Risk assessment of contaminant intake from traditional Greenland food items

Nielsen, Elsa; Larsen, John Christian; Ladefoged, Ole

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Prepared by:

Elsa Nielsen
John Christian Larsen
Ole Ladefoged

Department of Toxicology and Risk Assessment
Danish Institute for Food and Veterinary Research

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Danish Veterinary and Food Administration
Mørkhøj Bygade 19, DK-2860 Søborg
Tel. + 45 72 34 60 00, fax + 45 72 34 70 01

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Preface

People in Greenland have generally a higher intake of contaminants from their diet than people in the more westernised countries because marine traditional food items such as fish, seabirds, seals and whales are much more important dietary sources in Greenland, and because some of these food items have high levels of some heavy metals as well as of persistent organochlorines. It has recently been published that the intake of a number of these contaminants from the traditional diet exceeded the tolerable daily intake (TDI).

The scope of this report is to present the assessments of the risk to Greenlanders of experiencing adverse health effects from intake of contaminants from the traditional food items by using another approach for the risk characterisation than a comparison of the estimated intakes with the tolerable daily intake. Eleven contaminants have been evaluated in this report: lead, cadmium, mercury, selenium, PCB, DDT, chlordane, HCH, chlorobenzenes, dieldrin, and toxaphene.

The report has been prepared by the Danish Institute for Food and Veterinary Research, Department of Toxicology and Risk Assessment, as a contract work for the National Environmental Research Institute, Department of Arctic Environment and financed by the Danish Environmental Protection Agency as part of the environmental support program Dancea – Danish Cooperation for Environment in the Arctic.

The risk assessments of the selected contaminants have been presented and discussed at a meeting in the national human health advisory group on AMAP (Arctic Monitoring and Assessment Programme) activities in Greenland and The Faroe Islands held 7 January 2005, and at a national seminar on contaminants in traditional Greenland food items held 1 April 2005.

The authors are solely responsible for all the assessments and conclusions presented in the report, and do not necessarily reflect the position of the Danish Institute for Food and Veterinary Research, the National Environmental Research Institute, or the Danish Environmental Protection Agency.

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In addition, we thank David Boertmann, the National Environmental Research Institute, Department of Arctic Environment, for kindly providing the photo presented on the front page of the report.
Finally we would like to thank the National Environmental Research Institute, Department of Arctic Environment / the Danish Environmental Protection Agency for the financial support.

Summary

The traditional diet is a significant source of exposure to contaminants to people in Greenland. Some traditional food items contain very high levels of heavy metals as well as of persistent organic pollutants.

A risk assessment in order to evaluate the risk to Greenlanders of experiencing adverse health effects from intake of contaminants from traditional food items has been performed for eleven contaminants, including lead, cadmium, mercury, selenium, PCB, DDT, chlordane, HCH, chlorobenzenes, dieldrin and toxaphene.

Within the European Union, specific programs on risk assessment for new and existing chemical substances are on-going. A Technical Guidance Document[1] (TGD) supporting the risk assessment regulations has been prepared and provides the detailed methodology for the risk assessment process.

In this report, the risk to Greenlanders of experiencing adverse health effects from intake of the eleven selected contaminants from traditional food items has been characterised and evaluated by using the principles for the risk characterisation process according to the TGD as outlined in section 3.1, i.e., the MOS approach.

The risk characterisation has been carried out by quantitatively comparing the outcome of the effect assessment, i.e., the NOAEL (or LOAEL) established for the critical effect(s) of a given contaminant, to the outcome of the exposure assessment, i.e., the mean daily human intake of that specific contaminant from traditional Greenland food items as presented in Johansen et al. (2004a[2],b[3]). The ratio resulting from this comparison is called Margin of Safety (MOS); i.e., MOS = N(L)OAEL / mean daily human intake of the contaminant.

In the characterisation of the risk, the MOS has been compared with a reference MOS (MOSref). When the MOS value is clearly below the MOSref, a concern for adverse health effects among Greenlanders due to long-term intake of a specific contaminant from the traditional Greenland food items is identified and it is recommended to reduce the intake of the specific contaminant from the traditional Greenland food items as well as from other sources. Thus, it is not the value of the MOS in it self but the ratio 'MOS/MOSref', which is crucial for the identification of a concern. This means strictly spoken that the lower the ratio 'MOS/MOSref' is (or the higher the ratio 'MOSref/MOS' is), the higher the concern (or risk) is for adverse health effects following dietary intake of a specific contaminant. When the MOS value is in the range of the MOSref, a more careful overall evaluation on a case-by-case basis has been performed.

[1] Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances. European Chemicals Bureau, European Commission, Joint Research Centre, EUR 20418 EN.

[2] Johansen P, Muir D, Asmund G and Riget F (2004a). Human exposure to contaminants in the traditional Greenland diet. Sci Total Environ **331**, 189-206.

[3] Johansen P, Asmund G and Riget F (2004b). High human exposure to lead through consumption of birds hunted with lead shot. Environ Pollut **127**, 125-129.

The MOSref is, as addressed in section 3.1.2.2, an overall assessment (or uncertainty) factor (AF), as a numerical value, addressing the differences between the experimental data and the human situation, taking into account the uncertainties in the extrapolation procedure and in the available data set. When the available data for a given contaminant did not allow the derivation of substance-specific assessment factors for the various elements included in the uncertainties related to the establishment of a NOAEL for the critical effect(s) for that contaminant, default values have been applied.
A default value of 10 has been applied for the assessment factor (AF_I) accounting for the interspecies variation, i.e., to account for that humans are assumed to be more susceptible to a given effect of a contaminant than experimental animals.
A default value of 10 has been applied for the assessment factor (AF_{II}) accounting for the inter-individual variation, i.e., to account for that individuals in the human population such as children and the unborn child, pregnant women, elderly, or sick people may be more susceptible than the general population.
No default assessment factor (AF_{III}) has been applied to account for other uncertainties than the interspecies and inter-individual variation in the establishment of a NOAEL for the critical effect(s) for a specific contaminant, but a value from 1 to 10(100) has been applied dependent on the uncertainties in the various elements included in assessment factor (AF_{III}).

For contaminants, which are both genotoxic and carcinogenic, a quantitative risk characterisation has not been performed for this endpoint. The reason for this decision is that there currently is no clear consensus on an appropriate methodology for the estimation of a no-effect level. For these contaminants, it is recommended that the intake of the specific contaminant from the traditional Greenland food items as well as from other sources should be reduced.

A MOS in the range of 0.03 to 0.17 has been calculated for the mean daily human intake of cadmium from traditional Greenland diet in the spring and of 0.05 to 0.33 in the fall. These MOS values are well to far below the MOSref of 3 (about 9-100 times below) implicating a concern of experiencing adverse health effects in the kidney and bone among Greenlanders due to long-term intake of cadmium from the traditional Greenland food items. Furthermore, a carcinogenic and a genotoxic potential of cadmium for humans in relation to dietary exposure cannot be excluded. Consequently, it is recommended to reduce the intake of cadmium from the traditional Greenland food items as well as from other sources.

A MOS of 3 has been calculated for the mean daily human intake of lead from murre and of 0.4 for eider. The MOS value for murre is equal to the MOSref and the MOS value for eider is below the MOSref of 3 (about 7 times below) implicating a concern of experiencing adverse health effects in the developing nervous system among Greenland children due to long-term intake of lead through the consumption of eider hunted with lead shot. The concern of experiencing adverse health effects from the consumption of murre is low. A carcinogenic potential cannot be fully excluded in relation to dietary exposure to lead, but is not considered to be of any great significance through consumption of birds hunted with lead shot. However, as no threshold apparently exists for the critical effect, it is recommended to reduce the intake of lead from the traditional Greenland food items as well as from other sources.

Two sources, seal muscle and seal liver, dominate the intake of mercury. In meat from fish and marine animals, mercury is predominantly present in the form of methylmercury whereas in the liver, mercury is predominantly present in the form of inorganic mercury. Inorganic mercury in food is considered as being considerably less toxic than methylmercury. Therefore, the risk characterisation

has been performed for two scenarios: 1) the intake of total mercury was assumed as all of the mercury was methylmercury; and 2) the intake of total mercury was assumed as all of the mercury was inorganic mercury.
1) A MOS of 0.91 has been calculated for the mean daily human intake of methylmercury in the spring and of 1.4 in the fall. These MOS values are below the MOSref of 3 (about 3 times below in the spring and about 2 times below in the fall) implicating a concern of experiencing adverse health effects in the developing nervous system among Greenlanders due to long-term intake of methylmercury from the traditional Greenland food items. As no threshold has been identified for the critical effect, it is recommended to reduce the intake of methylmercury from the traditional Greenland food items as well as from other sources.
2) A MOS of 273 is calculated for the mean daily human intake of inorganic mercury in the spring and of 429 in the fall. These MOS values are below the MOSref of 1000 (about 3 times below in the spring and about 2 times below in the fall) implicating a concern of experiencing adverse health effects in the kidney among Greenlanders due to long-term intake of inorganic mercury from the traditional Greenland food items. Therefore, it is recommended to reduce the intake of inorganic mercury from the traditional Greenland food items as well as from other sources.

A MOS of 6.7 is calculated for the mean daily human intake of selenium in the spring and of 7.6 in the fall. These MOS values are below the MOSref of 10 implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of selenium from the traditional Greenland food items. However, as the calculated MOS values are very close to the MOSref and as the NOAEL used as the starting point for the risk characterisation was a conservative estimate, the concern is very low.

The toxicological evaluation of PCBs has included commercial and defined PCB mixtures as well as the individual congeners included in the sPCB10 (congeners CB 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180) analysed in the traditional Greenland food items, which represents most of the predominant congeners in fish and marine mammals. It has been considered reasonable to use commercial PCB mixtures as a surrogate for sPCB10 in the evaluation of adverse health effects from exposure to sPCB10 and therefore, the LOAEL established for commercial PBC mixtures has been used as the starting point in the risk characterisation of PCBs.
A MOS of 13 has been calculated for the mean daily human intake of sPCB10 in the spring and in the fall. This MOS value is far below the MOSref of 1000 (about 75 times below) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of PCBs from the traditional Greenland food items. Consequently, it is recommended to reduce the intake of PCBs from the traditional Greenland food items as well as from other sources.

The toxicological evaluation of DDT has included *p,p´*-DDT and technical DDT as well as the two isomeric forms of the metabolite DDE: *o,p*-DDE and *p,p*-DDE. DDE is the most persistent of the two metabolites and also more persistent than DDT, and DDE is usually found at higher levels than DDT (and DDD) in the tissues. It has been considered appropriate to use the NO(A)EL established for the sum of DDT and DDE as a starting point in the risk characterisation of DDT although the intake estimates of DDT also include the metabolite DDD.
A MOS of 94 has been calculated for the mean daily human intake of sDDT in the spring and of 120 in the fall. These MOS values are below the MOSref of 150 (about 1.6 times below in the spring and about 1.3 times below in the fall) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of DDT from the traditional Greenland food items. However, as the calculated MOS values are close to the MOSref and as the

NO(A)EL is a conservative estimate for minimal histological effects in the liver, the concern is low.

The toxicological evaluation of chlordane has included the seven compounds representing sCHL (sum of heptachlor, heptachlor epoxide, oxychlordane, cis- and trans-chlordane, and cis- and trans-nonachlor) analysed in the traditional Greenland food items. No data have been located regarding adverse health effects of oxychlordane (metabolite of chlordane) and nonachlor following repeated oral administration. Analytical grade chlordane used in the experimental studies consisted of 72% *cis* and 23% *trans* isomers. Technical grade chlordane is a mixture of more than 140 related substances and consists generally of 60-85% of *cis*- and *trans*-chlordane; other major constituents are chlordene, heptachlor, and *cis*- and *trans*-nonachlor. Technical grade heptachlor used in the experimental studies consisted of 73% heptachlor, 18% *trans*-chlordane, 2% *cis*-chlordane. Overall, specific toxicological evaluations could not be performed for analytical grades of chlordane / heptachlor versus the technical grades due to lack of data. Oxychlordane and heptachlor epoxide are the metabolites of chlordane and heptachlor, respectively, that are considered of primary toxicological significance. Based on the available data, it has been considered that the toxicological potency of chlordane and heptachlor, and consequently of oxychlordane and heptachlor epoxide, is quite similar, and the NOAEL established for heptachlor epoxide has been used as the starting point for the risk characterisation of chlordane.
A MOS of 83 has been calculated for the mean daily human intake of sCHL in the spring and of 100 in the fall. These MOS values are well below the MOSref of 1000 (about 12 times below in the spring and about 10 times below in the fall) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of chlordane from the traditional Greenland food items. Consequently, it is recommended to reduce the intake of chlordane from the traditional Greenland food items as well as from other sources.

The toxicological evaluation of chlorobenzenes has included the three isomers constituting the sCBz (sum of 1,2,3,4-tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene) analysed in the traditional Greenland food items.
The available data on chlorobenzenes in general indicate a trend for the toxicity to increase with increased chlorination of the benzene ring and the LOAEL established for hexachlorobenzene has been used as the starting point for the risk characterisation of chlorobenzenes.
A MOS of 150 has been calculated for the mean daily human intake of sCBz in the spring and in the fall. This MOS value is well below the MOSref of 1000 (about 6.6 times below) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of chlorobenzenes from the traditional Greenland food items. However, as the LOAEL is considered to be a conservative estimate, the concern is relatively low.

The toxicological evaluation of hexachlorocyclohexanes (HCH) has included the three HCH isomers constituting the sHCH (sum of α-, β- and γ-HCH) analysed in the traditional Greenland food items as well as technical HCH.
β-HCH is the most persistent isomer in the environment and accumulates to a greater degree in tissues and organs than the other isomers. γ-HCH is the most toxic isomer in acute toxicity studies, but the available data from intermediate- and long-term experimental studies do not indicate noteworthy differences in the general toxicity of the individual isomers; however, isomer specific differences have been indicated for reproductive/developmental and carcinogenic effects. The LOAEL established for γ-HCH has been used as the starting point for the risk characterisation of HCH.

A MOS of 150 has been calculated for the mean daily human intake of sHCH in the spring and of 200 in the fall. These MOS values are below the MOSref of 1000 (about 6.6 times below in the spring and about 5 times below in the fall) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of HCH from the traditional Greenland food items. However, as the LOAEL is considered to be a conservative estimate and to provide adequate protection against liver toxicity and probably also carcinogenicity, neurotoxicity, and reproductive and developmental effects of the three individual HCH isomers as well as of t-HCH, the concern is relatively low.

A MOS of 38 has been calculated for the mean daily human intake of dieldrin in the spring and of 43 in the fall. These MOS values are below the MOSref of 100 (about 2.6 times below in the spring and about 2.3 times below in the fall) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of dieldrin from the traditional Greenland food items. However, as the calculated MOS values are at the same order of magnitude as the MOSref and as the NOAEL is considered to be a conservative estimate for minimal effects in the liver, the concern is relatively low.

There are major difficulties in assessing the hazards of toxaphene and the risks related to toxaphene exposure due to the fact that toxaphene is a complex mixture containing a large number of individual compounds. Toxaphene levels in traditional Greenland food items have been analysed as "total" toxaphene quantified with a technical toxaphene standard and 22 chlorobornane congeners. Furthermore, toxaphene residues in biological samples show a different profile from that of technical toxaphene presumably due to metabolic transformation and degradation of some components. Humans may therefore be exposed via the diet to different components than those, which have been tested in experimental systems. Also the toxicological data set of toxaphene is rather scarce and most of the information is from older studies using poorly characterised test materials and lack of compliance with modern test guidelines.
A MOS of 400 has been calculated for the mean daily human intake of toxaphene from traditional Greenland food items in the spring and of 387 in the fall. These MOS values are far below the MOSref of 10000 (about 25-26 times below) implicating a significant concern of experiencing adverse health effects in the target organs and tissues among Greenlanders due to long-term intake of toxaphene from the traditional Greenland food items. Furthermore, a carcinogenic potential of toxaphene for humans in relation to dietary exposure cannot be excluded. In addition, based on the current limited database, it cannot be concluded whether toxaphene has a genotoxic potential *in vivo* or not. This implicates that a NOAEL cannot be derived for the carcinogenic effects for the time being. Consequently, it is recommended to reduce the intake of toxaphene from the traditional Greenland food items as well as from other sources.

Table 1 (see last page of section 4) summarises the outcome of the risk assessments as performed for the eleven contaminants evaluated in this report.

The MOSref derived for the selected contaminants has been compared with the overall uncertainty factor applied by e.g., FAO/WHO (JECFA/JMPR) in establishing an acceptable or tolerable daily intake (ADI/TDI), or by US-EPA in establishing an oral reference dose (RfD) when available in order to evaluate whether significant differences could be identified between the risk characterisation of the contaminants as performed in this report (the MOS approach) versus the more traditional approach (ADI/TDI or RfD approach).

No significant difference between the risk characterisations of cadmium, lead, mercury, DDT, and dieldrin as performed in this report (the MOS approach) versus the more traditional approach (TDI or RfD approach) has been identified.
The risk characterisations of selenium and PCBs as performed in this report (the MOS approach) are a little more conservative than the more traditional approach (TDI or RfD approach); however, a significant difference has not been identified.
The risk characterisations of chlordane, chlorobenzenes, and hexachlorocyclohexanes (HCH) as performed in this report (the MOS approach) are conservative compared to the more traditional approach (TDI or RfD approach). This is predominantly because the risk characterisations performed for these contaminants in this report include a number of isomers whereas the FAO/WHO (JMPR) and US-EPA evaluations in most cases only consider a specific isomer.
No comparison could be performed for toxaphene as no guidance values have been established.

According to the current practices, risk assessments of exposures to chemical substances are generally based upon data from studies on the individual substances. However, humans are simultaneously exposed to a large number of chemical substances that potentially possess a number of similar or different toxic effects, as is also the case for Greenlanders via consumption of traditional food items. A concern for additive effects is indicated when all the substances in the mixture act by the same mode of action, and thus differ only in their potencies.
A number of the contaminants evaluated in this report act on the same target organ and possibly by the same mode of action. It is beyond the scope of this report to evaluate the potential adverse effects of combined exposure to the contaminants in the traditional Greenland food items; however, the available data indicate a concern for additive effects of some of the individual contaminants. This concern should be taken into account in a consideration of risk reduction to be recommended for the contaminants for which the risk characterisation has indicated relatively low or low concern of experiencing adverse health effects among Greenlanders due to long-term intake of these contaminants from the traditional Greenland food items.

In conclusion, the risk characterisations of cadmium, lead, mercury, PCBs, chlordane, and toxaphene, as performed in this report indicate a concern of experiencing adverse health effects among Greenlanders due to long-term intake of these contaminants from the traditional Greenland food items. Risk reduction is therefore recommended, i.e., the intake of these contaminants from the traditional Greenland food items as well as from other sources should be reduced.
The risk characterisations, as performed in this report indicate a relatively low concern of adverse health effects of chlorobenzenes, HCH and dieldrin, and a low concern for selenium and DDT. No risk reduction has been recommended for these contaminants based on the risk characterisations. However, as a concern for additive effects of some of the individual contaminants is indicated, the intake of chlorobenzenes, HCH, dieldrin, and DDT from the traditional Greenland food items as well as from other sources should probably also be reduced.

Sammenfatning på dansk

Den grønlandske befolkning har generelt et højere indtag af kontaminanter via kosten end befolkningen i den vestlige verden generelt. Dette skyldes blandt andet, at traditionelle grønlandske kostemner så som fisk, havfugle, sæler og hvaler udgør en forholdsvis stor del af den daglige kost hos mange grønlændere. Endvidere indeholder disse kostemner ofte høje koncentrationer af visse tungmetaller samt visse persistente organiske kontaminanter som for eksempel chlorholdige pesticider. Det er således for nylig publiceret, at indtag af en række kontaminanter fra traditionel grønlandsk kost overskrider den tolerable daglige indtagelse (TDI) betydeligt (Johansen et al. 2004a[4],b[5]).

Formålet med denne rapport har været at vurdere den sundhedsmæssige risiko for grønlændere ved indtagelse af kontaminanter fra den traditionelle grønlandske kost. Følgende 11 kontaminanter er udvalgt: bly, cadmium, kviksølv, selen, PCB, DDT, chlordaner, hexachlorcyclohexaner, chlorbenzener, dieldrin og toxafen.

Rapporten er udarbejdet af Danmarks Fødevareforskning (DFVF), Afdeling for Toksikologi og Risikovurdering som et kontraktarbejde for Danmarks Miljøundersøgelser, Afdeling for Arktisk Miljø, og finansieret af Miljøstøtteordningen til Arktis Dancea' (Danish Cooperation for Environment in the Arctic).

Rapportens resultater har været præsenteret og diskuteret på et møde d. 7. januar 2005 i den nationale følgegruppe for AMAP delprogrammet 'Menneskets sundhed' for aktiviteter i Grønland og på Færøerne, samt på et nationalt seminar om kontaminanter i traditionel grønlandsk kost d. 1. april 2005.

I denne rapport er den sundhedsmæssige risiko for grønlændere ved indtagelse af kontaminanter fra den traditionelle grønlandske kost vurderet efter de principper, der anvendes i EU for risikovurdering af nye og eksisterende kemiske stoffer, og som beskrevet i vejledningen 'Technical Guidance Document'[6] (TGD).

Principperne er meget kort skitseret:
Først foretages en farlighedsvurdering (sundhedsmæssig vurdering) af det kemiske stof med udgangspunkt i de tilgængelige informationer om stoffet. Derefter vurderes den kritiske effekt, og der fastsættes et nul-effekt niveau (NOAEL) eller laveste effekt niveau (LOAEL) for den kritiske effekt. Principperne for sundhedsmæssig vurdering af kemiske stoffer er beskrevet i detaljer i en rapport udarbejdet af Nielsen et al. (2005) for Miljøstyrelsen[7].

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[4] Johansen P, Muir D, Asmund G and Riget F (2004a). Human exposure to contaminants in the traditional Greenland diet. Sci Total Environ **331**, 189-206.
[5] Johansen P, Asmund G and Riget F (2004b). High human exposure to lead through consumption of birds hunted with lead shot. Environ Pollut **127**, 125-129.
[6] Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances. European Chemicals Bureau, European Commission, Joint Research Centre, EUR 20418 EN.
[7] Nielsen E, Østergaard, G, Larsen JC og Ladefoged O. Principper for sundhedsmæssig vurdering af kemiske stoffer med henblik på fastsættelse af kvalitetskriterier for luft, jord og vand. Miljøprojekt Nr. 874 2005. Miljøstyrelsen.

Herefter foretages en såkaldt risikokarakterisering. Ved risikokarakteriseringen af kemiske stoffer i henhold til TGD vurderes den såkaldte Margin of Safety (MOS), der er forholdet mellem NOAEL (eller LOAEL) for en given effekt og et estimat for eksponering for et givent scenarie, det vil sige MOS = N(L)OAEL/eksponering. Med henblik på at karakterisere risikoen sammenlignes denne beregnede MOS med en reference værdi (MOSref). Når den beregnede MOS er lavere end MOSref, indikerer risikokarakteriseringen, at der er en risiko for udvikling af den givne effekt ved den estimerede eksponering i det givne scenarie, og der skal foretages en risikoreduktion. Det er således ikke selve størrelsen af den beregnede MOS værdi i sig selv, der indikerer, hvorvidt der er en risiko eller ej, men forholdet mellem den beregnede MOS og MOSref. Jo mindre forholdet mellem MOS og MOSref er (MOS/MOSref), jo højere vurderes risikoen at være.
MOSref er i princippet analog til den usikkerhedsfaktor, der ofte anvendes ved vurdering af kemiske stoffer i forskellige reguleringer. Usikkerhedsfaktorer samt anvendelse af disse er også beskrevet detaljeret i rapporten om principper for sundhedsmæssig vurdering af kemiske stoffer udarbejdet af Nielsen et al. (2005) for Miljøstyrelsen.

I denne rapport er der foretaget en sundhedsmæssig vurdering af hver enkelt af de 11 udvalgte kontaminanter, herunder en vurdering af stoffets kritiske effekt(er) samt en fastlæggelse af NOAEL eller LOAEL for den kritiske effekt. De sundhedsmæssige vurderinger er foretaget udelukkende med udgangspunkt i eksisterende nationale og internationale vurderinger af de enkelte kontaminanter. Risikokarakteriseringen er foretaget ved at sammenholde NOAEL (eller LOAEL) fastlagt for den kritiske effekt for den enkelte kontaminant med det estimerede daglige indtag af den pågældende kontaminant fra den traditionelle grønlandske kost præsenteret i Johansen et al. 2004a[8],b[9]. Det vil sige, at i denne rapport er MOS = N(L)OAEL divideret med det estimerede daglige indtag. Når denne beregnede MOS er lavere end MOSref, indikerer risikokarakteriseringen således, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af en given kontaminant fra den traditionelle grønlandske kost. I disse tilfælde anbefales det, at indtaget af den pågældende kontaminant fra traditionel grønlandsk kost, såvel som fra andre kilder, bør reduceres.
Som nævnt ovenfor, er MOSref i princippet analog til den usikkerhedsfaktor, der ofte anvendes ved vurdering af kemiske stoffer i forskellige reguleringer. I denne rapport er MOSref derfor vurderet ved anvendelse af de principper, der generelt anvendes ved vurdering af usikkerhedsfaktorer, som beskrevet i detaljer i rapporten om principper for sundhedsmæssig vurdering af kemiske stoffer udarbejdet af Nielsen et al. (2005) for Miljøstyrelsen[10].

Visse kemiske stoffer har en kræftfremkaldende virkning som følge af, at de kan forårsage skader på generne. For sådanne stoffer foretages generelt en kvantitativ risikovurdering, idet der for denne type effekter ikke anses at være en tærskel, det vil sige, en dosis (eller koncentration) hvorunder der ikke ses effekt. I denne rapport er der ikke foretaget en kvantitativ risikovurdering for denne type effekter. Grunden hertil er, at der endnu ikke er en klar konsensus med hensyn til, hvilke principper og metoder der bør anvendes ved den kvantitative risikovurdering. For kontaminanter med denne form for kræftfremkaldende virkning anbefaler denne

[8] Johansen P, Muir D, Asmund G and Riget F (2004a). Human exposure to contaminants in the traditional Greenland diet. Sci Total Environ **331**, 189-206.
[9] Johansen P, Asmund G and Riget F (2004b). High human exposure to lead through consumption of birds hunted with lead shot. Environ Pollut **127**, 125-129.
[10] Nielsen E, Østergaard, G, Larsen JC og Ladefoged O. Principper for sundhedsmæssig vurdering af kemiske stoffer med henblik på fastsættelse af kvalitetskriterier for luft, jord og vand. Miljøprojekt Nr. 874 2005. Miljøstyrelsen.

rapport derfor, at indtaget fra traditionel grønlandsk kost, såvel som fra andre kilder, bør reduceres.

I det efterfølgende summeres kort for hver enkelt af de 11 udvalgte kontaminanter den sundhedsmæssige vurdering og risikokarakteriseringen.

For cadmium vurderes den kritiske effekt at være påvirkning af nyrer og knogler. På baggrund af den nuværende viden, kan der ikke fastlægges et NOAEL for den kritiske effekt. LOAEL vurderes at ligge i intervallet fra ca. 10 til 60 µg Cd/dag. Det gennemsnitlige daglige indtag af cadmium fra traditionel grønlandsk kost er estimeret til 346 µg om foråret og til 182 µg om efteråret. MOS beregnes til at ligge i intervallet 0,03-0,17 (forår) og 0,05-0,33 (efterår). Disse MOS er betydeligt lavere end MOSref på 3 (ca. 9-100 gange lavere), hvilket indikerer, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af cadmium fra traditionel grønlandsk kost. Hertil kommer, at indtaget cadmium sandsynligvis kan medføre udvikling kræft, en effekt der formentlig skyldes en skadelig virkning på generne. Det anbefales derfor, at indtaget af cadmium fra traditionel grønlandsk kost, såvel som fra andre kilder, bør reduceres.

For bly vurderes den kritiske effekt at være påvirkning af det bloddannende system, nervesystemet og reproduktionssystemet. Børn under udvikling (i fostertilstanden samt i barndommen) er særligt følsomme. På baggrund af den nuværende viden kan det endnu ikke vurderes, hvorvidt der findes en tærskel for påvirkning af nervesystemet hos børn under udvikling, det vil sige, at der ikke kan fastlægges et NOAEL for den kritiske effekt. LOAEL vurderes til ca. 62,5 µg Pb/ dag. I traditionel grønlandsk kost findes bly primært i havfugle skudt med blyhagl, og det estimerede indtag af bly fra et måltid med edderfugl er 1220 µg svarende til ca. 174 µg/dag under forudsætning af, at der indtages et måltid edderfugl hver uge. MOS beregnes til 0,4. Denne MOS er lavere end MOSref på 3 (ca. 7 gange lavere), hvilket indikerer, at der er en sundhedsmæssig risiko for grønlandske børn ved indtagelse af bly fra traditionel grønlandsk kost. Hertil kommer, at der endnu ikke kan fastlægges et NOAEL for blys påvirkninger af nervesystemet hos børn under udvikling. Det anbefales derfor, at indtaget af bly fra traditionel grønlandsk kost, såvel som fra andre kilder, bør reduceres.

