Danish Ministry of the Environment
Environmental Protection Agency

Evaluation of health hazards by exposure to

Polychlorinated biphenyls (PCB)

and proposal of a health-based quality criterion for soil

Environmental Project No. 1485, 2014

Title:
Evaluation of health hazards by exposure to Polychlorinated biphenyls (PCB) and proposal of a health-based quality criterion for soil

Authors:
John Christian Larsen
Elsa Nielsen
Julie Boberg
Marta Axelstad Petersen

Published by:

The Danish Environmental Protection Agency
Strandgade 29
1401 Copenhagen K
Denmark
www.mst.dk/english

Year:

2014

ISBN no.

978-87-93026-17-9

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Preface

This report has been prepared by John Christian Larsen, Elsa Nielsen, Julie Boberg, and Marta Axelstad Petersen, Division of Toxicology and Risk Assessment, National Food Institute (DTU Food), Technical University of Denmark.

The Danish EPA has requested documentation for a health-based quality criterion in soil for PCB with focus on the PCB congeners of relevance in contaminated soil. It is stated that seven congeners (PCB 28, 52, 101, 118, 138, 153 and 180) are typically determined in soil. The Danish EPA has suggested that the present documentation should be based on the report 'Sundhedsmæssig vurdering af PCB-holdige bygningsfuger' (Gunnarsen et al. 2009) as well as on data regarding non-dioxin-like PCBs from the ATHON project (an EU FP6 project – full project title: Assessing the toxicity and hazard of non-dioxin-like PCBs present in food). From the information obtained it appears that several studies from the ATHON project still awaits publication, however, studies already published in the open literature have been considered. The health-based quality criterion in soil should be set for total-PCB with a correction factor taking into account that the seven PCB congeners typically determined in soil only constitute a few of the PCBs being present in contaminated soil.

The report has been elaborated according to the general practice laid down in the Danish EPA guidance document for the setting of health-based quality criteria for chemical substances in relation to soil, ambient air and drinking water.

The report has been subjected to review and discussion and has been endorsed by a steering committee consisting of the following representatives from Danish authorities:

- The Danish Nature Agency: Anne Christine Duer
- The Danish Ministry of Food, Agriculture and Fisheries (The Faculty of Agricultural Sciences): Niels Henrik Spliid
- The Danish Veterinary and Food Administration: Gudrun Hilbert
- The National Board of Health, Denmark, medical officer of health: Lene Garsdal
- Danish Regions: Arne Rokkjær,
- The Danish Environmental Protection Agency: Katrine Smith, Poul Bo Larsen, Finn Pedersen, Jette Rud Heltved.

1 General description

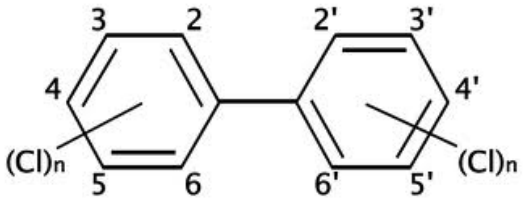
Polychlorinated biphenyls (PCBs) are synthetic chlorinated hydrocarbon compounds that consist of two benzene rings linked by a single carbon-carbon bond, with from 1 to all 10 of the hydrogen atoms replaced with chlorine atoms, see the structural formula. There are 209 possible PCB congeners, see Appendix 1. The PCB congeners are arranged in ascending numerical order using a numbering system developed by Ballschmiter and Zell (1980) that follow the IUPAC rules of substituent characterization in biphenyls. The resulting PCB numbers, also referred to as congener, IUPAC, or BZ numbers, are widely used for identifying individual congeners. PCBs can also be categorized by degree of chlorination. The term 'homolog' is used to refer to all PCBs with the same number of chlorines (e.g., trichlorobiphenyls). Homologs with different substitution patterns are referred to as isomers. For example, the dichlorophenyl homolog contains 12 isomers. (Larsen 2003, WHO 2003, ATSDR 2000).

The benzene rings can rotate around the bond connecting them; the two extreme configurations are planar (the two benzene rings in the same plane) and non-planar in which the benzene rings are at a 90° angle to each other. The co-planar PCBs are those where the benzene rings can rotate around the bond connecting them. This is mainly possible for congeners with no or only one chlorine atom in positions 2, 2', 6 and 6' (*ortho* positions) since the steric hindrance will be minimal. The degree of planarity is largely determined by the number of substitutions in the *ortho* positions. The replacement of hydrogen atoms in the *ortho* positions with larger chlorine atoms forces the benzene rings to rotate out of the planar configuration. The benzene rings of non-*ortho* substituted PCBs, as well as mono-*ortho* substituted PCBs, may assume a planar configuration and are referred to as planar or coplanar congeners. The benzene rings of the other congeners cannot assume a planar configuration and are referred to as non-planar congeners. (ATSDR 2000, Larsen 2003, Van den Berg et al. 1998, 2006; EFSA 2005).

1.1 Identity

Molecular formula:
$C_{12}H_{10-n}Cl_n$; n = 1-10

Structural formula:



Molecular weight:
189-499

CAS-no.:
1336-36-3 (for specific congeners, see Appendix 1)

Synonyms:
Commercial technical mixtures, marketed under trade names, such as Aroclor, Clophen, Phenochlor, Kanechlor, Pyralene, Fenclor, Delor.

The commercial PCB mixtures differ mainly in percentages of individual chlorinated biphenyls, method of production, and level of contaminants (e.g. polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs)). There is evidence to suggest that PCB congeners differ qualitatively and quantitatively in biological and toxicological activities and that several diverse mechanisms are involved in responses to PCB mixtures. Based on structural characteristics and toxicological effects, PCBs can be divided into two groups (Larsen 2003, ATSDR 2000, Van den Berg et al. 1998, 2006):

- The congeners considered to be most toxic, based on combined health effects considerations, are coplanar. Some co-planar PCBs (called dioxin-like PCBs, DL-PCBs, these congeners are marked with an * throughout

the document) have been shown to exert a number of toxic responses similar to those of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), the most toxic dioxin, and their effects are mediated through binding to the aryl hydrocarbon (Ah) receptor (effects on liver, thyroid, lung, immune function, reproduction and behaviour).

- The other PCBs do not show dioxin-like toxicity, but have a different toxicological profile. This group of PCBs is called 'non dioxin-like PCBs' (NDL-PCBs). Although NDL-PCBs generally are discussed as a group of PCB congeners, it is unlikely that all these congeners have identical toxicological profiles. Mixtures used to study the toxicity of PCB contain both NDL- and DL-PCBs and some PCB congeners may even possess both types of toxicity.

The dioxin-like PCBs only constitute a minor fraction of the total amount of a PCB mixture (total-PCB). Of the 209 theoretical PCB congeners only 12 are considered to be dioxin-like (the 12 congeners marked with '[DL]' in the table in Appendix 1).

For the purpose of assessing the human health risk from exposure to mixtures of dioxin-like PCBs, the concept of toxic equivalency factors (TEF) has been developed. According to the WHO, a TEF indicates an order of magnitude estimate of the toxicity of a dioxin-like compound relative to the toxicity of 2,3,7,8-TCDD and is determined based on available *in vitro* and *in vivo* data (Van den Berg et al. 1998, 2006).

WHO only assigned TEFs for compounds that:

a. Show a structural relationship to the PCDDs and PCDFs
b. Bind to the Ah receptor
c. Elicit Ah receptor-mediated biochemical and toxic responses
d. Are persistent and accumulate in the food chain.

The majority of studies assessing the combined effects of PCDD, PCDF and DL-PCB congeners in complex mixtures have supported the hypothesis that the toxic effects of combinations of congeners follow dose additivity. Therefore, the concentrations and TEFs of individual congeners in a mixture may be converted into a toxic equivalent (TEQ) concentration by multiplying the analytically determined amounts of each congener by the corresponding TEF and summing the contribution from each congener using the following equation (Van den Berg et al. 1998, 2006; WHO 2000):

$$TEQ = \sum (PCDD_i \times TEF_i) + \sum (PCDF_i \times TEF_i) + \sum (PCB_i \times TEF_i)$$

The use of the consensus TEF values for 2,3,7,8-TCDD and dioxin-like compounds published in 2005 by the WHO (Van den Berg et al. 2006) for all cancer and non-cancer effects mediated through aryl hydrocarbon receptor binding has also been recommended by the US-EPA (US-EPA 2010).

The nomenclature as well as the structure and the TEF assigned to the 12 dioxin-like congeners by WHO in 1997 (Van den Berg et al. 1998) and revised by WHO in 2005 (Van den Berg et al. 2006) are shown in Table 1. The shorthand nomenclature refers to the systematic numbering system proposed by Ballschmiter and Zell (1980) and modified by Ballschmiter et al. (1987, 1992) (see Appendix 1).

PCB number	Structure	TEF (1997)	TEF (2005)
non-*ortho* PCB			
77	3,3',4,4'-tetrachlorobiphenyl	0.0001	0.0001
81	3,4,4',5-tetrachlorobiphenyl	0.0001	0.0003
126	3,3',4,4',5-pentachlorobiphenyl	0.1	0.1
169	3,3',4,4',5,5'-hexachlorobiphenyl	0.01	0.03
mono-*ortho* PCB			
105	2,3,3',4,4'-pentachlorobiphenyl	0.0001	0.00003
114	2,3,4,4',5-pentachlorobiphenyl	0.0005	0.00003
118	2,3',4,4',5-pentachlorobiphenyl	0.0001	0.00003
123	2',3,4,4',5'-pentachlorobiphenyl	0.0001	0.00003
156	2,3,3',4,4',5-hexachlorobiphenyl	0.0005	0.00003
157	2,3,3',4,4',5'-hexachlorobiphenyl	0.0005	0.00003
167	2,3',4,4',5,5'-hexachlorobiphenyl	0.00001	0.00003
189	2,3,3',4,4',5,5'-heptachlorobiphenyl	0.0001	0.00003

Table 1.
Nomenclature of DL-PCB congeners and assigned TEF by WHO in 1997 and 2005, respectively (Van den Berg et al. 1998, 2006)

1.2 Physical / chemical properties

Description:
Commercial PCB mixtures are light yellow or dark yellow in colour. They do not crystallise, even at low temperatures, but turn into solid resins.

Purity:
Commercial PCB mixtures contain PCDFs at levels ranging from a few mg/kg up to 40 mg/kg.

Vapour pressure:
In general, PCBs have very low vapour pressure, which decreases with increasing numbers of chlorine atoms in the molecule.

Flash point:
PCBs are, in practice, fire resistant, with rather high flash points.

Solubility:
In general, PCBs are lipophilic and have a very low water solubility which decreases with increasing numbers of chlorine atoms in the molecule

$logP_{octanol/water}$:
6-8.4 for PCB 77, 138, 153, 169, 180 (ATSDR 2000)

Stability:
PCBs are chemically very stable under normal conditions; when heated, other toxic compounds, such as PCDFs, can be produced.

References:
ATSDR (2000), Van den Berg et al. (1998).

1.3 Production and use

Since the 1930s, PCBs have been produced commercially by chlorination of biphenyl with anhydrous chlorine under heated reaction conditions and in the presence of a catalyst (e.g. iron fillings or ferric chloride). The result is always a mixture of different congeners and impurities (mainly PCDFs). (ATSDR 2000).

Due to their physical and chemical properties, such as non-flammability, chemical stability, high boiling point, low heat conductivity and high dielectric constants, PCBs were widely used until the middle of the 1970es, in a number of industrial and commercial open and closed applications. Closed applications include the use of PCB in hydraulic and heat transfer systems as well as cooling and

insulating fluids in transformers and capacitors. Use of PCBs in pigments, dyes, repellents and carbonless copy paper or as plasticizers in paints, sealings, plastics and rubber products are typical open applications. For technical purposes, PCBs were never used as single compounds but as complex, technical mixtures. It is estimated, that more than one million tons of technical PCB mixtures were produced world-wide since their first commercial use in the late 1920s. The technical mixtures were liquids with different viscosity depending on the degree of chlorination (between 21 and 68 % chlorine). Depending on the chlorine content and the production process, the composition of the technical mixtures, marketed under trade names, such as Aroclor, Clophen, Phenochlor, Kanechlor, Pyralene, Fenclor, Delor, may differ significantly as to the number and content of individual PCB congeners. Even mixtures with a comparable chlorine content but from different manufacturers (e.g. Aroclor 1260 and Clophen A60) show varying composition. Technical mixtures are found to contain only about 100-140 individual congeners. (EFSA 2005).

In Denmark the first ban was introduced on the use of PCBs in open applications such as sealants, paints, glues and plastics with effect from 1977. The first ban on PCBs came as a result of an EU directive in 1976. Since 1986 all sales were banned of PCB and PCB-containing electrical equipment, such as transformers, capacitors (big industrial capacitors, but also small capacitors in household electrical appliances), heat transfer and hydraulic systems. In 1995 it was forbidden to use PCB-containing capacitors and transformers, weighing more than 1 kg or having a power greater than 2kVA. Smaller transformers and capacitors may be used until their lifetime expires and is subject to special rules for disposal of hazardous waste. Today PCBs are under the Stockholm Convention, which most countries have acceded and which came into force in 2004. The Convention prohibits the production of PCBs and regulates how PCB-containing wastes are handled and disposed. In 2006 a national plan for the Danish implementation of the Convention was developed. The plan describes the Danish efforts, including in relation to monitoring the amount of PCB in food and environment. The Stockholm Convention is implemented in, the EU in the so-called POPs Regulation. (MST 2012a).

1.4 Attempts to estimate total PCB concentrations

In principle, it is possible to determine all 209 PCB congeners by gas chromatography. However, this is a costly and very time consuming task and therefore, only a small number of PCB congeners are generally analysed. This raises the question to which extent these results can be used for estimation of the total PCB concentration in a given sample. In order to compare the results of the analyses of PCB and to perform toxicological evaluations, many attempts have been made to estimate the total PCB concentration from determinations of individual congeners.

Schulte and Malisch (1984 – as cited in EFSA 2005) found that the sum of PCB 138, 153 and 180 on average contributed 61% of the human body burden with PCB and suggested to multiply the sum of their concentrations in human samples with a factor of 1.64 in order to estimate the total PCB concentration. The factor of 1.64 is supported by the results of later analyses of PCB in human milk in which PCB 138, 153 and 180 were found to be the most predominant congeners, making up on average more than 65%. A factor of 1.7 has been used to multiply the sum of their concentrations in human milk in order to estimate the total PCB concentration and is considered a being reasonable precise to be used for the estimation of the human body burden with total-PCB (EFSA 2005).

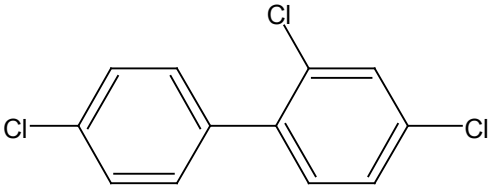
However, for foods of animal origin the factor of 1.64 was considered to be too uncertain. Beck and Mathar (1985 – as cited in EFSA 2005) suggested to focus the analyses mainly on six PCB congeners (PCB 28, 52, 101, 138, 153 and 180, see Figure 1). The six individual congeners were not selected from a toxicological point of view, but were considered as indicators for the different PCB pattern in various sample types. This concerns both technical mixtures as the main source of contamination as well as environmental and human samples where the pattern is significantly affected by biodegradation and photodegradation as well as bioaccumulation and metabolism. These six congeners are often termed 'indicator-PCB' or 'marker-PCB'. Other investigations, especially in connection with the Belgium PCB/dioxin case in 1999 also include the DL-PCB 118 (Figure 1) as a seventh congener into the group of indicator PCB.

Analyses of the six or seven indicator PCB, either as individual congeners or as a sum, are now used in numerous national regulations. (EFSA 2005).

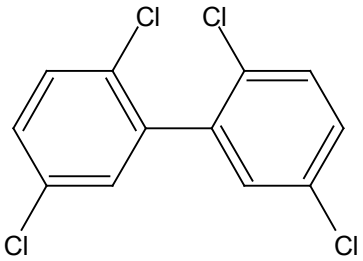
Studies from the Netherlands have indicated that a reasonable estimate of total-PCB in fatty foods can be obtained by multiplying the sum of the six or seven indicator PCB by a factor of 2 (Liem and Thelen 1997 – as cited in EFSA 2005).

The above-mentioned approaches are directed towards an estimation of the total PCB concentrations based on the determination of a limited number of congeners and subsequent transformation with empirical factors. Other approaches express the PCB concentration in a given sample as a sum of a number of selected congeners, often done as the sum of the three predominant PCB 138, 153 and 180, as the sum of the six or seven indicator PCB, or as the sum of all PCB congeners quantified in the respective sample. Often, the sum of PCB is reported as such without giving any details on the kind and number of congeners analysed.

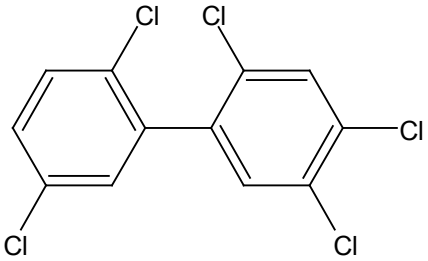
The situation becomes even more complicated when different matrices are evaluated with the aim of tracing back contamination pathways or assessing different routes of human PCB exposure. When analysing indoor air, for example, it is common practise to measure the six indicator PCB, add up their concentrations and multiply the sum by a factor of five to estimate the total PCB concentration (VDI 1997 – as cited in EFSA 2005). Depending on the chlorine content of the PCB-containing sealant as the most likely contamination source, the results of air measurements in the respective building will lead to erroneous estimation of the real PCB concentration. This is especially important because air samples are mostly dominated by the highly volatile, lower chlorinated PCB 28 and 52, while the more persistent PCB 138, 153 and 180 are normally of minor importance due to their lower volatility. In contrast, the latter ones bioaccumulate within the food chain and predominate in human samples, whereas PCB 52, and to some extent PCB 28, are normally found at considerably lower levels. (EFSA 2005).



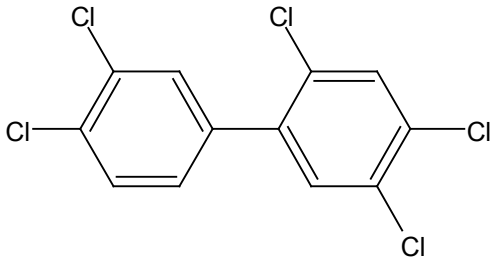
PCB 28 (2,4,4' – trichlorobiphenyl)



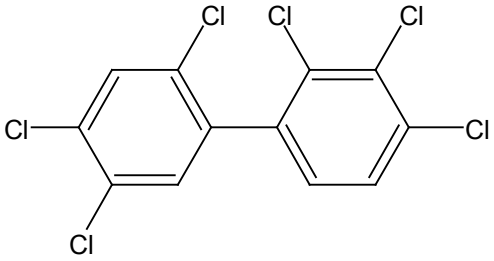
PCB 52 (2,2',5,5' – tetrachlorobiphenyl)



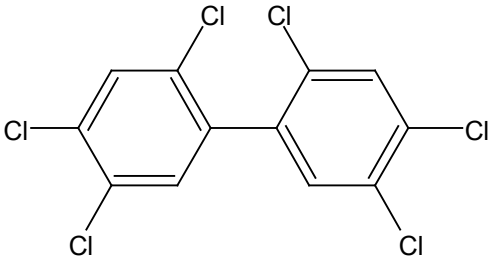
PCB 101 (2,2',4,5,5' – pentachlorobiphenyl)



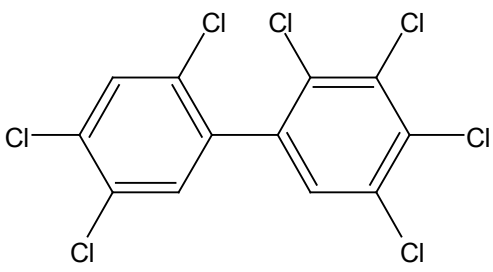
PCB 118 (2,3',4, 4',5, – pentachlorobiphenyl)



PCB 138 (2,2',3,4,4',5' – hexachlorobiphenyl)



PCB 153 (2,2',4,4',5,5' – hexachlorobipheny)l



PCB 180 (2,2',3,4,4',5,5' – heptachlorobiphenyl)

Figure 1.
Chemical structures of the seven indicator PCBs (EFSA 2005)

1.5 Environmental occurrence and fate

PCBs are synthetic compounds and have been released to the environment solely by human activity.

Although production and use of PCBs has been abandoned in almost all industrialised countries since the late 1980s, their continued release to the environment cannot be avoided, in particular due to improper disposal practices or leaks in electrical equipment and hydraulic systems still in use. PCBs are globally circulated and are present in all environmental media and are redistributed from one compartment to another, e.g. soil to water, water to air, air to water, and sediments to water. (EFSA 2005, ATSDR 2000).

Once released to the environment the individual PCB congeners in the released PCB mixtures undergo bio- and photodegradation. The ability of the individual congeners to be degraded or transformed in the environment depends on the degree of chlorination of the biphenyl molecule as well as on the isomeric substitution pattern. The result is that the congener patterns of the PCB mixtures in the environment over time differ from those in the original technical mixtures. The change in the PCB congener pattern is even more pronounced when the PCBs are taken up by fish and mammals, including humans. While some of the PCB congeners with lower chlorine contents are metabolised and excreted quickly, higher chlorinated congeners with certain substitution patterns are much more stable and accumulate within the food chains. (EFSA 2005, ATSDR 2000).

The vapour-phase reaction of PCBs with hydroxyl radicals is the dominant transformation process in the atmosphere, while photolysis appears to be the only viable chemical degradation process in water. Biodegradation has been demonstrated under both aerobic and anaerobic conditions and is the major degradation process for PCBs in soil and sediment. (ATSDR 2000).

1.5.1 Air

1.5.1.1 Ambient air

Atmospheric transport is the most important mechanism for global dispersion of PCBs. Biphenyls with 0-1 chlorine atom remain in the atmosphere, those with 1-4 chlorines gradually migrate toward polar latitudes, those with 4-8 chlorines remain in mid-latitudes, and those with 8-9 chlorines remain close to the source of release. PCBs enter the atmosphere from volatilisation from both soil and water surfaces with the majority coming from soil. Once in the atmosphere, PCBs are present both in the vapour phase and sorbed to particles. PCBs in the vapour phase appear to be more mobile and are transported further than particle-bound PCBs. At lower temperatures and with an increasing number of chlorine atoms in the molecule, the congeners will mostly be attached to particles and aerosols. PCBs are rather stable in the atmosphere. In general, persistence of congeners increases with the degree of chlorination. Photolysis may be the most important degradation process. However, the majority of PCBs is removed from the atmosphere by wet and dry deposition and the dominant source of PCBs to surface waters is atmospheric deposition. (ATSDR 2000, EFSA 2005, IPCS 1993, MST 1997).

Lower-chlorinated PCBs have a considerably higher vapour pressure than the higher-chlorinated ones. Therefore, the composition in air is dominated by the lower-chlorinated congeners such as PCB 18, 28, 66, 52 and 74 (vapour pressures in the range 0.001- 0.14 Pa compared to the vapour pressure reported for PCB 153 of 0.00012 Pa). (EFSA 2005).

PCB concentrations in outdoor air have been reported for different European regions. In rural areas levels up to 0.1 ng/m^3 have been reported whereas levels higher than 1 ng/m^3 have been reported in industrial areas (EFSA 2005). In Western European countries the reported PCB concentrations in ambient air ranged from 0.01 to 1 ng/m^3 and in Central and East European countries from 0.05 to 10 ng/m^3 (EC 2004 – quoted from EFSA 2005). According to EFSA, the data cannot be regarded as representative for the European region since most of the monitoring campaigns have focused on specific problem areas.

Monitoring studies conducted over the years have shown that atmospheric concentrations of PCBs have decreased since the late 1970s. (ATSDR 2000, IPCS 1993).

1.5.1.2 Indoor air

In 2011, indoor air concentrations of 24 PCB congeners (28, 52, 66, 74, 77*, 81*, 99, 101, 105*, 114*, 118*, 123*, 126*, 138, 153, 156*, 157*, 167*, 169*, 170, 180, 183, 187, 189*; congeners marked with an * are the dioxin-like congeners), were measured in 104 apartments (83 contaminated apartments and 21 non-contaminated apartments) in Farum Midtpunkt (Sundhedsstyrelsen 2012).

In the contaminated apartments (N = 83), the highest concentrations were found for the less chlorinated PCB congeners 28, 52, 66 and 74 (median: 61.4, 94.6, 33.7 and 23.7 ng/m^3, respectively; maximum: 296, 426, 148, and 106 ng/m^3, respectively). For the PCB congeners 77*, 81*, 99, 101, 105*, 114*, 118*, 123*, the median concentrations were below 10 ng/m^3 and the maximum concentrations were below 21 ng/m^3 except for PCB 101 (47.1 ng/m^3). For the remaining PCB congeners (126*, 138, 153, 156*, 157*, 167*, 169*, 170, 180, 183, 187, 189*), the median and maximum concentrations were below the 'Limit Of Quantification' (LOQ) except for PCB 153 and 183 for which the maximum concentration was 1.5 and 0.5 ng/m^3, respectively.

In the non-contaminated apartments (N = 21), the median concentrations were below the 'Limit Of Quantification' (LOQ) for all 24 PCB congeners. The highest maximum concentrations were found for PCB 28 and 52 (19.8 and 28.3 ng/m^3, respectively). For the PCB congeners 66, 74, 77*, 99, 101, 105*, 114*, the maximum concentrations were below 7 ng/m^3. For the remaining PCB congeners (81*, 118, 123, 126*, 138, 153, 156*, 157*, 167*, 169*, 170, 180, 183, 187, 189*), the maximum concentrations were below the LOQ.

1.5.2 Water

Wet and dry deposition remove PCBs from the atmosphere and the dominant source of PCBs to surface waters is atmospheric deposition; however, redissolution of sediment-bound PCBs also accounts for water concentrations. PCBs in water are transported by diffusion and currents. PCBs are removed from the water by sorption to suspended solids and sediments as well as by volatilisation from water surfaces. Higher chlorinated congeners are more likely to sorb, while lower chlorinated congeners are more likely to volatilise. PCBs also leave the water by concentrating in biota. (ATSDR 2000).

PCBs have a tendency to accumulate in sediments because of their low water solubility (IPCS 1993, MST 1997). Preliminary estimates of degradation half-lives in nature indicate half-lives in water and sediments ranging from around 30 years to around 200 years. (MST 2000).

Concentrations of PCBs in drinking water are generally lower than 0.1 µg/l (ATSDR 2000).

1.5.3 Soil

PCBs, particularly the highly chlorinated congeners, adsorb strongly to sediment and soil particles where they tendt to persist with half-lives in the order of months to years. PCBs are considered rather immobile in soil and will mainly concentrate in the topsoil layer unless they are mechanically spread. PCBs are unlikely to migrate to groundwater because of the strong binding to soil. The tendency to leach will be greatest among the least chlorinated congeners and is expected to be greatest in soil with low organic carbon. Volatilisation from soil appears to be an important loss mechanism; it is more important for the lower chlorinated congeners than for the higher chlorinated congeners. Soils with low organic carbon will have the greatest rate of volatilisation of PCBs. Photolysis of PCBs from the surface layer may occur, and PCBs may also undergo base-catalysed dechlorination; however, both of these processes are likely to be insignificant removal mechanisms. (ATSDR 2000, IPCS 1993, MST 1997).

Biodegradation has been shown to occur under both aerobic and anaerobic conditions and is a major degradation process for PCBs in soil and sediment. Numerous bacteria and some fungi have been reported to aerobically biodegrade PCBs. Experiments with both pure and mixed microbial cultures show that some congeners of PCBs, usually containing from one to four chlorines, are readily biodegraded aerobically; biodegradation of congeners containing up to six or seven chlorines has been shown under enrichment conditions. The most common process for the aerobic degradation of PCBs by bacterial cultures proceeds in two distinct steps: First bioconversion to the corresponding chlorinated benzoic acid and secondly, mineralisation of the chlorobenzoate to carbon dioxide and inorganic chlorides. Aerobic oxidation of PCBs has been identified in the environment. (ATSDR 2000).

PCB congeners with three or less chlorines (major components in Aroclors 1221 and 1232) are considered to be non-persistent, while those with five or more chlorines (major components in Aroclors 1248, 1254, and 1260) are not readily degraded and considered to be persistent. Tetrachlorobiphenyls (major components in Aroclors 1016 and 1242) are intermediate in persistence. Thus, the release of a PCB mixture to an aerobic environment results in a fractionating effect where the less chlorinated congeners biodegrade first and the more highly chlorinated congeners remain for long-term build-up. In addition to the degree of chlorination, the chlorine substitution pattern affects the biodegradation rate of PCBs. For example, PCBs containing chlorines on only one ring are degraded more quickly than PCBs containing an equivalent number of chlorines distributed between both rings. Additionally, PCBs with chlorines in *ortho* positions are more resistant to aerobic biodegradation than those with chlorines in either the *para* or *meta* positions. (ATSDR 2000).

Aerobic degradation rates of PCBs can be highly variable, depending not only on structural characteristics as outlined above, but also on a number of other factors including previous exposure to PCBs or PCB-like compounds, bioavailability, initial concentration, moisture, temperature, available nutrients such as carbon sources, and the presence of inhibitory compounds. Biodegradation of PCBs in aerobic soil is slow, especially in soils that have a high organic carbon content. PCBs that remain firmly bound in soil and sediment may not be bioavailable to the degrading organisms at sufficient concentrations. The efficiency of PCB degradation may also be controlled by the metabolite production pattern. For example, mono- and dichlorobenzoates, and possibly other higher chlorobenzoates formed from aerobic degradation of PCBs, have been shown to act as inhibitors towards further degradation of higher chlorinated PCBs. (ATSDR 2000).

PCBs are slowly biodegraded in anaerobic environments by reductive dechlorination resulting in the formation of less toxic mono- and dichlorobiphenyl congeners, which are aerobically biodegradable. However, the overall congener distribution profile is markedly different following anaerobic biodegradation. The profile shows a decrease in concentration of the more highly chlorinated congeners and a corresponding increase in the overall proportion of the less chlorinated congeners.

The most important structural factors determining whether a chlorine atom will be removed from a particular congener during anaerobic biodegradation include the position of the chlorine in relation to the opposite phenyl ring, the configuration of the surrounding chlorine atoms, the chlorine configuration of the opposite ring and the total number of chlorine atoms. There are at least eight distinct, documented, reductive dechlorination pathways or processes, each resulting in a different congener distribution profile. (ATSDR 2000).

PCBs are generally stable and stationary in soil. Therefore, the impact of local emission sources is of major importance for the contamination levels in soil. Consequently the location of sampling can strongly influence the levels found in the direct vicinity of PCB emission sources. Such data should therefore not be used for averaging national contamination levels. (EFSA 2005).

Based on data from national surveys provided to the European Commission (EC 2004) and data reviewed by Buckland et al. (1998), it can be concluded that levels for total PCB in agricultural and rural areas usually range from 0.1 to 10 ng/g dry matter. Levels reported for urban/industrial sites usually range from 10-100 ng/g dry matter. In contaminated areas total PCB levels ranging from about 1000 ng/g dry matter up to 35,000 ng/g dry matter have been reported. (EFSA 2005).

No Danish data on average concentrations of PCB in soils have been found.

The following text in this section is an MST contribution regarding measurements of PCB levels in Danish soils:

In 2004 the Danish EPA carried out a series of investigations of diffuse pollution in urban areas and reference levels for PCB were estimated (MST 2004a). For rural areas it was estimated that the reference level was 0.1-0.4 µg/kg dry weight (DW) and for urban areas it was estimated that the reference level was 10-200 µg/kg DW.

In 55 soil samples taken from areas with urban housing and analyzed for PCB-7, 52 of these

samples did not contain PCB-7. The highest level measured was 0.481 mg/kg DW, which was found in a depth of 30 cm. In two other samples levels of PCB-7 were 0.033 and 0.071 mg/kg DW, respectively. (MST 2004a).

In 20 samples from soil near roads analyzed for PCB-7, 3 samples contained PCB and the maximum level was 0.015 mg/kg DW PCB-7 (MST 2004b).

In a series of samples of soil taken around a former rolling mill and analyzed for PCB-7, PCBs were not detected in 90 % of the samples (in total 35 soil samples); the maximum level of PCB-7 was 0.110 mg/kg DW (MST 2004c).

Point sources may also contribute to elevated PCB levels in soil. One of these point sources may be buildings containing PCB, but other point sources with PCBs from industrial sites may also be present. Five sites with PCB have been registered as contaminated in 'DKjord' in December 2011 (the Danish data system for contaminated sites). On these sites there has for example been production of condenser and landfill. (MST 2012b).

In 10 soil samples taken from 10 buildings containing PCB, maximum levels of PCB-7 were 0.122 mg/kg DW and 0.185 mg/kg DW. The rest of the samples had content below 0.051 mg/kg DW. (MST 2009).

In an additional investigation of PCBs in soil, around 50 soil samples from 9 buildings were analyzed for PCB-7 in Roskilde and Copenhagen. The average concentrations of PCB-7 for each of the 9 locations were all below 0.022 mg/kg. (VJ 2009).

The Danish EPA is at present making further investigations on the contribution of PCB to soil near buildings containing PCB. These investigations are at present not finished and reports are not published yet, but preliminary results have been obtained. (MST 2012b).

In one project around 70 soil samples were obtained from 20 locations, 6 samples had a content of PCB-7 between 0.1 and 0.5 mg/kg DW. The rest of the samples had a content below 0.1 mg/kg DW PCB-7 and various samples did not contain PCB at all. (MST 2012b).

In another project soil from 3 locations are investigated in different depths. The maximum levels of the 3 sites are 0.105 mg/kg, 0.053 mg/kg and 4.45 mg/kg PCB-7, respectively. The high level of the latter location is unusual compared to the other investigations mentioned and the cause of this high level is at present not identified. (MST 2012b).

The view of Danish EPA based on current investigations of PCB in soil is, that the content of PCB-7 in soil seldom will exceed 0.5 mg/kg PCB-7 and very rarely 1 mg/kg PCB-7. Some point sources may exceed this level. (MST 2012b).

1.5.4 Sewage sludge

The text in this section is an MST contribution regarding measurements of PCB levels in Danish sewage sludge:

The first large survey of organic contaminants in Danish sludge was published in 1996 (Kristensen et al. 1996). The survey included PCB7 in sludge from 20 municipal waste water treatment plants (WWTPs) throughout Denmark. The overall data is presented in Table 2. It shows that all but two of the PCB congeners were detected in less than half of the plants. In 7 out of 20 WWTPs (35%), no PCB could be detected at all. The median and average concentration of the PCB7 was 27 and 42.6 µg/kg dry weight (dw), respectively. The highest sum value for the PCB7 was 140 µ/kg dw.

PCB	Mean	Median (=LOD)*	Maximum	% of samples > LOD
28	4.40	4	12	10%
52	9.01	5	39	50%
101	7.68	5	31	50%
118	5.75	3	28	20%
138	7.08	5	29	30%
153	8.64	5	34	35%
180	5.77	5	13	20%
Sum of PCB_7	42.6	27	140	65%

Table 2.
The concentration of PCB in 20 sludge samples taken from WWTPs in Denmark as reported by Kristensen et al. (1996). All data in µg/kg dw.

*** The median of the individual congener corresponds to the limit of detection, i.e. is at the LOD or lower.**

For a few years, a number of organic contaminants in sewage sludge were included in the national monitoring programme in Denmark called NOVANA. The monitoring data from 2003 and 2004 has been published and covers data from approximately 35 and 7 WWTPs, covering approximately 65 and 7 samples, respectively (Miljøstyrelsen 2005a,b). The following 10 PCB congeners were among these contaminants: 28, 31, 52, 101, 105, 118, 138, 153, 156, and 180. The 2003 data showed that some of the PCBs (28, 31, 105 and 156) were detected in less than 10% of the WWTPs, whereas others were detected in up to 46% of the WWTPs. In the smaller 2004 data set, the various congeners were detected in two of the seven WWTPs, but with two exceptions (PCB 138 and 153) which were detected in three of the plants. The 95% percentile of the observed concentrations of the sum of 10 congeners was 0.01µg/kg. It can, therefore, be concluded that in practical terms, no PCB was detected in any of the 7 WWTPs in 2004. In 2003, the average and the maximum (95% percentile) value of the individual congeners were found in the range of 1.7-5.1 and 8.1-18 µg/kg dw. However, the concentration level was only reported for six of the 10 congeners.

In 2010, a set of unpublished analytical data for PCB in Danish sludge destined for incineration in Germany was reported by Danbørs, a Danish sludge distribution company. The PCB content was measured by a German laboratory in connection with routine control of hazardous substance in sludge incinerated in Germany. PCB was detected in 8 out of 27 samples, with sum values of PCB6 in the range of 41-518 µg/kg dw. The average and median value of PCB6 in the eight samples were 183 and 99 µg/kg dw. Two of the eight samples were significantly higher than the others, with concentrations of 520 and 450 µg/kg. In both cases, the individual concentrations of congeners 101, 138 and 153 ranged between 110-150 µg/kg and, thus, constituted the great majority of the summed concentrations.

The Danish Association "Recycling of Organic Residual Products in Agriculture (BGORJ)" has recently collected a wider set of unpublished data on the level of PCB in Danish sludge from their members. It covers nation-wide data from 62 samples from more than 50 different Danish WWTPs collected in the years 2007-2011 with a dominance of data from 2010. A summary of the data is presented below in Table 3. The data shows that the PCB concentration in the vast majority of WWTPs, i.e. 5-95 percentiles, is found in the range of approximately 9 to 71 µg/kg dw. Only three of the 62 samples were unable to meet the new provisional Danish limit value of 80 µg/kg, whereas all samples were able to meet the lowest existing permanent international limit value of 400 µg/kg found in Sweden.

In 2002, a small Danish data set from two sludge samples (each being a mixtures of five sub-samples collected daily over the work-week) from the WWTP Lynetten in Copenhagen showed that no PCB could be detected in the sewage sludge (detection limit was 10 µg/kg) (Lynettefællesskabet 2003).

Recently, a Danish water company, Faxe Forsyning, analysed a single sample of sewage sludge from three individual WWTPs (Faxe Forsyning 2010,

unpublished). None of the samples contained any of the seven common PCB congeners in detectable amounts (LOD = 10 µg/kg).

In 2000, the Danish county Aarhus conducted an investigation regarding the fate of anthropogenic substances in sludge following application on agricultural soils (Aarhus Amt 2005). In this context, the PCB levels in three sludge samples from two WWTPs as well as the soil concentrations before and after sludge application were determined (see also section 5.4.3). The total concentration of PCB7 in the sludge samples ranged from 5.5-18.2 (Egå WWTP) and 120-145 µg/kg (Søholt WWTP), respectively.

Based on the data gathered on PCB in sewage sludge, it can be concluded that, except for the sludge samples from the Paris area and two samples from a smaller data set in Denmark, practically every sludge sample analysed complies with the limit values set out in most countries. In a screening survey conducted on 62 samples of Danish sludge from more than 50 WWTPs, three samples were unable to meet the temporary Danish criteria of 80 µg/kg, whereas all of them were able to meet the lowest existing permanent international limit value of 400 µg/kg found in Sweden. For risk assessment purposes, a PCB7 sludge concentration of 71 µg/kg is suggested, as this corresponds to the 95% percentile of the 62 Danish samples mentioned above.

1.5.5 Bioaccumulation

Since PCBs are very lipophilic and very resistant towards chemical and biological degradation processes, they persist in the environment and accumulate most in higher trophic levels through the consumption of contaminated food (ATSDR 2000).

Bioconcentrations factors of 200-70000 have been measured for various aquatic organisms (IPCS 1993, MST 1997).

Vapour-phase PCBs accumulate in the aerial parts of terrestrial vegetation and food crops by vapour-to-plant transfer. PCBs may also be absorbed by the plant roots, which accumulate more than the stems and foliage (ATSDR 2000, IPCS 1993, MST 1997).

All DL-PCB and many NDL-PCB congeners accumulate in animals and humans, predominantly deposited in the body fat, and maintain their biological activities long after the exposure has ceased. The biological half-life of the individual PCB congeners varies considerably depending on its chemical structure. While the elimination half-lives for a number of lower chlorinated PCBs in humans have been estimated to be from a few days up to six years, the half-lives for the total content of higher chlorinated PCBs (> 4 chlorine atoms) have been estimated from 8-24 years. (Wolff et al. 1992).

1.5.6 Food

PCBs in food derive from contamination of the environment where they, due to their lipophilicity, accumulate up through the food chain. Fish, meat, poultry, eggs and dairy products are the main foodstuffs containing PCBs.

	28	52	101	118*	138	153	180	Total
Mean	6.0	5.5	6.2	2.8	7.6	7.9	5.5	41.6
Median	5.0	5.0	5.0	2.8	5.0	6.0	5.0	35.0
5% Percentile	1.1	2.0	1.1	0.0	2.0	2.1	1.0	9.3
95% Percentile	10.0	11.0	12.9	10.0	19.7	18.8	10.0	71.0
Maximum	56.0	16.0	32.0	10.0	56.0	56.0	42.0	196.0

Table 3.
The concentration of PCB in 62 sludge samples from approximately 50 different Danish WWTPs. All data is in µg/kg dw and covers the period from 2007-2011 with the majority of data from 2010 and 2011. (BGORJ, unpublished).

* PCB_{118} were not analysed in 25 samples (40%) and are, hence, not included in the PCB_{total} for these samples. The PCB_6 analyses include mainly the older analyses. All statistical values listed above are, however, lower if based only on the 60% of samples where PCB_7 has been monitored.

In Denmark (1998-2003), ten individual indicator PCB congeners (PCB 28, 52, 101, 105, 118, 138, 153, 156, 170 and 180) were measured in 3552 samples taken from 35 food categories (Fromberg et al. 2005, Fromberg et al. 2011). In addition to the seven indicator PCBs presented in Figure 1, these ten indicator PCBs also include the NDL-PCB (170) and two DL-PCBs (105, 156). The mean indicator PCB-sum was calculated as the sum of the mean for the ten individual indicator congeners. The highest concentrations were found in fish with the mean PCB-sum ranging from about 3 to 56 µg/kg fish (highest concentrations in fatty fishes. The mean PCB-sum in animal fat and dairy products were generally low (2-7 µg/kg fat) as well as in eggs (4.7 µg/kg egg) (Fromberg et al. 2011).

As part of the EC recommended monitoring programme for dioxins and PCBs, 12 Member States as well as Iceland and Norway submitted data on the occurrence of these contaminants in various food products to the Commission. In almost all cases, the determination of PCBs was limited to the six or seven indicator PCBs.

Mean levels for the sum of the six indicator PCBs indicated low background contamination of food of plant origin ranging up to approximately 0.1 ng/g fresh weight. Considerably higher mean levels for the sum of the six indicator PCBs were found for food samples of animal origin ranging between 2.61 and 12.7 ng/g lipid. The food groups 'Fish oil' and 'Fish and fishery products' showed high contamination, with mean levels for the sum of the six indicator PCBs of 70.2 ng/g lipid, and 12.5 ng/g whole weight, respectively. (EFSA 2005).

The average concentrations of the individual congeners estimated for the various food groups (cereals and cereal products, fruit and vegetables, eggs, fats and oils, fish and fishery products, meat and meat products, and milk and dairy products) indicated that the congener profile in different food groups of animal origin were similar. PCB 138 and 153 were clearly the most prominent congeners, followed by PCB 101 and 180. PCB 28 and 52 generally contributed only minor amounts to the sum of the indicator PCB. In the food groups 'Cereals and cereal products', 'Fruit and vegetables', and in the subgroup 'Vegetable oil' (which in contrast to animals, lacks the ability to metabolise the lower chlorinated congeners) the congener profile was somewhat different profile with the lower chlorinated congeners (PCB 28, 52 and to a certain extent also PCB 101) being present at somewhat higher levels compared to food samples of animal origin. (EFSA 2005).

1.6 Human exposure

The general population is exposed to PCBs by ingestion of contaminated food and by inhaling contaminated air. Food consumption has and continues to be the major contributor to the body burden of PCBs in the general population and more than 90% comes from the dietary exposure. Several studies indicate that diets high in fish, from PCB-contaminated waters, can significantly increase a person's dietary intake of PCBs. In child-bearing women, this can be especially important since PCBs can concentrate in breast milk. Infants who are breast fed may therefore be at increased risk for PCB exposure if the mother has a diet high in contaminated fish. PCB exposure has also been attributed to inhalation of indoor air especially at locations which still use electrical equipment containing PCBs. (ATSDR 2000).

In Denmark, intake calculations have been made for ten individual indicator PCB congeners (PCB 28, 52, 101, 105, 118, 138, 153, 156, 170 and 180) as well as for their sum, see Table 4 (Fromberg et al. 2005, Fromberg et al. 2011). In addition to the seven indicator PCBs presented in Figure 1, these ten indicator PCBs also include the NDL-PCB (170) and two DL-PCBs (105, 156). The intake has apparently decreased in comparison with previous monitoring periods, with an intake for average indicator PCB-sum of 0.9 µg/day (1998-2003) compared to 2.2 µg/day (1993-1997).

Substance	Average	90th percentile	95th percentile
PCB 28	0.09	0.14	0.17
PCB 52	0.10	0.15	0.18
PCB 101	0.08	0.13	0-16
PCB 105	0.08	0.12	0.14
PCB 118	0.07	0.11	0.13
PCB 138	0.10	0.16	0.20
PCB 153	0.10	0.17	0.21
PCB 156	0.04	0.06	0.07
PCB 170	0.05	0.07	0.09
PCB 180	0.06	0.09	0.10
Indicator PCB-sum	0.90	1.41	1.66

Table 4.
Estimated dietary intakes for adults (aged 15-75), in µg/day (Fromberg et al. 2005, Fromberg et al. 2011).

The estimated contributions of individual food groups to the mean daily intake of PCB 153 showed that especially fish contributed to the mean daily intake for both adults and children (Fromberg et al. 2005, Fromberg et al. 2011).

When the estimated mean dietary intakes for the sum of the ten indicator congeners were expressed as ng/kg bw/day, children have approximately twice as high an exposure than adults, see Table 5. This is considered to be due to children's high intake of some food groups, e.g. milk, in relation to their body weight. (Fromberg et al. 2011).

Human exposure to PCBs from other sources includes air, outdoor as well as indoor air, and soil and dust particles. Water is not considered to contribute to the human exposure due to the very low water solubility of the PCBs.

EFSA has provided estimates of the contribution from ambient air and soil to the daily exposure of the general population to PCB calculated based on the data as presented above in section 1.5.1 (air) and 1.5.3 (soil) (EFSA 2005).

Based on ambient air levels ranging from about 0.1-1 ng/m^3, a body weight of 15 kg and an inhalation rate of 7.5 m^3/day, the intake of PCB by children from inhalation was calculated to be 0.05-0.5 ng/kg bw/day. For adults with a body weight of 70 kg and an inhalation rate of 20 m^3/day, the intake by inhalation was calculated to be about 0.03-0.3 ng/kg bw/day. On average the contribution from ambient air amounts to only a low percentage of the intake via food.

The contribution from indoor air was considered generally to be in the same order as that from ambient air, and therefore low. However, specific situations exist in which this contribution to the overall exposure could be considerable for certain PCB congeners.

The contribution from ingested soil or dust particles, particularly by children, was considered to be small. Based on soil concentrations of 10-100 ng PCB/g dry matter and assuming ingestion of 100 mg soil or dust per day, intake values of 0.06-0.6 ng/kg bw/day could be calculated for children. It has been suggested that PCB entering the body

Average		90th percentile		95th percentile	
Adults	Children	Adults	Children	Adults	Children
12.6	24.9	19.6	41.0	23.0	48.0

Table 5.
Estimated dietary intakes of PCB (sum of ten indicator congeners) for adults and children, in ng/kg bw/day (Fromberg et al. 2011).

from the airways and from the gastrointestinal tract is readily absorbed. This is in contrast to dermal exposure where the absorption rate is low, and depends on the degree of chlorination of the PCB. Using an average dermal absorption rate of 14%, the dose which is absorbed from the skin following contact with highly contaminated soil (1,000 ng PCB/g dry matter) was calculated to be about 5 pg/kg bw/day for children, and 0.76 pg/kg bw/day for adults. These values are about three to four orders of magnitude lower than the average intake via food.

2 Toxicokinetics

2.1 Absorption

PCBs in food are absorbed from the gastrointestinal tract by simple passive diffusion (ATSDR 2000). Studies in rats have shown that all PCB congeners are well absorbed from the gastrointestinal tract, with > 90 % absorption of lower chlorinated congeners (Albro and Fishbein 1972, Safe 1980, Bergman et al. 1982), and possibly lower absorption of higher chlorinated congeners, such as octachlorobiphenyls (75 %) (Tanabe et al. 1981). The reduced absorption of highly chlorinated PCBs is consistent with the data on dioxins, and probably arises from the inability of these compounds to form a molecular solution in the contents of the gut lumen. Factors such as dietary lipids and bile salts might enhance the extent of absorption, which probably involves incorporation into chylomicrons and uptake via the lymphatic system. The positive influence of bile has been shown by comparing normal and bile canulated rats treated with PCB (Bergman et al. 1982).

Absorption of NDL-PCBs in a nursing infant was estimated to be 96-98 % for the main congeners present in human milk based on the difference between the amount ingested and the unabsorbed PCBs excreted in the faeces (McLachlan 1993). Any variable that influences mobilisation of the PCB body burden, such as fasting, would alter the extent of faecal elimination of the pre-existing body burden.

Precise estimates of the oral bioavailability of PCB residues in soil are not available (ATSDR 2004).

In an *in vivo* study (Fries 1985 – as cited in ATSDR 2004), lambs fed soil spiked with polybrominated biphenyls in their diet were found to have a net absorption of 40-65% as measured in faecal matter; the bioavailability decreased with increasing organic content of the soil.

Another *in vivo* study in rats (Fries et al. 1989 – as cited in ATSDR 2004) demonstrated that the intestinal absorption of PCBs administered via spiked soil is lower than that of PCBs ingested via corn.

In an artificial digestive tract model to simulate *in vivo* digestion of soil-bound contaminants (Hack and Selenka 1996 – as cited in ATSDR 2004), eighteen PCB-contaminated soils and materials were evaluated. Thirty-three percent of PCBs in soil were mobilised in the gastrointestinal assay, while 64% were mobilised when dry whole milk was added; the presence of lipids and micelles were attributed to the increased mobilisation. The amount mobilised represented the fraction of PCBs in the gut that has desorbed from soil and was available to be absorbed; absorption across the intestinal lumen was not evaluated in this assay.

In another artificial digestive tract model (Oomen et al. 2000 – as cited in ATSDR 2004) the bioaccessible fraction of four PCB congeners in soil ranged from 30-47%. This assay utilised an *in vitro* artificial digestive system followed by an intestinal epithelial cell model to simulate mobilisation from soil and absorption and uptake from the gastrointestinal tract.

The Fries *in vivo* study reported a range of 40-65% for the oral bioavailability of PCBs. This is supported by the *in vitro* model used by Oomen et al. (range 30-47%) which simulated both mobilisation and absorption, attempting to account for the impact of other constituents in the digestive tract on bioavailability. A number of chemical and biological factors affect the bioavailability of PCBs from soil such as soil type, age and organic content, chlorination level of the compound, and dietary lipids present in the intestinal tract. Bioavailability decreases with aging in the soil, higher organic content and with greater levels of chlorination. Because PCBs are highly fat soluble, increasing dietary lipid levels can increase mobilisation of PCBs from soils and absorption from the digestive tract. It is therefore, important to take such factors into account in the evaluation of the oral bioavailability of PCBs in soil.

The uptake of PCDDs and PCDFs, and by inference of PCBs, by pulmonary absorption and dermal

permeation is considered to be more limited than the uptake after oral ingestion (IARC 1997). For occupational exposure to PCB, inhalation is a major route and it has been suggested that 80% of the levels commonly seen in adipose tissue of exposed capacitor workers may have been absorbed from the airways, with the remainder derived from dermal or oral exposure (Wolf 1985, Wilson et al. 2001 – as cited by EFSA 2005). Inhalation of dust from PCB contaminated soil may also be a relevant exposure route to PCB exposure. For combustion particles, such as fly ash, being a major source to PCDDs and PCDFs in ambient air, a bioavailability of 5 – 20 % has been proposed (IARC 1997). The pattern of PCB congeners in the body following occupational exposure is influenced both by the volatility of the congeners and by the particle content of the working environment, since PCBs are commonly adsorbed onto particles that can be inhaled. The PCBs on particles could either be taken up via the respiratory system or via the intestinal tract since particles may also be transported up the airways by mucociliary clearance and swallowed (EFSA 2005).

