychlorinated biphenyls in brain regions, blood, liver, and fat of adult rats following repeated exposure to Aroclor 1254. *Toxicol Appl Pharmacol*, 153(2):199–210. doi:10.1006/taap.1998.8534 PMID:9878591

Koga N, Beppu M, Yoshimura H (1990). Metabolism in vivo of 3,4,5,3′,4′-pentachlorobiphenyl and toxicological assessment of the metabolite in rats. *J Pharmacobiodyn*, 13(8):497–506. doi:10.1248/bpb1978.13.497 PMID:2132573

Koller LD (1977). Enhanced polychlorinated biphenyl lesions in Moloney leukemia virus-infected mice. *Clin Toxicol*, 11(1):107–16. doi:10.3109/15563657708989824 PMID:406114

Kopec AK, Burgoon LD, Ibrahim-Aibo D, Mets BD, Tashiro C, Potter D *et al.* (2010). PCB153-elicited hepatic responses in the immature, ovariectomized C57BL/6 mice: comparative toxicogenomic effects of dioxin and non-dioxin-like ligands. *Toxicol Appl Pharmacol*, 243(3):359–71. doi:10.1016/j.taap.2009.12.003 PMID:20005886

Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD (1988). Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. *Mol Pharmacol*, 33(1):120–6. PMID:3122017

Kornbrust D, Dietz D (1985). Aroclor 1254 pretreatment effects on DNA repair in rat hepatocytes elicited by in vivo or in vitro exposure to various chemicals. *Environ Mutagen*, 7(6):857–70. doi:10.1002/em.2860070607 PMID:3933969

Krutovskikh VA, Mesnil M, Mazzoleni G, Yamasaki H (1995). Inhibition of rat liver gap junction intercellular communication by tumor-promoting agents in vivo. Association with aberrant localization of connexin proteins. *Lab Invest*, 72(5):571–7. PMID:7745951

Kunz S, Schwarz M, Schilling B, Päpke O, Lehmler HJ, Robertson LW *et al.* (2006). Tumor promoting potency of PCBs 28 and 101 in rat liver. *Toxicol Lett*, 164(2):133–43. doi:10.1016/j.toxlet.2005.12.003 PMID:16426774

Kuratsune M (1989). Yusho, with reference to Yu-Cheng . In: Kimbrough RD, Jensen AA editors. 2nd Ed. *Topics in environmental Health 4: Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related*

products. Amsterdam, the Netherlands: Elsevier Science Publishers, pp. 381–400.
Kuratsune M, Yoshimura T, Matsuzaka J, Yamaguchi A (1971). Yusho, a poisoning caused by rice oil contaminated with polychlorinated biphenyls. *HSMHA Health Rep*, 86(12):1083–91. doi:10.2307/4594392 PMID:5157795
Kwon O, Lee E, Moon TC, Jung H, Lin CX, Nam KS *et al.* (2002). Expression of cyclooxygenase-2 and pro-inflammatory cytokines induced by 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) in human mast cells requires NF-κ B activation. *Biol Pharm Bull*, 25(9):1165–8. doi:10.1248/bpb.25.1165 PMID:12230110
Laden F, Ishibe N, Hankinson SE, Wolff MS, Gertig DM, Hunter DJ *et al.* (2002). Polychlorinated biphenyls, cytochrome P450 1A1, and breast cancer risk in the Nurses' Health Study. *Cancer Epidemiol Biomarkers Prev*, 11(12):1560–5. PMID:12496044
Langer P, Kočan A, Drobna B, Susienkova K, Radikova Z, Huckova M *et al.* (2010). Polychlorinated biphenyls and testosterone: age and congener related correlation approach in heavily exposed males. *Endocr Regul*, 44(3):109–14. doi:10.4149/endo_2010_03_109 PMID:20799853
Langer P, Kočan A, Drobna B, Susienkova K, Radikova Z, Huckova M *et al.* (2012). Blood testosterone in middle aged males heavily exposed to endocrine disruptors is decreasing more with HCB and p,p'-DDE related to BMI and lipids, but not with Σ15PCBs. *Endocr Regul*, 46(2):51–9. doi:10.4149/endo_2012_02_51 PMID:22540852
Lans MC, Klasson-Wehler E, Willemsen M, Meussen E, Safe S, Brouwer A (1993). Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-p-dioxins and -dibenzofurans with human transthyretin. *Chem Biol Interact*, 88(1):7–21. doi:10.1016/0009-2797(93)90081-9 PMID:8330325
Larsson C, Ellerichmann T, Hühnerfuss H, Bergman A (2002). Chiral PCB methyl sulfones in rat tissues after exposure to technical PCBs. *Environ Sci Technol*, 36(13):2833–8. doi:10.1021/es025512n PMID:12144255
Lawton RW, Ross MR, Feingold J, Brown JF Jr (1985). Effects of PCB exposure on biochemical and hematological findings in capacitor workers. *Environ Health Perspect*, 60:165–84. doi:10.1289/ehp.8560165 PMID:2863133
Lecavalier P, Chu I, Yagminas A, Villeneuve DC, Poon R, Feeley M *et al.* (1997). Subchronic toxicity of 2,2′,3,3′,4,4′-hexachlorobiphenyl in rats. *J Toxicol Environ Health*, 51(3):265–77. PMID:9183382
Lehmann L, Esch HL, Kirby PA, Robertson LW, Ludewig G (2007). 4-monochlorobiphenyl (PCB3) induces mutations in the livers of transgenic Fisher 344 rats. *Carcinogenesis*, 28(2):471–8. doi:10.1093/carcin/bgl157 PMID:16950798
Lehmler HJ, Harrad SJ, Hühnerfuss H, Kania-Korwel I, Lee CM, Lu Z *et al.* (2010). Chiral polychlorinated biphenyl transport, metabolism, and distribution: a review. *Environ Sci Technol*, 44(8):2757–66. doi:10.1021/es902208u PMID:20384371
Letcher RJ, Lemmen JG, van der Burg B, Brouwer A, Bergman A, Giesy JP *et al.* (2002). In vitro antiestrogenic effects of aryl methyl sulfone metabolites of polychlorinated biphenyls and 2,2-bis(4-chlorophenyl)-1,1-dichloroethene on 17beta-estradiol-induced gene expression in several bioassay systems. *Toxicol Sci*, 69(2):362–72. doi:10.1093/toxsci/69.2.362 PMID:12377985
Li Y, Millikan RC, Bell DA, Cui L, Tse CK, Newman B *et al.* (2005). Polychlorinated biphenyls, cytochrome P450 1A1 (CYP1A1) polymorphisms, and breast cancer risk among African American women and white women in North Carolina: a population-based case-control study. *Breast Cancer Res*, 7(1):R12–8. doi:10.1186/bcr941 PMID:15642161
Liebl B, Schettgen T, Kerscher G, Broding HC, Otto A, Angerer J *et al.* (2004). Evidence for increased internal exposure to lower chlorinated polychlorinated biphenyls (PCB) in pupils attending a contaminated school. *Int J Hyg Environ Health*, 207(4):315–24. doi:10.1078/1438-4639-00296 PMID:15471095
Lin PH, Sangaiah R, Ranasinghe A, Upton PB, La DK, Gold A *et al.* (2000). Formation of quinonoid-derived protein adducts in the liver and brain of Sprague-Dawley rats treated with 2,2′,5, 5′-tetrachlorobiphenyl. *Chem Res Toxicol*, 13(8):710–8. doi:10.1021/tx000030f PMID:10956058
Linderholm L, Park JS, Kocan A, Trnovec T, Athanasiadou M, Bergman K *et al.* (2007). Maternal and cord serum exposure to PCB and DDE methyl sulfone metabolites in eastern Slovakia. *Chemosphere*, 69(3):403–10. doi:10.1016/j.chemosphere.2007.04.081 PMID:17574648
Lindström G, Hooper K, Petreas M, Stephens R, Gilman A (1995). Workshop on perinatal exposure to dioxin-like compounds. I. Summary. *Environ Health Perspect*, 103:Suppl 2: 135–42. doi:10.1289/ehp.95103s2135 PMID:7614935
Liu S, Li S, Du Y (2010). Polychlorinated biphenyls (PCBs) enhance metastatic properties of breast cancer cells by activating Rho-associated kinase (ROCK). *PLoS ONE*, 5(6):e11272 doi:10.1371/journal.pone.0011272 PMID:20585605
Liu Y, Apak TI, Lehmler HJ, Robertson LW, Duffel MW (2006). Hydroxylated polychlorinated biphenyls are substrates and inhibitors of human hydroxysteroid sulfotransferase SULT2A1. *Chem Res Toxicol*, 19(11):1420–5. doi:10.1021/tx060160+ PMID:17112228
Liu Y, Smart JT, Song Y, Lehmler HJ, Robertson LW, Duffel MW (2009). Structure-activity relationships for hydroxylated polychlorinated biphenyls as substrates

and inhibitors of rat sulfotransferases and modification of these relationships by changes in thiol status. *Drug Metab Dispos*, 37(5):1065–72. doi:10.1124/dmd.108.026021 PMID:19196841

Loose LD, Silkworth JB, Mudzinski SP, Pittman KA, Benitz KF, Mueller W (1979). Modification of the immune response by organochlorine xenobiotics. *Drug Chem Toxicol*, 2(1–2):111–32. doi:10.3109/01480547908993185 PMID:121284

Lü YC, Wu YC (1985). Clinical findings and immunological abnormalities in Yu-Cheng patients. *Environ Health Perspect*, 59:17–29. doi:10.2307/3429869 PMID:3921359

Lu Z, Lee EY, Robertson LW, Glauert HP, Spear BT (2004). Effect of 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153) on hepatocyte proliferation and apoptosis in mice deficient in the p50 subunit of the transcription factor NF-kappaB. *Toxicol Sci*, 81(1):35–42. doi:10.1093/toxsci/kfh193 PMID:15201435

Lu Z, Tharappel JC, Lee EY, Robertson LW, Spear BT, Glauert HP (2003). Effect of a single dose of polychlorinated biphenyls on hepatic cell proliferation and the DNA binding activity of NF-kappaB and AP-1 in rats. *Mol Carcinog*, 37(4):171–80. doi:10.1002/mc.10135 PMID:12891626

Lubet RA, Lemaire BN, Avery D, Kouri RE (1986). Induction of immunotoxicity in mice by polyhalogenated biphenyls. *Arch Toxicol*, 59(2):71–7. doi:10.1007/BF00286726 PMID:3092783

Ludewig G (2001). Cancer initiation by PCBs . In: Robertson LW, Hansen LG editors. *PCBs: Recent advances in environmental toxicology and health effects*. Lexington (KY): The University of Kentucky Press, pp. 337–354.

Ludewig G, Esch H, Robertson LW (2007). Polyhalogenierte Bi- und Terphenyle . In: Dunkelberg H, Gebel T, Hartwig A editors. *Handbuch der Lebensmitteltoxikologie*. Weinheim: Wiley-VCH Weinheim, pp. 1031–1094.