I traditionel grønlandsk kost forekommer kviksølv primært i kød og lever fra sæl. I kødet forekommer kviksølv primært som organisk kviksølv (methylkviksølv), mens leveren primært indeholder uorganisk kviksølv. Indtaget af kviksølv er estimeret som kviksølv totalt. Da organisk og uorganisk kviksølv giver forskellige former for sundhedsmæssige påvirkninger, er risikokarakteriseringen i denne rapport foretaget i form af 2 forskellige scenarier: 1) indtaget kviksølv er organisk, og 2) indtaget kviksølv er uorganisk.
For organisk kviksølv (methylkviksølv) vurderes den kritiske effekt at være påvirkning af nervesystemet. Børn under udvikling (især i fostertilstanden) er særligt følsomme. På baggrund af den nuværende viden kan det endnu ikke vurderes, hvorvidt der findes en tærskel for påvirkning af nervesystemet hos børn under udvikling, det vil sige, at der ikke kan fastlægges et NOAEL for den kritiske effekt. LOAEL vurderes til 60 µg Hg/dag. Det gennemsnitlige daglige indtag af kviksølv totalt fra traditionel grønlandsk kost er estimeret til 66 µg om foråret og til 42 µg om efteråret. MOS beregnes til 0,9 (forår) og 1,4 (efterår). Disse MOS er lavere end MOSref på 3 (henholdsvis 3 og 2 gange lavere), hvilket indikerer, at der er en sundhedsmæssig risiko for grønlandske børn ved indtagelse af methylkviksølv fra traditionel grønlandsk kost hos gravide kvinder. Hertil kommer, at der endnu ikke kan fastlægges et NOAEL for methylkviksølvs påvirkninger af nervesystemet hos børn under udvikling. Det anbefales derfor, at indtaget af

methylkviksølv fra traditionel grønlandsk kost, såvel som fra andre kilder, bør reduceres.
For uorganisk kviksølv vurderes den kritiske effekt at være påvirkning af nyrerne. På baggrund af den nuværende viden, kan der ikke fastlægges et NOAEL for den kritiske effekt. LOAEL vurderes til 18000 µg Hg/dag. Det gennemsnitlige daglige indtag af kviksølv totalt fra traditionel grønlandsk kost er estimeret til 66 µg om foråret og til 42 µg om efteråret. MOS beregnes til 273 (forår) og 429 (efterår). Disse MOS er lavere end MOSref på 1000 (henholdsvis 3 og 2 gange lavere), hvilket indikerer, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af uorganisk kviksølv fra traditionel grønlandsk kost. Det anbefales derfor, at indtaget af uorganisk kviksølv fra traditionel grønlandsk kost, såvel som fra andre kilder, bør reduceres.

For selen vurderes den kritiske effekt at være selenosis samt påvirkning af leveren. NOAEL vurderes til ca. 850 µg Se/dag. Det gennemsnitlige daglige indtag af selen fra traditionel grønlandsk kost er estimeret til 127 µg om foråret og til 112 µg om efteråret. MOS beregnes til 6,7 (forår) og 7,6 (efterår). Disse MOS er lidt lavere end MOSref på 10, hvilket indikerer, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af selen fra traditionel grønlandsk kost. Men da de beregnede MOS er meget tæt på MOSref, og da NOAEL vurderes at være et forholdsvis konservativt estimat, vurderes risikoen som værende meget lav.

Den sundhedsmæssige vurdering af PCB har omfattet kommercielle blandinger, definerede blandinger såvel som enkelte af de congener, der indgår i sPCB10 (PCB 28, 31, 52, 101, 105, 118, 138, 153, 156 og 180) som analyseret i de traditionelle grønlandske kostemner. De 10 congener repræsenterer de hyppigst forekommende congener i fisk og marine pattedyr. I den sundhedsmæssig vurdering er de kommercielle blandinger valgt som surrogat for sPCB10. For PCB vurderes den kritiske effekt at være påvirkning af immunsystemet, hud og øjne samt det ufødte barn. På baggrund af den nuværende viden, kan der ikke fastlægges et NOAEL for den kritiske effekt. LOAEL vurderes til 300 µg/dag. Det gennemsnitlige daglige indtag af sPCB10 fra traditionel grønlandsk kost er estimeret til 23 µg for både forår og efterår. MOS beregnes til 13. Denne MOS er betydeligt lavere end MOSref på 1000 (ca. 75 gange lavere), hvilket indikerer, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af PCB fra traditionel grønlandsk kost. Det anbefales derfor, at indtaget af PCB fra traditionel grønlandsk kost, såvel som fra andre kilder, bør reduceres.

Den sundhedsmæssige vurdering af DDT har omfattet de isomerer og metabolitter, der indgår i sDDT (sum af *p,p'*-DDE, -DDD, -DDT + *o,p'*-DDE, -DDD, -DDT) som analyseret i de traditionelle grønlandske kostemner. Den kritiske effekt af disse DDT forbindelser vurderes at være påvirkning af nervesystemet, leveren og reproduktionssystemet. NOAEL vurderes til 3000 µg/dag. Det gennemsnitlige daglige indtag af sDDT fra traditionel grønlandsk kost er estimeret til 32 µg om foråret og til 25 µg om efteråret. MOS beregnes til 94 (forår) og 120 (efterår). Disse MOS er lidt lavere end MOSref på 150 (henholdsvis 1,6 og 1,3 gange lavere), hvilket indikerer, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af DDT fra den traditionelle grønlandske kost. Men da de beregnede MOS er meget tæt på MOSref, og da NOAEL vurderes at være et forholdsvis konservativt estimat, vurderes risikoen som værende meget lav.

Den sundhedsmæssige vurdering af chlordaner har omfattet de 7 forbindelser, der indgår i sCHL (sum af heptachlor, heptachlor epoxid, oxychlordan, cis- og trans-chlordan, og cis- og trans-nonachlor) som analyseret i de traditionelle grønlandske kostemner. Den kritiske effekt af chlordaner vurderes at være påvirkning af leveren. På baggrund af data for heptachlor epoxid vurderes NOAEL til 1500

µg/dag. Det gennemsnitlige daglige indtag af sCHL fra traditionel grønlandsk kost er estimeret til 18 µg om foråret og til 15 µg om efteråret. MOS beregnes til 83 (forår) og 100 (efterår). Disse MOS er betydeligt lavere end MOSref på 1000 (henholdsvis 12 og 10 gange lavere), hvilket indikerer, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af chlordaner fra traditionel grønlandsk kost. Det anbefales derfor, at indtaget af chlordaner fra traditionel grønlandsk kost, såvel som fra andre kilder, bør reduceres.

Den sundhedsmæssige vurdering af chlorbenzener har omfattet de 3 forbindelser, der indgår i sCBz (sum af 1,2,3,4-tetrachlorbenzen, pentachlorbenzen og hexachlorbenzen) som analyseret i de traditionelle grønlandske kostemner. Den kritiske effekt af chlorbenzener vurderes at være påvirkning af leveren, knoglerne, immunsystemet og reproduktionssystemet. På baggrund af den nuværende viden, kan der ikke fastlægges et NOAEL for den kritiske effekt. LOAEL vurderes til 600 µg/dag. Det gennemsnitlige daglige indtag af sCBz fra traditionel grønlandsk kost er estimeret til 4 µg for både forår og efterår. MOS beregnes til 150. Denne MOS er lavere end MOSref på 1000 (ca. 6,6 gange lavere), hvilket indikerer, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af chlorbenzener fra traditionel grønlandsk kost. Men da LOAEL vurderes at være et forholdsvis konservativt estimat, vurderes risikoen som værende relativt lav.

Den sundhedsmæssige vurdering af hexachlorcyclohexaner har omfattet de 3 isomere forbindelser, der indgår i sHCH (sum af α-, β- og γ-HCH) som analyseret i de traditionelle grønlandske kostemner. Den kritiske effekt af HCH vurderes at være påvirkning af immunsystemet. På baggrund af den nuværende viden, kan der ikke fastlægges et NOAEL for den kritiske effekt. LOAEL vurderes til 600 µg/dag. Det gennemsnitlige daglige indtag af sHCH fra traditionel grønlandsk kost er estimeret til 4 µg om foråret og til 3 µg om efteråret. MOS beregnes til 150 (forår) og 200 (efterår). Disse MOS er lavere end MOSref på 1000 (henholdsvis 6,6 og 5 gange lavere), hvilket indikerer, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af HCH fra traditionel grønlandsk kost. Men da LOAEL vurderes at være et forholdsvis konservativt estimat, vurderes risikoen som værende relativt lav.

For dieldrin vurderes den kritiske effekt at være påvirkning af leveren. NOAEL vurderes til 300 µg/dag. Det gennemsnitlige daglige indtag af dieldrin fra traditionel grønlandsk kost er estimeret til 8 µg om foråret og til 7 µg om efteråret. MOS beregnes til 38 (forår) og 43 (efterår). Disse MOS er lavere end MOSref på 100 (henholdsvis 2,6 og 2,3 gange lavere), hvilket indikerer, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af dieldrin fra traditionel grønlandsk kost. Men da de beregnede MOS er meget tæt på MOSref, og da NOAEL vurderes at være et forholdsvis konservativt estimat, vurderes risikoen som værende relativt lav.

Toxafen er en meget kompleks blanding af en lang række forskellige stoffer, og de sundhedsmæssige effekter af disse stoffer såvel som for blandingen toxafen er meget dårligt belyst. På baggrund af den nuværende viden vurderes den kritiske effekt at være påvirkning af lever, skjoldbruskkirtel og nyrer. NOAEL vurderes til 12000 µg/dag. Det gennemsnitlige daglige indtag af toxafen fra traditionel grønlandsk kost er estimeret til 30 µg om foråret og til 31 µg om efteråret. MOS beregnes til 400 (forår) og 387 (efterår). Disse MOS er betydeligt lavere end MOSref på 10000 (ca. 25 gange lavere), hvilket indikerer, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af toxafen fra traditionel grønlandsk kost. Hertil kommer, at indtaget toxafen muligvis kan medføre kræft, en virkning der muligvis skyldes en skadelig virkning på generne. Det anbefales

derfor, at indtaget af toxaphen fra traditionel grønlandsk kost, såvel som fra andre kilder, bør reduceres.

Resultaterne af risikovurderingerne for de 11 udvalgte kontaminanter er summeret i tabel 1, se sidste side i kapitel 4.

Som nævnt tidligere, er MOSref i princippet analog til den usikkerhedsfaktor, der ofte anvendes ved vurdering af kemiske stoffer i forskellige reguleringer. Derfor er MOSref for de udvalgte kontaminanter sammenlignet med de usikkerhedsfaktorer, der er anvendt af blandt andre FAO/WHO (JECFA/JMPR) ved fastsættelse af acceptabel eller tolerabel daglig indtagelse (ADI/TDI), eller af den amerikanske miljøstyrelse (US-EPA) ved fastsættelse af en såkaldt oral reference dosis (RfD). Denne sammenligning er foretaget med henblik på at vurdere, hvorvidt der er store forskelle mellem risikokarakteriseringen foretaget for de 11 kontaminanter i denne rapport versus den nuværende praksis anvendt ved fastsættelse af ADI/TDI og RfD.
For cadmium, bly, kviksølv, DDT og dieldrin er der praktisk taget ingen forskelle. For selen og PCB er risikokarakteriseringerne i denne rapport en lille smule mere konservative end de traditionelle vurderinger. For chlordaner, chlorbenzener og hexachlorcyclohexaner er risikokarakteriseringerne i denne rapport betydeligt mere konservative end de traditionelle vurderinger. Dette skyldes primært, at for disse kontaminanter omfatter risikokarakteriseringen i denne rapport en række isomerer, mens de traditionelle vurderinger har været foretaget for en specifik forbindelse. Der kunne ikke foretages en sammenligning for toxafen, da der ikke er fastsat TDI eller RfD for toxafen.

Generelt foretages sundhedsmæssige vurderinger for kemiske stoffer med udgangspunkt i konkret viden om det enkelte stof. Imidlertid udsættes mennesker samtidigt for en række forskellige kemiske stoffer med en række forskellige sundhedsmæssige effekter. Effekterne af forskellige kemiske stoffer kan i visse tilfælde lægges sammen (additiv effekter). Dette betyder i praksis, at der ved samtidig eksponering for flere stoffer kan ses effekter ved lavere niveauer i forhold til eksponering for det enkelte stof. Dette gælder for stoffer, der har den samme virkningsmekanisme eller – i nogle tilfælde – virker på det samme organ eller system.
En række af de kontaminanter, der er vurderet i denne rapport, virker på det samme organ eller system og har muligvis også samme virkningsmekanisme. Der kan således være grund til bekymring for, at nogle af effekterne af de forskellige kontaminanter i traditionel grønlandsk kost er additive. Det ligger imidlertid uden for denne rapports formål at vurdere sådanne samspilseffekter.

Sammenfattende indikerer risikokarakteriseringerne for de 11 kontaminanter vurderet i denne rapport, at der er en sundhedsmæssig risiko for grønlændere ved indtagelse af cadmium, bly, kviksølv, PCB, chlordaner og toxafen fra traditionel grønlandsk kost. For disse kontaminanter anbefales det derfor, at indtaget fra traditionel grønlandsk kost, såvel som fra andre kilder, bør reduceres. For chlorbenzener, HCH, og dieldrin indikerer risikokarakteriseringerne en relativt lav sundhedsmæssig risiko, og for selen og DDT er lav sundhedsmæssig risiko, hvorfor der ikke umiddelbart er anbefalet en reduktion af indtaget af disse kontaminanter. Imidlertid er der grund til bekymring for, at nogle af effekterne af disse kontaminanter (bortset fra selen) er additive, hvorfor det anbefales, at også indtaget af disse kontaminanter fra traditionel grønlandsk kost, såvel som fra andre kilder, bør reduceres.

1 Introduction

People in Greenland have generally a higher intake of contaminants from their diet than people in the more westernised countries because marine traditional food items such as fish, seabirds, seals and whales are much more important dietary sources in Greenland, and because some of these food items have high levels of heavy metals as well as of persistent organochlorines.

In a recently published paper by Johansen et al. (2004a)[11], the intake by Greenlanders of selected contaminants from a number of traditional food items has been estimated. The estimated intakes of the contaminants were compared with guideline values such as e.g., acceptable daily intake (ADI) or tolerable daily intake (TDI) established by FAO/WHO, and the significance of the exposure to the contaminants from the different food items has been assessed. The selected contaminants included selenium, the heavy metals cadmium and mercury, and the following organochlorines: polychlorinated biphenyls (PCBs), dichlorophenyl-trichloroethane (DDT), chlordanes, hexachlorocyclohexanes (HCHs), chlorobenzenes, dieldrin and toxaphene, and were analysed in the major species and tissues consumed by Greenlanders.

Generally, the levels of the selected contaminants were very low in terrestrial species and in the muscle of many marine species. High concentrations of organochlorines were typically found in the blubber of marine mammals and high levels of metals in the liver and kidneys of seals and whales. The evaluation of the contaminant intake indicated that the muscle, liver, kidneys and blubber from seals, and the blubber from whales are the dominant sources of contaminants in the traditional diet.

The estimated mean intakes of cadmium, chlordanes and toxaphene significantly exceeded the tolerable daily intake (TDI) by a factor of between 2.5 and 6. Mean intakes of mercury, PCBs and dieldrin also exceeded the TDI by up to approximately 50%. The intakes of these contaminants were expressed as mean intakes. As the estimated dietary intakes are mean values, and as the variation in both food intake and contaminant levels in the various traditional food items is large, the variation of contaminant intake among individuals is large as well. Implicitly, some individuals may have significantly higher intakes of these contaminants from traditional food items than the average general population and thereby may have a higher risk of experiencing adverse health effects from intake of these contaminants.
The estimated mean intakes of DDT, HCH and chlorobenzenes were well below the TDI and the authors concluded that it seems unlikely that the TDI for these contaminants normally is exceeded in the Greenland population.
According to the authors, a way to reduce the contaminant intake would be to avoid or limit the consumption of the traditional food items with high contaminant levels.

[11] Johansen P, Muir D, Asmund G and Riget F (2004a). Human exposure to contaminants in the traditional Greenland diet. Sci Total Environ **331**, 189-206.

In another recently published study, Johansen et al. (2004b)[12] have assessed the lead contamination of common eider and thick-billed murre killed with lead shot, the two most important Greenland seabirds in the diet. The authors documented that the lead concentration is very high in meat of lead shot eiders, about 44 times higher than in drowned eiders and eight times higher than in lead shot murres. The intake by Greenlanders of lead from lead shot eiders and murres was estimated and compared with the tolerable daily intake (TDI) established by FAO/WHO. It was concluded by the authors that in some cases, the lead intake by Greenland bird eaters will largely exceed the TDI for lead and that lead shot is a more important source of lead in the diet than previously estimated. The contribution of lead to the lead body burden from other traditional food items than lead shot birds was considered by the authors to be non-significant.

As outlined above, the significance of the human exposure to the selected contaminants from the different food items has been assessed by comparing the estimated intakes of the contaminants with guideline values such as e.g., acceptable daily intake (ADI) or tolerable daily intake (TDI) established by FAO/WHO.
The purpose of this report was to perform a more refined risk assessment, i.e., to evaluate the risk to Greenlanders of experiencing adverse health effects from intake of contaminants from the traditional food items by using another approach for risk characterisation.
The eleven contaminants included in the studies by Johansen et al. (2004a,b) have also been evaluated in this report, i.e., lead, cadmium, mercury, selenium, PCBs, DDT, chlordane, HCHs, chlorobenzenes, dieldrin, and toxaphene.

The first part of the report (chapter 2) briefly introduces the principles of effect assessment of chemical substances (section 2.1) and also includes the effect assessments of the selected contaminants (sections 2.2 to 2.12).
The second part of the report (chapter 3) introduces the principles of the approach used for the risk characterisation in this report (section 3.1) and also includes the risk characterisation of the selected contaminants (sections 3.2 to 3.12).
The exposure assessments of the selected contaminants are the mean intake of the specific contaminant from the traditional Greenland food items as presented in Johansen et al. (2004a,b).

[12] Johansen P, Asmund G and Riget F (2004b). High human exposure to lead through consumption of birds hunted with lead shot. Environ Pollut **127**, 125-129.

2 Effect assessment

2.1 Introduction

The scientific basis for an effect assessment (also known as hazard assessment) consists of a hazard identification and a dose-response / dose-effect assessment (hazard characterisation). Below, the principles of the hazard assessment process are briefly introduced based on a recent report[13], which has reviewed the current knowledge and experiences on the hazard assessment of chemical substances.

The hazard assessment is generally based on data elucidating the toxicological effects in humans and experimental animals of a given chemical substance. For the contaminants evaluated in this report, data have predominantly been collected from national and international criteria documents and monographs, i.e., original reports, papers and research articles have not been consulted.

Human data include information from case reports (e.g., poisoning cases), clinical examinations, studies in volunteers, experiences from the working environment, and epidemiological studies. However, for several of the selected contaminants, human data are not adequate or available. The effect assessments for these contaminants, predominantly the organochlorines, are therefore primarily based upon data from studies performed with experimental animals.

Ideally, a complete data set including information on toxicokinetics, and on the potential toxic effects (toxicological endpoints) such as acute and repeated dose toxicity, irritation, sensitisation, mutagenicity and genotoxicity, carcinogenicity, and toxicity to reproduction should be available for the effect assessment. However, for most chemical substances, a complete data set is generally not available, and this is also the case for a number of the contaminants selected in this report. In the risk characterisation for these contaminants, the so-called 'minimal Margin of Safety' (equivalent to an assessment or uncertainty factor) is increased in order to compensate for limitations in the data set (see section 3.1.2.2.3).

Exposure to a chemical substance can result in a broad spectrum of effects varying from mild effects to fatal poisonings. The type and severity of the effects observed is generally correlated with the dose or exposure concentration.
The first step in the hazard assessment is the hazard identification, i.e., an identification of the potential toxic effects, which a given substance has an inherent capacity to cause. The effects can be divided into two types: 1) Those effects, which are considered as having a threshold (dose or exposure concentration below which the effects is not observed) for the effect (threshold effects). 2) Those effects for which a threshold cannot be identified (non-threshold effects, e.g. genotoxic effects and carcinogenic effects, which are caused by damage of the genetic material), For these effects, it is assumed that there is a dose-dependent response at all doses above zero and thus, some risk is considered to exist at any exposure level.

[13] Nielsen E, Østergaard G, Larsen JC and Ladefoged O (2005). Principper for sundhedsmæssig vurdering af kemiske stoffer med henblik på fastsættelse af kvalitetskriterier for luft, jord og drikkevand. Miljøprojekt nr. 974 2005, Miljøstyrelsen, Miljøministeriet. In Danish, with an English summary.

The next step is the hazard characterisation, i.e., an estimation of the relationship between the dose (or exposure concentration), and the incidence and severity of an effect. Regarding the severity of a given effect, it is evaluated whether the effect can be considered as being 'adverse' or not. Generally, an effect is considered to be 'adverse' when there is a change in morphology, physiology, functional capacity, development, and/or life span in the exposed individuals, and when the incidence of the effect is statistically significantly different from that in the control group.

The hazard assessment also involves an evaluation of the 'no observed adverse effect level' (NOAEL) and 'the lowest observed adverse effect level' (LOAEL) for the various effects observed.
The 'NOAEL' is defined as "The greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development or life span of the target organism under defined conditions of exposure." Alterations of the above mentioned parameters may be detected, which are judged not to be adverse.
The 'LOAEL' is defined as "The lowest concentration or amount of a substance, found by experiment or observation, which causes an adverse alteration of morphology, functional capacity, growth, development or life span of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure."

When all the relevant effect data (the toxicological data set) have been evaluated, the hazard(s) considered most important, 'the critical effect(s)', is identified, i.e., the effect(s), which is considered as being the essential one(s) for the risk characterisation.
For threshold effects, a NOAEL (or LOAEL) is identified for the critical effect(s) and is taken forward to the risk characterisation. This approach is used for all the eleven contaminants selected in this report.
For non-threshold effects (e.g. genotoxic effects and carcinogenic effects, which are caused by damage of the genetic material), there is currently no clear consensus on an appropriate methodology for the estimation of a no-effect level. In the risk characterisation for such effects exerted by the eleven contaminants selected in this report, the so-called 'reference Margin of Safety' (equivalent to an assessment or uncertainty factor) is increased when appropriate in order to compensate for the severity of such effects (see section 3.1).

2.2 Cadmium

Cadmium is a metallic element, which displays two oxidation states: 0 and +2. Cadmium can form a number of salts. In this report, the term 'cadmium' refers to ionic cadmium, except when specific cadmium compounds are mentioned.

There is no evidence that organocadmium compounds, where the metal is covalently bound to carbon, occur in nature. Cadmium may occur bound to proteins and other organic molecules and form salts with organic acids; in these forms, cadmium is regarded as inorganic. Most of the cadmium found in mammals, birds, and fish is probably bound to protein molecules. (WHO 1992b).

The speciation of cadmium in plants, animal tissues, and foodstuffs may be of importance for the evaluation of the health hazards associated with areas of cadmium contamination or high cadmium intake. However, very few data on the occurrence and speciation of cadmium compounds in nature are available. (WHO 1992a).

The major route of exposure to cadmium for the non-smoking general population is via food, which provides 99 % of the cadmium intake (ECB 2003).

In Denmark, the estimated average dietary intake for adults (1993-1997) was 17 µg/day with the 95 percentile being 28 µg/day (FDIR 2000).

In the EU Member States, the mean intake of cadmium is less than 30% of the PTWI (0.49 mg for a person weighing 70 kg). Cereals and vegetables are the main sources of cadmium in the diet, representing approximately 2/3 of the mean cadmium intake. Data indicate that children have a lower intake of cadmium than adults. However, children have a larger burden/kg b.w., due to their lower body weight, and children 4-6 years old may reach 65% of the PTWI. (EC 2003).

2.2.1 Toxicokinetics

The systemic absorption of ingested cadmium is generally less than 5% but absorption rates of 10% has been reported. The bioavailability is subject to variations according to age, composition of diet, source of cadmium, presence of zinc, and iron status. Animal studies indicate that absorption is markedly higher during the first weeks of life. Low calcium, iron, zinc and protein contents tend to increase the absorption of cadmium. The bioavailability of cadmium in seafood is lower than that of ionic cadmium. The concomitant presence of zinc in contaminated food reduces the bioavailability of cadmium. Depleted iron status (mainly women) increases the absorption of cadmium by a factor of two. (ECB 2003, CSTEE 2004).

Cadmium is widely distributed in the body. In blood, most of the cadmium is found in the erythrocytes (about 90%). In plasma, cadmium is predominantly bound to proteins of high molecular weight (albumin or larger). The biological half-life of cadmium has been estimated to vary from about 10 to 40 years. Consequently, it accumulates over many years, mainly in the renal cortex and, to a smaller extent, in the liver and the lung. The maximum concentrations in kidney cortex are observed at about 50-60 years of age, after which the concentrations decrease. The average total body burden at age 50 has been estimated to range from 5 to 30 mg. In non-occupationally exposed subjects the cadmium concentration in the kidneys is

generally between 10 and 50 mg/kg (2-5 fold increased in smokers). (ECB 2003, CSTEE 2004).

The considerable age-related accumulation of cadmium in the body indicates that only a small part of cadmium absorbed from long-term low-level exposure will be excreted. The daily excretion, which takes place approximately equal via faeces and urine represents only about 0.005 - 0.02% of the total body burden of cadmium. After the development of severe cadmium-induced renal dysfunction, the cadmium level in the renal cortex decreases and urinary excretion increases. The reduction of renal cadmium is likely due to a release of cadmium from the kidney combined with a depressed re-absorption of circulating cadmium. This explains why in most severely poisoned individuals the concentration of cadmium in the renal cortex may be relatively low in contrast to the liver level. (ECB 2003).

The placenta provides a relative barrier protecting the foetus against cadmium exposure. There is some build up of cadmium in the placenta and levels are significantly higher in smokers than in non-smokers. Cadmium can cross the placenta and the cadmium concentration in the blood from new-borns is on average 40-50% lower than that in the maternal blood. (ECB 2003).

In humans with long-term high exposure, the concentration of cadmium in whole blood (Cd-B) may be about 30 times higher than that in the plasma. The Cd-B value is generally below 3 µg/l in European subjects not occupationally exposed to cadmium. Concentrations in the order of 5-10 µg/l are rare, unless in heavily contaminated areas. Cd-B is generally 2-5 fold higher in smokers than in non-smokers. (ECB 2003).

The excretion of cadmium in the urine (Cd-U) is correlated with the body burden and has been extensively used as a biomarker of long-term exposure in human studies. The mean urinary cadmium level in individuals neither occupationally exposed to cadmium nor living in a cadmium-polluted area is generally below 1-2 µg Cd/g creatinine. Several studies have shown that in the general population, the urinary excretion of cadmium increases with age and this increase coincides with an increased body burden. There is a close relationship between the cadmium concentration in urine and the amount in the renal cortex. By assuming a linear relationship between cadmium in urine and kidney, a Cd-U of 2.5 µg/g creatinine in urine corresponds to about 50 mg/kg in the kidneys cortex. (ECB 2003, CSTEE 2004).

2.2.2 Single dose toxicity

The target organ of ingested cadmium at acute toxic doses is the proximal parts of the intestinal tract with severe nausea, vomiting, and abdominal pain being the most characteristic symptoms. Recovery from acute poisoning appears to be rapid and complete. The amount of cadmium absorbed is probably very limited due to vomiting and the consequential short time of presence of cadmium in the gastrointestinal tract. Acute effects have been reported to occur following consumption of drinks with about 16 mg Cd/l. The no-effect level of a single oral dose for humans is estimated at 3 mg cadmium and the lethal doses range from 350 to 8900 mg. (ECB 2003, WHO 1992a).

In experimental animas, the LD_{50}-values reported for rat and mouse range from 50 to 400 mg Cd/kg for water-soluble compounds (ECB 2003, WHO 1992a).

2.2.3 Repeated dose toxicity

Most of the available epidemiological studies or group observations, as well as the clinical studies, have been performed either on occupationally exposed workers or on Japanese populations in cadmium-polluted areas. Many of the human studies have focused on the detection of early signs of kidney dysfunction. In Japan, particular attention has been given to the detection of and screening for bone disease, e.g., the so-called 'Itai-Itai disease' (WHO 1992a).

2.2.3.1 Effects on the kidney

Numerous studies in rats, mice, rabbits and rhesus monkeys indicate that exposure to cadmium compounds administered orally causes kidney damage including an increase or decrease in relative kidney weight, and histopathological (necrosis of the proximal tubules, interstitial renal fibrosis) and functional changes (reduced glomerular filtration rate, proteinuria). (ECB 2003, CSTEE 2004).

In humans, chronic exposure to cadmium can cause damage to the proximal tubules of the kidney, detected as increased urinary excretion of low molecular weight (LMW) proteins. In more severe cases, glomerular dysfunction can occur, which can be detected as increased urinary excretion of high molecular weight (HMW) proteins. While some of the effects are irreversible and progress to kidney disease even after a reduction of exposure, others as e.g., mild LMW proteinuria seem reversible after cessation of exposure. (ECB 2003, CSTEE 2004).

Cadmium nephrotoxicity has been studied in workers as well as in a number of large-scale epidemiological studies of general population cohorts living in regions with more or less cadmium pollution. In these studies, urinary LMW protein concentrations are used as markers of nephrotoxic effects which, in combination with corresponding Cd-U values, lead to the evaluation of the critical intake of cadmium. Studies in workers have shown clear adverse effects at levels of Cd-U of 5 µg Cd/g creatinine or greater and thus constitutes a LOAEL for LMW proteinuria in workers. Studies in the general population have suggested different levels of Cd-U at which effects were detectable, in the range of 0.5 to 2.6 µg Cd/g creatinine. The available mortality studies were not able to detect an excess of end-stage renal diseases in populations exposed to cadmium compounds, but a recent epidemiological study suggests that the incidence of renal replacement therapy is increased in a population with occupational/environmental exposure to cadmium. (ECB 2003, CSTEE 2004).

2.2.3.2 Effects on bone tissue

The bone tissue is another target following exposure to cadmium compounds.

The most severe form of bone disease caused by cadmium intoxication is the 'Itai-Itai disease', which has been observed in aged Japanese women and is associated with both kidney and bone lesions. The mechanism of bone toxicity in humans is not fully elucidated and types of bone lesions associated with cadmium exposure are not clearly identified. One likely mechanism is disturbance of bone metabolism but another explanation is that cadmium-induced kidney damage and/or hypercalciuria might promote osteoporosis and osteoporotic fractures. An inverse relationship between bone mineral density and Cd-U has been found in two recent studies. In one of these studies, no quantitative relationships were given; in the

other, the effect was observed above 3 µg Cd/g creatinine. LOAELs of 0.5 and 3 µg Cd/g creatinine were also discussed. (ECB 2003, CSTEE 2004).

In experimental animals, cadmium has been shown to affect bone metabolism. These effects have manifested themselves as osteopetrosis, osteosclerosis, osteomalacia and/or osteoporosis. In most of the experimental studies, bone effects were accompanied or preceded by renal damage induced by the treatment with cadmium. Young age (growing bones), gestation, lactation, and ovariectomy appeared to exacerbate the cadmium-induced bone toxicity. (ECB 2003, CSTEE 2004).

In vitro studies have demonstrated that cadmium compounds might exert a direct effect on bone affecting both bone resorption and formation, and inducing calcium release. (ECB 2003, CSTEE 2004).