Transdermal absorption may be of relevance for PCB exposure due to contaminated soil. Absorption across the skin depends on a number of variables, with the formulation/vehicle being particularly important. Absorption tends to be slow and incomplete, and there would be a significant accumulation in the adipose tissue within the dermis, a depot, that would slowly reach equilibrium with the concentrations in the general circulation. Generally, dermal uptake of PCBs is only a very minor pathway for their uptake (EFSA 2005). According to IARC the bioavailability of PCBs after dermal contact is probably less than 1 % (IARC 1997).

In Rhesus monkeys exposed to Aroclor 1260 dermally with PCB-spiked soil, percutaneous absorption was approximately 3.4 and 4.3 % following exposure for 12 and 24 hours, respectively, to PCBs aged in soil; following exposure for 24 hours to soil freshly spiked with PCBs, the percutaneous absorption was approximately 4.1 % (Mayes et al. 2002).

2.2 Distribution

Due to their high lipophilicity and resistance to biotransformation a number of DL- and NDL PCB congeners accumulate in the body, mainly in adipose tissue and liver (ATDSR 2000, EFSA 2005). Data on distribution between different types of lipids indicate that PCB seem to partition primarily to the triglycerides (Sandermann, 2003). PCB congeners can pass the placenta of pregnant animals and humans (IARC 1997, IPCS 1993).

The rate of distribution of PCBs from blood lipids into tissue lipids depends on the blood flow to the organs. As indicated by studies on PCB transfer between the maternal and foetal compartments transfer across cell membranes is by passive diffusion, and there is yet no evidence of specific transporters for PCB (Meironyté Guvenius et al. 2003, Soechitram et al. 2004 – as cited by EFSA 2005). This distribution process results in lower body burdens in the foetus since the relative blood lipid content is lower in the foetal compartment than in the maternal, approx. 0.3 % and 0.6 %, respectively. Also, the total lipid content is influencing the total body burden of the foetus.

While PCBs and also PCB methyl sulfone metabolites are mainly transported in blood lipids, the phenolic metabolites of PCBs (hydroxy-PCBs) have been shown to be associated with the lipoprotein depleted fraction in blood, the one mainly containing albumin (Norén et al. 1999 – as cited by EFSA 2005). Therefore, when retained in the body the hydroxy-PCBs are mainly bound to blood proteins and are in fact more efficiently transferred to the foetus than the parent congeners (Meironyté Guvenius et al. 2003, Soechitram et al. 2004 – as cited by EFSA 2005). On the other hand, hydroxy-PCBs are not transferred into mother's milk to any appreciable extent, i.e. the hydroxy-PCB concentrations are less than 1 % of the PCB levels in the milk (Fängström et al. 2005 – as cited by EFSA 2005). PCB methyl sulfones are distributed similarly as the PCB but the sulfones have generally strong tissue selectivity (EFSA 2005).

Activation of the Ah receptor by binding of DL-PCB congeners increases the amount of CYP1A2 in the liver (IARC 1997). Highly toxic congeners such as PCB 126 and the PCB contaminant 2,3,4,7,8-PCDF bind very tightly to CYP1A2 and subsequently

concentrate in the liver in many rodent species, even at low dose levels. The hepatic accumulation of PCBs decreases dramatically with the addition of one chlorine atom in ortho position. The distribution of PCBs between liver and adipose tissue thus vary significantly between different congeners (Van den Berg et al. 1998). The liver/adipose tissue distribution of DL-PCBs also varies between different species. In general, humans have greater fat stores than rats and will accumulate more of the very lipophilic congeners in adipose tissue and less in target tissues such as the liver. In humans, the liver concentration of congeners is about 1/10 of the level in adipose tissue. In rats, the tissue distribution of 2,3,7,8-TCDD was dose-dependent with about the same concentration in liver and fat at a single dose of 1 ng/kg bw but a five times higher concentration in the liver than in fat at a single dose of 1 µg/kg bw (Neubert 1997/98 – as cited by EFSA 2005).

Data on the concentrations in human tissues are available from occupational studies which largely reflect inhalation exposure (Maroni et al. 1981, Takamatsu et al. 1985, Brown Jr. et al. 1994 – as cited by EFSA 2005). Human tissue levels of PCB have also been reported from autopsy material (Schecter et al. 1989, Dewailly et al. 1999, Meironyté Guvenius et al. 2001 – as cited by EFSA 2005). The pattern of distribution is what would be predicted for highly lipid soluble compounds, with high concentrations in adipose tissue, and blood levels being associated with the lipid fraction. The pattern of different congeners present in the body depends on a number of variables including the extent of recent intake, the long term intake and most importantly the potential for metabolism of different congeners (EFSA 2005).

Human PCB body burdens have been investigated in many studies. Some of the most sensitive effects of PCBs are related to exposure of the foetus during pregnancy and maybe also exposure of the neonate during the nursing period. Therefore studies on human milk are particularly relevant for the risk assessment of PCBs as these provide insight in the actual body burden in the pregnant woman (EFSA 2005).

In 2001/2002 58 pooled samples of human milk from 18 European countries were analysed for both PCDD/PCDF and PCB in connection with the third 'WHO human milk field study'. The following 38 PCB congeners were determined:

- Non-*ortho* PCB congeners: 37, 77, 81, 126, 169
- Mono-*ortho* PCB congeners: 28, 33, 55, 60, 66, 74, 105, 110, 114, 118, 122, 123, 124, 156, 157, 167, 189
- Di-*ortho* PCB congeners: 18, 47, 52, 99, 101, 128, 138, 141, 153, 170, 180, 183, 187, 194, 206, 209

PCB 55, 122, 123 and 124 could only occasionally be detected at concentrations near the detection limit. On a concentration basis, the non-*ortho* congeners only amounted 0.04% of all the PCB congeners determined (present at pg/g fat levels), whereas the contribution of mono-*ortho* and di-*ortho* PCB congeners was 14..2% and 85.8%, respectively (present at ng/g fat levels). The dominating congener in all cases was PCB 153 (27%), followed by PCB 138 (22%) and PCB 180 (18%) and these three di-*ortho* congeners thus constituted about 67% of the PCB congeners determined in human milk. The DL-PCB 118 constituted about 5% of the PCB congeners determined. (EFSA 2005).

In 2011, plasma concentrations of 27 PCB congeners (28, 52, 66, 74, 77*, 81*, 99, 101, 105*, 114*, 118*, 123*, 126*, 138, 153, 156*, 157*, 167*, 169*, 170, 178, 180, 182, 183, 187, 189*, 190; congeners marked with an * are the dioxin-like congeners), were measured in 273 residents living in apartments in Farum Midtpunkt (139 in contaminated apartments and 134 in non-contaminated apartments) (SST 2012).

In residents living in the contaminated apartments (N = 139), the highest concentrations were found for PCB 28 and 74 (median: 1.4 and 1.1 µ/l, respectively; maximum: 10.7 and 7.6 µ/l, respectively). For the PCB congeners 52, 66, 74, 99, 101, 105*, 118*, 138, 153, 156*, 170, 178, 180, 183, 187, 190, the median concentrations were between 0.02 and 0.6 µ/l. For the PCB congeners 52, 66, 74, 99, 101, 105*, 114*, 118*, 123*, 138, 153, 156*, 157, 167*, 169*, 170, 178, 180, 183, 187, 189*, 190, the maximum concentrations were between 0.01 and 4.6 µ/l. The median and maximum concentrations were below the 'Limit Of Quantification' (LOQ) for the PCB congeners 77*, 81*, 114*, 123*, 126*, 157*, 167*, 169*, 182, 189* and PCB congeners 77*, 81*, 126*, 182, 189*, respectively.

In residents living in the non-contaminated apartments (N = 134), the median concentrations were below the 'Limit Of Quantification' (LOQ) for the PCB congeners 52, 66, 77*, 81*, 101, 105*, 114*, 123*, 126*, 157*, 167*, 169*, 182, 189*; the median concentrations for the remaining PCB congeners (28, 74, 99, 118*, 138, 153, 156*, 170, 178, 180, 183,

187, 190) were between 0.01 and 0.3 µ/l. The maximum concentrations were below the 'Limit Of Quantification' (LOQ) for the PCB congeners 77*, 81*, 126*, 169*, 182„; the maximum concentrations for the remaining PCB congeners (28, 52, 66, 74, 99, 101, 105*, 114*, 118*, 123*, 138, 153, 156*, 157*, 167*, 170, 178, 180, 183, 187, 189*190) were between 0.02 and 1.9 µ/l.

The plasma concentrations in men were a little bit higher than in women. The plasma concentrations increased with the age of the residents.

2.3 Metabolism and excretion

The metabolism of PCBs has been described by EFSA (2005):

The major determinant for metabolism of PCB congeners is the presence of two adjacent, unsubstituted carbon atoms on the lateral positions (3,4 and 4,5). These positions are preferentially oxidised by the cytochrome P450 system resulting in more polar metabolites (Van den Berg et al. 1998).

The initial step in the biotransformation of PCB involves oxidation by cytochrome P-450 (CYP) enzymes, including arene oxide (epoxide) formation and an alternative route for direct insertion of a hydroxyl group to PCB congeners less easily forming arene oxides (Letcher et al. 2000, Safe 2003 – as cited by EFSA 2005). A general scheme for the metabolic transformation possibilities of PCB congeners is shown in Figure 2 with an overarching description of enzymes involved in the transformations (Table 6). Also non-enzymatic pathways are indicated when relevant. More than one arene oxide intermediate may be formed for PCB congeners with structural features susceptible for oxidations at more than one site, such as congeners with two unsubstituted *meta* and *para* carbon atoms in the molecule (e.g. PCB 52, PCB 95 and PCB 136) (EFSA 2005).

Arene oxides of PCBs are reactive electrophilic PCB intermediates that may form dihydrodiol-PCB, polychlorobiphenylols (hydroxy-PCB), glutathione (GSH) conjugates or adducts to biomacromolecules (DNA and proteins) and to lipids (EFSA 2005). Dihydrodiol PCB can be aromatised and form catechol metabolites in equilibrium with their oxidized forms, the corresponding hydroquinone and quinone. It is not yet possible to assess human exposure to reactive intermediates of PCB although the neutral, lipophilic $MeSO_2$-PCB metabolites resulting from conjugation with glutathione, could be used as a measure of arene oxide formation (EFSA 2005).

The major PCB metabolites formed are the hydroxy-PCB (polychlorobiphenylols) leading to a very large number of different structures since hydroxy-PCB may be formed after 1,2-rearrangements of a chlorine atom as has been described for PCB 105 and PCB 118 both of which produce the same metabolite, 2,3,3',4',5-pentachloro-4-biphenylol (4-OH-PCB 107) (Sjödin et al. 1998 – as cited by EFSA 2005). The majority of all hydroxy-PCB formed are excreted in a non-conjugated form or as glucuronide and sulfate conjugates. The hydroxy-PCBs are excreted both in urine and faeces. A limited number of all potential hydroxy-PCBs are retained in blood, bound to proteins such as transthyretin (Brouwer et al. 1998, Purkey et al. 2004 – as cited by EFSA 2005). There are five major hydroxy-PCB congeners (4-OH-PCB 187 > 4-OH-PCB 146 > 4-OH-PCB 107 > 3´-OH-PCB 138 > 4-OH-PCB 153) retained in the blood among a total of approximately fifty hydroxy-PCBs. Due to their physico-chemical properties the hydroxy-PCB do not partition to the lipids very efficiently (Malmberg 2004, Fängström et al. 2005 – as cited by EFSA 2005). The major hydroxy-PCB congeners in blood are present in concentrations in the same range as, the most persistent PCB congeners (Letcher et al. 2000, Sjödin et al. 2000, Hovander et al. 2004, Soechitram et al. 2004 – as cited by EFSA 2005).

PCB congeners with non-chlorinated meta-/para-positions and chlorinated neighbouring ortho-/meta-positions are rapidly metabolised. PCB congeners with free meta-/para-positions in at least one of the phenyl rings (Figure 2) may form PCB methyl sulfone metabolites ($MeSO_2$-PCB) in a multi-step pathway involving GSH conjugation, mercapturic acid pathway degradation, enterohepatic circulation, methylation and oxidation (Bakke et al. 1982, Bakke and Gustafsson, 1984, Letcher et al. 2000 – as cited by EFSA 2005). $MeSO_2$-PCBs are neutral PCB metabolites with a lipophilicity only slightly lower than that of the parent PCB compound. This leads to a general distribution to lipids but in humans

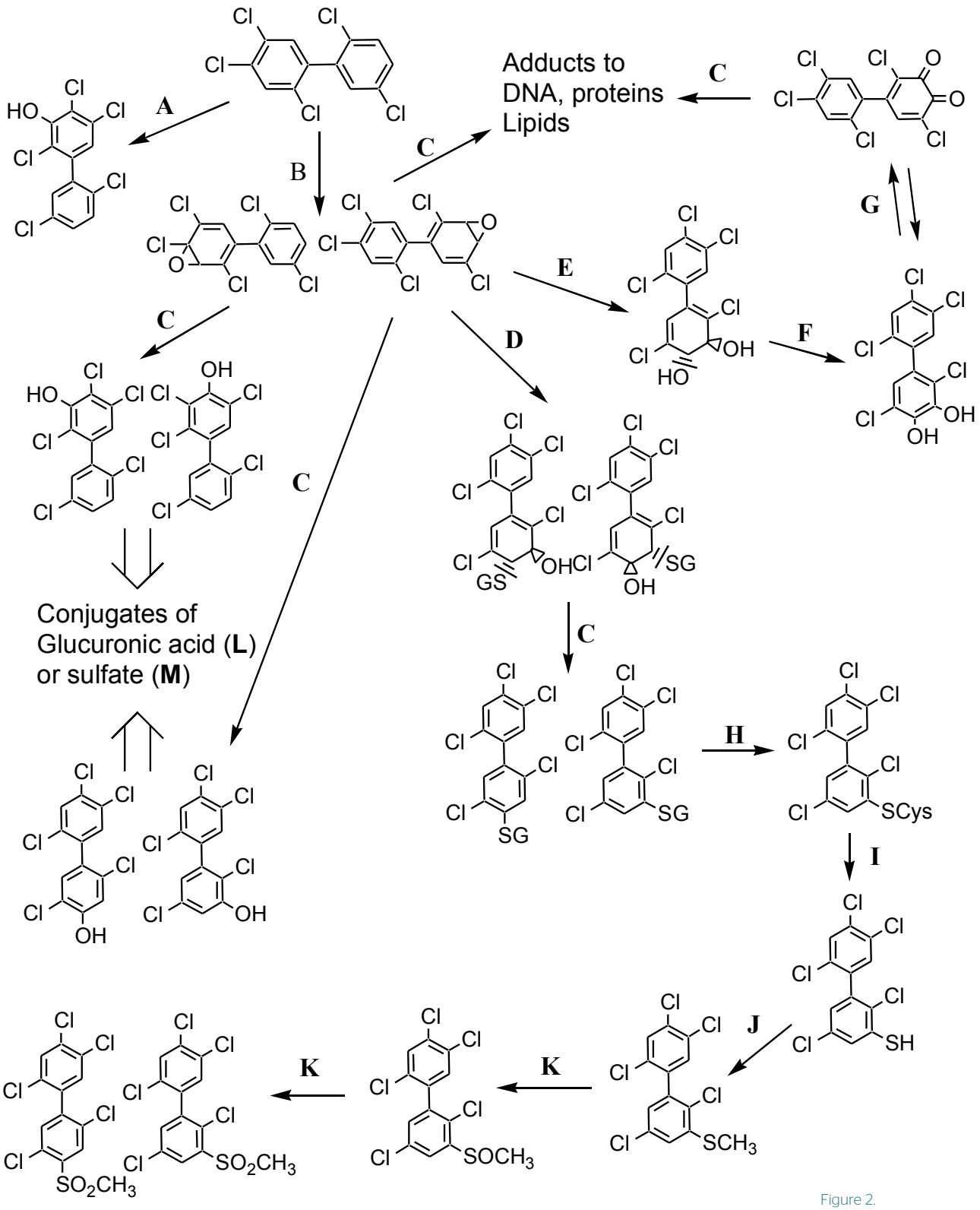


Figure 2.
General metabolic scheme for a PCB congener, PCB 101. Enzymes involved in the metabolism are indicated by the letters A, B and D-M with the letter C indicating non-enzymatic transformations. Enzymes for these transformations are listed in Table 4 (Taken from EFSA 2005).

the concentrations in blood are low (less than 1% the concentration of the most persistent PCB congeners) (Letcher et al. 2000, Hovander et al. 2004 – as cited by EFSA 2005). The $MeSO_2$-PCB concentration is however relatively high in comparison to their parent PCB. It is notable that some of the $MeSO_2$-PCBs are accumulated in a highly tissue-specific manner, with liver and lung as target tissues. Four $MeSO_2$-PCBs present in human tissues/fluids form atropisomer pairs (optically active forms) that are retained with high enantiomeric selectivity. Some $MeSO_2$-PCBs have strong tissue and cell specific retentions leading to higher local (cellular) concentrations than general tissue levels. It is relevant to assess human levels of $MeSO_2$-PCBs with the highest retention potential, since the corresponding parent PCB congeners are only present in trace concentrations or are non-detectable due to their rapid metabolism (EFSA 2005).

PCB congeners have very different pharmacokinetics depending on the rate of metabolism. The half-lives reported in humans vary considerably and the different values were scrutinised by Shirai and Kissel (1996). A set of half-lives, as reported in the literature, are presented in Table 7. It is clearly shown that the PCB congeners may have suggested half-lives from a week and up to several years in humans. Half-lives as reported in rhesus monkey and in rats are given for comparison (Table 7). In this case the lower chlorinated biphenyls have half-lives of a few days in rats (EFSA 2005).

For comparison it may be mentioned that the apparent half-life of the two abundant OH-PCB metabolites, 4-OH-CB107 and 4-OH-CB187, were reported in rats to 3.8 and 15 days, respectively (Malmberg et al. 2004 – as cited by EFSA 2005).

2.4 Interspecies and inter-individual differences

The interspecies differences in half-lives of NDL-PCBs are illustrated in Table 5.

In rats, the half-life of 2,3,7,8-TCDD ranged from 17 to 31 days. In rhesus monkeys, an average half-life of about 400 days has been determined for 2,3,7,8-TCDD. However, in humans the half-life of this congener has been reported to range from 5.5 to 11 years. Other PCDD, PCDF and DL-PCB congeners have been estimated to have half-lives between 3 and 50 years in humans. The apparent half-life is not absolute but may vary with dose, body composition, age and sex (IARC 1997, WHO 2000). In the risk assessments of PCDDs, PCDFs and dioxinlike-PCB the SCF (2001) and JECFA (2002) used a half-life of 7.5 years for 2,3,7,8-TCDD equivalents.

Enzyme "Letter"	Enzymes active in the metabolic process described
A	Cytochrome P450 enzyme system, Direct insertion in meta position; CYP2B (rodents)
B	Cytochrome P450 enzyme system CYP2B, CYP2C, CYP3A ; e.g. CYP2B1 (rodents); CYP3A4 (humans)
C	Non-enzymatic reaction
D	Glutathione -S-transferase
E	Epoxid hydrolase
F	Dihydrodiol dehydrogenase
G	Autooxidation and/or Peroxidases
H	Mercapturic acid pathway (MAP) 1 glutamyltransferase 2 cysteinyl glycinase
I	C-S-lyase
J	S-adenosylmethionine S-Methyltransferase (SAM)
K	CYP-mediated S-oxidation. P450 alt. FAD-containing mono-oxygenases
L	UDP-glucuronosyl transferase (UGT)
M	Sulfotransferase (SULT)

Table 6.
Enzymes in the metabolism of PCB. The letters given in the table correspond to the letters in Figure 2 (taken from EFSA 2005).

PCB #	$T_{½}$ Humans (Days - years)	Ref.	$T_{½}$ Rhesus monkey (Months)	Ref.	$T_{½}$ Rat (Days)	Ref.
28	18/44 days [a)] 182 days 16.8 months 4.8 years 3.0 years	A B C D E			1.4/6 [b)]	K
52	5.5 years [c)]	D			0.9/3.4	K
101	7/14 days 5.7 [d)]	A D			2.6/35	K
105	186/212 days 3.9 years	A C	4.8	I	98 5.6/>90	J K
118	9.4/10 months 3.6-9.7 months 1.1 year 5.8 years 9.6 years	F G H C D	15.6	I	117 6.6/>90	J K
138	10.7 months 3.4 years 6-7 years 16.3 years 16.7 years 20/32 years	G H C E D F	9.6	I	101 >90	J K
153	11.3 months 3.9 years 12.4 years 26/47 years 27 years	G H C F E	8.4	I	113 >90	J K
170	4.5 years 47/71 years	H F			83 >90	J K
180	4.1 months 4.8 years 9.9 years	G H D	8.4	I	81 >90	J K

Table 7.
Apparent half-lives of nine NDL-PCB congeners in a set of different studies in humans, in rhesus monkey and in rats (taken from EFSA 2005).

[a)] Two values given in the study depending on sample selections
[b)] The two values presented represent first and second phase half-lives for compounds with biphasic eliminations.
[c)] Co-elution between PCB 47, 49 and 52
[d)] Co-elution between PCB 99 and 101

References A-K:
A: (Luotamo et al. 1991) , B: (Wolff and Schecter 1991), C: (Brown Jr. et al. 1989), D: (Wolff et al. 1992), E: (Yakushiji et al. 1984), F: (Chen et al. 1982), G: (Bühler et al. 1988), H: (Ryan et al. 1993), I: (Mes et al. 1995), J: (Öberg et al. 2002), K: (Tanabe et al. 1981)

2.5 Mode of action

The PCB mixtures in environmental media, such as soil, contain both DL- and NDL-PCBs which will have different modes of biochemical and toxicological actions. DL-PCBs have dioxin-like effects via the intracellular aryl hydrocarbon (Ah) receptor (AhR) and principally include the coplanar PCBs such as PCBs 77, 81, 126, and 169. Dioxin-like effects include weight loss, thymic atrophy, enzyme induction, immunotoxicity, teratogenicity, dermatologic effects, carcinogenicity, and endocrine disruption. The mono-*ortho* coplanar DL-PCBs such as 105, 114, 118, 123, 156, 157, 167, and 189 have DL effects via the AhR and possibly other mechanisms of action, such as a phenobarbital-like spectrum of enzyme induction. *Ortho*-substituted nonplanar PCBs that do not bind to the AhR elicit enzyme induction, neurotoxicity, tumour promotion, and endocrine activity by different modes of action.

2.5.1 DL-PCBs

A broad variety of data primarily on 2,3,7,8-TCDD but also on other dioxin-like compounds in many experimental models using multiple species, including humans, have shown that binding to the intracellular AhR is important in mediating the biochemical and toxic effects of PCDDs, PCDFs and DL-PCBs (SCF 2000, WHO 2000).

After binding to the AhR in the cytoplasm, the receptor–ligand complex binds the AhR nuclear translocator (ARNT). The AhR–ARNT heterodimer acts as a nuclear transcription factor by binding to consensus sequences called "xenobiotic responsive elements" (XREs) located in the 5'-flanking region of responsive genes. The observed effects caused by "coplanar" PCBs are similar to effects caused by TCDD and related halogenated aromatic hydrocarbons (Hori et al. 1997, Safe 1990, NTP 2010).

The AhR is expressed in all tissues examined with a definite tissue specificity in terms of level of expression and diversity of response, indicating that dioxin-like compounds (DLCs) are likely to have some effect in every tissue. However, even with the same receptor and the same ligand, there are both qualitative and quantitative differences in response and these differences in response are likely to be involved in the tissue- and species-specificity of the response. It is still not known how alterations in gene expression ultimately lead to the development of pathologies and adverse health effects associated with dioxin-like compound exposure. However, it is generally accepted that most, if not all, responses require an initial step of binding to the AhR (NTP 2010).

The exact physiological role of the AhR is not known but it may be involved in the embryonic development. Studies using Ah-receptor-deficient mice have documented a spectrum of pathological lesions and indicated a role of the receptor in the normal growth and development of the liver and the immune system (SCF 2000). The AhR may also modulate biochemical and cellular responses via non-DNA dependent mechanisms. Resulting biochemical responses are (ATSDR 2000):

The AhR binding affinity of the individual congeners is dependent on the extent and pattern of chlorination. In general, the congeners most similar to 2,3,7,8-TCDD have the highest binding affinity and thus show the strongest toxicity (IARC 1997, IPCS 1993).

- Induction of drug-metabolising phase 1 and 2 enzymes such as cytochrome P450 (CYP) enzymes belonging to the CYP1A1, 1A2 and 1B1 families (CYP1A1, CYP1A2, and cCYP1B1), and aldehyde-3-dehydrogenase, glutathione S-transferase (GST), uridine diphosphate glucuronosyltransferase (UDP-GT), NAD(P)H:quinone oxidoreductase and prostaglandin endoperoxide H synthase-2.
- Modulation of growth factors, growth factor receptors, transcription factors, lymphokines and related factors.
- Modulation of thyroid hormones, vitamin A and retinoids.
- Modulation of protein phosphorylation.
- Modulation of biochemical responses associated with glucose metabolism and transport.
- Modulation of oestrogenic responses.
- Induction of oxidative stress in various tissues, probably related to the induction of cytochromes, resulting in for instance enhancement of lipid peroxidation.
- Modulation of cell cycle regulation and apoptosis.

These biochemical responses, some of which may be adaptive to dioxin exposure, may or may not lead to toxic effects at higher exposure levels (IARC 1997).

The most well studied response to DLCs is induction of the CYP1A cytochromes P450. CYP1A1 is induced in most tissues including liver, lung, kidney, nasal passages, and small intestine with the highest induction in rats occurring in the liver. Induction of CYP1A1 is a sensitive response and serves as a useful marker for exposure to DLCs. DLCs induce CYP1A1 *in vivo* and *in vitro* in human and animal models and induction of 7-ethoxyresorufin-O-deethylase (EROD) activity is a marker of CYP1A1 activity. CYP1A2 is constitutively expressed in the liver at low levels and is inducible by DLCs in liver and possibly the nasal turbinates of rats. Induction of acetanilide-4-hydroxylase (A4H) activity and 4- methoxyresorufin-O-deethylase (MROD) activity are markers of CYP1A2 activity. In addition to the well-characterized induction of CYP1A1 and CYP1A2, DLCs also induce another cytochrome P450, CYP1B1, in human cells and rodent tissues. CYP1B1 is active in the metabolism of numerous polycyclic aromatic hydrocarbons and arylamines and can catalyze the 4-hydroxylation of 17β-estradiol (NTP 2010).

DLCs are believed to disrupt thyroid hormone homeostasis via the induction of the phase II enzymes UDP-glucuronosyl transferases. Thyroxine (T4) production and secretion are controlled by thyroid stimulating hormone (TSH), which is under negative and positive regulation from the hypothalamus, pituitary gland, and thyroid gland by thyrotropin releasing hormone, TSH itself, T4, and triiodothyronine (T3). 2,3,7,8-TCDD induces the synthesis of UDP-glucuronosyltransferase-1 (UGT) mRNA by an AhR-dependent transcriptional mechanism. Consequently, a reduction in serumT4 levels via an induction of UGT may lead to a decrease in the negative feedback inhibition on the pituitary gland. This hypothyroidism would then lead to a rise in secreted TSH resulting in chronic hyperstimulation of the thyroid gland follicular cells (NTP 2010). Hypothyroidism is known to interfere with the development of the central nervous system (CNS).

Oxidative stress involves a depletion of the protective antioxidant defenses of the body. Oxidative stress from 2,3,7,8-TCDD exposure causes increased production of reactive oxygen species, enhanced lipid peroxidation, decreased glutathione content, decreased hepatic membrane fluidity, and DNA damage. Persistent organohalogen compounds are tumor promoters, and there is evidence that this promotion is mediated at least in part by reactive oxygen species such as superoxide or hydrogen peroxide. The mechanism by which this effect occurs remains to be elucidated, but it appears to be AhR mediated (Burgin et al. 2011).

2.5.2 NDL-PCBs

While DL-PCBs activate the AhR and induce e.g. cytochrome P450 (CYP) enzymes 1A1, 1A2 and 1B1, it has been suggested that NDL-PCBs activate the constitutive active (androstane) receptor (CAR) and the pregnane X receptor (PXR) (Kretschmer and Baldwin 2005 – as cited by EFSA 2005) resulting, e.g., in changed CYP2B1, CYP 2B2 and CYP3A1 gene expression with some overlap between both induction mechanisms (Sueyoshi and Negishi, 2001 – as cited by Ross et al. 2011). Pent-oxyresorufin-O-deethylase (PROD) activity is a marker of CYP2B. The most potent inducers of this type have at least two *ortho* chlorines and one or two *para* chlorines (Connor et al. 1995, Hansen 1998 – as cited by EFSA 2005).

Among the sulphonate PCB metabolites, 3-MeSO2 metabolites were strong phenobarbital type inducers of hepatic drug-metabolising enzymes, while 4-MeSO2 derivatives had almost no effect on both cytochrome content and enzyme activities (Kato et al. 1995 – as cited by EFSA 2005).

Mechanistic studies *in vivo* and *in vitro* have shown that NDL-PCBs can affect components of the nervous system in at least four different ways: i) by interference with intracellular sequestration of calcium and increased activation of protein kinase C (PKC), thereby altering intracellular signal transduction pathways (Tilson 1998, Kodavanti and Tilson 1997 – as cited by EFSA 2005), ii) through induction of apoptosis subsequent to activation of the ryanodine receptor and increased production of reactive oxygen species (Howard et al. 2003 – as cited by EFSA 2005), iii) by changing the levels of neurotransmitters such as dopamine and acetylcholine (Seegal 1989, Shain 1991 – as cited by EFSA 2005), the latter may be due to interference with PCBs on thyroid hormone levels because cholinergic fibres are particularly sensitive to

thyroid hormone deficiency (Juárez de Ku 1994 – as cited by EFSA 2005), and iiii) by increasing the release of arachidonic acid (Kodavanti and Derr-Yellin 2002 – as cited by EFSA 2005).

In vivo observations have confirmed changes in the PKC signalling pathway and calcium homeostasis (Yang 2003 – as cited by EFSA 2005) and reduced dopamine levels were observed in brain tissue from adult non-human primates (*Macaca nemestrina*) (Seegal et al. 1991 – as cited by EFSA 2005). For these endpoints NDL-PCBs were more potent than DL-PCBs. These endpoints are thought to be related to modulation of motor activity, learning and memory, neural damage and abnormal brain development.

Both estrogenic activity and anti-estrogenic activity have been observed for NDL-PCBs and hydroxylated metabolites of lower chlorinated NDL-PCBs. Structure activity relationships were complex and differed from one *in vitro* assay to another (Connor 1997 – as cited by EFSA 2005). *In vivo* animal studies, using single congeners, showed estrogenic effects such as increases in uterine weight, and changes in oestrogen and progesterone receptors. DL-PCBs however showed similar changes and were more potent: LOAEL in rats of 0.016 mg PCB126 /kg b.w per day versus a LOAEL of 8 mg PCB18 /kg bw per day for the same endpoint (Fisher et al. 1998, Li and Hansen 1995 – as cited by EFSA 2005). NDL-PCBs may also interfere with the binding of testosterone with the androgen receptor (Schrader and Cooke 2003 – as cited by EFSA 2005).

NDL- and DL-PCB interfere with thyroid hormone status through both distinct and similar mechanisms. NDL-PCB and hydroxy-PCBs may bind to the hormone receptor and affect thyroid hormone status by inhibiting the binding of T4 to transthyretin, which is an important transport protein for both T4 and T3 in rats (Chauhan 2000 – as cited by EFSA 2005). Some hydroxy-PCBs are potent inhibitors of thyroid hormone sulfation (Schuur 1998 – as cited by EFSA 2005). Furthermore, NDL-PCB can induce a UDP-glucuronosyltransferase which can enhance the elimination of T4 from the circulation via glucuronidation (Hood and Klaassen 2000 – as cited by EFSA 2005).

Immunological effects of PCDDs/PCDFs and PCBs include morphological changes in organs related to the immune system, as well as functional impairment of humoral- and cell-mediated immune responses. *In vitro* results indicate the existence of mechanisms of immunotoxic actions of PCBs that are independent of the Ah receptor: reduced lipopolysaccharide induced proliferative response in splenocytes, reduced antibody secretion (Smithwick et al. 2003) and impaired neutrophil function (Brown and Ganey 1995 – as cited by EFSA 2005).

NDL-PCBs may disrupt the immune system also, but by different modes of action: disruption of the endothelial barrier function, activation of oxidative stress-sensitive signalling pathways, and induction of subsequent proinflammatory events (interleukin-6) (Hennig 2002 – as cited by EFSA 2005), indicating a possible role in the pathology of atherosclerosis and cardiovascular disease. *In vivo* immune defects included decreases in thymic weight, reduced B cell numbers, reduced cytotoxic T-lymphocyte response, and reductions in plaque forming cell response and IgM (Sargent et al. 1991, Kerkvliet et al. 1990, Harper et al. 1995, Arnold et al. 1999 – as cited by EFSA 2005). From the data following systemic administration by Leece et al. (1987), Biegel et al. (1989) and Harper et al. (1995) as presented in Appendix 2, it can be concluded that the NDL-PCB tested (PCB 153, 170, 180) are much less potent *in vivo* than DL-PCB.

3 Human toxicity

3.1 Single dose toxicity

No specific human data are available regarding the acute toxicity in humans from PCB exposures (ATSDR 2000).

3.2 Repeated dose toxicity

3.2.1 Inhalation

It should be noted that in many of the occupational settings where exposure to PCBs have been reported by inhalation exposure via the dermal route may also have been significant.

3.2.1.1 Dermal effects

Chloracne is the most easily recognized effect of exposure to PCBs, PCDFs, and PCDD ("dioxins") Chloracne is a high-dose response in humans. Its presence in humans indicates exposure to PCBs and/or other chlorinated organic compounds, but its absence does not preclude such exposure. Furthermore, the variability of the response in more highly exposed individuals suggests that susceptibility varies greatly among individuals. Chloracne can first occur on the face, particularly under the eyes and behind the ears. With increasing exposure, the rest of the face and neck, upper arms, chest, back, abdomen, outer thighs, and genitalia may be affected. When severe, chloracne can cover the entire body. Clinically, changes vary from an eruption of comedones to the occurrence of papules and pustules. Histologically, the lesions consist of keratinous cysts caused by squamous metaplasia of sebaceous glands. The acute stage is followed by vermiculite skin atrophy (ATSDR 2000).

Chloracne has been observed in workers exposed to Aroclors or other PCB mixtures used as heat exchange materials for an average duration of 14.3 months (Meigs et al. 1954 cited by ATSDR 2000), among an unspecified number of autoclave operators exposed to Aroclor 1254 for 4-7 months (Bertazzi et al. 1987 – as cited by ATSDR 2000), and among persons engaged in impregnating capacitors with PCB. All of the workers with chloracne were employed in high exposure jobs. Their blood PCB concentrations ranged from 300 to 500 µg/kg.

Other dermal effects reported in workers include skin rashes, pigmentation disturbances of skin and nails, erythema and thickening of the skin, and burning sensations. Statistically significant associations were reported between dermatologic effects and plasma levels of higher chlorinated PCB congeners (Fischbein et al. 1979, 1982, Smith et al. 1982 – as cited by ATSDR 2000). No relationships between the incidence of skin rash or dermatitis and plasma levels of lower chlorinated PCBs were found.

Chloracne has occurred with or without other effects in at least a few workers in all reported accidents at trichlorophenol (TCP) production facilities and among individuals involved in production of 2,3,7,8-TCDD-contaminated products. Chloracne was also noted in the Seveso cohort (mostly children). Among Seveso residents, the chloracne resolved in all but one person within 7 years despite of high serum 2,3,7,8-TCDD levels. However, in TCP workers the mean duration of chloracne has been reported to be 26 years. A threshold level above which chloracne occurs has not been established, but a study has estimated that some TCP production workers with diagnosed chloracne had adipose levels of 2,3,7,8-TCDD greater than 200 ng/kg lipid (ATSDR 1997, IARC 1997, IPCS 1993, SCF 2000, WHO 2000).

3.2.1.2 Ocular effects

Ocular effects, including hypersecretion of the Meibomian glands, abnormal pigmentation of the conjunctiva, and swollen eyelids have been observed in humans occupationally exposed to PCBs and dioxins. The primary ocular effects reported by workers exposed to airborne PCBs were eye irritation, tearing, and burning (ATSDR 2000).

3.2.1.3 Effects on the liver

3.2.1.3.1 Liver enzymes

Increased serum levels of liver-related enzymes, particularly gamma-glutamyl transpeptidase (GTP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), and/or lactate dehydrogenase (LDH), were reported in a number of epidemiology studies and clinical surveys of PCB-exposed workers. Increases in levels of these serum enzymes have been correlated with serum PCB levels (ATSDR 2000).

For example, slightly increased serum levels of GTP, AST, and/or ALT were found in 14 of 80 capacitor manufacturing or repair workers who were exposed to PCB mixtures with a 42% chlorine content for an average of 12 years. PCB levels ranged from 41–1,319 µg/kg in the blood. The liver enlargement was considered indicative of hepatic microsomal enzyme induction (ATSDR 2000).

A significantly lower mean half-life of antipyrine clearance from blood, suggesting induced hepatic microsomal enzymes, was found in five workers exposed for an average of 9 years to various Aroclors, including Aroclor 1260, compared to five control subjects. Serum PCB levels were up to 125 µg/kg. Another study found no difference in antipyrine plasma half-life in transformer maintenance workers exposed to lower concentrations of Aroclor 1260 (serum PCB <15 µg/kg) for an average of 3.75 years.

Transient elevations in liver enzyme (GPT, AST, and ALT) and D-glucaric acid levels have been observed in TCP production workers as well as both children and adults among residents in areas of industrial accidents with dioxins or dioxin-like compounds (IARC 1997, IPCS 1993, SCF 2000, WHO 2000, JECFA 2002). Increased concentrations of urinary D-glucaric acid were found in adults and children exposed to 2,3,7,8-TCDD in Seveso in 1976. However, by 1981 the concentrations were within the normal range (JECFA 2002).

3.2.1.3.2 Serum lipids, triglycerides and cholesterol

A number of case reports and some epidemiological studies have described increased levels of serum lipid fractions, particularly total cholesterol and triglycerides in people exposed to PCDDs, PCDFs and PCBs in high doses. However, the majority of epidemiological studies of workers and community residents have reported no significant increases in total cholesterol or triglycerides levels among exposed populations compared with controls (IARC 1997, IPCS 1993, SCF 2000, WHO 2000).

Serum triglycerides, total cholesterol, ALT, and albumin/globulin ratio were increased in capacitor plant workers exposed to various Aroclor mixtures with a mean length of employment of 17 years. In other studies, no changes in serum cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and/or serum albumin levels were found in workers exposed primarily to Aroclor 1260 for a mean of 3.75 years or to an unspecified Aroclor mixture in transformer fluids for 4–17 years. Significant positive correlations between serum triglyceride or cholesterol levels and serum PCBs in the PCB-exposed workers were reported (ATSDR 2000).

3.2.1.3.3 Porphyria

PCB-exposed workers with a mean employment length of 10 and 12 years exhibited increased urinary excretion of total porphyrins and porphyrin homologues (coproporphyrin, pentaporphyrin, hexaporphyrin, heptaporphyrin, and uroporphyrin) as well as GGT compared to a control population of unexposed electrical workers (Colombi et al. 1982). However, in another study, urinary porphyrin excretion did not correlate with serum PCB levels in workers exposed to various Aroclors for >13 years (ATSDR 2000).

Evidence of alterations in porphyrin metabolism among populations exposed to 2,3,7,8-TCDD has been considered inconsistent (IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

3.2.1.4 Renal effects

No abnormalities were found in blood urea nitrogen (BUN) or other routinely-examined kidney function indices in two studies of PCB-exposed capacitor plant workers exposed to various Aroclor mixtures (ATSDR 2000).

3.2.1.5 Gastrointestinal effects

A statistically significant increase in loss of appetite was reported by PCB-exposed transformer workers (20%) as compared to the control group (4%). Gastrointestinal symptoms (anorexia, nausea, vomiting, and abdominal pain) and weight loss were also reported in 18% of capacitor workers exposed to various Aroclors. A significant

association was found between loss of appetite and increasing PCB blood levels in electrical equipment manufacturing workers who were exposed to various Aroclors (ATSDR 2000).

3.2.1.6 Pulmonary effects

Upper respiratory tract or eye irritation (48%), cough (14%), and tightness of the chest (10%) were noted in a limited study among 326 capacitor workers exposed to various Aroclors for >5 years. In another study, a correlation between coughing on the job or soon after work and PCB blood levels in electrical capacitor manufacturing workers has been reported. Changes in lung function (decrease in forced expiratory volume (FEV)) have also been reported in PCB workers. However, these effects could not be attributed solely to PCB exposure (ATSDR 2000).

3.2.1.7 Musculoskeletal effects

Joint pain was reported by 11% of workers exposed to various Aroclors. A higher prevalence was noted in female workers (15.2%) than in males (7.7%). Muscle pain was reported by <4% of the males and females. A number of study limitations were noted and the significance of these symptoms cannot be determined because a control group was not examined (ATSDR 2000).

3.2.1.8 Haematological effects

In general, haematological effects have not been observed in humans occupationally exposed to PCBs (ATSDR 2000).

3.2.1.9 Cardiovascular effects

A number of occupational exposure studies have investigated the possible relationship between PCB exposure and increased or decreased risk of cardiovascular disease or altered blood pressure. However, the inconsistency of the results precludes drawing conclusions from these studies (ATSDR 2000).

3.2.1.10 Immunological effects

Most epidemiological studies have not found a clear relationship between exposure to PCDDs, PCDFs and PCBs and impaired immunological status. However, alterations in the level and function of different antibodies, lymphocytes and complement proteins have been measured in some of the studies (IARC 1997, IPCS 1993, SCF 2000, WHO 2000).

A limited amount of information is available on immunological end points in PCB-exposed workers because assessments in most occupational studies were limited to routine clinical measurements of white blood cell (WBC) counts and serum proteins and did not include assessment of immunocompetence (ATSDR 2000).

Total and differential WBC counts were determined in workers who were exposed to Aroclors 1254, 1242, and/or 1016 for an average duration of 17 years. Clinical examinations of 194 capacitor plant workers (152 males, 42 females) in 1976 showed some elevations in total WBCs associated with decreased polymorphonuclear neutrophil (PMN) white cells and increased lymphocytes, monocytes, and eosinophils. All PCB use was discontinued in 1977 and in 1979, the WBC and lymphocyte counts were near normal and the increases in monocytes and eosinophils were marginal (Lawton et al. 1985 – as cited by EFSA 2005).

Other studies of PCB-exposed workers did not report any effects on total and differential WBC counts or changes in serum albumin, globulin, and/or total proteins (ATSDR 2000).

Delayed-type hypersensitivity was not affected in transformer repairmen exposed to Aroclor 1260. The mean length of employment of the exposed workers was 3.75 years (ATSDR 2000).

3.2.1.11 Neurological effects

Reports of neurological effects in workers exposed to PCBs are limited. Approximately 49% of workers (64 males, 94 females) exposed to various Aroclors at a capacitor manufacturing plant for more than 5 years complained of headache, dizziness, depression, fatigue, memory loss, sleeplessness, somnolence, and nervousness. The prevalence of these symptoms was not compared to a control group and routine neurological examination did not reveal any remarkable prevalence of abnormalities (ATSDR 2000).

An occupational cohort study involving 17,321 workers indicated that exposure to PCBs likely has an effect on neurodegenerative diseases for women but not men. The total cohort showed no excess of neurodegenerative diseases mortality compared to what would be expected in the U.S. population. However, the data do show mortality excesses of amyotrophic lateral sclerosis (ALS – also known as motor neuron disease) among

women, and of Parkinson disease and dementia (other than cerebrovascular dementia) among women in the high-exposure group. However, further investigations will be required to determine why women, and not men, would be susceptible to neurodegenerative disease from PCBs exposure (Steenland et al. 2006 – as cited by ATSDR 2011).

Viet Nam veterans who had serum levels of 2,3,7,8-TCDD above 33.3 ng/kg lipid tended to have a higher proportion of individuals with abnormal coordination than comparisons. A lot of other studies has not shown any relationship between exposure to 2,3,7,8-TCDD and neurological effects (ATSDR 1997, IARC 1997, IPCS 1993, SCF 2000, WHO 2000, JECFA 2002).

3.2.1.12 Endocrine effects

3.2.1.12.1 Thyroid

Total thyroxine (T4) and free T4 (T4 index) were significantly lower (approximately 10%) in a group of 55 transformer maintenance workers primarily exposed to Aroclor 1260 compared to a comparison control group of workers, even though thyroid stimulating hormone (THS) levels were in the normal range for adults in both groups. There was no correlation between PCB levels in serum or adipose tissue and serum T4 concentrations (ATSDR 2000).

Mean thyroid volume was significantly greater in 238 employees of a factory that produced PCBs than in 572 adult controls from "less polluted areas" of Slovakia. The workers also had elevated prevalence of antibodies for thyroid peroxidase (TPO Ab), thyroglobulin (Tg Ab) and TSH receptor (TSHR Ab). There were no differences between the worker and control groups with regard to serum total T4, TSH, or thyroglobulin concentrations (Langer et al. 1998 – as cited by ATSDR 2000).

3.2.1.12.2 Diabetes

No studies showing diabetes in humans after occupational exposure to PCBs were available

Marginally elevated mean fasting glucose levels were found in a study with German workers exposed to 2,3,7,8-TCDD. Among US production workers, the overall prevalence of diabetes mellitus was not significantly different from controls. However, an increased prevalence was found in workers with serum 2,3,7,8-TCDD concentrations in excess of 1500 ng/kg lipid. An increased risk for elevated fasting glucose levels and diabetes was found in the Viet Nam veterans who had levels of 2,3,7,8-TCDD above 94 ng/kg lipid (IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

3.2.2 Oral intake

Information on health effects in humans from oral intake of PCBs is in particular available from studies of people in Japan (the *Yusho* incident) and Taiwan (the *Yu-Cheng* incident) exposed by consumption of rice oil contaminated with heat-degraded Kanechlor, and from studies of people exposed by consumption of contaminated fish and other food products of animal origin. It is important to mention, however, that the findings from these studies cannot be attributed solely to exposure to PCBs since the victims of the *Yusho* and the *Yu-Cheng* groups also were exposed to PCDFs and other chlorinated chemicals such as polychlorinated quaterphenyls and -terphenyls, and since contaminated fish and food products of animal origin almost always contain a number of other contaminants, in particular PCDDs and PCDFs, in addition to PCBs.

For the *Yusho* incident, the daily intake was estimated at 0.33 mg PCBs/kg bw/day and 0.002 mg PCDFs/kg bw/day for about a month. For the *Yu-Cheng* incident, the daily intake was estimated at 0.06 mg PCBs/kg bw/day and 0.0002 mg PCDFs/kg bw/day for 10 months. In terms of DL-PCB and PCDFs exposure, it has been estimated that the *Yu-Cheng* cohort had body burdens of 2 – 3000 ng TEQ/kg bw (IARC 1997, IPCS 1993, SCF 2000, WHO 2000, JECFA 2002).

3.2.2.1 Dermal and ocular effects

Among victims of the *Yusho* and *Yu-Cheng* poisoning episodes, skin changes characteristic of chloracne most commonly developed on the face and other parts of the head, axillae, trunk, and external genitalia, with follicular plugging occurring in the axillae, groin, glenoid regions such as elbow and knee flexures, trunk, thigh, and outer aspect of the forearm. Dark-colored pigmentation frequently occurred in the gingival and buccal mucosa, lips, and nails, and improved only gradually in most patients (Fu 1984, Kuratsune 1989, Lu and Wu 1985, Rogan 1989 – as cited by ATSDR 2000).

Evaluation of *Yu-Cheng* subjects 14 years after the incident showed that men and women exposed to PCBs/PCDFs had a higher lifetime prevalence of

chloracne, abnormal nails, hyperkeratosis, and gum pigmentation (Guo et al. 1999 – as cited by ATSDR 2000).

Skin lesions including hyperpigmentation of the skin, nails and gingivae, deformed nails, and acne were commonly observed in children born to mothers with *Yusho* or *Yu-Cheng* exposure. The dermal changes were consistent with those observed in exposed adults (Funatsu et al. 1971, Gladen et al. 1990, Hsu et al. 1985, Rogan et al. 1988, Taki et al. 1969, Yamaguchi et al. 1971, Yoshimura 1974 – as cited by IARC 1997, IPCS 1993, WHO 2000, ATSDR 2000). These effects generally diminished as the babies grew older.

Ocular effects, such as hypersecretion of the Meibomian glands and abnormal pigmentation of the conjunctiva were commonly observed in *Yusho* and *Yu-Cheng* patients (Masuda 1994). Typical cases showed swollen Meibomian glands filled with yellow infarctlike contents. Abnormal changes in the Meibomian glands as well as eye discharge were still seen 10 years after the poisoning incident. The incidence of ocular signs was closely related to PCB concentrations and patterns in blood (ATSDR 2000).

Babies born to *Yusho* mothers also had increased eye discharge. Similar findings were seen in children born to *Yu-Cheng* mothers who also showed high incidence of conjunctivitis, swelling of the eyelid, and eye discharge (IARC 1997, IPCS 1993, ATSDR 2000).

3.2.2.2 Effects on the liver

3.2.2.2.1 Liver enzymes, enlargement and pathology

Serum GGT and cholesterol but not serum ALT or bilirubin, were positively correlated with serum PCB levels in 458 residents of Triana, Alabama. Consumption of contaminated fish was the only known source of PCB exposure. The mean serum concentration of PCBs (analyzed as Aroclor 1260) was 17.2 µg/kg. DDT was also increased in the serum of the people and in the fish, and serum DDT and serum PCB levels were highly correlated (Kreiss et al. 1981 – as cited by ATSDR 2000).

A comparison of 23 Swedish males with a high consumption of Baltic Sea fish and 20 men with virtually no fish consumption showed no statistically significant differences in serum levels of AST, ALT, GGT, AP, or bilirubin (Svensson et al. 1994 – as cited by ATSDR 2000). The fish eaters had elevated blood levels of PCBs and other organochlorines, as well as increased erythrocyte levels of methylmercury.

People exposed during the Yusho and Yu-Cheng PCB accidental ingestion incidents include increases in serum liver-related enzymes and triglycerides and urinary uroporphyrins. Elevations in serum AST and ALT are generally consistent findings in Yu-Cheng patients, whereas few abnormalities in AST and ALT and other basic liver function indices have been associated with Yusho exposure (Kuratsune 1989, Rogan 1989, Masuda 1994 – as cited by ATSDR 2000).

The predominant morphological finding in the liver of Yusho patients appears to be ultrastructural changes suggestive of microsomal enzyme induction, particularly alterations in the endoplasmic reticulum and pleomorphic and enlarged mitochondria (Kuratsune 1989, Masuda 1994 – as cited by ATSDR 2000). In a cohort of 1,940 Yu-Cheng victims (>95% of all registered cases) followed for 12 years after exposure, mortality from cirrhosis of the liver and from liver diseases excluding cirrhosis was increased in both sexes (Hsieh et al. 1996 – as cited by IARC 1997, WHO 2000, ATSDR 2000).

3.2.2.2.2 Serum lipids, triglycerides and cholesterol

Serum cholesterol, GGT, and blood pressure, but not serum HDL cholesterol or triglycerides, were positively correlated with serum PCB levels in 458 residents of Triana, Alabama. Consumption of contaminated fish was the only known source of PCB exposure, but PCB intake was not estimated. DDT was also increased in the serum of the people and in the fish, and serum DDT and serum PCB levels were highly correlated (Kreiss et al. 1981 – as cited by ATSDR 2000).

Serum cholesterol and triglycerides were increased in individuals with elevated serum PCB levels who had resided near waste sites for 5 years. The increases were not substantially greater than normal, however, and neither levels of cholesterol nor triglycerides correlated with serum PCB concentrations (Steer-Green et al. 1986a, 1986b; Steer-Green et al. 1986a, 1986b – as cited by ATSDR 2000).