Luecke S, Backlund M, Jux B, Esser C, Krutmann J, Rannug A (2010). The aryl hydrocarbon receptor (AHR), a novel regulator of human melanogenesis. *Pigment Cell Melanoma Res*, 23(6):828–33. doi:10.1111/j.1755-148X.2010.00762.x PMID:20973933

Lundgren K, Andries M, Thompson C, Lucier GW (1987). alpha-Naphthoflavone metabolized by 2,3,7,8-tetrachlorodibenzo(p)dioxin-induced rat liver microsomes: a potent clastogen in Chinese hamster ovary cells. *Cancer Res*, 47(14):3662–6. PMID:3594431

Lundgren K, Collman GW, Wang-Wuu S, Tiernan T, Taylor M, Thompson CL *et al.* (1988). Cytogenetic and chemical detection of human exposure to polyhalogenated aromatic hydrocarbons. *Environ Mol Mutagen*, 11(1):1–11. doi:10.1002/em.2850110103 PMID:3338440

Machala M, Bláha L, Vondrácek J, Trosko JE, Scott J, Upham BL (2003). Inhibition of gap junctional intercellular communication by noncoplanar polychlorinated biphenyls: inhibitory potencies and screening for potential mode(s) of action. *Toxicol Sci*, 76(1):102–11. doi:10.1093/toxsci/kfg209 PMID:12915713

Maddox C, Wang B, Kirby PA, Wang K, Ludewig G (2008). Mutagenicity of 3-methylcholanthrene, PCB3, and 4-OH-PCB3 in the lung of transgenic Big Blue rats. *Environ Toxicol Pharmacol*, 25(2):260–6. doi:10.1016/j.etap.2007.10.021 PMID:18438460

Maervoet J, Covaci A, Schepens P, Sandau CD, Letcher RJ (2004). A reassessment of the nomenclature of polychlorinated biphenyl (PCB) metabolites. *Environ Health Perspect*, 112(3):291–4. doi:10.1289/ehp.6409 PMID:14998742

Mahy BWJ, Barrett T, Evans S, Anderson EC, Bostock CJ (1988). Characterization of a seal morbillivirus. *Nature*, 336(6195):115–6. doi:10.1038/336115a0 PMID:3185731

Major J, Jakab MG, Tompa A (1999). The frequency of induced premature centromere division in human populations occupationally exposed to genotoxic chemicals. *Mutat Res*, 445(2):241–9. doi:10.1016/S1383-5718(99)00129-1 PMID:10575433

Marabini L, Calò R, Fucile S (2011). Genotoxic effects of polychlorinated biphenyls (PCB 153, 138, 101, 118) in a fish cell line (RTG-2). *Toxicol In Vitro*, 25(5):1045–52. doi:10.1016/j.tiv.2011.04.004 PMID:21504788

Marlowe JL, Puga A (2005). Aryl hydrocarbon receptor, cell cycle regulation, toxicity, and tumorigenesis. *J Cell Biochem*, 96(6):1174–84. doi:10.1002/jcb.20656 PMID:16211578

Maroni M, Colombi A, Arbosti G, Cantoni S, Foa V (1981a). Occupational exposure to polychlorinated biphenyls in electrical workers. II. Health effects. *Br J Ind Med*, 38(1):55–60. PMID:6451237

Maroni M, Colombi A, Cantoni S, Ferioli E, Foa V (1981b). Occupational exposure to polychlorinated biphenyls in electrical workers. I. Environmental and blood polychlorinated biphenyls concentrations. *Br J Ind Med*, 38(1):49–54. PMID:6781529

Martin LA, Wilson DT, Reuhl KR, Gallo MA, Klaassen CD (2012). Polychlorinated biphenyl congeners that increase the glucuronidation and biliary excretion of thyroxine are distinct from the congeners that enhance the serum disappearance of thyroxine. *Drug Metab Dispos*, 40(3):588–95. doi:10.1124/dmd.111.042796 PMID:22187485

Martin WJ 2nd, Gadek JE, Hunninghake GW, Crystal RG (1981). Oxidant injury of lung parenchymal cells. *J Clin Invest*, 68(5):1277–88. doi:10.1172/JCI110374 PMID:7298852

Mason ME, Okey AB (1982). Cytosolic and nuclear binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin to the Ah receptor in extra-hepatic tissues of rats and mice. *Eur J Biochem*, 123(1):209–15. doi:10.1111/j.1432-1033.1982.tb06518.x PMID:6279396

Masuda Y (2001). Fate of PCDF/PCB congeners and change of clinical symptoms in patients with Yusho PCB

poisoning for 30 years. *Chemosphere*, 43(4–7):925–30. doi:10.1016/S0045-6535(00)00452-5 PMID:11372885
Matthews HB, Anderson MW (1975a). The distribution and excretion of 2,4,5,2′,5′-pentachlorobiphenyl in the rat. *Drug Metab Dispos*, 3(3):211–9. PMID:238820
Matthews HB, Anderson MW (1975b). Effect of chlorination on the distribution and excretion of polychlorinated biphenyls. *Drug Metab Dispos*, 3(5):371–80. PMID:241618
Matthews HB, Dedrick RL (1984). Pharmacokinetics of PCBs. *Annu Rev Pharmacol Toxicol*, 24(1):85–103. doi:10.1146/annurev.pa.24.040184.000505 PMID:6428301
Matthews HB, Domanski JJ, Guthrie FE (1976). Hair and its associated lipids as an excretory pathway for chlorinated hydrocarbons. *Xenobiotica*, 6(7):425–9. doi:10.3109/00498257609151655 PMID:826029
Mayes BA, McConnell EE, Neal BH, Brunner MJ, Hamilton SB, Sullivan TM *et al.* (1998). Comparative carcinogenicity in Sprague-Dawley rats of the polychlorinated biphenyl mixtures Aroclors 1016, 1242, 1254, and 1260. *Toxicol Sci*, 41(1):62–76. PMID:9520342
McAuliffe ME, Williams PL, Korrick SA, Altshul LM, Perry MJ (2012). Environmental exposure to polychlorinated biphenyls and p,p′-DDE and sperm sex-chromosome disomy. *Environ Health Perspect*, 120(4):535–40. doi:10.1289/ehp.1104017 PMID:22189045
McCready D, Aronson KJ, Chu W, Fan W, Vesprini D, Narod SA (2004). Breast tissue organochlorine levels and metabolic genotypes in relation to breast cancer risk Canada. *Cancer Causes Control*, 15(4):399–418. doi:10.1023/B:CACO.0000027505.32564.c2 PMID:15141140
McGraw JE Sr, Waller DP (2006). Specific human CYP 450 isoform metabolism of a pentachlorobiphenyl (PCB-IUPAC# 101). *Biochem Biophys Res Commun*, 344(1):129–33. doi:10.1016/j.bbrc.2006.03.122 PMID:16616008
McGraw JE Sr, Waller DP (2009). The role of African American ethnicity and metabolism in sentinel polychlorinated biphenyl congener serum levels. *Environ Toxicol Pharmacol*, 27(1):54–61. doi:10.1016/j.etap.2008.08.008 PMID:20047000
McLean MR, Bauer U, Amaro AR, Robertson LW (1996a). Identification of catechol and hydroquinone metabolites of 4-monochlorobiphenyl. *Chem Res Toxicol*, 9(1):158–64. doi:10.1021/tx950083a PMID:8924585
McLean MR, Robertson LW, Gupta RC (1996b). Detection of PCB adducts by the 32P-postlabeling technique. *Chem Res Toxicol*, 9(1):165–71. doi:10.1021/tx9500843 PMID:8924587
McMahon RE, Cline JC, Thompson CZ (1979). Assay of 855 test chemicals in ten tester strains using a new modification of the Ames test for bacterial mutagens. *Cancer Res*, 39(3):682–93. PMID:371791
Meerts IA, Assink Y, Cenijn PH, Van Den Berg JH, Weijers BM, Bergman A *et al.* (2002). Placental transfer of a hydroxylated polychlorinated biphenyl and effects on fetal and maternal thyroid hormone homeostasis in the rat. *Toxicol Sci*, 68(2):361–71. doi:10.1093/toxsci/68.2.361 PMID:12151632
Meerts IA, Hoving S, van den Berg JH, Weijers BM, Swarts HJ, van der Beek EM *et al.* (2004). Effects of in utero exposure to 4-hydroxy-2,3,3′,4′,5-pentachlorobiphenyl (4-OH-CB107) on developmental landmarks, steroid hormone levels, and female estrous cyclicity in rats. *Toxicol Sci*, 82(1):259–67. doi:10.1093/toxsci/kfh251 PMID:15310862
Meigs JW, Albom JJ, Kartin BL (1954). Chloracne from an unusual exposure to arochlor. *J Am Med Assoc*, 154(17):1417–8.doi:10.1001/jama.1954.02940510017007 PMID:13151867
Meisner LF, Roloff B, Sargent L, Pitot H (1992). Interactive cytogenetic effects on rat bone-marrow due to chronic ingestion of 2,5,2′,5′ and 3,4,3′,4′ PCBs. *Mutat Res*, 283(3):179–83. doi:10.1016/0165-7992(92)90105-Q PMID:1383786
Melino G, Vernole P, Antinori M *et al.* (1992). Immunological and cytogenetic damage in workers accidentally exposed to polychlorinated biphenyls (pcb). *Clinical Chemistry and Enzymology Communications*, 4:341–353.
Mes J, Arnold DL, Bryce F (1994). Determination of polychlorinated biphenyls in postpartum blood, adipose tissue, and milk from female rhesus monkeys and their offspring after prolonged dosing with Aroclor 1254. *J Anal Toxicol*, 18(1):29–35. doi:10.1093/jat/18.1.29 PMID:8127081
Mes J, Arnold DL, Bryce F (1995a). Postmortem tissue levels of polychlorinated biphenyls in female rhesus monkeys after more than six years of daily dosing with Aroclor 1254 and in their non-dosed offspring. *Arch Environ Contam Toxicol*, 29(1):69–76. doi:10.1007/BF00213089 PMID:7794014
Mes J, Arnold DL, Bryce F (1995b). The elimination and estimated half-lives of specific polychlorinated biphenyl congeners from the blood of female monkeys after discontinuation of daily dosing with Aroclor 1254. *Chemosphere*, 30(4):789–800. doi:10.1016/0045-6535(94)00408-M PMID:7889352
Miller VM, Sanchez-Morrissey S, Brosch KO, Seegal RF (2012). Developmental coexposure to polychlorinated biphenyls and polybrominated diphenyl ethers has additive effects on circulating thyroxine levels in rats. *Toxicol Sci*, 127:76–83. doi:10.1093/toxsci/kfs089 PMID:22345314
Mills SA 3rd, Thal DI, Barney J (2007). A summary of the 209 PCB congener nomenclature. *Chemosphere*, 68(9):1603–12. doi:10.1016/j.chemosphere.2007.03.052 PMID:17499337

Mio T, Sumino K (1985). Mechanism of biosynthesis of methylsulfones from PCBs and related compounds. *Environ Health Perspect*, 59:129–35. doi:10.2307/3429885 PMID:3921355

Mitchell MM, Woods R, Chi LH, Schmidt RJ, Pessah IN, Kostyniak PJ *et al.* (2012). Levels of select PCB and PBDE congeners in human postmortem brain reveal possible environmental involvement in 15q11-q13 duplication autism spectrum disorder. *Environ Mol Mutagen*, 53(8):589–98. doi:10.1002/em.21722 PMID:22930557