2.2.3.3 Other effects

Evidence for cardiovascular toxicity resulting from oral exposure to cadmium compounds in experimental animals is suggestive of a slight effect on blood pressure. However, results from human studies do not indicate that cadmium may cause hypertension. (ECB 2003).

Exposure to cadmium compounds can cause liver damage in animals but generally only after high exposure levels. There is little evidence for liver damage in humans exposed to cadmium compounds. (ECB 2003).

Cadmium-induced haematological effects have been reported in experimental animals (anaemia) exposed to very high doses of cadmium compounds (ECB 2003).

There is evidence from animal studies, and limited evidence from studies in occupationally exposed humans, that cadmium may cause damage to the central and peripheral nervous systems, both in adults and in the developing organism. However, the evidence is relatively weak because of the limited data available. (ECB 2003, CSTEE 2004).

2.2.4 Genotoxicity

Studies on the genotoxicity of soluble cadmium compounds have giving conflicting results. Some *in vitro* studies are negative, especially in bacterial systems. Other studies have yielded positive results for the induction of DNA strand breaks, protein-DNA crosslinks, chromosome aberrations, and other markers of genotoxicity. Cadmium can induce genotoxicity by interacting directly with DNA and by inhibiting DNA repair.
Results on the *in vivo* genotoxicity of cadmium compounds are also conflicting as e.g., cadmium chloride has been found to cause micronuclei and chromosome aberrations in mice, while cadmium oxide is negative in similar tests, a result that may reflect the bioavailability of cadmium in each case.
(ECB 2003, CSTEE 2004).

With regard to human exposure to cadmium compounds, data are conflicting for populations exposed orally to high levels of cadmium. A number of studies have found no increase in biomarkers of genotoxicity. Other studies have reported increased levels of chromosome aberrations and sister-chromatid exchanges in

individuals who developed ‘Itai-Itai disease’. Increased levels of biomarkers of genotoxicity have also been found in populations with high environmental cadmium exposure in China and the Czech Republic. In some studies, a correlation between Cd-U and the levels of chromosome aberrations have been found. (ECB 2003, CSTEE 2004).

2.2.5 Carcinogenicity

Animal studies have clearly shown that cadmium compounds can cause lung cancer in rats and mice after inhalation. Furthermore, oral treatment of rats with cadmium chloride has resulted in the induction of leukaemia, interstitial cell tumours of the testis and proliferative lesions of the prostate. (CSTEE 2004).

Epidemiological studies in cadmium-exposed workers have provided evidence of increased risks of prostate and lung cancer. Evidence of possible prostate carcinogenesis has also been observed in populations living in cadmium-polluted regions who, as a result, had a high dietary intake of cadmium; however, the studies suffer from weaknesses in the assessment of exposure and adequate control of confounding factors. Recent case-control studies on renal-cell cancer and cadmium indicate a significant association between cadmium exposure and a carcinogenic effect on the kidney. (ECB 2003, CSTEE 2004).

2.2.6 Toxicity to reproduction

The few available epidemiological studies have not indicated an association between exposure to cadmium compounds and effects on fertility or reproductive organs. Based on the available human data, there is no clear evidence indicating that cadmium has a potential to induce developmental effects. Effects on birth weight, motor and perceptual abilities of offspring have been reported by some authors; however, these studies suffer from drawbacks. Furthermore, it is not clear whether the effects on psychomotor development were related to cadmium or simultaneous exposure to other substances such as lead. (ECB 2003).

In experimental animals, effects have been observed on male and female reproductive organs in rats and mice upon chronic exposure to cadmium by the oral route. In several studies, effects were reported to occur at dose levels, which also caused general toxicity (effects on the kidney, liver or body weights). A dose level of around 1 mg Cd/kg b.w./day has been reported to affect (histologically) the seminiferous tubuli and the reproductive capacity of male rats. Developmental effects including reduced foetal body weight and malformations (primarily of the skeleton) have been observed in offspring of animals after oral exposure to cadmium compounds; the reported developmental effects occur at dose levels that also cause maternal toxicity (about 0.4 mg Cd/kg b.w./day). Neurobehavioural effects or changes in electrophysiological parameters have been reported to occur at dose levels that did not induce maternal toxicity and the lowest dose reported to generate behavioural changes in pups is 0.04 mg Cd/kg b.w./day. (ECB 2003).

2.2.7 Evaluation

Repeated oral exposure to cadmium is associated with effects on the kidney and the bone, carcinogenicity, and reproductive and developmental effects.

The first sign of cadmium toxicity to the kidney is damage to the proximal tubules detected as increased urinary excretion of low molecular weight (LMW) proteins. Studies in workers have shown clear adverse effects at Cd-U levels of 5 µg Cd/g creatinine or greater and thus constitutes a LOAEL for LMW proteinuria in workers. There is consensus in the literature concerning the health significance of this threshold because of the frequent observation of irreversible tubular changes above this value and in view of its association with further renal alteration. Studies in the general population have suggested different levels of Cd-U at which effects were detectable, in the range of 0.5 to 2.6 µg Cd/g creatinine. There is, however, an on-going debate about the health significance of the changes observed at Cd-U levels < 5µg Cd/g creatinine. A LOAEL in the range of 0.5 to 2.6 µg Cd/g creatinine is considered for renal effects based on the occurrence of LMW proteinuria.

Both experimental and clinical studies have demonstrated that cadmium induces toxic effects in the bone (bone mineral density and increased risk of fractures) directly and/or secondary to kidney damage at relatively low exposure levels. Results from studies of the general Swedish population suggest a Cd-U LOAEL above 3 µg Cd/g creatinine for bone mineral density, but LOAELs of 0.5 and 3 µg Cd/g creatinine have also been discussed reflecting the uncertainty of the LOAEL. A LOAEL in the range of 0.5 to 3 µg Cd/g creatinine is considered for bone mineral density. This LOAEL is in line with the hypothesis that bone effects follow or are accompanied by kidney dysfunction, which appears within the same range of body burden (LOAEL in the range of 0.5 to 2.6 µg Cd/g creatinine).

Overall, a LOAEL in the range of 0.5 to 3 µg/g creatinine is considered for the kidney and bone effects. Assuming that 2.5 µg Cd/g creatinine corresponds to a long-term daily intake of 50 µg Cd/day (CSTEE 2004), the LOAEL ranges from about 10 to 60 µg Cd/day (0.0002 – 0.001 mg Cd/kg b.w./day for an adult person weighing 60 kg).

Other effects observed following long-term oral exposure to cadmium compounds include effects in the liver, the haematopoietic system, the cardiovascular system, and possibly also the nervous system. The effects in the liver, the haematopoietic system, and the cardiovascular system have been reported to occur at high exposure levels and are not considered to occur at the low dose levels at which the kidney and bone effects are induced. The effects in the nervous system have been observed following inhalation exposure and may probably not occur following oral exposure.

Data regarding genotoxicity are conflicting but seem to indicate a genotoxic potential of cadmium, in certain forms, in experimental systems and possibly also in humans, at least in occupational settings. It cannot be excluded, based on the available data, that cadmium might exert genotoxic effects in populations exposed via the oral route. In the EU, soluble cadmium compounds are classified for mutagenic effects in category 2 (Muta. Cat. 2, R46 – may cause heritable genetic damage) (EEC 2004).

Epidemiological studies and studies in experimental animals show that cadmium compounds are carcinogenic following inhalation exposure. Some studies also indicate that cadmium compounds may have a carcinogenic potential following oral intake. Cadmium is considered by IARC (1993) as carcinogenic to humans (Category 1) based on lung cancer following inhalation. In the EU, soluble cadmium compounds are classified for carcinogenic effects in category 2 (Carc. Cat. 2, R45 – may cause cancer) (EEC 2004), implicating that the carcinogenic

potential is not dependent on the exposure route. Therefore, a carcinogenic potential cannot be excluded in relation to dietary exposure to cadmium.

There is evidence from animal studies of reproductive and developmental toxicity of cadmium compounds. Corresponding epidemiological evidence in humans does not exist for reproductive effects and is limited or inconclusive for developmental effects. A LOAEL of 1 mg Cd/kg b.w./day has been identified for reproductive effects in male rats and a LOAEL of about 0.4 mg Cd/kg b.w./day for developmental effects. The effects in animals were generally observed at dose levels, which also caused general toxicity. Neurobehavioural changes were reported to occur at dose levels that did not induce maternal toxicity (LOAEL of 0.04 mg Cd/kg b.w./day); however, the significance of these changes and underlying mechanisms for the observed effects on behavioural endpoints are not completely elucidated yet. In the EU, soluble cadmium compounds are classified for reproductive effects in category 2 (Repr. Cat. 2, R60-61 – may impair fertility, may cause harm to the unborn child) (EEC 2004). Overall, the LOAEL of 0.0002 – 0.001 mg Cd/kg b.w./day derived for the kidney and bone effects is considered to provide adequate protection against reproductive and developmental effects of cadmium.

2.2.8 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of cadmium are the effects observed in the kidney and bone, and the carcinogenic effect.

A LOAEL in the range of 0.5 to 3 µg/g creatinine is considered for the kidney and bone effects corresponding to about 10 to 60 µg Cd/day (0.0002 – 0.001 mg Cd/kg b.w./day for a 60 kg adult person). It should be recognised that the clinical significance of the biochemical changes observed at this dose level is subject to an on-going scientific debate. The LOAEL is considered to provide adequate protection against reproductive and developmental effects of cadmium, and is taken forward to the risk characterisation.

A carcinogenic potential of cadmium for humans in relation to dietary exposure cannot be excluded. Furthermore, a genotoxic potential of cadmium cannot be excluded either.

2.2.9 References

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2.3 Lead

Lead is a metallic element, which displays three oxidation states: 0, +2 and +4. The usual oxidation state of lead in inorganic compounds is +2. Lead can form a number of salts. In this report, the term 'lead' refers to ionic lead, except when specific lead compounds are mentioned.

Lead in the environment exists almost entirely in the inorganic form. Environmental fate processes may transform one lead compound to another; however, lead is not degraded and is still available for human exposure, even though the compounds containing it vary enormously. (Nielsen 1999, WHO 1995).

In the general adult population and older children, the major source of lead is food with an estimated intake of around 10 µg/day (WHO 1995). In addition to exposure from the diet, some infants and young children receive high doses of lead through mouthing or swallowing non-food items such as soil, dust, or flakes of lead-based paint; these sources often constitute the major exposure with the intake of lead being influenced by the age and behavioural characteristics of the child and bioavailability of lead in the source material (Nielsen 1999, WHO 1995). A recent Swedish study has shown that food is now the main source of lead exposure even in young children living in areas with high soil lead concentrations (CSTEE 2000).

In Denmark, the estimated average dietary intake for adults (1988-1992) was 27 µg/day with the 95 percentile being 46 µg/day. The dietary intake has decreased during the recent 5-year period (1993-1997) with an average daily intake for an adult person of 18 µg/day and a 95 percentile of 28 µg/day (FDIR 2000).

In the EU Member States, the mean intake of lead is 14% of the PTWI (1.75 mg for a person weighing 70 kg). Specific foodstuffs from some Member States were reported to contain very high lead levels (wine, game, fish and meat). Data indicate that children have a lower intake of lead than adults. However, children have a larger burden/kg b.w., due to their lower body weight, and may reach 35% of the PTWI. (EC 2003).

2.3.1 Toxicokinetics

Absorption of lead after ingestion can range from 3 to 80% and is influenced predominantly by food intake with much higher absorption occurring after fasting than when lead is ingested with a meal. Absorption is also affected by age with about 40 to 50% of dietary lead being absorbed in infants and young children compared to around 5 to 10% in adults. (Nielsen 1999, JECFA 2000, WHO 1995, WHO 1996).

Following absorption, there is a rapid uptake of lead into blood and soft tissues, followed by a slower redistribution to bone. Blood lead (PbB) is distributed between the plasma and the erythrocyte with less than 1% in plasma for PbB levels of up to 100 µg/dl. In the erythrocytes lead is bound primarily to haemoglobin. Lead in plasma and in soft tissues binds predominantly to proteins. Bone accumulates lead over much of the human life span and may serve as an endogenous source of lead long after exposure has ended. About 95% of the body burden in adults is located in the bones, compared with about 70% in children. For adults, the half-life of lead is 36 days in blood, 40 days in soft tissues, and 27 years in the bone compartment; the biological half-life may be considerably longer in

children than in adults. (Nielsen 1999, WHO 1995, WHO 1996, ATSDR 1999, JECFA 2000).

Placental transfer of lead occurs in humans as early as week 12 of gestation, and uptake of lead by the foetus continues throughout development. The PbB level in umbilical cord blood is 80 to 100% of the maternal PbB level; the same applies to the PbB level in the foetus. (Nielsen 1999, WHO 1995, WHO 1996).

Inorganic lead is not metabolised in the body. Unabsorbed dietary lead is eliminated in the faeces. Lead that is absorbed but not retained in the body is excreted unchanged via the kidneys and to a lesser extent by biliary clearance. (Nielsen 1999, WHO 1995, WHO 1996, ATSDR 1999, JECFA 2000).

2.3.2 Single dose toxicity

Overt signs of acute intoxication include dullness, restlessness, irritability, poor attention span, headaches, muscle tremor, abdominal cramps, kidney damage, hallucinations, loss of memory, and encephalopathy occurring at PbB levels of 100 to 120 µg/dl in adults and 80 to 100 µg/dl in children (WHO 1996).

Health effects are generally not observed in laboratory animals after a single exposure, and no LD_{50}-values have been reported in the literature. The lowest observed lethal doses in animals after short-term oral exposure range from 300 to 4000 mg/kg b.w. (JECFA 2000).

2.3.3 Repeated dose toxicity

Lead is a cumulative general poison with pregnant women, the foetus, infants, and children up to 6 years of age being the most susceptible subgroups to adverse health effects (WHO 1995, WHO 1996).

The toxicity of lead may to some extent be explained by its inactivation of certain enzyme systems by binding to protein sulfhydryl groups or by displacing other essential metal ions. For this reason, almost all organs and organ systems may be considered as potential targets. A wide range of biological effects of lead has been documented including effects on the nervous and haematopoietic systems, and cardiovascular, hepatic, renal, and gastrointestinal effects. (Nielsen 1999).

2.3.3.1 Effects on the nervous system

Lead causes a continuum of nervous system effects in children and adults ranging from slowed nerve conduction, behavioural changes, and small decrements in cognitive ability, to mental retardation and encephalopathy. (Nielsen 1999, WHO 1995, ATSDR 1999, JECFA 2000, CSTEE 2000).

Peripheral neuropathy is a common sign of chronic, high level lead exposure, often manifested as weakness in the upper or lower limbs. Measurements of nerve conduction velocity (NCV) are generally used as a sensitive indicator of peripheral nerve dysfunction. In adults, a decrease in NCV has been observed at PbB levels of about 30 µg/dl. In children, a threshold for NCV at PbB levels of 20 to 30 µg/dl has been estimated.

Neurobehavioral testing has revealed a number of effects in adults at PbB levels from 40 to 60 µg/dl including disturbances in reaction time, visual motor performance, hand dexterity, IQ test and cognitive performance, impaired memory and learning ability, nervousness, mood, or coping ability.
A number of epidemiological studies have been carried out to investigate lead-induced neurobehavioral effects in children. Most studies have primarily addressed effects arising from exposure to low levels of lead (i.e., PbB levels below 40 µg/dl). The inconsistencies in the results of the studies has been the subject of many reviews. The overall conclusion is that there appears to be an association between indices of lead burden (usually PbB) and impairment of cognitive and behavioural development of the central nervous system. The epidemiological studies do not provide definitive evidence of a threshold; below the PbB range of 10 to 15 µg/dl, the effects of confounding variables and limits in the precision in analytical and psychometric measurements increase the uncertainty of any estimate of effect; however, there is some evidence of an association between lead exposure and cognitive deficits even below this range.

One Polish study has shown that auditory function in children was impaired at a PbB level even below 10 µg/dl.

High-level exposure to lead produces encephalopathy in children and adults. Severe lead encephalopathy is generally observed at extremely high PbB levels (>300 µg/dl), but may occur at PbB levels of 100 to 120 µg/dl in some adults and of 80 to 100 µg/dl in some children.

2.3.3.2 Effects on the haematological system

Lead is shown to affect several enzymatic reactions critical in haem synthesis, causing abnormal concentrations of haem precursors in blood and urine. Lead inhibits the activity of three enzymes (δ-aminolaevulinic acid dehydratase (ALAD), coproporphyrinogen oxidase, and ferrochelatase) involved in the haem synthesis resulting in decreased haem synthesis and subsequently increased activity of the rate limiting enzyme (δ-aminolaevulinate synthase) leading to an accumulation of δ-aminolaevulinate, a neurotoxin. (Nielsen 1999, WHO 1995, WHO 1996, ATSDR 1999, JECFA 2000).

General population studies indicate that the activity of ALAD is inhibited at very low PbB levels, with no threshold apparent. ALAD activity was inversely correlated with PbB levels (3 to 34 µg/dl) in urban subjects never exposed occupationally. A similar relationship was reported in a population of children (aged 10-13 years) having PbB levels of 4.7 to 41 µg/dl. Other reports have confirmed this correlation and apparent lack of a threshold in different age groups and exposure categories.

Lead-induced anaemia may result from either a decrease in haemoglobin production or an increase in the rate of destruction of erythrocytes. In adults, the estimated PbB level associated with a decrease in haemoglobin concentration is 50 µg/dl. Decreased haemoglobin concentrations in children have been reported to occur at a PbB level of 40 µg/dl. However, an epidemiological study of children (aged 1 to 5 years) living in close proximity to a primary lead smelter revealed that adverse effects on haematocrit may occur at lower PbB levels. Anaemia was defined as a haematocrit value below 35% and was not observed at PbB levels below 20 µg/dl. There was a strong non-linear dose-response relationship between PbB levels and haematocrit at higher PbB levels, which was influenced by age and the effect was strongest in the youngest children.

2.3.3.3 Other effects

There is currently considerable debate whether there is a causal relationship between lead exposure and hypertension. The possible relationship between PbB levels and blood pressure has been examined in several large-scale population studies; however, the studies do not provide conclusive evidence that lead exposure, as assessed by PbB levels, is positively associated with hypertension. (Nielsen 1999, WHO 1995, ATSDR 1999).

Qualitative evidence linking lead exposure to cardiac effects includes the finding of degenerative changes in cardiac muscle; however, according to WHO (1995), lead has no direct effect on the myocardium and the cardiac effects observed occurs via the autonomic nervous system. (Nielsen 1999, WHO 1995, ATSDR 1999).

In children, exposure to lead has been shown to inhibit formation of the haem-containing protein cytochrome P450, as reflected in decreased activity of hepatic mixed-function oxygenases, which consequently may result in impaired metabolism of a number of chemicals in the liver (Nielsen 1999, WHO 1995, ATSDR 1999).

Characteristics of chronic lead nephropathy include progressive interstitial fibrosis, dilation of tubules and atrophy or hyperplasia of the tubular epithelial cells, reduction in glomerular filtration rate, and azotaemia. The effects in the kidney are irreversible. The chronic form is reported mainly in lead workers. Increased risk from nephropathy was noted in workers with PbB levels ranging from 40 to >100 µg/dl. Renal effects have recently been seen among the general population when more sensitive indicators of function were measured. Results from one study showed that PbB levels from 10 µg/dl may impair renal function in middle-aged and older men. In another study, adverse effects of chronic low-level lead exposure on kidney function could be detected by measuring urinary markers in children with PbB levels of approximately 13 µg/dl; the pattern of effects was similar to that previously observed in adults. (Nielsen 1999, WHO 1995, WHO 1996, ATSDR 1999).

Colic is an early symptom of lead poisoning in individuals acutely exposed to high levels of lead. A PbB threshold of 60 to 100 µg/dl has been identified for children and of 100 to 200 µg/dl for adults. (Nielsen 1999, WHO 1995, ATSDR 1999).

2.3.4 Genotoxicity

Studies of chromosomal aberrations in humans exposed to lead (PbB > 40 µg/dl) have given conflicting results. Positive reports have been published concerning workers in lead-battery industries and lead smelters, but other studies of workers under comparable conditions have given negative results. Increased incidences of sister chromatid exchanges have been reported in the peripheral blood lymphocytes of workers exposed to lead, but not in those of children exposed to high levels of lead in the environment. A few studies in rodents treated with lead salts *in vivo* have shown small, but significant increases in the frequency of chromosomal aberrations and micronuclei in bone-marrow cells, but most studies have shown no increase. Lead salts caused morphological sperm abnormalities in mice, but not in rabbits. Sister chromatid exchanges and unscheduled DNA synthesis were not induced in cells of animals treated with lead salts *in vivo*, and chromosomal aberrations were not induced in human lymphocytes *in vitro*. Conflicting results

have been obtained in assays for transformation in cultured rodent cells. Lead salts did not cause aneuploidy in *Drosophila melanogaster*, mutation or gene conversion in yeast or mutation or DNA damage in bacteria. (IARC 1997, WHO 1995, WHO 1996, IRIS 2004).

2.3.5 Carcinogenicity

The carcinogenicity of lead in humans has been examined in several epidemiological studies, which either have been negative or have shown only very small excess mortalities from cancers. One major difficulty in many of the studies is the concurrent exposure to potential carcinogens such as arsenic and chromium. (Nielsen 1999, IARC 1997, WHO 1995, WHO 1996, ATSDR 1999, IRIS 2004).

There have been several experimental studies in rat and mice in which long-term administration of soluble lead compounds in food or drinking water has produced tumours of the kidney. Detectable increases in renal tumour frequency apparently required doses in excess of 10 mg/kg b.w./day; such concentrations also cause renal toxicity in rodents. (IARC 1997, WHO 1995, IRIS 2004, JECFA 2000).

2.3.6 Toxicity to reproduction

Reproductive effects of lead have been studied extensively and the studies clearly indicate that high levels of lead cause adverse effects on human reproduction, including increased incidences of spontaneous abortion, miscarriages, and stillbirths. The mechanisms responsible for these effects are unknown, but many factors may contribute to these results. These factors include indirect effects of lead on maternal nutrition or hormonal status before and during pregnancy to more direct gametogenic effects that could affect parental fertility in either sex. (Nielsen 1999, ATSDR 1999).

The available data do not permit any estimate of effect levels in women. One study has reported that women with a PbB close to 50 µg/dl have a greater risk of miscarriage. Two studies found no effect on the rate of spontaneous abortions at PbB levels of 10-15 µg/dl. Regarding male reproductive function, adverse effects such as lowered sperm counts and increases in the numbers of abnormal sperm may occur at PbB levels of 40 to 50 µg/dl. (Nielsen 1999, WHO 1995, WHO 1996, ATSDR 1999, CSTEE 2000).

Developmental effects observed in humans following exposure to low levels of lead include reduced birth weight, reduced gestational age and neurobehavioral deficits or delays. The evidence for an association between PbB levels and reduced birth weight and gestational age is inconsistent and the weight of evidence indicates that there may not be a direct association. There is a predominance of negative results, with the most recent studies showing no such association. The evidence in support of neurobehavioral deficits or delays is more consistent, with most of the studies indicating that there is an association between lead exposure at low levels and developmental neurobehavioral effects. No evidence of an association with major congenital malformations has been found. One study has reported an association between cord blood lead levels and minor anomalies; the relative risk doubled at blood lead levels of about 7 to 10 µg/dl. (Nielsen 1999, WHO 1995, WHO 1996, ATSDR 1999).

2.3.7 Evaluation

Repeated oral exposure to lead is associated with effects in the nervous, haematopoietic, and reproductive systems, and carcinogenicity.

Most of the human data on health effects of lead are expressed in terms of internal exposure (blood lead (PbB) levels), rather than external exposure levels (mg/kg b.w./day). The relationship between exposure and PbB appears to be curvilinear over a wide range of PbB values. For children, the relationship between PbB level and lead intake from food has, according to (WHO 1995), been determined to be 0.16 µg/dl per µg Pb/day for a median PbB level of approximately 10 µg/dl.

Lead causes a continuum of nervous system effects in children and adults ranging from slowed nerve conduction, behavioural changes, and small decrements in cognitive ability, to mental retardation and encephalopathy. The effects in the nervous system generally develop at lower PbB levels in children than in adults and the most critical effect of lead at low exposure levels is the association with impaired cognitive development and intellectual performance in children. The available epidemiological studies have shown inconsistency in the results, but the overall conclusion is that there appears to be an association between indices of lead burden (usually PbB) and impairment of cognitive and behavioural development of the central nervous system. The studies do not provide definitive evidence of a threshold and there is some evidence of an association between lead exposure and cognitive deficits below a PbB level of 10 µg/dl; a level at which the effects of confounding variables increase the uncertainty of any estimate of effect.

Lead is shown to affect several enzymatic reactions critical in haem synthesis. The available epidemiological studies indicate that the activity of one of the enzymes, ALAD, is inhibited at very low PbB levels (about 5 µg/dl), with no threshold apparent. The effects of lead in the haematopoietic system result in decreased haemoglobin synthesis. The estimated PbB associated with a decrease in haemoglobin level in children is 40 µg/dl and in adults 50 µg/dl. Anaemia, defined as a haematocrit below 35%, was not observed in one study of children at a PbB level below 20 µg/dl.

Other effects observed following long-term oral exposure to lead compounds include cardiovascular, hepatic, renal, and gastrointestinal effects. These effects have been reported to occur at higher exposure levels than those at which the effects in the nervous and haematopoietic systems have been observed.

Studies in experimental animals have shown that soluble lead compounds in food or drinking water has produced tumours in the kidneys at quite high doses (>10 mg/kg b.w./day), which also cause kidney toxicity in rodents. The epidemiological studies have either been negative or have shown only very small excess mortalities from cancers. Lead is considered by IARC (1987) and US-EPA (IRIS 2004) as probably carcinogenic to humans (Group B2; evidence in humans inadequate, evidence to animals sufficient). Data regarding genotoxicity are conflicting. Overall, a carcinogenic potential cannot be fully excluded in relation to dietary exposure to lead, but is not considered to be of great significance at the exposure levels at which the effects in the nervous and haematopoietic systems have been observed.

Epidemiological studies clearly indicate that lead causes adverse effects on human reproduction, including effects on sperm parameters, increased incidences of spontaneous abortion, miscarriages, and stillbirths. Developmental effects observed in humans following exposure to low levels of lead include reduced birth weight,

reduced gestational age, and neurobehavioral deficits or delays. Regarding male reproductive function, decreased sperm counts and increased number of abnormal sperm may occur at PbB levels of 40 to 50 µg/dl. The available data do not permit any estimate of effect levels in women, but is probably above PbB levels of 10 µg/dl. One study has reported an association between cord PbB levels and minor anomalies; the relative risk doubled at PbB levels of about 7 to 10 µg/dl. In the EU, lead compounds are classified for reproductive effects (Repr. Cat. 1, R61 – may cause harm to the unborn child; Repr. Cat. 3, R62 – possible risk of impaired fertility) (EEC 2004). Overall, data indicate that the neurodevelopmental effects might occur even below a PbB level of 10 µg/dl and no clear threshold for these effects has been identified. Other reproductive effects are not considered to occur at the exposure levels at which the effects in the nervous and haematopoietic systems in adults have been observed.

2.3.8 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of lead are the effects observed in the nervous, haematopoietic and reproductive systems, and carcinogenicity, and lead has particularly significant effects in children.

The most sensitive endpoints appear to be the neurodevelopmental effects and the effects on haem synthesis, effects for which no threshold apparently exists. There is some evidence of an association between lead exposure and cognitive deficits below a PbB level of 10 µg/dl. ALAD, one of the enzymes in the haem synthesis, is inhibited at very low PbB levels (about 5 µg/dl) although adverse effects are not associated with its inhibition at this level. Based on the available data, a PbB level of 10 µg/dl is considered as a LOAEL for effects on the developing nervous system. For children, the relationship between PbB level and lead intake from food has, according to (WHO 1995), been determined to be 0.16 µg/dl per µg Pb/day for a median PbB level of approximately 10 µg/dl and the LOAEL of 10 µg Pb/dl corresponds to 62.5 µg Pb/day (about 6 µg Pb/kg b.w./day for a child weighing 10 kg) based on this relationship. This LOAEL is considered to provide adequate protection against the other reproductive effects of lead as well as of the haematological effects, and is taken forward to the risk characterisation.

A carcinogenic potential cannot be fully excluded in relation to dietary exposure to lead, but is not considered to be of great significance at the exposure levels at which the effects in the nervous system have been observed.

2.3.9 References

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2.4 Mercury

Mercury is a metallic element, which displays three oxidation states: 0, +2 and +4. Elemental mercury has a very high vapour pressure. The mercurous (Hg_2^{++}) and mercuric (Hg^{++}) states form numerous inorganic and organic compounds. Organic forms are those in which mercury is attached covalently to at least one carbon atom. In this report, the term 'mercury' refers to mercury in general unless otherwise stated.

There is a well-recognised global cycle for mercury, whereby vapour of metallic mercury is converted to soluble forms and deposited by rain onto soil and water. The change in speciation of mercury from inorganic to methylated forms is the first step in the aquatic bioaccumulation process. Once methylmercury is released, it enters the food chain and binds to proteins. As a result of food-chain biomagnification, the highest levels of methylmercury are found in the tissues of large predatory fish, which are at the top of the food chain. (WHO 1990,1991, EFSA 2004, JECFA 2004).

In the general population, the major source of mercury is food. In most foodstuffs, mercury is largely in the inorganic form, which in food is considerably less toxic than methylmercury. Fish and seafood products are the dominant sources of methylmercury in the diet. Some individuals may have a significant contribution to the mercury body burden from dental amalgam. (EFSA 2004, EC 2003, JECFA 2000, WHO 1990,1991).

In Denmark, the estimated average dietary intake of total mercury for adults (1988-1992) was 5 µg/day with the 95 percentile being 9 µg/day. The dietary intake has decreased a little during the recent 5-year period (1993-1997) with an average daily intake for an adult person of 4 µg/day and a 95 percentile of 7 µg/day. The most significant sources are fish, beverages, grain products and dairy products. (FDIR 2000).

In the EU Member States, the mean intake of total mercury is less than 30% of the PTWI for total mercury (0.35 mg for a person weighing 70 kg). The mean intake of methylmercury from fish and shellfish, the main source of mercury in the diet, in the Member States is less than 30% of the PTWI for methylmercury (0.112 mg for a person weighing 70 kg). Data indicate that children have a lower intake of methylmercury than adults. However, children have a larger burden/kg b.w., due to their lower body weight, and may exceed the PTWI for methylmercury. (EC 2003).