Marked elevated serum triglyceride levels with unchanged total serum cholesterol was a characteristic finding of Yusho and Yu-Cheng exposures. The elevated triglycerides generally persisted for several years following exposure and subsequently declined to normal levels (Oxymora et al. 1979, Masuda et al. 1994, Uzawa et al. 1969 – as cited by ATSDR 2000).

3.2.2.2.3 Porphyria

Type B hepatic porphyria (i.e., a uroporphyrin/coproporphyrin ratio greater than 1) was a consistent finding in Yu-Cheng patients, including children born to exposed mothers (Chang et al. 1980, Gladen et al. 1988, Hsu et al. 1994, Lu et al. 1980 – as cited by ATSDR 2000). Abnormal urinary porphyrin levels have rarely been associated with Yusho exposure (Masuda et al. 1994 – as cited by ATSDR 2000).

3.2.2.3 Gastrointestinal effects

Transient nausea, vomiting and abdominal pain has been observed in Yusho and Yu-Cheng patients (IARC 1997).

3.2.2.4 Pulmonary effects

Potential respiratory effects have also been reported in Yusho and Yu-Cheng patients. More frequent or severe respiratory infections and chronic bronchitis accompanied by persistent cough and sputum production have been reported (Kuratsune 1989, Rogan 1989, Nakanishi et al. 1985, Shigematsu et al. 1971, 1977 – as cited by ATSDR 2000).

3.2.2.5 Musculoskeletal effects

No studies were located by ATSDR in 2000 regarding musculoskeletal effects in humans after oral exposure to PCBs (ATSDR 2000).

3.2.2.5.1 Effects on bone mineral density (BMD)

No statistically significant relationship between PCBs exposure and bone mineralization was observed in 115 Swedish men from the general population (Glynn et al. 2000 – as cited by ATSDR 2011).

In similar studies, Wallin et al. (2005), found no significant relationship between bone mineralization and exposure to PCB-153 in Swedish fishermen and their wives from the west coast (high exposure) compared to fishermen and their wives from the east coast (controls). Similar findings were reported by Weiss et al. (2006), who assessed serum levels of PCB-153, and hydroxylated PCB metabolites (OH-PCBs), in a group of Swedish middle-aged and elderly women. No associations between BMD or biochemical markers of bone metabolism and PCB-153 (median 260 ng/g fat) and OH-PCBs were found (both as cited by ATSDR 2011).

However, Hodgson et al. (2008 – as cited by ATSDR 2011) studied a population, 60–81 years of age, (154 males, 167 females) and showed that in males who lived near a PCBs-contaminated river PCB-118 (dioxin-like PCB) exposure was negatively associated with BMD. In females, PCB-118 was positively associated with BMD. In addition, the sum of the three most abundant non-dioxin-like PCBs (PCB-138, PCB-153, and PCB-180) was positively associated with BMD (ATSDR 2011).

3.2.2.6 Haematological effects

Mild normocytic anemia and leukocytosis have been reported in Yu-Cheng patients (Rogan 1989 – as cited by ATSDR 2000)

3.2.2.7 Cardiovascular effects

A 30% increase over the national average incidence of borderline and definite hypertension was observed in Triana, Alabama, residents, with increased systolic and diastolic blood pressure being significantly associated with serum PCB levels (Kreiss 1985 – as cited by ATSDR 2000). However, subsequent studies of environmentally exposed populations have failed to show an association between hypertension and PCB exposure (Stehr-Green et al. 1986a, Massachusetts Department of Public Health 1987 – as cited by ATSDR 2000).

3.2.2.8 Immunological effects

Marginally, not statistically significant, reduced natural killer (NK) cell activity was reported in a group of 23 Swedish men with high consumption of fatty fish species from the Baltic Sea. Data from some of the subjects obtained 3 years prior to the study showed weak negative correlations between numbers of NK cells and blood levels of PCB 126 and PCB 118, but a similar correlation was also found for p,p-DDT (Svensson et al. 1994 – as cited by ATSDR 2000).

In a group of 68 Latvian fishermen who consumed fatty fish from the Baltic Sea, high fish consumption was correlated positively with B cell numbers but

negatively with levels of cytotoxic (CD8+) T cells (Hagmar et al. 1995 – as cited by ATSDR 2000).

The number of infectious illnesses (colds, earaches, and flu symptoms) during the first 4 months of life in breast-fed infants whose mothers consumed contaminated Great Lakes fish was positively and significantly associated with maternal serum PCB levels. Possible associations between infectious illnesses and other chemicals in the fish were not investigated (Smith 1984 – as cited by ATSDR 2000).

Susceptibility to infections and immune status was studied in 98 breast-fed and 73 bottle-fed Inuit (Eskimo) infants from Arctic Quebec, Canada. Acute otitis media was the most frequent health problem during the first year of life, with 80.0% of ever breast-fed and 81.3% of bottle-fed infants experiencing at least one episode. Relative risk (RR) analysis by follow-up period and number of episodes showed associations between increasing prenatal exposure to organochlorine compounds and otitis media that were more consistent for hexachlorobenzene and p,p'-DDE than for PCBs. (Dewailly et al. 2000 – as cited by ATSDR 2000).

Clinical observations strongly suggest that Yusho and Yu-Cheng patients experienced frequent or more severe skin and respiratory infections and lowered resistance to illness (Kuratsune 1989, Nakanishi et al. 1985, Rogan 1989, Shigematsu et al. 1971 – as cited by ATSDR 2000). Total serum levels of IgA and IgM, but not IgG, were reduced in Yusho and Yu-Cheng patients (Chang et al. 1981, Shigematsu et al. 1971 – as cited by ATSDR 2000).

Other assessments of Yu-Cheng patients found various other immunologic changes, including lower percentages of monocytes and PMN leukocytes with immunoglobulin and complement receptors, reduced T lymphocytes and suppressed dermal delayed-type hypersensitivity responses to streptokinase/streptodornase antigen mixtures tested 1 year after exposure and tuberculin antigen tested 4 years after exposure (Chang et al. 1981, 1982a, 1982b; Lu and Wu 1985 – as cited by ATSDR 2000).

Children born to mothers who had Yu-Cheng disease had higher prevalence of bronchitis or pneumonia at 6 months of age, respiratory tract infections at 6 years of age, and middle ear infections at 6–14 years of age (Chao et al.1997, Yu et al. 1998 – as cited by ATSDR 2000).

In a subgroup of 55 infants from the Dutch Mother-Child study no correlation was found between pre- or postnatal exposure to PCBs and dioxins and the number of episodes of rhinitis, bronchitis, tonsillitis, and otitis during the first 18 months of life, or with humoral immunity as evaluated by antibody levels to mumps, measles, and rubella at 18 months of age. Pre- and postnatal PCB/dioxin exposure was estimated by the sum of PCB congeners 118, 138, 153, and 180 in maternal blood during the last month of pregnancy and the total TEQ level in maternal milk (17 dioxin and 8 dioxin-like PCB congeners). Although differences in the leukocyte subpopulation were observed between high and low PCB/dioxin-exposed infants, all values were within the normal range (Weisglas-Kuperus et al. 1995 – as cited by ATSDR 2000).

Follow-up evaluations at 42 months of age, reported as a study abstract, found that prenatal PCB exposure was associated with increased T cell numbers and lower antibody levels to mumps, measles, and rubella (Weisglas-Kuperus 2000). Additionally, a higher prevalence of recurrent middle ear infections and chicken pox and a lower prevalence of allergic reactions was reported to be associated with PCB body burden at 42 months of age. In further follow-up studies at school age, a higher postnatal PCB exposure through lactation was significantly associated with a greater prevalence of recurrent middle ear infections (Weisglas-Kuperus et al. 2004 – as cited by ATSDR 2011).

In a Faroese birth cohort, increased perinatal exposure to PCBs adversely impacted immune responses to childhood vaccinations. Sera from 119 children at 18 months and 129 children at 7 years of age were examined for antibody responses against diphtheria and tetanus vaccines. The antibody response to diphtheria vaccine decreased at age 18 months by 24.4% for each doubling of the PCB exposure at the time of examination. Antibody response to tetanus vaccine was decreased at age 7 years by 16.5% (Heilman et al. 2003, 2006 – as cited by EFSA 2005 and ATSDR 2011).

In a follow-up study by Heilmann et al. (2010), a total of 587 children participated in the examinations at ages 5 and/or 7 years. At age 5 years, before the booster vaccination, the

antidiphtheria antibody concentration was inversely associated with PCB concentrations in milk and 18-month serum. A simplified sum of PCB concentration was calculated as the sum of congeners CB-138, CB-153, and CB-180 multiplied by 2. Results obtained 2 years later showed an inverse association of concentrations of antibodies against both toxoids with PCB concentrations at 18 months of age. The strongest associations suggested a decrease in the antibody concentration by about 20% for each doubling in PCB exposure. At age 5 years, the odds of an antidiphtheria antibody concentration below a clinically protective level of 0.1 IU/L increased by about 30% for a doubling in PCB in milk and 18-month serum. It should be noted that the sum of PCB concentration used as exposure measure correlated with concentrations of DL-PCBs and of other organochlorines.

Prenatal PCB exposure was associated with a smaller thymic index at birth in neonates from Eastern Slovakia. District of residence and delivery also predicted thymic index. Male sex, later gestational age, larger birth weight z-score, and Roma ethnicity were associated with a larger thymic index, whereas respiratory illness was associated with a lower thymic index (Park et al. 2008 – as cited by ATSDR 2011).

3.2.2.9 Neurological effects

Various neurological symptoms, including numbness, weakness and neuralgia of limbs, hypesthesia, and headaches were common in Yusho and Yu-Cheng victims (Chia and Chu 1984, 1985; Kuratsune 1989, Rogan 1989 – as cited by ATSDR 2000). Reduced sensory and motor nerve conduction velocities also occurred in the patients, but disappearance of related symptoms and signs indicated that the effects on nerve conduction did not persist. (Chen et al. 1985, Chia and Chu 1984, 1985 – as cited by ATSDR 2000).

The neuropsychological functioning of a group of 101, 50–90-year-old fish eaters, exposed to PCBs through Great Lakes fish consumption was compared to a group of 78 age- and sex-matched non-fish eaters. Blood samples of the participants were analyzed for PCBs and 10 other contaminants which included PBBs, DDE, HCB, oxychlordane, dieldrin, mirex, mercury, and lead. Serum levels of PCBs (and DDE) were significantly elevated in the fisheaters (PCBs=16.0 µg/kg) relative to the non-fish eaters (PCBs=6.2 µg/kg). PCB/DDE exposure did not affect visual-motor coordination and hand steadiness. Age and gender were the strongest predictors of performance (Schantz et al. 1996a, 1999 – as cited by ATSDR 2000).

When 4-7 years old children from Yu-Cheng women were tested for cognitive development they had a consistent 5-point lower score than control children (Chen et al. 1992 – as cited by ATSDR 2000). When the children were tested for behavior and activity level they also consistently scored worse (more disorders) for emotional and behavioral disorders than control children. Similar results were observed for the activity scores (Yu-Cheng children had increased activity levels) (Chen et al. 1994 – as cited by ATSDR 2000). Yu-Cheng children also scored significantly lower than controls in a number of other neurobehavioral tests between the ages of 6 months and 2 years (Lai et al. 1994), and at ages 6, 7, or 9 (Guo et al. 1995) (both as cited by ATSDR 2000).

Infants born to mothers who had consumed moderate to large quantities of Lake Michigan fish (on average 2 or 3 salmon or lake trout/month) showed decreased neuromuscular maturity 3 days after birth compared to a control group. Infants of mothers eating contaminated fish were more likely to exhibit hypoactive reflexes, more motor immaturity, poorer lability of states, and a greater amount of startle (Fein et al. 1984a, b, Jacobson et al. 1984a – as cited by ATSDR 2000). However, the effects seemed not to be related to the mother's serum levels of PCBs. A follow-up at 7 months of age showed a correlation between poorer mean visual recognition memory in the infants and overall contaminated fish consumption by their mothers. Recognition memory performance was not related to postnatal exposure from breast-feeding (Jacobson et al. 1985 – as cited by ATSDR 2000).

Approximately 75% of the children were re-examined at age 4. Neurobehavioral testing showed that prenatal exposure (maternal exposure before and during pregnancy), assessed by cord serum PCB levels was associated with poorer performance on both verbal and memory abilities. There was no indication of perceptual motor deficits or alterations of long-term memory. Activity level was inversely related to 4-year serum PCB levels in a dose-dependent manner and also to maternal milk PCB levels. Multivariate analysis of variance indicated that the effect of maternal milk was strongest in children of women

with higher-than average milk PCB levels (>780 µg/kg) who breast-fed for at least 12 months. Cognitive performance was unrelated to exposure from breast-feeding, which, according to the investigators, suggested that the neurobehavioral deficits were due to foetal exposure (Jacobson et al. 1990a, 1990b – as cited by ATSDR 2000).

In a reanalysis of the assessment at 4 years of age additional findings indicated that memory cognitive was associated with prenatal PCB exposure only in the most highly exposed children (Jacobson et al. 1992 – as cited by ATSDR 2000).

At an 11-year follow-up linear regression modeling with confounder control, indicated that prenatal exposure to PCBs was significantly associated with lower full-scale and verbal IQ scores. Prenatal exposure to PCBs was associated with poorer word comprehension and overall reading comprehension. The associations of intellectual performance to lead and mercury were evaluated in separate multivariate linear regression models lacking terms for PCB exposure. Lower verbal IQ scores, lower verbal comprehension scores, and poorer word, passage, and reading comprehension were significantly associated with higher lead levels at 4 years of age. Poorer spelling was significantly associated with a higher mercury concentration at 11 years of age (Jacobson and Jacobson 1996a – as cited by ATSDR 2000).

A study similar to the one conducted in Michigan was initiated in Oswego County (New York) based on babies born between 1991 and 1994 of mothers having high, medium and low consumption of contaminated fish. The results indicated that newborns exposed to high concentrations of fish from Lake Ontario demonstrated a greater number of abnormal reflexes and less mature autonomic responses than newborns having lower exposure. (Lonky et al. 1996 – as cited by ATSDR 2000).

The North Carolina Breast Milk and Formula Project was a cohort study, initiated in 1978, designed to assess the relationship between exposure to prenatal and postnatal PCBs and growth and development in infants and children. The study included a cohort of 931 children born between 1978 and 1982. (Rogan et al. 1986a, 1986b, 1987 – as cited by ATSDR 2000). Maternal serum, cord blood, and placenta samples were collected as well as colostrum, breast milk, or formula. The first follow-up visit occurred at 6 weeks with subsequent evaluations at 3 and 6 months postpartum. Subsequent follow-up evaluations occurred at 12, 18, and 24 months, with yearly visits until the age of 5. The assessment at birth comprised 912 children with at least partial neonatal information. No associations were found between birth weight or head circumference and maternal PCB levels. Less muscle tone and activity and hypo-reflexia were associated with higher PCB levels, but only at the highest levels of PCBs. At the follow-up evaluations at 6 and 12 months linear regression analyses indicated that the psychomotor index scores declined with increasing prenatal PCB exposure whereas mental index scores were not related to either transplacental or postnatal PCB exposure (Gladen et al. 1988 – as cited by ATSDR 2000).

The children also were evaluated at 18 and 24 months. The effects of prenatal PCB exposure on the psychomotor index score at 18 and 24 months follow up were similar to those seen at 6 and 12 months; however, they were not significant. There was no evidence of an effect through postnatal PCB exposure in breast milk. An additional report in this series found that the deficits observed in children through 2 years of age were no longer apparent at ages 3, 4, and 5 years (Gladen and Rogan 1991 – as cited by ATSDR 2000). Finally, evaluation of third and higher grade children showed no significant relationship between the child's work habit or conduct grades and PCB exposure either prenatally or through breast milk, or between hyperactivity reported by parents and exposure (Rogan and Gladen 1992 – as cited by ATSDR 2000).

The Dutch Mother-Child Study was designed as a prospective study to assess the possible adverse health effects of prenatal and postnatal PCB and dioxin exposure. The study group consisted of 418 healthy mother-infant pairs recruited between June 1990 and June 1992 (Koopman-Esseboom et al. 1994b, 1996; Huisman et al. 1995a – as cited by ATSDR 2000). Two hundred seven pairs (105 breast-fed and 102 formula-fed) were from Rotterdam, a highly industrialized area, while 211 pairs (104 breast-fed and 107 formula-fed) were from Groningen, a semi-urban area in northern Holland. In breast milk, of the total TEQ value, PCDDs and PCDFs contributed 46%, coplanar PCBs 24%, mono ortho-substituted PCBs 23%, and di-ortho-substituted PCBs 7% (Patandin et al. 1998 – as cited by ATSDR 2000), however, the authors used four PCB congeners (PCB 118, 138, 153, and

180) as indicators of PCB and dioxin exposure of the developing foetus and breast-fed infant. The prenatal exposure variables (PCB levels in maternal and cord plasma) were not associated with either the reflex or postural cluster scores in the newborn period. However, a significantly higher percentage of hypotonia was shown with an increase in planar PCB TEQ in milk. No effect on the reflex cluster was found (Koopman-Esseboom et al. 1994b – as cited by ATSDR 2000).

At 18 months of age, the neurological condition of the infants was assessed using an age-specific neurological examination which focuses on the observation of motor functions (grasping, sitting, crawling, standing, and walking) in a standardized free field situation. The results showed that prenatal PCB exposure had a small negative effect on the neurological condition of 18-month-old infants whose fathers did not smoke; no such effect was observed in children of fathers who smoked. Neurological condition was unrelated to exposure to PCBs and dioxins via breast milk (Huisman et al. 1995b – as cited by ATSDR 2000).

The mental and psychomotor development of infants exposed to PCBs was investigated at 3, 7, and 18 months of age. Prenatal exposure to PCBs was significantly associated with a decrease in the psychomotor development index (PDI) score at 3 months of age but not after both 7 and 18 months of age. Decreased PDI scores at 7 months among infants who were breast fed for longer periods and had higher TEQ level were associated with postnatal total TEQ (PCB plus dioxin) exposure. The PDI score at 18 months was not associated with postnatal PCB-dioxin exposure. The mental development index (MDI) scores were not significantly associated with either prenatal or postnatal PCB exposure. Higher MDI scores at 7 months of age were positively associated with breast feeding per se. (Koopman-Esseboom et al. 1996 – as cited by ATSDR 2000).

At the age of 42 months, follow-up evaluations included both neurological and cognitive outcomes. The neurological evaluation focused on the observation of motor functions The plasma PCB levels among children in the Rotterdam group at 42 months (n=173), found that median plasma levels were 3.6 times higher in breast-fed children (0.75 µg/L) than in their formula-fed peers (0.21 µg/L) (Patandin et al. 1997 – as cited by ATSDR 2000).

The neurological assessment included 394 mother-infant pairs (94% of total participants) from both Rotterdam and Groningen. The clinical examination yielded a diagnosis of "neurologically normal" in 97% of the children. No associations were found between neurological outcomes and maternal PCB-cord sum, maternal PCB-serum sum, PCB breast milk levels (TEQs) or the child's PCB level at 42 months.

Cognitive abilities (sequential problem solving, simultaneous problem solving, and verbal comprehension) were also evaluated at 42 months (Patandin et al. 1999). In the group as a whole, a significant decline in scores was observed for maternal serum PCB levels. When the groups were divided into breast-fed and formula-fed, only formula-fed children showed a significant association between declines in the scores and maternal serum PCB levels. Further analyses indicated that cognitive performance at 42 months was not related to either lactational exposure or current exposure to PCBs and dioxins (Lanting et al. 1998c, Patandin et al. 1999 – as cited by ATSDR 2000).

Re-examination of the Rotterdam part of the Dutch cohort at age nine years revealed that prenatal PCB exposure was associated with longer response times, greater variability, and lower scores on an executive function test. The latter also appeared negatively affected by lactational exposure to PCBs, while breastfeeding was associated with better scores. Likewise, the latencies on event-related potentials of the brain were longer at higher PCB exposure levels, but breastfeeding was associated with a decrease. These results suggest that the neurobehavioural manifestations reported to be associated with PCB exposure are likely to be permanent, but that the appearance may change during development (Vreugdenhil et al. 2004a, Vreugdenhil et al. 2004b – as cited by EFSA 2005).

In the joint European study, which also included the Dutch cohort at 42 and 72 months of age, two additional cohorts each comprising about 170 healthy mother-infant pairs were formed, namely a Danish cohort from the Faroe Islands (Steuerwald et al. 2000) and a German cohort from Düsseldorf (Winneke et al. 1998, Walkowiak et al. 2001). An association was found between PCB concentrations and mental development at 30 months for the German and at 42 months in both the Dutch (Patandin et al. 1999) and German

cohorts. Upon reassessment between 72 and 77 months of most of the participants, no statistically significant developmental delay was observed in either the Dutch or German cohort (Vreugdenhil et al. 2002, Winneke et al. 2005). However, in the Dutch study negative PCB-associations were still reported for socially disadvantaged mothers (all studies as cited by EFSA 2005).

The Bayley Scales of Infant Development -II (BSID) were administered at 16 months of age to over 750 Sloviakian children who also had prenatal PCB measurements. Maternal mono-ortho-substituted DL PCBs were significantly associated with lower scores on both the psychomotor (PDI) and mental development indices (MDI). A significant association between cord mono-ortho-substituted PCBs and reduced PDI was also observed. Anti-estrogenic and di-ortho-substituted PCBs did not show any statistically significant association with cognitive scores (Park et al. 2010).

3.2.2.10 Endocrine effects

3.2.2.10.1 Thyroid

In a case-control study of the Taiwan *Yu-Cheng* cohort involving 795 exposed subjects and 693 controls increased risk of developing goiter was reported without sign of hypothyroidism or hyperthyroidism (Guo et al. 1999 – as cited by ATSDR 2000).

A positive association was found between concentrations of TSH in serum and PCB 118 in blood of children (671 children, ages 7–10 years) who lived near a hazardous waste incinerator. Significant negative associations were found between serum T3 and PCBs 138, 153, 180, 183, and 187 (Osius et al. 1999 – as cited by ATSDR 2000).

Several studies have examined relationships between thyroid hormone levels in infants and maternal or neonatal PCB concentrations, or mixed PCB and PCDD concentrations (Koopman-Esseboom et al. 1994a, Longnecker et al. 2000, Nagayama et al. 1998a, Winneke et al. 1998a). Hormone levels were within normal ranges in these studies. Concentrations of T4 and TSH in cord blood at delivery were not related to breast milk PCB concentrations in breast milk of 880 mothers from the North Carolina Breast Milk and Formula Project cohort (Longnecker et al. 2000). However, a positive correlation was found between TSH concentrations in cord blood and total serum PCBs in 170 infants from the German cohort in the European Background PCB Study (Winneke et al. 1998b). Negative correlations were found for serum total T4 and T3 in infants and their estimated intakes of 2,3,7,8-TCDD TEQ in breast milk during the first year of postnatal life. No relationship was apparent between infant serum TSH or thyroxine binding globulin (TBG) and TEQ intake. The mean total TEQ intake was 34 ng/kg bw (Nagayama et al. 1998) (all studies as cited by ATSDR 2000).

In the Dutch Mother-Child Study cohort higher levels of total PCB-dioxin TEQ, dioxin TEQ, and PCB TEQ in milk were significantly correlated with lower maternal plasma total T3 concentrations in the last month of pregnancy, lower maternal plasma total T3 and T4 concentrations in the 2nd week after delivery, and higher plasma TSH concentrations in the infants at 2 weeks and 3 months of age (Koopman-Esseboom et al. 1994a – as cited by ATSDR 2000).

Mean thyroid volume was significantly greater in 454 adolescents in Slovakia who lived near a factory that produced PCBs, than in 956 adolescents who lived in "less polluted areas" of Slovakia. There were no differences between the groups with regard to serum TSH or TPO Ab concentrations (Langer et al. 1998 – as cited by ATSDR 2000)

3.2.2.10.2 Diabetes

In an epidemiologic study investigating the potential for health effects in Native Americans from exposure to persistent toxic substances (POPs), preliminary results indicated that elevated serum PCB levels (mean was 3.7 µg/kg and the maximum was 9.6 µg/kg) were correlated with self-reported diabetes and liver disease in two of the cohorts (Dellinger et al. 1997, Tarvis et al. 1997 – as cited by ATSDR 2000).

However, Turyk et al. (2009) did not find a relationship between POPs, including several PCBs, body burdens and the incidence of diabetes mellitus in a cohort of Great Lakes sport fish consumers that was established in 1990 and followed through 2005. The incidence of diabetes (Type 2) was not associated with mono-ortho PCB-118, total PCBs, or years of sport fish consumption (Turyk et al. 2009 – as cited by ATSDR 2011).

Serum from 196 men (median age 60 years) and 184 women (median age 64 years) was measured for PCB 153 concentrations in Swedish fishermen

and their wives. Elevated PCB 153 concentrations were significantly associated with diabetes mellitus type 2 prevalence. An increase of 100 ng PCB-53/g lipids was related to an odds ratio of 1.16 (Rylander et al. 2005 – as cited by ATSDR 2011). Other studies have also reported associations between incidences of type 2 diabetes mellitus and exposure to PCBs (Vasiliu et al. 2006, Chen et al. 2008, Cordru et al. 2007, Wang et al. 2005 – as cited by ATSDR 2011).

A cross-sectional study showed an association between diabetes mellitus prevalence and the concentrations of PCB-153 and p, p'-DDE in 544 serum samples among Swedish fishermen's wives (Rignell-Hydborn et al. 2007 – as cited by ATSDR 2011). Similar findings were reported by Lee et al. (2006, 2007a, b – as cited by ATSDR 2011).

3.2.2.10.3 Other effects

No significant association between PCB levels and time to menopause was observed in Michigan women (n=874, age 24y and older) interviewed in 1997 about their menstrual periods. Serum PCBs were measured as Aroclor 1254 at enrollment between 1976 and 1978 (Blanck et al. 2004 – as cited by ATSDR 2011).

3.2.3 Dermal contact

Serum enzyme (AST, ALT, LDH, and AP) and bilirubin levels were within normal limits in 16 workers exposed to PCBs (type not reported) primarily via dermal contact with used transformer oil containing 600,000 mg/kg PCBs or secondary contact with contaminated clothes or shoes. No correlation between serum triglyceride levels and serum PCB levels was found. Physical examinations showed no dermal or other abnormalities consistent with PCB exposure. Serum PCB concentrations in the study group were low (generally <10 µg/l) in comparison to other occupational studies (ATSDR 2000).

3.3 Toxicity to reproduction

3.3.1 Inhalation

Evaluation of birth data on 172 high-exposure (Aroclors 1254, 1242, and/or 1016 for a minimum of 3 months) and 184 low-exposure female workers showed no significant difference in the mean number of pregnancies. Decreased birth weights and gestational ages in the exposed women were associated with increased serum PCB levels (Taylor et al. 1989 – as cited by ATSDR 2000).

Sperm counts, fertility history, and testicular abnormalities as determined by physical examination were normal in 38 current exposed transformer repairmen compared to 56 unexposed workers. The mean length of employment of the exposed workers was 3.75 years and the predominant exposure was from Aroclor 1260 with lesser exposure to Aroclor 1242. Geometric mean PCB concentrations in the current-exposed and comparison workers were 2.08 and 0.60 mg/kg adipose tissue (Emmett et al. 1988a, 1988b – as cited by ATSDR 2000).

3.3.2 Oral intake

The following summary of reproductive and developmental effects of PCBs is based on ATSDR (2000) with additional studies cited by EFSA (2005) and by ATSDR in an update from 2010.

Menstrual irregularities (altered intervals, duration, and flow) were observed in women exposed during the *Yusho* poisoning incident. Sexual maturation was not delayed, and testicular and scrotal development was not altered in boys born to *Yu-Cheng* women, and sex ratio was not altered in children born to Yu-Cheng women during or after the poisoning began. Fertility, fecundity, and rates of spontaneous abortion have not been studied in *Yusho* and *Yu-Cheng* patients. Decreased birth weight and reduced growth during early life, and neurodevelopmental alterations have been reported among children born to *Yusho* and *Yu-Cheng* women (ATSDR 2000).

In women, suggestive evidence that consumption of PCB-contaminated fish may be associated with a slightly shorter menstrual cycle length

was found. Two studies of fish consumption and conception have demonstrated no effect whereas a third study found that fish consumption of 3-6 year duration was associated with a reduction in fecundity (biological capacity for reproduction) in females. No increased risk for spontaneous foetal death has been related to consumption of fish. Associations between conception delay and consumption of PCB-contaminated fish of exposed men, but not their wives have been reported in one cohort whereas there was no clear association between paternal exposure to consumption of contaminated fish and conception delay or reduced fecundity in another cohort (ATSDR 2000).

Several environmental studies have indicated decreased birth weight and early postnatal growth as possible indicators of adverse developmental effects of mixtures containing PCB. Some studies reported significant negative associations between anthropometric measures at birth (and at early ages) and exposure to PCB (Fein et al. 1984, Jacobson et al. 1990a b, Rylander et al. 1998, Hertz-Picciotto et al. 2005 – as cited by EFSA 2005), others found equivocal or no-significant results (Sauer et al. 1994, Patandin et al. 1998, Vartiainen et al. 1998, Grandjean et al. 2001a, Longnecker et al. 2005 – as cited by EFSA 2005). The wide range of results may reflect the different degree of controlling for confounders and/or different ways of measuring exposure.

In women occupationally exposed to technical PCB mixtures through the manufacture of capacitors, Taylor et al. (1989 – as cited by EFSA 2005) found a significant association between the increased PCB exposure and decreased birth weight and gestational age. Similar findings were reported following high-level exposure to PCB and related chemicals during the Yusho and Yu-Cheng poisoning incidents (Funatsu et al. 1971, Lan et al. 1987, Rogan 1989, Yamaguchi et al. 1971 – as cited by EFSA 2005).

In regard to postnatal growth, the Dutch study (Patandin et al. 1998) found no significant association with PCB exposure and growth up to age 42 months. In a US study, prenatal PCB exposure was associated with increased height and weight at age five years in girls (Hertz-Picciotto et al. 2005). However, several confounders may have affected these associations. The tendency towards lower body weight when a child had been breastfed for a longer time (Dewey et al. 1995) was replicated in a prospective cohort study in the Faroe Islands, but the duration of breastfeeding as such was found to be an unimportant predictor of growth. Reduced body size was primarily associated with the calculated transfer of contaminants via human milk (Grandjean et al. 2003 – as cited by EFSA 2005).

A total of 34,457 Finnish infants born were examined for natal and neonatal teeth. Exposure of the infants to PCBs was evaluated by measuring the levels of 36 congeners in their mothers' milk when they were 4–8 weeks old. The median PCB level in milk was 7.24 picograms/gram (pg/g) in fat (measured as 2,3,7,8-TCDD toxicity equivalents: TEQs). A total of 34 infants with teeth were observed (29 infants had one or two natal teeth and five neonates had neonatal teeth). The prevalence of natal and neonatal teeth was 1:1000, and therefore, no association was found between pollutant levels and occurrence of natal and neonatal teeth (Alaluusua et al. 2002 – as cited by ATSDR 2011).

Four hundred thirty-two Slovenian children, 8–9 years of age, were evaluated for long-term exposure to PCBs. The total PCB serum concentrations in the children were <200, 200–600, and >600 ng PCBs/g serum lipids. Standard dental indices were used to evaluate caries susceptibility, gingival health, and enamel defects. The proportion of deciduous and permanent teeth affected with enamel defects were significantly higher in the highest exposed children (>600 ng PCB/g group). Caries susceptibility, gingival health, or number of teeth was not affected significantly compared to controls; however, a dose-response relationship between PCB exposure and developmental enamel defects of permanent teeth in children was observed (Jan and Vrdic, 2000, Jan et al. 2007 – as cited by ATSDR 2011).

A prospective case-control study of 151 cord bloods (67 cryptorchid/84 matched control) and 125 colostrums (56 cryptorchid/69 matched control) was initiated to assess the incidence of cryptorchidism in male children who were exposed to PCBs during prenatal and postnatal life. The results of this study suggest a positive association (p=0.045) between high total PCB concentrations (perinatal exposure) and cryptorchidism in boys (Brucker-Davis et al. 2008 – as cited by ATSDR 2011).

A cohort of 615 children who were born during the period 1959–1965 was selected at random from 12 U.S study centers. A complete data set was available for 195 children with sensorineural hearing loss. Exposures to PCBs were measured as total PCB concentration in maternal serum, with the median measured as 2.8 µg/L during the third trimester. This level was about twofold higher than in recent background levels in the United States. Hearing evaluation was carried out when the children were about 8 years old. No significant adverse effects on the average hearing threshold across the frequencies required for speech recognition were observed (Longnecker et al. 2004 – as cited by ATSDR 2011).

In a prospective cohort of 25 randomly selected full-term children who breastfed exclusively for at least 4 months, levels of PCB congeners (PCBs 105, 118, 138, 153, 156, and 180) were measured in colostrums and breast milk at 1 and 3 months after delivery. Visual function evaluations were carried out by P100 with latency visual evoked potentials (VEPs) being measured starting at 12 months of age. At 15 months of age, impaired VEP was significantly correlated with all PCB congeners except for PCB-105. However, VEP at one hour was correlated with PCB-180 only. This study suggests a weak correlation between PCB levels and impaired visual functions at 12 months of age (Riva et al. 2004 – as cited by ATSDR 2011).

Pregnant women (n=118, age 25–34 years of age) were selected to participate in a study to examine the association between transplacental exposure to dioxins/PCBs and thyroid and growth hormones in newborns. Cord sera from 118 newborns were analyzed for 12 DL-PCB congeners, other related compounds such as dioxins, and thyroid and growth hormones. Statistical analyses showed independently and significantly decreased concentrations of free T4 (FT4) x TSH with increasing non-ortho PCBs (Wang et al. 2005 – as cited by ATSDR 2011).

A cohort of 232 pregnant German women, 18–42 years of age, was recruited in a study from 2000–2002. The authors investigated the effects of PCBs and dioxins on gonadal hormones. Maternal blood and milk samples were collected from a subset of 104 mother-infant pairs for chemical analyses of the sum of 6 PCBs (28, 52, 101, 138, 153, and 180) as indicator PCBs, and 17 PCDD/F congeners. The median concentrations in maternal blood fat and milk fat for the sum of the indicator PCBs were 149 and 177 ng/g. The median concentrations of PCDD/F in maternal blood fat and milk fat were 15.3 and 13.1 pg WHO-TEQ/g, respectively. Maternal sera and cord sera were also analyzed for testosterone and estradiol. The adjusted means ratio (MR) for testosterone hormonal level in cord serum samples was significantly reduced in girls for the sum of non-o-PCBs; sum of mono-o-PCBs, sum of 6 PCBs; and for sum of PCDD/F, but not in boys. On other hand, estradiol levels were significantly reduced in boys but not in girls after PCB exposures. Estradiol levels were not significantly associated with any PCB category level. This study suggested that even low levels of PCBs had a robust negative impact on gonadal hormones in newborns (Cao et al. 2007 – as cited by ATSDR 2011).

However in another study, no strong association was found between umbilical cord PCB levels and testicular sizes, serum testosterone concentrations, or spermaturia in boys at 7 and 14 years of age (Mol et al. 2002 – as cited by ATSDR 2011).

A cohort of 138 girls (10–16.9 years old) from the Akwesasne Mohawk Nation, New York was studied by comparing blood PCB levels against attainment of menses. The cohort was exposed to PCBs via food, and 16 PCB congeners were detected in more than 50% of the blood samples. In this study, the presence or absence of menses at the time of the interview was recorded. The geometric mean (0.12 µg/l) of estrogenic PCBs (PCB 52, 70, 90/101, 187) was associated with a significantly greater probability of having started menarche early, where 86% of 12-year-old girls were predicted to have reached menarche at the 75th percentile of estrogenic PCBs levels. The study suggested that even at low levels of estrogenic PCBs, the time to menarche attainment was decreased (Denham et al. 2005 – as cited by ATSDR 2011). The median age at menarche for this cohort (138 girls) was 12.2 years. However, Vasiliu et al. (2004 – as cited by ATSDR 2011) reported no association with maternal PCB exposure.

The serum concentrations of PCBs 138, 153, and 180 and other related contaminants were measured in 200 individuals who lived in highly-, moderately-, lowly-, or non-contaminated areas, with a mean age of 17.4 years. Significantly fewer boys had reached the adult stage of genital and pubic hair development in the highest contaminated areas in comparison to controls. Fewer girls reached the

adult stage of breast development in the highest contaminated area (Den Hond et al. 2002 – as cited by ATSDR 2011).

Most studies on reproductive effects of dioxins in humans have concerned paternal exposure of workers or Viet Nam veterans. Some studies have shown alterations in sex hormone levels (elevated serum levels of luteinizing hormone and follicle-stimulating hormone and a decreased level of testosterone) and sperm characteristics (lower concentration, percentage of motile cells and percentage of morphological normal cells) after PCDD exposure. Discordant results exist for an increase in the risk of spontaneous abortions among the wives of the exposed men. In most of the studies an elevation in birth defects were not detected (IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

An alteration of the sex ratio was observed between 1977 and 1984 in children born to parents highly exposed to 2,3,7,8-TCDD after the industrial accident in Seveso in 1976. Paternal serum lipid concentrations of 2,3,7,8-TCDD higher than 118 ng/kg lipid at the time of conception were associated with the birth of significantly more girls than boys (twice as many). The decreased male/female sex ratio might already be apparent at serum concentration between 15 and 80 ng/kg lipid in the father. During 1985 – 1994 the sex ratio reverted to normal. An explanation of this phenomenon has not been offered but a possible role of hormonal disruption cannot be ruled out. However, no changes in sex ratio have been observed in other studies including a study of the Taiwanese cohort having very heavy maternal body burdens (estimated levels at 2 – 3000 ng TEQ/kg bw) (IARC 1997, IPCS 1993, SCF 2000, WHO 2000, JECFA 2002).

3.4 Mutagenic and genotoxic effects

3.4.1 Inhalation

Available information on *in vivo* genotoxic effects of PCB in humans is limited by confounding exposures that involved mixtures of chemicals. Workers exposed for 2-25 years during the production of PCB-based products named Delor 103 and Delor 106 showed increased chromosome aberrations and sister chromatid exchanges (SCE) in their lymphocytes, compared to controls matched for alcohol consumption and smoking. The length of exposure over 10 years was associated with increased frequencies of aberrant cells with chromosome aberrations and SCE (ATSDR 1997, EFSA 2005).

A slight increase in sister chromatid exchanges and chromosomal aberrations in lymphocytes were also seen in workers exposed to PCBs following a fire in an electric station (ATSDR 1997, EFSA 2005).

No statistically significant difference was found in the frequencies of chromosomal aberrations or sister chromatid exchanges in peripheral lymphocytes in male workers exposed to 2,3,7,8-TCDD in a German accident (IARC 1997).

In the Seveso accident, the frequency of aberrant cells in maternal peripheral lymphocytes, and placental and umbilical cord tissues was not increased in women exposed to 2,3,7,8-TCDD compared to unexposed controls. However, significant increases in aberrations were noted in foetal tissues.

3.4.2 Oral intake

No statistically significant difference was found in the frequencies of chromosomal aberrations or sister chromatid exchanges in peripheral lymphocytes in Taiwanese women exposed to PCBs and PCDFs in contaminated rice oil compared to unexposed controls. However, exposure to PCBs and PCDFs enhanced the sensitivity of lymphocytes to sister chromatid exchanges induced by α-naphthoflavone. No DNA-adducts were detected in placentas from non-smoking Taiwanese women exposed to PCBs and PCDFs in contaminated rice oil (IARC 1997).

3.5 Carcinogenic effects

The carcinogenicity of PCBs in humans has been investigated in retrospective cohort mortality studies of workers, and in case-control studies of environmental exposure that examined

associations between serum or adipose tissue levels of PCBs and occurrence of cancer.

3.5.1 Inhalation

Some of the mortality studies, but not all, suggest that occupational exposures to PCBs were associated with cancer at several sites, particularly the liver, biliary tract and/or gall bladder, intestines, and skin.

There was no clear association between occupational exposures to PCBs and cancer in other tissues, including the brain and breast, and haematopoietic and lymphatic tissues (ATSDR 2000).

3.5.2 Oral intake

A report of liver cancer in *Yusho* victims appears to support the occupational hepatocarcinogenicity data. However, no statistically significantly increased mortality from cancer of the stomach or esophagus, rectum, sigmoid colon, and anus, from leukemia and from cancer of the pancreas was found (ATSDR 2000).

In *Yu-Cheng* victims, mortality from Hodgkin's disease was increased in comparison to Taiwan national or local rates in the males, whereas no increased mortality from cancer of the liver and intrahepatic bile ducts, of the stomach or small intestine was found (Hsieh et al. 1996 – as cited by ATSDR 2000).

A number of case-control studies have investigated possible associations between breast cancer and concentrations of PCBs in breast tissue or blood in the general population. Breast adipose levels of total PCBs or individual congeners were increased in women with breast cancer in some but not all studies. Other environmental studies used serum PCB concentrations as the marker of exposure with blood samples taken after the diagnosis of breast cancer, or prospectively collected prior to diagnosis. None of the serum studies found significantly different mean blood levels of PCBs in breast cancer cases and controls. None of the prospective studies found that PCBs were associated with the occurrence of breast cancer. However, increased risks of breast cancer associated with increased tissue levels of some congeners was suggested in subgroups of women that were postmenopausal and who were parous and had never breast-fed, or in postmenopausal women with a putative high-risk *CYP1A1* variant genotype (ATSDR 2000).

The incidence of stomach cancer was reported significantly elevated in Swedish fisherman that had high intake of PCBs in fish, but the effect cannot be definitely attributed to PCBs because consumption included smoked fish and PCBs were not the only contaminants in the fish (ATSDR 2011).

Certain PCBs congeners, particularly the higher chlorinated PCBs (PCB 156, 180, 194) were associated with increased risk of non-Hodgkin's lymphoma development (De Roos et al. 2005 – as cited by ATSDR 2011).

Hardell et al. (2004) found that blood concentrations of PCBs were higher in mothers of patients with testicular cancer than in controls. Hardell et al. (2006) also reported an association between POPs and prostate cancer, in particular for PCB 153 in a total study population of 58 cases (ATSDR 2011).

4 Animal toxicity

Most studies in experimental animals have investigated the commercial PCB mixtures that were produced in the USA before 1977 under Aroclor trade names. Studies are also available for PCB mixtures produced in other countries and among the most common tested of these mixtures are Kanechlors produced in Japan and Clophens produced in Germany. The technical PCB mixtures may contain other chlorinated compounds as impurities, such as polychlorinated naphthalenes (PCN) and polychlorinated dibenzofurans (PCDF). The different composition as well as the presence of toxicological relevant impurities may have a significant impact on the results of toxicological studies on technical mixtures. A reliable interpretation of results of those studies, especially a differentiation of effects caused by NDL-PCB and DL-PCB, respectively, is only achievable if the congener composition of the technical PCB mixture applied is known. Unfortunately, this is not the case for most of the animal studies performed. Moreover, it has to be considered that the manufacture of technical PCB mixtures was mostly ceased already in the late 1970s, before many of the analytical techniques, necessary for performing congener specific analyses were developed. Therefore, almost all congener specific analyses of technical mixtures were performed after the cessation of production (EFSA 2005).

The variation of the congener composition as well as the amount of impurities in different technical mixtures was discussed by the EFSA in its evaluation of NDL-PCB in food (EFSA 2005). Two Aroclor 1254 lots, lot number 6024 and lot number 124-191 were analysed for DL-PCB and PCDF by Kodavanti et al. (2001) and concentrations were transferred by EFSA into toxic equivalents (TEQ) using the TEF values proposed by WHO in 1997 (Van den Berg et al. 1998), see Table 8.

The proportions of non-ortho (0.02% in Lot 124-191 vs. 2.9% in Lot 6024) as well as mono-ortho PCB (24% vs. 38%) differ significantly between the two lots. As a consequence, the resulting TEQ values (38.3 vs 395.1 µg WHO-TEQ/g) differ more than one order of magnitude. The amount of PCDF formed as by-products during the production process are also somewhat higher in Aroclor 1254 lot 6024 (Kodavanti et al. 2001). While lot 124-191 has the typical PCB congener distribution of "early" Aroclors 1254 types (G4 type), lot 6024 represents the "late" A4 type (1974-1976) production of Aroclor 1254. A4 and G4 type Aroclor 1254 are produced by different chlorination procedures (two steps versus single step chlorination) (Frame, 1996).

It appears that the TEQ contents in both of these two Aroclor 1254 lots have been rather high. Burkhard and Lukasewycz (2008) systematically collected data from the literature on TEQs in a number of commercial PCB mixtures (Aroclor, Clophen, Kanechlor, Chlorofen, Sovol, Delor, and Phenoclor) and reported a value of 7.9 µg TEQ/g for Aroclor 1254, when WHO TEFs from 2005 were used. TEQs were similar across the different PCB product lines for mixtures of similar chlorine content. The TEQ content varied from 0.034 µg/g in Aroclor 1221 to 11.8 µg/g in Aroclor 1248.

Parameter	Lot 124-191	Lot 6024
Σ Non-ortho PCB congeners	17.1 µg WHO TEQ/g	327 µg WHO-TEQ/g
Σ Mono-ortho PCB congeners	20.5 µg WHO TEQ/g	65 µg WHO-TEQ/g
Σ PCDF	0.7 µg WHO TEQ/g	3.1 µg WHO-TEQ/g
Total PCDF/PCB	38.3 µg WHO TEQ/g	395.1 µg WHO-TEQ /g

Table 8.
Dioxin-like PCBs and PCDF in two different Aroclor 1254 lots (from EFSA 2005).

While production records suggest that the A4 pattern lots of Aroclor 1254 (e.g. lot 6024) represented less than 1% of the total Aroclor 1254 production, their availability during the final years of production resulted in their disproportionate use by researchers into Aroclor 1254 toxicity. For example, the major chronic 2-year rat carcinogenicity study of Aroclors (Mayes et al. 1998) and an in vivo neurotoxicity study (Kodavanti et al. 1998) both employed an Aroclor lot of this type (Frame 1999).

Therefore, the presence of DL-PCB and PCDF in different technical PCB mixtures (expressed as WHO-TEQ) must be considered when interpreting toxicological animal studies aiming at assessing the toxic effects of NDL-PCB based on technical PCB mixtures.

Burgin et al. (2001) studied the two above mentioned lots of Aroclor 1254 in order to determine if the difference in the TEQ could account for different *in vivo* responses seen on a weight basis. Male Long-Evans rats (70 days old) were treated orally with a single dose of 0–1,000 mg/kg bw of each lot. Hepatic ethoxy-, methoxy-, and pentoxyresorufin *O*-deethylase (EROD, MROD, and PROD, respectively) activities as well as serum thyroxine (T4) concentrations and measures of oxidative stress were determined 4 days after treatment. Results, on a weight basis, indicate that lot 6024 led to a greater induction of EROD, MROD, and PROD but not total T4 reduction. The differences in TEQ between the lots explained the differential induction of EROD and MROD but did not account for the induction of PROD nor decreases in T4. PROD induction is not due to dioxin-like congeners, whereas the decrease in serum T4 levels may involve multiple mechanisms. Effects on the antioxidants ascorbic acid and uric acid were seen only at the highest mass dose for both lots and were not explained by the difference in TEQ. These results illustrate that the differences in the TEQ explain the differences in the strict dioxin-like effects, but the non-dioxin-like congeners cause other effects that are not associated with the aryl hydrocarbon receptor (e.g., PROD).

4.1 Single dose toxicity

4.1.1 Inhalation

No data were available regarding lethality or decreased longevity of animals due to acute or chronic inhalation of PCBs (ATSDR 2000).

4.1.2 Oral intake

In experimental animals, the acute oral toxicity of PCBs varies for different commercial mixtures, but is in general low to moderate with oral LD_{50}-values between 1010 and 4250 mg/kg bw in rats. Signs of toxicity include diarrhoea, respiratory depression, dehydration, decreased response to pain stimuli, unusual gait and stance, oliguria, coma, and pathological changes in organs. (WHO 2003, ATSDR 2000).

4.1.2.1 NDL-PCB

Adverse effects reported in laboratory animals following exposure to individual NDL-PCB (PCB 18, 28, 47, 52, 95, 101, 110, 128, 132, 149, 153, 170, 180, 206, 209, see Appendix 2) were effects on the thyroid, liver, brain biochemistry, immunotoxicity, oestrogenicity, reproductive and neurodevelopmental effects, in particular in the offspring of rodents following *in utero* exposure.

In acute and subacute rodent studies (single dose or a few days of dosing) the NOAELs for these effects for the individual NDL-PCB generally exceeded 1 mg/kg bw per day (see Table in Appendix 3). For the NDL-PCB tested for reproductive and developmental effects in rodents, including oestrogenic effects and effects on the thyroids as well as on the developing nervous system (PCB 18, 28, 47, 52, 101, 110, 153), the NOAELs ranged from 1 mg to >50 mg/kg bw per day. In most studies, the dams were treated on gestational days (GDs) 10-16, and the test compounds administered by either gavage or intraperitoneal injection (see Appendix 2 and 3).

4.1.2.2 DL-PCB

There is limited data on the individual PCB congeners in mammals. The acute toxicity of 2,3,7,8-TCDD and related PCDDs and PCDFs substituted in at least the 2, 3, 7, and 8 positions varies widely between and among species. For

example, the oral LD_{50} in guinea-pigs was 0.6 µg/kg bw, while that in hamsters was greater than 5000 µg/kg bw.

In all mammalians species tested so far, lethal doses of PCDDs, PCDFs and DL-PCBs result in a generalised delayed wasting syndrome that precedes death. It is characterised by inhibition of gluconeogenesis, reduced feed intake, and excessive loss of body weight. Although some species differences exist, other toxic effects observed after acute exposure to PCDDs include haemorrhages in a number of organs, thymic atrophy, hypertrophy/ hyperplasia of hepatic, gastrointestinal, urogenital and cutaneous epithelia, atrophy of the gonads, subcutaneous oedema and systemic haemorrhage, and reduced bone-marrow cellularity (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

In rhesus monkeys, a single dose of 1 µg/kg bw of 2,3,7,8-TCDD induced chloracne (IARC 1997). Chloracne has also been seen in cows, horses, rabbits and hairless mice (WHO 2000).

High, near lethal doses of 2,3,7,8-TCDD alter cardiac function and morphology in several animal species (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000).

4.1.3 Dermal contact

A single topical dose of 2,273 mg/kg bw Aroclor 1254 was fatal to hairless mice within 24 hours. Median lethal doses for single dermal applications of PCBs to rabbits were between 794 and 1,269 mg/kg bw for Aroclors 1242 and 1248, between 1,260 and 3,169 mg/kg bw for Aroclors 1221 and 1262, and between 1,260 and 2,000 mg/kg bw for Aroclors 1232 and 1260. Cause of death was not reported, and there was no clear trend of toxicity with degree of chlorination (ATSDR 2000).

4.2 Irritation

4.2.1 Respiratory irritation

No studies are available regarding respiratory effects in animals after inhalation exposure to PCBs.

Only few studies have examined the respiratory system in animals after oral exposure to PCDDs. Haemorrhage and hyperplasia of the bronchial epithelium (as well as at other organ sites that had mucous-secreting cells) developed in monkeys fed 2,3,7,8-TCDD. In rodents conflicting evidence exist. (ATSDR 1998).

4.3 Repeated dose toxicity

4.3.1 Inhalation

No studies were located regarding respiratory effects in animals after inhalation exposure to PCBs.

Intermittent exposure to near-saturation vapor concentrations of heated Aroclor 1242 (8.6 mg/m^3) over 24 days was not lethal in rats, mice, rabbits, or guinea pigs, and no signs of intoxication were reported. Similar exposures to lower concentrations of heated Aroclors 1242 and 1254 were also found not to produce lethality in these species (ASTDR 2000).

4.3.2 Oral intake

The preponderance of toxicity data for PCBs is available from experimental animals exposed to commercial mixtures of PCBs in the diet in intermediate-duration studies whereas fewer studies with chronic oral exposure have been performed. Studies have been performed with various species, but the rat and monkey have been tested most extensively. Generally, Aroclor 1254 has been used in most of the studies, particularly in the studies with monkeys.

Data on defined experimental mixtures are available from a study in monkeys (Cynomolgus and Rhesus). Infant monkeys ingested from birth until 20 weeks old a defined PCB mixture (mostly mono- and di-*ortho*-substituted congeners) analogous to the congener composition in human milk (Canadian women), and were observed until they were at least 66 weeks old. The total daily intake of 0.0075 mg/kg bw/day represented the approximate daily intake of a nursing human infant whose mother's milk contained 50 µg/kg

PCBs (the Health Canada guideline for maximum concentration in human milk).