Miyazaki W, Iwasaki T, Takeshita A, Kuroda Y, Koibuchi N (2004). Polychlorinated biphenyls suppress thyroid hormone receptor-mediated transcription through a novel mechanism. *J Biol Chem*, 279(18):18195–202. doi:10.1074/jbc.M310531200 PMID:14985366

Morales NM, Matthews HB (1979). In vivo binding of 2,3,6,2′,3′,6′-hexachlorobiphenyl and 2,4,5,2′,4′,5′-hexachlorobiphenyl to mouse liver macromolecules. *Chem Biol Interact*, 27(1):99–110. doi:10.1016/0009-2797(79)90153-4 PMID:113111

Morse DC, Van Bladeren PJ, Klasson-Wehler E, Brouwer A (1995). beta-Naphthoflavone- and self-induced metabolism of 3,3′,4,4′-tetrachlorobiphenyl in hepatic microsomes of the male, pregnant female and foetal rat. *Xenobiotica*, 25(3):245–60. doi:10.3109/00498259509061849 PMID:7618351

Morse DC, Wehler EK, Wesseling W, Koeman JH, Brouwer A (1996). Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicol Appl Pharmacol*, 136(2):269–79. doi:10.1006/taap.1996.0034 PMID:8619235

Moser GA, McLachlan MS (2001). The influence of dietary concentration on the absorption and excretion of persistent lipophilic organic pollutants in the human intestinal tract. *Chemosphere*, 45(2):201–11. doi:10.1016/S0045-6535(00)00551-8 PMID:11572612

Moysich KB, Shields PG, Freudenheim JL, Schisterman EF, Vena JE, Kostyniak P *et al.* (1999). Polychlorinated biphenyls, cytochrome P4501A1 polymorphism, and postmenopausal breast cancer risk. *Cancer Epidemiol Biomarkers Prev*, 8(1):41–4. PMID:9950238

Muangmoonchai R, Smirlis D, Wong SC, Edwards M, Phillips IR, Shephard EA (2001). Xenobiotic induction of cytochrome P450 2B1 (CYP2B1) is mediated by the orphan nuclear receptor constitutive androstane receptor (CAR) and requires steroid co-activator 1 (SRC-1) and the transcription factor Sp1. *Biochem J*, 355(Pt 1):71–8. doi:10.1042/0264-6021:3550071 PMID:11256950

Murk A, Morse D, Boon J, Brouwer A (1994). In vitro metabolism of 3,3′,4,4′-tetrachlorobiphenyl in relation to ethoxyresorufin-O-deethylase activity in liver microsomes of some wildlife species and rat. *Eur J Pharmacol*, 270(2–3):253–61. PMID:8039554

Murphy KA, Quadro L, White LA (2007). The intersection between the aryl hydrocarbon receptor (AhR)- and retinoic acid-signalling pathways. *Vitam Horm*, 75:33–67. doi:10.1016/S0083-6729(06)75002-6 PMID:17368311

Murray GI, Melvin WT, Greenlee WF, Burke MD (2001). Regulation, function, and tissue-specific expression of cytochrome P450 CYP1B1. *Annu Rev Pharmacol Toxicol*, 41(1):297–316. doi:10.1146/annurev.pharmtox.41.1.297 PMID:11264459

Nagayama J, Nagayama M, Haraguchi K, Kuroki H, Masuda Y (1995). Influence of five methylsulphonyl PCB congeners on frequency of micronucleated cells in cultured human lymphocytes by cytokinesis block method. *Fukuoka Igaku Zasshi*, 86(5):190–6. PMID:7628807

Nagayama J, Nagayama M, Haraguchi K, Kuroki H, Masuda Y (1999). Induction of sister chromatid exchanges in cultured human lymphocytes with methylsulphonyl PCB congeners. *Fukuoka Igaku Zasshi*, 90(5):238–45. PMID:10396880

Nagayama J, Nagayama M, Iida T, Hirakawa H, Matsueda T, Masuda Y (1994). Effects of highly toxic organochlorine compounds retained in human body on induction of sister chromatid exchanges in cultured human lymphocytes. *Chemosphere*, 29(9–11):2349–54. doi:10.1016/0045-6535(94)90403-0 PMID:7850383

Nagayama J, Nagayama M, Iida T, Hirakawa H, Matsueda T, Ohki M *et al.* (2001). Comparison between "Yusho" patients and healthy Japanese in contamination level of dioxins and related chemicals and frequency of sister chromatid exchanges. *Chemosphere*, 43(4–7):931–6. doi:10.1016/S0045-6535(00)00453-7 PMID:11372886

Nakanishi Y, Nomoto Y, Matsuki A, Kunitake R, Hara N (1995). Effect of polychlorinated biphenyls and polychlorinated dibenzofurans on leukocyte in peripheral blood and bronchoalveolar lavage fluid. *Fukuoka Igaku Zasshi*, 86(5):261–6. PMID:7628818

Nakanishi Y, Shigematsu N, Kurita Y, Matsuba K, Kanegae H, Ishimaru S *et al.* (1985). Respiratory involvement and immune status in yusho patients. *Environ Health Perspect*, 59:31–6. doi:10.2307/3429870 PMID:3921360

Narayanan PK, Carter WO, Ganey PE, Roth RA, Voytik-Harbin SL, Robinson JP (1998). Impairment of human neutrophil oxidative burst by polychlorinated biphenyls: inhibition of superoxide dismutase activity. *J Leukoc Biol*, 63(2):216–24. PMID:9468280

Nath RG, Randerath E, Randerath K (1991). Short-term effects of the tumor promoting polychlorinated biphenyl mixture, Aroclor 1254, on I-compounds in liver, kidney and lung DNA of male Sprague-Dawley rats. *Toxicology*, 68(3):275–89. doi:10.1016/0300-483X(91)90075-C PMID:1896999

Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM *et al.* (2007). Gender disparity in liver cancer due to sex differences in MyD88-dependent

IL-6 production. *Science*, 317(5834):121–4. doi:10.1126/science.1140485 PMID:17615358
Nesaretnam K, Corcoran D, Dils RR, Darbre P (1996). 3,4,3′4′-Tetrachlorobiphenyl acts as an estrogen in vitro and in vivo. *Mol Endocrinol*, 10(8):923–36. doi:10.1210/me.10.8.923 PMID:8843409
Ng CH, Janoo-Gilani R, Sipahimalani P, Gallagher RP, Gascoyne RD, Connors JM *et al.* (2010). Interaction between organochlorines and the AHR gene, and risk of non-Hodgkin lymphoma. *Cancer Causes Control*, 21(1):11–22. doi:10.1007/s10552-009-9429-5 PMID:19821039
Nilsson B, Ramel C (1974). Genetic tests on Drosophila melanogaster with polychlorinated biphenyls (PCB). *Hereditas*, 77(2):319–22. doi:10.1111/j.1601-5223.1974.tb00944.x PMID:4217330
Nims RW, Fox SD, Issaq HJ, Lubet RA (1994). Accumulation and persistence of individual polychlorinated biphenyl congeners in liver, blood, and adipose tissue of rats following dietary exposure to Aroclor 1254. *Arch Environ Contam Toxicol*, 27(4):513–20. doi:10.1007/BF00214843 PMID:7811109
Nordlund-Möller L, Andersson O, Ahlgren R, Schilling J, Gillner M, Gustafsson JA *et al.* (1990). Cloning, structure, and expression of a rat binding protein for polychlorinated biphenyls. Homology to the hormonally regulated progesterone-binding protein uteroglobin. *J Biol Chem*, 265(21):12690–3. PMID:2115524
NTP; National Toxicology Program(2006a). NTP toxicology and carcinogenesis studies of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (CAS No. 57465–28–8) in female Harlan Sprague-Dawley rats (Gavage Studies). *Natl Toxicol Program Tech Rep Ser*, 520(520):4–246. PMID:16628245
NTP; National Toxicology Program(2006b). NTP technical report on the toxicology and carcinogenesis studies of 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) (CAS No. 35065–27–1) in female Harlan Sprague-Dawley rats (Gavage studies). *Natl Toxicol Program Tech Rep Ser*, 529(529):4–168. PMID:16835634
NTP; National Toxicology Program(2006c). Toxicology and carcinogenesis studies of a binary mixture of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (Cas No. 57465–28–8) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) (CAS No. 35065–27–1) in female Harlan Sprague-Dawley rats (gavage studies). *Natl Toxicol Program Tech Rep Ser*, 530(530):1–258. PMID:17160104
NTP; National Toxicology Program(2006d). Toxicology and carcinogenesis studies of a binary mixture of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (Cas No. 57465–28–8) and 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118) (Cas No. 31508–00–6) in female Harlan Sprague-Dawley rats (gavage studies). *Natl Toxicol Program Tech Rep Ser*, 531(531):1–218. PMID:17342196
NTP; National Toxicology Program(2006e). NTP technical report on the toxicology and carcinogenesis studies of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746–01–6) in female Harlan Sprague-Dawley rats (Gavage Studies). *Natl Toxicol Program Tech Rep Ser*, 521(521):4–232. PMID:16835633
NTP; National Toxicology Program(2006f). Toxicology and carcinogenesis studies of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (Cas No. 57117–31–4) in female Harlan Sprague-Dawley rats (gavage studies). *Natl Toxicol Program Tech Rep Ser*, 525(525):1–198. PMID:17160103
NTP; National Toxicology Program(2006g). Toxicology and carcinogenesis studies of a mixture of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Cas No. 1746–01–6), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (Cas No. 57117–31–4), and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (Cas No. 57465–28–8) in female Harlan Sprague-Dawley rats (gavage studies). *Natl Toxicol Program Tech Rep Ser*, 526(526):1–180. PMID:17342195
NTP; National Toxicology Program(2010). Toxicology and carcinogenesis studies of 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118) (CAS No. 31508–00–6) in female harlan Sprague-Dawley rats (gavage studies). *Natl Toxicol Program Tech Rep Ser*, 559(559):1–174. PMID:21383778
Oakley GG, Devanaboyina U, Robertson LW, Gupta RC (1996a). Oxidative DNA damage induced by activation of polychlorinated biphenyls (PCBs): implications for PCB-induced oxidative stress in breast cancer. *Chem Res Toxicol*, 9(8):1285–92. doi:10.1021/tx960103o PMID:8951230
Oakley GG, Robertson LW, Gupta RC (1996b). Analysis of polychlorinated biphenyl-DNA adducts by 32P-postlabeling. *Carcinogenesis*, 17(1):109–14. doi:10.1093/carcin/17.1.109 PMID:8565118
Öberg M, Sjödin A, Casabona H, Nordgren I, Klasson-Wehler E, Håkansson H (2002). Tissue distribution and half-lives of individual polychlorinated biphenyls and serum levels of 4-hydroxy-2,3,3′,4′,5-pentachlorobiphenyl in the rat. *Toxicol Sci*, 70(2):171–82. doi:10.1093/toxsci/70.2.171 PMID:12441362
Odashima S (1976). The cooperative development in Japan of methods for screening chemicals for carcinogenicity. In: Bartsch R, Tomatis L editors. *Screening tests for chemical carcinogenesis*. Scientific Publication Number 12. Lyon, France: IARC, pp. 61–75.
Oesterle D, Deml E (1981). Promoting effect of various PCBs and DDT on enzyme-altered islands in rat liver. *Naunyn Schmiedebergs Arch Pharmacol*, 316:R16
Osius N, Karmaus W, Kruse H, Witten J (1999). Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. *Environ Health Perspect*, 107(10):843–9. doi:10.1289/ehp.99107843 PMID:10504153
Osterhaus ADME, Vedder EJ (1988). Identification of virus causing recent seal deaths. *Nature*, 335(6185):20 doi:10.1038/335020a0 PMID:3412456