2.4.1 Toxicokinetics

The absorption in humans of inorganic mercuric mercury compounds is less than 10% on average, but there is considerable individual variation. Absorption in young children may be considerably greater than in adults. (WHO 1991). Methylmercury in the diet is almost completely absorbed (WHO 1990, IRIS 2004a).

The kidney is the main depository after administration of inorganic mercury salts and 50-90% of the body burden is found in the kidney (based on animal data). The erythrocyte to plasma ratio in humans is generally below 1 after administration of inorganic mercury salts. (WHO 1991).
Following absorption, methylmercury is distributed to all tissues within about 4 days for most tissues and 5-6 days for the brain. In humans, methylmercury is

distributed between the plasma and the erythrocyte with a ratio of 1 to 20. In erythrocytes, methylmercury is complexed with glutathione (GSH). High concentrations of methylmercury are found in the brain. The blood-to-hair ratio in humans is about 1 to 250, but appreciable individual differences have been found. (WHO 1990, JECFA 2000).

Only a small fraction of administered divalent inorganic mercury is transferred to the foetus (WHO 1991).
Placental transfer of methylmercury occurs readily and a strong correlation exists between maternal-blood mercury concentrations and foetal-blood mercury concentrations, as shown by cord-blood. A number of studies have reported cord-blood mercury and maternal-blood mercury data; overall, these studies indicate that cord-blood mercury is higher than maternal-blood mercury and that the cord-blood-to-maternal-blood ratio is around 1.7. (IRIS 2004a).
The cord-blood-to-maternal-blood ratio in Inuits with a high consumption of marine foods and in Swedish women who ate large amounts of fish has been reported to be 3.2 (JECFA 2000).

Several forms of metabolic transformation may occur: 1) reduction of divalent mercury to metallic mercury, 2) methylation of inorganic mercury, and 3) conversion of methylmercury to divalent inorganic mercury (WHO 1991).
The reduction of divalent mercury to metallic mercury has been demonstrated both in humans and in experimental animals and a small amount of exhaled mercury vapour is the result of this reduction (WHO 1991).
According to WHO (1991) there is no evidence in the literature for the syntesis of organomercury compounds in human or mammalian tissues; however, a slight increase in the concentration of methylmercury in blood and/urine has been reported among dentists and workers in the chloralkali industry.
Methylmercury is converted to inorganic mercury in the body and this conversion is considered to be a key step in the process of excretion of mercury after exposure to methylmercury. The main site of decomposition of methylmercury is the intestinal tract, where the portion secreted with bile or with cells shed from the intestinal wall is decomposed and the remainder is reabsorbed. The fraction of total mercury present in the tissues as inorganic mercury depends on the duration of exposure to methylmercury and the time after cessation of exposure. (WHO 1990,1991, JECFA 2000).

Urine and faeces are the main pathways for the elimination of inorganic mercury in humans, although some elemental mercury is exhaled. Mercury is also excreted in milk. In humans, inorganic mercury compounds have two half-times: one lasts for days or weeks and accounts for most of the absorbed mercury; the other is much longer, probably years, and accounts for only a fraction of the mercury. (WHO 1991, IARC 1993).
Following exposure to methylmerucy, mercury is excreted via the urine, faeces and hair with faecal excretion predominating over urinary excretion. The rate of excretion of mercury is directly proportional to the simultaneous body burden. The half-time in humans has been estimated to be 39 to 70 days in blood (average approximately 50 days). The half-time of mercury in hair closely follow that in blood, but with wider variation (35-100 days, average 65 days). (WHO 1990, JECFA 2000).

Selenium has been found to affect the distribution of mercuric mercury in rats, mice, rabbits and pigs. Mercury forms a mercury-protein complex with selenium, which can be identified in plasma and blood cells. When given with selenium, mercury is retained longer in blood and accumulation in the kidney is decreased. Mercury taken up by the kidney is bound to a protein-selenium complex, and, on

administration of equivalent amounts of selenium, the binding to metallothionein is diminished and may be negligible. A consequence of the changed binding of mercury in blood brought about by selenium is that transport of selenium and mercury through the placenta is inhibited. Only selenate or selenite compounds, and not the naturally occurring selenium compounds in food, have been studied. However, one study compared the distribution and form of mercury and protection against the nephrotoxic effects of mercury after exposure to different forms or compounds of selenium. It was concluded that dietary selenium is less efficient than selenite as an antidote against mercurial nephrotoxicity. Studies of selenium interaction with mercuric mercury have mainly been carried out in rodents. Selenium metabolism in humans is different from that in most animals, and selenium dependency in humans is comparatively less than that in rodents. (WHO 1991).

Methylmercury has a strong affinity for sulphur and selenium. Selenium alters the tissue distribution and excretion of methylmercury and also the inorganic-to-methylmercury ratio in tissues. However, selenite increases the brain concentration of methylmercury. (WHO 1990, JECFA 2000).
Selenium has been suggested to provide protection against the toxic effects of methylmercury; however, according to JECFA (2000), no such effect has been demonstrated.

2.4.2 Single dose toxicity

2.4.2.1 Inorganic mercury

Human poisoning has been reported following oral ingestion of mercuric chloride and lethal doses ranging from 29 to 50 mg/kg b.w have been reported. The most common autopsy findings were gastrointestinal lesions and renal lesions that had resulted in renal failure. (WHO 1991).

The oral LD_{50}-value for inorganic mercury has been reported to be between 10 and 40 mg/kg b.w. in rodents. The effects of acute toxicity observed include shock, cardiovascular collapse, acute renal failure, and severe gastrointestinal damage. (WHO 1991).

2.4.2.2 Methylmercury

In experimental animals, an LD_{50}-value of 25 mg/kg b.w. has been reported for methylmercury in old rats (450 g b.w.) and of 40 mg/kg b.w. in young rats (200 g b.w.). The clinical effects observed in the animals reflect the corrosive effect of methylmercury at the site of contact. (JECFA 2000).

2.4.3 Repeated dose toxicity

2.4.3.1 Inorganic mercury

A few workers exposed occupationally to mercuric oxide, mercuric acetate and probably mercury vapours have experienced a nephrotic syndrome, which was considered to be an idiosyncratic reaction since many other workers with similarly high levels of urine mercury did not develop proteinuria (IRIS 2004b).

In experimental animals, the kidney is the critical organ, and the most sensitive adverse effect caused by mercuric mercury is the formation of mercuric-mercury-induced auto-immune glomerulonephritis. The production and deposition of IgG antibodies to the glomerular basement membrane can be considered the first step in the formation of this glomerulonephritis. (IRIS 2004b, WHO 1991).

The Brown Norway rat is the most sensitive species to this effect (due to a genetic susceptibility). Following oral administration of mercuric chloride in the feed (3 mg/kg b.w. per week for up to 60 days, corresponding to about 320 µg Hg/kg b.w./day), 4/5 exposed rats showed IgG deposition in the glomeruli after 15 days and 5/5 exposed rats after 60 days; weak proteinuria was observed in 3/5 rats exposed for 60 days. Following gavage with mercuric chloride (3 mg/kg b.w. twice a week for 60 days, corresponding to about 630 µg Hg/kg b.w./day), deposits of IgG were observed in the glomeruli; morphological lesions of the ileum and the colon with abnormal deposits of IgA and IgG in the basement membranes were also observed. Following subcutaneous injection of mercuric chloride (0, 100, 250, 500, 1000 or 2000 µg/kg b.w. 3 times per week for 8 weeks; or 50 µg/kg b.w. three times per week for 12 weeks, corresponding to about 15.8 µg Hg/kg b.w./day), tubular lesions were observed at the higher dose levels, proteinuria from 100 µg/kg b.w., and IgG deposition in the glomeruli at all dose levels. (IRIS, 2004b, WHO 1991).

One chronic ingestion study is available in which rats were administered mercuric acetate in their food for up to 2 years at dietary concentrations corresponding to about 0, 0.025, 0.125, 0.50, 2.0, or 8.0 mg/kg b.w./day. Kidney weights were significantly increased at the two highest dose levels and pathological changes originating in the proximal convoluted tubules of the kidneys were also noted, with more severe effects in females than in males. (IRIS 2004b).

Subchronic and chronic gavage studies with mercuric chloride have been performed by NTP in rats and mice. In rats, increased relative kidney weights were observed in the 6-month study from about 0.33 mg Hg/kg b.w./day, and nephropathy (minimal to mild) in males from about 0.16 mg Hg/kg b.w./day (lowest dose level) and in females at about 2.6 mg Hg/kg b.w./day (highest dose level); in the 2-year study, nephropathy (moderate to marked) was observed in males only from about 1.3 mg Hg/kg b.w./day (lowest dose level). Similar effects were observed in mice but at much higher dose levels. (IRIS 2004b).

2.4.3.2 Methylmercury

Methylmercury is a highly toxic substance and a number of adverse health effects associated with exposure to it have been identified in humans and in animal studies. Most extensive are the data on neurotoxicity, particularly in developing organisms. Some of the effects observed in experimental animals, such as renal damage and anorexia, have not been observed in humans although exposed to high doses. (WHO 1990, JECFA 2000, IRIS 2004a).

The nervous system, especially the central nervous system, is the most sensitive target organ. Information on dose-effect and dose-response relationships in humans has been derived from numerous studies on populations exposed to methylmercury through mass poisonings or through high consumption of fish containing varying levels of methylmercury (Japan, Iraq, Canada, New Zealand, Peru, Seychelles, Faroe Islands, Amazon Basin).

The effects of methylmercury on the adult brain differ both quantitatively and qualitatively from the effects seen after prenatal or, possibly, early postnatal exposures (WHO 1990).

2.4.3.2.1 Effects on the adult nervous system

In adult human beings, areas of damage to the brain are highly localised (focal), and the sensory, visual, and auditory functions as well as those of the brain areas (especially the cerebellum) concerned with coordination, are the most common functions to be affected. A long latent period usually lasting several months is a characteristic feature. The earliest clinical effects are non-specific symptoms, such as complaints of paraesthesia, malaise, and blurred vision. Subsequently, signs appear such as concentric constriction of the visual field, deafness, dysarthria, and ataxia. Inhibition of protein synthesis is one of the earliest detectable biochemical effects in the adult brain; however, the sequence of events leading to overt damage is not yet understood. Effects in severe cases are irreversible due to destruction of neuronal cells. In less severe cases, some degree of recovery in each symptom occurs. At high doses, methylmercury also affects the peripheral nervous system and it has been shown that methylmercury can react directly with acetylcholine receptors in the peripheral nerves. (WHO 1990).

According to WHO (1990), no adverse effects have been detected in adults with long-term daily intake of methylmercury of 3 to 7 µg/kg b.w. (hair mercury concentrations of approximately 50-125 µg/g). Clinical observations in Iraq suggested that pregnant women are more sensitive to the toxic effects of methylmercury and effects may be observed at lower methylmercury exposure.

A cross-sectional study of neurotoxic effects in adults has reported significant mercury-assocciated neurobehavioural deficits in persons whose current hair-mercury concentration was below 15 µg/g (JECFA 2004).

2.4.3.2.2 Effects on the developing nervous system

Observations on human subjects as well as information from studies in experimental animals indicate that the developing central nervous system is more sensitive to damage from methylmercury than the adult nervous system. In case of prenatal exposure, the effects of methylmercury seem to be of a much more general basic nature. The clinical picture is dose dependent. In infants who have been exposed to high maternal blood levels of methylmercury, cerebral palsy indistinguishable from that caused by other factors has been observed. Microcephaly, hyperreflexia, and gross motor and mental impairment, sometimes associated with blindness or deafness, is the main pattern. The affected infants show mainly psychomotor impairment and persistence of pathological reflexes. Milder cases have findings quite similar to the findings in the minimal brain damage syndrome. In the developing brain, methylmercury affects normal neuronal development, leading to altered brain architecture, heterotopic cells, and decreased brain size. Methylmercury may also be exerting an effect, perhaps through inhibition of the microtubular system, on cell division during critical stages in the formation of the central nervous system. (WHO 1990).

According to WHO (1990), the lowest level (maximum maternal hair mercury concentration during pregnancy) at which severe effects were observed was 404 µg/g in the Iraqi poisoning outbreak, and the highest no observed effect level (NOEL) for severe effects was 399 µg/g. Evidence of psychomotor retardation was

seen at lower maternal hair mercury levels (maximum concentration during pregnancy) of about 10 to 20 µg/g. (WHO 1990).

Since the evaluation by WHO (1990), a considerable amount of data have become available on the possible neurobehavioural effects of prenatal and postnatal exposure.

According to JECFA (2000), the most relevant data are those from two large prospective epidemiological studies of cohorts assembled from the populations of the Faroe Islands and the Seychelles, who eat large amounts of seafood. The prenatal exposure of the two cohorts to mercury appears to have been similar. The geometric mean concentration of mercury in the hair of mothers during pregnancy was 4.3 µg/g (interquartile range, 3-8 µg/g) in the Faroe Islands and 6.8 µg/g (range, 0.5-27 µg/g) in the Seychelles. In the Faroe Islands, the geometric mean concentration in umbilical cord blood was 23 µg/l (interquartile range, 13-41 µg/l). In the Faroe Islands, no association was seen between the extent of prenatal exposure to mercury and performance in clinical or neuropsychological tests, whereas significant decrements were observed in the children's scores in tests of functions such as fine motor skill, attention, language, visual-spatial skills, and memory. In the study in the Seychelles, no adverse effects associated with exposure to mercury were observed. JECFA concluded that the data did not provide consistent evidence of neurodevelopmental effects in children of mothers whose intake of methylmercury yielded hair burdens of 20 µg/g or less.

In 2003, JECFA (JECFA 2004) reviewed new data and analyses from the Seychelles Islands cohort and concluded that no adverse effects of prenatal methylmercury exposure had been detected in this cohort. The results from the Seychelles Islands cohort were combined with those from the Faroe Islands cohort and JECFA used the average from the two studies, 14 µg Hg/g maternal hair-mercury, as an estimate of the concentration of methylmercury in maternal hair that reflects exposures that would have no appreciable adverse effects in children born to mothers in these two study populations. The maternal hair concentration was converted to a blood concentration of 0.056 mg/l using the average hair-to-blood ratio from a number of studies, and the maternal blood concentration was converted to a daily intake of 1.5 µg/kg b.w. using an equation, which incorporated the rate of elimination.
EFSA (2004) pointed out that the maternal hair level of 14 µg Hg/g was not a NOAEL in the data from the Faroe Islands.

According to US-EPA, there are three epidemiological studies for which quantitative analyses are available for evaluation. The Seychelles study yielded scant evidence of impairment related to *in utero* methylmercury exposure. The studies from the Faroe Islands and from New Zealand found dose-related effects on a number of neuropsychological endpoints. US-EPA considered the Faroe Islands studies as being the most appropriate ones although there was co-exposure to PCBs, but statistical analyses indicated that the effects of PCBs and methylmercury were independent. US-EPA chose benchmark dose (BMD) analysis as the most appropriate method of quantifying the dose-effect relationship in these studies. The benchmark dose lower limit ($BMDL_{05}$, the lower 95% confidence limit of the BMD_{05}) was calculated and ranged from 46 to 79 ppb in maternal blood for different neuropsychological effects in the offspring at 7 years of age, corresponding to a range of maternal daily intakes of 0.857 to 1.472 µg/kg b.w./day. US-EPA stressed that it is important to note that no evidence of a threshold arose for methylmercury-related neurotoxicity within the range of exposures in the Faroe Islands study. (IRIS 2004a).

EFSA (2004) agreed with the JECFA and US-EPA evaluations that the developing brain should be considered the most sensitive target organ for methylmercury toxicity. EFSA noted that the health based guidance values set by JECFA and US-EPA differed by a factor of approximately two, largely because of different interpretations of the main epidemiology studies and because of the different uncertainty factors used. It was beyond the scope of the present EFSA opinion to perform a refinement of the hazard characterisation for methylmercury.

2.4.3.2.3 Other effects

A recent study has shown an association between low-level methylmercury exposure and cardiovascular effects. Diastolic and systolic blood pressure increased with increasing cord-blood mercury in 7-year-old Faroese children; there was also a decrease in heart rate variability (an indication of cardiac autonomic control). (IRIS 2004a).
In a cohort study, Finnish men with hair mercury concentrations of 2 µg/g or higher had a greater risk of acute myocardial infarction and, over a 4-year follow-up interval, with an increased risk for atherosclerotic disease (IRIS 2004a, JECFA 2004).
The results of two large case-control studies of mercury exposure and coronary heart disease are conflicting, one study reporting significantly higher concentrations of mercury in the toenails of cases than of controls, whereas the other reported similar concentrations in the two groups (JECFA 2004).
JECFA (2004) determined that the available evidence for the potential cardiotoxicity of methylmercury was not conclusive, but noted that further studies are needed.

2.4.4 Genotoxicity

Humans ingesting methylmercury-contaminated foods have been reported to experience chromosomal aberrations (IRIS 2004a, WHO 1990); however, according to US-EPA (IRIS 2004a), the interpretation of the studies is limited by methodological deficiencies.

According to IARC (1993), the findings of 14 studies of cytogenetic effects, such as sister chromatid exchange, micronucleus formation, structural chromosomal aberrations, aneuploidy and polyploidy, in peripheral lymphocytes of individuals exposed to various mercury compounds are controversial and uncertain.

According to IARC (1993), several organomercury compounds were assayed in a variety of short-term tests. Tests for unscheduled DNA synthesis, sister chromatid exchange, chromosomal aberrations and dominant lethal mutations in mammals *in vivo* gave conflicting results. Tests for clastogenicity in fish and amphibians gave more convincingly positive results. All studies of induction of c-mitosis (spindle disturbances), sister chromatid exchange, structural chromosomal aberrations and aneuploidy in cultured human lymphocytes gave positive results. The results of the majority of studies of the induction of forward mutations, c-mitosis and polyploidy in cultured mammalian (non-human) cells were positive, and those of one study on micronucleus induction in cultured fish cells were also positive. In *Drosophila melanogaster* and other insects, the majority of mercury compounds induced sex-linked recessive lethal mutation and nondisjunction (aneuploidy) but did not induce dominant lethal mutation. The assessment of nuclear or mitochondrial DNA mutations, mitotic recombination and gene conversion in the yeast *Saccharomyces cerevisiae* led to conflicting results. Most of the few studies available in bacteria

(investigating differential killing in *rec*$^-$ *Bacillus subtilis* or reversion in *his*$^-$ *Salmonella typhimurium* or *trp*$^-$ *Escherichia coli*) gave negative results. (IARC 1993).
According to WHO (1990) and US-EPA (IRIS 2004a) *"methylmercury is not a potent mutagen"*.

According to IARC (1993), there were fewer studies of inorganic mercury compounds (mostly mercuric chloride), and a minority compared inorganic and organic compounds. As in studies with organomercury compounds, studies in rodents treated *in vivo* with mercuric chloride gave weakly positive results for dominant lethal mutation. Studies on the induction of chromosomal aberrations in rodents yielded conflicting results. One study on chromosomal effects in amphibians gave positive results for mercuric chloride and methylmercury chloride at similar doses. Chromosomal alterations were reported in cultured human lymphocytes. The dose of mercuric chloride required to induce sister chromatid exchange in cultured human lymphocytes was 5-25 times higher than those needed of methylmercury chloride. Mercuric acetate did not induce anchorage-independent growth in human cells. Five to ten times higher doses of mercuric chloride than methylmercury chloride were required to induce polyploidy. DNA damage has been induced repeatedly in mammalian cells by mercuric chloride. Although the information comes from single studies, this compound also induced sister chromatid exchange, chromosomal aberrations, aneuploidy (spindle disturbances) and enhancement of virus-induced morphological transformation. Unlike organomercury compounds, mercuric chloride failed to enhance the frequency of micronuclei in cultured fish cells. Mercuric chloride failed to enhance lethality in a DNA repair-deficient strain of *E. coli*. (IARC 1993, IRIS 2004b).
According to US-EPA (IRIS 2004b) *"the effects of mercuric chloride on genetic material has been suggested to be due to the ability of mercury to inhibit the formation of the mitotic spindle, and event known as c-mitosis."*

2.4.5 Carcinogenicity

2.4.5.1 Inorganic mercury

According to US-EPA (IRIS 2004b), no data are available on the carcinogenic effects of mercuric chloride in humans, and no human data on inorganic mercury compounds have been included in IARC (1993); according to WHO (1991), inorganic mercury is generally not considered to be carcinogenic in humans.

Mercuric chloride has been tested for carcinogenicity in two studies in mice, by oral gavage and by administration in the drinking water; and in one study in rats by oral gavage. In the rat study, a few renal adenomas occurred in females, and there was a dose-related increase in the incidence of squamous-cell papillomas of the forestomach in males, and a few papillomas in females; dose-related hyperplasia of the forestomach was seen in both males and females. According to US-EPA (IRIS 2004b), a high mortality in both male dose groups indicates that the maximum tolerable dose was exceeded and therefore limits the value of the study for assessment of carcinogenic risk. Only the gavage study was, according to IARC (1993) and US-EPA (IRIS 2004b), adequate for an evaluation of carcinogenicity; the combined incidence of renal adenomas and adenocarcinomas was increased in high-dose males only. (IRIS 2004c, IARC 1993).

2.4.5.2 Methylmercury

Three epidemiological studies are available regarding the relationship between methylmercury exposure and cancer. No evidence of increased carcinogenicity attributable to methylmercury exposure was observed in any of the studies. (IRIS 2004a, IARC 1993). According to US-EPA (IRIS 2004a), the interpretation of these studies was limited by poor study design and incomplete descriptions of methodology and/or results.

In three dietary studies in two different strains of mice, methylmercury chloride induced an increased incidence of renal tumours in male mice. No increase in tumour incidence was observed in four dietary studies in rats with methylmercury chloride or in a drinking water study in mice with methylmercury acetate. (IRIS 2004a, IARC 1993). According to US-EPA (IRIS 2004a), the studies are difficult to interpret either because tumours were only observed at doses that exceeded the maximum tolerated dose (MTD), or because of failure to achieve an MTD or deficiencies in study design.

2.4.6 Toxicity to reproduction

2.4.6.1 Inorganic mercury

The available epidemiological studies in male workers have not revealed any reproductive effects even following high exposures to elemental mercury vapour; no data have been located for inorganic mercury compounds. (WHO 1991).

There have been reports of increased menstrual disturbances in women exposed to elemental mercury vapour but no conclusion can be drawn based on these reports. There have also been reports, which suggest that occupational exposure to inorganic mercury compounds may cause spontaneous abortion, stillbirth and congenital malformations, while other studies have not indicated such effects. (WHO 1991).

In experimental animals, decreased fertility has been observed in male mice, alterations in testicular tissue in male rats, and reproductive effects in female hamsters following parenteral administration of mercuric chloride. Elemental mercury vapour or parenteral administration of various inorganic mercuric salts to pregnant rodents have induced foetal growth retardation, malformations and deaths. (WHO 1991, IRIS 2004b).

2.4.6.2 Methylmercury

According to US-EPA (IRIS 2004a), no studies are available regarding reproductive deficits in humans exposed to low-dose methylmercury. Among Iraqi women exposed to methylmercury in treated grain, the number of pregnant women seemed to be lower than generally.
In a case-control study, higher blood mercury concentrations have been found in infertile compared with in fertile couples (JECFA 2004).
Neurodevelopmental effects are discussed in section 2.3.3.2.

Short-term, high-dose studies in rodents and guinea pigs have reported low sperm counts, testicular tubule atrophy, reduced litter size, decreased foetal survival, resorptions, and foetal malformations following exposure to methylmercury. There are no standard, two-generation reproductive studies available. In monkeys treated with methylmercury, decreased conception rates, early abortions, and stillbirths

have been reported, but at higher doses than those at which behavioural deficits were observed in the offspring. (IRIS 2004a, JECFA 2000, WHO 1990).

2.4.7 Evaluation

2.4.7.1 Inorganic mercury

A few workers exposed occupationally to mercuric oxide, mercuric acetate and probably mercury vapours have experienced toxic effects in the kidneys, a nephrotic syndrome, probably due to an idiosyncratic reaction.

The kidney is the critical organ in experimental animals following repeated oral exposure, and the most sensitive adverse effect caused by mercuric mercury is the formation of mercuric-mercury-induced auto-immune glomerulo nephritis, the first step being the production and deposition of IgG antibodies to the glomerular basement membrane. This effect has been observed following oral administration of mercuric chloride at 317 or 633 µg Hg/kg b.w./day for 60 days to Brown Norway rats, the most sensitive species (due to a genetic susceptibility), and following subcutaneous injection of mercuric chloride (15.8 µg Hg/kg b.w./day for 12 weeks).
Subchronic and chronic oral studies are also available for other strains of rats as well as for mice showing nephropathy following administration of mercuric chloride and mercuric acetate. However, according to US-EPA (IRIS 2004b), the Brown Norway rat should be used for mercury risk assessment as it is a good surrogate for the study of mercury-induced kidney damage in sensitive humans. Based on the subcutaneous animal LOAEL of 15.8 µg Hg/kg b.w./day, US-EPA (IRIS 2004b) and WHO (1991) has estimated an equivalent oral LOAEL of 226 µg Hg/kg b.w./day by assuming 100% absorption following subcutaneous injection and 7% absorption following oral administration.

No human data regarding carcinogenic effects of inorganic mercury compounds are available. In experimental animals, tumours have been observed in the kidneys in male mice and female rats following oral administration of mercuric chloride, and an increased incidence of forestomach tumours in rats. Metallic mercury and inorganic mercury compounds are considered by IARC (1993) as not classifiable as to their carcinogenicity to humans (Group 3; inadequate evidence in humans, limited evidence in experimental animals). Mercuric chloride is considered by US-EPA (IRIS 2004b) as a possible human carcinogen (Group C; no data in humans, limited evidence in experimental animals). Genotoxicity test data on inorganic mercury compounds (mostly mercuric chloride) have shown both positive and negative results. Overall, a conclusion regarding the carcinogenic potential in relation to dietary exposure to inorganic mercury compounds cannot be drawn based on the available data, but is not considered to be of any significance at the dose levels at which IgG deposition in the renal glomeruli of Brown Norway rats has been observed.

There are indications from animal studies of reproductive and developmental toxicity of elemental mercury vapour and inorganic mercuric salts administered par-enterally. The human data regarding reproductive effects following occupational exposure to elemental mercury vapour and inorganic mercury compounds are conflicting. Overall, no conclusion regarding reproductive toxicity can be drawn based on the available data, but is not considered to be of any significance at the dose levels at which IgG deposition in the renal glomeruli of Brown Norway rats has been observed.

2.4.7.2 Methylmercury

Repeated oral exposure to methylmercury is associated predominantly with effects in the nervous system, especially in the developing nervous system, but also with effects on the cardiovascular and reproductive systems, and carcinogenicity.

The two biomarkers most frequently used for quantifying the burden of methylmercury in the human body are blood and hair concentrations. JECFA (2000, 2004) has concluded that a ratio of 250 is a reasonable central estimate of the ratio of hair-to-blood concentration. The approximate relationship between weekly intake and blood concentration of mercury at steady state indicates that an intake of 1 µg Hg/kg b.w./week in the form of methylmercury corresponds to a concentration of 10 µg Hg/l of blood and of 2.5 µg Hg/g of hair (JECFA 2000).

The effects of methylmercury in the adult brain differ both quantitatively and qualitatively from effects seen after pre-natal and, possibly, post-natal exposure. Damage is generalised throughout the brain in the case of pre-natal exposure, in contrast to adult exposure where focal lesions are predominant. The clinical and epidemiological evidence indicates that the developing central nervous system is more sensitive to damage from methylmercury than the adult nervous system. According to WHO (1990), no adverse effects have been detected in adults with long-term daily intake of methylmercury of 3 to 7 µg/kg b.w. (hair-mercury concentrations of approximately 50-125 µg/g).

There is a general agreement that the developing nervous tissue is the most sensitive target organ for methylmercury toxicity; however, no consensus has been achieved regarding a threshold for the neurodevelopmental effects.
According to WHO (1990), evidence of psychomotor retardation was seen at maternal hair mercury levels (maximum concentration during pregnancy) of about 10 to 20 µg/g.
In 2003, JECFA (2004) concluded, based on the results from the Faroe Islands cohort and the Seychelles Islands cohort, that the average from the two studies, 14 µg Hg/g maternal hair, should be used as an estimate of the concentration of methylmercury in maternal hair that reflects exposures that would have no appreciable adverse effects in children born to mothers in these two study populations, corresponding to a blood concentration of 0.056 mg/l or a daily intake of 1.5 µg/kg b.w.
According to US-EPA (IRIS 2004a), the Faroe Islands studies were considered as being the most appropriate ones. The calculated benchmark dose lower limit ($BMDL_{05}$, the lower 95% confidence limit of the BMD_{05}) ranged from 46 to 79 ppb in maternal blood for different neuropsychological effects in the offspring at 7 years of age, corresponding to a range of maternal daily intakes of 0.857 to 1.472 µg/kg b.w./day. US-EPA stressed that it is important to note that no evidence of a threshold arose for methylmercury-related neurotoxicity within the range of exposures in the Faroe Islands study.
EFSA (2004) noted that the health based guidance values set by JECFA and US-EPA differed by a factor of approximate two, largely because of different interpretations of the main epidemiology studies and because of the different uncertainty factors used and pointed out that the maternal hair level of 14 µg Hg/g as a NOAEL in the JECFA (2004) evaluation was not a NOAEL in the data from the Faroe Islands. It was beyond the scope of the present EFSA opinion to perform a refinement of the hazard characterisation for methylmercury.

In the three available epidemiological studies, no evidence of increased incidences of tumours attributable to methylmercury exposure was observed. In experimental

animals, three dietary studies in two strains of mice indicate that methylmercury is carcinogenic as it induced an increased incidence of tumours in the kidneys; however, tumours were observed only in those dose groups in which the maximum tolerated dose (MTD) was exceeded or in conjunction with toxicity in the kidneys. A drinking water study in mice and four dietary studies in rats failed to indicate any carcinogenic effects associated with methylmercury exposure. Methylmercury is considered by US-EPA (IRIS 2004a) and by IARC (1993) as possible carcinogenic to humans (US-EPA: Group C; evidence in humans inadequate, evidence to animals limited – IARC: Group 2B; inadequate evidence in humans, sufficient evidence in experimental animals). Although genotoxicity test data indicate that methylmercury is capable of producing chromosomal and nuclear damage, there are also non-positive genotoxicity data. Overall, a carcinogenic potential cannot be fully excluded in relation to dietary exposure to methylmercury, but is not considered to be of any significance at the exposure levels at which the effects in the nervous system have been observed.