Data on individual congeners are available from comparative 13-week oral toxicity studies in rats in which 7 individual PCB congeners (DL-PCBs 77, 105, 118, and 126, and NDL-PCBs 28, 128, 153) were selected based on frequent occurrence in environmental samples and human tissues, or toxic potency. A broad spectrum of health effects have been observed in experimental animals including effects on the liver, stomach, thyroid and adrenal glands, skin and eyes, and the haematopoietic, immune and nervous systems (ATSDR 2000). In addition more recent long-term toxicity and carcinogenicity studies on PCBs 118, 126 and 153 have become available.

The DL-PCBs share the toxicological properties of the "dioxins" (with 2,3,7,8-TCDD being the prototype) and were previously evaluated together with PCDDs, PCDFs in the MST Report "Evaluation of health hazards by exposure to PCDDs, PCDFs and dioxin-like PCBs" by Larsen and Nørhede (2004). The toxicological studies on DL-PCBs are therefore not described in details in this report, except for a few never studied that have been included to complement the previous evaluation. Description of the toxicological studies on individual DL-PCBs were also included in an Annex to the EFSA evaluation of NDL-PCB (EFSA 2005).

As regards studies on individual NDL-PCBs these are briefly summarised in the following sections, and more detailed descriptions can be found in Appendix 2 'Toxicological studies *in vivo* on individual NDL-PCB congeners'.

4.3.2.1 Effects on the liver

Liver toxicity induced by PCBs is well documented in experimental animals exposed to commercial mixtures or single congeners. PCB-induced liver effects in animals seem to be reversible when mild and include microsomal enzyme induction, increased serum levels of liver-related enzymes and lipids, liver enlargement, altered porphyrin and vitamin A metabolism, and histopathological alterations that progress to non-neoplastic degenerative lesions and/or tumours with higher doses or longer duration exposures. Monkeys appear to be more sensitive than rats to PCB hepatotoxicity (ATSDR 2000).

Induction of microsomal enzymes (EROD activity) appears to be the most sensitive hepatic effect in rats and has been observed following dietary administration of Aroclor 1242, 1248, 1254, or 1260 for 4 weeks at dose levels from 0.03 mg/kg bw/day (lowest dose level tested). Increased urinary coprophophyrin levels, increased liver weight, and lipid deposition in the liver have been observed in rats fed Aroclor 1242 from 0.25 mg/kg bw/day for 2-6 months (ATSDR 2000).

In a recent comprehensive comparative 24-months oral toxicity study in rats, microscopic liver lesions (hepatocellular hypertrophy and vacuolisation) were observed following dietary administration of Aroclor 1016, 1242, 1254, or 1260 at dose levels from 1-2 mg/kg bw/day, and increased serum cholesterol in females exposed to Aroclor 1242, 1254 and 1260 from about 1.4-5.7 mg/kg bw/day. The effects were usually much more severe in females than in males and showed the following pattern of Aroclor toxicity: 1254 > 1260 ≈ 1242 > 1016 (ATSDR 2000).

In the comparative 13-week oral toxicity studies in rats with individual congeners, hepatic effects included increased liver weight, biochemical changes (increased serum enzymes and cholesterol, increased liver porphyrins, decreased liver vitamin A), and histopathology (cytoplasmic vacuolation and fatty alterations). The most toxic congener was PCB 126 with a LOAEL of 0.00074 mg/kg bw/day, the next most toxic congener was PCB 105 with a LOAEL of 0.039 mg/kg bw/day, and the least toxic congener was PCB 128 with a LOAEL of 0.425 mg/kg bw/day. Considering dose-response and severity of liver effects, the order of toxicity was PCB 126 > 105 > 118 ≈ 77 > 153 ≈ 28 > 128 (ATSDR 2000).

In a recent study, highly purified PCB 180 (dioxinlike impurities: 2.7 ng TEQWHO/g PCB 180) was tested in a 28-day toxicity study in young adult Sprague-Dawley rats. Groups of five male and female rats were given total doses of 3, 10, 30, 100, 300, 1000 or 1700 mg/kg bw PCB 180 in corn oil by gavage. Loading doses were administered on days 0-5 and maintenance doses on days 10, 17, and 24. At the end of the treatment period (males on study day 28-32, females on days 28-32) blood samples were obtained and the rats were killed by exsanguination. A complete necropsy (macroscopic observations, tissue sampling for molecular biology, biochemistry, histopathology, analytical chemistry and organ weights) was

performed on each rat. Tissue samples were stored at -80 °C for further analysis. In addition, perirenal adipose tissue and liver were stored at -20 °C for determination of PCB 180 tissue concentration. In the present study only liver tissue samples, as the most sensitive organ for PCB exposure, and blood serum samples were analyzed. Increased liver weights were observed at ≥300 mg/kg bw in males and females. No increases in serum ALT or ALP activities were found. A significant increase in liver pentoxyresorufin O-dealkylase (PROD) activity was found in males at ≥10 mg/kg bw and in females at ≥30 mg/kg bw. A significant induction of hepatic 7-ethoxyresorufin O-deethylase (EROD) activity was also observed in males at ≥10 mg/kg bw and in females at ≥300 mg/kg bw. Western blotting showed that mainly cytochromes P450 (CYPs) 2B1/2 and 3A1 were induced while only slight effects were seen on CYP1A1, CYP1A2 and CYP1B1. However, no induction of CYP1A1, 1A2 and 1B1 was found on the mRNA level, except for a slight effect in females at 1000 mg/kg bw. Furthermore, hepatic UDP-glucuronosyltransferases (UGTs) 1A1 and 1A6 were markedly induced in males at ≥300 mg/kg bw but only slightly induced in females at ≥1000 mg/kg bw. The hepatic concentrations of apolar retinoids were decreased in males at ≥30 mg/kg bw and in females at ≥300 mg/kg bw. Centrilobular liver hypertrophy (staged in three classes, mild (1), moderate (2), and severe (3)) was observed on histopathology in both genders. A benchmark dose (BMD) approach was used to model lowest effective dose levels for these effects. For centrilobular liver hyperthrophy the BMD5s in males for progressing to respectively stage 1, 2, and 3 were 15, 17, and 62 mg/kg bw, and in females 205 and 617 mg/kg bw (females would not progress to stage 3). The corresponding BMDL5s for progressing to stage 1 were 9.4 mg/kg bw in males and 139 mg/kg bw in females. The findings show that pure PCB 180 leads to hepatic changes in a dose range which did not cause CYP1A1 induction but causes centrilobular liver hypertrophy, affects drug-metabolising enzymes involved in the metabolism of exogenous and endogenous substrates and leads to changes in liver retinoid levels. Comparison of PCB 180 liver level related to BMDL5 for hepatic hypertrophy in rats with human data on hepatic PCB levels in individuals without history of specific exposure suggests a relatively small margin of tissue burden in the range of 37-fold. The results show that the highly pure non-dioxin-like PCB 180 exerted strong effects different to dioxin-like compounds and that the low TEQ contamination allowed a characterization of the PCB as non-dioxin-like (Ross et al. 2011).

In Rhesus monkeys, hepatic effects (liver enlargement, fatty degeneration, hepatocellular necrosis, and changes in the bile duct) were observed after 12-28 months of dietary exposure to 0.2 mg/kg bw/day of Aroclor 1254. Increased liver weight and serum triglycerides, and decreased serum bilirubin and cholesterol have been observed in Rhesus monkeys that ingested 0.08 mg/kg bw/day of Aroclor 1254 for 72 months; no effects were observed at dose levels of up to 0.04 mg/kg bw/day. In another study, monkeys receiving Aroclor 1254 for 37 months had decreased plasma cholesterol from 0.04 mg/kg bw/day and increased plasma triglycerides from 0.005 mg/kg bw/day (ATSDR 2000).

In the study on infant monkeys treated with the defined PCB mixture (0.0075 mg/kg bw/day) analogous to that in human milk, reported hepatotoxicity-related endpoints were limited to alterations (not significantly) in serum biochemical indices, including liver enzymes, bilirubin, triglycerides, and cholesterol.

The LOAEL of the commercial PCB mixture Aroclor 1254 for effects on the liver in monkeys was 0.08 mg/kg bw/day administered in the diet for 72 months. The critical effect was increased liver weight.

A number of biochemical changes such as liver enzyme induction, enhanced expression of growth factors and enhanced oxidative stress have been noted in experimental animals at 2,3,7,8-TCDD doses above 100 pg/kg bw per day equivalent to body burdens of 3-10 ng/kg bw (IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

Liver hyperplasia, fatty infiltration, and necrosis have been observed in a number of species fed 2,3,7,8-TCDD in the µg/kg bw dose range. Liver toxicity is associated with increased serum transaminases and dehydrogenases, and impaired biliary clearance. Altered lipid metabolism results in elevated serum triglycerides and cholesterol, as well as decreased serum glucose levels. Accumulation of porphyrins has also been observed (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

4.3.2.2 Effects on the gastro-intestinal tract

Administration of PCBs in the diet to monkeys at dose levels from 1.3 mg/kg bw/day of Aroclor 1248 or from 0.12 mg/kg bw/day of Aroclor 1242 for 2 months has produced gastritis with hypertrophy and hyperplasia of the gastric mucosa, which progressed to ulceration of the gastric mucosa and haemorrhage. Effects on stomach tissue have also been observed in Cynomolgus monkeys administered 0.2 mg/kg bw/day Aroclor 1254 in the diet for 12-13 months and in Rhesus monkeys treated similarly for 28 months, but not in Rhesus monkeys receiving Aroclor 1254 at a dose level of 0.08 mg/kg bw/day for 72 months (ATSDR 2000).

Gastric ulcers also occurred in minks at similar dietary doses of Aroclor 1016, 1242, or 1254 (Bleavins et al. 1980, Hornshaw et al. 1986 – as cited by ATSDR 2000), and there is evidence of gastric erosion and necrosis in pigs treated with 9.2 mg/kg/day Aroclor 1242 or 1254 for 91 days (Hansen et al. 1976 – as cited by ATSDR 2000).

There were no histological changes in the stomach or intestines of rats treated with 100 mg/kg/day Aroclor 1242 by gavage 3 times/week for 3 weeks (Bruckner et al. 1973 – as cited by ATSDR 2000).

Re-examination of the National Cancer Institute (NCI 1978) cancer bioassay showed Aroclor 1254-induced intestinal metaplasia and some adenocarcinoma in the glandular stomach of Fischer 344 rats following chronic dietary treatment (Morgan et al. 1981, Ward 1985 – as cited by ATSDR 2000). The intestinal metaplasia appeared to be dose-related. Non-proliferative gastric lesions were not observed.

No histopathological changes were observed in the gastrointestinal tract of male or female rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at dose levels of 8.0–11.2, 4.0–5.7, 4.3–6.1, or 4.1–5.8 mg/kg/day, respectively (Mayes et al. 1998).

In the comparative 13-week oral toxicity studies in rats with individual congeners, no histological alterations were observed in the gastrointestinal tract; doses ranged from 0.01 µg/kg bw/day to approximately 4 mg/kg bw/day (ATSDR 2000).

The NOAEL of the commercial PCB mixture Aroclor 1242 for effects on the gastro-intestinal tract in monkeys was 0.08 mg/kg bw/day administered in the diet for72 months. The observed effect on the gastric mucosa in monkeys is considered to be specific for the PCDFs and DL-PCBs in the mixture.

A significant increase in the serum level of gastrin as well as hyperplasia of the stomach epithelia in response to toxic doses of 2,3,7,8-tetraCDD has been observed in several species. Monkeys are more sensitive than rodents to gastrointestinal effects of 2,3,7,8-TCDD (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

4.3.2.3 Musculoskeletal effects

Little information exists regarding musculoskeletal effects of PCBs in animals. Changes in femur bone morphology resulting in weaker bones occurred in growing (28-day-old) rats (10 per dose) that were treated with Aroclor 1254 by gavage for 10–15 weeks at doses from 0.1 mg/kg bw/day to 25 mg/kg bw/day. Serum and urinary calcium levels were increased, but there were no treatment-related alterations in serum parathyroid hormone concentration (ATSDR 2000).

No histopathological changes were observed in skeletal muscle of male or female rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at dose levels of 8.0-11.2, 4.0-5.7, 4.3-6.1, or 4.1-5.8 mg/kg/day, respectively (Mayes et al. 1998). Similarly, there were no histological alterations in skeletal muscle of rats in the comparative 13-week oral toxicity studies with individual congeners; doses ranged from 0.17 mg/kg bw/day to approximately 4 mg/kg bw/day (ATSDR 2000).

Goat dams were exposed to 98 µg/kg bw/day of PCB-153 or 49 ng/kg/day of PCB-126 in corn oil on gestation day 60 until delivery. The offspring were also exposed to PCBs during the lactation period of 6 weeks. The peri-natal exposure to PCB 153, but not PCB 126, resulted in altered bone composition in female goat offspring. The biomechanical testing showed no significant differences between the exposed and control groups for either congener (Lundberg et al. 2006 – as cited by ATSDR 2011).

4.3.2.4 Effects on the thyroid gland

Various effects on the thyroid gland and thyroid hormone system have been observed in studies in experimental animals. Effects include disruption of the production and levels of thyroid hormones, interference with thyroid hormone transport, and acceleration of the metabolic clearance of thyroid hormones; hyperplasia, hypertrophy and increased vacuolisation of follicular cells; depletion of follicular

colloid and reduced follicular size; and thyroid enlargement (ATSDR 2000).

In rats, decreased serum levels of the thyroid hormones T4 and T3 were observed following dietary administration of Aroclor 1254 at 0.09 mg/kg bw/day (lowest dose level tested) for 5 months. Histological alterations have been observed in rats following dietary administration of Aroclor 1254 at 0.25 mg/kg bw/day for 5 weeks, but not at 0.025 mg/kg bw/day (ATSDR 2000).

In the comparative 13-week oral toxicity studies in rats with individual congeners, histopathological lesions were observed in the thyroid to varying degrees of severity for the individual congeners. The most toxic congener was PCB 126 with a LOAEL of 0.00074 mg/kg bw/day, the next most toxic congener was PCB 105 with a LOAEL of 0.039 mg/kg bw/day, and the least toxic congener was PCB 128 with a LOAEL of 0.425 mg/kg bw/day (ATSDR 2000).

In Rhesus monkeys, no effects on thyroid tissue and serum hormone levels were observed following exposure to Aroclor 1254 at dose levels up to 0.08 mg/kg bw/day for up to 72 months. In one study of Rhesus monkeys, enlarged thyroid glands and histological alterations were observed following dietary administration of Aroclor 1254 at 0.2 mg/kg bw/day for 28 months, whereas in another study, Cynomolgus monkeys treated similarly for 12 months did not show histological alterations in the thyroid (ATSDR 2000). In the study on Rhesus monkeys developmental effects were seen at the NOAEL for thyroid effects.

Study summaries of the most relevant new studies are provided below, starting with a few summaries of studies examining single NDL PCB congeners (52, 153 and 180), followed by summaries of studies examining the effects of Aroclors and other mixtures on the thyroid hormone system.

In a study from 2011, pregnant SD rats were exposed to PCB 52 in doses of 0, 30, 100, 300, 1000 or 3000 mg/kg bw (total dose levels) from GD 7 to GD 16 and again from PND 1 to PND 10 every second day (total of 10 doses; listed doses are total dose levels). For PCB 180 exposure was from GD 7 to GD 10 in total doses of 0, 30, 100, 300, 1000 or 3000 mg/kg bw. Due to the longer half life of PCB 180 than PCB 52 dosing was spread out over a longer interval for PCB 52 than for PCB 180.T3 but not T4 was reduced in dams from 100 mg/kg by PCB 52 and offspring T3 was reduced from 30 mg/kg bw. Free T4 was reduced at higher levels. A LOAEL for thyroid effects could thus be 30 mg/kg bw. PCB180 reduced free T3 slightly from 30 mg/kg bw in dams and had no effects in offspring at PND 35 but were reduced at PND 84 from 30 mg/kg bw (males). For PCB180 a LOAEL for thyroid effects could also be set to 30 mg/kg bw. The lowest benchmark doses for effects on hearing thresholds were 50 mg/kg bw, i.e. thyroid effects may be considered critical among the reported endpoints. (Lilienthal et al. 2011).

Xiao et al. (2010) exposed pregnant SD rats by gavage to PCB 153 from PND 3 to PND 7 at doses of 0, 0.025, and 2.5 mg/kg bw/day (total doses 0.125 and 12.5 mg/kg bw). T4 was decreased on PND 8 in both exposure groups, yielding a LOAEL for thyroid effects of 0.025 mg/kg bw/day (total dose 0.125 mg/kg bw). The dose levels reported to cause effects in this study were very low compared to a study by Kobayashi et al. (2008). Here pregnant SD rats were orally exposed to PCB 153 at 0, 16 or 64 mg/kg bw/day from GD 10 to 16, and a significant decrease in T4 and T3 was observed at 64 mg/kg bw/day (total dose of 448 mg/kg bw), i.e. the NOAEL was 16 mg/kg bw/day (total dose 112 mg/kg bw).
This low-dose effect on the thyroid hormone level reported by Xiao et al. (2010) needs to be confirmed by others before it can be considered for use in the risk assessment of PCB 153.

A number of animal studies have also evaluated the effects of different mixtures of PCBs on thyroid function. A mixture of PCB 77, 126, 105, 118, 138 and 153 was administered to pregnant SD rats from GD 6 to 16 at mixture doses of 2.46 and 4 mg/kg bw/day. Thyroid hormone levels were reduced at GD 16 in both dose groups (Gauger et al. 2007). In another study female rat dams were exposed to a mixture of four Aroclors (called the Fox River Mix) at the following doses 0.51, 1.5, 3, 6, 12 and 18 mg/kg bw/day from gestational day 6 through postnatal day 21, and T4 levels in the male offspring were examined. Dose dependant T4 reductions were seen with a NOAEL of 1.5 mg/kg bw/day and a LOAEL of 3 mg/kg/day (Miller et al. 2012). Pereira et al. (2007) treated male and female Wistar rats with 2.85 mg Clophen A60/kg bw/day from 100 days before mating and until PND 21. The F1 generation was further treated for 150 days after weaning with 1.43 mg/kg bw/day. Thyroid histology revealed

changes in parental and F1 animals indicating impaired thyroid function (Pereira et al. 2007).

Most mixture studies have been performed using Aroclor 1254, and in the open literature studies show that thyroid effects in rats start after doses of around 1 mg/kg bw/day, depending on the experimental setup. Bansal et al. (2005) exposed pregnant SD rats to Aroclor 1254 from GD 6 to GD 16 at doses of 0, 1, or 4 mg/kg bw/day. Dam T4 levels were reduced at both doses though this was only statistically significant at 4 mg/kg bw/day (Bansal et al. 2005). In papers by Gauger et al. (2004) and Zoeller et al. (2000), the reported results are probably from the same study, as T4 levels are exactly the same. However, the study by Gauger 2004 also shows significant effect on T3 at 1 mg/kg bw/day, i.e. a LOAEL of 1 mg/kg bw/day. In the Zoeller et al. study a LOAEL of 1 mg/kg bw/day for dam T4 reduction following exposure GD 6 to GD 16 was seen.

More recent studies from the open literature have shown that when pregnant SD rats were exposed from GD 7 to PND 15 to 5 mg/kg bw/day of Aroclor 1254 (total dose 140 mg/kg bw) (with or without co-treatment with methimazole to induce hypothyroidism), T4 levels were reduced in pups at PND 15. However, the other effects of PCBs were somewhat different than those of methimazole (Bansal et al. 2008).

In a study by Yang et al. (2009) pregnant LE rats were exposed to 1 or 6 mg/kg bw/day of aroclor 1254 from 2 weeks before breeding and until PND 21 (56 days). Significant decreases in T3 and T4 levels were seen in both dose groups at PND 21. Structural changes in the brain and subtle deficits in learning and memory were observed, and a LOAEL of 1 mg/kg bw/day (56 days) for thyroid effects was seen (Yang et al. 2009).

Combinations of five PCB congeners (105, 118, 126, 138, 153) with or without actions on the thyroid receptor were recently examined *in vivo* by Giera et al. (2011) and compared to effects of Aroclor 1254. Pregnant SD rats were exposed from GD 6 to PND 16 to chemical mixtures (PCB mixture doses of 0.5 to 1 mg/kg bw/day; Aroclor mixture of 5 mg/kg/day. Thyorid hormone levels and thyroid hormone target genes were examined in different tissues. The Aroclor mixture and mixtures of PCBs reduced T4 levels on PND 15 (Giera et al. 2011).

The NOAEL of the commercial PCB mixture Aroclor 1254 for effects on the thyroids in monkeys was 0.08 mg/kg bw/day administered in the diet for 72 months. The critical effect was increased liver weight.

For NDL-PCBs, the lowest NOAELs for thyroid effects were obtained in 90-day studies in rats showing effects at approximately 340-420 µg/kg bw/day (LOAEL) of PCB 28, 128 or PCB 153. This results in body burdens from about 4000 µg/kg bw (PCB 28) to 14,000 µg/kg bw (PCB 128). The NOAELs are 34, 42, or 36 µg/kg bw/day for PCB 28, 128 and 153, respectively. In the 2-year long-term toxicity and carcinogenicity study on PCB 153 performed by the NTP (2006) the NOAEL for effects on thyroid hormones was 70 µg/kg bw/day (body burden 16,000 µg/kg bw) and the LOAEL was 210 µg/kg bw/day (body burden 52,000 µg/kg bw).

In Appendix 3 is given a tabular overview of the NOAELs and LOAELs found in a wide range of animal studies examining the effects on the thyroid system in rats exposed to different NDL-PCB congeners and Aroclors and other PCB mixtures. It appears that in the reproductive and developmental studies on Aroclors with exposures during gestation and/or lactation effects on thyroid hormones are seen at doses from 1000 µg/kg bw/day. This is generally lower than the LOAEL doses of single NDL PCB congeners. Only few studies were designed to establish NOAELs for effects on thyroid hormone levels.

Yang et al. (2010) suggested a scheme to calculate TEF values for PCBs based on thyroid effects (TEF_{TH}) in an approach including both dioxin-like and non-dioxin-like PCBs. TEF calculations are based on an *in vivo* effect, i.e. reduction of serum T4, and may therefore cover a number of different mechanisms of actions leading to that effect. For dioxin-like PCBs, the authors considered that TEFs for thyroid effects are comparable to WHO-TEFs. In addition, this approach includes TEFs for some non-dioxin-like PCBs. The selected TEFs are mainly based on a study by Crofton et al. 2005, who compared the effects on T4 levels in rats of 12 different PCBs as well as 2,3,7,8-TCDD. The authors find that this new TEF_{TH} method takes a step forward to address the deficiency in the current methods for assessing risk of PCBs which only account for DL-PCBs.

However, the potencies for thyroid effects of NDL-PCBs are relatively low compared to the potencies for thyroid effects of DL-PCBs according to this paper, i.e. higher doses of NDL-PCBs are required to see effects (Yang et al. 2010). To use this TEF_{TH} method for risk assessment a 2,3,7,8-TCDD-based NOAEL for thyroid effect would need to be determined. Studies in rats have indicated that thyroid effects are seen at 3-fold higher doses (body burdens) than those causing reproductive effects. Thus, the LOAEL for effects on the thyroids in the most recent long-term toxicity and carcinogenicity study on 2,3,7,8-TCDD in female rats was 22 ng/kg bw five days per week for two years, resulting in an estimated body burden of 140 ng/kg bw (NTP 2006), whereas the lowest LOAEL body burden in rat dams for effects on the reproductive organs the in male offspring was estimated at 40 ng/kg bw (Faqi et al. 1998, Mably et al. 1992, Hurst et al. 2000a, 2000b, Gray et al. 1997a, 1997b – as evaluated by SCF 2000, 2001).

Overall, the finding of developmental effects of Aroclor mixtures at lower dose levels than those inducing thyroid effects also indicates that studies on developmental effects rather than thyroid effects should be considered for risk assessment of complex PCB mixtures.

4.3.2.5 Effects on the adrenal glands

Other effects of PCBs on endocrine function observed in experimental animals include effects on the adrenal glands and serum adrenal steroid levels.

In rats, alterations in hormone levels have been observed following dietary administration of Aroclor 1254 at 0.1 mg/kg bw/day for 15 weeks, and from 0.25 mg/kg bw/day, but not at 0.05 mg/kg bw/day for 5 months. No histological alterations in the adrenals of rats were observed at dose levels of up to 25 mg/kg bw/day Aroclor 1254 by gavage for 15 weeks (ATSDR 2000).

In the comparative 13-week oral toxicity studies in rats with individual PCB congeners, no histopathological alterations in the adrenal glands were observed; doses ranged from 0.01 µg/kg bw/day for the dioxin-like PCB 126 to approximately 4 mg/kg bw/day for some non-dioxin-like congeners (ATSDR 2000).

In monkeys, no effects on the adrenal tissue were observed following dietary administration of Aroclor 1254 at dose levels of up to 0.2 mg/kg bw/day for 12 months, or up to 0.08 mg/kg bw/day for 72 months; and no effects on serum hormone levels at dose levels of up to 0.08 mg/kg bw/day for up to 22 months (ATSDR 2000).

The NOAEL of the commercial PCB mixture Aroclor 1254 for effects on the adrenals in monkeys was 0.08 mg/kg bw/day administered in the diet for72 months. The NOAEL for effect on the level of serum adrenal steroids in rats was 0.05 mg Aroclor 1254/kg bw/day administered in the diet for 5 months, whereas the LOAEL was 0.1 mg Aroclor 1254/kg bw/day administered in the diet for 5 weeks.

4.3.2.6 Pulmonary effects

No histological alterations were seen in the lungs of rats administered a single 4,000 mg/kg dose of Aroclor 1242 by gavage and evaluated 24 hours posttreatment or in rats treated with 100 mg/kg/day Aroclor 1242 by gavage every other day for 3 weeks (Bruckner et al. 1973 – as cited by ATSDR 2000).

Mice fed a diet that provided .22 mg Aroclor/kg/day for 6 weeks had no changes in lung weight or histology (Loose et al. 1978a, 1978b – as cited by ATSDR 2000).

Lung inflammation was observed in rats that died following dietary exposure to Phenoclor DP6 at .25 mg/kg/day for 8 days or .50 mg/kg/day for 6 days (Narbonne et al. 1978 – as cited by (ATSDR 2000).

Other respiratory end points were not examined in these studies.

No histopathologic changes were observed in the trachea or lungs of male or female rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at intake levels of 8.0-11.2, 4.0-5.7, 4.3-6.1, or 4.1-5.8 mg/kg/day, respectively (Mayes et al. 1998).

In the comparative 13-week oral toxicity studies in rats with individual congeners, no histopathological alterations in the lungs were observed; doses ranged from 0.01 µg/kg bw/day for the dioxin-like PCB 126 to approximately 4 mg/kg bw/day for some non-dioxin-like congeners (ATSDR 2000).

Rhesus monkeys receiving daily doses of 0.005, 0.020, 0.040, or 0.080 mg/kg/day Aroclor 1254

for 72 months showed no effects on lung tissue (Arnold et al. 1997 – as cited by ATSDR 2000).

4.3.2.7 Dermal effects

Dermal effects including facial oedema, acne, folliculitis, and alopecia have been observed in monkeys exposed to 0.1 mg/kg bw/day Aroclor 1248 or 0.12 mg/kg bw/day Aroclor 1242 for 2 months. Chronic dietary treatment with 0.1 mg/kg bw/day Aroclor 1248 for 12 months, or 0.2 mg/kg bw/day Aroclor 1254 for 12-28 months produced progressive dermal effects in monkeys including alopecia, facial oedema, acne, fingernail loss, and gingival hyperplasia and necrosis of varying severity. Fingernail and toenail changes have been observed in monkeys following administration of 0.005 mg/kg bw/day Aroclor 1254 for 37 months, or 0.04 mg/kg bw/day for 72 months (ATSDR 2000).

In the comparative 13-week oral toxicity studies in rats with individual congeners, no histopathological alterations in the skin were observed. Doses ranged from 0.01 µg/kg bw/day for the dioxin-like PCB 126 to approximately 4 mg/kg bw/day for some non-dioxin-like congeners (ATSDR 2000).

LOAEL for effects of commercial PCB mixtures (Aroclor 1254) on skin and eyes was 0,005 mg/kg bw/day in monkeys for 35 months. The effects on skin and eyes have only been observed in monkeys (and humans) and are specific for PCDDs, PCDFs and DL-PCBs (ATSDR 2000).

4.3.2.8 Ocular effects

Ocular effects including swelling and reddening of the eyelid and eyelid discharge have been observed in monkeys exposed to 0.1 mg/kg bw/day Aroclor 1248 or 0.12 mg/kg bw/day Aroclor 1242 for 2 months. Monkeys exposed to 0.005-0.08 mg/kg bw/day Aroclor 1254 for 37 months showed ocular effects including eye exudates and inflammation and/or prominence of the tarsal glands. Conjunctivitis was observed in Rhesus monkeys following dietary administration of 0.2 mg/kg bw/day Aroclor 1254 for 12 months (ATSDR 2000).

No histopathological changes were observed in the eyes of rats administered Aroclor 1016, 1242, 1254, or 1260 in the diet for 24 months at dose levels from about 4 to about 11 mg/kg bw/day (ATSDR 2000).

In the comparative 13-week oral toxicity studies in rats with individual congeners, no histopathological alterations in the eye or optic nerve were observed. Doses ranged from 0.01 µg/kg bw/day for the dioxin-like PCB 126 to approximately 4 mg/kg bw/day for some non-dioxin-like congeners (ATSDR 2000).

4.3.2.9 Haematological effects

Anaemia, manifested by decreased haemoglobin content, decreased haematocrit and hypocellularity of erythrocytic and other precursor cells in the bone marrow, has been observed in monkeys treated with Aroclor 1248 or 1254 at dose levels from 4 mg/kg bw/day for 2 months, or from 0.2 mg/kg bw/day for 12-28 months. In one study, haematological changes consistent with a picture of anaemia have been observed in monkeys treated with 0.08 mg/kg bw/day of Aroclor 1254 for 37 months; however, in another study, no effects on haematological parameters were observed in monkeys receiving Aroclor 1254 from 0.08 mg/kg bw/day for 72 months (ATSDR 2000).

Red blood cell count and haemoglobin concentration were reduced in female rats that were fed Aroclor 1016 or 1260 for 24 months from dose levels of 2.7 or 1.4 mg/kg bw/day, respectively, whereas no haematological effects were observed in female rats that were similarly exposed to Aroclor 1242 from 5.7 mg/kg bw/day or Aroclor 1254 from 6.1 mg/kg bw/day; or in male rats exposed to Aroclor 1016, 1242, 1254, or 1260 at dose levels from 8.0, 5.7, 8.1, or 4.1 mg/kg bw/day, respectively (Mayes et al. 1998).

In the comparative 13-week oral toxicity studies in rats with individual congeners, decreases in several haematological parameters were observed for PCB 105 at about 4 mg/kg bw/day, and for PCB 126 at about 0.74 µg/kg bw/day, but not for the other congeners (ATSDR 2000).

NOAEL for haematological effects of commercial PCB mixtures (Aroclor 1254) was 0.08 mg/kg bw/day in monkeys for 72 months. The LOAEL was 0.2 mg/kg bw/day for 12-28 months.

4.3.2.10 Effects on the immune system

Immunotoxicity of PCBs in animals has been documented in various species that were orally exposed via commercial mixtures and single congeners. Morphological and functional alterations observed in the immune system of rats,

mice, guinea pigs, rabbits, and monkeys include thymic and splenic atrophy, reduced antibody production against foreign antigens, increased susceptibility to infections by viruses and other microbes, reduced skin reaction to tuberculin, and increased proliferation of splenic lymphocytes in response to mitogenic stimulation. The available data indicate that the immune system of monkeys is more sensitive to PCBs than that of the other species, and reduced IgM and IgG antibody responses to sheep red blood cells (SRBC) are the parameters most consistently affected by PCBs in monkeys (ATSDR 2000).

In a comprehensive comparative 24-months oral toxicity study in rats, no changes in white blood cell counts or histology of the thymus, spleen or lymph nodes were observed following dietary administration of Aroclor 1016, 1242, 1254, or 1260 at dose levels up to about 4-8 and 6-11 mg/kg bw/day in males and females, respectively (Mayes et al. 1998).

In the comparative 13-week oral toxicity studies in rats with individual congeners, histopathological lesions were observed in the thymus with PCB 126 (LOAEL of 0.00074 mg/kg bw/day) and with PCB 28, 105 and 153 (LOAEL of 4 mg/kg bw/day). No effects were observed on the spleen, lymph nodes and bone marrow, or on white blood cell count with these four congeners, and no changes in the immunological endpoints were induced by PCB 77, 118, or 128 (ATSDR 2000).

In monkeys, decreased antibody response to SRBC, increased susceptibility to bacterial infections, and/or histopathological changes in the thymus, spleen, and lymph nodes have been observed at dose levels from 0.1 to 0.3 mg/kg bw/day Aroclor 1248 and 1254 from 238-267 days and up to about 28 months. In the most comprehensive study in monkeys, Rhesus monkeys administered Aroclor 1254 orally in capsules showed significant dose-related decreases in IgM and IgG antibody titers to SRBC at dose levels from 0.005 mg/kg bw/day (the lowest dose level tested) after 23 months, and alterations in lymphocyte T-cell subsets at 0.08 mg/kg bw/day (ATSDR 2000).

In the study on infant monkeys treated with the defined PCB mixture (0.0075 mg/kg bw/day) analogous to that in human milk, anti-SRBC titers were reduced (not significantly) in treated animals (ATSDR 2000).

Thymic atrophy and suppression of humoral immunity occur at doses causing other overt signs of toxicity in multiple animal species. 2,3,7,8-TetraCDD has caused suppression of cell-mediated immunity after exposure to doses as low as 10 ng/kg bw in rats. The most sensitive immunological effect reported in mice is enhanced mortality due to influenza after exposure to a single dose of 10 ng/kg bw. However, no dose-response relationship existed. In studies in monkeys, alterations in the ratio of different subsets of T-lymphocytes have been observed at the same low doses. However, some doses lead to increases and other to decreases of a certain subset of T-cells with no clear pattern. Neonates and young animals are much more sensitive than adults to most of the immunological responses (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

The LOAEL for commercial PCB mixtures (Aroclor 1254) for effects on the immune system was 0,005 mg/kg bw/day. The effects on the immune system are considered to be dioxin-like effects.

4.3.2.11 Effects on the nervous system

Adult animals exposed to relatively high 2,3,7,8-tetraCDD doses exhibit behavioural signs indicative of effects on the central nervous system. Minor changes in the brain neurotransmitter system, a slowing of sensory and motor conduction velocities as well as a progressive neuropathy have been reported in rats. (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

LOEL for commercial PCB mixtures (Aroclor 1016 and 1260) on neurotransmitter levels in the brain of monkeys was 0,8 mg/kg bw/day administered in the diet for 20 weeks (ATSDR 2000).

Neurobehavioural alterations (effects on motor activity and effects on higher cognitive functions, i.e. learning, memory and attention) have been observed in rats and monkeys following pre- and/or postnatal exposure to PCBs in commercial mixtures, defined experimental congener mixtures, single congeners, and contaminated fish. Both dioxin-like and non-dioxin-like PCB congeners have been shown to induce neurobehavioural alterations in animals. It appears that *ortho*-substituted congeners are more active than coplanar PCBs in modifying cognitive processes (ATSDR 2000).

In the study on infant monkeys treated with the defined PCB mixture (0.0075 mg/kg bw/day) analogous to that in human milk, learning deficits (impaired performance in both nonspatial and spatial discrimination reversal tasks) and inability to inhibit inappropriate responding were reported when testing were performed at an age of 3 years (ATSDR 2000).

Changes in levels of neurotransmitters in various brain areas have also been observed in monkeys, rats and mice and the most consistent result is a decrease in dopamine concentrations in different areas of the brain. Decreased dopamine concentrations have been observed in adult male rats following dietary administration of Aroclor 1254 at dose levels from 39 mg/kg bw/day for 30 days and in monkeys receiving Aroclor 1016 or 1260 at dose levels from 0.8 mg/kg bw/day in the diet for 20 weeks (ATSDR 2000).

Coburn et al. (2005 – as cited in ATSDR 2011) assessed the impact of PCBs on brain mechanisms of body fluid regulation, both in central and systemic vasopressin (VPs) release in response to acute dehydration after oral exposure to Aroclor 1254 in adult male rats. Central vasopressin release from magnocellular neuroendocrine cells in the supraoptic nucleus (SON) occurs within several hours after acute dehydration, and is an important autoregulatory mechanism. SON prepared from dehydrated PCB-exposed rats released significantly more VP than did SON from control rats. In contrast, while PCB exposure had no effect on baseline water intake, weight gain, or plasma osmolality responses to dehydration in PCB-fed rats, the SON failed to respond with increased VP release during dehydration. Dehydrated PCB-fed rats had an exaggerated increase in plasma VP. This indicates a limited inhibitory effect of central VP on plasma VP output.

Rat pups were exposed to Aroclor 1254 from conception to age of 16, 30, and 60 days. Results from morphological analyses of brain tissue confirmed that, in continuously PCB-treated rats at doses of 125 ppm, the relative size of the intra- and infra-pyramidal (II-P) mossy fiber was smaller than in control rats in all ages tested. Furthermore, this reduction in growth was selective for the II-P mossy fibers (Pruitt et al. 1999).

Dziennis et al. (2008 – as cited by ATSDR 2011) exposed rats to Aroclor 1254 (A1254) at 0.1 or 1 mg/kg/day in the maternal diet throughout gestation and lactation. Focal cerebral ischemia was induced at 6–8 weeks of age via middle cerebral artery occlusion, and infarct size was measured in the cerebral cortex and striatum at 22 hr of reperfusion. Exposure during the development period resulted in significantly decreased striatal infarct in females and males at 0.1 and 1 mg/kg/day, respectively. Effects of A1254 exposure during development on Bcl2 and Cyp2C11 expression did not correlate with effects on infarct volume.

In the comparative 13-week oral toxicity studies in rats with individual congeners, decreased dopamine concentrations were observed for PCB 105 at about 4 mg/kg bw/day, for PCB 118 at 0.2 mg/kg bw/day, and for PCB 153 at 0.34 mg/kg bw/day; no biologically relevant changes were observed for the other congeners (ATSDR 2000).

Cromwell et al. (2007 – as cited by ATSDR 2011) examined the impact of PCBs on maternal odor conditioning in rat pups 12-14 days of age. PCB-77 exposure changed aspects of maternal-offspring interaction in rodents. The results suggest that exposure to PCBs decreases the preference for the maternal-associated cue but did not impair discrimination for a novel odor. Pups exposed to perinatal PCBs did not remain in the cue-associated location longer relative to the non-cue-related location and for the lower maternal dose of PCB (12.5 ppm). The pups actually spent significantly longer time in the non-cue location. These shifts in maternal cue preference were observed without significant changes in body weight, feeding, or olfactory function. Similarly, Cumming et al. (2005 – as cited by ATSDR 2011) reported that behavioral changes discerned through use of a cross-fostering paradigm suggest that changes in maternal behavior are likely to emerge from direct effects of PCB-77 on the dams as well as in response to effects of the PCB on the litter.

Pregnant rats were exposed from GD 6 to PND 18 to 2 mg/kg bw/day of PCB 77 (total dose 26 mg/kg bw). On the day of birth offspring was cross-fostered or left with their own mothers to examine the influence of prenatal versus postnatal exposure. Prenatal exposure alone did not affect partner preference but postnatal exposure did alter female partner preference (Cummings et al. 2008 – as cited by ATSDR 2011).

Exposure to PCB-77 during gestation and lactation can have a significant effect on the maternal behavior of rat dams, as reported by Simmons et al. (2005 – as cited by ATSDR 2011). Exposure to 2 and 4 mg/kg bw of PCB-77 during GD 6–18 reduced the amount of nursing time in which the dams displayed high-crouch posture over postnatal days 1–6. The amount of maternal licking and grooming of the litters, the amount of time the dams spent on the nest, and pup mortality were increased at the high dose. At both the lower and the higher doses, the weight gain of the litters during the first 6 days of life was reduced.

Developmental neurochemical studies and prepubertal uterotrophic studies were performed for 4 PCBs in SD rats. For developmental studies on PCB 77 dams were orally exposed to PCB 77 (TCB) at 0.1 or 1 mg/kg bw/day from GD 6 to PND 21 (36 doses). Elevated dopamine levels were observed in cortex of rats from the highest dose group at age 35 and 60 days and in both dose groups at 90 days. LOAEL 0.1 mg/kg bw/day. For developmental studies on PCB 126 dams were orally exposed to PCB 126 (PtCB) at 0.25 or 1 ug/kg bw/day from GD 6 to PND 21 (36 doses). Elevated dopamine levels were observed in cortex of rats from the highest dose group at age 35 and 90 days. NOAEL 0.25 µg/kg bw/day, LOAEL 1 µg/kg bw/day (Seegal et al. 2005).

Orito et al. (2007 as cited by ATSDR 2011) exposed female rat dams orally at GD15 to 30 µg/kg bw/day of PCB-126 in corn oil. At 4-5 weeks of age, male offspring were assessed by use of an open field test. Intrauterine exposure to PCBs resulted in a reduction in time spent in the center of an open field, a reduction in the number of rearings, and an extension of grooming duration. Interaction behaviour, which is an index of anxiety level, was shortened in social interaction. The results suggest that exposure to PCBs may exert anxiogenic behaviour in rats.

Purified PCBs 52, 138 and 180 were administered in a sweet jelly bit to pregnant Wistar rats from GD 7 to PND 21 at a dose of 1 mg/kg bw/day. Behavioural studies were performed on offspring and learning ability was found to be impaired by PCB 138 and 180, but not PCB 52. PCB 52 impaired motor coordination. Differences between the congeners were thus observed and were consistent with reduced amounts of NMDA receptors in cerebellum of pups exposed to PCB 138 and 180 but not PCB 52. In contrast, PCB 52 increased GABA (Boix et al. 2010).

4.3.2.12 Cardiovascular effects

Data on the cardiovascular toxicity of PCBs in animals are limited to several oral exposure studies conducting histological examinations of the heart and blood vessels. Pericardial oedema occurred in four of six monkeys given 12 mg/kg/day Aroclor 1248 in the diet for 3 months (Allen et al. 1973 – as cited by ATSDR 2000). However, Rhesus monkeys receiving daily doses of 0.005, 0.020, 0.040, or 0.080 mg/kg/day Aroclor 1254 for 25 months showed no effects on cardiac tissue (Arnold et al. 1997 – as cited by ATSDR 2000).

Histological examination of the heart was normal in rats evaluated 24 hours following a single 4,000 mg/kg dose of Aroclor 1242 or 100 mg/kg/day Aroclor 1242 every other day for 3 weeks administered by gavage (Bruckner et al. 1973 – as cited by ATSDR 2000).

No histopathologic changes were observed in the heart of male or female rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at dose levels of 8.0-11.2, 4.0-5.7, 4.3-6.1, or 4.1-5.8 mg/kg/day, respectively (Mayes et al. 1998).

In the comparative 13-week oral toxicity studies in rats with individual congeners, no histopathological alterations in the heart or thoracic aorta were observed. Doses ranged from 0.01 µg/kg bw/day for the dioxin-like PCB 126 to approximately 4 mg/kg bw/day for some non-dioxin-like congeners (ATSDR 2000).

Chronic exposure to 1000 ng PCB-126/kg bw/day by gavage 5 days per week for two years to Sprague-Dawley rats increased the incidence of spontaneous cardiomyopathy and arthritis of the coronary vessel (Jokinen et al. 2003 – as cited by ATSDR 2011). Female rats treated with a total dose of 224 µg PCB-126/kg bw by five intraperitoneal injections once every second week had raised levels of serum cholesterol, increased blood pressure, and increased myocardial mass. The first two doses were 64 µg/kg bw each and the remaining three doses were 32 g/kg bw each (Lind et. Al., 2004 – as cited by ATSDR 2000).

4.4 Toxicity to reproduction

Information on reproductive and developmental effects in experimental animals due to oral intake of PCBs is available from studies of commercial PCB mixtures, of a defined PCB mixture, and of individual congeners.

PCDDs, PCDFs and PCBs are developmental and reproductive toxicants in experimental animals. Most perturbations of the reproductive system in adult animals require overtly toxic doses. In contrast, effects on the developing organism occur at doses more than 100 times lower than those required in the mother. Adverse developmental effects observed after dioxin-like compounds include structural malformations (cleft palate, hydronephrosis), accelerated tooth eruption and impairment of dentin and enamel formation, growth retardation, gastrointestinal haemorrhage and oedema. Sensitive targets include the developing reproductive, nervous and immune systems. Reproductive effects include delayed puberty, altered mating behaviour, decreased sperm count, and genital malformations. Effects on the nervous system include hearing deficits, changes in locomotor activity and rearing behaviour, depression of core body temperature and deficits in object learning. Immuntoxic effects include thymic and splenic atrophy, changes in cell surface markers and suppression of delayed type hypersensitivity. Perturbations of multiple hormonal systems may play a role in these events (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

4.4.1 Reproductive effects

Reproductive effects in female animals administered commercial PCB mixtures have been observed in various species, including rats (prolonged oestrus, decreased sexual receptivity, and reduced implantation rate in adults and/or their offspring exposed via gestation and lactation), mice (decreased conception), and monkeys (prolonged menstruation, decreased fertility). Monkeys appear to be particularly sensitive to the reproductive effects of PCBs. In adult male animals, a few studies have examined reproductive effects of PCBs showing changes in reproductive organ weights and histology and impaired semen quality (ATSDR 2000).

Here, studies on developmental effects of Aroclor mixtures are described followed by descriptions of studies on other mixtures and of single PCB congeners. In general, mainly studies using more than one dose group is applied whereas a number of studies using one dose group only have been reviewed but are not included here.

4.4.1.1 Fertility effects in adult animals

Increased menstrual duration (5-7 days) and bleeding were observed in Rhesus monkeys exposed to 0.1 mg/kg bw/day Aroclor 1248 in the diet from 7 months prior to breeding and throughout pregnancy, and conception rate was decreased at 0.2 mg/kg bw/day; resorptions or abortions occurred at both dose levels. Similar effects occurred in Rhesus monkeys that were mated after 38 weeks of dietary exposure to 0.2 mg/kg bw/day Aroclor 1248 (ATSDR 2000).

Reduced conception rates and increased incidences of abortions, resorption, or stillbirths were observed in Rhesus monkeys that were fed encapsulated Aroclor 1254 at dose levels of 0.02 to 0.08 mg/kg bw/day for 37 months before breeding and subsequently throughout mating and gestation until the breeding phase of the study (29 months) was completed; there were no clear effects on reproduction at 0.005 mg/kg bw/day (the lowest dose level in the study) (ATSDR 2000).

One of four male monkeys that were fed 0.1 mg/kg bw/day Aroclor 1248 for 17 months developed decreased libido and absence of mature spermatozoa after the first year of exposure while no effects were observed in the other 3 animals (ATSDR 2000).

In the above mentioned studies, the NOAEL for fertility effects of commercial PCB mixtures (Aroclor 1254) in monkeys was 0.005 mg/kg bw/day for 37 months before mating and during the pregnancy. The LOAEL was 0.02 mg/kg bw/day.

Male weanling rats that were treated with 25 mg/kg bw/day Aroclor 1254 by gavage for 15 weeks had significant reductions in seminal vesicle and epididymal weights, and epididymal sperm counts. These effects were not observed at lower dose levels of 0.1 to 10 mg/kg bw/day, and there were no changes in other testicular end-points, including sperm count and motility, testicular weight, and serum levels of testosterone. None of the studies

in adult rats have evaluated reproductive capability (Gray et al. 1993 – as cited by ATSDR 2000).

Adult male SD rats were exposed intraperitoneally to 2 mg/kg bw/day of aroclor 1254 for eight weeks (alone or in combination with either lycopene or ellagic acid). Body weight, epididymal sperm concentration, and testicular superoxide dismutase activity were decreased, and histological changes were observed in testes. Co-exposure to lycopene or ellagic acid partly counteracted the effects of aroclor (Atessahin et al. 2010).

Adult male Wistar rats were exposed intraperitoneally to aroclor 1254 (0, 0.75, 1.5, 3 mg/kg bw/day) for 20 days. Decreased body weight, testes weight, epididymal and relative epididymal weights and decreased activities of testicular mitochondrial CAT, GPx and GR were observed at 1.5 and 3 mg/kgbw/day. SOD activity decreased at 1.5 mg/kg bw/day. GSH and Vit C decreased at all doses. The authors conclude that induction of oxidative stress in testicular mitochondria may result in impaired spermatogenesis (Aly et al. 2009).

Young male C57 mice were gavaged with aroclor 1254 every 3 days for a total of 50 days (not clear if 17 or 50 exposures were applied) at low doses of 0.5, 5, 50 and 500 ug/kg bw/day. Sperm count was reduced and sperm mortality was increased dose-dependently in all doses. At the three highest doses, the number of abnormal sperm was increased. Testicular histology showed degenerative changes at 500 ug/kg bw/day and a decreased number of proliferating cells (PCNA staining) and an elevated number of apoptotic cells (TUNEL staining). No significant changes of serum testosterone levels were observed whereas 17beta estradiol levels were decreased dose dependently and this was statistically significant in the highest dose group only (Cai et al. 2011).

The findings in the study by Cai et al. (2011) may indicate low-dose effects of the Aroclor mixture. However, the dose levels applied are considerably lower than those being without effect in comparable rat studies (Gray et al. 1993, Aly et al. 2009) or in the monkey study described above. It is possible that mice are more sensitive than rats or monkeys to effects on sperm quality; however, the finding needs to be substantiated before it can be used for risk assessment of PCBs. In this connection, it is noteworthy that the US EPA, in its recent re-evaluation of 2,3,7,8-TCDD discharge results from mouse studies (although some of them show very sentitive effects) and states that EPA has less confidence in the reference dose (RfD) estimates based on mouse data than those based on either the rat or human data (US EPA 2012).

Overall, the NOAEL of 0,005 mg/kg bw/day for effects of Aroclor 1254 in monkeys can be considered relevant for effects of commercial PCB mixtures on fertility of adult animals.

A series of toxicity studies has been performed in rats, which were given diets containing four dose levels of 7 individual PCB congeners (28, 77, 105, 118, 126, 128, 153) for 13 weeks. The congeners were selected based on frequent occurrence in environmental samples and human tissues, or toxic potency. Mild changes were observed in the ovaries of female rats exposed to PCB 126 at about 0.009 mg/kg bw/day, but not at about 0.0008 mg/kg bw/day; no effects were observed in male reproductive tissues at about 0.0007 mg/kg bw/day. No effects in reproductive tissues were found in males and females following exposure to PCB 28 at about 4 mg/kg bw/day, PCB 77 at about 0.8 mg/kg bw/day, PCB 105 at about 4 mg/kg bw/day, PCB 118 at about 0.7 or about 0.2 mg/kg bw/day (males and females, respectively), PCB 128 at about 4 mg/kg bw/day, or PCB 153 at about 4 mg/kg bw/day (ATSDR 2000).

Uterotrophic studies were performed for four PCBs: PCB 47, 77, 126 and 169. For PCB 47, prepubertal female rats were exposed ip to 8, 16 or 32 mg/kg bw/day of PCB 47 for 2 days (total dose 16, 32, 64 mg/kg). No uterotrophic effect was observed for PCB 47. For PCB 77, prepubertal female rats were exposed ip to 3, 9 or 27 mg/kg bw/day of PCB 77 for 2 days (total dose 6, 18 or 54 mg/kg). A NOAEL of 6 or 18 mg/kg total dose was observed ($P<0.1$ at dose 9, dose response curve), LOAEL 18 or 54 mg/kg total dose. For PCB 126, prepubertal female rats were exposed ip to 4, 16, 64, 100, 200, 300 or 400 ug/kg bw/day of PCB 126 for 2 days (total dose 8, 32, 128, 200, 400, 600, 800 ug/kg). A NOAEL of 8 or 128 ug/kg total dose was observed (uneven dose response curve), LOAEL 32 or 200 ug/kg total dose for uterotrophic effect. For PCB 169, prepubertal female rats were exposed ip to 200, 400 or 800 ug/kg bw/day of PCB 169 for 2 days (total dose 400, 800 or 1600 ug/kg). No

uterotrophic effects were seen for PCB 169. (Seegal et al. 2005).