Ota K, Hammock BD (1980). Cytosolic and microsomal epoxide hydrolases: differential properties in mammalian liver. *Science*, 207(4438):1479–81. doi:10.1126/science.7361100 PMID:7361100

Otake T, Yoshinaga J, Enomoto T, Matsuda M, Wakimoto T, Ikegami M *et al.* (2007). Thyroid hormone status of newborns in relation to in utero exposure to PCBs and hydroxylated PCB metabolites. *Environ Res*, 105(2):240–6. doi:10.1016/j.envres.2007.03.010 PMID:17490634

Ouw HK, Simpson GR, Siyali DS (1976). Use and health effects of Aroclor 1242, a polychlorinated biphenyl, in an electrical industry. *Arch Environ Health*, 31(4):189–94. doi:10.1080/00039896.1976.10667218 PMID:821401

Parkinson A, Robertson L, Safe L, Safe S (1980). Polychlorinated biphenyls as inducers of hepatic microsomal enzymes: structure-activity rules. *Chem Biol Interact*, 30(3):271–85. doi:10.1016/0009-2797(80)90050-2 PMID:6769597

Parkinson A, Safe SH, Robertson LW, Thomas PE, Ryan DE, Reik LM *et al.* (1983). Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. A study of structure-activity relationships. *J Biol Chem*, 258(9):5967–76. PMID:6304102

Pearce EN, Braverman LE (2009). Environmental pollutants and the thyroid. *Best Pract Res Clin Endocrinol Metab*, 23(6):801–13. doi:10.1016/j.beem.2009.06.003 PMID:19942155

Pereg D, Robertson LW, Gupta RC (2002). DNA adduction by polychlorinated biphenyls: adducts derived from hepatic microsomal activation and from synthetic metabolites. *Chem Biol Interact*, 139(2):129–44. doi:10.1016/S0009-2797(01)00292-7 PMID:11823002

Pereg D, Tampal N, Espandiari P, Robertson LW (2001). Distribution and macromolecular binding of benzo[a]pyrene and two polychlorinated biphenyl congeners in female mice. *Chem Biol Interact*, 137(3):243–58. doi:10.1016/S0009-2797(01)00256-3 PMID:11566292

Persky V, Turyk M, Anderson HA, Hanrahan LP, Falk C, Steenport DN *et al.* ; Great Lakes Consortium(2001). The effects of PCB exposure and fish consumption on endogenous hormones. *Environ Health Perspect*, 109(12):1275–83. doi:10.1289/ehp.011091275 PMID:11748036

Persson I, Johansson I, Ingelman-Sundberg M (1997). In vitro kinetics of two human CYP1A1 variant enzymes suggested to be associated with interindividual differences in cancer susceptibility. *Biochem Biophys Res Commun*, 231(1):227–30. doi:10.1006/bbrc.1997.6051 PMID:9070254

Pessah IN, Hansen LG, Albertson TE, Garner CE, Ta TA, Do Z *et al.* (2006). Structure-activity relationship for noncoplanar polychlorinated biphenyl congeners toward the ryanodine receptor-Ca2+ channel complex type 1 (RyR1). *Chem Res Toxicol*, 19(1):92–101. doi:10.1021/tx050196m PMID:16411661

Petersen MS, Halling J, Damkier P, Nielsen F, Grandjean P, Weihe P *et al.* (2007). Polychlorinated biphenyl (PCB) induction of CYP3A4 enzyme activity in healthy Faroese adults. *Toxicol Appl Pharmacol*, 224(2):202–6. doi:10.1016/j.taap.2007.07.002 PMID:17692354

Pienta RJ (1980). Transformation of Syrian hamster embryo cells by diverse chemicals and correlation with their reported carcinogenic and mutagenic activities. In: de Serres FJ, Hollander A editors. *Chemical Mutagens: Principles and methods for their detection*. Volume 6:. New York (NY): Plenum Press; pp. 175–202. doi:10.1007/978-1-4613-3072-1_7

Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S *et al.* (2004). NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature*, 431(7007):461–6. doi:10.1038/nature02924 PMID:15329734

Plísková M, Vondrácek J, Canton RF, Nera J, Kocan A, Petrík J *et al.* (2005). Impact of polychlorinated biphenyls contamination on estrogenic activity in human male serum. *Environ Health Perspect*, 113(10):1277–84. doi:10.1289/ehp.7745 PMID:16203234

Porta M, López T, Pumarega J, Jariod M, Crous-Bou M, Marco E *et al.* ; PANKRAS II Study Group(2009). In pancreatic ductal adenocarcinoma blood concentrations of some organochlorine compounds and coffee intake are independently associated with KRAS mutations. *Mutagenesis*, 24(6):513–21. doi:10.1093/mutage/gep037 PMID:19797353

Portigal CL, Cowell SP, Fedoruk MN, Butler CM, Rennie PS, Nelson CC (2002). Polychlorinated biphenyls interfere with androgen-induced transcriptional activation and hormone binding. *Toxicol Appl Pharmacol*, 179(3):185–94. doi:10.1006/taap.2002.9371 PMID:11906248

Price NO, Young RW, Dickinson JK, Bunce GE (1972). Pesticide residues and polychlorinated biphenyl levels in diets, urine, and fecal matter of preadolescent girls. *Proc Soc Exp Biol Med*, 139(4):1280–3. doi:10.3181/00379727-139-36347 PMID:4623415

Probst GS, McMahon RE, Hill LE, Thompson CZ, Epp JK, Neal SB (1981). Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: a comparison with bacterial mutagenicity using 218 compounds. *Environ Mutagen*, 3(1):11–32. doi:10.1002/em.2860030103 PMID:7021142

Puga A, Ma C, Marlowe JL (2009). The aryl hydrocarbon receptor cross-talks with multiple signal transduction pathways. *Biochem Pharmacol*, 77(4):713–22. doi:10.1016/j.bcp.2008.08.031 PMID:18817753

Rallis GN, Sakkas VA, Boumba VA, Vougiouklakis T, Albanis TA (2012). Determination of organochlorine pesticides and polychlorinated biphenyls in post-mortem human lung by matrix solid-phase dispersion

with the aid of response surface methodology and desirability function. *J Chromatogr A*, 1227:1–9. doi:10.1016/j.chroma.2011.12.083 PMID:22265777

Ramamoorthy K, Vyhlidal C, Wang F, Chen I, Safe S, McDonnell DP *et al.* (1997). Additive estrogenic activities of a binary mixture of 2′,4′,6′-trichloro- and 2′3′,4′,5′-tetrachloro-4-biphenylol. *Toxicol Appl Pharmacol*, 147(1):93–100. doi:10.1006/taap.1997.8281 PMID:9356311

Rasmussen TH, Nielsen F, Andersen HR, Nielsen JB, Weihe P, Grandjean P (2003). Assessment of xenoestrogenic exposure by a biomarker approach: application of the E-Screen bioassay to determine estrogenic response of serum extracts. *Environ Health*, 2(1):12 doi:10.1186/1476-069X-2-12 PMID:14613489

Ravoori S, Ayotte P, Srinivasan C, Pereg D, Robertson LW, Russell GK *et al.* (2008). DNA damage associated with PCBs in the whole blood cells of Inuit. *Environ Toxicol Pharmacol*, 25(2):273–6. doi:10.1016/j.etap.2007.10.027 PMID:21783863

Ravoori S, Srinivasan C, Pereg D, Robertson LW, Ayotte P, Gupta RC (2010). Protective effects of selenium against DNA adduct formation in Inuit environmentally exposed to PCBs. *Environ Int*, 36(8):980–6. doi:10.1016/j.envint.2009.08.001 PMID:19735942

Rayne S, Forest K (2010). pK(a) values of the monohydroxylated polychlorinated biphenyls (OH-PCBs), polybrominated biphenyls (OH-PBBs), polychlorinated diphenyl ethers (OH-PCDEs), and polybrominated diphenyl ethers (OH-PBDEs). *J Environ Sci Health A Tox Hazard Subst Environ Eng*, 45(11):1322–46. doi:10.1080/10934529.2010.500885 PMID:20658412

Rice RH, Cohen DE (1996). Toxic responses of the skin . In: Klaassen CD editor. *Cassarett and Doull's toxicology: The basic science of poisons*. New York: McGraw-Hill.

Richardson JR, Miller GW (2004). Acute exposure to Aroclor 1016 or 1260 differentially affects dopamine transporter and vesicular monoamine transporter 2 levels. *Toxicol Lett*, 148(1-2):29–40. doi:10.1016/j.toxlet.2003.12.006 PMID:15019086

Rignell-Hydbom A, Rylander L, Giwercman A, Jönsson BA, Lindh C, Eleuteri P *et al.* (2005). Exposure to PCBs and p,p′-DDE and human sperm chromatin integrity. *Environ Health Perspect*, 113(2):175–9. doi:10.1289/ehp.7252 PMID:15687046

Ritter R, Scheringer M, MacLeod M, Moeckel C, Jones KC, Hungerbühler K (2011). Intrinsic human elimination half-lives of polychlorinated biphenyls derived from the temporal evolution of cross-sectional biomonitoring data from the United Kingdom. *Environ Health Perspect*, 119(2):225–31. doi:10.1289/ehp.1002211 PMID:20934951

Robertson LW, Berberian I, Borges T, Chen LC, Chow CK, Glauert HP *et al.* (2007). Suppression of peroxisomal enzyme activities and cytochrome P450 4A isozyme expression by congeneric polybrominated and polychlorinated biphenyls. *PPAR Res*, 2007:15481 doi:10.1155/2007/15481 PMID:18274624

Robertson LW, Ludewig G (2011). Polychlorinated Biphenyl (PCB) carcinogenicity with special emphasis on airborne PCBs. *Gefahrst Reinhalt Luft*, 71(1–2):25–32. PMID:21686028

Rogan WJ (1989). Yu-Cheng . In: Kimbrough RD, Jensen AA editors. 2nd Ed. *Topics in environmental Health 4: Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products*. Amsterdam, the Netherlands: Elsevier Science Publishers, pp. 401–415.