There is evidence from animal studies of reproductive and developmental toxicity of methylmercury, including developmental neurotoxicity. No adequate human data on reproductive effects are available; neurodevelopmental effects are discussed above. The developing nervous system is the most sensitive target organ for methylmercury toxicity and other reproductive and developmental effects in experimental animals have been observed at higher doses than those at which the neurodevelopmental effects were observed in the offspring.

2.4.7.3 Protective effect of selenium

Some selenium compounds affect the kinetics of inorganic mercury and of methylmercury and it has been claimed that selenium has a protective effect against the toxicity of mercury compounds. However, according to JECFA (2000), no such effect has been demonstrated for methylmercury. According to WHO (1991), only selenate or selenite compounds, and not the naturally occurring selenium compounds in food, have been studied in experimental animals, mainly in rodents. One study comparing the protective effect of different forms or compounds of selenium against mercurial nephrotoxicity concluded that dietary selenium is less efficient than selenite as an antidote. Furthermore, selenium metabolism in humans is different from that in most animals, and selenium dependency in humans is comparatively less than that in rodents. Based on these data, it is considered that dietary selenium possibly has no or only a limited protective effect against mercurial nephrotoxicity in humans.

2.4.8 Critical effect(s) and NOAEL / LOAEL

2.4.8.1 Inorganic mercury

The critical effect following dietary intake of inorganic mercury compounds is the effects observed in the kidney.

According to US-EPA (IRIS 2004b), the Brown Norway rat should be used for mercury risk assessment as it is a good surrogate for the study of mercury-induced kidney damage in sensitive humans. The most sensitive adverse effect is the formation of auto-immune glomerulo nephritis, the first step being the production and deposition of IgG antibodies to the glomerular basement membrane. An oral LOAEL of 317 µg Hg/kg b.w./day has been estimated for this effect. Based on the subcutaneous animal LOAEL of 15.8 µg Hg/kg b.w./day, US-EPA (IRIS 2004b)

and WHO (1991) has estimated an equivalent oral LOAEL of 226 µg Hg/kg b.w./day (by assuming 100% absorption following subcutaneous injection and 7% absorption following oral administration). A LOAEL of 300 µg Hg/kg b.w./day is taken forward to the risk characterisation.

2.4.8.2 Methylmercury

The critical effect following dietary intake of methylmercury is the effects observed in the developing nervous system.

There is a general agreement that the developing nervous system is the most sensitive target organ for methylmercury toxicity; however, no consensus has been achieved regarding a threshold for neurodevelopmental effects. Based on the evaluations performed by US-EPA (IRIS 2004a), a maternal daily intake of 1.0 µg/kg b.w./day (range, 0.857-1.472 µg/kg b.w./day) is considered as a LOAEL for neurodevelopmental effects (see section 2.4.7.2 for further details), and is taken forward to the risk characterisation.

2.4.9 References

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2.5 Selenium

Selenium is a metalloid element, which displays five oxidation states: -2, 0, +2, +4, and +6. All of the oxidation states of the element listed are commonly found in nature except +2. In this report, the term 'selenium' refers to ionic selenium, except when specific selenium compounds are mentioned.

Selenium is an essential trace element. Selenium plays an important role in scavenging active oxygen species and this action is mediated through selenium-containing proteins (selenoproteins). (Beltoft & Nielsen 1999, Lu 1997).

The general population is exposed to selenium mainly through the diet. Foods contain a number of different selenium forms. In animal foods, there are specific selenium proteins where selenium is incorporated via selenide as selenocysteine, while selenomethionine, and possibly also selenocysteine to some extent, are non-specifically incorporated as analogues to methionine and cysteine in foods both of animal and plant origin. It is uncertain if the inorganic forms of selenium occur in foods. In addition to these forms, a number of uncharacterised forms exist, e.g., in fish. (SCF 2000).

The intakes reported from non-endemic areas in China probably represent the minimum human requirements for selenium (19.4 and 14.1 µg/day for males and females, respectively). In Denmark, a daily intake of 50 µg selenium is recommended. (Beltoft & Nielsen 1999).

Selenium toxicity (selenosis), is caused by both organic and inorganic forms of selenium. The toxicity of selenium varies according to the valence state of selenium when incorporated into biomolecules; however, there is general agreement that sodium selenite, sodium selenate, and selenomethionine are among the more toxic selenium species. (Beltoft & Nielsen 1999).

The molecular mechanisms of selenium toxicity remain unclear. Several mechanisms have been suggested: redox cycling of auto-oxidisable selenium metabolites, glutathione depletion, protein synthesis inhibition, depletion of S-adenosyl-methionine, general replacement of sulphur and reactions with critical sulphydryl groups of proteins and cofactors. No unifying hypothesis is possible and it is likely that several mechanisms may operate and vary among different selenium compounds. (Beltoft & Nielsen 1999, SCF 2000).

A wide variety of interactions of selenium with essential and nonessential elements, vitamins, xenobiotics, and sulphur-containing amino-acids have been demonstrated in numerous studies. Selenium has been reported to reduce the toxicity of many metals such as mercury, cadmium, lead, silver, and to some extent, copper. The reduction of the toxicity of the metals by selenium seems to be due to the forming of inert metal selenide complexes (Beltoft & Nielsen 1999).

2.5.1 Toxicokinetics

Most forms of selenium salts and organic bound selenium are generally readily absorbed from the gastrointestinal tract of humans and animals. Absorption of 80-100% of selenium administered as sodium selenite and sodium selenate in the diet has been reported for both humans and animals. The bioavailability of ingested selenium can be affected by the physical state of the compound, the chemical form of selenium, and the dosing regimen. In general, it appears that the degree of

selenium absorption is independent of the exposure level, but in some cases is greater when selenium deficiency exists. (Beltoft & Nielsen 1999, ATSDR 2003, Alexander & Meltzer 1995).

After absorption, selenium compounds are generally distributed rapidly to most organs with the highest concentrations in the liver and kidney followed by the spleen and testes (Beltoft & Nielsen 1999, WHO 1996, Alexander & Meltzer 1995, ATSDR 2003).

Selenium can pass the placenta and is also excreted into the milk. Selenium levels in human milk have been shown to correlate with dietary intake. (Beltoft & Nielsen 1999, ATSDR 2003, Alexander & Meltzer 1995).

Selenium-containing amino acids and probably other selenium forms, such as selenite and selenate, can be converted to selenide in mammals. Selenide is a central metabolic form of selenium, which is utilised for the formation of selenocysteine, incorporated into specific selenoproteins, and in case of high exposure, into excretory products such as dimethyl selenide (which is exhaled) and trimethylselenonium ions (which are excreted into urine). (SCF 2000, ATSDR 2003).

Selenium is primarily eliminated in the urine in both humans and laboratory animals. Excretion of selenium in faeces constitutes a minor pathway immediately following exposure, but the amount excreted can be equal to that excreted in the urine depending on the chemical form of selenium administered, the dose level, and time after exposure. The form of selenium excreted is dependent on the form of selenium that was ingested. In case of acute exposure to toxic concentrations of selenium or selenium compounds, significant amounts of selenium can be eliminated in the breath, causing the characteristic “garlic breath”. (Beltoft & Nielsen 1999, ATSDR 2003).

2.5.2 Single dose toxicity

Acute toxicity in humans is predominantly related to high doses of dietary supplement. Intake of 250 mg selenium as a single dose or multiple doses of 27-31 mg selenium have resulted in nausea, vomiting, nail changes, dryness of hair, hair loss, tenderness and swelling of fingertips, fatigue, irritability, and “garlic breath”. (Beltoft & Nielsen 1999, Alexander & Meltzer 1995, SCF 2000).

In experimental animals, the most acutely toxic selenium compounds are the soluble sodium selenate and sodium selenite. The LD_{50}-values reported for orally administered selenite are in the range of 1.0 mg Se/kg (rabbits) to 4.8-7.0 mg Se/kg (rats). (Beltoft & Nielsen 1999, ATSDR 2003).

2.5.3 Repeated dose toxicity

Long-term oral intake results in gastrointestinal disturbances, icteroid discoloration of the skin, pathological deformation and loss of nails, hair loss, and excessive tooth decay and discoloration have also been reported. These clinical symptoms are usually referred to as selenosis. Selenium also affects the liver. (Beltoft & Nielsen 1999, WHO 1996, ATSDR 2003, Alexander & Meltzer 1995, SCF 2000).

In a study of Chinese people living in an area with high environmental selenium concentrations, a NOAEL for clinical symptoms indicative of selenosis was

reported to be about 825 µg Se/day and a LOAEL to be about 1270 µg Se/day. Biochemical changes (increase in prothrombin time and decrease in the concentration of glutathione in blood) were seen at a dietary intake of about 850 µg Se/day. (Beltoft & Nielsen 1999, ASTDR 2003, WHO 1996, IRIS 2004, Alexander & Meltzer 1995, SCF 2000).

In another study (USA), no clinical or biochemical signs of selenium toxicity were reported at an average daily selenium intake around 239 µg/day (range 68-724 µg Se/day) (Beltoft & Nielsen 1999, SCF 2000, Longnecker et al. 1991).

In rats, oral intakes for a longer time period may result in growth retardation, liver changes, anaemia, and pancreatic enlargement. Rats fed 2.5 mg/kg diet of sodium selenite or sodium selenate for 6 weeks showed normal growth whereas rats fed 5 mg/kg diet exhibited reduced body weight gain; rats fed 10 mg/kg diet died within 29 days. Liver cirrhosis has been observed in rats fed 4.3 mg Se/kg diet for 16 weeks but not at 2.4 mg Se/kg diet. (Beltoft & Nielsen 1999).

2.5.4 Genotoxicity

Inorganic selenium compounds have been observed to have both genotoxic and anti-genotoxic effects. The anti-genotoxic effects generally occur at lower selenium exposure levels than the frank genotoxicity. In general, selenite and selenate have produced mixed results in bacterial mutagenicity test systems. Results with mammalian cell systems are also mixed, although selenite is more consistently genotoxic in these systems. Results of *in vivo* genotoxicity tests have been both negative and positive. The underlying mechanisms responsible for the varying genotoxicity results remain to be elucidated. (ATSDR 2003, Beltoft & Nielsen 1999, SCF 2000).

2.5.5 Carcinogenicity

The majority of studies of humans and animals have revealed no association between selenium intake and the incidence of cancer. Some epidemiological studies and experimental evidence suggests that selenium exposure, under certain conditions, may contribute to a reduction in cancer risk. (ATSDR 2003, Beltoft & Nielsen 1999, SCF 2000).

2.5.6 Toxicity to reproduction

One study in humans indicated no correlation between sperm count or motility and selenium concentrations in sperm samples; no other human data are available (ATSDR 2003). No indication of teratogenicity of selenium has been shown in humans even in the areas of high selenium intake in China (SCF 2000).

A few reproductive studies in animals indicate that oral exposure to selenium can reduce female fertility whereas male fertility appears not to be affected. Failure of about half of the F_3 generation pairs to breed successfully was observed in a three-generation reproduction study on mice given sodium selenate (0.57 mg Se/kg b.w./day) in the drinking water; male fertility was not reduced. No effects were observed on the fertility in female mice following administration of sodium selenite at doses of 0.34 mg Se/kg b.w./day in drinking water for 30 days before mating and for 18 days during gestation. Selenium administered in the diet or in drinking water over short exposure periods (e.g., 1 month) does not appear to affect

the fertility of female animals unless the intake is sufficiently high to cause general toxicity. (Beltoft & Nielsen 1999, ATSDR 2003).

Developmental studies using the oral route of administration indicate that excessive levels of selenite or selenate can result in foetal toxicity and reduced growth in experimental animals, but generally only at doses that produce maternal toxicity. A slight reduced foetal growth was observed in mice following administration of sodium selenite at doses of 0.34 mg Se/kg b.w./day in drinking water for 30 days before mating and for 18 days during gestation, but not at 0.17 mg Se/kg b.w./day. Teratogenic effects have not been observed at levels that are not maternally toxic. (Beltoft & Nielsen 1999, ATSDR 2003).

2.5.7 Evaluation

Repeated oral exposure to selenium is predominantly associated with clinical signs of selenosis and alterations in biochemical parameters indicative of possible liver dysfunction (increased prothrombin time).

One epidemiological study has reported that no biochemical or clinical signs of selenium toxicity were observed at an average dietary intake of 239 µg Se (range 68-724 µg Se/day; 239 µg Se/day corresponds to about 4 µg/kg b.w./day for an adult person weighing 60 kg).
Another epidemiological study has reported a NOAEL for clinical symptoms indicative of selenosis to be about 825 µg Se/day (corresponding to about 14 µg/kg b.w./day for an adult person weighing 60 kg) with clinical signs of selenosis occurring from around 1200 µg Se/day (corresponding to about 20 µg/kg b.w./day). Biochemical changes (increase in prothrombin time and decrease in the concentration of glutathione in blood) were observed at a dietary intake of about 850 µg Se/day (corresponding to about 14 µg/kg b.w./day).
Overall, a NOAEL of about 850 µg Se/day (corresponding to about 14 µg/kg b.w./day) is considered for clinical signs of selenosis and alterations in biochemical parameters indicative of possible liver dysfunction.

The available data do not suggest that inorganic selenium salts or organic selenium compounds relevant in food and nutrition are associated with an increased risk of cancer. Selenium compounds have shown equivocal results regarding genotoxicity, however, the effects may be dose-dependent *in vivo* and are not considered to occur at the recommended dietary intakes (50 µg Se/day for adults in Denmark).

Effects of selenium on reproduction and offspring in rodents have generally been associated with overt maternal toxicity or nutritional deprivation. No indication of teratogenicity of selenium has been shown in humans even in the areas of high selenium intake.

2.5.8 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of selenium are selenosis and the effects in the liver. A NOAEL of about 850 µg Se/day (corresponding to about 14 µg/kg b.w./day for an adult person weighing 60 kg) is considered for the critical effects, and is taken forward to the risk characterisation.

2.5.9 References

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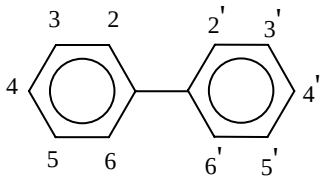
2.6 PCBs

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. (Larsen 2003, WHO 2003, ATSDR 2000, EFSA 2005).



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 the non-planar in which the benzene rings are at a 90° angle to each other. 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. (WHO 2003, ATSDR 2000).

The health effects of PCBs have been extensively studied. The mechanisms of toxicity are not completely understood, but there is evidence to suggest that PCB congeners differ qualitatively and quantitatively in biological activities and that multiple and diverse mechanisms are involved in responses to PCB mixtures. The congeners considered as being the most toxic ones, based on combined health effects considerations, are coplanar. Some co-planar PCBs 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 the Ah receptor. These PCBs, termed ‘dioxin-like PCBs’, only constitute a minor fraction of the total amount of a PCB mixture (total-PCB) and, of the 209 theoretical PCBs only 12 congeners are considered to be dioxin-like. The remaining PCBs are generally referred to as ‘non dioxin-like PCBs’. (ATSDR 2000, Larsen 2003, Van den Berg et al. 1998).

Most studies have investigated 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 commonly tested of these mixtures are Kanechlors produced in Japan and Clophens produced in Germany. The commercial (technical) PCB mixtures contain both dioxin-like and non dioxin-like PCB congeners and may vary considerably with respect to their congener composition due to differences in the amount of chlorine and the method of production. Moreover, these PCB mixtures contain other dioxin-like compounds as impurities, such as polychlorinated dibenzofurans (PCDFs). The different compositions of the PCB mixtures as well as the presence of toxicologically relevant impurities may have a significant impact on the results of toxicological

studies with the mixtures. (ATSDR 2000, Larsen 2003, Van den Berg et al. 1998, EFSA 2005).

PCB levels in traditional Greenland food items have been presented as the sum of 10 congeners (sPCB10). These 10 congeners are CB 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180. This group represents most of the predominant congeners in fish and marine mammals. (Johansen et al. 2004).
Of these 10 congeners, only PCB 105, 118 and 156 are dioxin-like PCBs, but not among the most toxic of the dioxin-like PCBs. Therefore, this evaluation primarily focuses on commercial and defined PCB mixtures, and on the individual congeners included in the sPCB10 as well. The term 'PCB' in this evaluation refers to PCB mixtures in general unless otherwise stated.

PCBs have been produced commercially since 1929 and have been used extensively in various products for example in electric equipment and in hydraulic systems. Many countries have severely restricted or banned the production of PCBs and since 1986 no new products containing PCBs have been allowed in Denmark. (Larsen 2003, WHO 2003, ATSDR 2000).

Due to their high persistency, PCBs are present in the environment all over the world. The composition of the mixtures of congeners found in the environment differs from the commercial mixtures originally released into the environment because of differences in the rate of degradation of the individual congeners. Consequently, the toxicity of environmental PCB mixtures may be increased or decreased compared to commercial mixtures. (Larsen 2003, Van den Berg 1998, ATSDR 2000).

PCBs accumulate in the food chain and fish, meat, poultry and milk are the main foodstuffs containing PCBs. More than 90% of the general human exposure to PCBs is estimated to occur through the diet. Furthermore, human milk can be an important source of PCBs in breastfeed infants. In the USA, the dietary intake of PCBs for adults and infants continually has decreased from 1978 (0.027 / 0.011 µg/kg b.w./day) to 1986-1991 (< 0.001 µg/kg b.w./day). (WHO 2003, ATSDR 2000).

In Denmark, the estimated average dietary intake of PCB-sum (congeners 28, 52, 101, 105, 118, 138, 153, 156, 170 and 180) and total-PCB for adults (1993-1997) was 2.2 and 4.0 µg/day, respectively, and the 95 percentile was 3.6 and 6.0 µg/day, respectively. (FDIR 2000).

2.6.1 Toxicokinetics

Humans and experimental animals can absorb PCBs administered by the oral route. 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. (Larsen 2003, WHO 2003, ATSDR 2000).

PCBs distribute first to the liver and muscle, and are subsequently translocated to adipose tissue and skin for storage. Due to the high lipophilicity and resistance to biotransformation, PCBs, especially the highly chlorinated congeners, accumulate, particularly in the adipose tissue and liver. The hepatic accumulation of PCBs decreases dramatically with the addition of one chlorine atom in *ortho* position. The liver/adipose tissue distribution can thus vary significantly between different congeners. In humans, the liver concentration of congeners is about 1/10 of the

level in adipose tissue. (Larsen 2003, Van den Berg 1998, WHO 2003, ATSDR 2000).

PCBs are metabolised by microsomal monooxygenase system catalysed by cytochrome P450 to polar metabolites that can undergo conjugation with glutathione and glucuronic acid. Many PCBs are relatively resistant to metabolism. The major determinant for metabolism of the congeners is the presence of two adjacent, unsubstituted carbon atoms on the lateral positions. These positions are preferentially oxidised by the cytochrome P450 system. The major routes of excretion of PCBs are faecal and, especially for metabolites, urinary. For higher chlorinated congeners, the predominant route of excretion is via the faeces (up to 60% of total excretion); for lower chlorinated congeners, the situation seems to reverse. Mainly metabolites are found in urine and bile, although small amounts of parent compound may appear in the faeces, in particular congeners that are poorly metabolised. (Larsen 2003, WHO 2003, ATSDR 2000).

Estimates for the half-lives for elimination of PCBs from humans have been based on body burden measurements and estimates as low as 0.02 years and as long as infinity have been reported for individual congeners. For PCB mixtures, estimated half-lives between 0.5 years and infinity have been reported. According to ATSDR, estimated half-lives of 2.6-4.8 years are the best estimates for PCB mixtures based on data from two different occupational cohorts. (ATSDR 2000).

PCBs can pass the placenta of pregnant animals and humans and are excreted in human milk. (Larsen 2003, WHO 2003).

2.6.2 Single dose toxicity

No specific human data are available regarding the acute toxicity of PCBs.

In experimental animals, the acute 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 b.w. in rats. Signs of toxicity include diarrhoea, respiratory depression, dehydration, decreased response to pain stimuli, unusual gait and stance, oliguria, coma, pathological changes in organs. (WHO 2003, ATSDR 2000).

2.6.3 Repeated dose toxicity

Information on health effects in humans due to oral intake of PCBs is 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 by consumption of contaminated fish and other food products of animal origin. 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. Epidemiological studies suggest an association between PCB exposure and thyroid hormone anomalies in humans; among the Yu-Cheng cohort, a significantly elevated odds ratio for goiter occurred indicating the possibility of excess thyroid disease. Chloracne and other dermal alterations, and ocular effects have been reported in individuals exposed during the Yusho and Yu-Cheng

incidents; no adverse dermal or ocular effects have been reported in subjects with high consumption of contaminated fish. 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 in children born to exposed mothers, decreased total serum IgA and IgM antibody levels, and/or changes in T lymphocyte subsets. Infants exposed *in utero* and/or via breast-feeding seem to be particularly sensitive. Sensory and motor nerve alterations have been observed in highly exposed Yusho and Yu-Cheng patients; however, there is no evidence that PCBs are neurotoxic to adults at the levels generally found in the environment. (ATSDR 2000).
Carcinogenic and reproductive and developmental effects are addressed in sections 2.6.5 and 2.6.6, respectively.

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 relatively few 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 b.w./day represented the approximate daily intake of a nursing human infant whose mother's milk contained 50 ppb 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 (28, 77, 105, 118, 126, 128, 153) were selected based on frequent occurrence in environmental samples and human tissues, or toxic potency; four of the congeners included in sPCB10 was tested (28, 105, 118, 153). 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).

The following summary of effects of PCBs in experimental animals is exclusively based on ATSDR (2000), the most recent review.

2.6.3.1 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 (see section 2.6.5) with higher doses or longer duration exposures. Monkeys appear to be more sensitive than rats to PCB hepatotoxicity.

Induction of microsomal enzymes 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 b.w./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 b.w./day for 2-6 months.

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 b.w./day, and increased serum cholesterol in females exposed to Aroclor 1242, 1254 and 1260 from about 1.4-5.7 mg/kg b.w./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.

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 b.w./day, the next most toxic congener was PCB 105 with a LOAEL of 0.039 mg/kg b.w./day, and the least toxic congener was PCB 128 with a LOAEL of 0.425 mg/kg b.w./day. Considering dose-response and severity of liver effects, the order of toxicity was PCB 126 > 105 > 118 ≈ 77 > 153 ≈ 28 > 128.

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 b.w./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 b.w./day of Aroclor 1254 for 72 months; no effects were observed at dose levels of up to 0.04 mg/kg b.w./day. In another study, monkeys receiving Aroclor 1254 for 37 months had decreased plasma cholesterol from 0.04 mg/kg b.w./day and increased plasma triglycerides from 0.005 mg/kg b.w./day.

In the study on infant monkeys treated with the defined PCB mixture (0.0075 mg/kg b.w./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.

2.6.3.2 Effects on the gastro-intestinal tract

Administration of PCBs in the diet to monkeys at dose levels from 1.3 mg/kg b.w./day of Aroclor 1248 or from 0.12 mg/kg b.w./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 b.w./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 dose levels from 0.08 mg/kg b.w./day for 72 months. 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 0.009 µg/kg b.w./day to approximately 4 mg/kg b.w./day.

2.6.3.3 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.

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 b.w./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 b.w./day for 5 weeks, but not at 0.025 mg/kg b.w./day.

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 b.w./day, the next most toxic congener was PCB 105 with a LOAEL of 0.039 mg/kg b.w./day, and the least toxic congener was PCB 128 with a LOAEL of 0.425 mg/kg b.w./day.

In Rhesus monkeys, no effects on thyroid tissue and serum hormone levels were observed following exposure to Aroclor 1254 at dose levels of up to 0.08 mg/kg b.w./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 b.w./day for 28 months, whereas in another study, Cynomolgus monkeys treated similarly for 12 months did not show histological alterations in the thyroid.

2.6.3.4 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 b.w./day for 15 weeks, and from 0.25 mg/kg b.w./day, but not at 0.05 mg/kg b.w./day, for 5 months. No histological alterations in the adrenals of rats were observed at dose levels of up to 25 mg/kg b.w./day Aroclor 1254 by gavage for 15 weeks.

In the comparative 13-week oral toxicity studies in rats with individual congeners, no histopathological alterations in the adrenal glands were observed; doses ranged from 0.009 µg/kg b.w./day for the dioxin-like PCB 126 to approximately 4 mg/kg b.w./day for some non-dioxin-like congeners.

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 b.w./day for 12 months, or up to 0.08 mg/kg b.w./day for 72 months; and no effects on serum hormone levels at dose levels of up to 0.08 mg/kg b.w./day for up to 22 months.

2.6.3.5 Dermal effects

Dermal effects including facial oedema, acne, folliculitis, and alopecia have been observed in monkeys exposed to 0.1 mg/kg b.w./day Aroclor 1248 or 0.12 mg/kg b.w./day Aroclor 1242 for 2 months. Chronic dietary treatment with 0.1 mg/kg

b.w./day Aroclor 1248 for 12 months, or 0.2 mg/kg b.w./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 b.w./day Aroclor 1254 for 37 months, or 0.04 mg/kg b.w./day for 72 months.

In the comparative 13-week oral toxicity studies in rats with individual congeners, no histopathological alterations in the skin were observed. Doses ranged from 0.009 µg/kg b.w./day for the dioxin-like PCB 126 to approximately 4 mg/kg b.w./day for some non-dioxin-like congeners.

2.6.3.6 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 b.w./day Aroclor 1248 or 0.12 mg/kg b.w./day Aroclor 1242 for 2 months. Monkeys exposed to 0.005-0.08 mg/kg b.w./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 b.w./day Aroclor 1254 for 12 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 from about 4 to about 11 mg/kg b.w./day.

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 0.009 µg/kg b.w./day for the dioxin-like PCB 126 to approximately 4 mg/kg b.w./day for some non-dioxin-like congeners.

2.6.3.7 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 b.w./day for 2 months, or from 0.2 mg/kg b.w./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 b.w./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 b.w./day for 72 months.

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 b.w./day, respectively, whereas no haematological effects were observed in female rats that were similarly exposed to Aroclor 1242 from 5.7 mg/kg b.w./day or Aroclor 1254 from 6.1 mg/kg b.w./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 b.w./day, respectively.

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 b.w./day, and for PCB 126 at about 7.4 µg/kg b.w./day, but not for the other congeners.

2.6.3.8 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.

In a recent 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 b.w./day in males and females, respectively.

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 b.w./day) and with PCB 28, 105 and 153 (LOAEL of 4 mg/kg b.w./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.

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 b.w./day Aroclor 1248 and 1254 for 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 b.w./day (the lowest dose level tested) after 23 months, and alterations in lymphocyte T-cell subsets at 0.08 mg/kg b.w./day.

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

2.6.3.9 Effects on the nervous system

Neurobehavioural alterations (effects on motor activity and effects on higher cognitive functions, i.e., learning, memory, attention) have been observed in rats and monkeys following pre- and/or postnatal exposure to commercial mixtures, defined experimental congener mixtures, single congeners, and contaminated fish, see section 2.6.6. 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.

In the study on infant monkeys treated with the defined PCB mixture (0.0075 mg/kg b.w./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.

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 b.w./day for 30 days and in monkeys receiving Aroclor 1016 or 1260 at dose levels from 0.8 mg/kg b.w./day in the diet for 20 weeks.

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 b.w./day, for PCB 118 at 0.2 mg/kg b.w./day, for PCB 128 at 0.005 mg/kg b.w./day, and for PCB 153 at 0.01 mg/kg b.w./day; no changes were observed for the other congeners.

2.6.4 Genotoxicity

Chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes were increased in workers involved in the manufacturing of PCBs; however, exposures involved mixtures of chemicals including benzene and formaldehyde in one study and PCDDs and PCDFs in another study.

The genotoxicity of PCBs has been tested in several *in vitro* and *in vivo* studies. End-points that have been examined include gene mutations in bacteria and Chinese hamster V79 cells, chromosomal aberrations in human lymphocytes and rat and mouse bone marrow cells and spermatogonia, micronuclei in mouse bone marrow cells, and dominant lethal mutations in rat sperm cells. (ATSDR 2000).

Aroclor 1242 and 1254 did not induce chromosome abnormalities in rat bone marrow cells or in spermatogonial cells following a single or repeated administration. Dominant lethal mutations were not induced in male rats following treatment with Aroclor 1242 or 1254. Aroclor 1260 did not produce detectable DNA adducts in the liver of rats. One study in rats given a single dose of Aroclor 1254 showed evidence of DNA damage in liver cells 4-12 hours after treatment, but was no longer detectable 48 hours after treatment due to DNA repair. Aroclor did not induce micronuclei in mouse bone marrow cells or chromosomal abnormalities in mouse sperm cells. (ATSDR 2000, IARC 1987).

Aroclor 1254 was not mutagenic in bacteria (*Salmonella typhimurium*) with or without exogenous metabolic activation, and Aroclor 1242 or Clophen A60 did not induce gene mutations in Chinese hamster V79 cells. Aroclor 1254 induced chromosomal damage in one study with human lymphocytes, but not in another study, and induced unscheduled DNA synthesis in rat liver cells in one study. (ATSDR 2000, IARC 1987).

2.6.5 Carcinogenicity

The following summary of carcinogenicity of PCBs is exclusively based on ATSDR (2000), the most recent review.

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.
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. There is no clear association between occupational exposures to PCBs and cancer in other tissues, including the brain and breast, and haematopoietic and lymphatic tissues.
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.

A number of oral cancer studies have been performed in experimental animals with commercial PCB mixtures.
The most comprehensive and adequately performed study (published in 1998) compared the four most widely used commercial Aroclor mixtures (1016, 1242, 1254 and 1260) administered in the diet to rats at dose levels of 2.0-11.2, 2.0-5.7, 1.0-6.1, or 1.0-5.8 mg/kg b.w./day, respectively, for 24 months. Increased tumour incidences were found 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 found that commercial mixtures with 60% chlorine content (Aroclor 1260 and Clophen A60) induced liver tumours with indications of a sex-dependent response (stronger in females). With commercial mixtures containing less than 60% chlorine, liver tumours were reportedly induced by Aroclor 1254 and Clophen A30.
Oral carcinogenicity studies of commercial PCB mixtures in mice are limited to two studies that were less than lifetime in duration and only investigated the liver. Liver tumours, predominantly benign, were observed at relatively high dietary dose levels.