An overall evaluation of the above studies leads to the conclusion that a NOAEL for fertility effects of commercial PCB mixtures (Aroclor 1254) can be set to 0.005 mg/kg bw/day with a LOAEL of 0.02 mg/kg bw/day based on effects in female monkeys exposed for 37 months before mating and during the pregnancy. These values are higher than the LOAEL of 0.005 mg/kg bw/day for developmental effects in the same study (see section 4.4.2).

4.4.1.2 Reproductive effects in offspring

Gupta (2000 – as cited by ATSDR 2011) investigated the fetal long-term effect on the reproductive parameters of male mice offspring of pregnant mice fed 50 µg/kg bw/day of Aroclor 1016 during GDs 16-18. The male offspring were examined at 3, 21, and 60 days after birth. The effects of PCB exposure were an increased anogenital distance, increased prostate size, and decreased epididymal weight, however, no effects were observed on testicular weight or size compared to control.

Other studies on perinatal exposure to Aroclor mixtures applied higher doses (above 1 mg/kg bw/day) and longer dosing periods (often GD 6 to PND 21), as described below.

Fertility was markedly reduced in male offspring of rats that were lactationally exposed to 8 mg/kg bw/day Aroclor 1254 whereas fertility was not impaired in the male offspring of rats that were administered 30 mg/kg bw/day of Aroclor 1221, 1242, or 1260 by gavage during gestation days 12 to 20 (ATSDR 2000).

Pregnant SD rats were gavaged with A1254 (0, 10, 50 mg/kgbw) from GD 8 to PND 21. Exposed offspring were mated with controls (males/females). Vaginal opening and time of first estrous was delayed and the presence of irregular estrous cycles was increased. Reproductive function of males and female offspring was impaired at the high dose. KAP3 gene expression in hypothalamus was reduced at GD 18, PD5 and PD 28 (Lee et al. 2007).

Pregnant LE rats were exposed to 1 or 6 mg/kg bw/day of aroclor 1254 from 2 weeks before breeding and until PND 21 (56 days). Significant decreases in T3 and T4 levels were seen in both dose groups at PND 21. Vaginal opening was delayed at the highest dose of aroclor 1254. Structural changes in brain and subtle deficits in learning and memory were observed. The LOAEL was 1 mg/kg bw/day (56 days) for thyroid effects (Yang et al. 2009).

Pregnant rats were exposed from two weeks before breeding to PND 21 to 0.1 or 1 mg/kg bw/day of aroclor 1254. The size of an induced brain infarct was measured and was decreased in size by both doses of aroclor 1254 (Dziennis et al. 2008).

When pregnant Sprague-Dawley rats were administered 0, 0.1, 1, or 10 mg/kg Aroclor 1221 (A1221) on GD 16 and 18, the litter sex ratio was skewed toward females in both the F1 and F2 generation. The F2 generation showed more profound alterations than F1, particularly with respect to fluctuations in hormones and reproductive tract tissues across the oestrous cycle.

An increased serum level of luteinizing hormone (LH) was found in F1 but not F2 animals at 1 but not 10 mg/kg of aroclor 1221. The authors describe reduced progesterone and LH levels in pro-oestrus of all exposed groups but this was only seen in the F2 animals and only when pooling the PCB groups. No changes in anogenital distance, no changes in ovary or uterus weights at different cycle stages were induced by PCBs and no changes in maturational in vivo markers were observed. The authors interpret the lower LH and progesterone levels in pro-oestrous as a block of the pre-ovulatory GnRH/LHsurge. They find that is associated with reduced ovarian and uterine weight at oestrus, but organ weight reductions are not statistically significant, although the expected peak at oestrus is absent in PCB exposed groups. This study shows subtle effects on the examined markers of female reproduction. A NOAEL cannot be determined as statistics appear to be based on the combined data for the PCB exposed groups versus controls. Validity of results should be further examined (Steinberg et al. 2008).

Female rat dams were exposed to a Fox River Mixture of different Aroclors at the following doses 0.51, 1.5, 3, 6, 12 and 18 mg/kg/day from gestational day 6 through postnatal day 21. Dose dependant T4 reductions were seen with a NOAEL of 1.5 mg/kg/day and a LOAEL of 3 mg/kg/day (Miller et al. 2012).

Pregnant rats were given a single dose of 375 µg of PCB-118/kg bw by gavage on GD 6. The offspring were reported to be hyperactive at PND 70-74. At adulthood (PND 170) the male offspring had smaller testes, epididymides, and seminal vesicles; and showed decreases in sperm and spermatid numbers; and impairment of daily sperm production (Kuriyama and Chahoud 2004).

Pregnant SD rats were exposed to 25 pg, 2.5 ng, 250 ng or 7.5 ug of PCB126/kg bw/day from GD 13 to 19. Spermatogenesis was affected in offspring at the 250 ng and the 7.5 ug dose groups at age 90 weeks (i.e. aging rats). This may be associated with accelerated spermatogenic senescence. This study is supported by similar findings in a later study by Wakui et al. (2010) using the same doses but with smaller intervals between doses (Wakui et al. 2007).

Pregnant SD rats were exposed intragastrically to 2.5, 25, or 250 ng/kg bw of PCB 126 on GD 13 to 19, i.e. 7 doses and a total dose of 17.5, 175 and 1750 ng/kg bw. Spermatid retention was observed in seminiferous tubules at age 7, 10, and 13 weeks in animals exposed to 250 ng/kg bw/day (i.e. 1750 ng/kg bw cumulated dose). Spermatid retention was not present at age 17 weeks. Epididymal sperm count was reduced at the highest dose at age 7, 10 and 13 weeks but not at age 17 weeks. A NOAEL of 175 ng/kg bw and a LOAEL of 1750 ng/kg bw can be determined (Wakui et al. 2010).

Female mink were exposed to 0.24, 2.4 or 24 ug/kg feed of PCB 126 (total exposure over 133 days in the low dose group: 4.8 ug/kg bw (0.036 ug/kg bw/day). Total exposure over 54 days in the middle and high dose group: 19 ug/kg bw (0.35 ug/kg bw/day) and 233 ug/kg bw (4.3 ug/kg bw/day), respectively) from 21 days before breeding until 6 weeks of age. Impaired reproductive performance was observed from 0.35 ug/kg bw/day of PCB 126 (total 19 ug/kg bw) (Beckett et al. 2008).

Overall, the doses of PCB mixtures inducing reproductive effects in offspring are higher than the doses inducing other developmental effects as described below.

4.4.2 Developmental effects

Developmental effects have been observed in experimental animals including rats (reduced growth, changes in the thyroid gland and thyroid hormones, neurobehavioural alterations, and changes in the reproductive system), mice (neurobehavioural alterations), and monkeys (reduced growth, neurobehavioural alterations, and changes in the immune system). In general, studies in rodents have used relatively high doses of PCBs. Studies in rodents with commercial PCB mixtures have shown that developmental toxicity can occur in the absence of overt signs of maternal toxicity and that PCBs are not teratogenic unless very high doses are used. The available studies suggest that primates are much more sensitive to the developmental effects than rodents (ATSDR 2000).

Here, studies on developmental effects of Aroclor mixtures are described followed by descriptions of studies on other mixtures and of single PCB congeners.

Reduced birth weight was observed in offspring from Rhesus monkeys treated with 0.03 mg/kg bw/day Arochlor 1016 in the diet for a total of 12 months (before mating and during gestation) but not at 0.007 mg/kg bw/day. At weaning, body weight in the high-dose group was still lower, but not significantly different, than in controls. Neurobehavioural alterations were observed at both dose levels. Both groups of neonates showed hyperpigmentation (ATSDR 2000).

In offspring from female Rhesus monkeys fed a diet that provided 0.1 or 0.2 mg/kg bw/day Aroclor 1248 for 15 months, mean birth weight was reduced in both groups and remained low for the next 12 weeks. At 2 months of age, the infants had signs of PCB intoxication (facial acne, swollen eyelids, loss of eyelashes, and hyperpigmentation of the skin) and three of six infants died between days 44 and 329. Pathological changes in lymphoid tissues (thymus, spleen, and bone marrow) were observed in deceased infants (ATSDR 2000).

Neurobehavioural alterations have been observed in monkeys born to mothers fed a diet providing approximately 0.1 mg/kg bw/day Aroclor 1248 for 16 to 21 months (feeding terminated at the end of 3 months of nursing), and in monkeys born to mothers fed 0.08 mg/kg bw/day for 18 months and allowed to breed 32 months post-exposure (ATSDR 2000).

Rhesus monkeys were fed encapsulated Aroclor 1254 at dose levels of 0.005, 0.02, 0.04 or 0.08 mg/kg bw/day for 37 months before breeding and

subsequently throughout mating and gestation until the breeding phase of the study (29 months) was completed. A significant increasing dose-related trend for foetal mortality incidence rates (combined foetal and postpartum deaths) was observed; a significant increase rate was noted for only the highest dose group (0.08 mg/kg bw/day). Mean birth weight was not significantly affected at any dose level. Clinical findings in the offspring included skin, nail, and gum lesions and were observed from 0.005 m/kg bw/day. Immunosuppresive effects (decreased IgM and IgG antibody titers to SRBC and decreased lymphocyte proliferation) were observed in the offspring and occurred from 0.005 m/kg bw/day (decreased IgM titers) (ATSDR 2000).

The LOAEL for developmental effects of commercial PCB mixtures (Aroclor 1254) in offspring of monkeys was 0.005 mg/kg bw/day administered to the mothers for 37 months before mating and for the following 29 months throughout pregnancy, birth and childhood. The effects were related to skin, nails and the immune system and considerd to be mainly dioxin-like effects.

Neurobehavioural alterations were reported in the offspring of rats treated with 2.4 mg/kg bw/day Clophen A30 premating and during gestation, and in offspring from rats fed approximately 1 mg/kg bw/day Aroclor 1254 during gestation and lactation, but not following treatment with 0.4 mg/kg bw/day Clophen 42. In a cross-fostering study, exposure *in utero* resulted in neurobehavioural alterations, whereas postnatal-only exposure resulted in no detectable behavioural changes (ATSDR 2000).

Depressed serum levels of T4 and T3 have been observed in pups born to female rats that were exposed orally to Aroclor 1254 from 0.1 mg/kg bw/day during gestation and lactation, and lesions in thyroid were observed from 2.5 mg/kg bw/day (ATSDR 2000).

In rats that were exposed to 4 mg/kg bw/day of a PCB congener mixture (simulating the congener content of human milk) from 50 days prior to mating until birth, pup body weight was significantly reduced at birth and on post-natal days 7, 14, and 21 (ATSDR 2000).

When a PCB mixture of congeneric composition similar to that found in Canadian beast milk was administered to Rhesus and Cynomolgus monkeys during the first 20 weeks of life (0.0075 mg/kg bw/day), no significant difference between the control and treated groups for body weight gain was observed throughout the study (ATSDR 2000).

Steinberg et al. (2007) exposed pregnant female rats by intraperitoneal injection to low levels of Aroclor 1221 (0, 0.1, 1, or 10 mg/kg) on embryonic day 16 of F1. The exposure of offspring to Aroclor 1221 resulted in a significant reduction in their mating trial pacing, vocalizations, ambulation, and the female's likelihood to mate. Similar results on the reproductive developmental effects of female rats by PCB 126 were reported by Shirota et al. (2006).

Crofton et al. (2000 – as cited by ATSDR 2011) compared the impact of prenatal versus postnatal exposure of rats to Aroclor 1254. In this study, primiparous rats received 0 or 6 mg/kg Aroclor 1254 (po in corn oil) from GD 6 to PND 21. On the day of birth, half of treated litters and half of the control litters were cross-fostered. As a result, the experiment consisted of the following groups: Ctrl/Ctrl, A1254/A1254 (perinatal exposure), A1254/Ctrl (prenatal exposure only), and Ctrl/A1254 (postnatal exposure only). Serum thyroid hormone concentrations, liver and brain concentration of PCBs, body weight, mortality, age of eye opening, auditory startle amplitudes, and auditory thresholds for 1 kHz and 40 kHz tones were assessed. The results demonstrated that postnatal exposure alone was responsible for ototoxicity. These cross-fostering experiments also showed that prenatal-only exposure led to small postnatal hypothyroxinemia, which recovered by the end of lactation, whereas the hypothyroxinemia that occurred following postnatal-only exposure matched that seen with perinatal exposure within a few days after birth.

Pregnant LE rats were exposed from GD 6 to PND 21 to three doses of mixed PCBs in the diet (Fox River PCBs). Specific changes in IL-6, myelin basic protein and glial fibrillary acidic protein were observed. PCB exposure lowered serum T4 in male and female offspring (all doses). The specific composition of the mixture was not specified (Miller et al. 2010).

Pregnant LE rats were exposed orally to 1 or 3 mg/kg bw/day of a mixture of PCB (Fox river mixture composed of 3 different Aroclor mixtures) from 28

days before breeding until PND 21 (or MeHg or a mixture of PCBs and MeHg). Hearing deficits were measured in both PCB groups; mixture LOAEL of 1 mg/kg bw/day (total dose 70 mg/kg bw) (Powers et al. 2009).

A mixture of PCB 77, 126, 105, 118, 138 and 153 was administered to pregnant SD rats from GD 6 to 16 at mixture doses of 2.46 and 4 mg/kg bw/day. Thyroid hormone levels were reduced at GD 16 in both dose groups (Gauger et al. 2007).

Combinations of PCB congeners (105, 118, 126, 138, 153) with or without actions on the thyroid receptor were examined in vivo and compared to effects of Aroclor 1254. Pregnant SD rats were exposed from GD 6 to PND 16 to the chemical mixtures (PCB mixture doses of 0.5 to 1 mg/kg/day; Aroclor mixture of 5 mg/kg/day. Thyroid hormone levels and thyroid hormone target genes were examined in different tissues. The Aroclor mixture and mixtures of PCBs inhibited T4 levels on PD 15 in pups (Giera et al. 2011).

Female rat dams were exposed to a Fox River Mix of different Aroclors at the following doses 0.51, 1.5, 3, 6, 12 and 18 mg/kg/day from gestational day 6 through postnatal day 21, and T4 levels in the male offspring were examined. Dose dependant T4 reductions were seen with a NOAEL of 1.5 mg/kg/day and a LOAEL of 3 mg/kg/day (Miller et al. 2012).

Three papers have described studies on thyroid hormones in offspring of rats exposed to Aroclor 1254 from GD 6 to 16. Pregnant SD rats were administered Aroclor 1254 on a wafer at doses of 0, 1, or 4 mg/kg bw/day. Dam T4 levels were reduced at both doses, but this was only statistically significant at 4 mg/kg bw/day (Bansal et al. 2005). In a paper by Gauger et al. 2004, the same doses were used (may be the same study as Bansal et al. 2005, as T4 levels are exactly the same), however, a significant reduction of T3 was seen at 1 mg/kg bw/day, i.e. the LOAEL was 1 mg/kg bw/day. A previous study by the same group found a LOAEL of 1 mg/kg bw/day for dam T4 reduction following exposure from GD 6 to GD 16 (Zoeller et al. 2000).
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An overall LOAEL for developmental effects of commercial PCB mixtures (Aroclor 1254) can be set to 0.005 mg/kg bw/day based on effects in offspring of monkeys exposed for 37 months before mating and for the following 29 months throughout pregnancy, birth and childhood. The effects were related to skin, nails and the immune system and considered to be mainly dioxin-like effects.

Differences in potency between NDL-PCB and DL-PCB congeners on female reproduction and neurobehavioral development of the offspring have been shown in studies performed in rats in the same laboratory, using the same dosing protocol (gavage dosing from GD 10-16) and measuring the same endpoints (Schantz et al. 1995, 1996). The LOAEL for PCB 126 was <0.001 mg/kg bw per day, for PCB 118 the LOAEL was 4 mg/kg bw per day; for PCB 77 the NOAEL was at 2 mg/kg bw per day. For NDL-PCB 28 and 153 the NOAEL was 8 and 16 mg/kg bw per day, respectively. For all endpoints examined, the dioxin-like congener PCB-126 was by far the most potent, whereas the potencies of the other DL-PCB tested were more similar or slightly higher than the potencies of the NDL-PCB (EFSA 2005).

No effects on birth weight or pup weight at weaning was observed following administration of 0.001 mg/kg bw/day PCB 126 or 8 mg/kg bw/day PCB 77 to pregnant rats on gestation days 10-16, or following administration of 0.001 mg/kg bw/day PCB 126 beginning 5 weeks before and continuing through gestation and lactation (ATSDR 2000).

Serum T4 levels were depressed in 21-day-old pups, but not in 60-day-old pups, born to pregnant rats administered 0.00025 or 0.001 mg/kg bw/day PCB 126 beginning 5 weeks before and continuing through gestation and lactation; no significant neurobehavioural alterations were observed when pups were tested in different tests at up to about 400 days of age (ATSDR 2000).

Effects of *in utero* exposure to 4-hydroxy-2,3,3',4',5-pentachlorobiphenyl (4-OH-PCB107) on development, sex steroid hormone levels, and female reproduction in rats were investigated by Meerts et al. (2004 – as cited by EFSA 2005). The developmental effects observed following exposure to 4-OH-PCB 107 were a dose-related prolongation of the estrous cycle in female offspring, measured between PNDs 210 and 231, and increased oestradiol:progesterone ratios. The effects of 4-OH-PCB 107 were considered to be sex-related, because no effects could be detected on male accessory sex organ weights or testosterone levels at PNDs 310 to 325.

4.5 Mutagenic and genotoxic effects

4.5.1 In vitro studies

Text taken from EFSA (2005):

Aroclor 1254 and PCB 52 did not induce gene mutations in *S. typhimurium* with or without exogenous metabolic activation. Aroclor 1254 was not clastogenic in an *in vitro* chromosome aberration test carried out with human lymphocytes. No clastogenicity was observed in human peripheral lymphocytes exposed *in vitro* to Delor 103. PCB 52 did not induce chromosome damage. PCB 77 caused dose-related chromosome breakage in human lymphocytes in a range of non toxic concentrations (10-1-10-4 µg/mL), and an interactive effect of PCB 77 with PCB 52 was reported. However, others did not find any increase in micronucleus frequencies or DNA single strand breaks in human lymphocytes treated with PCB 77 (EFSA 2005).

Aroclor 1254, in contrast to TCDD, was able to induce unscheduled DNA synthesis (UDS) in cultured rat hepatocytes. Aroclor 1242 and Clophen A60 were found to be negative in a gene mutation assay carried out in Chinese hamster V79 cells in the absence of metabolic activation (EFSA 2005).

In vitro covalent binding of radiolabelled PCB 153 to calf thymus DNA and to proteins, measured by liquid scintillation spectrometry has been reported and using 32P-postlabelling it was shown that some lower chlorinated PCB produced modified DNA bases, revealed as "spots" on the TLC plate, and that guanine was the preferential site of attack (EFSA 2005).

Metabolism of PCB generates electrophilic metabolites and reactive oxygen species that can damage DNA. Thus, dihydroxylated PCBs and PCB quinones, after reaction with glutathione, produced reactive oxygen species in HL-60 human cell lines and oxidative DNA damage in the form of DNA strand breaks in a supercoiled plasmid DNA from *E. coli*. Modified DNA bases formed after bioactivation of PCB with rat, mouse and human hepatic microsomes were compared using the 32P-postlabeling assay. Eight congeners ranging from mono- to hexachlorinated biphenyls were tested. Modified DNA bases were formed with mono-, di- and tri-chlorinated congeners, but not with higher chlorinated congeners. Similar adduct patterns were observed for 2-monochlorobiphenyl (2-CB) activated with all microsomal systems, while 4-CB, 3,4-CB and 3,4,5-CB showed similar patterns for two out of the three microsomal systems used. 4,4'-CB showed different adduct patterns in all microsomal systems. Higher adduct levels were obtained with the rodent microsomes compared with human microsomes. Using the same technique, low levels of DNA damage were also observed in human hepatocytes exposed to Aroclors 1016 and 1254Formation of a guanine-adduct was reported after incubation of calf thymus DNA with quinones of lower chlorinated PCB. These studies have shown that liver enzymes metabolise lower chlorinated PCB congeners to reactive intermediates that can produce DNA damage *in vitro* and that para-quinone metabolites of PCB may in part be involved (EFSA 2005).

By using a modified 32P-postlabeling technique it was shown that the incubation of PCB 1, PCB 12, PCB 28, PCB 38 and PCB 52 with calf thymus DNA and liver microsomes from rats treated with Phenobarbital produced five to eight different spots. For some higher chlorinated congeners (PCB 138, PCB 153 and PCB 180) a single dominant spot was detected after butanol enrichment. Both higher and lower chlorinated PCB congeners were unable to significantly increase oxidative DNA damage in calf thymus DNA measured as 8-oxo-7,8-dihydro-2'-deoxyguanosine (EFSA 2005).

4.5.1.1 DL-PCB

2,3,7,8-TCDD was mainly negative when tested *in vivo* for chromosomal aberrations, sister chromatid exchanges and increases in the frequency of micronuclei in bone marrow or peripheral lymphocytes in mice, rats or monkeys. A dominant lethal test and tests for DNA adduct formation in rat liver as well as a test for covalent binding of 2,3,7,8-tetraCDD to DNA in mice liver *in vivo* were negative. However, DNA-single strand breaks were observed in rat liver. *In vitro*, tests for reverse mutations in *Salmonella typhimurium* (Ames test) and *Escherichia coli* with and without metabolic activation were predominantly negative. Unscheduled DNA synthesis in human mammary epithelial cells was negative. Test for gene mutations in *Saccharomyces cerevisiae*, for sister chromatid exchanges in Chinese hamster cells and in human lymphocytes and for micronuclei in human lymphocytes were positive. (ATSDR 1998, IARC 1997).

2,3,7,8-TCDD did not bind covalently to DNA in mouse liver *in vitro*, and 2,3,4,7,8-PCDF as well as 1,2,3,7,8-PCDF and 2,3,4,6,7,8-HCDF did not bind to DNA in rat liver *in vivo*. Mixtures of PCDDs, PCDFs and PCBs were negative *in vivo* when tested in the mouse spot test but positive in studies of sister chromatid exchange in human lymphocytes *in vitro*. (IARC 1997).

4.5.2 In vivo studies

Clophen 30 and Clophen 50 did not induce chromosomal non-disjunction in *Drosophila melanogaster*. Aroclor 1254 produced chromosome aberrations in embryos and in fish erythrocytes. It also induced DNA fragmentation in rat hepatocytes, but was unable to induce micronuclei in mouse bone marrow cells or chromosomal abnormalities in mouse sperm cells. Aroclor 1254 was not able to induce chromosomal abnormalities in rat sperm cells and bone marrow cells either. Aroclor 1242 did not induce chromosomal abnormalities in rat bone marrow cells and rat spermatogonia or dominant lethal mutations in rat sperm cells. A mixture of PCB was found to be unable to induce micronuclei in mouse bone marrow cells. PCB 52 and 77 given individually in the feed were unable to produce chromosome aberrations in the bone marrow cells of rats. However, there was a significant increase in chromosomal damage in rats fed both PCB 52 and PCB 77 in combination (EFSA 2005).

An early *in vivo* study in mice showed covalent binding of PCB 136 to DNA, RNA and proteins in liver, muscle and kidneys and of PCB 153 to RNA and proteins in the liver and another study showed binding of PCB 153 to nuclear proteins and DNA in the livers of treated rats. In rats orally treated with Aroclor 1242 no DNA adducts nor oxidative DNA damage were detected in any of several organs (EFSA 2005).

4.5.3 Conclusion on genotoxicity

In conclusion, the overall negative results of *in vitro* and *in vivo* genotoxicity studies indicate that technical PCB mixtures are not mutagenic at gene or chromosome level.

Some lower chlorinated PCB formed modified DNA bases revealed as spots by the ^{32}P-postlabeling assay. By using the same technique it was shown that PCB 153 also produced modified DNA bases, *in vitro* as well as *in vivo*. Furthermore it has been shown that PCB-derived paraquinones can bind to DNA *in vitro* to form specific adducts at the N2-position.

The results obtained by ^{32}P-postlabeling are difficult to interpret, because this technique does not provide structural information on the analytes, but only detects unidentified spots. Reactive oxygen species might be responsible for their formation. Although it is difficult to draw a general conclusion from the present data, they raise the possibility that oxidative DNA damage may be involved in the carcinogenicity of PCB in rodents.

4.6 Carcinogenic effects

Many studies of complex PCB mixtures have shown liver and thyroid neoplasms in rats. The most comprehensive and adequately performed study compared the four most widely used commercial Aroclor mixtures (1016, 1242, 1254 and 1260) administered in the diet to male and female Sprague-Dawley rats at dose levels of 2.0-11.2, 2.0-5.7, 1.0-6.1, or 1.0-5.8 mg/kg bw/day, respectively, for 24 months (Mayes et al. 1998). In females, increased survival was observed for all Aroclor

treatment groups. Liver toxicity was distinctly more severe in females than in males. The incidence of hepatocellular neoplasms (primarily adenomas) was highly sex-dependent (females much more susceptible than males), differed among mixtures and, for females, was dependent on the level of chlorination (Aroclor 1254 > Aroclor 1260 > Aroclor 1242 > Aroclor 1016). A significant increased incidence in liver tumours in males was only observed for the high dose group administered Aroclor 1260. For males, a small increase in the incidence of thyroid gland follicular cell adenomas was noted for Aroclors 1242, 1254 and 1260, with similar incidences across dose groups and mixtures. For females receiving Aroclors 1242, 1254 or 1260, a significantly decreased trend in the incidence of mammary gland tumours was observed (EFSA 2005).

The interpretation of the carcinogenicity studies with technical mixtures is complicated by the fact that these mixtures contain both dioxin-like and non dioxin-like congeners. Since liver carcinogenicity has been shown in female rats as the dominant carcinogenic effect for the prototype dioxin 2,3,7,8-TCDD, and also for 2,3,4,7,8-PCDD and for the most potent DL-PCB congener PCB 126, the possibility that the liver carcinogenicity of technical PCB mixtures is due to the dioxin-like compounds present in these mixtures was evaluated by EFSA (2005). The results from the chronic carcinogenicity study in rats described above (Mayes et al. 1998) indicates that the total TEQ-doses, associated with dioxin-like constituents within the technical mixtures, but not the doses of total PCB, are mainly, if not exclusively, responsible for the development of liver neoplasms. A quantitative comparison with the chronic carcinogenicity study with TCDD in female rats (Kociba et al. 1978), revealed similar dose-response curves for the total TEQs in the various technical PCB mixtures as for TCDD as inducers of hepatic neoplasms in female rats. These findings suggest that in rats, NDL-PCB administered together with DL-PCB in technical mixtures play a minor – if any – role as carcinogens (EFSA 2005).

The administration of 2,3,7,8-TCDD to rodents significantly increased the incidence of benign as well malignant tumours in various tissues (e.g. liver, thyroid gland, lymphatic system, skin, lungs) in both sexes in several chronic studies. The lowest effective dose causing tumours was 10 ng/kg bw/day for two years at which female rats developed hepatic adenomas. The NOEL was 1 ng/kg/day. In the long-term study in rats in which the incidence of liver tumours was increased, the LOEL (10 ng/kg of body weight per day) corresponded to a steady-state body burden of 294 ng/kg of body weight. In order for humans to attain a similar steady-state body burden, they would have to have a daily intake of 150 pg/kg of body weight (JECFA 2002).

Tumour promotion experiments have shown that after initiation with a genotoxic carcinogen, technical PCB mixtures and individual dioxin-like and non dioxin-like congeners as well as TCDD act as liver tumour promoters in rats (EFSA 2005). The promoting activity of di-*ortho* PCBs such as PCB 47, 52, 49, and 153 has been demonstrated in short-term assays in which orally administered congeners promoted development of pre-neoplastic lesions in rat liver following induction by various initiators. In this respect NDL-PCBs appear to be less effective than DL-PCBs (EFSA 2005).

Based on the determination of the number and volume of gamma-glutamyl transpeptidase (GGT)-positive foci (pre-neoplastic lesions) it was shown that PCB 3, PCB 15, PCB 52 and PCB 77 significantly increased the number of the GGT-positive foci per cm3 of liver and per liver in male rats. Only PCB 3 and PCB 15 in particular, also increased the volume of the GGT positive foci (EFSA 2005).

Knowledge on the mechanisms of action underlying the tumour-promoting capacity of NDL-PCB is limited to data obtained from *in vitro* studies. Many tumour promoters such as TCDD have been shown to suppress apoptosis of pre-neoplastic cells and/or inhibit intercellular gap junctional communication. This cellular function is thought to prevent uncontrolled growth of cells bearing an intrinsic deficiency in single-cell growth regulation. Induction of oxidative stress, inhibition of cellular communication, and inhibition of apoptosis are mechanisms which have also been observed after PCB exposure, and which may be of relevance for PCB-related tumour promotion. In addition, sulphonated and hydroxylated PCB metabolites have also been shown to inhibit gap junction intracellular communication *in vitro* (EFSA 2005).

The development of thyroid tumours in male rats was attributed to the ability of Aroclor treatment to decrease the thyroid hormone levels in peripheral blood. As a result the release of thyroid stimulating

hormone (TSH) from the pituitary gland is enhanced. This effect is considered a risk factor in the development of thyroid cancer in rodents, but not in humans. This is based on the fact that the transport of thyroid hormones in the blood follows a different mechanism in humans than in rodents (EFSA 2005).

4.6.1 DL-PCBs

As part of the US National Toxicology Program's dioxin TEF evaluation in female Harlan Sprague-Dawley long-term carcinogenicity studies have been performed on PCBs 118, 126, and 153 as well as 2,3,7,8-TCDD.

Groups of 80 female Harlan Sprague-Dawley rats were administered 100, 220,460, 1,000, or 4,600 µg PCB 118/kg bw in corn oil : acetone (99:1) by gavage, 5 days per week, for up to 105 weeks; a group of 80vehicle control female rats received the corn oil/ acetone vehicle alone. Groups of 30 female rats received 10 or 30 µg/kg bw for up to 53 weeks only. Up to 10 rats per group were evaluated at 14, 31, or 53 weeks. A stop-exposure group of 50 female rats was administered 4,600 µg/kg PCB 118 in corn oil : acetone (99:1) by gavage for 30 weeks then the vehicle for the remainder of the study. Survival of all dosed groups of rats was similar to that of the vehicle control group. Mean body weights of 1,000 µg/kg bw rats were 7% less than those of the vehicle controls after week 36, and those of the 4,600 µg/kg core study and stop-exposure groups were 7% less than those of the vehicle controls after week 7. Following cessation of treatment, the body weight gain in the stop-exposure group was similar to that of the vehicle control group (NTP 2010).

In general, exposure to PCB 118 lead to dose-dependent decreases in the concentrations of serum total thyroxine(T4) and free T4 in all groups administered doses of 220 µg PCB 118/ kg bw and higher , 5 days per week. . The NOEL was 100 µg/kg bw leading to fat concentrations of 33 and 117 µg/g PCB 118 after 53 weeks and two years, respectively. There were no effects on triiodothyronine or thyroid stimulating hormone levels in any dosed groups evaluated at the 14-, 31-, and 53-week interim evaluations. There were increases in hepatic cell proliferation in the 4,600 µg/kg group at 14, 31, and 53 weeks. Administration of PCB 118 led to dose-dependent increases in CYP1A1-associated 7-ethoxyresorufin-O-deethylase, CYP1A2-associated acetanilide-4-hydroxylase, and CYP2B-associated pentoxyresorufin-O-deethylase activities at the 14-, 31-, and53-week interim evaluations. The NOEL was 10 µg/kg bw leading to a concentration of 15 µg/g PCB 118 in fat after 53 weeks. Analysis of PCB118 concentrations in dosed groups showed dose- and duration of dosing-dependent increases in fat, liver, lung, and blood. The highest concentrations were seen in fat at 2years with lower concentrations observed in the liver, lung, and blood.

At the 53-week interim evaluation, three 4,600 µg/kg bw rats had liver cholangiocarcinoma and one had hepatocellular adenoma. At 2 years, there were significant treatment-related increases in the incidences of cholangiocarcinoma and hepatocellular adenoma. Four incidences of hepatocholangioma occurred in the 4,600 µg/kg bw core study group.

At 2 years, a significant dose-related increase in hepatictoxicity was observed and was characterized by increased incidences of numerous lesions including hepatocyte hypertrophy, inflammation, oval cell hyperplasia, pigmentation, multinucleated hepatocytes, eosinophilic and mixed cell foci, diffuse fatty change, toxic hepatopathy, nodular hyperplasia, necrosis, bile duct hyperplasia and cyst, and cholangiofibrosis. The incidences of these lesions were often decreased in the 4,600 µg/kg bw stop-exposure group compared to the 4,600 µg/kg bw core study group.

In the lung at 2 years, a significantly increased incidence of cystic keratinising epithelioma occurred in the 4,600 µg/kg bw core study group compared to the vehicle control group incidence. Incidences of bronchiolar metaplasia of the alveolar epithelium were significantly increased in the groups administered 460 µg/kg bw or greater, and the incidence of squamous metaplasia was significantly increased in the 4,600 µg/kg bw core study group.

The incidence of carcinoma of the uterus in the 4,600 µµg/kg bw stop-exposure group was significantly greater than those in the vehicle control and 4,600 µg/kg bw core study groups at 2 years. A marginal increase in squamous cell carcinoma occurred in the 220 µg/kg bw group. At 2 years, there were marginally increased incidences

of exocrine pancreatic adenoma or carcinoma in the 460, 1,000, and 4,600 µg/kg bw core study groups.

Numerous non-neoplastic effects were seen in other organs including: adrenal cortical atrophy and cytoplasmic vacuolisation, pancreatic acinar cell cytoplasmic vacuolisation and arterial chronic active inflammation, follicular cell hypertrophy of the thyroid gland, inflammation and respiratory epithelial hyperplasia of the nose, and kidney pigmentation.

Under the conditions of this 2-year gavage study, there was clear evidence of carcinogenic activity of PCB118 in female Harlan Sprague-Dawley rats based on increased incidences of neoplasms of the liver (cholangiocarcinoma, hepatocholangioma, and hepatocellular adenoma) and cystic keratinising epithelioma of the lung. Occurrences of carcinoma in the uterus were considered to be related to the administration of PCB 118. Occurrences of squamous cell carcinoma of the uterus and acinar neoplasms of the pancreas may have been related to administration of PCB118. Administration of PCB 118 caused increased incidences of nonneoplastic lesions in the liver, lung, adrenal cortex, pancreas, thyroid gland, nose, and kidney (NTP 2010).

Groups of 81 female Harlan Sprague-Dawley rats were administered 30, 100, 175, 300, 550, or 1,000 ng PCB 126/kg bw in corn oil : acetone (99:1) by gavage, 5 days per week, for up to 104 weeks; a group of 81 vehicle control female rats received the vehicle alone. A group of 28 rats received 10 ng/kg bw for up to 53 weeks only. Up to 10 rats per group were evaluated at 14, 31, or 53 weeks. A stop-exposure group of 50 female rats was administered 1,000 ng/kg bw PCB 126 in corn oil : acetone (99:1) by gavage for 30 weeks then the vehicle for the remainder of the study. Mean body weights of 30 bw and 100 ng/kg bw rats were similar to those of the vehicle controls during most of the study, mean body weights of 175 and 300 ng/kg bw rats were less than those of the vehicle controls during year 2 of the study, and mean body weights of 550 ng/kg bw, 1,000 ng/kg bw core study, and 1,000 ng/kg bw stop-exposure rats were less than those of the vehicle controls after week 17 (NTP 2006a).

Alterations in serum thyroid hormone levels were evaluated at the 14-, 31- and 53-week interim evaluations. In the 550 and 1,000 ng/kg bw rats, total thyroxine (T4) and free T4 levels were significantly lower than those of vehicle controls and serum triiodothyronine (T3) and thyroid stimulating hormone (TSH) levels were significantly higher than vehicle controls at the 14-week interim evaluation. Serum T3 was also significantly higher in the 300 ng/kg bw rats compared to vehicle controls at 14 weeks. At 31 weeks, T3 was significantly higher at doses of 100 ng/kg bw or greater compared to vehicle controls. TSH levels were higher in 550 and 1,000 ng/kg bw rats than in vehicle controls. At 53 weeks, significantly lower serum concentrations of total T4 and free T4 were observed compared to vehicle controls in groups administered 175 ng/kg bw or greater and 30 ng/kg bw or greater, respectively. Serum T3 levels were significantly higher at doses of 175 ng/kg bw or greater compared to vehicle controls. No changes in TSH levels were observed between vehicle controls and dosed rats at 53 weeks.

To evaluate hepatocyte replication, analysis of labelling of replicating hepatocytes with 5-bromo-2N-deoxyuridine was conducted at the 14-, 31-, and 53-week interim evaluations. The hepatocellular labeling index was significantly higher at doses of 300 ng/kg bw or greater at 14 weeks and 175 ng/kg bw or greater at 31 weeks compared to vehicle controls. No statistically significant differences were observed between vehicle controls and PCB 126 dosed rats at 53 weeks. However, at 53 weeks, a 5.8-fold increase above the vehicle controls was observed in the 1,000 ng/kg bw group.

To evaluate the expression of known dioxin-responsive genes, CYP1A1-associated 7-ethoxyresorufin-*O*-deethylase (EROD) activity and CYP1A2-associated acetanilide 4-hydroxylase (A4H) activity were evaluated at the 14-, 31-, and 53-week interim evaluations. In addition, CYP2B associated pentoxyresorufin-*O*-deethylase (PROD) activity was analysed. Hepatic PROD (CYP2B1) and hepatic and pulmonary EROD (CYP1A1) activities were significantly greater in all dosed groups than in vehicle controls at weeks 14, 31, and 53. Hepatic A4H (CYP1A2) activity was significantly greater in the 30 ng/kg bw and greater groups compared to vehicle controls at weeks 14, 31, and 53.

The tissue disposition of PCB 126 was analysed in the liver, lung, fat, and blood of all rats in vehicle controls and all dosed groups at the 14-, 31-, and 53-week interim evaluations and in 10 rats per group

including vehicle controls at the end of the 2-year study (104 weeks). Detectable concentrations of PCB 126 were observed in the liver, fat, lung, and blood. Measurable concentrations of PCB 126 were present in the liver and fat at weeks 31, 53, and 104. Hepatic and fat concentrations increased with increasing doses of PCB 126. Measurable concentrations of PCB 126 were present in vehicle control lung tissue at 53 and 104 weeks. No PCB 126 was observed in the blood from the vehicle control rats. Lung and blood concentrations tended to increase with increasing doses of PCB 126, with a few exceptions. In the stop-exposure group, PCB 126 concentrations in liver and fat were lower than the levels observed in the 30 ng/kg bw group. In the stop-exposure group, lung tissue PCB 126 concentrations were equivalent to the levels observed in the 30 ng/kg bw group. In blood from the stop-exposure group, PCB 126 concentrations were equivalent to the levels observed in the 100 ng/kg bw group.

Absolute and relative liver weights were significantly increased at all time points and correlated with increased incidences of hepatocellular hypertrophy. At 2 years, there were significant treatment-related increases in the incidences of cholangiocarcinoma and hepatocellular adenoma. Three hepatocholangiomas were seen in the 1,000 ng/kg bw core study group and a single incidence of cholangioma each occurred in the 550 and 1,000 ng/kg bw core study groups.

At 2 years, a significant dose-related increase in hepatic toxicity was observed and was characterized by increased incidences of numerous lesions including hepatocyte hypertrophy, multinucleated hepatocytes, diffuse fatty change, bile duct hyperplasia, bile duct cyst, oval cell hyperplasia, necrosis, pigmentation, inflammation, nodular hyperplasia, portal fibrosis, cholangiofibrosis, and toxic hepatopathy. The incidences of these lesions were generally decreased in the 1,000 ng/kg bw stop-exposure group compared to the 1,000 ng/kg bw core study group.

The lung weights of 1,000 ng/kg bw rats were generally significantly increased at weeks 14, 31, and 53. At 2 years, treatment related increases in the incidences of cystic keratinising epithelioma and squamous cell carcinomas were observed. In addition, dose-related increases in the incidences of bronchiolar metaplasia of the alveolar epithelium and squamous metaplasia were also observed.

The incidence of gingival squamous cell carcinoma of the oral mucosa was significantly increased in the 1,000 ng/kg bw core study group at 2 years. Gingival squamous cell carcinoma, although reduced in incidence as compared to the 1,000 ng/kg bw core study group, was still present in the 1,000 ng/kg bw stop-exposure group.
At 2 years, adenomas and/or carcinomas were present in the adrenal cortex of most core study groups and in the 1,000 ng/kg bw stop-exposure group. Dose-related effects on the incidences of adrenal cortex atrophy and cytoplasmic vacuolisation were also seen.

There were dose-related increases in the incidences of numerous nonneoplastic responses including: chronic active inflammation, acinar atrophy, and acinar cytoplasmic vacuolation of the pancreas and chronic active inflammation of the pancreatic arteries; nephropathy; cardiomyopathy; follicular cell hypertrophy of the thyroid gland; thymic atrophy; clitoral gland cystic ducts; chronic active inflammation of the mesenteric artery; and, lymphoid follicular atrophy of the spleen.

Under the conditions of this 2-year gavage study there was clear evidence of carcinogenic activity of PCB 126 in female Harlan Sprague-Dawley rats based on increased incidences of cholangiocarcinoma of the liver, squamous neoplasms of the lung (cystic keratinising epithelioma and squamous cell carcinoma), and gingival squamous cell carcinoma of the oral mucosa. Hepatocellular adenoma and hepatocholangioma of the liver were also considered to be related to the administration of PCB 126. Neoplasms of the adrenal cortex and cholangioma of the liver may have been related to administration of PCB 126. PCB 126 administration caused increased incidences of non-neoplastic lesions of the liver, lung, adrenal cortex, pancreas, kidney, heart, thyroid gland, thymus, spleen, clitoral gland, and mesenteric artery in female rats (NTP 2006a).

In the NTP study of 2,3,7,8-TCDD carried out as part of the dioxin TEF evaluation in female Harlan Sprague-Dawley rats there was clear evidence of carcinogenicity up to 100 ng/kg bw based on increased incidences of cholangiocarcinoma of the liver, hepatocellular adenoma, cystic

keratinising epithelioma of the lung, and gingival squamous cell carcinoma of the oral mucosa (NTP, 2006b). Increased incidences of squamous cell carcinoma of the uterus were also considered to be related to 2,3,7,8-TCDD exposure, and marginal increased incidences of pancreatic neoplasms and hepatocholangioma and cholangioma of the liver may have been related to 2,3,7,8-TCDD exposure. In addition, there were increased incidences of non-neoplastic lesions in the liver, lung, adrenal cortex, pancreas, kidney, heart, thyroid gland, thymus, spleen, clitoral gland, forestomach, and mesenteric artery that were due to treatment (NTP 2006b). The NOAEL was 2.14 ng/kg bw/day based on increased absolute and relative liver weights, increased incidence of hepatocellular hypertrophy, and increased incidence of alveolar to bronchiolar epithelial metaplasia.

In contrast to the effects seen with the DL-PCBs in the NTP studies, the NDL-PCB PCB 153, at doses up to 3 mg/kg only showed equivocal evidence of carcinogenic activity female Harlan Sprague-Dawley rats (NTP 2006f).

Groups of female Harlan Sprague-Dawley rats were treated by gavage with PCB-153 in corn oil : acetone (99:1) at doses of 10, 100, 300, 100, or 3,000 µg/kg 5 days/week for up to 105 weeks. Exposure at the highest dose (3,000 µg/kg) continued for 30 weeks, then treatment changed to vehicle only for the rest of the study. The NTP stated that there was equivocal evidence of carcinogenic activity of PCB 153 in female Harlan Sprague-Dawley rats based on the occurrences of cholangioma of the liver. The NOAEL was 300 µg PCB 153/kg bw/day administered five days per week for two years corresponding to 210 µg/kg bw/day. In addition, increased incidences of non-neoplastic lesions of the liver, thyroid gland (NOAEL of 100 µg/kg bw/day five days per week for two years, corresponding to 70 µg/kg bw/day), ovary, oviduct, and uterus (NOAEL of 300 µg/kg bw/day five days per week for two years, corresponding to 210 µg/kg bw/day) were induced in female rats. The tissue concentrations of PCB 153 in fat and liver after 70 µg/kg bw/day for 105 weeks were 158,434 ng/g and 3699 ng/g, respectively (NTP 2006f).

The NOEL for liver microsomal enzyme induction and sporadic increased incidences of minimal to mild follicular cell hypertrophy of the thyroid gland was 10 µg/kg bw/day five days per week for 53 weeks, corresponding to 7 µg/kg bw/day. The tissue concentrations of PCB 153 in fat and liver after 7 µg/kg bw/day for 53 weeks were 8,880 ng/g and 218 ng/g, respectively (NTP 2006f). For further details see Appendix 2.

In another 2-year oral gavage study, female rats were exposed to ratios of one part 2,3,7,8-TCDD, two parts PeCDF, and ten parts PCB-126. The dose formulation was intended to give approximately equal toxic contributions from each substance. The administered doses were 10, 22, 46, or 100 ng toxic equivalents/kg body weight in corn oil : acetone (99:1) by gavage, daily for 5 days /week, for up to 105 weeks. There was clear evidence of carcinogenic activity of the mixture of 2,3,7,8-TCDD, PeCDF, and PCB-126 in female Harlan Sprague-Dawley rats; the evidence was based on increased incidences of hepatocellular adenoma and cholangiocarcinoma of the liver and cystic keratinising epithelioma of the lung. Neoplasms of the pancreatic acinus may have been related to administration of this mixture. This is a rare cancer, forming only 1% of all pancreatic tumors. In this case cancer arises from acinar cell of the pancreas and secretes pancreatic enzymes, mostly lipase. Also, this mixture caused increased incidences of non-neoplastic lesions of the liver, lung, pancreas, adrenal cortex, oral mucosa, uterus, thymus, ovary, kidney, heart, bone marrow, urinary bladder, mesenteric artery, and thyroid gland in female rats (NTP 2006c).

Walker et al. (2005) used statistically based dose-response modelling to indicate that the shape of the dose-response curves for hepatic, lung, and oral mucosal neoplasms was the same in the NTP studies of the three individual chemicals and the above-mentioned the mixture of TCDD, PCDF, and PCB-126. In addition, the dose response for the mixture could be predicted from a combination of the potency-adjusted doses of the individual compounds. Finally, they showed that use of the current WHO dioxin TEF values (Van den Berg et al. 1998) adequately predicted the increased incidence of liver tumours (hepatocellular adenoma and cholangiocarcinoma) induced by exposure to the mixture.

Similarly, in the binary mixture study of PCB126 and PCB118 in female Harlan Sprague-Dawley rats that was conducted as part of the dioxin TEF evaluation, there was clear evidence of carcinogenicity of the mixture based on increased incidences of cholangiocarcinoma

and hepatocellular neoplasms (predominantly hepatocellular adenomas) of the liver and cystic keratinising epithelioma of the lung (NTP, 2006d). The marginally increased incidences of gingival squamous cell carcinoma of the oral mucosa were also considered to be related to administration of the mixture of PCB 126 and PCB 118. Occurrences of cholangioma and hepatocholangioma of the liver may have been related to administration of the mixture of PCB 126 and PCB 118. Administration of the mixture of PCB 126 and PCB 118 caused increased incidences of non-neoplastic lesions in the liver, lung, oral mucosa, thymus, thyroid gland, adrenal cortex, pancreas, kidney, heart, lymph nodes, mesenteric artery, brain, forestomach, spleen, and nose. (NTP 2006d)

Another two-year oral gavage NTP study showed clear evidence of carcinogenic activity of a constant ratio binary mixture of PCB-126 and PCB-153 in female Harlan Sprague-Dawley rats. The daily doses were 10, 100, 300, or 1,000 ng of PCB-126, each with 1,000 times more PCB-153, per kilogram body weight. The evidence was based on increased incidences of cholangiocarcinoma, hepatocholangioma, and hepatocellular neoplasms (predominantly adenomas) of the liver, squamous neoplasms of the lung (predominantly cystic keratinising epithelioma), and gingival squamous cell carcinoma of the oral mucosa. Increased incidences of pancreatic acinar neoplasms were also considered to be related to the administration of the binary mixture of PCB-126 and PCB153. The increased incidences of uterine squamous cell carcinoma may have been related to administration of the binary mixture of PCB-126 and PCB-153. The uterine squamous cell carcinoma is a rare type of cancer. Administration of the binary mixture of PCB-126 and PCB-153 caused increased incidences of non-neoplastic lesions in the liver, lung, oral mucosa, pancreas, adrenal cortex, thyroid gland, thymus, kidney, nose, and fore stomach (NTP 2006e). The carcinogenicity profile appears to be dominated by the content of PCB 126.

A number of hypotheses addressing the mechanism of tumour promotion exist. The PCDDs, PCDFs and DL-PCBs are not acting as initiators of carcinogenesis as evidenced by the mainly negative results in the genotoxicity tests including the lack of covalent binding to DNA. Several indirect mechanism of carcinogenicity are suggested including Ah receptor-mediated alteration in expression of networks of genes involved in cell growth and differentiation, DNA damage mediated by oxidative stress due to induction of cytochrome P450-catalysed metabolic activation pathways, expansion of pre-neoplastic cell populations via inhibition of apoptosis, positive modulation of intra- or extracellular growth stimuli, or suppression of immune surveillance. Thyroid tumours are probably induced through a mechanism involving induction of hepatic UDP-GT resulting in enhanced elimination of thyroid hormones from the circulation, and consequently elevated levels of circulating thyroid stimulating hormone which results in a chronic proliferative stimulation of thyroid follicular cells. (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000).

5 Regulations

5.1 Ambient air

Denmark (C-value): –

Denmark (emission value):
0.0001 mg/normal m^3 (MST 2001).

5.2 Indoor air

Denmark (action levels[1]):
3000 ng PCB/m^3 air: Intervention without any unnecessary delay.
300-3000 ng PCB/m^3 air: Intervention at sight in order to reduce the concentration below 300 ng PCB/m^3.
(Sundhedsstyrelsen 2011)

5.3 Drinking water

Denmark: –

EU: –

WHO: –

US-EPA:
Polychlorinated biphenyls (PCBs) CAS No 1336-36-3:
MCLG[2]: 0 mg/l (zero)
MCL[3]: 0.0005 mg/l
(US-EPA 2012a)

The MCLG was based on the best available science to prevent potential health problems.

Potential health effects: Some people who drink water containing polychlorinated biphenyls in excess of the MCL over many years could experience changes in their skin, problems with their thymus gland, immune deficiencies, or reproductive or nervous system difficulties, and may have an increased risk of getting cancer (US-EPA 2012).

5.4 Soil

Denmark:
There is no need to set a health-based soil quality criterion based on children's direct exposure to soil for PCDDs, PCDFs and dioxin-like PCBs (expressed as WHO-TEQ) (Larsen and Nørhede 2004).

The Netherlands:
Sum-PCBs in mg/kg dry matter (VROM 2012):
Background value: 0.02
Maximum value function class 'Residence': 0.02
Maximum value function class 'Industry': 0.5
Intervention value (in the Soil Protection Act): 1

UK:
PolyChloroBiphenyls (total) (UK 2012):
Optimum: 0.02 mg/kg dry weight
Action: 1 mg/kg dry weight

Canada:
Polychlorinated biphenyls (total) (Canada 1999):
Soil quality guideline (SQG), agricultural: 0.5 mg/kg
Soil quality guideline, residential / parkland: 1.3 mg/kg
Guidelines determined for environmental health as data are insufficient and inadequate to calculate SQGs for human health.

1 These action levels are the German action levels for PCB in indoor air (SST 2011).
2 Maximum Contaminant Level Goal (MCLG) – The level of a contaminant in drinking water below which there is no known or expected risk to health.
3 Maximum Contaminant Level (MCL) – An enforceable regulation set for most contaminants, based on the MCLG. MCLs are set as close to the MCLGs as possible, considering cost, benefits and the ability of public water systems to detect and remove contaminants using suitable treatment technologies.

5.5 Occupational Exposure Limits

Denmark:
0.01 mg/m^3, notation HK, (1996) (At 2007).