Roos R, Andersson PL, Halldin K, Håkansson H, Westerholm E, Hamers T *et al.* (2011). Hepatic effects of a highly purified 2,2′,3,4,4′,5,5′-heptachlorbiphenyl (PCB 180) in male and female rats. *Toxicology*, 284(1–3):42–53. doi:10.1016/j.tox.2011.03.013 PMID:21458519

Ross P, De Swart R, Addison R, Van Loveren H, Vos J, Osterhaus A (1996). Contaminant-induced immunotoxicity in harbour seals: wildlife at risk? *Toxicology*, 112(2):157–69. doi:10.1016/0300-483X(96)03396-3 PMID:8814345

Ross PS, De Swart RL, Reijnders PJH, Van Loveren H, Vos JG, Osterhaus AD (1995). Contaminant-related suppression of delayed-type hypersensitivity and antibody responses in harbor seals fed herring from the Baltic Sea. *Environ Health Perspect*, 103(2):162–7. doi:10.1289/ehp.95103162 PMID:7737064

Ruch RJ, Klaunig JE (1986). Effects of tumor promoters, genotoxic carcinogens and hepatocytotoxins on mouse hepatocyte intercellular communication. *Cell Biol Toxicol*, 2(4):469–83. doi:10.1007/BF00117849 PMID:2477123

Ruiz P, Faroon O, Moudgal CJ, Hansen H, De Rosa CT, Mumtaz M (2008). Prediction of the health effects of polychlorinated biphenyls (PCBs) and their metabolites using quantitative structure-activity relationship (QSAR). *Toxicol Lett*, 181(1):53–65. doi:10.1016/j.toxlet.2008.06.870 PMID:18662755

Rusiecki JA, Baccarelli A, Bollati V, Tarantini L, Moore LE, Bonefeld-Jørgensen EC (2008). Global DNA hypomethylation is associated with high serum-persistent organic pollutants in Greenlandic Inuit. *Environ Health Perspect*, 116(11):1547–52. doi:10.1289/ehp.11338 PMID:19057709

Sacco JC, Lehmler HJ, Robertson LW, Li W, James MO (2008). Glucuronidation of polychlorinated biphenylols and UDP-glucuronic acid concentrations in channel catfish liver and intestine. *Drug Metab Dispos*, 36(4):623–30. doi:10.1124/dmd.107.019596 PMID:18180271

Safe S (1989). Polychlorinated biphenyls (PCBs): mutagenicity and carcinogenicity. *Mutat Res*, 220(1):31–47. doi:10.1016/0165-1110(89)90007-9 PMID:2492077

Safe S (1990). Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic

considerations which support the development of toxic equivalency factors (TEFs). *Crit Rev Toxicol*, 21(1):51–88. doi:10.3109/10408449009089873 PMID:2124811

Safe S (1993). Toxicology, structure-function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. *Environ Health Perspect*, 100:259–68. doi:10.1289/ehp.93100259 PMID:8354174

Safe S, Bandiera S, Sawyer T, Robertson L, Safe L, Parkinson A *et al.* (1985). PCBs: structure-function relationships and mechanism of action. *Environ Health Perspect*, 60:47–56. PMID:2992927

Sandal S, Yilmaz B, Carpenter DO (2008). Genotoxic effects of PCB 52 and PCB 77 on cultured human peripheral lymphocytes. *Mutat Res*, 654(1):88–92. doi:10.1016/j.mrgentox.2008.05.005 PMID:18573685

Sargent L, Dragan YP, Erickson C, Laufer CJ, Pitot HC (1991). Study of the separate and combined effects of the non-planar 2,5,2′,5′- and the planar 3,4,3′,4′-tetrachlorobiphenyl in liver and lymphocytes in vivo. *Carcinogenesis*, 12(5):793–800. doi:10.1093/carcin/12.5.793 PMID:1827616

Sargent L, Roloff B, Meisner L (1989). In vitro chromosome damage due to PCB interactions. *Mutat Res*, 224(1):79–88. doi:10.1016/0165-1218(89)90006-2 PMID:2505070

Sargent LM, Sattler GL, Roloff B, Xu YH, Sattler CA, Meisner L *et al.* (1992). Ploidy and specific karyotypic changes during promotion with phenobarbital, 2,5,2′,5′-tetrachlorobiphenyl, and/or 3,4,3′4′-tetrachlorobiphenyl in rat liver. *Cancer Res*, 52(4):955–62. PMID:1737357

Sartor MA, Schnekenburger M, Marlowe JL, Reichard JF, Wang Y, Fan Y *et al.* (2009). Genomewide analysis of aryl hydrocarbon receptor binding targets reveals an extensive array of gene clusters that control morphogenetic and developmental programs. *Environ Health Perspect*, 117(7):1139–46. doi:10.1289/ehp.0800485 PMID:19654925

Sasaki YF, Sekihashi K, Izumiyama F, Nishidate E, Saga A, Ishida K *et al.* (2000). The comet assay with multiple mouse organs: comparison of comet assay results and carcinogenicity with 208 chemicals selected from the IARC monographs and U.S. NTP Carcinogenicity Database. *Crit Rev Toxicol*, 30(6):629–799. doi:10.1080/10408440008951123 PMID:11145306

Scheele J, Teufel M, Niessen KH (1992). Chlorinated hydrocarbons in the bone marrow of children: studies on their association with leukaemia. *Eur J Pediatr*, 151(11):802–5. doi:10.1007/BF01957928 PMID:1468452

Schiestl RH, Aubrecht J, Yap WY, Kandikonda S, Sidhom S (1997). Polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin induce intrachromosomal recombination in vitro and in vivo. *Cancer Res*, 57(19):4378–83. PMID:9331101

Schilderman PA, Maas LM, Pachen DM, de Kok TM, Kleinjans JC, van Schooten FJ (2000). Induction of DNA adducts by several polychlorinated biphenyls. *Environ Mol Mutagen*, 36(2):79–86. doi:10.1002/1098-2280(2000)36:2<79::AID-EM1>3.0.CO;2-E PMID:11013405

Schlezinger JJ, Keller J, Verbrugge LA, Stegeman JJ (2000). 3,3′4,4′-Tetrachlorobiphenyl oxidation in fish, bird and reptile species: relationship to cytochrome P450 1A inactivation and reactive oxygen production. *Comp Biochem Physiol C Toxicol Pharmacol*, 125(3):273–86. PMID:11790349

Schlezinger JJ, Struntz WDJ, Goldstone JV, Stegeman JJ (2006). Uncoupling of cytochrome P450 1A and stimulation of reactive oxygen species production by co-planar polychlorinated biphenyl congeners. *Aquat Toxicol*, 77(4):422–32. doi:10.1016/j.aquatox.2006.01.012 PMID:16500718

Schlezinger JJ, White RD, Stegeman JJ (1999). Oxidative inactivation of cytochrome P-450 1A (CYP1A) stimulated by 3,3′,4,4′-tetrachlorobiphenyl: production of reactive oxygen by vertebrate CYP1As. *Mol Pharmacol*, 56(3):588–97. PMID:10462547

Schlummer M, Moser GA, McLachlan MS (1998). Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: mass balances and mechanistic considerations. *Toxicol Appl Pharmacol*, 152(1):128–37. doi:10.1006/taap.1998.8487 PMID:9772208

Schmidt JV, Bradfield CA (1996). Ah receptor signaling pathways. *Annu Rev Cell Dev Biol*, 12(1):55–89. doi:10.1146/annurev.cellbio.12.1.55 PMID:8970722

Schoeny R (1982). Mutagenicity testing of chlorinated biphenyls and chlorinated dibenzofurans. *Mutat Res*, 101(1):45–56. doi:10.1016/0165-1218(82)90164-1 PMID:7043248

Schoeny RS, Smith CC, Loper JC (1979). Non-mutagenicity for Salmonella of the chlorinated hydrocarbons Aroclor 1254, 1,2,4-trichlorobenzene, mirex and kepone. *Mutat Res*, 68(2):125–32. doi:10.1016/0165-1218(79)90140-X PMID:92763

Schrader TJ, Cooke GM (2003). Effects of Aroclors and individual PCB congeners on activation of the human androgen receptor in vitro. *Reprod Toxicol*, 17(1):15–23. doi:10.1016/S0890-6238(02)00076-X PMID:12507654

Schrenk D, Schmitz HJ, Bohnenberger S, Wagner B, Wörner W (2004). Tumor promoters as inhibitors of apoptosis in rat hepatocytes. *Toxicol Lett*, 149(1–3):43–50. doi:10.1016/j.toxlet.2003.12.019 PMID:15093247

Schuetz EG, Brimer C, Schuetz JD (1998). Environmental xenobiotics and the antihormones cyproterone acetate and spironolactone use the nuclear hormone pregnenolone X receptor to activate the CYP3A23 hormone response element. *Mol Pharmacol*, 54(6):1113–7. PMID:9855641

Schuur AG, Brouwer A, Bergman A, Coughtrie MW, Visser TJ (1998b). Inhibition of thyroid hormone sulfation by hydroxylated metabolites of polychlorinated biphenyls.