Oral exposure to commercial PCBs or to single congeners can promote pre-neoplastic lesions and tumours in the liver and lung of rats and mice following induction with various initiators.

According to a very recent EFSA opinion on non-dioxin like PCBs, evaluation of the cancer studies in rats with commercial PCB mixtures (Arochlors), and comparison with data obtained with TCDD, indicate that the dioxin-like components in the commercial PCB mixtures are likely to be responsible for the carcinogenic response of these mixtures (EFSA 2005).

2.6.6 Toxicity to reproduction

The following summary of reproductive and developmental effects of PCBs is exclusively based on ATSDR (2000), the most recent review.

Information on reproductive and developmental effects in humans due to oral intake of PCBs is 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 by consumption of contaminated fish and other food products of animal origin; and for developmental effects, also from studies of the general population with no known high exposure to PCBs. 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.
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 a 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. There is mounting evidence from epidemiological studies of populations with high consumption of contaminated fish as well as of the general population that perinatal exposure to PCBs have induced adverse developmental effects in humans, specifically, but not limited to, neurobehavioural alterations in newborn and children exposed during gestation and/or via breast milk. Neurodevelopmental effects associated with PCB exposure included abnormal reflexes and more motor immaturity in newborns, altered PDI scores at 1-2 years of age, and alterations in memory functions at 7 months and 4 years of age and in cognitive abilities at 42 months of age. Other effects observed include lower birth weight and reduced growth during early life; however, the results of the studies have varied, with some studies finding significant negative associations between birth weight and exposure to PCBs, some studies finding positive associations, and some studies finding no significant associations.

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.

2.6.6.1 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. Limited information is available on reproductive effects of PCBs in male animals.

Increased menstrual duration (5-7 days) and bleeding were observed in Rhesus monkeys exposed to 0.1 mg/kg b.w./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 b.w./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 b.w./day Aroclor 1248.
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 b.w./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 b.w./day (the lowest dose level in the study).
One of four male monkeys that were fed 0.1 mg/kg b.w./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.

Male weanling rats that were treated with 25 mg/kg b.w./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 b.w./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.
Fertility was markedly reduced in male offspring of rats that were lactationally exposed to 8 mg/kg b.w./day Aroclor 1254 whereas fertility was not impaired in the male offspring of rats that were administered 30 mg/kg b.w./day of Aroclor 1221, 1242, or 1260 by gavage during gestation days 12 to 20.

There were no significant effects on number of implantation sites, litter size, or offspring sex ratio in rats that were exposed to 4 mg/kg b.w./day of a PCB congener mixture simulating the congener content of human milk from 50 days prior to mating until birth.

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; four of the congeners included in sPCB10 were tested (28, 105, 118, 153). Mild changes were observed in the ovaries of female rats exposed to PCB 126 at about 0.009 mg/kg b.w./day, but not at about 0.0008 mg/kg b.w./day; no effects were observed in male reproductive tissues at about 0.0007 mg/kg b.w./day. No effects in reproductive tissues were found in males and females following exposure to PCB 28 at about 4 mg/kg b.w./day, PCB 77 at about 0.8 mg/kg b.w./day, PCB 105 at about 4 mg/kg b.w./day, PCB 118 at about 0.7 or about 0.2 mg/kg b.w./day (males and females, respectively), PCB 128 at about 4 mg/kg b.w./day, or PCB 153 at about 4 mg/kg b.w./day.

2.6.6.2 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.

Reduced birth weight was observed in offspring from Rhesus monkeys treated with 0.03 mg/kg b.w./day Arochlor 1016 in the diet for a total of 12 months (before mating and during gestation) but not at 0.007 mg/kg b.w./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.
In offspring from female Rhesus monkeys fed a diet that provided 0.1 or 0.2 mg/kg b.w./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.
Neurobehavioural alterations have been observed in monkeys born to mothers fed a diet providing approximately 0.1 mg/kg b.w./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 b.w./day for 18 months and allowed to breed 32 months post-exposure.
Rhesus monkeys were fed encapsulated Aroclor 1254 at dose levels of 0.005, 0.02, 0.04 or 0.08 mg/kg b.w./day for 37 months before breeding and subsequently throughout mating and gestation until the breeding phase of the study (29 months) was completed. A significantly increasing dose-related trend for foetal mortality incidence rates (combined foetal and postpartum deaths) was observed; a significant increased rate was noted for only the highest dose group (0.08 mg/kg b.w./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 mg/kg b.w./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 b.w./day (decreased IgM titers).

Neurobehavioural alterations were reported in the offspring of rats treated with 2.4 mg/kg b.w./day Clophen A30 premating and during gestation, and in offspring from rats fed approximately 1 mg/kg b.w./day Aroclor 1254 during gestation and lactation, but not following treatment with 0.4 mg/kg b.w./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.

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 b.w./day during gestation and lactation, and lesions in thyroid were observed from 2.5 mg/kg b.w./day.

In rats that were exposed to 4 mg/kg b.w./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.

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 b.w./day), no significant difference between the control and treated groups for body weight gain was observed throughout the study.

No effects on birth weight or pup weight at weaning was observed following administration of 0.001 mg/kg b.w./day PCB 126 or 8 mg/kg b.w./day PCB 77 to pregnant rats on gestation days 10-16, or following administration of 0.001 mg/kg b.w./day PCB 126 beginning 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, born to pregnant rats administered 0.00025 or 0.001 mg/kg b.w./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.

2.6.7 Evaluation

Repeated oral exposure to PCBs is associated with effects in the liver, stomach, thyroid, adrenal glands, skin and eyes, the haematopoietic, immune, and nervous systems, and carcinogenic, reproductive and developmental effects.

Information on effects in humans due to oral intake of PCBs is available from studies of people in the Yusho and the Yu-Cheng incidents exposed by consumption of contaminated rice oil, and of people exposed by consumption of contaminated fish and other food products of animal origin. Unlike commercial PCB mixtures, the Yusho and Yu-Cheng Kanechlors were heated resulting in the production of relatively high concentrations of PCDFs, and PCDFs are generally considered to be the main causal agents of the adverse health effects observed among the patients. Contaminated fish often contain other persistent organochlorine compounds, which also have the potential to induce similar effects. Consequently, the effects observed in the people exposed to PCBs in contaminated fish or in heated rice oil cannot be attributed solely to PCBs and therefore, the human data cannot be used in the risk characterisation.

The 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 the most sensitive animal species 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 indicates, 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 (0.0075 mg/kg b.w./day).
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) including four of the congeners included in sPCB10 (28, 105, 118, 153); of these individual congeners in sPCB10, PCB 105 (a dioxin-like congener) appears to be the most toxic of these four congeners.

The mechanisms of toxicity are not completely understood, but there is evidence to suggest that PCB congeners differ qualitatively and quantitatively in biological activities and that multiple and diverse mechanisms are involved in responses to PCB mixtures.
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 congeners included in the sPCB10 based on the results for PCB 105 in studies with rats unless otherwise stated.

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 b.w./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 b.w./day for 24 months and in Rhesus monkeys after 12-28 months of dietary exposure to 0.2 mg/kg b.w./day of Aroclor 1254. In one study of monkeys, increased liver weight and biochemical changes were observed following 0.08 mg/kg b.w./day of Aroclor 1254 for 72 months with no effects at dose levels of up to 0.04 mg/kg b.w./day, whereas, in another study, biochemical changes were observed from 0.005 mg/kg b.w./day for 37 months. For individual congeners tested in the 13-week oral toxicity studies in rats, PCB 105 was the next most toxic congener with a LOAEL of 0.039 mg/kg b.w./day for liver effects.
Overall, a LOAEL of 0.08 mg/kg b.w./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 b.w./day is considered for effects in the liver. A LOAEL of 0.04 mg/kg b.w./day is considered for liver effects of individual PCB10 congeners.

Effects in stomach tissue have been observed in monkeys administered 0.2 mg/kg b.w./day Aroclor 1254 in the diet for up to 28 months, and by dietary exposure to Aroclor 1248 from 1.3 mg/kg b.w./day or to Aroclor 1242 from 0.12 mg/kg b.w./day for 2 months. No effects were observed in monkeys administered Aroclor 1254 at 0.08 mg/kg b.w./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 b.w./day (PCB 105) for 13 weeks.
Overall, a NOAEL of 0.08 mg/kg b.w./day is considered for effects in stomach tissue of commercial PCB mixtures, and a NOAEL of 4 mg/kg b.w./day for individual PCB10 congeners.

Various effects in the thyroid gland and in the 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 b.w./day for 5 months, and histological alterations at 0.25 mg/kg b.w./day for 5 weeks, but not at 0.025 mg/kg b.w./day. In monkeys, no effects in thyroid tissue and in serum hormone levels were observed following exposure to Aroclor 1254 up to 0.08 mg/kg b.w./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 b.w./day for 28 months. For individual congeners tested in the 13-week oral toxicity studies in rats, PCB 105 was the next

most toxic congener with a LOAEL of 0.039 mg/kg b.w./day for effects on the thyroid.
Overall, a NOAEL of 0.08 mg/kg b.w./day is considered for effects in the thyroid of commercial PCB mixtures, and a LOAEL of 0.04 mg/kg b.w./day for individual PCB10 congeners.

Other effects of PCBs on the endocrine function observed in experimental animals include effects in the adrenal glands and in serum adrenal steroid levels. In rats, alterations in hormone levels have been observed following dietary administration of Aroclor 1254 at 0.1 mg/kg b.w./day for 15 weeks, but not at 0.05 mg/kg b.w./day, for 5 months; no histological alterations have been observed up to 25 mg/kg b.w./day for 15 weeks. In monkeys, no effects in the adrenal tissue have been observed following dietary administration of Aroclor 1254 up to 0.2 mg/kg b.w./day for 12 months, or up to 0.08 mg/kg b.w./day for 72 months; and no effects in serum hormone levels up to 0.08 mg/kg b.w./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 b.w./day is considered for effects of commercial PCB mixtures, and a NOAEL of about 4 mg/kg b.w./day for individual PCB10 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 b.w./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 b.w./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 b.w./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 b.w./day is considered for dermal and ocular effects of commercial PCB mixtures, and a NOAEL of about 4 mg/kg b.w./day for individual PCB10 congeners.

Anaemia has been observed in monkeys treated with Aroclor 1248 or 1254 at dose levels from 4 mg/kg b.w./day for 2 months, from 0.2 mg/kg b.w./day for 12-28 months, and at 0.08 mg/kg b.w./day of Aroclor 1254 for 37 months; however, in another study, no effects in haematological parameters were observed in monkeys receiving Aroclor 1254 from 0.08 mg/kg b.w./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 b.w./day.
Overall, a NOAEL of 0.08 mg/kg b.w./day is considered for haematological effects of commercial PCB mixtures, and a LOAEL of about 4 mg/kg b.w./day for individual PCB10 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 b.w./day (the lowest dose level tested) Aroclor 1254. Other effects in 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 b.w./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 b.w./day.
Overall, a LOAEL of 0.005 mg/kg b.w./day is considered for immunotoxic effects of commercial PCB mixtures, and a LOAEL of about 4 mg/kg b.w./day for individual PCB10 congeners.

Neurobehavioural alterations have been observed in rats and monkeys following pre- and/or post-natal exposure. It appears that *ortho*-substituted congeners (i.e., non-dioxin-like PCBs) are more active than coplanar PCBs in modifying cognitive processes. 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 b.w./day, the lowest dose level tested in intermediate-duration studies of any PCB mixture in any species. 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 b.w./day in the diet for 20 weeks. 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 b.w./day, for PCB 118 at 0.2 mg/kg b.w./day, and for PCB 153 at 0.01 mg/kg b.w./day.
Overall, a LOAEL of 0.0075 mg/kg b.w./day is considered for neurotoxic effects following post-natal exposure to PCB mixtures based on the study in monkeys with a defined PCB mixture, and a LOAEL of 0.01 mg/kg b.w./day for individual PCB10 congeners based on the 13-week oral toxicity study in rats with PCB 153.

A number of oral cancer 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 have 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). 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 in 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).
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 damage to the genes. 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 b.w./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.
Overall, a carcinogenic potential of PCB cannot be excluded for humans in relation to dietary exposure to PCB, but is not considered to be significant at dose levels not producing liver toxicity.

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. There is mounting evidence that peri-natal exposure to PCBs have induced adverse developmental effects in humans, specifically, but not limited to, neurobehavioural alterations in newborn and children exposed during gestation and/or via breast milk. Other effects observed include lower birth weight and reduced growth during early life; however, according to ATSDR (2000), no firm conclusions can be made regarding growth and development of children and exposure to PCBs. It must be kept in mind, however, that there is a possibility that other lipophilic compounds, such as PCDDs and PCDFs may have contributed to the observed effects.

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 b.w./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 b.w./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 b.w./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 b.w./day.
Overall, a NOAEL of 0.005 mg/kg b.w./day is considered for reproductive effects of commercial PCB mixtures, and a NOAEL of about 4 mg/kg b.w./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 b.w./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 administered Aroclor 1248 at dietary dose levels from 0.1 mg/kg b.w./day Aroclor 1248 for 15 months. Neurobehavioural alterations have been reported in the offspring of rats fed about 1 mg/kg b.w./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 b.w./day during gestation and lactation.
Reduced birth weight and postnatal weight gain were observed in pups of rats exposed to 4 mg/kg b.w./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 b.w./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 b.w./day is considered for developmental effects of commercial PCB mixtures, and a NOAEL of 0.001 mg/kg b.w./day for individual PCB10 congeners based on studies with PCB 126 (a congener which is not included in PCB10).

2.6.8 Critical effect(s) and NOAEL / LOAEL

Effects observed following dietary intake of PCBs include effects in the liver, stomach, thyroid, adrenals, and skin and eyes; in the haematological, immune and nervous systems; carcinogenicity, and reproductive and developmental effects. The N/LOAELs considered for commercial mixtures are based on the results for Aroclor 1254 in studies with monkeys unless otherwise stated, and for individual congeners included in the sPCB10 based on the results for PCB 105 in studies with rats unless otherwise stated.

For commercial PCB mixtures, a LOAEL of 0.08 mg/kg b.w./day is considered for liver effects based on increased liver weight; 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 b.w./day is a LOEL for effects in the liver. A LOAEL of 0.005 mg/kg b.w./day is considered for dermal, ocular, immunotoxic, and developmental effects.
A NOAEL of 0.08 mg/kg b.w./day is considered for effects in stomach tissue, the thyroid, the adrenal glands, and the haematological system, and a NOAEL of 0.005 mg/kg b.w./day is considered for reproductive effects.

A LOAEL of 0.0075 mg/kg b.w./day is considered for neurotoxic effects following postnatal exposure to a defined PCB mixture analogous to the congener composition found in human milk based on the study in monkeys. This LOAEL is, according to ATSDR (2000), the lowest dose level tested in intermediate-duration studies of any PCB mixture in any species.

For individual congeners included in the sPCB10, a LOAEL of 0.04 mg/kg b.w./day is considered for effects in the liver and the thyroid, of 0.01 mg/kg b.w./day for neurotoxic effects, and of 4 mg/kg b.w./day for haematological and immunotoxic effects based on the data for PCB 105. A NOAEL of 4 mg/kg b.w./day is considered for effects in stomach tissue, adrenal glands, and skin and eyes, and for reproductive effects based on the data for PCB 105. A NOAEL of

0.001 mg/kg b.w./day is considered for developmental effects of PCB 126 (a congener which is not included in PCB10); no data for developmental effects of PCB 105 have been located.

Overall, a LOAEL of 0.005 mg/kg b.w./day is considered for adverse health effects of commercial PCB mixtures, a LOAEL of 0.0075 mg/kg b.w./day for a defined PCB mixture analogous to the congener composition found in human milk, and a LOAEL of 0.01 mg/kg b.w./day for individual PCB10 congeners. These LOAELs are taken forward to the risk characterisation.

A carcinogenic potential of PCBs for humans in relation to dietary exposure cannot be excluded, but is not considered to be significant at dose levels not producing liver toxicity, i.e., at the LOAEL of 0.005 mg/kg b.w./day considered for adverse health effects of commercial PCB mixtures.

2.6.9 References

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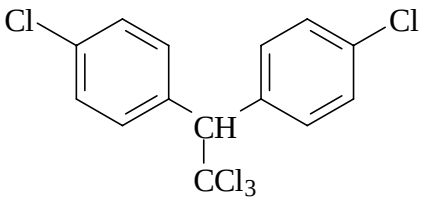
2.7 DDT

DDT is a synthetic organochlorine compound. The term DDT generally refers to 1,1´-(2,2,2-trichloroethylidene)bis[4-chlorobenzene] (*p,p´*-DDT) or dichloro-diphenyl-trichloroethane with the structural formula (Beltoft et al. 2000):



Several different isomeric forms exist and the term DDT is also applied to commercial products consisting predominantly of *p,p´*-DDT with smaller amounts of other compounds mainly *o,p´*-DDT and the main metabolites DDE (dichloro-diphenyl-dichloroethylene) and DDD (dichloro diphenyl dichloroethane). (Beltoft et al. 2000).

This evaluation considers the compound *p,p´*-DDT and technical DDT as well as the two isomeric forms of the metabolite DDE: *o,p*-DDE and *p,p*-DDE.

DDT has been used world-wide in agriculture for the control of insects. In the early 1970's, the use of DDT was banned in several countries; in Denmark for agricultural purposes in 1969 and in 1984 for all purposes. (Beltoft et al. 2000, ATSDR 1994).

The general population is exposed to DDT and its metabolites mainly through the diet and it has been estimated that over 90% of the DDT stored in the general population is derived from the food. However, the residues in food have decreased significantly since the ban on most uses of DDT. In foodstuff, particularly the DDT-metabolite DDE is detected. (Beltoft et al. 2000, WHO 1996).

In Denmark, the estimated average dietary intake for adults (1988-1992) was less than 2 µg/day with the 95 percentile being 5.3 µg/day (LST 1995). In the period from 1993 to 1997, the average daily intake for an adult person was estimated at 0.5 µg/day and the 95 percentile at 1.0 µg/day (FDIR 2000).

2.7.1 Toxicokinetics

DDT is absorbed following ingestion. Absorption of the relatively small amounts in food is virtually complete and is facilitated by the presence of fat in the food. (Beltoft et al. 2000, WHO 1996).

Once absorbed, DDT, DDE, and DDD are readily distributed to all body tissues. DDT storage in organs and other tissues following repeated intake is proportional to their neutral fat content. Storage in adipose tissue increases rapidly at first and then more gradually until a steady state is reached. In humans, the time necessary to reach storage equilibrium is at least one year. There is a gradual reduction in the amount of DDT stored in the tissues if exposure to the compound is discontinued and it has been reported that the mean concentrations of DDT in the population

have declined in much of the world over the last three decades. Because DDE is more persistent than DDT, DDE is usually found at higher levels than DDT in tissues. (Beltoft et al. 2000, WHO 1996, ATSDR 1994, JMPR 2000).

DDT is initially metabolised in the liver to DDD and DDE, which are further metabolised to DDA (dichloro-diphenyl-acetic acid) by different metabolic pathways. DDT metabolites are excreted in conjugated form in the urine. Some excretion of DDT also occurs in the faeces, which may be a major route of excretion following exposure to high doses. The metabolism of DDT in humans is apparently similar to that in experimental animals including the rat. (Beltoft et al. 2000, ATSDR 1994).

DDT metabolites are also excreted in human milk. In a Danish investigation from 1993-94, the median concentration of *p,p´*-DDE was reported to be 178 ng/g milk fat (76-647 ng/g milk fat). Comparing this investigation with an earlier one (1982) showed a pronounced reduction of the concentration of *p,p'*-DDE of approximately 80%. (SST/FDIR 1999).

The half-lives of both DDT and the metabolites DDD and DDE are very long and can be ranked as follows: DDE>DDT>DDD. The half-life of DDE is more than three years in human adipose tissue. (Beltoft et al. 2000, ATSDR 1994).

2.7.2 Single dose toxicity

The nervous system appears to be the primary target system for DDT toxicity in humans after acute, high doses and both central and peripheral parts are affected. Signs and symptoms reported following oral intake (accidental or intentional) include prickling sensation of the tongue and around the mouth and nose, paraesthesia of the extremities, nausea, vomiting, dizziness, ataxia, confusion, headache, restlessness, tremor, and, in severe cases convulsions. Sweating, headache, and nausea have been observed following a dose of 6 mg DDT/kg b.w., vomiting (reported to be of central origin) at doses of 10 mg DDT/kg b.w., and convulsions at doses from around 16 mg DDT/kg b.w. A dose as high as 285 mg DDT/kg b.w. has been ingested accidentally with no fatal results. Volunteers have achieved almost complete recovery within 24 hours after exposure to DDT at oral doses from around 3.5 to 22 mg/kg b.w. (Beltoft et al. 2000, ATSDR 1994, WHO 1979, JMPR 2000).

In experimental animals, the nervous system and the liver also appear to be the primary targets following acute administration of DDT. DDT is moderately acutely toxic to experimental animals following oral administration with LD_{50}-values of 113-450 mg/kg b.w. reported for rats, 100-800 mg/kg b.w. for mice, 250-560 mg/kg b.w. for guinea pigs, 300-1770 mg/kg b.w. for rabbits, and >300 mg/kg b.w. for dogs when DDT was given in oil solution. DDT is less toxic when given as a water suspension or powder. (Beltoft et al. 2000, WHO 1979, ATSDR 1994).

2.7.3 Repeated dose toxicity

No adverse effects, including neurological effects and effects on the liver function, were observed in volunteers given DDT at dose levels of 0.05 or 0.5 mg/kg b.w./day for 12 to 18 months; in workers who had daily intakes of up to 0.25 mg/kg b.w./day for 11 to 19 years; or in workers exposed at 0.2 to 0.6 mg/kg b.w./day for 0.4 to 6.5 years. (Beltoft et al. 2000, ATSDR 1994, WHO 1979, JMPR 2000).

In experimental animals, the nervous system and the liver appear to be the primary targets following subchronic and chronic administration of DDT.

The hepatic effects of DDT in rats include increased liver weight, hypertrophy, hyperplasia, induction of microsomal enzymes, cellular necrosis, increased activity of serum liver enzymes, and mitogenic effects, which might be related to a regenerative liver response to DDT. DDT and DDE are well known inducers of the hepatic cytochrome P450 mixed function oxidase system. In this respect, DDT/DDE resemble phenobarbital by inducing activities related to isozymes of the CYP2B and CYP3A subfamilies in rodents. Induction of these enzyme activities may result in interference with the biotransformation of steroid sex hormones. The effects on CYP2B and associated enzymes in the livers of rats indicated that males have a lower threshold than females, which induced these enzymes to a greater extent. (Beltoft et al. 2000, JMPR 2000, Nims et al. 1998).
No hepatic effects were reported when DDT was given in doses up to 32 mg/kg b.w./day for 78 weeks to rats or up to 8 mg/kg b.w./day for 3.5 -7.5 years to rhesus monkeys. Other studies in rats have reported a NOAEL of 0.05 mg/kg b.w./day for liver effects when commercial DDT was given in the diet for 15-27 weeks or a LOAEL of 0.5 mg/kg b.w./day when administered in the diet for 2 years. In rats, centrilobular necrosis was observed when DDE was administered at 12 mg/kg b.w./day for 78 weeks. (Beltoft et al. 2000, ATSDR 1994, IRIS 2004, JMPR 2000).

Effects on the central nervous system, such as hyperactivity and tremors, have been reported in chronic studies in rats at doses from 11 mg/kg b.w./day, in mice from 6.5 mg/kg b.w./day, and in hamsters fed 41.5 mg/kg b.w./day. One study has reported no clinical signs of neurotoxicity in hamsters fed up to 40 mg/kg b.w./day for life. (Beltoft et al. 2000, ATSDR 1994).

2.7.4 Genotoxicity

In most studies, DDT did not induce genotoxic effects in rodent or human cell systems, nor was it mutagenic to fungi or bacteria. Conflicting results have been obtained in tests for chromosomal aberration in cultured rodent and human cells, in cell transformation assays, and in dominant lethal tests in mice and rats. *p,p´*-DDE weakly induced chromosomal aberrations in cultured rodent cells and mutations in mammalian cells and insects, but not in bacteria. (Beltoft et al. 2000, IARC 1991, JMPR 2000).

2.7.5 Carcinogenicity

Epidemiological studies on the association between exposure to DDT and cancer risk have been reviewed extensively. These studies have revealed small and inconsistent excesses on cancer risks associated with exposure to DDT; however, most of the studies have limitations (e.g., in the assessments of exposure, confounding factors), which complicate an evaluation. (Beltoft et al. 2000, JMPR 2000).

Oral administration of DDT to rats and mice has resulted in increased incidences of liver tumours. When DDT was administered orally to hamsters at concentrations similar to or higher than those found to cause liver tumours in mice and rats, some increase in the incidence of adrenocortical adenomas was observed. DDE also produced liver tumours in mice and in hamsters. (Beltoft et al. 2000).

JMPR (2000) has established an overall NOAEL for carcinogenicity in rats of 6.25 mg/kg b.w./day.

2.7.6 Toxicity to reproduction

Reduced sperm counts have been observed in aviation crop dusters handling DDT (one study). No correlation between exposure to DDT and stillbirths, miscarriage, or premature rupture of foetal membranes has been observed in the few studies available. Exposure to DDT transplacentally or during breast-feeding did not affect phychomotor or mental behavioural patterns or measures of school performance in English and mathematics in 859 children in USA who were tested at the age of 3, 4, or 5 years. (Beltoft et al. 2000, JMPR 2000).

DDT has been reported to impair reproduction and/or development in mice, rats, rabbits, dogs, and avian species. Acute oral exposure (100-500 mg/kg b.w./day) has been associated with decreased male fertility. Longer term exposure of male and female rodents to lower doses (0.35-39 mg/kg b.w./day) has resulted in decreased fertility, stillbirths, and increased foetal mortality. One study has reported a 75% depression of fertility in rats fed diets providing an intake of 0.35 mg DDT/kg b.w./day for 60 days before mating; the F1 pups from these dams were completely infertile when mated. (Beltoft et al. 2000, ATSDR 1994).

Exposure of the developing foetus may result in reproductive effects later in life. Embryotoxicity and foetotoxicity have been reported in animals in the absence of maternal toxicity. Embryolethality, decreased foetal growth, and prematurity have been observed in rabbits and dogs following administration of diets providing a dose of 5 mg/kg b.w./day. In rodents, decreased ovarian weights, cystic ovaries, loss of corpora lutea, infertility, premature puberty, altered onset of vaginal opening, tail anomalies, and increased pup mortality have been observed and a NOAEL of 1 mg/kg b.w./day has been reported for rats. Exposure during gestation and in the neonatal period may cause developmental neurotoxicity: a single oral dose of DDT (0.5 mg/kg b.w.) given to 10-day old mice was reported to affect the cholinergic system in the neonatal brain and lead to permanent changes in the cholinergic system in animals reaching an adult age of 4 months and was further reported to lead to permanent functional disturbances in the adult animals. The available data do not indicate that DDT is a teratogen. (Beltoft et al. 2000, ATSDR 1994, JMPR 2000).

DDT is weakly oestrogenic (feminising) and the isomer *o,p'*-DDT has been identified as the biologically active isomer. The DDT metabolite *p,p'*-DDE specifically binds to the androgen receptor and inhibits testosterone-induced transcriptional activity *in vitro*. In rat-studies, DDE had a potent anti-androgenic (demasculinising) effect. (Beltoft et al. 2000, JMPR 2000).

2.7.7 Evaluation

Repeated oral exposure to DDT and its metabolites is predominantly associated with effects in the nervous system, the liver, and the reproductive system.

No adverse effects, including neurological effects and effects in the liver function, were observed in volunteers given DDT at levels of 0.05 or 0.5 mg/kg b.w./day for 12 to 18 months; in workers who had daily exposures of up to 0.25 mg/kg b.w./day for 11 to 19 years; or in workers exposed at 0.2 to 0.6 mg/kg b.w./day for 0.4 to 6.5 years. Thus, the human studies indicate a NOAEL for effects in the nervous system

and liver of approximately 0.2 to 0.6 mg/kg b.w./day. Effects in the central nervous system have been reported in chronic studies in rats at doses from 11 mg/kg b.w./day, and in mice from 6.5 mg/kg b.w./day. In rats, a NOAEL of 0.05 mg/kg b.w./day has been identified for minimal histological effects observed in the liver of rats at the next dose level (0.25 mg/kg b.w./day). Overall, a NOAEL of 0.05 mg/kg b.w./day is considered for the effects in the nervous system and the liver.

The histological changes in the rodent liver are thought to precede the liver carcinogenicity of DDT observed in mice and rats following prolonged administration of higher doses. Several studies have shown that DDT is a promoter of liver tumours in rodents. DDT does not bind covalently to DNA and in most studies, DDT did not induce genotoxic effects in rodent or human cell systems, nor was it mutagenic to fungi or bacteria. Although genotoxic effects have been reported in tests for chromosomal aberration in cultured rodent and human cells, in cell transformation assays, and in dominant lethal tests in mice and rats, the weight of evidence points to a dominating non-genotoxic mechanism for the liver carcinogenicity of DDT in rodents. The available epidemiological data have revealed small and inconsistent excesses on cancer risks associated with exposure to DDT, however, the data are considered to be inadequate for the evaluation of the carcinogenicity of DDT in humans. DDT is considered by IARC (1991) as possibly carcinogenic to humans (Group 2B; evidence in humans inadequate, evidence to animals sufficient). US-EPA (IRIS 2004) has considered DDT as a probable human carcinogen (Group 2B; evidence in humans inadequate, evidence to animals sufficient). In the EU, DDT is classified for carcinogenic effects in category 3 (Carc. Cat. 3, R40 – limited evidence of a carcinogenic effect) (EEC 2004), implicating that the carcinogenic potential is independent on the exposure route. Overall, the NOAEL of 0.05 mg/kg b.w./day for minimal histological effects on the liver is considered to be protective for liver carcinogenicity as well.