5.6 Classification, EU

Both the classification according to the Dangerous Substances Directive 67/548/EEC (DSD) in the former chemical regulation as well as the classification according to the present CLP-Regulation (EC) No 1272/2008 (CLP: Classification, Labelling and Packaging) are reproduced from the European chemical Substances Information System (ESIS 2012):

PCB / polychlorobiphenyls (CAS No 1336-36-3):
DSD: R33, N; R50-53
CLP: STOT RE 2 H373, Aquatic Acute 1 H400, Aquatic Chronic 1 H410

5.7 Maximum levels in food, EU

Maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs have been established by the European Commission in foodstuffs (EC 2011).

For the sum of dioxins (expressed as WHO-PCDD/F-TEQ), the maximum levels range from 1- 2.5 pg/g fat in meat and meat products, milk, egg and animal fat. In fish and fish products, the maximum level is set at 3.5 pg/g wet weight.

For foods for infants and young children, the maximum level is set at 0.1 pg/g wet weight.

For the sum of dioxins and dioxin-like PCBs (expressed as WHO-PCDD/F-PCB-TEQ), the maximum levels range from 1- 5.5 pg/g fat in meat and meat products, milk, egg and animal fat. In fish and fish products, the maximum level is set at 6.5 pg/g wet weight.

For foods for infants and young children, the maximum level is set at 0.2 pg/g wet weight.

For the sum of non dioxin-like PCBs (expressed as the sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180), the maximum levels range is set at 40 ng/g fat in meat and meat products, milk, egg and animal fat, and in fish and fish products at 75 ng/g wet weight.

For foods for infants and young children, the maximum level is set at 1.0 ng/g wet weight.

5.8 IARC

Polychlorinated biphenyls (PCBs, CAS No 1336-36-3) are probably carcinogenic to humans, Group 2A (limited evidence in humans, sufficient evidence in experimental animals) (IARC 1987).

2,3,7,8-TCDD is carcinogenic to humans (group 1). Other PCDDs and PCDFs are not classifiable as to their carcinogenicity to humans (group 3) (IARC 1997).

5.9 US-EPA

US-EPA has not set an oral Reference Dose (RfD) for polychlorinated biphenyls (PCBs, CAS No 1336-36-3), but it was noted in the document that the reader should check the individual files for Aroclor 1216, Aroclor 1248 and Aroclor 1264 (US-EPA 2012b).

US-EPA has set an oral RfD for Aroclor 1016 of 0.07 µg/kg bw/day based on a NOAEL of 0.007 mg/kg bw/day for reduced birth weights in a monkey reproductive study (last revised 11/01/1996). A total uncertainty factor of 100 was used with a factor of 3 to account for sensitive individuals, a factor of 3 for extrapolation from rhesus monkeys to humans, a factor of 3 because of limitations in the data base, and a factor of 3 for extrapolation from a subchronic exposure to a chronic RfD. (US-EPA 2012c).

US-EPA has not set an oral RfD for Aroclor 1248 because the database at the time of review was considered as being insufficient as a frank effect (death of an infant) was noted at the lowest dose tested in a sensitive animal species, rhesus monkeys (US-EPA 2012d).

US-EPA has set an oral RfD for Aroclor 1254 of 0.02 µg/kg bw/day based on a LOAEL of 0.005 mg/kg bw/day for ocular, dermal and immunological effects in monkeys (last revised 11/01/1996). A total uncertainty factor of 300 was used with a factor of 10 to account for sensitive individuals, a factor of 3 for extrapolation from rhesus monkeys to humans, a factor of 3 for the use of a minimal LOAEL, and a factor of 3 to account for extrapolation from a sub-chronic exposure to a chronic RfD. (US-EPA 2012e).

US-EPA has set an oral RfD for 2,3,7,8-TCDD of 0.7 pg/kg bw/day (7 x 10^{-10} mg/ kg bw/day) based a LOAEL of 20 pg/kg bw/day for 2,3,7,8-TCDD in humans (last revised 02/17/2012). The US EPA carried out a systematic evaluation of the peer-reviewed epidemiological studies and rodent bioassays available relevant to 2,3,7,8-TCDD dose-response analysis and performed dose-response analyses using a 2,3,7,8-TCDD physiologically based pharmacokinetic model that simulates 2,3,7,8-TCDD blood concentrations following oral intake. The LOAEL was based on two epidemiological studies of the Seveso population that had been exclusively exposed to high levels of 2,3,7,8-TCDD. One study associated TCDD exposures with decreased sperm concentration (20%) and sperm motility (11%) in men (22-31 year-old) who were exposed during childhood (1 to 9 year-old) (Mocarelli et al. 2008); the other study associated increased thyroid-stimulating hormone (TSH) levels in newborn infants born to mothers who were exposed to 2,3,7,8-TCDD (Bacarrelli et al. 2008). In the latter study the LOAEL was defined in terms of the maternal 2,3,7,8-TCDD serum level corresponding to neonatal TSH levels above 5 µU/mL of serum. A total uncertainty factor of 30 was used with a factor of 3 to account for sensitive individuals and a factor of 10 for LOAEL to NOAEL extrapolation as a NOAEL could not be identified for either study. (US-EPA 2012f).

Carcinogenicity assessment for polychlorinated biphenyls (PCBs, CAS No 1336-36-3): Group B2, probable human carcinogen (US-EPA 2012b).

Carcinogenicity assessment for 2,3,7,8-TCDD: Not applicable, is currently underway (US-EPA 2012f).

5.10 WHO / JECFA

PCDDs, PCDFs and dioxin-like PCBs:
70 pg/kg bw, provisional tolerable monthly intake (PTMI) (JECFA 2002).

The FAO/WHO Joint Expert Group on Food Additives (JECFA) and the EU Scientific Committee on Food (SCF) have performed risk assessments of PCDDs, PCDFs and dioxin-like PCBs. Although these assessments were performed independently, they used the same approach (body burden approach) and essentially the same pivotal toxicological studies and reached similar conclusions. In contrast to the SCF (see below), JECFA expressed the health guidance value as a provisional tolerable monthly intake.

5.11 The EU Scientific Committee on Food (SCF)

PCDDs, PCDFs and dioxin-like PCBs:
14 pg TEQs/kg bw, tolerable weekly intake (TWI) (SCF 2000, 2001).

The evaluation was based on the most sensitive effects of 2,3,7,8-TCDD in animals (decreased sperm production and altered sexual behaviour in male offspring of the exposed rat dams). For these effects, a steady state LOAEL body burden in the dams was estimated to 39 ng/kg bw. In order for humans to achieve a LOAEL body burden of 39 ng/kg bw at steady state a daily intake of 20 picogram (pg) 2,3,7,8-TCDD would be needed for 30-40 years. The SCF applied an uncertainty factor of 10 to the LOAEL of 20 pg/kg bw/day and established a tolerable weekly intake (TWI) of 14 pg/kg bw for 2,3,7,8-TCDD. This TWI was extended to include the PCDDs, PCDFs, and DL-PCB that had been allocated 2,3,7,8-TCDD toxic equivalence factors (TEFs) by WHO in 1998 (Van den Berg et al. 1998). The TWI was then expressed as 14 pg TEQs/kg bw/week.

5.12 European Food Safety Authority (EFSA)

Non dioxin-like PCBs:
No health based guidance value for humans can be established (EFSA 2005).

The rationale behind this conclusion was that simultaneous exposure to NDL-PCB and dioxin-like compounds hampers the interpretation of the results of the toxicological and epidemiological studies as well as the data regarding effects of individual NDL-PCB congeners are rather limited. The Panel noted that there are indications that subtle developmental effects, being caused by NDL-PCBs, DL-PCBs, or polychlorinated dibenzo-p-dioxins/dibenzofurans alone, or in combination, may occur at maternal body burdens that are only slightly higher than those expected from the average daily intake in Europeans. Because some individuals and some sub-populations may be exposed to considerably higher average intakes, the Panel concluded that a continuous effort to lower the levels of NDL-PCBs in food is warranted.

6 Summary and evaluation

6.1 Description

Polychlorinated biphenyls (PCBs) are synthetic chlorinated hydrocarbon compounds that consist of two benzene rings linked by a single carbon-carbon bond, with from 1 to all 10 of the hydrogen atoms replaced with chlorine atoms. There are 209 possible PCB congeners, see Appendix 1.

The commercial PCB mixtures differ mainly in percentages of individual chlorinated biphenyls, method of production, and level of contaminants (e.g., polychlorinated dibenzofurans (PCDFs)). The PCB congeners differ qualitatively and quantitatively in biological and toxicological activities and several diverse mechanisms are involved in responses to PCB mixtures. Based on structural characteristics and toxicological effects, PCBs can be divided into two groups:

1). The congeners considered to be most toxic, based on combined health effects considerations, are coplanar. Some co-planar PCBs, called dioxin-like PCBs (DL-PCBs) have been shown to exert a number of toxic responses similar to those of 2,3,7,8-TCDD, the most toxic dioxin and their effects are mediated through binding to the aryl hydrocarbon receptor (AhR). Of the 209 theoretical PCB congeners only 12 are considered to be dioxin-like, the 12 congeners marked with '[DL]' in the table in Appendix 1.

2). The other PCBs that do not show dioxin-like toxicity via the AhR have a different toxicological profile. This group of PCBs is called 'non dioxin-like PCBs' (NDL-PCBs).

The PCB mixtures in environmental media, such as soil, contain both DL- and NDL-PCBs which will have different modes of biochemical and toxicological actions. DL-PCBs principally include the coplanar PCBs such as PCBs 77, 81, 126, and 169. Dioxin-like effects include weight loss, thymic atrophy, enzyme induction, liver and thyroid toxicity, immunotoxicity, reproductive, developmental and neurobehavioral toxicity, dermatologic effects, carcinogenicity, and endocrine activity. The mono-*ortho* coplanar PCBs such as 105, 114, 118, 123, 156, 157, 167, and 189 have both dioxin-like effects via the AhR and possibly other mechanisms of action, such as a phenobarbital-like spectrum of enzyme induction. *Ortho*-substituted non-planar PCBs that do not bind to the AhR elicit enzyme induction, liver and thyroid toxicity, neurotoxicity, tumour promotion, and endocrine activity by different modes of action.

When two different lots of Aroclor 1254 (Lot 124-191 and Lot 6024) was analysed for PCDFs and NDL-PCBs is was shown that Lot 6024 had 10 times higher TEQ content that Lot 124-191 (395.1 versus 38.3 µg WHO-TEQ/g) While lot 124-191 has the typical PCB congener distribution of "early" Aroclors 1254 types (G4), lot 6024 represents the "late" A4 type (1974-1976) production of Aroclor 1254. While production records suggest that the A4 pattern lots of Aroclor 1254 (e.g. lot 6024) represented less than 1% of the total Aroclor 1254 production, their availability during the final years of production resulted in their disproportionate use by researchers into Aroclor 1254 toxicity. For example, the major chronic 2-year rat carcinogenicity study of Aroclors employed an Aroclor lot of this type.

It appears that the TEQ contents in both of these two Aroclor 1254 lots have been rather high. Burkhard and Lukasewycz (2008) systematically collected data from the literature on TEQs in a number of commercial PCB mixtures (Aroclor, Clophen, Kanechlor, Chlorofen, Sovol, Delor, and Phenoclor) and reported a value of 7.9 µg TEQ/g for Aroclor 1254, when WHO TEFs from 2005 were used. TEQs were similar across the different PCB product lines for mixtures of similar chlorine content. The TEQ content varied from 0.034 µg/g in Aroclor 1221 to 11.8 µg/g in Aroclor 1248.

Due to their physical and chemical properties, such as non-flammability, chemical stability, high boiling point, low heat conductivity and high dielectric constants, PCBs were widely used until the middle of the 1970es, in a number of industrial and commercial open and closed applications.

Since 1986 no new products containing PCBs have been allowed in Denmark and continued use of old products has only been allowed in closed systems.

6.2 Environment

PCBs are synthetic compounds and have been released to the environment solely by human activity. PCBs are globally circulated and are present in all environmental media and are redistributed from one compartment to another, e.g. soil to water, water to air, air to water, sediments to water.

PCBs, particularly the highly chlorinated congeners, adsorb strongly to sediment and soil particles where they tend to persist with half-lives in the order of months to years. PCBs are considered rather immobile in soil and will mainly concentrate in the topsoil layer unless they are mechanically spread. PCBs are unlikely to migrate to groundwater because of the strong binding to soil. Volatilisation from soil appears to be an important loss mechanism and is more important for the lower chlorinated congeners than for the higher chlorinated congeners. Soils with low organic carbon will have the greatest rate of volatilisation of PCBs. Biodegradation has been shown to occur under both aerobic and anaerobic conditions and is a major degradation process for PCBs in soil and sediment.

Once released to the environment the individual PCB congeners in the released PCB mixtures undergo bio- and photodegradation. The ability of the individual congeners to be degraded in the environment depends on the degree of chlorination of the biphenyl molecule. PCB congeners with three or less chlorines are considered to be non-persistent, while those with five or more chlorines are not readily degraded and considered to be persistent; tetrachlorobiphenyls are intermediate in persistence. Thus, the release of a PCB mixture to an aerobic environment results in a fractionating effect where the less chlorinated congeners biodegrade first and the more highly chlorinated congeners remain for long-term build-up. In addition to the degree of chlorination, the chlorine substitution pattern affects the biodegradation rate of PCBs. For example, PCBs containing chlorines on only one ring are degraded more quickly than PCBs containing an equivalent number of chlorines distributed between both rings. Additionally, PCBs with chlorines in *ortho* positions are more resistant to aerobic biodegradation than those with chlorines in either the *para* or *meta* positions. The result is that the congener patterns of the PCB mixtures in the environment over time differ from those in the original technical mixtures.

Aerobic degradation rates of PCBs can be highly variable, depending not only on structural characteristics as outlined above, but also on a number of other factors including previous exposure to PCBs or PCB-like compounds, bioavailability, initial concentration, moisture, temperature, available nutrients such as carbon sources, and the presence of inhibitory compounds. PCBs that remain firmly bound in soil and sediment may not be bioavailable to the degrading organisms at sufficient concentrations. In general, biodegradation of PCBs in aerobic soil is slow, especially in soils that have a high organic carbon content.

PCBs are slowly biodegraded in anaerobic environments by reductive dechlorination resulting in the formation of less toxic mono- and dichlorobiphenyl congeners, which are aerobically biodegradable. However, the overall congener distribution profile is markedly different following anaerobic biodegradation. The profile shows a decrease in concentration of the more highly chlorinated congeners and a corresponding increase in the overall proportion of the less chlorinated congeners.

Levels for total PCB in agricultural and rural areas usually range from 0.1 to 10 ng/g dry matter and for urban/industrial sites usually from 10-100 ng/g dry matter. In contaminated areas total PCB levels ranging from about 1000 ng/g dry matter up to 35,000 ng/g dry matter have been reported.

No Danish data on average concentrations of PCB in soils have been found.

The view of Danish EPA based on current investigations of PCB in soil is, that the content of PCB-7 in soil seldom will exceed 0.5 mg/kg PCB-7 and very rarely 1 mg/kg PCB-7. Some point sources may exceed this level.

6.3 Human exposure

The general population can be exposed to PCBs by ingestion of contaminated food, by inhaling contaminated air and by contact with contaminated soil and dust particles. Food consumption is by far the major contributor to the body burden of PCBs and more than 90% comes from the dietary exposure.

In Denmark, intake calculations have been made for ten individual indicator PCB congeners (PCB 28, 52, 101, 105, 118, 138, 153, 156, 170 and 180) as well as for their sum, see Table 2 (in section 1.6). The intake has apparently decreased in comparison with previous monitoring periods, with an intake for average indicator PCB-sum of 0.9 µg/day (1998-2003). The estimated contributions of individual food groups to the mean daily intake showed that especially fish contributed for both adults and children. Children had approximately twice as high an exposure than adults; this is considered to be due to children's high intake of some food groups, e.g. milk, in relation to their body weight.

EFSA has provided estimates of the contribution from air and soil to the daily exposure of the general population to PCB. On average the contribution from ambient air amounts to only a low percentage of the intake via food. The contribution from indoor air was considered generally to be in the same order as that from ambient air, and therefore low. The contribution from ingested soil or dust particles, particularly by children, was considered to be small, about three to four orders of magnitude lower than the average intake via food.

6.4 Toxicokinetics

The three main factors that govern the toxicokinetics of individual PCB congeners are their lipophilicity, the rate of metabolism of the compound, and for DL-PCBs the degree of binding of to cytochrome P4501A2 (CYP1A2) in the liver.

The lipophilicity controls the rate and extent of absorption, tissue distribution and passive elimination and the chlorine substitution pattern determines the metabolic rate of PCBs. For DL-PCBs, binding to CYP1A2 results in hepatic sequestration of these congeners. The degree of this binding is also dependent on the chlorine substitution pattern.

Humans and experimental animals absorb PCBs from the gastrointestinal tract after oral exposure. Molecular size and solubility of a congener are the rate limiting factors for the absorption from the gastrointestinal tract. Congeners having 4-6 chlorine atoms are well absorbed (50-90 %) while hepta- and octa-chlorinated congeners are absorbed to a lesser extent. A bioavailability of 5-20% from dust has been proposed for PCDDs and PCDFs and would likely be similar for PCBs.

Absorption after inhalation is much less investigated, but is considered to be more limited than the uptake after oral ingestion. Transdermal absorption may also be of relevance for PCB exposure due to contaminated soil; however, the bioavailability of PCBs after dermal contact is probably less than 1 %.

In Rhesus monkeys exposed to Aroclor 1260 dermally with PCB-spiked soil, percutaneous absorption was approximately 3-4 % following exposure for 12-24 hours.

Initially, PCBs distribute to liver and muscle but are subsequently translocated to adipose tissue and skin for storage. Due to the high lipophilicity and resistance to biotransformation, some PCBs, especially the highly chlorinated congeners, accumulate, particularly in the adipose tissue and for DL-PCBs also in the liver. The dominating PCB congeners in human adipose tissue are PCB 153 (27%), followed by PCB 138 (22%) and PCB 180 (18%). These three di-ortho congeners constituted about 67% of the PCB in human milk, whereas the DL PCB 118 constituted about 5 % of the total PCB content.

PCBs can pass the placenta of pregnant animals and humans and are excreted in human milk.

Many PCB are metabolized to polar metabolites, mainly in the liver by the microsomal monooxygenase system catalysed by certain cytochrome P450 species. The initial step in the biotransformation of PCBs involves oxidation by cytochrome P-450 (CYP) enzymes, including intermediate arene oxide (epoxide) formation. The major PCB metabolites formed

are the hydroxy-PCBs and the neutral, lipophilic methylsulfone metabolites ($MeSO_2$-PCBs) resulting from conjugation with glutathione. The polar metabolites undergo conjugation with glutathione and glucuronic acid before they are excreted. However, many PCBs are relatively resistant to metabolism. The major determinant for metabolism of a congener is the presence of two adjacent, unsubstituted carbon atoms on the lateral positions (3,4 or 4,5 positions). These positions are preferentially oxidised by the cytochrome P450 system. PCBs are mainly excreted in faeces whereas the metabolites mainly appear in urine.

Elimination half-lives for PCBs in humans have been reported to vary from 0.02 years to infinity for individual congeners. For PCB mixtures, estimated half-lives between 0.5 years and infinity have been reported. According to ATSDR (200), estimated half-lives of 2.5-5 years are considered the best estimates for PCB mixtures based on data from two different occupational cohorts. Elimination half-lives for "dioxins" and DL-PCB were estimated by the SCF (2000) and JRCFA (2002) to be 5-11 years (7½ year for 2,3,7,8-TCDD).

6.5 Human toxicity

Information on health effects of PCBs is available from studies of people exposed in the workplace, by consumption of contaminated rice oil in Japan (the *Yusho* incident) and Taiwan (the *Yu-Cheng* incident), by consumption of contaminated fish, and via general environmental exposures, as well as food products of animal origin. The health effects that have been associated with exposure to PCBs in humans and/or animals include liver, thyroid, dermal and ocular changes, immunological alterations, neurodevelopmental changes, reduced birth weight, reproductive and developmental toxicity, and cancer.

6.5.1 Single dose toxicity

No specific studies are available.

6.5.2 Repeated dose toxicity

Chloracne and other dermal alterations, and ocular effects have been reported in individuals exposed to PCBs via contaminated rice oil during the Yusho and Yu-Cheng incidents; no adverse dermal or ocular effects have been reported in subjects with high consumption of contaminated fish. Ultrastructural changes indicative of microsomal enzyme induction are predominant hepatic findings in *Yusho* patients. Increased serum cholesterol, but not triglycerides, has been reported for consumers of contaminated fish, whereas increased serum triglycerides, but not cholesterol, were reported for Yusho and Yu-Cheng patients. Hepatic porphyria was commonly observed in people exposed during the Yu-Cheng incident, but was not a usual finding in Yusho patients.

A number of studies have examined the relationships between PCB exposure and thyroid hormone status in both children and adults. The results suggest that PCBs can induce a variety of changes in thyroid hormone levels as well as thyroid toxicity. Differing results have been reported for differing Aroclor mixtures and PCB congeners, as well as for differing exposure scenarios and differing ages at the time of exposure. Increased thyroid gland volume has been found among workers at a PCB production facility as well as among nearby residents. An elevated odds ratio for goiter has been found among the *Yu-Cheng* cohort. In addition, numerous statistically significant positive and/or negative correlations (for a number of different age groups) have been reported between circulating levels of TSH, T4, and T3, and varying measures of PCB exposure. However, in most of the environmental studies thyroid hormone levels were within the normal ranges.

Studies in humans who consumed high amounts of fish contaminated with environmentally persistent chemicals, including PCBs, have provided evidence that PCBs are important contributors to subtle neurobehavioral alterations observed in newborn children and that some of these alterations persist during childhood. Some consistent observations at birth have been motor immaturity and hyporeflexia and lower psychomotor scores between 6 months and 2 years old. In a recent study, maternal mono-ortho-substituted DL PCBs were significantly associated with lower scores on both psychomotor (PDI) and

mental development indices (MDI) while di-ortho-substituted PCBs did not show any statistically significant association with cognitive scores.

Immunological alterations associated with consumption of contaminated rice oil in the Yusho and Yu-Cheng incidents, consumption of contaminated fish and other marine foods, and general environmental exposures include increased susceptibility to respiratory tract infections in adults and their children, increased prevalence of middle ear infections decreased total serum IgA and IgM antibody levels, and/or changes in T lymphocyte subsets and decreased antibody response to diphtheria and tetanusvaccine in children born to exposed mothers,

The human studies of occupational exposures to PCBs, the *Yusho* and *Yu-Cheng* poisoning incidents, contaminated fish consumption, and general populations are complicated by the mixture nature of PCB exposure and possible interactions between the congeneric components and other chemicals. Therefore, although PCBs may have contributed to adverse health effects in these human populations, it cannot be determined with certainty which congeners may have caused the effects. Therefore the human studies on PCBs are not suitable for use in the health risk characterisation of PCB exposure from different environmental media, including soil.

6.5.3 Toxicity to reproduction

Limited information is available on reproductive effects of PCBs in humans. Despite the variation in results between studies, the available data support a possible association between PCBs and menstrual irregularities, and conception delay in males and females. According to ATSDR (2000), the strength of the human evidence that consumption of contaminated fish may or may not be associated with adverse effects on conception and other reproductive abilities is weak.

6.5.4 Mutagenic and genotoxic effects

There are no indications of genotoxicity of PCBs in humans

6.5.5 Carcinogenic effects

Human studies provide suggestive evidence that PCBs are carcinogenic and some of the mortality studies suggest that occupational exposures to PCBs were associated with cancer at several sites, particularly the liver, biliary tract, intestines, and skin. Case-control studies of the general population are inconclusive with respect to associations between environmental exposure to PCBs and risk of breast cancer. PCBs are considered by IARC (1987) as probably carcinogenic to humans (Group 2A; limited evidence in humans, sufficient evidence in experimental animals). PCBs are considered by US-EPA (IRIS 2004d) as probable human carcinogen (Group B2; inadequate evidence in humans and sufficient evidence in experimental animals).

6.6 Animal toxicity

6.6.1 Single dose toxicity

The acute oral toxicity of PCBs varies for different commercial mixtures, but is in general low to moderate with oral LD_{50}-values between 1010 and 4250 mg/kg bw in rats. Signs of toxicity include diarrhoea, respiratory depression, dehydration, decreased response to pain stimuli, unusual gait and stance, oliguria, coma, and pathological changes in organs.

6.6.2 Repeated dose toxicity

The health effects of PCBs have been extensively studied in experimental animals. The preponderance of toxicity data is available from experimental animals exposed to commercial mixtures of PCBs in the diet in intermediate-duration studies whereas relatively few studies with chronic oral exposure have been performed. The rat and monkey have been tested most extensively; monkeys appear to be more sensitive than rats to the various effects induced by PCBs and some effects have been observed in monkeys only. Aroclor 1254 has been used in most of the studies, particularly in the studies with monkeys, and the 24-month comparative study in rats with Aroclor 1016, 1242, 1254, or 1260 indicate, that Aroclor 1254

is the most toxic of these PCB mixtures in the rat. Data on defined experimental mixtures are available from a study in infant monkeys exposed from birth until 20 weeks old to a PCB mixture analogous to the congener composition in human milk. Data on individual congeners are available from comparative 13-week oral toxicity studies in rats with 7 individual PCB congeners (28, 77, 105, 118, 126, 128, 153), from a 28-day toxicity study in rats with PCB 180, and from long-term toxicity and carcinogenicity studies of PCBs (118, 126, 153).

The mechanisms of toxicity are not completely understood. DL-PCBs have dioxin-like effects via the intracellular aryl hydrocarbon (Ah) receptor (AhR) and principally include the coplanar PCBs such as PCBs 77, 81, 126, and 169. Dioxin-like effects include weight loss, thymic atrophy, enzyme induction, immunotoxicity, teratogenicity, dermatologic effects, carcinogenicity, and endocrine disruption. The mono-*ortho* coplanar DL-PCBs such as 105, 114, 118, 123, 156, 157, 167, and 189 have DL effects via the AhR and possibly other mechanisms of action, such as a phenobarbital-like spectrum of enzyme induction. *Ortho*-substituted nonplanar PCBs that do not bind to the AhR elicit enzyme induction, neurotoxicity, tumour promotion, and endocrine activity by different modes of action.

In the evaluation of the various toxicological endpoints, N/LOAELs are considered for commercial mixtures based on the results for Aroclor 1254 in studies with monkeys unless otherwise stated, and for individual NDL-PCB congeners included in the sPCB based on the results in studies with rats unless otherwise stated.

N/LOAELs for DL-PCBs are not considered here because risk assessment of DL-PCBs was already an integral part of the the SCF (2000, 2001) and JECFA (2002) evaluations of "dioxins" in food which include PCDDs, PCDFs and DL-PCBs.

Liver toxicity induced by PCBs is well documented in experimental animals exposed to commercial mixtures or single congeners. Induction of microsomal enzymes appears to be the most sensitive hepatic effect in rats and induction has been observed following dietary administration of Aroclor 1242, 1248, 1254, or 1260 for 4 weeks at dose levels from 0.03 mg/kg bw/day (lowest dose level tested). Histopathological changes have been observed in the liver of rats following administration of Aroclor 1016, 1242, 1254, or 1260 at dose levels from 1-2 mg/kg bw/day for 24 months and in Rhesus monkeys after 12-28 months of dietary exposure to 0.2 mg/kg bw/day of Aroclor 1254. In one study of monkeys, increased liver weight and biochemical changes were observed following 0.08 mg/kg bw/day of Aroclor 1254 for 72 months with no effects at dose levels of up to 0.04 mg/kg bw/day, whereas, in another study, biochemical changes were observed from 0.005 mg/kg bw/day for 37 months. For the individual NDL congeners tested in 28-days (PCB 180), 13-week (PCB 28, 128, 153), and 2-year chronic toxicity and carcinogenicity (PCB 153) oral studies in rats the NOAELs were in the range of 0.034-0.07 mg/kg bw/day (Table in Appendix 3).

Overall, a LOAEL of 0.08 mg/kg bw/day is considered for liver effects of commercial PCB mixtures based on increased liver weight in the 72-month study in monkeys with Aroclor 1254. Biochemical changes indicative of liver effects have been observed at lower dose levels, but are not considered as being adverse effects; a LOEL of 0.005 mg/kg bw/day is considered for effects on the liver. NOAELs of 0.034-0.07 mg/kg bw/day is considered for liver effects of individual NDL PCB congeners. This resulted in body burdens from about 400 ug/kg bw (PCB 28) to 1,200 ug/kg bw (PCB 128) in the 13-week studies. The NOAELs were 34, 42, or 36 ug/kg bw/day for PCB 28, 128 and 153, respectively. The LOAELs and associated body burdens were in all cases 10 times higher. In the 2-year long-term toxicity and carcinogenicity study on highly purified PCB 153 performed by the NTP (2006) the NOAEL for effects on the liver was 70 µg/kg bw/day (body burden 16,000 µg/kg bw) and the LOAEL was 210 µg/kg bw/day (body burden 52,000 µg/kg bw).

Effects on stomach tissue have been observed in monkeys administered 0.2 mg/kg bw/day Aroclor 1254 in the diet for up to 28 months, and by dietary exposure to Aroclor 1248 from 1.3 mg/kg bw/day or to Aroclor 1242 from 0.12 mg/kg bw/day for 2 months. No effects were observed in monkeys administered Aroclor 1254 at 0.08 mg/kg bw/day for 72 months. No effects have been observed in the gastrointestinal tract of rats following administration of individual PCB congeners at dose levels from 0.17 to approximately 4 mg/kg bw/day (PCB 105) for 13 weeks.

Overall, a NOAEL of 0.08 mg/kg bw/day is considered for effects on stomach tissue of

commercial PCB mixtures, and a NOAEL of 4 mg/kg bw/day for individual PCB10 congeners.

Various effects on the thyroid gland and thyroid hormone system have been observed in studies in experimental animals. In rats, decreased serum levels of the thyroid hormones have been observed following dietary administration of Aroclor 1254 at 0.09 mg/kg bw/day for 5 months, and histological alterations at 0.25 mg/kg bw/day for 5 weeks, but not at 0.025 mg/kg bw/day. In monkeys, no effects on thyroid tissue and serum hormone levels were observed following exposure to Aroclor 1254 up to 0.08 mg/kg bw/day for up to 72 months; enlarged thyroid glands and histological alterations have been observed following dietary administration of Aroclor 1254 at 0.2 mg/kg bw/day for 28 months. For individual NDL PCB congeners tested in the oral toxicity studies in rats, the lowest NOAELs for thyroid effects were obtained in 90-day studies in rats showing effects at approximately 340-420 ug/kg bw/day (LOAEL) of PCB 28, 128 or PCB 153. This resulted in body burdens from about 4000 ug/kg bw (PCB 28) to 14,000 ug/kg bw (PCB 128). The NOAELs were 34, 42, or 36 ug/kg bw/day for PCB 28, 128 and 153, respectively. In the 2-year long-term toxicity and carcinogenicity study on PCB 153 performed by the NTP (2006) the NOAEL for effects on thyroid hormones was 70 µg/kg bw/day (body burden 16,000 µg/kg bw) and the LOAEL was 210 µg/kg bw/day (body burden 52,000 µg/kg bw).

Overall, a NOAEL of 0.08 mg/kg bw/day is considered for effects on the thyroid of commercial PCB mixtures, and a LOAEL of 0.04 mg/kg bw/day for individual PCB10 congeners.

Other effects of PCBs on endocrine function observed in experimental animals include effects on the adrenal glands and serum adrenal steroid levels. In rats, alterations in hormone levels have been observed following dietary administration of Aroclor 1254 at 0.1 mg/kg bw/day for 15 weeks, but not at 0.05 mg/kg bw/day, for 5 months; no histological alterations have been observed up to 25 mg/kg bw/day for 15 weeks. In monkeys, no effects on the adrenal tissue have been observed following dietary administration of Aroclor 1254 up to 0.2 mg/kg bw/day for 12 months, or up to 0.08 mg/kg bw/day for 72 months; and no effects on serum hormone levels up to 0.08 mg/kg bw/day for up to 22 months. For individual congeners tested in the 13-week oral toxicity studies in rats, no histopathological alterations in the adrenal glands were observed.

Overall, a NO(A)EL of 0.08 mg/kg bw/day is considered for effects of commercial PCB mixtures, and a NOAEL of about 4 mg/kg bw/day for individual NDL PCB congeners.

Dermal effects (including facial oedema, acne, folliculitis, and alopecia) and ocular effects (including swelling and reddening of the eyelid and eyelid discharge) have been observed in monkeys exposed to about 0.1 mg/kg bw/day of Aroclor 1242 or 1248 for 2 months. Fingernail and toenail changes and ocular effects have been observed in monkeys following administration of 0.005 mg/kg bw/day Aroclor 1254 for 37 months. No histopathological changes were observed in the eyes of rats administered Aroclor 1016, 1242, 1254, or 1260 in the diet for 24 months at dose levels up to about 11 mg/kg bw/day. For individual congeners tested in the 13-week oral toxicity studies in rats, no histopathological alterations in the skin, eye or optic nerve were observed.

Overall, a LO(A)EL of 0.005 mg/kg bw/day is considered for dermal and ocular effects of commercial PCB mixtures, and a NOAEL of about 4 mg/kg bw/day for individual NDL PCB congeners.

Anaemia has been observed in monkeys treated with Aroclor 1248 or 1254 at dose levels from 4 mg/kg bw/day for 2 months, from 0.2 mg/kg bw/day for 12-28 months, and at 0.08 mg/kg bw/day of Aroclor 1254 for 37 months; however, in another study, no effects on haematological parameters were observed in monkeys receiving Aroclor 1254 from 0.08 mg/kg bw/day for 72 months. For individual congeners tested in the 13-week oral toxicity studies in rats, decreases in several haematological parameters were observed for PCB 105 at about 4 mg/kg bw/day.

Overall, a NOAEL of 0.08 mg/kg bw/day is considered for haematological effects of commercial PCB mixtures, and a LOAEL of about 4 mg/kg bw/day for individual NDL PCB congeners.

Immunotoxicity of PCBs has been documented in experimental animals. The available data indicate that the immune system of monkeys is more sensitive to PCBs than that of other animal species. Decreased IgM and IgG antibody responses to

sheep red blood cells (SRBC) are the parameters most consistently affected by PCBs in monkeys and have been observed at chronic oral dose levels from 0.005 mg/kg bw/day (the lowest dose level tested) Aroclor 1254. Other effects on the immune system of monkeys including increased susceptibility to bacterial infections and/or histopathological changes in the thymus, spleen, and lymph nodes have been observed at dose levels from 0.1 to 0.3 mg/kg bw/day Aroclor 1248 and 1254 from 238-267 days and up to about 28 months. In the comparative 13-week oral toxicity studies in rats with individual congeners, histopathological lesions were observed in the thymus with PCB 28, 105 and 153 at about 4 mg/kg bw/day.

Overall, a LOAEL of 0.005 mg/kg bw/day is considered for immunotoxic effects of commercial PCB mixtures, and a LOAEL of about 4 mg/kg bw/day for individual NDL PCB congeners.

Neurobehavioural alterations have been observed in rats and monkeys following pre- and/or postnatal exposure. Monkeys exposed from birth to 20 weeks of age with a defined PCB mixture analogous to the congener composition found in human milk showed learning deficits long after exposure had ceased; effects occurred at 0.0075 mg/kg bw/day. Changes in levels of neurotransmitters in various brain areas have also been observed in monkeys, rats and mice; the most consistent result is decreased dopamine concentrations and have been observed in monkeys receiving Aroclor 1016 or 1260 at dose levels from 0.8 mg/kg bw/day in the diet for 20 weeks. In the comparative 13-week oral toxicity studies in rats with individual NDL PCB congeners, decreased dopamine concentrations were observed for PCB 105 at about 4 mg/kg bw/day, for PCB 118 at 0.2 mg/kg bw/day, and for PCB 153 (non-significantly) at 0.034 mg/kg bw/day.

Overall, a LOAEL of 0.0075 mg/kg bw/day is considered for neurotoxic effects following postnatal exposure to PCB mixtures based on the study in monkeys with a defined PCB mixture, and a LOAEL of 0.01 mg/kg bw/day for individual PCB10 congeners based on the 13-week oral toxicity study in rats with PCB 153.

6.6.3 Toxicity to reproduction

Oral studies with animals provide conclusive evidence for reproductive toxicity of commercial PCB mixtures in females of various species and effects include oestrus changes and reduced implantation rate in adult rats and/or their offspring, decreased conception in mice, and menstrual alterations and decreased fertility in monkeys. The monkey is the most sensitive species tested and effects were observed with Aroclor 1254 at dose levels of 0.02 to 0.08 mg/kg bw/day for 37 months before breeding and subsequently throughout mating and gestation (29 months); there were no clear effects on reproduction at 0.005 mg/kg bw/day (the lowest dose level in the study). There is limited evidence for reproductive effects in male adult animals whereas marked effects on morphology and production of sperm, and on fertility have been noted in male offspring of rats exposed to relatively high doses of Aroclor 1254 during gestation and lactation.

There were no significant reproductive effects in rats that were exposed to 4 mg/kg bw/day of a PCB congener mixture simulating the congener content of human milk from 50 days prior to mating until birth.

For individual congeners tested in the 13-week oral toxicity studies in rats, mild changes were observed in the ovaries only following administration of PCB 126 at about 0.009 mg/kg bw/day.

Overall, a NOAEL of 0.005 mg/kg bw/day is considered for reproductive effects of commercial PCB mixtures, and a NOAEL of about 4 mg/kg bw/day for individual PCB10 congeners.

Developmental effects observed in experimental animals support the findings in humans and include neurobehavioural alterations in rats, mice and monkeys; reduced growth in rats and monkeys; changes in the thyroid gland, thyroid hormones and in the reproductive system in rats; and changes in the immune system and clinical effects in monkeys. PCBs are not teratogenic unless very high doses are used. The monkey is the most sensitive species tested and effects were observed in the offspring with Aroclor 1254 at the lowest dose level of 0.005 mg/kg bw/day (clinical signs such as skin, nail, and gum lesions; immunological effects indicated by decreased IgM titers) for 37 months before breeding and

subsequently throughout mating and gestation (29 months). Reduced birth weight, clinical signs (facial acne and hyperpigmentation of the skin), neurobehavioural alterations, and pathological changes in lymphoid tissues (thymus, spleen, and bone marrow) have been observed in offspring from female monkeys with Aroclor 1248 at dietary dose levels from 0.1 mg/kg bw/day Aroclor 1248 for 15 months. Neurobehavioural alterations have been reported in the offspring of rats fed about 1 mg/kg bw/day Aroclor 1254 during gestation and lactation. In a cross-fostering study in rats, exposure *in utero* resulted in neurobehavioural alterations, whereas postnatal-only exposure resulted in no detectable behavioural changes indicating that pre-natal exposure is more critical for neurodevelopmental effects than post-natal exposure. Depressed serum levels of T4 and T3 have been observed in pups born to female rats that were exposed orally to Aroclor 1254 from 0.1 mg/kg bw/day during gestation and lactation.

Reduced birth weight and postnatal weight gain were observed in pups of rats exposed to 4 mg/kg bw/day of a PCB congener mixture (simulating the congener content of human milk) from 50 days prior to mating until birth.

No effects on birth weight or pup weight at weaning, and no significant neurobehavioural alterations were observed in offspring of rats following administration of 0.001 mg/kg bw/day PCB 126 from 5 weeks before and continuing through gestation and lactation; serum T4 levels were depressed in 21-day-old pups, but not in 60-day-old pups.

Overall, a LOAEL of 0.005 mg/kg bw/day is considered for developmental effects of commercial PCB mixtures, and a NOAEL of 0.001 mg/kg bw/day for individual PCB10 congeners based on studies with PCB 126 (a congener which is not included in PCB10).

6.6.4 Mutagenic and genotoxic effects

The genotoxicity of PCBs has been tested in several *in vitro* and *in vivo* studies with generally negative results implying that PCBs induce tumours primarily through modes of action that do not involve gene mutation. Oral exposure to commercial PCB mixtures or to single congeners can promote pre-neoplastic lesions and tumours in the liver of rats and mice following induction with various initiators. Liver toxicity induced by PCBs is well documented in experimental animals exposed to commercial mixtures or single congeners. Microsomal enzyme induction appear to be the most sensitive hepatic effect in rats and have been observed at dose levels from 0.03 mg/kg bw/day for 4 weeks; at higher doses or longer duration exposures, histopathological alterations that progress to non-neoplastic degenerative lesions and/or tumours have been observed.

6.6.5 Carcinogenic effects

A number of oral cancinogenicity studies have been performed in rats with commercial PCB mixtures. The most comprehensive and adequately performed study (published in 1998) revealed increased tumour incidences in the liver and thyroid, while decreased incidences occurred in the mammary gland. The response in the liver was both Aroclor- and sex-dependent (much greater in females than males), consisted primarily of benign tumours, and, for females, increased with dose in the general incidence potency pattern of Aroclor 1254 > Aroclor 1260 > Aroclor 1242 > Aroclor 1016. Previous lifetime dietary studies in rats also reported increased incidences of liver tumours with indications of a sex-dependent response (stronger in females) for commercial mixtures with 60% chlorine content (Aroclor 1260 and Clophen A60).

The interpretation of the carcinogenicity studies with technical mixtures is complicated by the fact that these mixtures contain both dioxin-like and non dioxin-like congeners. Since liver carcinogenicity has been shown in female rats as the dominant carcinogenic effect for the prototype dioxin 2,3,7,8-TCDD,and for the most potent DL-PCB congener PCB 126, the possibility that the liver carcinogenicity of technical PCB mixtures is due to the dioxin-like compounds present in these mixtures was evaluated by EFSA (2005). The results from the chronic carcinogenicity studies with Aroclors in rats indicates that the total TEQ-doses, associated with dioxin-like constituents within the technical mixtures, but not the doses of total PCB, are mainly, if not exclusively, responsible for the development of liver neoplasms.

In a recent long-term toxicity and carcinogenicity study on PCB 153 there was equivocal evidence of carcinogenic activity in female Harlan Sprague-Dawley rats based on the occurrences of a few cholangioma of the liver. The NOAEL was 300 µg PCB 153/kg bw/day administered five days per week for two years corresponding to 210 µg/kg bw/day.

Overall, a carcinogenic potential of PCBs cannot be excluded for humans in relation to dietary exposure to PCB, but is not considered to present a significant risk at dose levels protecting against liver toxicity.

6.7 Evaluation

6.7.1 Critical effects, NOAEL / LOAEL

Effects observed following dietary intake of PCBs include effects on the liver, stomach, thyroid, adrenals, and skin and eyes; on the haematological, immune and nervous systems; carcinogenicity, and reproductive and developmental effects.

For commercial PCB mixtures, a LOAEL of 0.08 mg/kg bw/day is considered for liver effects based on increased liver weight in long-term studies in Rhesus monkeys; biochemical changes indicative of liver effects have been observed at lower dose levels, but are not considered as being adverse effects and thus, 0.005 mg/kg bw/day is a LOEL for effects on the liver. A LOAEL of 0.005 mg/kg bw/day is considered for dermal, ocular, immunotoxic, and developmental effects in long-term studies in Rhesus monkeys.

A NOAEL of 0.08 mg/kg bw/day is considered for effects on stomach tissue, the thyroid, the adrenal glands, and the haematological system, and a NOAEL of 0.005 mg/kg bw/day is considered for reproductive effects in long-term studies in Rhesus monkeys.

A LOAEL of 0.0075 mg/kg bw/day is considered for neurotoxic effects following postnatal exposure of monkeys to a defined PCB mixture analogous to the congener composition found in human milk.

Overall, a LOAEL of 0.005 mg/kg bw/day is considered for adverse health effects of commercial and defined PCB mixtures in monkeys.

For individual NDL-PCB congeners, NOAELs of 0.034-0.07 mg/kg bw/day is considered for liver and thyroid effects. This resulted in body burdens from about 0.4 mg/kg bw (PCB 28) to 1.2 mg/kg bw (PCB 128) in comparative 13-week studies. The NOAELs were 0.034, 0.042, or 0.036 mg/kg bw/day for PCB 28, 128 and 153, respectively. The LOAELs and associated body burdens were in all cases 10 times higher. In the 2-year long-term toxicity and carcinogenicity study on highly purified PCB 153 performed by the NTP (2006) the NOAEL for effects on the liver was 0.070 mg/kg bw/day (body burden 16 mg/kg bw after two years) and the LOAEL was 0.21 mg/kg bw/day (body burden 52 mg/kg bw after two years). In the 28-day study on PCB 180 the NOAEL was estimated at 0.34 mg/kg bw/day with no tissue levels given, but an estimated body burden would be 9.4 mg/kg bw after 28 days. The LOAEL and associated body burden was considered to be three times higher than the NOAEL.

Overall, a NOAEL of 0.035 mg/kg bw/day is considered for adverse health effects of individual NDL-PCB congeners.

A carcinogenic potential of PCBs for humans in relation to dietary exposure cannot be excluded, but is not considered to present a significant risk at dose levels protecting against liver and thyroid toxicity, i.e., at the LOAEL of 0.005 mg/kg bw/day considered for adverse health effects of commercial PCB mixtures.

6.7.2 Tolerable Daily Intake (TDI)

The health-based quality criterion in soil should be set for total-PCB with a correction factor taking into account that the seven PCB congeners typically determined in soil only constitute a few of the PCBs being present in contaminated soil. These seven PCB congeners are PCB 28, 52, 101, 118, 138, 153 and 180, i.e. six NDL-PCBs and one DL-PCB (PCB 118). A correction factor of 5 is used by most investigators. However, it should be noted that based on the analysis performed by Gunnarsen et al. (2009) of a total of 22 detectable PCBs in soil samles from the vicinity of a few (10) selected buildings in Denmark it appears that a factor of 2 would have been more appropriate.

An approach to establish a TDI for the setting of a health-based quality criterion for total-PCB as well as for PCB-7 in soil could be to consider separate assessment of the two types of PCBs in soil: DL-PCBs and NDL-PCBs.

6.7.2.1 Dioxin-like PCBs

Risk assessment of DL-PCBs was an integral part of the SCF (2000, 2001) and JECFA (2002) evaluations of 'dioxins' in food which include PCDDs, PCDFs and DL-PCBs. The evaluation was based on the most sensitive effects of 2,3,7,8-TCDD reported in animals (decreased sperm production and altered sexual behaviour in male offspring of the exposed rat dams). For this effect, the SCF estimated a steady state LOAEL body burden in the dams at 39 ng/kg bw. In order for humans to achieve a LOAEL body burden of 39 ng/kg bw at steady state a daily intake of 20 picogram (pg) 2,3,7,8-TCDD/kg bw would be needed for 30-40 years. The SCF applied a uncertainty factor of 10 to the LOAEL of 20 pg/kg bw/day and established a tolerable weekly intake (TWI) of 14 pg/kg bw for 2,3,7,8-TCDD. This TWI was extended to include all the PCDDs, PCDFs, and DL-PCB that had been allocated 2,3,7,8-TCDD toxic equivalence factors (TEFs) by WHO in 1998 (Van den Berg et al. 1998). The TWI was then expressed as 14 pg TEQs/kg bw/week.

The JECFA (2002) reached similar conclusions as the SCF, but expressed the health guidance value as a provisional monthly tolerable intake (PMTI) of 70 pg/kg bw/month.

In a recent reanalysis of dioxin toxicity the US-EPA also established a LOAEL of 20 pg/kg bw/day for 2,3,7,8-TCDD in humans (US EPA 2012f). The US-EPA carried out a systematic evaluation of the peer-reviewed epidemiological studies and rodent bioassays available relevant to 2,3,7,8-TCDD dose-response analysis and performed dose-response analyses using a 2,3,7,8-TCDD physiologically based pharmacokinetic model that simulates 2,3,7,8-TCDD blood concentrations following oral intake. The LOAEL was based on two epidemiological studies of the Seveso population that had been exclusively exposed to high levels of 2,3,7,8-TCDD. One study associated TCDD exposures with decreased sperm concentration (20%) and sperm motility (11%) in men (22-31 year-old) who were exposed during childhood (1 to 9 year-old) (Mocarelli et al. 2008); the other study associated increased thyroid-stimulating hormone (TSH) levels in newborn infants born to mothers who were exposed to 2,3,7,8-TCDD (Bacarrelli et al. 2008). In the latter study the LOAEL was defined in terms of the maternal 2,3,7,8-TCDD serum level corresponding to neonatal TSH levels above 5 µU/mL of serum. In contrast to the SCF and JECFA the US EPA used an uncertainty factor of 30 to establish an RfD of 0.7 pg/kg bw/day.

The discrepancy between the three organisations is the use of an uncertainty factor of 10 (US-EPA) instead of 3 (SCF and JECFA) for going from a LOAEL to a NOAEL.

In the report 'Evaluation of health hazards by exposure to PCDDs, PCDFs and dioxin-like PCBs' prepared for the Danish EPA (Larsen and Nørhede 2004), the SCF TWI of 14 pg WHO-TEQs/kg bw/week (corresponding to a TDI of 2 pg WHO-TEQ/kg bw/day) / JECFA PTMI of 70 pg WHO-TEQ /kg bw/month (corresponding to a TDI of 2.3 pg WHO-TEQ/kg bw/day) was considered as the health-based guidance value for all the PCDDs, PCDFs, and DL-PCB that had been allocated a TEF value.

According to the REACH Guidance Document, it is suggested to use an assessment factor between 3 (as minimum/majority of cases) and 10 (as maximum/exceptional cases) when the starting point for the DNEL (Derived No Effect Level) calculation is a LOAEL. This is what the SCF/JECFA did. Therefore, the SCF/JECFA TDI of around 2 pg WHO-TEQ/kg bw/day for all the PCDDs, PCDFs, and DL-PCB that had been allocated a TEF value is still considered valid for the present assessment.

6.7.2.2 Non-dioxin-like PCBs

The toxicological evaluation of the NDL-PCBs considered commercial and defined PCB mixtures, and individual NDL-PCB congeners. A LOAEL of 5 µg/kg bw/day is considered for adverse health effects of commercial and defined PCB mixtures (both containing also DL-PCBs and PCDFs considered to be the most important toxic constituents) and a NOAEL of 35 µg/kg bw/day for individual NDL-PCB congeners.

The comprehensive toxicological database on health effects of technical PCB mixtures is not suitable for a separate assessment of NDL-PCB, and the human data on exposure to environmental mixtures containing PCB do also not allow for distinguishing between effects of NDL- and DL-PCB and PCDD/PCDF either. Therefore, EFSA

(2005) performed an assessment based on the toxicological information available for individual NDL-PCB congeners.

Although the absence of mutagenicity indicates that a threshold approach is appropriate for the hazard characterisation, the toxicological database was considered to be too limited to allow the establishment of a health based guidance value for NDL-PCB. Therefore the health risk characterisation for PCBs in food was performed by EFSA (2005) on the basis of a margin of exposure approach. In the following the hazard characterisation is updated to also include newer relevant study information.

As a provisional approach to the assessment of the effects of exposure to NDL-PCBs, it is assumed that all the NDL-PCBs in food have toxicological potencies similar to PCB 28, 128, and 153, and that the effects for the individual PCB congeners are additive. An overall NOAEL for liver and thyroid toxicity of NDL-PCB of 35 µg/kg bw per day as found in the 90-day rat studies, would provide a margin of exposure ranging from 800 to 4,000 when compared with the estimated daily intake of total NDL-PCB by humans (10-45 ng/kg bw per day). Such a margin of exposure would appear to be comfortable for most non-genotoxic compounds, when based on sub-chronic and chronic studies.

However, for compounds that accumulate in the body, such as the toxicologically most significant NDL-PCBs, it is considered, by analogy to the risk assessments of PCDD, PCDF and DL-PCBs, that the body burdens (BB) in experimental animals and humans would be more appropriate dose metrics for the assessment of NDL-PCBs.

The NDL-PCBs found in human milk are the congeners that accumulate in the human body: PCB 18, 28, 33, 37, 52, 60, 66, 74, 99, 101, 110, 128, 138, 141, 153, 170, 180, 183, 187, 194, 206, and 209. The median total concentration of all the NDL-PCB measured in human milk was about 240 ng/g fat, which would correspond to an estimated median human body burden of about 48 µg/kg bw, assuming 20% fat content in the human body.