Chem Biol Interact, 109(1–3):293–7. doi:10.1016/S0009-2797(97)00140-3 PMID:9566753
Schuur AG, Legger FF, van Meeteren ME, Moonen MJ, van Leeuwen-Bol I, Bergman A *et al.* (1998a). In vitro inhibition of thyroid hormone sulfation by hydroxylated metabolites of halogenated aromatic hydrocarbons. *Chem Res Toxicol*, 11(9):1075–81. doi:10.1021/tx9800046 PMID:9760282
Schuur AG, van Leeuwen-Bol I, Jong WM, Bergman A, Coughtrie MW, Brouwer A *et al.* (1998c). In vitro inhibition of thyroid hormone sulfation by polychlorobiphenylols: isozyme specificity and inhibition kinetics. *Toxicol Sci*, 45(2):188–94. PMID:9848125
Schwenk M, Gabrio T, Päpke O, Wallenhorst T (2002). Human biomonitoring of polychlorinated biphenyls and polychlorinated dibenzodioxins and dibenzofuranes in teachers working in a PCB-contaminated school. *Chemosphere*, 47(2):229–33. doi:10.1016/S0045-6535(01)00307-1 PMID:11993638
Seegal RF, Brosch KO, Okoniewski RJ (2005). Coplanar PCB congeners increase uterine weight and frontal cortical dopamine in the developing rat: implications for developmental neurotoxicity. *Toxicol Sci*, 86(1):125–31. doi:10.1093/toxsci/kfi174 PMID:15843507
Seelbach M, Chen L, Powell A, Choi YJ, Zhang B, Hennig B *et al.* (2010). Polychlorinated biphenyls disrupt blood-brain barrier integrity and promote brain metastasis formation. *Environ Health Perspect*, 118(4):479–84. doi:10.1289/ehp.0901334 PMID:20064788
Sells DM, Brix AE, Nyska A, Jokinen MP, Orzech DP, Walker NJ (2007). Respiratory tract lesions in noninhalation studies. *Toxicol Pathol*, 35(1):170–7. doi:10.1080/01926230601059969 PMID:17325986
Semple-Roberts E, Hayes MA, Armstrong D, Becker RA, Racz WJ, Farber E (1987). Alternative methods of selecting rat hepatocellular nodules resistant to 2-acetylaminofluorene. *Int J Cancer*, 40(5):643–5. doi:10.1002/ijc.2910400512 PMID:3679591
Senthilkumar PK, Klingelhutz AJ, Jacobus JA, Lehmler H, Robertson LW, Ludewig G (2011). Airborne polychlorinated biphenyls (PCBs) reduce telomerase activity and shorten telomere length in immortal human skin keratinocytes (HaCat). *Toxicol Lett*, 204(1):64–70. doi:10.1016/j.toxlet.2011.04.012 PMID:21530622
Senthilkumar PK, Robertson LW, Ludewig G (2012). PCB153 reduces telomerase activity and telomere length in immortalized human skin keratinocytes (HaCaT) but not in human foreskin keratinocytes (NFK). *Toxicol Appl Pharmacol*, 259(1):115–23. doi:10.1016/j.taap.2011.12.015 PMID:22210444
Shaddock JG, Heflich RH, McMillan DC, Hinson JA, Casciano DA (1989). Pretreatment with mixed-function oxidase inducers increases the sensitivity of the hepatocyte/DNA repair assay. *Environ Mol Mutagen*, 13(4):281–8. doi:10.1002/em.2850130402 PMID:2737181
Shahin MM, Andrillon P, Goetz N, Boré P, Bugaut A, Kalopissis G (1979). Studies on the mutagenicity of p-phenylenediamine in Salmonella typhimurium. Presence of PCB's in rat-liver microsomal fraction induced by Aroclor. *Mutat Res*, 68(4):327–36. doi:10.1016/0165-1218(79)90165-4 PMID:118383
Shain W, Overmann SR, Wilson LR, Kostas J, Bush B (1986). A congener analysis of polychlorinated biphenyls accumulating in rat pups after perinatal exposure. *Arch Environ Contam Toxicol*, 15(6):687–707. doi:10.1007/BF01054916 PMID:3098189
Shimada T, Gillam EM, Sutter TR, Strickland PT, Guengerich FP, Yamazaki H (1997). Oxidation of xenobiotics by recombinant human cytochrome P450 1B1. *Drug Metab Dispos*, 25(5):617–22. PMID:9152602
Shimada T, Sawabe Y (1984). Comparative studies on distribution and covalent tissue binding of 2,4,2′,4′- and 3,4,3′,4′-tetrachlorobiphenyl isomers in the rat. *Arch Toxicol*, 55(3):182–5. doi:10.1007/BF00316125 PMID:6437376
Silberhorn EM, Glauert HP, Robertson LW (1990). Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. *Crit Rev Toxicol*, 20(6):440–96. doi:10.3109/10408449009029331 PMID:2165409
Silkworth JB, Antrim L, Kaminsky LS (1984). Correlations between polychlorinated biphenyl immunotoxicity, the aromatic hydrocarbon locus, and liver microsomal enzyme induction in C57BL/6 and DBA/2 mice. *Toxicol Appl Pharmacol*, 75(1):156–65. doi:10.1016/0041-008X(84)90086-3 PMID:6431639
Silkworth JB, Grabstein EM (1982). Polychlorinated biphenyl immunotoxicity: dependence on isomer planarity and the Ah gene complex. *Toxicol Appl Pharmacol*, 65(1):109–15. doi:10.1016/0041-008X(82)90368-4 PMID:6815831
Silkworth JB, Koganti A, Illouz K, Possolo A, Zhao M, Hamilton SB (2005). Comparison of TCDD and PCB CYP1A induction sensitivities in fresh hepatocytes from human donors, sprague-dawley rats, and rhesus monkeys and HepG2 cells. *Toxicol Sci*, 87(2):508–19. doi:10.1093/toxsci/kfi261 PMID:16049271
Silkworth JB, Loose LD (1981). Assessment of environmental contaminant-induced lymphocyte dysfunction. *Environ Health Perspect*, 39:105–28. doi:10.1289/ehp.8139105 PMID:7016518
Šimečková P, Vondrácek J, Procházková J, Kozubík A, Krcmár P, Machala M (2009b). 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) induces degradation of adherens junction proteins and inhibits beta-catenin-dependent transcription in liver epithelial cells. *Toxicology*, 260(1-3):104–11. doi:10.1016/j.tox.2009.03.014 PMID:19464575
Šimečková P, Vondrácek J, Andrysík Z, Zatloukalová J, Krcmár P, Kozubík A *et al.* (2009a). The 2,2′,4,4′,5,5′-hexachlorobiphenyl-enhanced degradation of connexin 43 involves both proteasomal and lysosomal activities.

Toxicol Sci, 107(1):9–18. doi:10.1093/toxsci/kfn202 PMID:18832185

Sina JF, Bean CL, Dysart GR, Taylor VI, Bradley MO (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. *Mutat Res*, 113(5):357–91. doi:10.1016/0165-1161(83)90228-5 PMID:6877265

Singh V, Parmar D, Singh MP (2008). Do single nucleotide polymorphisms in xenobiotic metabolizing genes determine breast cancer susceptibility and treatment outcomes? *Cancer Invest*, 26(8):769–83. doi:10.1080/07357900801953196 PMID:18798070

Sinjari T, Klasson-Wehler E, Oskarsson A, Darnerud PO (1996). Milk transfer and neonatal uptake of coplanar polychlorinated biphenyl (PCB) congeners in mice. *Pharmacol Toxicol*, 78(3):181–6. doi:10.1111/j.1600-0773.1996.tb00201.x PMID:8882352

Sipka S, Eum SY, Son KW, Xu S, Gavalas VG, Hennig B *et al.* (2008). Oral administration of PCBs induces proinflammatory and prometastatic responses. *Environ Toxicol Pharmacol*, 25(2):251–9. doi:10.1016/j.etap.2007.10.020 PMID:18438459

Sipos E, Chen L, András IE, Wrobel J, Zhang B, Pu H *et al.* (2012). Proinflammatory adhesion molecules facilitate polychlorinated biphenyl-mediated enhancement of brain metastasis formation. *Toxicol Sci*, 126(2):362–71. doi:10.1093/toxsci/kfr349 PMID:22240979

Smart J, Daly AK (2000). Variation in induced CYP1A1 levels: relationship to CYP1A1, Ah receptor and GSTM1 polymorphisms. *Pharmacogenetics*, 10(1):11–24. doi:10.1097/00008571-200002000-00003 PMID:10739168

Smialowicz RJ, Andrews JE, Riddle MM, Rogers RR, Luebke RW, Copeland CB (1989). Evaluation of the immunotoxicity of low level PCB exposure in the rat. *Toxicology*, 56(2):197–211. doi:10.1016/0300-483X(89)90133-9 PMID:2499955

Smith AB, Schloemer J, Lowry LK, Smallwood AW, Ligo RN, Tanaka S *et al.* (1982). Metabolic and health consequences of occupational exposure to polychlorinated biphenyls. *Br J Ind Med*, 39(4):361–9. PMID:6128023

Smith SH, Sanders VM, Barrett BA,, Borzelleca JF, Munson AE (1978). Immunotoxicological evaluation of mice exposed to polychlorinated biphenyls. *Toxicol Appl Pharmacol*, 45:330–337.

Solt DB, Medline A, Farber E (1977). Rapid emergence of carcinogen-induced hyperplastic lesions in a new model for the sequential analysis of liver carcinogenesis. *Am J Pathol*, 88(3):595–618. PMID:18937

Spanò M, Toft G, Hagmar L, Eleuteri P, Rescia M, Rignell-Hydbom A *et al.* ; INUENDO(2005). Exposure to PCB and p, p′-DDE in European and Inuit populations: impact on human sperm chromatin integrity. *Hum Reprod*, 20(12):3488–99. doi:10.1093/humrep/dei297 PMID:16223788

Srinivasan A, Lehmler HJ, Robertson LW, Ludewig G (2001). Production of DNA strand breaks in vitro and reactive oxygen species in vitro and in HL-60 cells by PCB metabolites. *Toxicol Sci*, 60(1):92–102. doi:10.1093/toxsci/60.1.92 PMID:11222876

Srinivasan A, Robertson LW, Ludewig G (2002). Sulfhydryl binding and topoisomerase inhibition by PCB metabolites. *Chem Res Toxicol*, 15(4):497–505. doi:10.1021/tx010128+ PMID:11952335

Stadnicki SS, Allen JR (1979). Toxicity of 2,2′,5,5′-tetrachlorobiphenyl and its metabolites, 2,2′,5,5′-tetrachlorobiphenyl-3.4-oxide and 2,2′,5,5′-tetrachlorobiphenyl-4-ol to cultured cells in vitro. *Bull Environ Contam Toxicol*, 23(6):788–96. doi:10.1007/BF01770043 PMID:117862

Stadnicki SS, Lin FS, Allen JR (1979). DNA single strand breaks caused by 2,2′,5,5′-tetrachlorobiphenyl and its metabolites. *Res Commun Chem Pathol Pharmacol*, 24(2):313–27. PMID:111326

Stenberg M, Hamers T, Machala M, Fonnum F, Stenius U, Lauy AA *et al.* (2011). Multivariate toxicity profiles and QSAR modeling of non-dioxin-like PCBs–an investigation of in vitro screening data from ultra-pure congeners. *Chemosphere*, 85(9):1423–9. doi:10.1016/j.chemosphere.2011.08.019 PMID:21890175

Strathmann J, Schwarz M, Tharappel JC, Glauert HP, Spear BT, Robertson LW *et al.* (2006). PCB 153, a non-dioxin-like tumor promoter, selects for beta-catenin (Catnb)-mutated mouse liver tumors. *Toxicol Sci*, 93(1):34–40. doi:10.1093/toxsci/kfl041 PMID:16782779

Street JC, Sharma RP (1975). Alteration of induced cellular and humoral immune responses by pesticides and chemicals of environmental concern: quantitative studies of immunosuppression by DDT, Aroclor 1254, carbaryl, carbofuran, and methylparathion. *Toxicol Appl Pharmacol*, 32(3):587–602. doi:10.1016/0041-008X(75)90123-4 PMID:50651

Stronati A, Manicardi GC, Cecati M, Bordicchia M, Ferrante L, Spanò M *et al.* (2006). Relationships between sperm DNA fragmentation, sperm apoptotic markers and serum levels of CB-153 and p,p’-DDE in European and Inuit populations. *Reproduction*, 132(6):949–58. doi:10.1530/rep.1.01034 PMID:17127755

Sugimura T, Sato S, Nagao M *et al.* (1976). Overlapping of carcinogens and mutagens. In: Magnee PN editor. *Fundamentals in cancer prevention*. Baltimore (MD): University Park Press, pp. 191–215.