The available data do not indicate consistent effects of DDT exposure on human reproductive performance. DDT has been reported to impair reproductive performance and development in mice, rats, dogs, and avian species. One study has reported a 75% depression of fertility in rats fed diets providing an intake of 0.35 mg/kg b.w./day; however, other studies including multigeneration long-term carcinogenicity studies using higher dose levels have not reported such dramatic effects. Embryotoxicity and foetotoxicity have been reported in animals in the absence of maternal toxicity; a NOAEL of 1 mg/kg b.w./day has been reported for developmental effects in rats. Exposure during gestation and in the neonatal period may cause developmental neurotoxicity; in mice, a single oral dose of 0.5 mg/kg b.w. has been reported to affect the cholinergic system in the neonatal brain leading to permanent changes. The available data do not indicate that DDT is a teratogen. Technical DDT is weakly oestrogenic and the isomer *o,p'*-DDT has been identified as the biologically active component. However, the major DDT metabolite *p,p'*-DDE is anti-androgenic with a dose of 10 mg/kg b.w./day having a marginal effect. Overall, the NOAEL of 0.05 mg/kg b.w./day for minimal histological effects in the liver is considered to provide adequate protection against the reproductive and developmental effects of DDT and DDE.

2.7.8 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of DDT and its metabolites are the effects observed in the nervous system, the liver, and the reproductive system.

A NOAEL of 0.05 mg/kg b.w./day has been identified for minimal histological effects in the liver of rats at the next dose level (0.25 mg/kg b.w./day). This

NOAEL is considered to be sensitive and to provide adequate protection against liver toxicity and carcinogenicity, neurotoxicity, and reproductive and developmental effects of DDT and DDE, and is taken forward to the risk characterisation.

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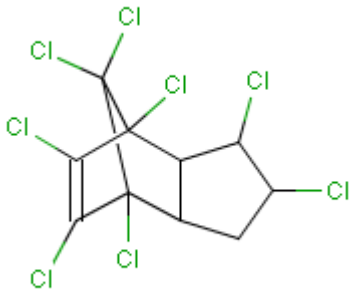
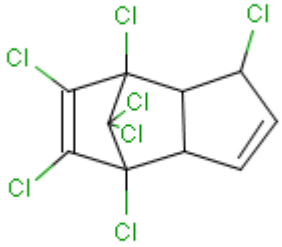
2.8 Chlordane

Chlordane is the common name for 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene (see the structural formula) and is a mixture of isomers, mainly *cis*- and *trans*-chlordane. Technical chlordane is a mixture of more than 140 related substances and consists of 60-85% of *cis*- and *trans*-chlordane; other major constituents are chlordene, heptachlor, and *cis*- and *trans*-nonachlor. (WHO 1996a, US-EPA 1997, ATSDR 1994).



Chlordane



Heptachlor

Chlordane levels in traditional Greenland food items have been presented as sCHL (sum of heptachlor, heptachlor epoxide, oxychlordane, cis- and trans-chlordane, and cis- and trans-nonachlor) (Johansen et al. 2004). Therefore, this evaluation will focus on these substances.

Oxychlordane is the epoxide of chlordane.
Heptachlor is the common name for 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene; heptachlor epoxide is the “2,3-epoxy” derivative of heptachlor (WHO 1996b, ATSDR 1993).
Nonachlor is the common name for 1,2,3,4,5,6,7,8,8-nonachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene.

Chlordane and heptachlor are organochlorine insecticides and have been used since the 1950s for termite control, on agricultural crops, on lawns, on livestock, and for other purposes. Their use has currently been banned or severely restricted in many countries, but human exposure is still possible due to their persistence in the environment. (IARC 2001).

The general population is exposed to chlordane and heptachlor mainly through the diet and chlordane and heptachlor has mainly been detected in milk and dairy products, meat products, fish, poultry, and vegetables (ATSDR 1994, 1993). The results for the 1991 US-FDA total diet study indicated a dietary intake of chlordane of 0.0013 µg/kg b.w./day for infants and of 0.0005-0.0015 µg/kg b.w./day for teenagers and adults (ATSDR 1994). For heptachlor, the 1988 US-FDA total diet study indicated a dietary intake of 0.004 µg/kg b.w./day for infants and 0.0007-0.017 µg/kg b.w./day for teenagers and adults (ATSDR 1993).

In Denmark, the estimated average dietary intake of heptachlor epoxide for adults (1993-1997) was 0.2 µg/day and the 95 percentile was 0.4 µg/day (FDIR 2000). No data are available regarding the other substances included in this evaluation.

2.8.1 Toxicokinetics

2.8.1.1 Chlordane

Chlordane appears to be absorbed readily from the gastrointestinal tract in experimental animals following oral administration. It has been estimated that about 80% of an orally administered dose is absorbed in rats and about 30-50% in rabbits. Absorption in the mouse is slower than in the rat. No human data are available on absorption; however, case reports on toxicity indicate that chlordane is absorbed following ingestion. (ATSDR 1994, US-EPA 1997, WHO 1996a, WHO 1984a).

Following absorption, chlordane is initially distributed to the liver and kidney and subsequently, chlordane and its metabolites (oxychlordane, trans-nonachlor, heptachlor epoxide) are redistributed to body fat, where they persist for a long time. Oxychlordane appears to be the most persistent chlordane residue in both human and animal tissues. The pattern of tissue distribution in experimental animals does not appear to depend on the dose level or whether exposure is to single or to multiple doses, whereas the accumulation in fat appears to depend on exposure duration. (ATSDR 1994, US-EPA 1997, WHO 1996a, WHO 1984a).

Chlordane is probably metabolised via four different routes to a number of different metabolites. One proposed route is via hydroxylation resulting in the formation of e.g., the epoxide of chlordane, oxychlordane. Another is via dehydrochlorination to form heptachlor. A third one is via dehalogenation and the fourth one involves hydrolytic removal of a chlorine atom. In rats, 70-90% of a single oral dose was excreted in the faeces and 2-8% in the urine; longer-term administration in the diet did not change the excretion pattern significantly from that observed following a single oral dose. Biliary excretion is significant in rats and is the source of faecal excretion in this species. Rabbits tend to excrete larger percentages of the administered dose in the urine (28-47%) with faecal excretion accounting for 22-48%. Lactation is a route of transfer of chlordane in females and chlordane is present in human milk mainly as the metabolite, oxychlordane. (ATSDR 1994, US-EPA 1997, WHO 1996a, WHO 1984a).

2.8.1.2 Heptachlor

Heptachlor and heptachlor epoxide are absorbed from the gastrointestinal tract of rats after oral administration. One study indicates that up to about 70% of an oral dose of heptachlor is absorbed in rats. Elevated serum levels of heptachlor metabolites in humans indicate absorption following ingestion. (ATSDR 1993, JMPR 1991, WHO 1996b, WHO 1984b).

Following absorption, heptachlor and heptachlor epoxide is distributed throughout the body of rats and dogs. A high concentration of heptachlor epoxide, but no heptachlor, has been detected in the body fat of rats and dogs; accumulation of heptachlor epoxide was directly related to the administered dose of heptachlor. Much lower amounts were found in liver, kidney, and muscle; and none was found in the brain. Heptachlor epoxide appears to be the most persistent heptachlor residue in both human and animal tissues. (ATSDR 1993, JMPR 1991, WHO 1996b, WHO 1984b).

Animal studies have shown that heptachlor is metabolised to heptachlor epoxide, which is further metabolised and excreted. In rats, about 62% of a single oral dose

was excreted in the faeces and about 6% in the urine; about 72% of the total amount in faeces was in the form of metabolites and about 26% as the parent compound. Biliary excretion is significant in rats and is the source of faecal excretion in this species. An *in vitro* study with human and rat liver microsomes showed the same metabolites, but in different proportions. Heptachlor and heptachlor epoxide have been detected in human milk. (ATSDR 1993, JMPR 1991, WHO 1996b, WHO 1984b).

2.8.1.3 Nonachlor

No data have been located regarding absorption, distribution, and elimination of nonachlor.

2.8.2 Single dose toxicity

2.8.2.1 Chlordane

Most data on the health effects of acute exposure to chlordane come from case reports of accidental or intentional ingestion. The data indicate that the central nervous system and liver are the target organs in humans by the oral route. The estimated acute oral lethal dose for chlordane is between 25 and 50 mg/kg b.w. (ATSDR 1994, WHO 1984a).

Chlordane is of moderate acute toxicity in experimental animals with reported oral LD_{50}-values in the rat ranging from 83 to 290 mg/kg b.w. Toxic symptoms include ataxia, convulsions, respiratory failure, and cyanosis. For technical chlordane, the reported oral LD_{50}-values in the rat range from 173 to 590 mg/kg b.w. Most of the chlordane metabolites are slightly to moderately toxic, with the exception of oxychlordane, which is highly toxic with an oral LD_{50}-value in the rat of 19 mg/kg b.w. (ATSDR 1994, WHO 1996a, WHO 1984a).

2.8.2.2 Heptachlor

No data have been located regarding acute oral toxicity of heptachlor in humans.

Acute oral LD_{50}-values for heptachlor in rodents range from 40 to 162 mg/kg b.w. and for heptachlor epoxide from 39-144 mg/kg b.w. Toxic symptoms include hypoactivity, tremor, convulsions, ataxia, and respiratory failure; histologically, severe liver damage has been reported. The oral LD_{50}-values for other metabolites of heptachlor were reported to be grater than 4600 mg/kg b.w. in rats. (ATSDR 1993, JMPR 1991, WHO 1996b, WHO 1984b).

2.8.2.3 Nonachlor

No data have been located regarding acute oral toxicity of nonachlor.

2.8.3 Repeated dose toxicity

2.8.3.1 Chlordane

No data have been located regarding adverse health effects of chlordane in humans following repeated oral administration.

For chlordane, data from studies in experimental animals indicate that the liver and the central nervous system are the major target organs. Chlordane induces its own metabolism to toxic intermediates and the epoxide oxychlordane is the metabolite that is considered of primary toxicological significance. (ATSDR 1994, US-EPA 1997, IRIS 2004a, JMPR 1986).

Effects observed in the liver of rodents following oral administration of chlordane include increased liver microsomal enzyme activity, increased relative liver weight, liver cell inclusion bodies, liver cell hypertrophy, and liver cell necrosis. In one study with pure analytical grade chlordane (72% *cis* and 23% *trans* isomers), no effects were observed in the liver of rats at dietary dose levels of up to 20.4/12.1 mg/kg b.w./day for males/females, respectively, for 80 weeks; and no non-neoplastic effects in the liver of mice at dose levels of up to 8.0/9.1 mg/kg b.w./day for males/females, respectively, (for neoplastic lesions is referred to section 2.8.5). In a 30-month study with technical chlordane, hepatocellular hypertrophy was observed in female rats from 5 mg/kg in the diet (0.273 mg/kg b.w./day) but not in male rats at dose levels of up to 25 mg/kg in the diet (1.175 mg/kg b.w./day); the NOAEL for liver effects in this study was 1 mg/kg in the diet (0.055 mg/kg b.w./day) based on the findings in female rats. In mice administered technical chlordane in the diet for 24 months, histopathological alterations were observed in the liver of both sexes from 5 mg/kg in the diet (0.75 mg/kg b.w./day); the NOAEL in this study was 1 mg/kg in the diet (0.15 mg/kg b.w./day). (ATSDR 1994, US-EPA 1997, IRIS 2004a, JMPR 1986).

Central nervous system effects in form of tremors and convulsions, and inhibited brain ATPase activity has been observed in rodents. In a 12-week dietary study with rats administered technical chlordane, brain ATPase activity was reduced from 1.25 mg/kg b.w./day and convulsions at 5 mg/kg b.w./day. In a study with pure analytical grade chlordane (72% *cis* and 23% *trans* isomers), tremors were observed in female rats at dietary dose levels from 12.1 mg/kg b.w./day for 80 weeks but not at 6.0 mg/kg b.w./day, and in both sexes of mice at 8.0/9.1 mg/kg b.w./day for males/females, respectively, but not at 4.3 mg/kg b.w./day; tremors were not observed in male rats at dose levels of up to 20.4 mg/kg b.w./day, and no brain lesions were found in rats or mice of either sex. Neither central nervous system signs nor histopathological lesions of the nervous system were found following dietary administration of technical chlordane for 30 months in rats at dose levels of up to 25 mg/kg in the diet, or for 24 months in mice at dose levels of up to 12.5 mg/kg in the diet. (ATSDR 1994, US-EPA 1997, IRIS 2004a, JMPR 1986).

2.8.3.2 Heptachlor

In a study of 45 individuals exposed for an unspecified period of time to contaminated raw milk products from cattle fed heptachlor-contaminated feed, 23-31% were found to have significantly elevated serum levels of heptachlor metabolites. In a follow-up study of the same families approximately 18 months later, relatively high concentrations of heptachlor epoxide was found in the blood of 7 out of 39 subjects. No evidence of related hepatic effects was found in the exposed subjects. (ATSDR 1993, JMPR 1991).

Data from studies in experimental animals indicate that the liver is the major target organ following repeated oral administration of heptachlor or heptachlor epoxide.

Effects observed in the liver of rats, mice and dogs include increased liver microsomal enzyme activity, relative liver weight and lipid content; proliferation of the smooth endoplasmic reticulum and increased number of mitochondria; and histological changes. Heptachlor induces its own metabolism to heptachlor epoxide, which is the metabolite that is considered of primary toxicological significance. (ATSDR 1993, JMPR 1991).

In a 2-year feeding study in rats with heptachlor, lesions were observed in the liver from 7 mg/kg diet and increased relative liver weight from 5 mg/kg diet; the NOAEL was 3 mg/kg diet (corresponding to 0.15 mg/kg b.w./day) (IRIS 2004b, JMPR 1991); however, according to JMPR (1991), organ weights of the heptachlor-treated rats were not markedly different from those of controls.

Following dietary administration of heptachlor epoxide to rats for 2 years, increased relative liver weight was observed in females from 5 mg/kg diet (the lowest dose level) and in males from 10 mg/kg diet (WHO 1984b, WHO 1996b). In dogs given diets containing heptachlor epoxide for 60 weeks, increased relative liver weight was observed in both sexes from 0.5 mg/kg diet (the lowest dose level; (corresponding to 0.0125 mg/kg b.w./day according to IRIS) (IRIS 2004c; JMPR 1991). According to WHO (1996b, 1984b) citing the same study, liver weights were increased from 5 mg/kg diet and degenerative liver changes were seen in only one dog at 7.5 mg/kg diet (highest dose level in the study); the NOAEL was 2.5 mg/kg diet (equivalent to 0.06 mg/kg b.w./day). According to JMPR (1991), pathology data were not included in the report.
In a 2-year feeding study in dogs with heptachlor epoxide, increased incidences of histopathological changes in the liver were observed from 3 mg/kg diet and increased liver weight at 10 mg/kg diet; the NOAEL was 1 mg/kg diet (corresponding to 0.025 mg/kg b.w./day) (JMPR 1991, WHO 1996b).

In mice fed a mixture of heptachlor and heptachlor epoxide (25:75) for 30 days, histopathological alterations were observed from 5 mg/kg diet; the NOAEL was 1 mg/kg diet (corresponding to 0.15 mg/kg b.w./day). When the mixture was administered for 18 months, histopathological alterations were observed from 1 mg/kg diet (the lowest dose level). (JMPR 1991).

2.8.3.3 Nonachlor

No data have been located regarding adverse health effects of nonachlor following repeated oral administration.

2.8.4 Genotoxicity

2.8.4.1 Chlordane

Chlordane has been tested in several *in vitro* and *in vivo* genotoxicity studies. The majority of the studies have yielded negative results, but positive results have been reported as well. Chlordane gave negative results for the ability to induce gene mutations in bacteria and in yeast, and for unscheduled DNA synthesis in rodent hepatocytes, but induced unscheduled DNA synthesis in human fibroblasts. Chlordane with metabolic activation reportedly caused mitotic gene conversion in yeast and prophage induction in *E. coli*, sister chromatid exchange in human lymphoid cells, and gene mutations in mouse lymphoma cells. Chlordane inhibited gap-junctional intercellular communication in rodent cells. (IARC 2001, ATSDR 1994, IRIS 2004a, WHO 1996a).

Chlordane did not cause dominant lethal mutations in mice and did not induce sex-linked recessive mutations in *Drosophila melanogaster*, but induced DNA damage in liver cells of rats treated *in vivo*. Dermal application of chlordane to mice induced micronuclei formation in the bone marrow cells and nuclei aberrations in the hair follicles. (IARC 2001, ATSDR 1994, IRIS 2004a, WHO 1996a).

2.8.4.2 Heptachlor

Heptachlor and heptachlor epoxide have been tested in several *in vitro* studies. The majority of the studies have yielded negative results, but positive results have been reported as well. Both compounds gave negative results for the ability to induce gene mutations in bacteria and in yeast, and for unscheduled DNA synthesis in rodent hepatocytes, but induced unscheduled DNA synthesis in human fibroblasts. Heptachlor without metabolic activation reportedly caused gene mutations in mouse lymphoma cells but not in rat liver cells. Chromosome aberrations and sister chromatid exchange have been observed in Chinese hamster ovary cells following exposure to heptachlor with metabolic activation but not without. Heptachlor inhibited gap-junctional intercellular communication in rodent cells. (IARC 2001, JMPR 1991, ATSDR 1993, IRIS 2004b,c, WHO 1996b).

Heptachlor and heptachlor epoxide did not cause dominant lethal mutations in mice, and heptachlor did not induce mutations in hepatocytes of *lacI* transgenic mice treated *in vivo* and did not induce sex-linked recessive mutations in *Drosophila melanogaster* (IARC 2001, JMPR 1991, ATSDR 1993, IRIS 2004b,c, WHO 1996b).

2.8.4.3 Nonachlor

No data have been located regarding mutagenicity or genotoxicity of nonachlor.

2.8.5 Carcinogenicity

2.8.5.1 Chlordane

No data are available regarding the carcinogenicity of chlordane following ingestion by humans (ATSDR 1994).

An increased incidence of liver tumours has been observed in mice following administration of analytical grade chlordane in the diet at dose levels from 25 mg/kg diet (about 3.6 mg/kg b.w./day) for 18 months. A dose-related increased in the incidence of liver tumours has also been observed in mice given a mixture of analytical *cis* (72%) and *trans* (23%) isomers of chlordane in the diet (males: from about 4.3 mg/kg b.w./day; females: at about 9.1 mg/kg b.w./day) for 80 weeks. In a special strain of mice, which are historically resistant to spontaneous liver tumours, an increased incidence of liver tumours was observed in males administered 50 mg/kg diet for 18 months. Administration of technical chlordane at 12.5 mg/kg diet (about 1.9 mg/kg b.w./day) for 2 years resulted in an increased incidence of liver tumours in male mice but not in female mice.
No evidence of carcinogenicity of analytical grade chlordane (72% *cis* and 23% *trans* isomers) was provided in an 80-week dietary study in rats at dose levels of up to 20.4/12.1 mg/kg b.w./day for males/females, respectively, whereas a marginal

increase of liver adenomas has been observed in male rats given technical grade chlordane at 25 mg/kg diet (about 1.2 mg/kg b.w./day) for 130 weeks. (ATSDR 1994, US-EPA 1997, IRIS 2004a, IARC 2001, JMPR 1986, WHO 1996a, WHO 1984a).

Dietary administration of technical chlordane at 3.25 mg/kg b.w./day for 25 weeks promoted the development of liver tumours in male mice previously initiated with diethylnitrosamine in drinking water for 14 weeks. (ATSDR 1994, JMPR 1986, IARC 2001).

2.8.5.2 Heptachlor

No data are available regarding the carcinogenicity of heptachlor or heptachlor epoxide following ingestion by humans (ATSDR 1993).

An increased incidence of liver tumours has been observed in mice following dietary administration of technical-grade heptachlor (73% heptachlor, 18% *trans*-chlordane, 2% *cis*-chlordane) at about 14 and 18 mg/kg diet for male and females, respectively (about 1.8 mg/kg b.w./day for males and 2.3 mg/kg b.w./day for females) for 80 weeks, but not at about 6 and 9 mg/kg diet for male and females, respectively. Increased incidences of liver tumours were also observed in mice following dietary administration of heptachlor (females only according to JMPR 1991, both sexes according to IRIS 2004b) or heptachlor epoxide at 10 mg/kg diet for 24 months.
In rats fed technical-grade heptachlor (73% heptachlor, 18% *trans*-chlordane, 2% *cis*-chlordane) for 80 weeks, an increase in thyroid tumours was observed at about 51 mg/kg diet (about 2.6 mg/kg b.w./day) but not at about 26 mg/kg diet (about 1.3 mg/kg b.w./day), and not in males at dose levels of up to about 78 mg/kg diet (about 3.9 mg/kg b.w./day). In 2-year dietary studies, no increased incidences of liver tumours were observed in rats given heptachlor or heptachlor epoxide at dose levels of up to 10 mg/kg diet (about 0.25 mg/kg b.w./day).
(ATSDR 1993, JMPR 1991, IRIS 2004b,c, WHO 1984b, IARC 2001).

In mice fed a mixture of heptachlor and heptachlor epoxide (25:75) for 18 months, an increased incidence of combined liver tumours and nodular changes was observed at 10 mg/kg diet but not at lower dose levels; the NOAEL was 5 mg/kg diet (JMPR 1991). According to US-EPA (IRIS 2004c), increased incidences of liver tumours were observed at both dose levels. According to WHO (1984b), an increased incidence of liver tumours were observed in females at 10 mg/kg diet and in males from 5 mg/kg diet.
In rats, no malignant lesions of the liver were observed following dietary administration of the mixture at dose levels of up to 12.5 mg/kg diet for 2 years (IRIS 2004b,c, WHO 1984b).

Dietary administration of technical heptachlor at 0.65 or 1.3 mg/kg b.w./day for 24 weeks promoted the development of liver tumours in male mice previously initiated with diethylnitrosamine in drinking water for 14 weeks. (ATSDR 1993, JMPR 1986, WHO 1996b, IARC 2001).

2.8.5.3 Nonachlor

No data have been located regarding carcinogenic effects of nonachlor.

2.8.6 Toxicity to reproduction

2.8.6.1 Chlordane

No data are available regarding toxicity to reproduction of chlordane following ingestion by humans (ATSDR 1994).

No histopathological lesions have been noted in the reproductive organs of rats in an 80-week dietary study of analytical grade chlordane (72% *cis* and 23% *trans* isomers) at dose levels up to 20.4/12.1 mg/kg b.w./day for males/females, respectively; or in mice at dose levels up to 7.3/8.31 mg/kg b.w./day for males/females, respectively. Likewise, no treatment-related histopathological lesions were noted in the reproductive organs following administration of technical chlordane to rats at dose levels of up to 25 mg/kg diet (about 1.2/1.4 mg/kg b.w./day for males/females, respectively) for 130 weeks or to mice at dose levels of up to 25 mg/kg diet (about 1.2/1.4 mg/kg b.w./day for males/females, respectively) for 2 years. Reduced fertility, reflected as a reduction in the number of mated females that delivered litters, was observed when male and female rats were fed a diet that provided chlordane at about 16 mg/kg b.w./day from weaning of the parental generation and throughout lactation; no histopathological lesions were found in the reproductive organs. (ATSDR 1994, US-EPA 1997).

In one study of rats, no effect on the incidence of malformations and no evidence of foetal toxicity, including retarded skeletal development was observed in the foetuses of rats given chlordane at dose levels of up to 80 mg/kg b.w./day during gestation. In another study, an increase in percent loss of pups per litter was seen following administration of chlordane from 21 mg/kg b.w./day during gestation days 6 to 19; maternal toxicity in the form of decreased body weight gain was noted as well. No effects on viability and postnatal growth were observed in the offspring of mice treated with chlordane at 50 mg/kg b.w./day during gestation days 8-12. The offspring of mice treated with chlordane at 1 or 2.5 mg/kg b.w./day during gestation days 12 to 19 exhibited subtle behavioural effects at both dose levels. (ATSDR 1994, US-EPA 1997, IRIS 2004a).

Studies conducted with pregnant mice indicate that *in utero* and/or neonatal exposure to chlordane at dose levels from about 8 mg/kg b.w./day during gestation days 1 to 19 may affect the developing immune system reflected as suppressed cell-mediated immunity in the offspring; there was no effect on humoral-mediated immunity. In one of the studies, the NOAEL was 0.16 mg/kg b.w./day. (ATSDR 1994, US-EPA 1997, IRIS 2004a).

2.8.6.2 Heptachlor

No adverse effects on reproduction (no decrease in fertility, no increase in foetal or neonatal deaths) were reported among women of child-bearing age following ingestion of heptachlor-containing milk in excess of 0.1 mg/l for 27-29 months (ATSDR 1993).

Increased numbers of resorptions were observed following dietary administration of heptachlor at 0.25 mg/kg b.w./day (the only dose level in the study) to rats for 60 days prior to mating and for females also during gestation; the number of abnormal embryos was not increased. During the second phase of the study, rats receiving 0.25 mg/kg b.w./day for two generations showed a marked decrease in pregnancy rates (18/25 in the first generation; 0/12 in the second generation); treatment seemed to affect male rats rather than female rats. Postnatal survival was

significantly reduced as only 19/122 offspring of treated rats survived 21 days postpartum. Mice fed heptachlor at dose levels from 6.5 mg/kg b.w./day (the lowest dose level in the study) for 10 weeks failed to produce a new generation; no histological alterations were found in ovaries or testes. In a 2-generation reproduction and teratology study in dogs with heptachlor epoxide, a significant increase in the mortality rate of pups of the first generation was observed following dietary administration at 10 mg/kg diet, and slight increases in mortality rates were observed among pups of the second generation from 3 mg/kg diet; the NOAEL was 1 mg/kg diet (corresponding to 0.025 mg/kg b.w./day). (ATSDR 1993, JMPR 1991, WHO 1996b, WHO 1984b).

At high dietary exposure levels, heptachlor can interfere with reproduction and the viability of offspring; cataracts have been observed in both parents and offspring in some of the older and rather limited rat studies. No indications of teratogenicity have been found in rats, rabbits, or beagle dogs exposed to heptachlor. (WHO 1996b, WHO 1984b).

2.8.6.3 Nonachlor

No data have been located regarding reproductive toxicity of nonachlor.

2.8.7 Evaluation

2.8.7.1 Chlordane

No data have been located regarding adverse health effects of chlordane in humans following repeated oral administration. In experimental animals, repeated exposure to chlordane is associated with effects in the liver and the central nervous system, carcinogenicity, and developmental effects.

The liver is the major target organ in rodents following repeated oral administration and the effects observed include increased liver microsomal enzyme activity, increased relative liver weight, liver cell inclusion bodies, liver cell hypertrophy, and liver cell necrosis. Following chronic dietary administration (30-months) of technical chlordane to rats and mice, no effects were observed in the liver of rats and mice at 1 mg/kg in the diet (corresponding to 0.055 mg/kg b.w./day for rats and to 0.15 mg/kg b.w./day for mice). For pure analytical grade chlordane (72% *cis* and 23% *trans* isomers), no effects were observed in the liver of male and female rats at dose levels of up to 20.4 and 12.1 mg/kg b.w./day, respectively, for 80 weeks, and no non-neoplastic effects in the liver of mice at dose levels of up to 8.0 and 9.1 mg/kg b.w./day for males and females, respectively. Overall, a NOAEL of 1 mg/kg in the diet is considered for liver toxicity of chlordane (corresponding to 0.055 mg/kg b.w./day based on the study of rats with technical chlordane).

Central nervous system effects in form of tremors have been observed in female rats and in both sexes of mice following chronic dietary administration of pure analytical grade chlordane (72% *cis* and 23% *trans* isomers); no effects were observed in rats at 6.0 mg/kg b.w./day or in mice at 4.3 mg/kg b.w./day. No histopathological lesions in the central nervous system were observed at relatively high dose levels. A NOAEL of 4.3 mg/kg b.w./day is considered for neurotoxic effects based on tremors observed in a 80-week study of mice.

Studies in different strains of mice, including one strain of mice that are historically resistant to spontaneous liver tumours, have shown that dietary administration of

chlordane is associated with the development of liver tumours. No evidence of carcinogenicity has been provided in rats. One study in mice indicates that chlordane may act as a liver tumour promoter. The available studies may indicate that the carcinogenic effect of chlordane in mice may be due to non-genotoxic mechanisms.
Chlordane is considered by IARC (2001) as possibly carcinogenic to humans (Group 2B; evidence in humans inadequate, evidence in animals sufficient). US-EPA has considered chlordane as a probable human carcinogen (Group B2; evidence in humans inadequate, evidence to animals sufficient) (IRIS 2004a). In the EU, chlordane is classified for carcinogenic effects in category 3 (Carc. Cat. 3, R40 – limited evidence of a carcinogenic effect) (MM 2002).
Chlordane has been tested in several *in vitro* and *in vivo* genotoxicity studies. The vast majority of the studies have yielded negative results, but positive results have been reported as well. Chlordane appears to have no genotoxic potential *in vivo*, but the data provide no conclusive evidence. Overall, a genotoxic potential of chlordane *in vivo* cannot be fully excluded.
Overall, a carcinogenic potential of chlordane cannot be excluded for humans in relation to dietary exposure, but is not considered to be significant at the exposure levels at which other effects in the liver have been observed.

No histopathological lesions have been noted in the reproductive organs of rats or mice following repeated oral administration of chlordane (analytical grade; technical grade) at relatively high dose levels for life-time. Reduced fertility has been observed in a limited study of rats fed a diet that provided chlordane at about 16 mg/kg b.w./day from weaning of the parental generation and throughout lactation. Embryo-foetal effects, but no malformations, were only noted in rats, at dose levels, which also gave rise to maternal effects. In one study, offspring of mice treated during late gestation exhibited subtle behavioural effects; the LOAEL was 1 mg/kg b.w./day. Some studies in mice indicate that chlordane may be an immunosuppressive agent following exposure *in utero*; a NOAEL of 0.16 mg/kg b.w./day. is indicated based on one of the studies.

2.8.7.2 Heptachlor

No adequate data have been located regarding adverse health effects of heptachlor or heptachlor epoxide in humans following repeated oral administration. In experimental animals, repeated exposure to heptachlor is associated with effects in the liver, carcinogenicity, and reproductive and developmental effects.