The estimated median human BB was also calculated for each congener from the median lipid-based contents reported for human milk (see Table in Appendix 3). The table also includes the NOAELs and/or LOAELs for various toxicological effects, derived from the experimental studies on individual NDL-PCB described in Appendix 2 as well as the corresponding estimated animal body burdens NOAEL BB and/or LOAEL BB. These body burdens were estimated as follows: In the case of the sub-chronic feeding studies on PCB 28, 128, 153, and the chronic toxicity and carcinogenicity study on PCB 153 the body burdens were calculated from the reported measured accumulated concentrations of the respective NDL-PCB in the fat tissue of the rats, assuming that the rats contained 10% fat as reported by Geyer et al. (1990). For the 28-day toxicity study on PCB 180 the body burdens at study termination were calculated assuming that no PCB 180 had been eliminated.

From studies where only a single dose was administered, this dose was considered equal to the body burden, assuming 100% bioavailability, irrespective of the route of administration. In the studies using more than one day of administration, the body burden at study termination was estimated assuming 100% bioavailability and one compartment, first order kinetics, using the half-lives in rats reported for individual NDL-PCB congeners by Tanabe et al. (1981).

Finally, a comparison is given of the estimated body burden at the NOAEL or LOAEL in animals with the estimated median human body burden, expressed as the margin of body burden (MoBB) by dividing the estimated animal body burdens with the estimated median human body burden.

For the NOAELs in the 90-day sub-chronic rat studies on PCB 28 (36 µg/kg bw per day), PCB 128 (43 µg/kg bw per day) and PCB 153 (34 µg/kg bw per day), the long-term toxicity and carcinogenicity study on PCB-153 (70 µg/kg bw per day), for effects on liver and thyroid, and the 28-day toxicity study on PCB-180 (340 µg/kg bw per day) for effects on the liver, and using the reported accumulated concentrations of the respective NDL PCB in the fat tissue, corresponding BB of 400, 800, 1200, 16,000 and 9,400 µg/kg bw respectively, were calculated. Comparison of these BB with the estimated human BB for these congeners results in NOAEL MoBBs of 900, 6,500, 85, 1,200 and 1025 for PCB 28, 128, 153, 153 and 180 respectively (Table in Appendix 3). Although PCB 28, 128, 153, and 180 showed similar potencies based on the daily doses in the toxicological studies, PCB 153 have by far the lowest MoBB due to its higher abundance in human tissues.

For the NDL-PCBs tested (PCB 18, 28, 52, 101, 110, 138, 153, and 180) for reproductive and developmental effects, oestrogenicity, thyroid effects and effects on the immune system and the developing nervous system in rats, the NOAEL-MoBBs were consistently higher than 1,000.

The most sensitive effects seen in the studies with NDL-PCB in experimental animals are liver and thyroid toxicity. The NOAELs in 90-day rat studies were in the range of 30-40 µg/kg bw per day. The effects seen in these studies occurred at considerably lower external dose levels than many other effects observed in studies of shorter duration with different NDL-PCB. However, when a comparison is made on the basis of estimated body burdens it appears that the NOAELs for all these effects are found at rather similar body burdens, ranging from about 400-1200 µg/kg bw or higher.

The available toxicological database on NDL-PCBs covers a range of the possible congeners that accumulate in the human body. In considering that the LOAELs for the most sensitive effects were 3-10 times higher than the NOAELs, it can be suggested, that an overall body burden of 500 µg/kg bw is a representative, conservative NOAEL BB (body burden at the NOAEL) for the sum of all individual NDL-PCBs occurring in human tissues.

The median total concentration of all NDL-PCBs measured in human milk was about 240 ng/g fat, which would, assuming 20% fat content in the human body, correspond to an estimated median human body burden of about 48 µg/kg b.w (EFSA 2005). Consequently a MoBB of about 10 to the NOAEL BB can be calculated.

It should be stressed, however, that thyroid and liver toxicity in rats can also be observed after treatment with PCDD/F or DL-PCB. Since a number of these compounds exhibit relatively high potencies for these effects in rats minor levels of potent dioxin-like contaminants (in the range of 0.1%) in the NDL-PCB test preparations might be sufficient to explain the effects observed. Thus, any estimate of a NOAEL for NDL-PCB is hampered by the uncertainty as to what extent the individual congeners were contaminated with PCDF and/or DL-PCB.

It is therefore concluded, in agreement with the EFSA Panel (EFSA 2005), that no health based guidance value (TDI / TWI / PTMI) for humans can be established for the NDL-PCBs because simultaneous exposure to NDL-PCB and dioxin-like compounds hampers the interpretation of the results of the toxicological and epidemiological studies.

6.7.2.3 Considerations on the toxicological significance of PCBs in soil

The representative NOAEL BB (body burden at the NOAEL) of 500 µg/kg bw for the sum of the individual NDL-PCBs occurring in human tissues, which are the most toxic and bioacumulative congeners among the NDL-PCBs, can be extended, as a conservative approach, to cover the sum of the NDL-PCBs in environmental media such as soil.

PCBs, particularly the highly chlorinated congeners, adsorb strongly to soil particles and both oral and dermal absorption from soil is therefore considered to be lower than the absorption of PCB from food. For the purpose of these considerations the oral bioavailability of PCBs from soil is assumed to be 50%. The dermal bioavailability is estimated to be only 5%, and is not further considered.

In order to reach a steady state body burden[4] of 500 µg/kg bw NDL-PCBs in humans a daily intake of 250 ng/kg bw/day of NDL-PCBs from soil is needed for a period of 30 – 40 years, assuming 50% oral bioavailability and one compartment, and an average elimination half-life of 7.5 years. The half-lives in humans may vary considerably between the NDL-PCBs, however, for consistency the half-life of 7.5 years used in the risk assessments of PCDDs, PCDFs, and DL-PCBs was chosen. Therefore a NOAEL of 250 ng/kg bw/day can be suggested for the sum of NDL-PCBs in soil.

Burkhard and Lukasewycz (2008) reported that the 2,3,7,8-TCDD equivalent (WHO 2005 TEQ) content in a large number of commercial PCB mixtures varied from 0.034 µg/g (in Aroclor 1221) to 11.8 µg/g (in Aroclor 1248), whereas EFSA (2005) calculated 40 µg TEQ/g (WHO 1998) in an Aroclor 1254 thought to be representative for the

4 Steady state body burden (ng/kg bw) = intake (ng/kg bw/day) x T½ (days) / ln2 x f
T½ = elimination half-life – set at 7½ year for persistent dioxins, DL-PCBs and NDL-PCBs
f = bioavailability – set at 1 / 0.5 for food and soil, respectively, for persistent dioxins, DL-PCBs and NDL-PCBs

Aroclor 1254 that had been in commercial use. If, as a conservative scenario, it is assumed that the concentration of TEQs in "aged" total PCBs in soil can be as high as 40 TEQ µg/g an intake of 250 ng total PCB/kg bw/day would result in an intake of 5 pg TEQ/kg bw. This is above the "TDI" of 2 pg TEQ/kg bw established by the SCF (2001).

However, the general population is mainly exposed to PCBs from food and the estimated average dietary exposures to PCB (sum of 10) in Denmark are estimated at 12.5 and 25 ng/kg bw/day for children and adults, respectively (Fromberg et al. 2010). This provides average margins of exposures (MOE) of 20 and 10 respectively, to the estimated NOAEL of 250 ng/kg bw/day. Such MOEs are considered sufficient when based on a body burden approach.

The average body burden in Danish adults is not known, but it may be assumed that it is similar to the "European" body burden of approximately 50 µg/kg bw estimated by the EFSA (2005). It is this (tolerable) body burden that should not be significantly exceeded in adult people including pregnant women in order to protect against detrimental effects of PCBs.

The following considerations are based on the PCB 7 content in soil samples. Any decisions on correction factors to estimate total PCB concentrations are outside the expertise of the authors of this evaluation.

For ease of calculation, it is assumed that a typical concentration of PCB 7 in soil in Denmark is 0.5 mg/kg soil. If it is anticipated that a child having a body weight of 10 kg will ingest 0.1 g soil per day, a background level of 0.5 mg/kg soil will provide an additional intake of 5 ng/kg bw/day of which 2.5 ng/kg bw/day would be orally bioavailable. (When using a conversion factor of 2 to convert PCB 7 concentrations into total PCB levels, this would correspond to 5 ng/kg bw/day of bioavailable PCBs; the following figures in brackets represent the total PCB levels). This is 10% (20%) of the estimated daily exposure to total PCBs from food. Intake of 2.5 (5) ng/kg bw/day "aged" PCB from soil would result in a TEQ exposure of 0.05 (0.1) pg/kg bw/day which can be considered to be without any dioxin-like toxicity when compared to the TWI of 14 pg TEQ/kg bw/week.

If this additional intake of PCBs from soil continues for 30-40 years (which is highly unlikely), this would result in an additional steady state body burden of 5 (10) µg/kg bw PCB/kg bw on top of the body burden of 50 µg/kg bw obtained from mainly food intake, resulting in a total body burden of 55 (60) µg/kg bw.

These scenaria shows that the contribution from soil to the intake of PCB7 will have a minor impact on the daily intake of PCBs by children and on the PCB body burden in adulthood.

Using the NOAEL BBs for NDL-PCBs as described above, the following calculation can be made: For children who ingest 0.1 g of soil containing 0.5 mg PCB 7/kg daily for two years (2.5 ng bioavailable PCB7/kg bw/day) the body burden can be (conservatively) estimated to reach approximately 18 µg/kg bw assuming no elimination and no weight gain. This provides a MOE BB of approximately 30 to the NOAEL BB of 500 µg/kg bw.

7 Health-based quality criterion

The health-based quality criteria for chemicals in soil are primarily aimed at protecting children who may ingest soil or come into dermal contact with the soil.

PCBs, particularly the highly chlorinated congeners, adsorb strongly to soil particles and both oral and dermal absorption from soil is therefore considered to be lower than the absorption of PCB from food. For the purpose of this assessment the oral bioavailability of PCBs from soil is assumed to be 50% whereas the dermal bioavailability is considered to be 5% as a maximum conservative figure.

The critical effects seen in experimental animals and used in the derivation of a NOAEL for the NDL-PCBs and the TWI for the DL-PCBs (and PCDDs and PCDFs) are related to the body burden in the adult. In order to reach a steady state body burden for the critical effects to occur in humans a daily intake is needed for a period of 30-40 years assuming an elimination half-life of 7.5 years. Therefore, elevated exposures within a shorter time period, i.e. in toddlers ingesting PCB contaminated soil for a short period in their life, will not have any significant impact on the overall steady state body burden obtained after 30-40 years.

The general population is mainly exposed to PCBs from food and the major contribution to the body burden (> 90%) of PCBs comes from the dietary exposure. EFSA has provided estimates of the contribution from soil to the daily exposure of the general population to PCB; the contribution from ingested soil or dust particles, particularly by children, was considered to be small.

In conclusion, a health-based soil quality criterion based on children's ingestion of soil contaminated with PCBs is not considered to be relevant. This conclusion is in agreement with the conclusion in the report 'Evaluation of health hazards by exposure to PCDDs, PCDFs and dioxin-like PCBs' prepared for the Danish EPA (Larsen and Nørhede 2004).

The following scenario may serve to illustrate this:

The general population is mainly exposed to PCBs from food and the average intake by adults and children in Denmark has been estimated at 12.5 and 25 ng/kg bw/day (sum of 10 PCBs), respectively (Fromberg et al. 2010). The average body burden in Danish adults is not known, but it may be assumed that it is similar to the "European" body burden of approximately 50 µg/kg bw estimated by the EFSA (2005). It is this (tolerable) body burden that should not be significantly exceeded in the adult people including pregnant women in order to protect against detrimental effects of PCBs.

For ease of calculation, a concentration of PCB 7 in soil in Denmark of 0.1 mg/kg soil is assumed (according to information from the Danish EPA, the concentration of PCB 7 in soil seldom will exceed 0.5 mg/kg soil and very rarely 1 mg/kg soil, see Section 1.5.3 last paragraph) and that the bioavailability of PCBs from soil is 50% (and 100% from food). If it is anticipated that a child having a body weight of 10 kg will ingest 0.1 g soil per day, a background level of 0.1 mg/kg soil will provide an additional intake of 1 ng/kg bw/day of which 0.5 ng/kg bw/day would be bioavailable. This is 2% of the estimated daily intake of PCB with food (and 70,000 times lower than the NOAELs for NDL-PCBs). If this additional intake of PCBs from soil continues for 30-40 years (which is highly unlikely), this would result in an additional steady state body burden[5] of 2 µg/kg bw PCB/kg bw on top of the body burden of 50 µg/kg bw obtained from mainly food intake, resulting in a total body burden of 52 µg/kg bw.

This scenario shows that the contribution from soil to the intake of PCBs for young children during

5 Steady state body burden (ng/kg bw) = intake (ng/kg bw/day) x T½ (days) / ln2 x f
T½ = elimination half-life – set at 7½ year for persistent dioxins, DL-PCBs and NDL-PCBs
f = bioavailability – set at 1 / 0.5 for food and soil, respectively, for persistent dioxins, DL-PCBs and NDL-PCBs

their two first years of life will have a negligible impact on the daily intake of PCB by children and on the PCB body burden in adulthood.

7.1.1. Health-based quality criterion in soil

A health-based soil quality criterion based on children's ingestion of soil contaminated with PCBs is not considered to be relevant.

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Appendix 1

Chemical identity of polychlorinated biphenyl congeners

The congeners are arranged in ascending numerical order using a numbering system developed by Ballschmiter and Zell (1980) that follow the IUPAC rules of substituent characterization in biphenyls. The resulting PCB numbers, also referred to as congener, IUPAC, or BZ numbers, are widely used for identifying individual congeners (ATSDR 2000).

PCB No.	Structure	CAS No.
	Biphenyl	92-52-4
	Monochlorobiphenyl	27323-18-8
1	2	2051-60-7
2	3	2051-61-8
3	4	2051-62-9
	Dichlorobiphenyl	25512-42-9
4	2,2’	13029-08-8
5	2,3	16605-91-7
6	2,3’	25569-80-6
7	2,4	33284-50-3
8	2,4’	34883-43-7
9	2,5	34883-39-1
10	2,6	33146-45-1
11	3,3’	2050-67-1
12	3,4	2974-92-7
13	3,4’	2974-90-5
14	3,5	34883-41-5
15	4,4’	2050-68-2
	Trichlorobiphenyl	25323-68-6
16	2,2’,3	38444-78-9
17	2,2’,4	37680-66-3
18	2,2’,5	37680-65-2
19	2,2’,6	38444-73-4
20	2,3,3’	38444-84-7
21	2,3,4	55702-46-0
22	2,3,4’	38444-85-8
23	2,3,5	55720-44-0
24	2,3,6	55702-45-9
25	2,3’,4	55712-37-3
26	2,3’,5	38444-81-4
27	2,3’,6	38444-76-7
28	2,4,4’	7012-37-5
29	2,4,5	15862-07-4

PCB No.	Structure	CAS No.
30	2,4,6	35693-92-6
31	2,4',5	16606-02-3
32	2,4',6	38444-77-8
33	2',3,4	38444-86-9
34	2',3,5	37680-68-5
35	3,3',4	37680-69-6
36	3,3',5	38444-87-0
37	3,4,4'	38444-90-5
38	3,4,5	53555-66-1
39	3,4',5	38444-88-1
	Tetrachlorobiphenyl	26914-33-0
40	2,2',3,3'	38444-93-8
41	2,2',3,4	52663-59-9
42	2,2',3,4'	36559-22-5
43	2,2',3,5	70362-46-8
44	2,2',3,5'	41464-39-5
45	2,2',3,6	70362-45-7
46	2,2',3,6'	41464-47-5
47	2,2',4,4'	2437-79-8
48	2,2',4,5	70362-47-9
49	2,2',4,5'	41464-40-8
50	2,2',4,6	62796-65-0
51	2,2',4,6'	68194-04-7
52	2,2',5,5'	35693-99-3
53	2,2',5,6'	41464-41-9
54	2,2',6,6'	15968-05-5
55	2,3,3',4	74338-24-2
56	2,3,3',4'	41464-43-1
57	2,3,3',5	70424-67-8
58	2,3,3',5'	41464-49-7
59	2,3,3',6	74472-33-6
60	2,3,4,4'	33025-41-1
61	2,3,4,5	33284-53-6
62	2,3,4,6	54230-22-7
63	2,3,4',5	74472-35-8
64	2,3,4',6	52663-58-8
65	2,3,5,6	33284-54-7
66	2,3',4,4'	32598-10-0
67	2,3',4,5	73575-53-8
68	2,3',4,5'	73575-52-7
69	2,3',4,6	60233-24-1
70	2,3',4',5	32598-11-1
71	2,3',4',6	41464-46-4
72	2,3',5,5'	41464-42-0
73	2,3',5',6	74338-23-1

PCB No.	Structure	CAS No.
74	2,4,4',5	32690-93-0
75	2,4,4',6	32598-12-2
76	2',3,4,5	70362-48-0
77 [DL]	3,3',4,4'	32598-13-3
78	3,3',4,5	70362-49-1
79	3,3',4,5'	41464-48-6
80	3,3',5,5'	33284-52-5
81 [DL]	3,4,4',5	70362-50-4
	Pentachlorobiphenyl	25429-29-2
82	2,2',3,3',4	52663-62-4
83	2,2',3,3',5	60145-20-2
84	2,2',3,3',6	52663-60-2
85	2,2',3,4,4'	65510-45-4
86	2,2',3,4,5	55312-69-1
87	2,2',3,4,5'	38380-02-8
88	2,2',3,4,6	55215-17-3
89	2,2',3,4,6'	73575-57-2
90	2,2',3,4',5	68194-07-0
91	2,2',3,4',6	68194-05-8
92	2,2',3,5,5'	52663-61-3
93	2,2',3,5,6	73575-56-1
94	2,2',3,5,6'	73575-55-0
95	2,2',3,5',6	38379-99-6
96	2,2',3,6,6'	73575-54-9
97	2,2',3',4,5	41464-51-1
98	2,2',3',4,6	60233-25-2
99	2,2',4,4',5	38380-01-7
100	2,2',4,4',6	39485-83-1
101	2,2',4,5,5'	37680-73-2
102	2,2',4,5,6'	68194-06-9
103	2,2',4,5',6	60145-21-3
104	2,2',4,6,6'	56558-16-8
105 [DL]	2,3,3',4,4'	32598-14-4
106	2,3,3',4,5	70424-69-0
107	2,3,3',4',5	70424-68-9
108	2,3,3',4,5'	70362-41-3
109	2,3,3',4,6	74472-35-8
110	2,3,3',4',6	38380-03-9
111	2,3,3',5,5'	39635-32-0
112	2,3,3',5,6	74472-36-9
113	2,3,3',5',6	68194-10-5
114 [DL]	2,3,4,4',5	74472-37-0
115	2,3,4,4',6	74472-38-1
116	2,3,4,5,6	18259-05-7
117	2,3,4',5,6	68194-11-6

PCB No.	Structure	CAS No.
118 [DL]	2,3',4,4',5	31508-00-6
119	2,3',4,4',6	56558-17-9
120	2,3',4,5,5'	68194-12-7
121	2,3',4,5',6	56558-18-0
122	2',3,3',4,5	76842-07-4
123 [DL]	2',3,4,4',5	65510-44-3
124	2',3,4,5,5'	70424-70-3
125	2',3,4,5,6'	74472-39-2
126 [DL]	3,3',4,4',5	57465-28-8
127	3,3',4,5,5'	39635-33-1
	Hexachlorobiphenyl	26601-64-9
128	2,2',3,3',4,4'	38380-07-3
129	2, 2',3,3',4,5	55215-18-4
130	2,2',3,3',4,5'	52663-66-8
131	2,2',3,3',4,6	61798-70-7
132	2,2',3,3',4,6'	38380-05-1
133	2,2',3,3',5,5'	35694-04-3
134	2,2',3,3',5,6	52704-70-8
135	2,2',3,3',5,6'	52744-13-5
136	2,2',3,3',6,6'	38411-22-2
137	2,2',3,4,4',5	35694-06-5
138	2,2',3,4,4',5'	35065-28-2
139	2,2',3,4,4',6	56030-56-9
140	2,2',3,4,4',6'	59291-64-4
141	2,2',3,4,5,5'	52712-04-6
142	2,2',3,4,5,6	41411-61-4
143	2,2',3,4,5,6'	68194-15-0
144	2,2',3,4,5',6	68194-14-9
145	2,2',3,4',6,6'	74472-40-5
146	2,2',3,4',5,5'	51908-16-8
147	2,2',3,4',5,6	68194-13-8
148	2,2',3,4',5,6'	74472-41-6
149	2,2',3,4',5',6	38380-04-0
150	2,2',3,4',5,6'	68194-08-1
151	2,2',3,5,5',6	52663-63-5
152	2,2',3,5,6,6'	68194-09-2
153	2,2',4,4',5,5'	35065-27-1
154	2,2',4,4',5,6'	60145-22-4
155	2,2',4,4',6,6'	33979-03-2
156 [DL]	2,3,3',4,4',5	38380-08-4
157 [DL]	2,3,3',4,4',5'	69782-90-7
158	2,3,3',4,4',6	74472-42-7
159	2,3,3',4,5,5'	39635-35-3
160	2,3,3',4,5,6	41411-62-5
161	2,3,3',4,5',6	74472-43-8

PCB No.	Structure	CAS No.
162	2,3,3',4',5,5'	39635-34-2
163	2,3,3',4',5,6	74472-44-9
164	2,3,3',4',5',6	74472-45-0
165	2,3,3',5,5',6	74472-46-1
166	2,3,4,4',5,6	41411-63-6
167 [DL]	2,3',4,4',5,5'	52663-72-6
168	2,3',4,4',5',6	59291-65-5
169 [DL]	3,3',4,4',5,5'	32774-16-6
	Heptachlorobiphenyl	28655-71-2
170	2,2',3,3',4,4',5	35065-30-6
171	2,2',3,3',4,4',6	52663-71-5
172	2,2',3,3',4,5,5'	52663-74-8
173	2,2',3,3',4,5,6	68194-16-1
174	2,2',3,3',4,5,6'	38411-25-5
175	2,2',3,3',4,5',6	40186-70-7
176	2,2',3,3',4,6,6'	52663-65-7
177	2,2',3,3',4',5,6	52663-70-4
178	2,2',3,3',5,5',6,	52663-67-9
179	2,2',3,3',5,6,6'	52663-64-6
180	2,2',3,4,4',5,5'	35065-29-3
181	2,2',3,4,4',5,6	74472-47-2
182	2,2',3,4,4',5,6'	60145-23-5
183	2,2',3,4,4',5',6	52663-69-1
184	2,2',3,4,4',6,6'	74472-48-3
185	2,2',3,4,5,5',6	52712-05-7
186	2,2',3,4,5,6,6'	74472-49-4
187	2,2',3,4',5,5',6	52663-68-0
188	2,2',3,4',5,6,6'	74487-85-7
189 [DL]	2,3,3',4,4',5,5'	39635-31-9
190	2,3,3',4,4',5,6	41411-64-7
191	2,3,3',4,4',5',6	74472-50-7
192	2,3,3',4,5,5',6	74472-51-8
193	2,3,3',4',5,5',6	69782-91-8
	Octachlorobiphenyl	31472-83-0
194	2,2',3,3',4,4',5,5'	35694-08-7
195	2,2',3,3',4,4',5,6	52663-78-2
196	2,2',3,3',4,4',5,6'	42740-50-1
197	2,2',3,3',4,4',6,6'	33091-17-7
198	2,2',3,3',4,5,5',6	68194-17-2
199	2,2',3,3',4,5,5',6'	52663-75-9
200	2,2',3,3',4,5,6,6'	52663-73-7
201	2,2',3,3',4,5',6,6'	40186-71-8
202	2,2',3,3',5,5',6,6'	2136-99-4
203	2,2',3,4,4',5,5',6	52663-76-0
204	2,2',3,4,4',5,6,6'	74472-52-9

PCB No.	Structure	CAS No.
205	2,3,3',4,4',5,5',6	74472-53-0
	Nonachlorobiphenyl	53742-07-7
206	2,2',3,3',4,4',5,5',6	40186-72-9
207	2,2',3,3',4,4',5,6,6'	52663-79-3
208	2,2',3,3',4,5,5',6,6'	52663-77-1
	Decachlorobiphenyl	2051-24-3
209	2,2',3,3',4,4',5,5',6,6'	2051-24-3

[DL] Dioxin-like PCBs, see section xxx for further explanation.

Appendix 2

Toxicological studies *in vivo* on individual NDL-PCB congeners

In the following brief summaries are given of toxicological studies performed with individual NDL-PCB in experimental animals. Indication is also given whether the specific NDL-PCB has been detected in human milk according to EFSA (2005).

PCB 1 (2-chlorobiphenyl), PCB 2 (3-chlorobiphenyl), PCB 3 (4 chlorobiphenyl), PCB 4 (2,2'-dichlorobiphenyl), PCB 8 (2,4'-dichlorobiphenyl), PCB 11 (3,3'-dichlorobiphenyl), and PCB 15 (4,4')

These PCB congeners were not found as contaminants in human milk (EFSA 2005).

Estrogenic effects
Groups of 8-17 immature female Wistar rats were given a single intraperitoneal injection of 8 mg of the above-mentioned PCBs, corresponding to 160 mg/kg bw PCB 1 and PCB 4 increased the uterine weights of the rats, whereas the others had no effect (Ecobichon and MacKenzie 1974).

PCB 18 (2,2',5-trichlorobiphenyl)

PCB 18 is a minor contaminant in human milk (EFSA 2005). A median concentration of 0.09 ng PCB 18/g fat in human milk would correspond to an estimated median human body burden of 0.018 µg/kg bw assuming that the human body contains 20% fat.

Tanabe et al. (1981) reported the elimination half-life in male Wistar rats to be 0.18 days. However, Saghir et al. (1999) using intravenous administration of radiolabelled PCB 18 found elimination half-lives of 37.5 and 49.2 hours for radioactivity in pre-pubertal male and female rats, respectively.

Estrogenic effect and effect on liver enzymes and the thyroid
Groups of 4-5 pre-pubertal female SD rats were injected intraperitoneally with PCB 18 after weaning on days 20 and 21 of life at dose levels of 0, 8, 32, or 64 mg/kg bw per day (total doses 0, 16, 64, or 128 mg/kg bw). On day 22, a significant increase was seen in the relative uterus weights in all dosed groups. The LOAEL was 8 mg/kg bw per day. PCB 18 was a very weak inducer of liver pentoxyresorufin O-deethylase (PROD) acitivity, but had no effect on ethoxyresorufin O-deethylase (EROD) activity and serum thyroxine (T4) levels (Li and Hansen 1995).

Assuming an elimination half.life of 2 days in the pre-pubertal female rat, the LOAEL body burden for estrogenic effect in the female rat would be 16 mg/kg bw and the NOAEL body burden for effect on serum thyroxine (T4) would be 128 mg/kg bw.

PCB 28 (2,4,4'-trichlorobiphenyl)

PCB 28 is a contaminant in human milk (EFSA 2005). A median concentration of 2.2 ng PCB 28/g fat in human milk would correspond to an estimated median human body burden of 044 µg/kg bw assuming that the human body contains 20% fat.

Tanabe et al. (1981) reported first and second phase elimination half-lives in male Wistar rats to be 1.4 days and 6 days, respectively.

Effects on reproduction and neurobehavioral development (learning)
Male NMRI mice received a single oral dose of 0.18, 0.36, or 3.6 mg PCB 28/kg bw on postnatal day 10 and were examined for changes in spontaneous motor behaviour, learning, memory, and brain biochemistry after 4 months. The highest dose changed the spontaneous motor behaviour but did not affect learning and memory. PCB 28 did not affect brain muscarinic- and nicotinic receptors and biogenic amines. Thus, the NOAEL was 0.36 mg/kg bw and the LOAEL was 3.6 mg/kg bw (Eriksson and Fredriksson 1996a).

Groups of time-mated Sprague-Dawley rats were exposed to PCB 28, 8 or 32 mg/kg bw per day; or corn oil vehicle via gavage on Gestation Days 10-16. Per group, 6-9 litters were culled to eight on Day 2 and weaned on Day 21. At weaning, no effects were seen on serum thyroxine (T4) or triiodothyronine (T3) in pups and dams. In a histological evaluation of the thyroids, no effects of PCB 28 were noted. Decreased birth weight and weight gain was observed in female pups from the dams given 32 mg/kg bw per day. The NOAEL was 8 mg/kg bw per day (Ness et al. 1993).

In the same offspring spatial learning and memory was assessed on a working/reference memory task on an eight-arm maze beginning on Day 90, for seven consecutive weeks. No differences in working or reference memory errors were observed. The same animals were later tested on a T arm-maze in a delayed spatial alternation (DSA) task. At 32 mg/kg bw per day a slower acquisition was seen for female rats. Males were not affected. The NOAEL was 8 mg/kg bw per day (Schantz et al. 1995).

In a similarly designed study, but using PCB 77 (3,3',4,4'-tetrachlorobiphenyl), 2 or 8 mg/kg/day; PCB 126 (3,3',4,4',5-pentachlorobiphenyl), 0.25 or 1.0 µg/kg bw per day or TCDD (2,3,7,8-tetrachloro-p-dioxin), 0.025 or 0.1 µg/kg bw per day it was shown that the TCDD-exposed rats displayed pronounced decreases in errors relative to controls in the eight arm maze. PCB 77- and PCB 126-exposed rats showed similar, but less pronounced, decreases in errors. The same animals were later tested on a T-maze DSA task, but no differences among groups were observed. These findings suggest that coplanar and ortho-substituted PCBs may have different mechanisms of action on the central nervous system (Schantz et al. 1996).

Short-term toxicity

Groups of 10 male and 10 female weanling Sprague-Dawley rats were administered PCB 28 in the diet at 0, 0.05, 0.50, 5.0, or 50.0 mg/kg for 13 weeks. (0, 2.8, 36, 359, or 3783 µg/kg/day in males and 2.9, 37, 365, or 3856 µg/kg/day in females). Growth rate and food consumption were not affected by treatment, and no clinical signs of toxicity were observed. Increased urinary ascorbic acid and hepatic microsomal ethoxyresorufin O-deethylase (EROD) activity were observed in the 50.0 mg/kg diet group of both sexes. The vitamin A content in liver, lung, and kidney was not significantly affected by treatment. Analysis of brain biogenic amines showed a decreased dopamine concentration in the substantia nigra region of female rats receiving 0.5 mg PCB 28/kg diet and higher doses. Female rats appeared to be more sensitive than males to the neurochemical effects of PCB 28, which was considered to be week. Dose-dependent histological changes were observed in the thyroid and liver, with biologically significant changes occurring at 5.0 mg/kg diet and above. Based on these data, the no observable-adverse-effect level (NOAEL) for PCB 28 was considered to be 0.5 mg/kg in diet equal to 36 µg/kg bw per day (Chu et al. 1996a). Based on a measured content of PCB 28 in fat of female rats of 4.0 mg/kg a body burden of 0.4 mg/kg bw can be estimated assuming that a rat contains 10% fat (Geyer et al. 1990). At the LOAEL the concentration in fat was 40 mg/kg bw corresponding to a body burden of 4 mg/kg bw.

Several special studies on these male and female rats administered PCB 28 in the diet at 0, 0.05, 0.50, 5.0, or 50.0 mg/kg for 13 weeks have been reported.

In one study, effects on luteinizing hormone, follicle-stimulating hormone, and testosterone concentrations were studied in the male rats, as well as the levels of thyroid-stimulating hormone, thyroxine (T4) and uridine diphosphate-glucuronyl transferase (UDP-GT) activity in both genders were examined. The only effect observed was a tendency for the highest dose of PCB 28 to decrease serum T4 concentrations in the female rats (Desaulniers et al. 1997).

In another study, electron microscopy of liver specimens revealed hepatocyte architectural modifications, which included an augmentation of SER profiles and an elevation of peroxisome numbers in animals regardless of gender, and mitochondrial abnormalities in the females only (abnormal shapes and cristae in atypical orientation). The alterations were seen in animals of the 5-and 50- mg/kg diet groups and were more extensive in the females. Ethoxyresorufin-O-de-ethylase (EROD) activity was significantly higher in the animals of the 50-mg/kg diet group. The results suggest that the female rats were more sensitive than the males to PCB 28, and the NOAEL was considered to be 0.5 mg/kg in the diet equal to 36 µg/kg bw per day (Singh et al. 1996). Use of transmission electron microscopy and stereology

revealed significant elevations in the mean volume fraction (VF) of liver smooth endoplasmic reticulum (SER) profiles in the female Sprague-Dawley rats administered PCB 28 via the diets 13 weeks in concentrations of 5 and 50 mg/kg diet. Also, the hepatocytes of the male rats contained a significantly greater baseline VF of SER compared to those of the females. No effect was seen after 0.05 or 0.5 mg/kg diet. Statistically significant alterations were not detected in mitochondria, rough endoplasmic reticulum, peroxisomes or lipid droplets. The NOAEL was 0.5 mg/kg diet, equal to 36 µg/kg bw per day (Connel et al. 2001).

PCB 33 (2,3',4'-trichlorobiphenyl)

PCB 33 is a minor contaminant in human milk (EFSA 2005). A median concentration of 0.06 ng PCB 33/g fat in human milk would correspond to an estimated median human body burden of 0.012 µg/kg bw assuming that the human body contains 20% fat.

No *in vivo* toxicological data are available on PCB 33.

PCB 37 (3,4,4'-trichlorobiphenyl)

PCB 37 is a minor contaminant in human milk (EFSA 2005). A median concentration of 12.7 pg PCB 37/g fat in human milk would correspond to an estimated median human body burden of 0.0025 µg/kg bw assuming that the human body contains 20% fat.

No *in vivo* toxicological data are available on PCB 37.

PCB 47 (2,4,2',4'-tetrachlorobiphenyl)

PCB 47 was not found as a minor contaminant in human milk (EFSA 2005).

Tanabe et al. (1981) reported the elimination half-life in male Wistar rats to be 3 days. For mice an elimination half-life of 9.2 days was cited by Hany et al. (1999).

Effects on reproduction and development, neurotransmitters, thyroid, and neurobehavioral development

Pregnant Sprague-Dawley dams were administered PCB 47 in the diet at dose levels of 1, 10, or 20 mg/kg bw per day from gestational day 6 through weaning. No effects were seen on weight of dams or pups, and on number of pups per litter. Male and female offspring were sacrificed on postnatal days 35, 60, and 90, and brain concentrations of dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, were determined in the frontal cortex, caudate nucleus and substantia nigra. *In utero* and lactational exposure to PCB 47 resulted in significant decreases in concentrations of dopamine in the frontal cortex and caudate nucleus that persisted into adulthood in the groups given 10 or 20 mg/kg bw per day. This was in contrast to the persistent elevations in brain dopamine and metabolite concentrations following perinatal exposure to PCB 77. In additional experiments, no effects were reported after PCB 47 doses of 0.01 or 0.1 mg/kg bw per day (Seegal et al. 1997). Assuming a half life of 3 days in the rat and close to steady state at delivery of the offspring, the NOAEL of 20 mg/kg bw per day for reproductive and developmental effect would correspond to a maternal body burden of 84 mg/kg bw, whereas the NOAEL of 1 mg/kg bw per day for effects on brain neurotransmitters would correspond to a maternal body burden of 4.2 mg/kg bw.

Groups of 10 pregnant Long-Evans rats were given daily intraperitoneal injections with either PCB 47 at 1 or 20 mg/kg bw or PCB 77 at 0.25 or 1 mg/kg bw or sesame oil (control group) from gestational days 7 to 18. Offspring were then tested for sexual behavior as adults. Exposure to 20 mg PCB 47/kg bw per day reduced the level of sexual receptivity in the female offspring, but had no detectable effects on the sexual behavior of the male offspring. In addition, a significant increase was seen in the female anogenital distance at that dose level. Similar effects were not seen in the males. PCB 77 produced these effects at both dose levels tested. The NOAEL for PCB 47 was 1 mg/kg bw per day (Wang et al. 2002). Assuming a half life of 3 days in the rat the NOAEL of 1 mg/kg bw per day would correspond to a body burden of 4.2 mg/kg bw at nearly steady state at delivery of the offspring, whereas the LOAEL of 20 mg/kg bw per

day would correspond to a body burden of 84 mg/kg bw.

Pregnant Long-Evans rats were treated by subcutaneous injection with 1.5 mg PCB 47/kg bw per day, 0.5 mg PCB 77/kg bw per day, 1.5 mg PCB 77/kg bw per day, or a mixture of 1.0 mg PCB 47 and 0.5 mg PCB 77 per kg bw per day from GD 7-18. The PCB levels in brain and perirenal fat of dams and offspring were determined on gestational day 19 (GD 19), postnatal day 21 (PND 21), and PND 45. PCB 77 was accumulated to a smaller degree than PCB 47. On GD19 the reported levels in the adipose tissue of the dams were 0.74 mg/kg lipid for PCB 77 and 39 mg/kg lipid for PCB 47. Assuming a fat content of 10% in rats this would correspond to a body burden of 4 mg/kg bw for PCB 47. On GD 19, PCB 77 was found to a greater extent in the brains of the offspring (0.13 mg/kg lipid) than in the brains of the dams (0.07 mg/kg lipid), whereas the level of PCB 47 was almost the same in dams and offspring (6.4 and 6.9 mg/kg lipid, respectively). At PND19 PCB 47 was present in the brain lipid tissue at a 450 fold higher concentration than PCB 77 (9 mg/kg lipid versus 0.02 mg/kg lipid). Pups from 7-18 litters per group were used for behavioural testing. The testing of open-field behaviour in male rats on PND 18 and PND 70 revealed an altered distribution of activity with enhanced activity in the inner zone in PCB 77-treated rats compared to all other groups, while the overall activity was not changed. The only effect seen after PCB 47 exposure was increased activity (distance travelled and rearing behaviour) on PND 340. A step-down passive avoidance task revealed decreased latencies in the PCB 77 and combined exposure groups on PND 80. Only PCB 77-treated animals showed increased latencies on PND 100 on the haloperidol-induced catalepsy test. These results indicate long-term effects of maternal exposure to PCB 77 on emotional and motor functions. The two congeners given in combination did not cause additive or synergistic effects. Instead, concurrent exposure to PCB 47 seemed to counteract PCB 77-induced changes in the pattern of activity (Hany et al. 1999).

In the same experiment, pups from 9-10 litters were used for measurements of the flash-evoked electroretinogram (ERG), which started in the offspring at an age of about 200 days. The scotopic b-wave, the maximum potential, and oscillatory potentials were recorded after dark adaptation. Amplitudes of these potentials were reduced in female rats exposed to PCB 77. No differences from controls were found in females of other groups or male rats (Kremer et al. 1999). Altmann et al. (1998) used pups from 9-10 litters eposed by maternal treatment by subcutaneous injection with 1.5 mg PCB 47/kg bw per day, or 0.5 mg PCB 77/kg bw per day from GD 7-18. They measured the amount of long-term potentiation (LTP) at postnatal days 11-19 in the visual cortex and hippocampus. PCB-77 exposure affected LTP statistically significantly in cortical but not hippocampal slices. PCB-47 was present in the brain lipid tissue at PND19 at a 450 fold higher concentration than PCB 77 (9 mg/kg lipid versus 0.02 mg/kg lipid) without having significant effects.

Groups of 4-7 weanling (20-21 days old) female Sprague-Dawley rats were given either 2 or 5 consecutive daily doses of 30 mg PCB 47/kg bw or a total dose of 120 mg/kg bw of Aroclor 1242 divided into 2, 3, or 5 daily doses by intraperitoneal injection. Serum thyroxine (T4) increased between 20 and 25 days of age in control rats, but declined to 35-52% of controls by day 25 in PCB-treated rats. In rats receiving only 2 doses of PCB 47, the declines in serum T4 were more modest but the thyroid follicular epithelial cell height increased and the colloid area decreased. In the Aroclor 1242-treated rats, follicular cell height increases and colloid area decreases were somewhat greater (Saeed and Hansen 1997).

Developmental neurochemical studies and prepubertal uterotrophic studies were performed for 4 PCBs in SD rats. For developmental studies dams were orally exposed to PCB 47 at 1, 10 or 20 mg/kg bw/day from GD 6 to PND 21 (36 doses). Decreased dopamine levels were observed in cortex of rats from the highest dose group. NOAEL 10 mg/kg bw/day, LOAEL 20 mg/kg bw/day. Assuming a half life of 3 days in the rat and close to steady state at delivery of the offspring, the NOAEL of 10 mg/kg bw per day for effects on brain neurotransmitters would correspond to a maternal body burden of 42 mg/kg bw. For the uterotrophic study prepubertal female rats were exposed ip to 8, 16 or 32 mg/kg bw/day of PCB 47 for 2 days (total dose 16, 32, 64 mg/kg). No uterotrophic effect was observed (Seegal et al. 2005).

Male NMRI mice received a single oral dose of 0.41 or 4.1 mg PCB 47/kg bw on postnatal day 10 and were examined for changes in spontaneous motor behaviour, learning, memory, and brain

biochemistry after 2, 4, and 6 months. PCB 47 did not change the spontaneous motor behaviour and did not affect learning and memory. Thus, the NOAEL was 4.1 mg PCB 47/kg bw (Eriksson 1998).

PCB 52 (2,2',5,5'-tetrachlorobiphenyl)

PCB 52 is a minor contaminant in human milk (EFSA 2005). A median concentration of 0.32 ng PCB 52/g fat in human milk would correspond to an estimated median human body burden of 0.064 µg/kg bw assuming that the human body contains 20% fat.

Tanabe et al. (1981) reported the elimination half-life in male Wistar rats to be 0.89 (3.4) days.

Estrogenic effects

Immature (20-day) Sprague Dawley rats at age of 20 days were treated intraperitoneally with corn oil (vehicle), or 14 mg PCB 52/kg bw per day for two consecutive days. PCB 52 produced a significant increase in uterine weights and increases in uterine 3H-thymidine labelling (Jansen et al. 1993). Increased uterine weight was also reported in immature female Wistar rats given an intraperitoneal injections of 8 mg PCB 52, corresponding to 160 mg/kg bw (Ecobichon & MacKenzie, 1974), whereas no effects were reported in immature female CD1 mice treated topically with 0.146, 1.46, 14.6, or 146 mg PCB 52/kg bw per day for 3 days (Nesaretnam and Darbre 1997).

Effects on the immune system

No effects were seen on the immune system in female Sprague Dawley rats receiving a diet containing 10 mg PCB 52/kg (equivalent to 1 mg/kg bw per day) for 12 months. A trend in reduction of B-cells was not significant. However, in combination with 0.1 mg PCB 77/kg diet, which alone had no effect, the combined effect of the PCB gave a greater than additive effect with a decrease in lymphocytes and B-cells (Sargent et al. 1991).

Neurobehavioral effects

Male NMRI mice received a single oral dose of 0.2, 0.42, or 3.6 mg PCB 52/kg bw on postnatal day 10 and were examined for changes in spontaneous motor behaviour, learning, memory, and brain biochemistry after 4 months. The highest dose changed the spontaneous motor behaviour, and did also affect learning, memory, and brain muscarinic- and nicotinic receptors. Brain biogenic amines were not affected. Thus, the NOAEL was 0.42 mg PCB 52/kg bw and the LOAEL 4.1 mg PCB 52/kg bw (Eriksson and Fredriksson 1996a, 1996b).

Purified PCBs 52, 138 and 180 were administered in a sweet jelly bit to pregnant Wistar rats from GD 7 to PND 21 at a dose of 1 mg/kg bw/day. Behavioural studies were performed on offspring and learning ability was found to be impaired by PCB 138 and 180, but not PCB 52. PCB 52 impaired motor coordination. Differences between the congeners were thus observed and were consistent with reduced amounts of NMDA receptors in cerebellum of pups exposed to PCB 138 and 180 but not PCB 52. In contrast, PCB 52 increased GABA (Boix et al. 2010).

Pregnant SD rats were exposed to PCB 52 in doses of 0, 30, 100, 300, 1000 or 3000 mg/kg bw (total dose levels) from GD 7 to GD 16 and again from PND 1 to PND 10 every second day (total of 10 doses; listed doses are total dose levels). Auditory function was examined. Developmental changes are mentioned but reported in details elsewhere. It is mentioned that T3 but not T4 is reduced in dams from 100 mg/kg by PCB 52 and that offspring T3 was reduced from 30 mg/kg bw corresponding to an average dose of 3 mg/kg bw/day. Free T4 was reduced at higher dose levels. A LOAEL for thyroid effects could thus be 3 mg/kg bw/day. The lowest benchmark dose for effects on hearing thresholds was 50 mg/kg bw corresponding to 5 mg/kg bw/day (Lillienthal et al. 2011).

PCB 54 (2,2',6,6'-tetrachlorobiphenyl)

PCB 54 has not been detected in human milk (EFSA 2005)

Tanabe et al. (1981) reported the elimination half-life in male Wistar rats to be 0.21 days.

Estrogenic effect

PCB 54 has been tested for estrogenic effect in the immature female Sprague Dawley rat. In a symposium overview Fischer et al. (1998) reported on groups of rats given intraperitoneal injections of 0, 10, 20, or 30 mg PCB 54/kg bw per day for two days. Increased wet uterine weights were observed at 20 mg/kg bw per day and higher. The NOAEL was reported to be 10 mg/kg bw per day. However, in a similar (probably the same) study reported by Arcora et al. (1999) where groups of rats were given

intraperitoneal injections of 0, 3, 10, 20, or 30 mg PCB 54/kg bw per day for two days, increased wet uterine weights were reported at 10 mg/kg bw per day and higher. The NOAEL in this study was 3 mg/kg bw per day.

PCB 60 (2,3,4,4'-tetrachlorobiphenyl)

PCB 60 is a minor contaminant in human milk (EFSA 2005). A median concentration of 0.41 ng PCB 60/g fat in human milk would correspond to an estimated median human body burden of 0.082 µg/kg bw assuming that the human body contains 20% fat.

No *in vivo* toxicological data are available on PCB 60.

PCB 66 (2,4,3',4'-tetrachlorobiphenyl)

PCB 66 is a contaminant in human milk (EFSA 2005). A median concentration of 1.2 ng PCB 60/g fat in human milk would correspond to an estimated median human body burden of 0.24 µg/kg bw assuming that the human body contains 20% fat.

No *in vivo* toxicological data are available on PCB 66.

PCB 74 (2,4,5,4'-tetrachlorobiphenyl)

PCB 74 is a contaminant in human milk (EFSA 2005). A median concentration of 6.8 ng PCB 74/g fat in human milk would correspond to an estimated median human body burden of 1.36 µg/kg bw assuming that the human body contains 20% fat.

No *in vivo* toxicological data are available on PCB 75.

PCB 80 (3,5,3',5'-tetrachlorobiphenyl)

PCB 80 was not reported to be present in human milk (EFSA 2005).

Estrogenic effects
PCB 80 was considered to be a weak oestrogen agonist based on assays on ligand regulation of oestrogen-sensitive gene expression; ligand regulation of cell growth in oestrogen-dependent human breast cancer cell lines MCF7, McGrath, and ZR-75-1; and ligand activity in the mouse uterine weight bioassay using immature female CD1 mice treated topically with 0.146, 1.46, 14.6, or 146 mg PCB 80/kg bw per day for 3 days (Nesaretnam and Darbre 1997).

PCB 95 (2,3,6,2',5'-pentachlorobiphenyl)

PCB 95 was not detected in human milk (EFSA 2005).

Effects on neurotransmission
PCB possessing two or more ortho-chlorine substituents were able to activate the ryanodine receptors *in vitro* in mammalian brain tissues, revealing an Ah-receptor-independent mechanism through which non-coplanar PCBs may disrupt neuronal Ca2+ signalling. Of the congeners assayed, PCB 95 exhibits the highest potency toward activating high affinity [3H]ryanodine-binding in rat hippocampus, cerebellum, and cerebral cortex *in vitro*. PCB 66 and PCB 126 had no ryanodine receptor activity in all brain regions examined (Wong et al. 1997).

Effects on reproduction, development, and learning
Time-mated Sprague-Dawley rats were dosed with PCB 95 (8 or 32 mg/kg bw per day) or corn oil vehicle via gavage on gestation days 10-16. No effects were seen on gestation length, litter size, percent live births, birth weight and postnatal growth. The NOAEL was 32 mg/kg bw per day (Schantz et al. 1996). Locomotor activity was evaluated in an automated open field at 35 and 100 days of age. Spatial learning and memory was assessed using an eight arm radial maze working memory task at 60 days of age and a T-maze delayed spatial alternation task at 140 days of age. The animals were then euthanized and [3H] ryanodine binding was assayed in homogenates of cerebral cortex, hippocampus and cerebellum. Rats exposed to PCB 95 showed normal levels of activity as juveniles, but were hypoactive in adulthood. They also showed a faster acquisition of the working memory task on the radial arm maze, but did not differ from controls on the T-maze delayed spatial alteration task. Region-specific changes in ryanodine binding to Ca2+ channels were also observed, with decreased binding in the

hippocampus, increased binding in the cerebral cortex and a biphasic effect in the cerebellum. The LOAEL was 8 mg/kg bw per day (Schantz et al. 1997).

Kenet et al. (2007) exposed pregnant rats orally to 6 mg/kg bw/day of PCB-95 during the gestational period and throughout 3 subsequent suckling weeks. Exposure to PCB 95 resulted in abnormal development of the primary auditory cortex in pups; however, the hearing sensitivity and brainstem auditory response of the pups were normal (Kenet et al. 2007).

Endocrine effects

The effects of PCB 95 on selected endocrine parameters were studied in weanling female Sprague-Dawley rats given a single intraperitoneal dose of 4, 8, 16, and 32 mg/kg bw per day for 2 consecutive days and killed 24 hours after the last dose. PCB 95 exposure caused a dose-dependent decrease in serum thyroxine (T4) levels. Serum thyroid stimulating hormone (TSH) concentrations and prolactin (PRL) levels did not change significantly. No significant changes were seen in thyroid gland morphology and pituitary lactotroph number. The NOAEL was 4 mg PCB 95/kg bw per day. In a second study weanling female rats received a single intraperitoneal dose of PCB 95 or PCB 101 at 16 and 32 mg/kg bw per day for 2 days and were killed 48 hours after the last dose. Both PCB 95 and PCB 101 decreased serum T4 and hypothalamic dopamine levels. No changes were seen in serum triiodothyronine (T3), TSH, and PRL concentrations. Morphological analysis of the thyroid gland showed a decrease in colloid area and increased epithelial cell height. Thyroid epithelial cell proliferation increased following exposure to PCB 95, but not PCB 101. The results suggest that the HPT axis appears to be a target of ortho-substituted PCBs. The LOAEL was 16 mg/kg bw per day with PCB 95 being more effective than PCB 101 in causing these changes (Khan et al. 2002).

The functionality of the HPT-axis was evaluated by using the thyrotropin releasing hormone (TRH) test following acute exposure to PCBs 95. Weanling female rats received PCBs 95 intraperitoneally at 32 mg/kg bw per day for 2 consecutive days and synthetic TRH was given 48 hours after the last dose. Serum thyroxine (T4) levels decreased following the exposure and serum thyroid stimulating hormone (TSH) levels were elevated in response to TRH, but were only 40% of the control response to TRH. No significant changes were seen in serum prolactin (PRL), hypothalamic dopamine (DA), thyroid gland morphology, or epithelial cell proliferation (Khan and Hansen 2003).

PCB 99 (2,4,5,2',4'-pentachlorobiphenyl)

PCB 99 is a contaminat in human milk (EFSA 2005). A median concentration of 6.2 ng PCB 99/g fat in human milk would correspond to an estimated median human body burden of 1.24 µg/kg bw assuming that the human body contains 20% fat.

No *in vivo* toxicological data are available on PCB 99.

PCB 101 (2,4,5-2',5'-pentachlorobiphenyl)

PCB 101 is a minor contaminant in human milk (EFSA 2005). A median concentration of 0.69 ng PCB 101/g fat in human milk would correspond to an estimated median human body burden of 0.138 µg/kg bw assuming that the human body contains 20% fat.

Endocrine effects

Weanling female SD rats received a single intraperitoneal dose of PCB 101 at 16 and 32 mg/kg bw per day for 2 days and were killed 48 hours after the last dose. PCB 101 decreased serum T4 and hypothalamic dopamine levels. No changes were seen in serum triiodothyronine (T3), TSH, and PRL concentrations. Morphological analysis of the thyroid gland showed a decrease in colloid area. However, no increase was seen in the epithelial cell height. The LOAEL was 16 mg/kg bw per day (Khan et al. 2002).