Svensson B-G, Hallberg T, Nilsson A, Schütz A, Hagmar L (1994). Parameters of immunological competence in subjects with high consumption of fish contaminated with persistent organochlorine compounds. *Int Arch Occup Environ Health*, 65(6):351–8. doi:10.1007/BF00383243 PMID:8034358

Swierenga SHH, Yamasaki H, Piccoli C, Robertson L, Bourgon L, Marceau N *et al.* (1990). Effects on intercellular communication in human keratinocytes

and liver-derived cells of polychlorinated biphenyl congeners with differing in vivo promotion activities. *Carcinogenesis*, 11(6):921–6. doi:10.1093/carcin/11.6.921 PMID:2112060

Talcott PA, Koller LD (1983). The effect of inorganic lead and/or a polychlorinated biphenyl on the developing immune system of mice. *J Toxicol Environ Health*, 12(2–3):337–52. doi:10.1080/15287398309530431 PMID:6418890

Talcott PA, Koller LD, Exon JH (1985). The effect of lead and polychlorinated biphenyl exposure on rat natural killer cell cytotoxicity. *Int J Immunopharmacol*, 7(2):255–61. doi:10.1016/0192-0561(85)90034-7 PMID:3924847

Tampal N, Lehmler HJ, Espandiari P, Malmberg T, Robertson LW (2002). Glucuronidation of hydroxylated polychlorinated biphenyls (PCBs). *Chem Res Toxicol*, 15(10):1259–66. doi:10.1021/tx0200212 PMID:12387623

Tanabe S, Nakagawa Y, Tatsukawa R (1981). Absorption efficiency and biological half-life of individual chlorobiphenyls in rats treated with Kanechlor products. *Agric Biol Chem*, 45(3):717–26. doi:10.1271/bbb1961.45.717

Tharappel JC, Lee EY, Robertson LW, Spear BT, Glauert HP (2002). Regulation of cell proliferation, apoptosis, and transcription factor activities during the promotion of liver carcinogenesis by polychlorinated biphenyls. *Toxicol Appl Pharmacol*, 179(3):172–84. doi:10.1006/taap.2001.9360 PMID:11906247

Thomas PT, Hinsdill RD (1978). Effect of polychlorinated biphenyls on the immune responses of rhesus monkeys and mice. *Toxicol Appl Pharmacol*, 44(1):41–51. doi:10.1016/0041-008X(78)90282-X PMID:79241

Thomas PT, Hinsdill RD (1980). Perinatal PCB exposure and its effect on the immune system of young rabbits. *Drug Chem Toxicol*, 3(2):173–84. doi:10.3109/01480548009108281 PMID:6785064

Tian Y, Rabson AB, Gallo MA (2002). Ah receptor and NF-kappaB interactions: mechanisms and physiological implications. *Chem Biol Interact*, 141(1–2):97–115. doi:10.1016/S0009-2797(02)00068-6 PMID:12213387

Toborek M, Barger SW, Mattson MP, Espandiari P, Robertson LW, Hennig B (1995). Exposure to polychlorinated biphenyls causes endothelial cell dysfunction. *J Biochem Toxicol*, 10(4):219–26. doi:10.1002/jbt.2570100406 PMID:8568836

Tretjak Z, Volavsek C, Beckmann SL (1990). Structural chromosome aberrations and industrial waste. *Lancet*, 335(8700):1288 doi:10.1016/0140-6736(90)91362-E PMID:1971364

Tryphonas H, Feeley M (2001). Polychlorinated Biphenyl-induced Immunomodulation and Human Health Effects. In: Robertson LW, Hansen LG editors. *PCBs Recent Advances in Environmental Toxicology and Health Effects*. Lexington (KY): The University Press of Kentucky, pp. 193–209.

Tryphonas H, Hayward S, O'Grady L, Loo JC, Arnold DL, Bryce F *et al.* (1989). Immunotoxicity studies of PCB (Aroclor 1254) in the adult rhesus (Macaca mulatta) monkey–preliminary report. *Int J Immunopharmacol*, 11(2):199–206. doi:10.1016/0192-0561(89)90072-6 PMID:2495254

Tryphonas H, Luster MI, Schiffman G, Dawson LL, Hodgen M, Germolec D *et al.* (1991b). Effect of chronic exposure of PCB (Aroclor 1254) on specific and nonspecific immune parameters in the rhesus (Macaca mulatta) monkey. *Fundam Appl Toxicol*, 16(4):773–86. doi:10.1016/0272-0590(91)90163-X PMID:1884915

Tryphonas H, Luster MI, White KL Jr, Naylor PH, Erdos MR, Burleson GR *et al.* (1991a). Effects of PCB (Aroclor 1254) on non-specific immune parameters in rhesus (Macaca mulatta) monkeys. *Int J Immunopharmacol*, 13(6):639–48. doi:10.1016/0192-0561(91)90176-8 PMID:1721612

Tryphonas L, Charbonneau S, Tryphonas H, Zawidzka Z, Mes J, Wong J *et al.* (1986). Comparative aspects of Aroclor 1254 toxicity in adult cynomolgus and rhesus monkeys: a pilot study. *Arch Environ Contam Toxicol*, 15(2):159–69. doi:10.1007/BF01059965 PMID:3085600

Tryphonas L, Truelove J, Zawidzka Z, Wong J, Mes J, Charbonneau S *et al.* (1984). Polychlorinated biphenyl (PCB) toxicity in adult cynomolgus monkeys (M. fascicularis): a pilot study. *Toxicol Pathol*, 12(1):10–25. doi:10.1177/019262338401200103 PMID:6436955

Tsai PC, Huang W, Lee YC, Chan SH, Guo YL (2006). Genetic polymorphisms in CYP1A1 and GSTM1 predispose humans to PCBs/PCDFs-induced skin lesions. *Chemosphere*, 63(8):1410–8. doi:10.1016/j.chemosphere.2005.08.012 PMID:16580705

Tsuda H, Lee G, Farber E (1980). Induction of resistant hepatocytes as a new principle for a possible short-term in vivo test for carcinogens. *Cancer Res*, 40(4):1157–64. PMID:6101993

Tsukimori K, Morokuma S, Hori T, Takahashi K, Hirata T, Otera Y *et al.* (2013). Characterization of placental transfer of polychlorinated dibenzo-p-dioxins, dibenzofurans and polychlorinated biphenyls in normal pregnancy. *J Obstet Gynaecol Res*, 39(1):83–90. doi:10.1111/j.1447-0756.2012.01906.x PMID:22672617

Turyk ME, Anderson HA, Freels S, Chatterton R Jr, Needham LL, Patterson DG Jr *et al.* ; Great Lakes Consortium(2006). Associations of organochlorines with endogenous hormones in male Great Lakes fish consumers and nonconsumers. *Environ Res*, 102(3):299–307. doi:10.1016/j.envres.2006.01.009 PMID:16563369

Umannová L, Zatloukalová J, Machala M, Krcmár P, Májková Z, Hennig B *et al.* (2007). Tumor necrosis factor-alpha modulates effects of aryl hydrocarbon receptor ligands on cell proliferation and expression of cytochrome P450 enzymes in rat liver "stem-like" cells.

Toxicol Sci, 99(1):79–89. doi:10.1093/toxsci/kfm149 PMID:17557910

van Birgelen APJM, Ross DG, DeVito MJ, Birnbaum LS (1996). Interactive effects between 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,2′,4,4′,5,5′-hexachlorobiphenyl in female B6C3F1 mice: tissue distribution and tissue-specific enzyme induction. *Fundam Appl Toxicol*, 34(1):118–31. doi:10.1006/faat.1996.0182 PMID:8937899

van den Berg KJ, Zurcher C, Brouwer A (1988). Effects of 3,4,3′,4′-tetrachlorobiphenyl on thyroid function and histology in marmoset monkeys. *Toxicol Lett*, 41(1):77–86. doi:10.1016/0378-4274(88)90010-0 PMID:3128898

Van den Berg M, Birnbaum L, Bosveld ATC, Brunström B, Cook P, Feeley M *et al.* (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect*, 106(12):775–92. doi:10.1289/ehp.98106775 PMID:9831538

Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M *et al.* (2006). The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol Sci*, 93(2):223–41. doi:10.1093/toxsci/kfl055 PMID:16829543

Van Den Heuvel RL, Koppen G, Staessen JA, Hond ED, Verheyen G, Nawrot TS *et al.* (2002). Immunologic biomarkers in relation to exposure markers of PCBs and dioxins in Flemish adolescents (Belgium). *Environ Health Perspect*, 110(6):595–600. doi:10.1289/ehp.02110595 PMID:12055051

Van Der Burght AS, Kreikamp AP, Horbach GJ, Seinen W, Van Den Berg M (1998). Characterization of CYP1A in hepatocytes of cynomolgus monkeys (Macaca fascicularis) and induction by different substituted polychlorinated biphenyls (PCBs). *Arch Toxicol*, 72(10):630–6. doi:10.1007/s002040050553 PMID:9851678

van der Plas SA, Haag-Grönlund M, Scheu G, Wärngård L, van den Berg M, Wester P *et al.* (1999). Induction of altered hepatic foci by a mixture of dioxin-like compounds with and without 2,2′4,4′,5,5′-hexachlorobiphenyl in female Sprague-Dawley rats. *Toxicol Appl Pharmacol*, 156(1):30–9. doi:10.1006/taap.1999.8629 PMID:10101096

van Pelt FN, Haring RM, Overkamp MJ, Weterings PJ (1991). Micronucleus formation in cultured human keratinocytes following exposure to mitomycin C and cyclophosphamide. *Mutat Res*, 252(1):45–50. doi:10.1016/0165-1161(91)90250-C PMID:1899911

Venkatesha VA, Kalen AL, Sarsour EH, Goswami PC (2010). PCB-153 exposure coordinates cell cycle progression and cellular metabolism in human mammary epithelial cells. *Toxicol Lett*, 196(2):110–6. doi:10.1016/j.toxlet.2010.04.005 PMID:20394812

Venkatesha VA, Venkataraman S, Sarsour EH, Kalen AL, Buettner GR, Robertson LW *et al.* (2008). Catalase ameliorates polychlorinated biphenyl-induced cytotoxicity in nonmalignant human breast epithelial cells. *Free Radic Biol Med*, 45(8):1094–102. doi:10.1016/j.freeradbiomed.2008.07.007 PMID:18691649

Vezina CM, Walker NJ, Olson JR (2004). Subchronic exposure to TCDD, PeCDF, PCB126, and PCB153: effect on hepatic gene expression. *Environ Health Perspect*, 112(16):1636–44. doi:10.1289/ehp.7253 PMID:15598615

Visser IKG, van Bressem MF, Barrett T, Osterhaus ADME (1993). Morbillivirus infections in aquatic mammals. *Vet Res*, 24(2):169–78. PMID:8343804

Voie OA, Wiik P, Fonnum F (1998). Ortho-substituted polychlorinated biphenyls activate respiratory burst measured as luminol-amplified chemoluminescence in human granulocytes. *Toxicol Appl Pharmacol*, 150(2):369–75. doi:10.1006/taap.1998.8438 PMID:9653068

Vondráček J, Machala M, Bryja V *et al.* (2005). Aryl hydrocarbon receptor-activating polychlorinated biphenyls and their hydroxylated metabolites induce cell proliferation in contact-inhibited rat liver epithelial cells. *Toxicol Sci*, 83:53–63. doi:10.1093/toxsci/kfi009 PMID:15483185

Vos JG, Beems RB (1971). Dermal toxicity studies of technical polychlorinated biphenyls and fractions thereof in rabbits. *Toxicol Appl Pharmacol*, 19(4):617–33. doi:10.1016/0041-008X(71)90294-8 PMID:5132032

Vos JG, Notenboom-Ram E (1972). Comparative toxicity study of 2,4,5,2′,4′,5′-hexachlorobiphenyl and a polychlorinated biphenyl mixture in rabbits. *Toxicol Appl Pharmacol*, 23(4):563–78. doi:10.1016/0041-008X(72)90097-X PMID:4630164

Vos JG, van Driel-Grootenhuis L (1972). PCB-induced suppression of the humoral and cell-mediated immunity in guinea pigs. *Sci Total Environ*, 1(3):289–302. doi:10.1016/0048-9697(72)90024-1 PMID:4633048

Vos JG, Van Genderen H (1973). Toxicological aspects of immunosuppression. Pesticides and the environment: a continuing controversy. Symposia Specialists. 527–545

Wakui S, Muto T, Suzuki Y, Takahashi H, Hano H (2012). Sertoli cells proliferate in adult rats with prenatal exposure to 3,3′,4,4′,5-pentachlorobiphenyl. *Arch Toxicol*, 86(1):159–62. doi:10.1007/s00204-011-0736-8 PMID:21789670

Waldner MJ, Foersch S, Neurath MF (2012). Interleukin-6–a key regulator of colorectal cancer development. *Int J Biol Sci*, 8(9):1248–53. doi:10.7150/ijbs.4614 PMID:23136553

Walker NJ, Crockett PW, Nyska A, Brix AE, Jokinen MP, Sells DM *et al.* (2005). Dose-additive carcinogenicity of a defined mixture of "dioxin-like compounds". *Environ Health Perspect*, 113(1):43–8. doi:10.1289/ehp.7351 PMID:15626646

Waller DP, Presperin C, Drum ML, Negrusz A, Larsen AK, van der Ven H *et al.* (1996). Great Lakes fish as a source of maternal and fetal exposure to chlorinated

hydrocarbons. *Toxicol Ind Health*, 12(3–4):335–45. doi:10.1177/074823379601200306 PMID:8843551

Wang LQ, James MO (2007). Sulfonation of 17beta-estradiol and inhibition of sulfotransferase activity by polychlorobiphenylols and celecoxib in channel catfish, Ictalurus punctatus. *Aquat Toxicol*, 81(3):286–92. doi:10.1016/j.aquatox.2006.12.011 PMID:17239972

Wang LQ, Lehmler HJ, Robertson LW, Falany CN, James MO (2005). In vitro inhibition of human hepatic and cDNA-expressed sulfotransferase activity with 3-hydroxybenzo[a]pyrene by polychlorobiphenylols. *Environ Health Perspect*, 113(6):680–7. doi:10.1289/ehp.7837 PMID:15929889

Wang LQ, Lehmler HJ, Robertson LW, James MO (2006). Polychlorobiphenylols are selective inhibitors of human phenol sulfotransferase 1A1 with 4-nitrophenol as a substrate. *Chem Biol Interact*, 159(3):235–46. doi:10.1016/j.cbi.2005.12.004 PMID:16413005

Wangpradit O, Teesch LM, Mariappan SV, Duffel MW, Norstrom K, Robertson LW *et al.* (2009). Oxidation of 4-chlorobiphenyl metabolites to electrophilic species by prostaglandin H synthase. *Chem Res Toxicol*, 22(1):64–71. doi:10.1021/tx800300t PMID:19105592

Warner NA, Martin JW, Wong CS (2009). Chiral polychlorinated biphenyls are biotransformed enantioselectively by mammalian cytochrome P-450 isozymes to form hydroxylated metabolites. *Environ Sci Technol*, 43(1):114–21. doi:10.1021/es802237u PMID:19209593

Watanabe M, Honda S, Hayashi M, Matsuda T (1982). Mutagenic effects of combinations of chemical carcinogens and environmental pollutants in mice as shown by the micronucleus test. *Mutat Res*, 97(1):43–8. doi:10.1016/0165-1161(82)90018-8 PMID:6799823

Wei W, Zhang C, Liu AL, Xie SH, Chen XM, Lu WQ (2009a). Effect of PCB153 on BaP-induced genotoxicity in HepG2 cells via modulation of metabolic enzymes. *Mutat Res*, 675(1–2):71–6. doi:10.1016/j.mrgentox.2009.02.013 PMID:19386251

Wei W, Zhang C, Liu AL, Xie SH, Chen XM, Lu WQ (2009b). PCB126 enhanced the genotoxicity of BaP in HepG2 cells by modulating metabolic enzyme and DNA repair activities. *Toxicol Lett*, 189(2):91–5. doi:10.1016/j.toxlet.2009.03.009 PMID:19553035

Weisglas-Kuperus N, Sas TC, Koopman-Esseboom C, van der Zwan CW, De Ridder MA, Beishuizen A *et al.* (1995). Immunologic effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. *Pediatr Res*, 38(3):404–10. doi:10.1203/00006450-199509000-00022 PMID:7494667

Wen S, Yang FX, Gong Y, Zhang XL, Hui Y, Li JG *et al.* (2008). Elevated levels of urinary 8-hydroxy-2′-deoxyguanosine in male electrical and electronic equipment dismantling workers exposed to high concentrations of polychlorinated dibenzo-p-dioxins and dibenzofurans, polybrominated diphenyl ethers, and polychlorinated biphenyls. *Environ Sci Technol*, 42(11):4202–7. doi:10.1021/es800044m PMID:18589988

Wens B, De Boever P, Verbeke M, Hollanders K, Schoeters G (2013). Cultured human peripheral blood mononuclear cells alter their gene expression when challenged with endocrine-disrupting chemicals. *Toxicology*, 303:17–24. doi:10.1016/j.tox.2012.10.019 PMID:23146750

Wester RC, Maibach HI, Bucks DA, McMaster J, Mobayen M, Sarason R *et al.* (1990). Percutaneous absorption and skin decontamination of PCBs: in vitro studies with human skin and in vivo studies in the rhesus monkey. *J Toxicol Environ Health*, 31(4):235–46. doi:10.1080/15287399009531453 PMID:2254950

Wester RC, Maibach HI, Sedik L, Melendres J, Wade M (1993). Percutaneous absorption of PCBs from soil: in vivo rhesus monkey, in vitro human skin, and binding to powdered human stratum corneum. *J Toxicol Environ Health*, 39(3):375–82. doi:10.1080/15287399309531758 PMID:8350383

Westerink WM, Stevenson JC, Schoonen WG (2008). Pharmacologic profiling of human and rat cytochrome P450 1A1 and 1A2 induction and competition. *Arch Toxicol*, 82(12):909–21. doi:10.1007/s00204-008-0317-7 PMID:18493746

Whysner J, Montandon F, McClain RM, Downing J, Verna LK, Steward RE 3rd *et al.* (1998). Absence of DNA adduct formation by phenobarbital, polychlorinated biphenyls, and chlordane in mouse liver using the 32P-postlabeling assay. *Toxicol Appl Pharmacol*, 148(1):14–23. doi:10.1006/taap.1997.8311 PMID:9465259

Wierda D, Irons RD, Greenlee WF (1981). Immunotoxicity in C57BL/6 mice exposed to benzene and Aroclor 1254. *Toxicol Appl Pharmacol*, 60(3):410–7. doi:10.1016/0041-008X(81)90325-2 PMID:6794184

Wilhelm M, Wittsiepe J, Lemm F *et al.* (2008). The Duisburg birth cohort study: influence of the prenatal exposure to PCDD/Fs and dioxin-like PCBs on thyroid hormone status in newborns and neurodevelopment of infants until the age of 24 months. *Mutat Res*, 659:83–92. doi:10.1016/j.mrrev.2007.11.002 PMID:18093869

Wolff MS (1985). Occupational exposure to polychlorinated biphenyls (PCBs). *Environ Health Perspect*, 60:133–8. doi:10.1289/ehp.8560133 PMID:3928344

Wolff MS, Fischbein A, Selikoff IJ (1992). Changes in PCB serum concentrations among capacitor manufacturing workers. *Environ Res*, 59(1):202–16. doi:10.1016/S0013-9351(05)80240-3 PMID:1425510

Wong A, Basrur P, Safe S (1979). The metabolically mediated DNA damage and subsequent DNA repair by 4-chlorobiphenyl in Chinese hamster ovary cells. *Res Commun Chem Pathol Pharmacol*, 24(3):543–50. PMID:451338

Wu X, Pramanik A, Duffel MW, Hrycay EG, Bandiera SM, Lehmler HJ *et al.* (2011). 2,2′,3,3′,6,6′-Hexachlorobiphenyl (PCB 136) is

enantioselectively oxidized to hydroxylated metabolites by rat liver microsomes. *Chem Res Toxicol*, 24(12):2249–57. doi:10.1021/tx200360m PMID:22026639

Wuu KD, Wong CK (1985). A chromosomal study on blood lymphocytes of patients poisoned by polychlorinated biphenyls. *Proc Natl Sci Counc Repub China B*, 9(1):67–9. PMID:3939618

Wyndham C, Devenish J, Safe S (1976). The in vitro metabolism, macromolecular binding and bacterial mutagenicity of 4-chloribiphenyl, a model PCB substrate. *Res Commun Chem Pathol Pharmacol*, 15(3):563–70. PMID:825937

Xie W, Wang K, Robertson LW, Ludewig G (2010). Investigation of mechanism(s) of DNA damage induced by 4-monochlorobiphenyl (PCB3) metabolites. *Environ Int*, 36(8):950–61. doi:10.1016/j.envint.2009.12.004 PMID:20129669

Xu YH, Campbell HA, Sattler GL, Hendrich S, Maronpot R, Sato K *et al.* (1990). Quantitative stereological analysis of the effects of age and sex on multistage hepatocarcinogenesis in the rat by use of four cytochemical markers. *Cancer Res*, 50(3):472–9. PMID:1967547

Yamazaki K, Suzuki M, Itoh T, Yamamoto K, Kanemitsu M, Matsumura C *et al.* (2011). Structural basis of species differences between human and experimental animal CYP1A1s in metabolism of 3,3′,4,4′,5-pentachlorobiphenyl. *J Biochem*, 149(4):487–94. doi:10.1093/jb/mvr009 PMID:21258071

Yoshimura H, Yamamoto H (1975). A novel route of excretion of 2, 4, 3′, 4′-tetrachlorobiphenyl in rats. *Bull Environ Contam Toxicol*, 13(6):681–8. doi:10.1007/BF01721936 PMID:806316

Yoshizawa K, Brix AE, Sells DM, Jokinen MP, Wyde M, Orzech DP *et al.* (2009). Reproductive lesions in female Harlan Sprague-Dawley rats following two-year oral treatment with dioxin and dioxin-like compounds. *Toxicol Pathol*, 37(7):921–37. doi:10.1177/0192623309351721 PMID:19843953

Yoshizawa K, Walker NJ, Nyska A, Kissling GE, Jokinen MP, Brix AE *et al.* (2010). Thyroid follicular lesions induced by oral treatment for 2 years with 2,3,7,8-tetrachlorodibenzo-p-dioxin and dioxin-like compounds in female Harlan Sprague-Dawley rats. *Toxicol Pathol*, 38(7):1037–50. doi:10.1177/0192623310382560 PMID:20924081

Zettner MA, Flor S, Ludewig G, Wagner J, Robertson LW, Lehmann L (2007). Quinoid metabolites of 4-monochlorobiphenyl induce gene mutations in cultured Chinese hamster v79 cells. *Toxicol Sci*, 100(1):88–98. doi:10.1093/toxsci/kfm204 PMID:17686921

Zhang Y, Wise JP, Holford TR, Xie H, Boyle P, Zahm SH *et al.* (2004). Serum polychlorinated biphenyls, cytochrome P-450 1A1 polymorphisms, and risk of breast cancer in Connecticut women. *Am J Epidemiol*, 160(12):1177–83. doi:10.1093/aje/kwh346 PMID:15583370

Zhao S, Narang A, Ding X, Eadon G (2004). Characterization and quantitative analysis of DNA adducts formed from lower chlorinated PCB-derived quinones. *Chem Res Toxicol*, 17(4):502–11. doi:10.1021/tx034245b PMID:15089092