The functionality of the HPT-axis was evaluated by using the thyrotropin releasing hormone (TRH) test following acute exposure to PCBs 101. Weanling female rats received PCBs 101 intraperitoneally at 32 mg/kg bw per day for 2 consecutive days and synthetic TRH was given 48 h after the last dose. Serum thyroxine (T4) levels decreased following the exposure. Serum thyroid stimulating hormone (TSH) levels were not elevated in response to TRH. No significant changes were seen in serum

prolactin (PRL), hypothalamic dopamine (DA), thyroid gland morphology, or epithelial cell proliferation (Khan and Hansen 2003).

PCB 110 (2,3,6,3',4'-pentachlorobiphenyl).

PCB 110 is a minor contaminant in human milk (Table x). A median concentration of 0.21 ng PCB 110/g fat in human milk would correspond to a median human body burden of 0.042 µg/kg bw assuming that the human body contains 20% fat.

Endocrine effects

PCB 110 was investigated in weanling female rats dosed intraperitoneally with 4, 16, 24, or 48 mg/kg bw per day on days 21 and 22 and killed on day 23 of age. PCB 110 induced pentoxyresorufin O-dealkylase (PROD), was weakly uterotropic by increasing the relative uterus weight, and was a modestly lowered serum thyroxine (T4) at 16 mg/kg bw per day. The NOAEL was 4 mg PCB 110/kg bw per day (Li et al. 1998).

PCB 114 (2,3,4,5,4'-pentachlorobiphenyl).

PCB 114 is a minor contaminant in human milk (EFSA 2005). A median concentration of 0.53 ng PCB 114/g fat in human milk would correspond to an estimated median human body burden of 0.106 µg/kg bw assuming that the human body contains 20% fat.

No *in vivo* toxicological data are available on PCB 114.

PCB 128 (2,2', 3,3', 4,4'-hexachlorobiphenyl)

PCB 128 is a minor contaminant in human milk (EFSA 2005). A median concentration of 0.63 ng PCB 128/g fat in human milk would correspond to an estimated median human body burden of 0.126 µg/kg bw assuming that the human body contains 20% fat.

Short-term toxicity

The short-term toxicity of PCB 128 was investigated in rats following dietary exposure at 0, 0.05, 0.5, 5, or 50 mg/kg (equal to 0, 4.2, 42, 425, and 4210 µg/kg bw per day for males and 0, 4.5, 45, 441, and 4397 µg/kg bw per day for females) for 13 weeks. The growth rate was not affected by treatment and no apparent clinical signs of toxicity were observed. There was a significant increase in liver weight in the females administered 50 mg/kg diet. The liver EROD activity was increased by five- and fourfold in the highest dose males and females, respectively, while aminopyrine demethylase (ADPM) activity was significantly increased only in the highest dose females. Liver vitamin A was significantly reduced in the highest dose females. No other biochemical or hematological effects were observed. Treatment-related histopathological changes were seen in both sexes in the thyroid and liver, and to a lesser extent in the bone marrow and thymus. Residue data showed a dose-dependent accumulation of PCB 128 in the following tissues: fat, liver, kidney, brain, spleen, and serum, with the highest concentration being found in fat followed by liver and kidney. Based on these data, the NOAEL was 0.5 mg PCB 128/kg in the diet equal to 42 µg/kg bw per day. (Lacavalier et al. 1997). In a separate report Walker et al. (2000) reported a significant increase in the volume-fraction of smooth endoplasmic reticulum (SER) from the female rats fed diets containing 5 or 50 mg/kg of the congener and a significant increase was revealed in the male rats at doses of 0.5 and 50 mg/kg. The volume-fraction values of mitochondria, peroxisomes or lipid droplets of the hepatocytes in either the males or the females were not significantly different. Based on transmission electron microscopy Singh et al. (2000) reported that the architecture of the liver parenchymal cell was indistinguishable in the animals of the lowest concentration group from those in the controls. At the higher dose levels, smooth endoplasmic reticulum profiles increased, and abnormal mitochondria were noted in the liver of rats, regardless of gender. Based on a measured content of PCB 128 in fat of male rats of 8.12 mg/kg at the NOAEL a body burden of 0.8 mg/kg bw can be estimated assuming that a rat contains 10% fat (Geyer et al. 1990). At the LOAEL the concentration in fat was 69.8 mg/kg bw corresponding to a body burden of 7 mg/kg bw.

PCB 132 (2,2', 3,3', 4,6'-hexachlorobiphenyl) (2,3,6-substitution)

PCB 132 was not detected in human milk (EFSA 2005).

Endocrine effects
Weanling female Sprague Dawley rats were dosed intraperitoneally on days 21 and 22 with 0, 4, 16, or 48 mg PCB 132/kg bw per day and killed on day 24 of age for examination of uterotropic response, serum thyroid hormone, and hepatic enzyme induction. PCB 132 did not cause any significant increase in any of these endpoints (Li et al. 2001)

Effects on male reproductive organs
Pregnant rats were treated with a single dose of PCB-132 at 1 or 10 mg/kg bw on GD15. When the male offspring were assessed at adulthood (PND 84) a decrease in cauda epididymal weight, epididymal sperm count, and motile epididymal sperm count was observed. The spermatozoa in the PCB-132-exposed offspring produced significantly higher levels of reactive oxygen species (ROS) than in the controls. In the 1 mg/kg bw dose group, p53 was significantly induced and caspase-3 was inhibited, while in the 10 mg/kg bw dose group, activation of caspase-3 and -9 was significantly increased; whereas at the same time the expressions of Fas, Bax, bcl-2, and p53 genes were significantly decreased. The LOAEL was 1 mg/kg bw (Hsu et al. 2007).

PCB 138 (2,2',3,4,4',6-hexachlorobiphenyl)

PCB 138 is a major contaminant in human milk (EFSA 2005). A median concentration of 55.5 ng PCB 138/g fat in human milk would correspond to an estimated median human body burden of 11.1 µg/kg bw assuming that the human body contains 20% fat.

Purified PCBs 52, 138 and 180 were administered in a sweet jelly bit to pregnant Wistar rats from GD 7 to PND 21 at a dose of 1 mg/kg bw/day. Behavioural studies were performed on offspring and learning ability was found to be impaired by PCB 138 and 180, but not PCB 52. PCB 52 impaired motor coordination. Differences between the congeners were thus observed and were consistent with reduced amounts of NMDA receptors in cerebellum of pups exposed to PCB 138 and 180 but not PCB 52. In contrast, PCB 52 increased GABA (Boix et al. 2010).

PCB 141 (2,2',34,5,5'-hexachlorobiphenyl)

PCB 141 is a minor contaminant in human milk (EFSA 2005). A median concentration of 0.17 ng PCB 141/g fat in human milk would correspond to an estimated median human body burden of 0.0.034 µg/kg bw assuming that the human body contains 20% fat.

No *in vivo* toxicological data were available on PCB 141.

PCB 149 (2,2',3,4',5',6-hexachlorobiphenyl)

PCB 148 was not reported in human milk (EFSA 2005).

Endocrine effects
Weanling female Sprague Dawley rats were dosed intraperitoneally on days 21 and 22 with 0, 4, 16, or 48 mg PCB 149/kg bw per day and killed on day 24 of age for examination of uterotropic response, serum thyroid hormone, and hepatic enzyme induction. PCB 149 was not estrogenic and did not induce EROD activity, but was a weak inducer of PROD and BROD activities and a modestly lowered serum thyroxine levels in prepubertal female rats. The NOAEL was 8 mg/kg bw per day (Li et al. 2001).

PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl)

PCB 153 is a major contaminant in human milk (EFSA 2005). A median concentration of 67.8 ng PCB 153/g fat in human milk would correspond to an estimated median human body burden of 13.6 µg/kg bw assuming that the human body contains 20% fat.

Special studies on disposition in the brain of rats
Weanling male and female Long-Evans rats were given a single intraperitoneal dose of 8 mg radiolabeled [14C] PCB 153/kg bw or PCB 169/kg bw Excretion and tissue retention were determined

after 48 hours. Both congeners remained sequestered predominantly in mesenteric fat (compared to subcutaneous fat) and less than 1% of the doses were excreted. Excretion was 4- to 8-fold lower than in adult rats. PCB 169 did not accumulate appreciably in the brain, but was retained at 3-fold higher levels in the liver than was PCB 153. Accumulation of 14C-PCB 153 in brains was 4- to 9-fold higher than that of 14C-PCB 169. Autoradiography showed a higher CB 153-derived radioactivity associated with fiber tracts throughout the brain. Specifically, the corpus callosum, internal and external capsules, medial lemniscus, tegmentum of the mesencephalon and metencephalon, and cerebellar peduncles showed significantly higher 14C-PCB 153 than the other structures. The 14C-PCB 153 was not found in the ventricular system and vascular spaces. Thus the di-ortho-substituted PCB 153 accumulated preferentially in brain in a structure-specific manner when compared to the non-ortho-substituted PCB 169 (Saghir et al. 2000).

Uterothropic effect

PCB 153 was administered to immature Sprague Dawley rats at postnatal days 20 and 21 by intraperitoneal injections at doses of 8, 11, 25, 51 or 59 mg/kg bw per day. The relative uterus weights were increased after 25 mg/kg bw per day and higher doses. The NOAEL was 11 mg PCB 153/kg bw per day (Li et al. 1994).

Effects on thyroid hormones

Four groups of 10 male Sprague-Dawley rats received two intraperitoneal injections, one day apart, of corn oil, PCB 153 (25 mg/kg bw per day), estradiol-17 beta (E2; 20 µg/kg bw per day), or PCB 126 (0.1 mg/kg bw per day). Serum thyroxine (T4) levels were higher in the E2 and PCB 153 groups, and slightly reduced in the PCB 126-treated group, compared to controls. Simultaneous purification of pituitary follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH) isoforms was performed by HPLC, and the amounts of FSHs isoform eluted were lower in E2 and PCB 153-treated rats than in control or PCB 126-treated rats. The similarity between the effects of E2 and PCB 153 on T4 and FSH isoforms supports the contention that PCB 153 possesses estrogenic properties (Desaulniers et al. 1999).

Male and female C57BL/6J mice and Long-Evans rats were dosed orally for 4 consecutive days with either PCB 126 (0.03-300.0 µg/kg bw per day) or PCB 153 (0.3-300 mg/kg bw per day). PCB 126 did not affect total serum thyroxine (T4) in the mouse but decreased T4 in the rat. PCB 153 decreased T4 in both the rat and the mouse. PCB 126 increased hepatic microsomal ethoxyresorufin-O-deethylase (EROD) activity in both rats and mice. PCB 153 induced hepatic pentoxyresorufin-O-deethylase (PROD) activity in both rats and mice. Hepatic microsomal T4 glucuronidation was increased approximately 2- to 3-fold in both rats and mice treated with PCB153. PCB126 increased T4 glucuronidation in rats but only marginally in mice at the highest doses (Craft et al. 2002).

Effects on reproduction and development, including thyroid and neurobehavioral development

Even at high doses, PCB 153 was found not to cause foetal cleft palate, suppress the splenic plaque-forming cell response to sheep red blood cells, or induce hepatic microsomal ethoxyresorufin O-deethylase (EROD) activity in C57BL/6J mice. Despite the lack of activity of PCB 153 in eliciting any of these aryl hydrocarbon receptor-mediated responses, competitive binding studies indicated that PCB 153 competitively displaced 2,3,7,8-TCDD from the murine hepatic cytosolic receptor. Thus, when C57BL/6J mice were co-treated with TCDD and PCB 153, PCB 153 partially antagonized TCDD-mediated cleft palate and immunotoxicity, and antagonized TCDD-mediated hepatic microsomal EROD induction (Biegel et al. 1989).

C57BL/6N mice used to model induction of cleft palate and kidney malformations in offspring following maternal treatment with 2,3,7,8-TCDD, were dosed on gestation day (GD) 9 with PCB 153 (62.5, 125, 250, 500, 1000 mg/kg bw) and/or GD 10 with TCDD (15 or 18 µg/kg bw). There was no effect on number of live or dead offspring. Foetal body weight was slightly decreased in all groups dosed with greater than or equal to 250 mg PCB 153/kg bw PCB 153 did not induce cleft palate at a dose of 1000 mg/kg bw, but did induce increases in hydronephrosis and hydroureter at 500 and 1000 mg/kg bw. Combinations of PCB 153 and TCDD decreased the incidence of cleft palate induced by TCDD alone, but only at doses of 15 µg TCDD/ kg bw combined with 125-500 mg PCB 153/kg bw. The antagonism of hydronephrosis (incidence and severity) appeared over a narrower dose range (15 µg TCDD/kg bw + 500 mg PCB 153/kg bw). PCB 153 induced increases (3- fold) in ethoxyresorufin-O-deethylase (EROD) activity at doses of 500

and 1000 mg/kg bw, suggesting that the limited antagonism of TCDD teratogenicity could be under the control of the Ah-receptor (Morrissey et al. 1992).

A single dose of 271 mg PCB 153/kg bw on gestation day 10 did not induce foetal cleft palate in offspring from pregnant C57BL/6 mice, but significantly inhibited foetal cleft palate formation after 783 or 1044 µg PCB 126/kg bw (a DL-PCB). At doses as high as 72 mg/kg bw, PCB 153 was not immunotoxic; however, in mice co-treated with a immunotoxic dose of PCB 126 plus different doses of PCB 153 (18, 36 and 72 mg/kg bw), there was a dose-dependent inhibition of PCB 126-induced immunotoxicity (Zhao et al. 1997).

Groups of time-mated Sprague-Dawley rats were exposed to PCB 153 or PCB 118 (a DL-PCB), 16 or 64 mg/kg bw per day, or corn oil vehicle via gavage on Gestation Days 10-16. Per group, 6-9 litters were culled to eight on Day 2 and weaned on Day 21. At weaning, serum thyroxine (T4) was markedly depressed in pups, but not dams, exposed to PCB 118 or 153. Triiodothyronine (T3) was unchanged in pups and dams. In a histological evaluation of thyroids, no effects of PCB 153 were noted, whereas the PCB 118 pups revealed changes suggestive of sustained TSH stimulation, including increased follicular cell vacuolization and height, increased nuclear vesiculation, and decreased colloid area. Decreases in body and brain weights and increases in liver weights were observed in some groups, with the high dose PCB 118 pups showing the greatest effect (Ness et al. 1993).

In the same offspring spatial learning and memory was assessed on a working/reference memory task on an eight-arm maze beginning on day 90, and continued for seven consecutive weeks. No differences in working or reference memory errors were observed. The same animals were later tested on a T arm-maze in a delayed spatial alternation task. A slower acquisition was seen for female rats at 64 mg PCB 153/kg bw per day. Males were not affected. The NOAEL was 16 mg/kg bw per day (Schantz et al. 1995).

Pregnant SD rats were orally exposed to PCB 153 at 0, 16 or 64 mg/kg bw/day from GD 10 to 16. A significant decrease in T4 and T3 was observed at 64 mg/kg bw/day but no changes in growth, anogenital distance or organ weights were observed. A NOAEL of 16 mg/kg bw/day (total dose 112 mg/kg bw) and a LOAEL of 64 mg/kg bw/day (total doses 112 and 448 mg/kg bw) could be obtained for T3 and T4 reductions (Kobayashi et al. 2008).

Pregnant SD rats were orally exposed to PCB 153 at 0, 1 or 4 mg/kg bw/day from GD 10 to 16. No changes in thyroid hormone levels were seen at several ages except for an increase in T3 levels in 1 week old males. No changes in organ weights or anogenital distance of offspring were seen. The NOAEL was 4 mg/kg bw/day (total dose of 28 mg/kg bw/day) (Kobayashi et al. 2009).

Pregnant SD rats were exposed from GD 10 to 16 to 16 and 64 mg/kg bw/day of PCB 153 (total doses 112 and 448 mg/kg bw). Brain monoamine content was increased in the offspring at the highest dose compared to controls. Long term effects were observed as altered monoamine levels were found at 1 year after birth (Honma et al. 2009).

Rat pups were exposed through mother's milk to either PCB 153 or PCB 126. Groups of six mothers were dosed via gavage with corn oil vehicle, 5 mg/kg bw of PCB 153 or 2 µg/kg bw of PCB 126 every second day from day 3 to 13 after delivery. The exposure did not affect the body weight of the dams or the physical development of the pups. It was shown that the PCB- exposed offspring were hyperactive. The PCB 153-exposed male pups showed a behavioural pattern (increased fixed interval responding) similar to that observed in spontaneously hypertensive rats, an animal model of attention-deficit hyperactivity disorder (Holene et al. 1998). In the female offspring there were no statistically significant differences in this test. However, the female offspring showed slower acquisition of time discrimination in another intermittent schedule of reinforcement (Holene et al. 1999).

Anestrous, juvenile female mink and pregnant adult mink were given a single intraperitoneal injection of 20 mg/kg bw PCB 153. PCB 153 impaired 17 beta- estradiol-stimulated up-regulation of uterine nuclear estrogen receptors in anestrous mink. Embryotoxicity and reduced embryo growth were first observed 14 days after exposure. In pregnant mink, serum estrogen and progesterone receptor levels were increased. Progesterone concentrations were also increased. PCB 153 exposure resulted in decreased P450 concentration in anestrous juveniles, but had no effect on P450 during

gestation or EROD activity at any time (Patnode et al. 1994).

Lyche et al. (2004) examined the possible adverse effects on the hypothalamic-pituitary-gonadal axis by measuring gonadotrophins and gonadal steroid hormone concentrations in goat offspring exposed during gestation and lactation to PCB-153 at a dose level of 98 µg/kg bw/day. The results indicate that maternal exposure to PCB 153 during gestation and lactation suppressed prepubertal plasma luteinizing hormone concentrations and delayed the onset of puberty of the female offspring. Thus the LOAEL was 98 µg/kg bw/day. The resulting concentration in adipose tissue 9 months post-partum in the goat offspring was 5.8 µg/g (fat weight) for PCB 153.

Male NMRI mice received a single oral dose of 0.51 or 5.1 mg PCB 153/kg bw on postnatal day 10 and were examined for changes in spontaneous motor behaviour, learning, and memory after 4 months. The highest dose changed the spontaneous motor behaviour and affected learning and memory. Thus, the NOAEL was 0.51 mg/kg bw and the LOAEL 5.1 mg/kg bw (Eriksson 1998).

No statistically significant effects were seen in the offspring when pregnant mice were exposed to 0.5, 6.5 and 1500 µg/kg feed of PCB 153. Behavioural effects and thyroid hormones were analyzed in adulthood. Exposure of dams was estimated to 0.73 µg/kg bw/day during gestation and 1.72 µg/kg bw/day during lactation in the group receiving 6.5 µg/kg feed of PCB 153 (Haave et al. 2011).

Pregnant Wistar rats were exposed to 1 or 5 mg/kg bw/day of PCB 153 by gavage (or MeHg or a mixture of both) from GD 7 to PND 21. Adult offspring exposed to 5 mg/kg bw/day of PCB 153 had increased locomotor activity and impaired rotarod performance (sensorimotor coordination). MeHg had opposite effects of PCB 153 and no effects were seen with mixed exposure. A NOAEL of 1 mg/kg bw/day (total dose 35 mg/kg bw) and a LOAEL of 5 mg/kg bw/day (total dose 175 mg/kg bw) could be determined for PCB 153 (Gralewiez et al. 2009).

Pregnant SD rats were gavaged with PCB 153 from PND 3 to PND 7 (5 doses) at doses of 0, 0.025, and 2.5 mg/kg bw/day (total doses 0.125 and 12.5 mg/kg bw). T4 was decreased on PND 8 in both exposure groups and daily sperm production was decreased at PND 77 in the highest dose group. Gene expression studies showed changes in Sertoli cell markers at PND 77 in both exposure groups. A NOAEL of 0.025 mg/kg bw/day and a LOAEL of 2.5 mg/kg bw/day could be determined based on testicular effects and impaired sperm production. For thyroid effects a LOAEL of 0.025 mg/kg bw/day (total dose 0.125 mg/kg bw) could be determined based on T4 reduction (Xiao et al. 2010).

Pregnant rats were exposed orally from GD 7 to PND 21 to PCB 153 (with or without MeHg). No effects were observed at 1 mg/kg bw/day of PCB 153, but altered growth and development in offspring was observed at 5 mg/kg bw/day (total dose 175 mg/kg bw) (Sitarek and Gralewicz 2009).

Immunotoxicity

Groups of Ah-responsive male C57BL/6 mice were given single doses of 0, 30, 100, 300 mg PCB 153/kg bw by gavage 1-2 days prior to antigen challenge with P815 mastocytoma. The animals were examined 11 days after the dosing with PCB 153. In contrast to PCBs 156 and 169 (DL-PCB), PCB 153 had no effect on cytotoxic T lymphocyte (CTL) response. Spleen weight and cellularity as well as thymus weight were decreased at the highest dose level. The NOAEL was 100 mg/kg bw (Kerkvliet et al. 1990).

Intraperitoneal injections of a single dose of 100 mg PCB 153/kg bw to Wistar rats did not produce thymic atrophy (Leece et al. 1987).

Short-term toxicity

Groups of 10 male and 10 female Sprague-Dawley rats were administered PCB 153 in their diet at levels of 0.05, 0.50, 5.0 or 50 mg/kg (equal to 0, 3.6, 34, 346, or 3534 µg/kg bw per day for males and 0, 4.2, 42, 428, or 4125 µg/kg bw per day for females) for 13 weeks. Growth rate and dietary consumption were not affected by treatment. Clinical signs of toxicity were not observed. Enlarged, fatty liver was observed in treated animals at necropsy, but most were confined to the two highest dose groups. Increased hepatic microsomal ethoxyresorufin-O-deethylase (EROD), aminopyrine-N-demethylase and aniline hydroxylase activities occurred in high-dose groups of both sexes, with increased EROD activity being observed starting at 0.05 mg/kg diet in females and at 0.5 mg/kg diet in males. Treatment-related reduction in hepatic and pulmonary vitamin A was seen in the highest dose group of both sexes. Changes in brain

biogenic amines and intermediate products were observed mainly in females; these included decreased dopamine and 5- hydroxytryptamine concentrations in the frontal cortex region, and dihydroxyphenylacetic acid in the caudate nucleus region at 5.0 and 50 mg/kg diet. Female rats appeared to be more sensitive to the neurotoxic effects of PCB 153 than males. Dose-dependent histological changes were observed in the thyroid and liver of rats of both sexes and significant changes occurred at 5.0 and 50 mg/kg diet. Based on these data, the no- observable-adverse-effect level (NOAEL) of PCB 153 was judged to be 0.5 mg/kg in the diet or 34 µg/kg bw per day (Chu et al. 1996b). Based on a measured content of PCB 153 in fat of male rats of 11.6 mg/kg at the NOAEL a body burden of 1.2 mg/kg bw can be estimated assuming that a rat contains 10% fat (Geyer et al. 1990). At the LOAEL the concentration in fat was 89 mg/kg bw corresponding to a body burden of 9 mg/kg bw

Morphological effects on the liver of Sprague-Dawley rats administered PCB 153 according to the above mentioned regimen was examined by electron microscopy. Animals exposed to PCB 153 showed (in a dose-related manner) a marked increase in smooth endoplasmic reticulum profiles, and in the number of lipid droplets in many parenchymal cells. Mitochondrial abnormalities were also present. The magnitude of morphologic alterations did not reveal gender differences (Maclellan et al. 1994).

Long-term toxicity and carcinogenicity studies

Groups of 80-82 female Harlan Sprague-Dawley rats were administered PCB 153 (greater than 99% pure) in corn oil:acetone (99:1) by gavage at doses of 0, 10, 100, 300, 1,000, or 3,000 µg/kg bw/day 5 days per week. Ten animals from each group were sacrificed for interim evaluation after 14, 31, and 53 weeks. The remainder animals continued on the dosing for up to 105 weeks; a group of 81 female rats received the corn oil:acetone (99:1) vehicle alone. A stop-exposure group of 50 female rats was administered 3,000 µg/kg for 30 weeks and then the vehicle for the remainder of the study (NTP 2006b).

The survival of the dosed groups was similar to that of the vehicle control group. Mean body weights of 3,000 µg/kg bw core study rats were less than those of the vehicle controls after week 69 of the study.

Serum total thyroxine (T4), free T4, and total triiodothyronine (T3) concentrations in the 3,000 µg/kg bw group were significantly lower than those in the vehicle controls at the 14-week and 53-week interim evaluations. No effect was seen on thyroid stimulating hormone concentrations. At the 31-week interim evaluation, no significant differences were observed in serum total T4, free T4, T3, or thyroid stimulating hormone concentrations. No significant differences in hepatocellular labelling index were observed between the vehicle control and dosed groups at any of the interim evaluations.

Hepatic pentoxyresorufin-O-deethylase activities were dose-dependently increased over comtrol values at doses of 100 µg/kg bw and higher. Maximum increases over controls in the 3,000 µg/kg bw rats at 14, 31, and 53 weeks were 136-, 140-, and 40-fold, respectively. Hepatic 7-ethoxyresorufin-O-deethylase (EROD) and acetanilide-4-hydroxylase (A4H) activities were similarly elevated over controls at 14 and 31 weeks; however, increases were less than twofold. At 14 weeks, EROD activities in the lung were dose-dependently reduced compared to vehicle controls.

In the fat from vehicle controls, detectable levels of PCB 153 were observed at 14, 31, and 53 weeks and at the end of the 2-year study. Fat concentrations of PCB 153 increased with increasing doses of PCB 153 and tended to increase with the longer exposure durations. In the fat of the 3,000 µg/kg stop-exposure group, PCB 153 concentrations were between the levels observed in the 300 and 1,000 µg/kg groups. In the liver of vehicle controls, no measurable concentrations of PCB 153 were observed at any time point. In dosed groups, hepatic concentrations of PCB 153 increased with increasing dose and longer exposure duration.

Absolute liver weights of 1,000 µg/kg bw rats and absolute and relative liver weights of 3,000 µg/kg rats were significantly greater than those of vehicle controls at week 14. At week 31, relative liver weights of 1,000 µg/kg rats and absolute and relative liver weights of 3,000 µg/kg rats were significantly greater than those of vehicle controls. At week 53, absolute and relative liver weights were significantly greater in rats administered 100 µg/kg or greater compared to vehicle controls. Absolute kidney weights of all exposed groups and the relative kidney weight of 3,000 µg/kg rats were significantly increased at week 53.

The incidences of hepatocyte hypertrophy were significantly increased in the 1,000 and 3,000 µg/kg groups at 14 weeks and in all groups administered 300 µg/kg or greater at 31 and 53 weeks.

At 2 years, the incidences of hepatocyte hypertrophy were significantly increased in all dosed groups. The incidences of diffuse fatty change in the 300 µg/kg or greater groups and bile duct hyperplasia of the liver in 300 µg/kg and 3,000 µg/kg (core and stop-exposure) groups were significantly increased. The incidences of oval cell hyperplasia and pigmentation of the liver were significantly increased in the 3,000 µg/kg core study group.

At 2 years, two cholangiomas were seen in the 1,000 µg/kg group and two cholangiomas were seen in the 3,000 µg/kg stop-exposure group. A single hepatocellular adenoma was observed in the 3,000 µg/kg core study group.

At 53 weeks, sporadic incidences of minimal to mild follicular cell hypertrophy of the thyroid gland occurred in all groups (except 10 µg/kg). At 2 years, the incidences of minimal to mild follicular cell hypertrophy were significantly increased in the 300 µg/kg and 3,000 µg/kg (core and stop-exposure) groups.

At 2 years, significantly increased incidences of chronic active inflammation in the ovary and oviduct occurred in the 1,000 and 3,000 µg/kg core study groups. Incidences of suppurative inflammation of the uterus in the 1,000 µg/kg group and chronic active inflammation in the 3,000 µg/kg core study group were significantly greater than those in the vehicle control group. The NOAEL for these effects was 300 µg/kg bw.

The NTP stated that there was equivocal evidence of carcinogenic activity of PCB 153 in female Harlan Sprague-Dawley rats based on the occurrences of cholangioma of the liver. The NOAEL was 300 µg PCB 153/kg bw/day administered five days per week for two years corresponding to 210 µg/kg bw/day. The tissue concentrations of PCB 153 in fat and liver after 210 µg/kg bw/day for 105 weeks were 519,000 ng/g and 13,940 ng/g, respectively.

In addition, increased incidences of non-neoplastic lesions of the liver, thyroid gland (NOAEL of 100 µg/kg bw/day five days per week for two years, corresponding to 70 µg/kg bw/day), ovary, oviduct, and uterus (NOAEL of 300 µg/kg bw/day five days per week for two years, corresponding to 210 µg/kg bw/day) were induced in female rats. The tissue concentrations of PCB 153 in fat and liver after 70 µg/kg bw/day for 105 weeks were 158,434 ng/g and 3699 ng/g, respectively.

The NOEL for liver microsomal enzyme induction and sporadic increased incidences of minimal to mild follicular cell hypertrophy of the thyroid gland was 10 µg/kg bw/day five days per week for 53 weeks, corresponding to 7 µg/kg bw/day.

The tissue concentrations of PCB 153 in fat were 20,039 ng/g after 7 µg/kg bw/day for 2-years.

Based on the measured contents of PCB 153 in fat of male rats burdens burdens of 2, 16 and 52 mg/kg bw can be estimated for the dose levels of 7, 70, and 210 µg/kg bw/day, respectively, assuming that a rat contains 10% fat (Geyer et al. 1990).

PCB 170 (2,2',3,3',4,4',5-heptachlorobiphenyl)

PCB 170 is a contaminant in human milk (EFSA 2005). A median concentration of 17.9 ng PCB 170/g fat in human milk would correspond to an estimated median human body burden of 3.6 µg/kg bw assuming that the human body contains 20% fat.

Female B3C3F1mice were administered single intraperitoneal injections of various PCB mixtures and congeners 2 days before immunization with the antigen trinitrophenyl-lipopolysaccharide (TNP-LPS) and the immunosuppressive activity was measured after 4 day using the splenic plaque-forming cell (PFC) response and serum IgM units to TPN-LPS. PCB 170 was given at doses of 50,100,200 mg/kg bw The NOAEL was 50 mg PCB 170/ kg bw (Harper et al. 1995).

PCB 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl)

PCB 180 is a major contaminant in human milk. A median concentration of 45.8 ng PCB 180/g fat in human milk (EFSA 2005) would correspond to an estimated median human body burden of 9.16 µg/

kg bw assuming that the human body contains 20% fat.

Female B3C3F1 mice were administered single intraperitoneal injections of various PCB mixtures and congeners 2 days before immunization with the antigen trinitrophenyl-lipopolysaccharide (TNP-LPS) and the immunosuppressive activity was measured after 4 day using the splenic plaque-forming cell (PFC) response and serum IgM units to TPN-LPS. PCB 180 was given at doses of 50,100,200 mg/kg bw The NOAEL was 50 mg PCB 180/ kg bw The ED50 values for Aroclor 1260-, 1254-, 1248-, and 1242-induced immunotoxicity varied by less than twofold from 355 to 699 mg/ kg bw. The range of ED50 values were for TCDD, 4.6 to 4.9 µg/kg bw; PCB 77, 134 to 245 µg/kg bw; PCB 126, 4.7 to 7.0 µg/kg bw; PCB 169, 6.9 to 11.1 µg/ kg bw; PCB 105, 88,000 to 121,000 µg/kg bw; PCB 118, 122,000 to 132,000 µg/kg bw; PCB 156, 99,000 to 157,000 µg/kg bw; PCB 189, 89,000 to 129,000 µg/kg bw; PCB 170, 117,000 to 240,000 µg/kg bw; and PCB 180, 132,000 to 238,000 µg/kg bw. Based on the known concentrations of these congeners in the PCB mixtures, TCDD equivalents (TEQs) in the mixture were calculated for Aroclors 1260, 1254, 1248, and 1242 to be 16.0, 54.4, 260.4, and 197 mg/kg, respectively. Based on the ED50 value for the immunosuppressive activity of TCDD (4.8 µg/ kg bw), the calculated ED50 values for immune suppression by Aroclors 1260, 1254, 1248, and 1242 were 300, 88, 18, and 24 mg/kg, respectively. The ED50 (observed)/ED50 (calculated) ratios were 1.2, 5.9, 21, and 22.0 for Aroclors 1260, 1254, 1248 and 1242, respectively (Harper et al. 1995).

Purified PCBs 52, 138 and 180 were administered in a sweet jelly bit to pregnant Wistar rats from GD 7 to PND 21 at a dose of 1 mg/kg bw/day. Behavioural studies were performed on offspring and learning ability was found to be impaired by PCB 138 and 180, but not PCB 52. PCB 52 impaired motor coordination. Differences between the congeners were thus observed and were consistent with reduced amounts of NMDA receptors in cerebellum of pups exposed to PCB 138 and 180 but not PCB 52. In contrast, PCB 52 increased GABA (Boix et al. 2010).

Pregnant SD rats were exposed to PCB 180 from GD 7 to GD 10 in total doses of 0, 30, 100, 300, 1000 or 3000 mg/kg bw. PCB180 reduced free T3 slightly from 30 mg/kg bw in dams and had no effects in offspring at PND 35 but were reduced at PND 84 from 30 mg/kg bw (males). For PCB180 a LOAEL for thyroid effects could also be set to 30 mg/kg bw. Auditory function was examined in the offspring. The benchmark dose for effects on hearing thresholds was 292 mg/kg bw, corresponding to 73 mg/kg bw/day (Lilienthal et al. 2011).

Subacute toxicity studies

Highly purified PCB 180 (dioxinlike impurities: 2.7 ng TEQWHO/g PCB 180) was tested in a 28-day toxicity study in young adult Sprague-Dawley rats. Groups of five male and female rats were given total doses of 3, 10, 30, 100, 300, 1000 or 1700 mg/kg bw PCB 180 in corn oil by gavage. Loading doses were administered on days 0-5 and maintenance doses on days 10, 17, and 24. At the end of the treatment period (males on study day 28-32, females on days 28-32) blood samples were obtained and the rats were killed by exsanguination. A complete necropsy (macroscopic observations, tissue sampling for molecular biology, biochemistry, histopathology, analytical chemistry and organ weights) was performed on each rat. Tissue samples were stored at −80 ÅC for further analysis. In addition, perirenal adipose tissue and liver were stored at −20 ÅC for determination of PCB 180 tissue concentration. In the present study only liver tissue samples, as the most sensitive organ for PCB exposure, and blood serum samples were analyzed. Increased liver weights were observed at ≥300 mg/kg bw in males and females. No increases in serum ALT or ALP activities were found. A significant increase in liver pentoxyresorufin O-dealkylase (PROD) activity was found in males at ≥10 mg/kg bw and in females at ≥30 mg/kg bw. A significant induction of hepatic 7-ethoxyresorufin O-deethylase (EROD) activity was also observed in males at ≥10 mg/ kg bw and in females at ≥300 mg/kg bw. Western blotting showed that mainly cytochromes P450 (CYPs) 2B1/2 and 3A1 were induced while only slight effects were seen on CYP1A1, CYP1A2 and CYP1B1. However, no induction of CYP1A1, 1A2 and 1B1 was found on the mRNA level, except for a slight effect in females at 1000 mg/kg bw. Furthermore, hepatic UDP-glucuronosyltransferases (UGTs) 1A1 and 1A6 were markedly induced in males at ≥300 mg/kg bw but only slightly induced in females at ≥1000 mg/kg bw. The hepatic concentrations of apolar retinoids were decreased in males at ≥30 mg/kg bw and in females at ≥300 mg/kg bw. Centrilobular liver hypertrophy (staged in three classes, mild (1), moderate (2), and severe (3)) was

observed on histopathology in both genders. A benchmark dose (BMD) approach was used to model lowest effective dose levels for these effects. For centrilobular liver hyperthrophy the BMD5s in males for progressing to respectively stage 1, 2, and 3 were 15, 17, and 62 mg/kg bw, and in females 205 and 617 mg/kg bw (females would not progress to stage 3). The corresponding BMDL5s for progressing to stage 1 were 9.4 mg/kg bw in males and 139 mg/kg bw in females. The findings show that pure PCB 180 leads to hepatic changes in a dose range which did not cause CYP1A1 induction but causes centrilobular liver hypertrophy, affects drug-metabolizing enzymes involved in the metabolism of exogenous and endogenous substrates and leads to changes in liver retinoid levels. Comparison of PCB 180 liver level related to BMDL5 for hepatic hypertrophy in rats with human data on hepatic PCB levels in individuals without history of specific exposure suggests a relatively small margin of tissue burden in the range of 37-fold. The results show that the highly pure non dioxin-like PCB 180 exerted strong effects different to dioxin-like compounds and that the low TEQ contamination allowed a characterization of the PCB as non-dioxinlike (Ross et al. 2011).

In conclusion PCB 180 causes liver enlargement, liver enzyme induction, decreased hepatic apolar retinoid levels and centrilobular hepatocellular hypertrophy in rats in a dose range which does not cause characteristic AhR-mediated responses such as induction of CYP1A1 mRNA suggesting an AhR-independent mode of action with a biologically relevant outcome. CYP2B1 and CYP3A1 induction are the most sensitive parameters for exposure to PCB 180 and male rats are more sensitive than females (Ross et al. 2011).

PCB 183 (2,2',3,4,4',5',6-heptachlorobiphenyl)

PCB 183 is a contaminant in human milk (EFSA 2005). A median concentration of 6.0 ng PCB 183/g fat in human milk would correspond to an estimated median human body burden of 1.2 µg/kg bw assuming that the human body contains 20% fat.

No *in vivo* toxicological data were available on PCB 183.

PCB 187 (2,2',3,4',5,5',6-heptachlorobiphenyl)

PCB 187 is a contaminant in human milk (EFSA 2005). A median concentration of 9.6 ng PCB 187/g fat in human milk would correspond to an estimated median human body burden of 1.92 µg/kg bw assuming that the human body contains 20% fat.

No *in vivo* toxicological data were available on PCB 187.

PCB 194 (2,2',3,3',4,4',5,5'-octachlorobiphenyl)

PCB 194 is a contaminant in human milk (EFSA 2005). A median concentration of 3.2 ng PCB 194/g fat in human milk would correspond to an estimated median human body burden of 0.64 µg/kg bw assuming that the human body contains 20% fat.

No *in vivo* toxicological data were available on PCB 194.

PCB 206 (2,2',3,3',4,4',5,5',6-nonachlorobiphenyl)

PCB 206 is a minor contaminant in human milk (Table in chapter 6.3 of the Opinion). A median concentration of 0.30 ng PCB 183/g fat in human milk would correspond to an estimated median human body burden of 0.06 µg/kg bw assuming that the human body contains 20% fat.

The dose-dependent effects of PCB 206 on the suppression of the splenic plaque-forming cell (PFC) response to the T-cell-dependent antigen, sheep red blood cells (SRBCs) and the T-cell-independent antigen, trinitrophenyl-lipopolysaccharide (TNP-LPS), were determined in male C57BL/6 (10, 25, and 100 µmol/kg bw, single intraperitoneal dose) and DBA/2 mice (25,100, and 400 µmol/kg bw, single intraperitoneal dose). The induction of hepatic microsomal ethoxyresorufin O-deethylase (EROD) activity was also measured. PCB 206 suppressed the splenic PFC response to SRBCs in C57BL/6 and in DBA/2 mice at the two higher dose levels. It was relatively more active in the C57BL/6 mice, which are more responsive to aryl hydrocarbon (Ah) receptor agonists than

DBA/2 mice. The NOAEL was 10 µmol (4.6 mg)/kg bw. PCB 206 had no effect on TNP-LPS response, and only marginally induced hepatic microsomal EROD activity in C57BL/6 mice, not in DBA/2 mice (Harper et al. 1993).

PCB 207 (2,2',3,3',4,4',5,6,6'-nonachlorobiphenyl)

PCB 207 was not reported in human milk (EFSA 2005).

The dose-dependent effects of PCB 207 on the suppression of the splenic plaque-forming cell (PFC) response to the T-cell-dependent antigen, sheep red blood cells (SRBCs) and the T-cell-independent antigen, trinitrophenyl-lipopolysaccharide (TNP-LPS), were determined in male C57BL/6 (10,25, and 100 µmol/kg bw, single intraperitoneal dose) and DBA/2 mice (25,100, and 400 µmol/kg bw, single intraperitoneal dose). The induction of hepatic microsomal ethoxyresorufin O-deethylase (EROD) activity was also measured. PCB 207 suppressed the splenic PFC response to SRBCs in C57BL/6 at all doses, and in the DBA/2 mice at the highest dose. It was relatively more active in the C57BL/6 mice, which are more responsive to aryl hydrocarbon (Ah) receptor agonists than DBA/2 mice. The LOAEL was 10 µmol (4.6 mg)/kg bw. PCB 207 only marginally (non-significant) induced hepatic microsomal EROD activity in C57BL/6 mice (Harper et al. 1993).

PCB 208 (2,2',3,3',4,5,5',6,6'-nonachlorobiphenyl)

PCB 208 was not reported in human milk (EFSA 2005).

The dose-dependent effects of PCB 208 on the suppression of the splenic plaque-forming cell (PFC) response to the T-cell-dependent antigen, sheep red blood cells (SRBCs) and the T-cell-independent antigen, trinitrophenyl-lipopolysaccharide (TNP-LPS), were determined in male C57BL/6 (10,25, and 100 µmol/kg bw, single intraperitoneal dose) and DBA/2 mice (25,100, and 400 µmol/kg bw, single intraperitoneal dose). The induction of hepatic microsomal ethoxyresorufin O-deethylase (EROD) activity was also measured. PCB 208 suppressed the splenic PFC response to SRBCs in C57BL/6 at all doses, and in the DBA/2 mice at the two higher doses. It were relatively more active in the C57BL/6 mice, which are more responsive to aryl hydrocarbon (Ah) receptor agonists than DBA/2 mice. The LOAEL was 10 µmol (4.6 mg)/kg bw. PCB 208 only marginally (non-significant) induced hepatic microsomal EROD activity in C57BL/6 mice and not in DBA/2 mice (Harper et al. 1993).

PCB 209 (decachlorobiphenyl)

PCB 209 is a minor contaminant in human milk (EFSA 2005). A median concentration of 0.14 ng PCB 209/g fat in human milk would correspond to an estimated median human body burden of 0.028 µg/kg bw assuming that the human body contains 20% fat.

The dose-dependent effects of PCB 209 on the suppression of the splenic plaque-forming cell (PFC) response to the T-cell-dependent antigen, sheep red blood cells (SRBCs) and the T-cell-independent antigen, trinitrophenyl-lipopolysaccharide (TNP-LPS), were determined in male C57BL/6 (10,25, and 100 µmol/kg bw, single intraperitoneal dose) and DBA/2 mice (25,100, and 400 µmol/kg bw, single intraperitoneal dose). The induction of hepatic microsomal ethoxyresorufin O-deethylase (EROD) activity was also measured. PCB 209 suppressed the splenic PFC response to SRBCs in C57BL/6 at the highest dose, and in the DBA/2 mice at the two highest doses. In both strains the NOAEL was 25 µmol (11.5 mg)/kg bw and the LOAEL was 100 µmol (46 mg)/kg bw. PCB 209 did not induce hepatic microsomal EROD activity in C57BL/6 mice and DBA/2 mice (Harper et al. 1993).

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Appendix 3

Comparison of animal NOAEL/LOAEL body burden (BB) for NDL-PCBs tested *in vivo* with estimated human body burdens of NDL-PCB, expressed as margin of body burden (MoBB). Congeners in gray shade are found in human milk. The references are included in Appendix 2.

PCB No	Human BB [a] (µg/kg)	T½ (days)	Effect	NOAEL µg/kg /day	NOAEL BB (µg/kg)	LOAEL µg/kg /day	LOAEL BB (µg/kg)	MOBB[b] NOAEL	MOBB[b] LOAEL	Reference
1			Increased uterus weight, immature rat			160,000 i.p.	160,000[e]			Ecobichon(1974)
2			Increased uterus weight, immature rat	160,000 i.p.	160,000[e]					Ecobichon (1974)
3			Increased uterus weight, immature rat	160,000 i.p.	160,000[e]					Ecobichon (1974)
4		0.15	Increased uterus weight, immature rat			160,000 i.p.	160,000[e]			Ecobichon (1974)
8		0.18	Increased uterus weight, immature rat	160,000 i.p.	160,000[e]					Ecobichon (1974)
11			Increased uterus weight, immature rat	160,000 i.p.	160,000[e]					Ecobichon (1974)
15			Increased uterus weight, immature rat	160,000 i.p.	160,000[e]					Ecobichon (1974)
18	0.018	2.0	Increased uterus weight, immature rat Serum thyroxine, weanling rat	 128,000	 190,000[c]	8,000 i.p.	12,000[c]	 $>10^7$	670,000	Li (1995) Li (1995)
28	0.44	1.4	90-day toxicity, rat, liver and thyroid Repro rat, decreased body weight and spatial learning in female offspring	36 p.o. 8,000 p.o. .	400[d] 14,000[c]	360 p.o. 32,000 p.o.	4000[d] 56,000[c]	900 32,000	9,000 127,000	Chu (1996a) Ness(1993)
33	0.012	0.2	-							
37	0.0025	0.34	-							
47	0.28	3	Repro, rat, decreased dopamin in offspring Repro, rat, sexual behaviour Thyroid, weanling rat, Increased uterus weight, immature rat Repro, rat, decreased dopamin in offspring	1,000 p.o. 1,000 i.p. 4,100 p.o. 3,000 10,000	4,200[c] 4,200[c] 4,100[d] 6,000[c] 42,000[c]	10,000 20,000 9,000 20,000	42,000[c] 84,000[c] 18,000[c] 84,000[c]	15,000 15,000 15,000 21,000 150,000	150,000 300,000 64,000 300,000	Seegal (1997) Wang (2002) Saeed (1997) Seegal (2005) Seegal (2005)
52	0.064	0.9	Increased uterus weight, immature rat Immunotoxicity, rat Thyroid, T3 in dams, rat Thyroid, T3 in offspring, rat Hearing threshold, rat offspring Learning, rat offspring	 1,000 p.o. 3,000 1,000	 5,000[c] 4,000[c] 1,300	14,000 i.p. 10,000 3,000 5,000	20,000[c] 13,000[c] 4,000[c] 6,500[c]	 78,000 63,000 20,000	312,500 200,000 63,000 100,000	Jansen (1993) Sargent (1991) Lillienthal (2011) Lillienthal (2011) Lillienthal (2011) Boix (2010)
54		0.2	Increased uterus weight, immature rat	3,000	3,600[c]	10,000	12,000[c]			Arcaro(1999)
60	0.082	0.3	-							
66	0.24		-							
74	1.36	3.1 (37)	-							
95		1.4	Repro, rat Repro, behaviour, rat Thyroid, rat Repro, auditory cortex changes,rat	32,000 po 4,000 i.p.	64,000[c] 7,000[c]	 8,000 p.o. 8,000 i.p. 6000 p.o.	 16,000[c] 14,000[c] 12,000[c]			Schantz (1996) Schants (1997) Khan (2002) Kenet (2007)
99	1,24	>90	-							
101	0.138	2.6	Thyroid, rat			16,000 i.p.	30,000[c]		220,000	Khan (2002, 2003)
110	0.042	2.5 (64)	Oestrogenicity and thyroid hormone, rat	4,000 i.p.	8,000[c]	16,000 i.p.	32,000[c]	190,000	762,000	Li (1998)
128	0.126	6.3	90 day toxicity, rat, liver and thyroid	42 p.o.	800[d]	420 p.o.	7,000[d]	6,500	55,000	Lecavalier (1997)
132			Oestrogenicity, thyroid hormones, immature rat Sperm count, rat offspring	48,000 i.p.	96,000[c]	 1,000	 1,000[e]			Li (2001) Hsu (2007)
138	11.1	>90	Learning, rat offspring			1,000	15,000[c]		1,400	Boix (2010)
141	0.034	>90								
149			Oestrogenicity, immature rat Thyroid hormones, immature rat	48,000 i.p. 8,000	96,000[c] 16,000[c]	 32,000	 64,000[c]			Li (2001) Li (2001)

PCB No	Human BB [a] (µg/kg)	T½ (days)	Effect	NOAEL µg/kg /day	NOAEL BB (µg/kg)	LOAEL µg/kg /day	LOAEL BB (µg/kg)	MOBB[b] NOAEL	MOBB[b] LOAEL	Reference
153	13.56	>90	90 day toxicity, rat, liver and thyroid	34 p.o.	1,200[d]	340	9,000[d]	85	660	Chu (1996b)
			Reproduction, rat	125,000	125,000[c]	250,000	250,000[c]	9200	18,400	Morrisey (1992)
			Oestrogenicity, rat, ip	11,000 i.p.	22,000[c]	25,000 i.p.	50,000[c]	1,600	3,700	Li (1994)
			Thyroid, rat offspring			16,000	32,000[c]		2,300	Ness (1993)
			Learning, rat offspring	16,000 po.	32,000[c]	32,000	64,000[c]	2,300	4,700	Schantz (1995)
			Hyperactivity, rat offspring			5,000	50,000[c]		3,687	Holene (1998)
			Immunotoxicity, mouse, po	100,000	100,000[e]			7500		Kerkvliet (1990)
			Thyroid, rat offspring	16,000	112,000[c]	64,000	448,000[c]	8,300	33,000	Kobayashi (2008)
			Growth, rat offspring	1,000	35,000[c]	5,000	175,000[c]	2,600	13,000	Sitarek (2009)
			Elevated monoamine in brain, offspring, rats	16,000	112,000[c]	64,000	448,000[c]	8,300	33,000	Honma (2009)
			Testis/sperm, rat offspring	25	125[c]	2,500	12,500[c]	10	50	Xiao (2010)
			Thyroid, rat offspring			25	125[c]		10	Xiao (2010)
			2-Year toxicity, liver and thyroid, rat	70	16,000[d]	210	52,000[d]	1,200	3,800	NTP, (2006)
170	3.58	>90	Immunotoxicity, mice, ip	50,000	50,000[e]	100,000	100,000[e]	14,000	28,000	Harper (1995)
180	9.16	>90	Immunotoxicity, mice, ip	50,000	50,000[e]	100,000	100,000[e]	5,500	11,000	Harper (1995)
			Learning, rat offspring			1,000	15,000[c]		1650	Boix (2010)
			Thyroid, T3, rat offspring			7,500	30,000[c]		3,300	Lilienthal (2011)
			Hearing threshold, rat offspring, BMD			73,000	73,000[c]		8,000	Lilienthal (2011)
			28-Days toxicity, liver hypertrophy, rat	340	9,400[c]	500	15,000[c]	1025	1650	Ross (2011)
183	1.2		-							
187	1.92	>90	-							
194	0.64	>90	-							
206	0.06		Immunotox, mice, ip	4,600	4,600[e]	11,500	11,500[e]	77,000	192,000	Harper (1993)
207			Immunotox, mice, ip	4,600	4,600[e]	11,500	11,500[e]			Harper (1993)
208			Immunotox, mice, ip	4,600	4,600[e]	11,500	11,500[e]			Harper (1993)
209	0.028		Immunotox, mice, ip	11,500	11,500[e]	46,000	46,000[e]	410,000	1,600,000	Harper (1993)

a) Human body burdens calculated from the median PCB concentrations found in human milk (EFSA 2005)) assuming 20% lipid in the human body.

b) MoBB (Margin of body burdens) is calculated by dividing the estimated body burden in animals at the NOAEL or LOAEL with the calculated median human body burden

c) The body burden at study termination was estimated assuming 100% bioavailability and one compartment, first order elimination kinetics, using the half-lives in rats reported for individual PCB by Tanabe *et al.* (1981).

d) The body burdens were calculated from the reported measured accumulated concentrations of the respective NDL-PCB in the fat tissue of the rats. It was assumed that the rats contained 10% fat as reported by Geyer *et al.*, (1990).

e) Single dose study. The dose was considered equal to the body burden, assuming 100% bioavailability, irrespective of the route of administration.

Evaluation of health hazards by exposure to Polychlorinated biphenyls (PCB) and proposal of a health-based quality criterion for soil

This report describes an evaluation of health hazards by exposure to polychlorinated biphenyls (PCB), in order to get documentation for a health-based quality criterion in soil for PCB with focus on the PCB congeners of relevance in contaminated soil.

The report has been elaborated according to the general practice laid down in the Danish EPA guidance document for the setting of a health-based quality criterion for chemical substances in relation to soil, which is based on children's ingestion of soil. The conclusion of this evaluation was that a health-based soil quality criterion based alone on children's ingestion of soil contaminated with PCBs is not considered to be relevant.

Danish Ministry of the Environment
Environmental Protection Agency

Strandgade 29
1401 Copenhagen K, Denmark
Tel.: (+45) 72 54 40 00
www.mst.dk