The liver is the major target organ in rodents and dogs following repeated oral administration of heptachlor or heptachlor epoxide and the effects observed include increased liver microsomal enzyme activity, relative liver weight and lipid content; proliferation of the smooth endoplasmic reticulum and increased number of mitochondria; and histological changes. In a 2-year feeding study in rats with heptachlor, the NOAEL for increased liver weight was 3 mg/kg diet (0.15 mg/kg b.w./day). For heptachlor epoxide, a LOAEL of 5 mg/kg diet (the lowest dose level) has been reported for increased liver weight in a 2-year dietary study with rats, a NOAEL of 1 mg/kg diet (0.025 mg/kg b.w./day) for histopathological changes in the liver of dogs in a 2-year feeding study, and a LOAEL of 0.5 mg/kg diet (0.0125 mg/kg b.w./day) for increased liver weight in a 60-week dietary study in dogs. In mice fed a mixture of heptachlor and heptachlor epoxide (25:75) for 18 months, histopathological alterations were observed from 1 mg/kg diet (the lowest dose level, 0.15 mg/kg b.w./day). Most of the available long-term studies were conducted more than 30 years ago and have, according to JMPR (1991), severe methodological deficiencies; furthermore, the results or interpretations of some of

the studies are presented in different ways in various international evaluations. Overall, a NOAEL of 1 mg/kg in the diet is considered for liver toxicity of heptachlor and heptachlor epoxide (corresponding to 0.025 mg/kg b.w./day) based on the 2-year study of dogs with heptachlor epoxide as this study is considered to be the most valid one of the available studies.

Heptachlor and heptachlor epoxide have produced liver tumours in mice when administered orally, but not in rats. In one study of rats fed technical-grade heptachlor (73% heptachlor, 18% *trans*-chlordane, 2% *cis*-chlordane), an increase in thyroid tumours was observed. One study in mice indicates that heptachlor may act as a liver tumour promoter. The available studies may indicate that the carcinogenic effect of heptachlor and heptachlor epoxide in mice may be due to non-genotoxic mechanisms.
Heptachlor is considered by IARC (2001) as possibly carcinogenic to humans (Group 2B; evidence in humans inadequate, evidence in animals sufficient. US-EPA has considered heptachlor and heptachlor epoxide as probable human carcinogens (Group B2; evidence in humans inadequate, evidence to animals sufficient) (IRIS 2004b,c). In the EU, heptachlor and heptachlor epoxide are classified for carcinogenic effects in category 3 (Carc. Cat. 3, R40 – limited evidence of a carcinogenic effect) (MM 2002).
Heptachlor and heptachlor epoxide have been tested in several *in vitro* and in two *in vivo* genotoxicity studies. The vast majority of the studies have yielded negative results, but positive results have been reported as well. Heptachlor and heptachlor appear to have no genotoxic potential *in vivo*, but the data provide no conclusive evidence. Overall, a genotoxic potential of heptachlor and/or heptachlor epoxide *in vivo* cannot be fully excluded.
Overall, a carcinogenic potential of heptachlor and heptachlor epoxide cannot be excluded for humans in relation to dietary exposure, but is not considered to be significant at the exposure levels at which other effects on the liver have been observed.

No adverse effects on reproduction were reported among women of child-bearing age following ingestion of heptachlor-containing milk. Reduced fertility (rats) and pup survival (rats, mice, dogs) have been reported in generation studies following dietary administration of heptachlor (rats, mice) and heptachlor epoxide (dogs). A LOAEL of 0.25 mg/kg b.w./day is established for rats and of 6.5 mg/kg b.w./day for mice, while a NOAEL of 0.025 mg/kg b.w./day can be established for dogs. No indications of teratogenicity have been found in rats, rabbits, or beagle dogs exposed to heptachlor.

2.8.7.3 Nonachlor

No data have been located regarding adverse health effects of nonachlor following repeated oral administration.

2.8.8 Critical effect(s) and NOAEL / LOAEL

2.8.8.1 Chlordane

The critical effects following dietary intake of chlordane are the effects observed in the liver, the carcinogenic effect, and the developmental effects.
The liver is the major target organ in rats and mice; a NOAEL of 1 mg/kg in the diet (corresponding to 0.055 mg/kg b.w./day for rats; 0.15 mg/kg b.w./day for mice) can be established based on histopathological alterations in the liver of

female rats and mice of both sexes at higher dietary dose levels. Developmental effects in form of subtle behavioural effects (LOAEL 1 mg/kg b.w./day) and suppressed immune function (NOAEL of 0.16 mg/kg b.w./day) have been observed in the offspring of mice following exposure *in utero*. A NOAEL of 0.055 mg/kg b.w./day for effects in the liver is considered to be sensitive and to provide adequate protection against neurotoxicity and developmental effects as well, and is taken forward to the risk characterisation.

A carcinogenic potential of chlordane for humans in relation to dietary exposure cannot be excluded, but is not considered to be significant at the exposure levels at which other effects in the liver have been observed. Furthermore, a genotoxic potential of chlordane cannot be excluded either.

2.8.8.2 Heptachlor

The critical effects following dietary intake of heptachlor and heptachlor epoxide are the effects observed in the liver, the carcinogenic effect, and reproductive and developmental effects.
The liver is the major target organ in rats, mice and dogs; a NOAEL of 1 mg/kg in the diet for liver toxicity of heptachlor and heptachlor epoxide (corresponding to 0.025 mg/kg b.w./day) can be established based on histopathological alterations in the liver of dogs in the 2-year study with heptachlor epoxide. For reproductive and developmental effects, a NOAEL of 0.025 mg/kg b.w./day can be established based on the 2-generation study in dogs with heptachlor epoxide. A NOAEL of 0.025 mg/kg b.w./day is considered to be sensitive, and is taken forward to the risk characterisation.

A carcinogenic potential of heptachlor and heptachlor epoxide for humans in relation to dietary exposure cannot be excluded, but is not considered to be significant at the exposure levels at which other effects in the liver have been observed. Furthermore, a genotoxic potential of heptachlor and heptachlor epoxide cannot be excluded either.

2.8.8.3 Nonachlor

No data have been located regarding adverse health effects of nonachlor following repeated oral administration.

2.8.9 References

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2.9 Chlorobenzenes

Chlorobenzenes are formed by the addition of 1-6 atoms of chlorine to the benzene ring. This yields 12 compounds: monochlorobenene, three isomeric forms each of di-, tri, and tetrachlorobenzenes, as well as penta- and hexachlorobenzenes. (WHO 1991).

Chlorobenzene levels in traditional Greenland food items have been presented as sCBz (sum of 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB), pentachlorobenzene (PeCB) and hexachlorobenzene (HCB)) (Johansen et al. 2004). Therefore, this evaluation primarily focuses on these three chlorobenzenes; the term 'CBz' in this evaluation refers to these compounds in general. Data on the two other isomers of tetrachlorobenzene (TeCB) as well as on chlorobenzenes in general are included in this evaluation when considered relevant.

Chlorobenzenes are used mainly as intermediates in the synthesis of other chemicals, and as pesticides. 1,2,3,4-TeCB is used as a component in dielectric fluids and in the synthesis of fungicides. PeCB is used as an intermediate and has formerly been used in a pesticide used to combat oyster drills. HCB has had many uses in industry and agriculture. The major agricultural application for HCB was as a fungicide on the seeds of crops. The use of HCB in such applications was discontinued in many countries in the 1970s. (WHO 1991, WHO 1997, WHO 1996, ATSDR 2002).

Chlorobenzenes are found in fish, animal products and crops, and also in human milk. In the general population, the intake from food compared with that from other sources increases with increasing degree of chlorination and for TeCB, PeCB and HCB, food is the major source. The total daily intake of HCB by adults has been estimated to be between 0.0004 and 0.003 µg/kg b.w./day; for TeCB and PeCB, the total daily intake is considered to be less than 0.05 µg/kg b.w./day. The exposure of the general population to HCB declined from the 1970s to the mid-1990s. (WHO 1991, WHO 1997, WHO 1996).

In Denmark, the estimated average dietary intake of HCB for adults (1993-1997) was 0.2 µg/day and the 95 percentile was 0.4 µg/day (FDIR 2000). No data are available regarding 1,2,3,4-TeCB and PeCB.

2.9.1 Toxicokinetics

All chlorobenzenes appear to be absorbed readily from the gastrointestinal tract in humans and experimental animals following oral administration, with absorption being influenced by the position of the chlorine in different isomers of the same congener. Data from studies with HCB in experimental animals indicate that absorption is much more extensive when HCB was administered in oil vehicles or a high fat diet (up to about 82%) than from aqueous vehicles (about 6%). (WHO 1991, ATSDR 2002, WHO 1997, WHO 1996).

Following absorption, chlorobenzenes are initially rapidly distributed throughout the body to highly perfused tissues, but then accumulate in adipose fat, and to a lesser extent, to other lipid-rich organs and tissues. In general, accumulation is greatest for the more highly chlorinated congeners, but it can vary considerably for different isomers of the same congener. Results from studies in experimental animals indicate that HCB levels increase in a dose-dependent manner in all tissues, at least at dose levels up to around 100 mg/kg b.w./day. Human data

indicate that the body burden of HCB tends to increase with increasing age. (WHO 1991, ATSDR 2002, WHO 1997, WHO 1996).

The chlorobenzenes are metabolised by microsomal oxidation in the liver either directly or through the formation of a metastable arene oxide intermediate, to form the corresponding chlorophenols. These chlorophenols can be excreted in the urine as mercapturic acids, formed by conjugation with glutathione, or as glucuronic acid or sulfate conjugates. The metabolic transformation of chlorobenzenes decreases with increasing degree of chlorination and a greater proportion of the compound is eliminated unchanged in the faeces. The position of the chlorine atoms on the benzene ring is also an important determinant of the rates of metabolism and elimination, with chlorobenzenes with 2 adjacent un-substituted carbon atoms (e.g., 1,2,3,4-TeCB) being more rapidly metabolised and eliminated than congeners having a similar degree of chlorination, but without 2 adjacent un-substituted carbon atoms (e.g., 1,2,3,5-TeCB and 1,2,4,5-TeCB).
TeCBs are slowly metabolised; the corresponding tetrachlorophenols are the major metabolites in rats.
PeCB is metabolised primarily to pentachlorophenol by direct oxidation, or to 2,3,4,5-tetrachlorophenol. Approximately 12% of an oral dose was eliminated in the urine of rhesus monkeys and 24% in the faeces (99% as parent compound) within 40 days. In rhesus monkeys, the estimated half-life of PeCB was 2 to 3 months.
Elimination of absorbed HCB is slow and occurs primarily via the faeces, with smaller amounts being excreted in the urine; both biliary and intestinal excretion contributes to faecal excretion. HCB eliminated in the faeces is predominantly unchanged parent compound, although small amounts of various metabolites have also been found. Conversely, HCB in the urine is almost all in the form of metabolites. HCB is metabolised to 1) pentachlorophenol, which is subsequently converted to tetrachlorohydroquinone; 2) conjugated with glutathione to yield pentachlorothiophenol; or 3) reductively dechlorinated to form PeCB, TeCB, and lesser chlorinated benzenes. The major metabolites detected in the urine are pentachlorophenol, tetrachlorohydroquinone, pentachlorothiophenol, and PeCB. Studies that monitored elimination of HCB for an extended period of time noted that the rate of elimination decreased over time suggesting that elimination of HCB could continue for years. Elimination half-lives reported for HCB range from approximately one month in rats and rabbits to 2 or 3 years in monkeys.
Some of the chlorobenzenes induce a wide range of enzyme systems including those involved in oxidative, reductive, conjugation, and hydrolytic pathways. (WHO 1991, ATSDR 2002, WHO 1997, WHO 1996).

Chlorobenzenes have been shown to cross the placenta, and have been found in the foetal brain and liver tissues. They are also excreted in the human milk. (WHO 1991, WHO 1997, WHO 1996, ATSDR 2002).

2.9.2 Single dose toxicity

Data on the health effects of acute exposure to chlorobenzenes are restricted to case reports mainly concerning accidental exposure to products containing the lower chlorinated benzenes (mono-, di- and trichlorobenzenes) (WHO 1991).
No specific human data are available regarding the acute toxicity of 1,2,3,4-TeCB, PeCB, or HCB.

In experimental animals, the acute toxicity of chlorobenzenes is in general moderate with oral LD_{50}-values greater than 1000 mg/kg b.w. For 1,2,3,4-TeCE, the reported oral LD_{50}-value is 1167/1470 mg/kg b.w. in rats; for PeCB between

940 and 1370 mg/kg b.w. in mice and rats; and for HCB between 3500 and > 10000 mg/kg b.w. in rats. Signs of toxicity include convulsions, tremors, weakness, ataxia, paralysis, and pathological changes in organs. (WHO 1991, WHO 1997, WHO 1996, ATSDR 2002).

2.9.3 Repeated dose toxicity

Data on the health effects of exposure of the general population to chlorobenzenes are restricted to case reports mainly concerning accidental exposure to products containing the lower chlorinated benzenes (mono-, di-, and trichlorobenzenes) (WHO 1991) as well as HCB.

Most data on the effects of HCB on humans originate from accidental poisonings that took place in Turkey in 1955-1959, in which 3000-4000 people ingested bread prepared from grain treated with fungicides composed of 10% HCB. In this incident, more than 600 cases of porphyria cutanea tarda (PCT) were identified, with a mortality of 10%. In the liver, HCB inhibits the activity of an enzyme in the haeme biosynthesis pathway, leading to increased formation and excretion of haeme precursors (porphyrins). The activity of other enzymes in the haeme biosynthesis partway may also be altered. In addition to disturbances in porphyrin metabolism, clinical manifestations of PCT included skin lesions and ulcerations, hyperpigmentation, hypertrichosis, enlarged liver, weight loss, enlargement of the thyroid gland and lymph nodes, neurological effects, and a characteristic port wine colour of the urine (from increased excretion of porphyrins). In roughly half the cases, osteoporosis of extremities, deformation of the fingers, or arthritis were also observed, primarily in children. No specific information of exposure (dose and duration) is available, but a dose level of 50-200 mg/day (0.8-3.3 mg/kg b.w./day for a 60-kg person) for a number of months has been estimated for manifestations of the disease to become apparent. In 20-30-year follow-ups of exposed individual, neurological, dermatological, and orthopaedic abnormalities persisted, and there were elevated levels of porphyrins in excreta of some individuals. Breast-fed infants of mothers exposed to HCB in this incident developed a disorder called pembe yara (pink sore) and most of these children died within a year after birth. (WHO 1997, ATSDR 2002).

HCB is the most thoroughly tested chlorobenzene in intermediate- and long-term experimental studies and a broad spectrum of health effects have been observed in experimental animals including porphyria and other effects in the liver, effects in the kidney, thyroid, lung, heart, haematopoietic system, bone, thymus, spleen, immune system, and nervous system. (WHO 1997, ATSDR 2002).

The available repeated dose toxicity studies on chlorobenzenes other than HCB in experimental animals indicate a trend for the toxicity of chlorobenzenes to increase with increased chlorination of the benzene ring for many of the endpoints examined. However, variations in the toxicity of different isomers of the same congener are, in some cases, considerable. Data indicate that there is a good correlation between toxicity and the degree of accumulation of the compound in the tissues; female animals tend to be less sensitive than males. The major target organs following exposure to chlorobenzenes are the liver and kidney; at higher dose levels, effects on the haematopoietic system were reported, and thyroid toxicity was noted in studies with 1,2,4,5-TeCB and PeCB. (WHO 1991).

For TeCB, a 90-day dietary study to compare the toxicities of the TeCB isomers in rats has shown that 1,2,4,5-TeCB is the most toxic isomer and 1,2,3,4-TeCB the least toxic isomer. For 1,2,3,4-TeCB, the NOAEL for renal and hepatic toxicity

was 3.4 mg/kg b.w./day for male rats and 42 mg/kg b.w./day for female rats. No other data are available for 1,2,3,4-TeCB. (WHO 1991).

For PeCB, a 13-week dietary study is available for both rats and for mice. In rats, effects in the liver were observed from 33 mg/kg feed, in the kidney from 100 mg/kg feed, in the thyroid from 33 mg/kg feed, and changes in haematological parameters from 330 mg/kg feed; the NOAEL was 33 mg/kg feed (corresponding to about 2.2 mg/kg b.w./day) for female rats; for male rats, effects were observed at the lowest dose level of 33 mg/kg feed (corresponding to about 2.0 mg/kg b.w./day) thus constituting a LOAEL. In mice, effects in the liver were observed from 33 mg/kg feed, in the kidney from 1000 mg/kg feed, and in the thyroid from 33 mg/kg feed; the LOAEL was 33 mg/kg feed (corresponding to about 6 mg/kg b.w./day) for both sexes. (WHO 1991).

The available data indicate that the pathway for the biosynthesis of haem in the liver is a major target of HCB toxicity in several species of experimental animals following oral exposure, leading to porphyria. Rats, especially female rats, seem to be more sensitive than other species to the porphyrinogenic effects of HCB. Porphyria has been reported in rats following oral exposure at dose levels between 2.5 and 15 mg/kg b.w./day for 8 to 15 weeks and from 4 mg/kg b.w./day for 9-10 months; a NOAEL cannot be determined based on these studies. Other effects in the liver include induction of microsomal enzymes, increased liver weight, hepatocellular hypertrophy, and cellular damage. The relationship between porphyria and these other hepatic effects is uncertain. In some instances, the other effects occurred in rats at lower dose levels than porphyria, or in absence of porphyria. Enzyme induction and histopathological changes have been observed in rats exposed to HCB in the diet at dose levels between 0.25 and 0.6 mg/kg b.w./day for 3 to 12 months; the NOAELs were between 0.05 and 0.07 mg/kg b.w./day. (WHO 1997, ATSDR 2002).

Renal effects of HCB include increased kidney weight, accumulation of porphyrins (as in the liver), and renal tissue damage. Dietary dose levels of 19 to 32 mg/kg b.w./day for 12 to 16 weeks have resulted in increased kidney weights and histopathological changes in both male and female rats; the NOAELs ranged from 5 to 9.5 mg/kg b.w./day. Renal accumulation of porphyrins has been observed in female rats given 5 mg/kg b.w./day in the diet for 56 days, but not in male rats at dietary dose levels up to 25 mg/kg b.w./day for 15 weeks. (ATSDR 2002).

Effects in the thyroid exerted by HCB are indicated by decreased serum levels of thyroid hormones, which are, in some instances, accompanied by increases in thyroid weight, circulating levels of thyroid-stimulating hormone, or iodine uptake by the thyroid. In rats, decreased thyroid hormone levels have been observed following dietary administration at dose levels from 9.5 mg/kg b.w./day for 13 weeks. (WHO 1997, ATSDR 2002).

Pathological effects in the lungs (hypertrophy, proliferation of the lining endothelial cells of the pulmonary venules, and intra-alveolar accumulation of foamy-looking macrophages) have been observed in different strains of rats and in both sexes following oral administration of HCB at dose levels from 3 to 5 mg/kg b.w./day for up to 4 months. Such effects have also been observed from 1 mg/kg b.w./day in rats that received both pre- and postnatal exposure; the NOAEL was 0.2 mg/kg b.w./day. More severe pulmonary effects have also been observed in rats at higher doses. No pulmonary effects have been reported in studies on monkeys, dogs, or mice. (ATSDR 2002).

Fibrosis and degeneration of muscle fibres in the heart have been observed in rats exposed to HCB at dose levels from 25 mg/kg b.w./day in the diet for 4 months. No treatment-related lesions in cardiovascular tissues have been observed in dogs and monkeys. (ATSDR 2002).

Changes in haematological parameters indicative of anaemia and leucocytosis have been observed in rats at dose levels from 5 to 32 mg/kg b.w./day for approximately 4 months; female rats were much more sensitive than male rats to these effects (ATSDR 2002).

Repeated oral exposure to HCB has caused changes in calcium homeostasis and bone morphometry in rats at dose levels from 0.7 mg/kg b.w./day for 5 and 15 weeks, respectively, but not at 0.07 mg/kg b.w./day (WHO 1997, ATSDR 2002).

A number of studies have indicated that oral exposure to HCB affects the immune system, with immunosuppressive effects observed in mice and monkeys, and immunostimulatory effects in rats and dogs. Immunosuppressive effects have been observed in mice exposed to approximately 0.6 mg/kg b.w./day for 3 to 18 weeks. In rats, immunostimulation as indicated by increased spleen and lymph node weights, increased neutrophil counts, and increased serum immunoglobulins has been observed at relatively high dietary dose levels of 25-50 mg/kg b.w./day for 3 weeks. Atrophy of the thymus has been observed in male rats fed 100 mg/kg b.w./day for 3 weeks and in female rats from 15 mg/kg b.w./day for 13 weeks. The developing immune system of the rat seems to be particularly vulnerable to the immunotoxic action of HCB as stimulation of both humoral and cell-mediated immunity has been observed in rat pups of mothers fed from 0.2 mg/kg b.w./day from early pregnancy through lactation; weaning pups were fed the same dose level up to 7 months of age. (WHO 1997, ATSDR 2002).

HCB induces serious neurological effects such as convulsions, tremors, lethargy, and progressive weakness in experimental animals. The lowest dose to cause these effects was 8 mg/kg b.w./day and was observed in rats in a multigeneration study. (ATSDR 2002).

2.9.4 Genotoxicity

In a single study of workers exposed to a number of chlorinated compounds, including HCB, an increased frequency of micronucleated peripheral lymphocytes was found. There was no association with the concentrations of HCB in the blood. (IARC 2001, ATSDR 2002).

HCB has been tested in several *in vitro* and *in vivo* genotoxicity studies. HCB was negative for gene mutations and DNA repair in bacteria; did not produce chromosomal aberrations in human peripheral lymphocytes or in Chinese hamster cells *in vitro*; did not bind strongly to rat DNA *in vitro* or *in vivo*; did not produce dominant lethal mutations in orally exposed rats; did not increase the frequency of sister chromatid exchanges in the bone marrow of mice exposed orally; and did not show evidence of genotoxicity in mouse liver, lung, kidney, spleen, or bone marrow after oral dosing. HCB was positive in a gene mutation assay in *Saccharomyces cerevisiae* and in an assay for replicative DNA synthesis in mouse hepatocytes; and produced weak positive results in assays for DNA fragmentation and micronuclei formation in primary cultures of human and rat hepatocytes, minimal formation of DNA adducts in cultured human hepatoma cells, and DNA adducts in foetal hepatocytes from rats. (ATSDR 2002, IARC 2001, WHO 1997, IARC 1987, IRIS 2004b, WHO 1996).

PeCB did not show a mutagenic potential in *Salmonella typhimurium* and was also negative in Chinese hamster ovary cell assays for induction of sister chromatid exchanges and chromosomal aberrations *in vitro*. In 13-week rat and mouse micronucleus assays, PeCB was negative in all exposed groups. The metabolites of PeCB were all negative for gene mutation in *Salmonella typhimurium*, but some of the metabolites (e.g., pentachlorophenol), have shown evidence of clastogenic activity *in vitro*. (IRIS 2004a).

No data are available regarding mutagenic and genotoxic effects of 1,2,3,4-TeCB.

Generally, chlorobenzenes do not appear to be mutagenic (WHO 1991).

2.9.5 Carcinogenicity

A follow-up of 204 patients from the Turkish poisoning incident (see section 2.9.3) 25 to 30 years after the onset of HCB induced porphyria showed that the majority of the patients still had symptoms of adverse effects, including enlarged thyroids. However, no increased incidence of liver tumours were reported. (ATSDR 2002, IRIS 2004b, IARC 1987, WHO 1997, WHO 1996).

The risk for breast cancer has been investigated in relation to life-long exposure to HCB in nine studies. Five small case-control studies that included fewer than 50 cases of breast cancer each showed no overall association with the concentration of HCB in samples of adipose breast tissue. A secondary subgroup analysis in one of the studies revealed a significant association in post-menopausal women with oestrogen receptor positive cancer based on a small number of cases. In three large case-control studies, no consistent increase in the risk for breast cancer was found in women with elevated concentrations of HCB. In a fourth large case-control study, the risk for breast cancer in women whose serum concentration of HCB was in the upper three quartiles was twice that of those whose serum concentration of HCB was in the lower quartile; however, there was no evidence of a dose-response relationship. (IARC 2001, ATSDR 2002).

HCB has been tested for carcinogenicity by oral administration in rats, mice, and hamsters. In rats, increased incidences of tumours in the liver, bile duct (females only), kidney, and adrenals (females only) were observed following dietary administration from 4-5 mg/kg b.w./day for up to 2 years. An increased incidence of liver cell tumours has also been observed in mice following dietary exposure from 12 mg/kg b.w./day for 120 weeks. In hamsters fed diets containing HCB for lifetime, the incidence of liver cell tumours was increased from 4 mg/kg b.w./day and the incidence of thyroid tumours in males at 16 mg/kg b.w./day. In a 2-generation feeding study in mice, increased incidences of tumours in the liver and the adrenals were observed in females at about 1.9 mg/kg b.w./day and in the parathyroids of males at about 1.5 mg/kg b.w./day. In addition, rats, mice, and hamsters have developed tumours in the liver, bile duct, kidney, thymus, spleen and lymph nodes after subchronic dietary exposure to HCB. Results from a number of studies in which HCB was administered together with other compounds have indicated that HCB is a co-carcinogen or promoter of cancer. The available studies indicate that female rats appear to be more susceptible than males to the hepatocarcinogenic effects and that males are more susceptible to renal cancer. (WHO 1997, ATSDR 2002, IRIS 2004b, IARC 2001, WHO 1996).

No data are available regarding carcinogenic effects of 1,2,3,4-TeCB and PeCB.

2.9.6 Toxicity to reproduction

Studies of patients from the Turkish poisoning incident (see section 2.9.3) have suggested that HCB may cause spontaneous abortion, miscarriages and stillbirths among women with previous oral exposure to HCB (estimated intake: 0.8-3.3 mg/kg b.w./day for a number of months). In addition, serious developmental toxicity was observed. A 95% mortality rate was observed for infants under 2 years of age who had been breast-fed by exposed mothers. Among older children, between the ages of 6 and 15 years, the mortality rate was 10%. Follow-up studies have found persistent symptoms of developmental toxicity in a cohort of 252 adults (162 men and 90 women) who had been exposed as children (average age of 7.6 years). Short stature was seen in 42.1% of the patients, osteoporosis of bones in the hands in 66.6%, paraesthesia in 53.6%, and graded sensory loss indicative of polyneuropathy in 60.6% of the patients. Two follow-up studies conducted 20-30 years after initial exposure found potential reproductive effects in women exposed as children; 42/57 mothers who had suffered from porphyria, with 188/276 pregnancies were identified. Of these pregnancies, 15/23 were foetal deaths and 31/54 produced children who died in the first years of life; however, it is not clear from the studies that the rate of miscarriages was significantly higher than normal for this population. A subsequent retrospective study conducted 40 years after initial exposure suggests an association between elevated blood levels of HCB and increased risk for spontaneous abortion, but other studies did not. (ATSDR 2002).

Studies in experimental animals using oral exposure have shown that exposure to HCB is associated with reproductive effects and that the ovary is a sensitive target organ for HCB. Ninety-day studies in female Cynomolgus monkeys have revealed dose-related degenerative changes in oocytes and ovaries at all dose levels tested (0.01-10 mg/kg b.w./day) and hormonal and menstrual changes from 1.0 mg/kg b.w./day and at 10 mg/kg b.w./day, respectively. Similar effects have been observed in rats at the lowest dose level tested (1.0 mg/kg b.w./day). Testicular damage has been observed in male rats from dietary dose levels of 10 mg/kg b.w./day. HCB also adversely affects reproductive performance. In a multigeneration study in rats, decreased fertility and increased number of stillborns were observed at dietary dose levels from 16 mg/kg b.w./day, and decreased average litter size from 8 mg/kg b.w./day. Decreased male reproductive performance (mating index), but no changes in fertility (numbers of inseminated females made pregnant) was observed in a study in which only male rats were dosed (70 mg/kg b.w./day for 5 days). In contrast, no reproductive toxicity was observed in two studies in which only females were dosed (female rats fed 7 mg/kg b.w./day for 96 days; female Cynomolgus monkeys given 10 mg/kg b.w./day orally for 90 days). Similarly, no reproductive toxicity was observed in rats fed doses of up to 2 mg/kg b.w./day from 3 months prior to mating through weaning. (ATSDR 2002, WHO 1997).

HCB is also a developmental toxicant in experimental animals following oral administration. Effects on the developing immune system have been observed in rat pups born to dams exposed to 0.2 mg/kg b.w./day through gestation, lactation and 2 weeks or 7 months post-weaning; and in pups of mice fed doses from 0.5 mg/kg b.w./day on gestation days 1 to 18. Neurodevelopmental effects have been observed in pups of rats given 2.5 mg/kg b.w./day for 4 days at 2 weeks prior to mating and in rat pups in a 2-generation study following exposure at 1.3 mg/kg b.w./day but not at 0.6 mg/kg b.w./day. Other effects, including reduced neonatal viability and growth, and organ weight changes have generally been observed in rat pups born to dams exposed at dose levels of 4-5 mg/kg b.w./day. In a 2-generation study in rats, peribiliary lymphocytosis and fibrosis were observed in adult males

of the first generation (F_1 adult males, exposed *in utero*, during lactation and from their diets for the remainder of their lifetime – 130 weeks) at the lowest dose level tested (0.016 mg/kg b.w./day). Some evidence of teratogenic effects have been reported in studies of rats and mice at rather high exposure levels (40 and 100 mg/kg b.w./day, respectively) during gestation. (ATSDR 2002, IRIS 2004b, WHO 1997).

No human data are available regarding reproductive and developmental effects of 1,2,3,4-TeCB and PeCB. In experimental animals, some data indicate that TeCBs and PeCB are embryotoxic and foetotoxic at dose levels that are not toxic for the mother.
In one study of 1,2,3,4-TeCB in rats, a decreased number of live foetuses was observed following oral administration of 200 mg/kg b.w./day during gestation days 6-15 but not at 100 mg/kg b.w./day; no effects were noted in the dams. In another study in rats, embryonic growth retardation was observed at 300 mg/kg b.w./day during gestation days 9-13 but not at 100 mg/kg b.w./day; maternal toxicity was observed at 300 mg/kg b.w./day as well.
For PeCB, an increased incidence of extra ribs was observed in foetuses of pregnant rats administered oral doses from 50 mg/kg b.w./day during gestation days 6-15; no effects were noted in the dams. No embryotoxic, foetotoxic, or teratogenic effects were observed in offspring of mice exposed at dose levels of up to 100 mg/kg b.w./day during gestation days 6-15; maternal toxicity was observed from mg/kg b.w./day. When rats were administered PeCB in the diet from 4 to 5 weeks of age, and during gestation and lactation, maternal toxicity was noted at dose levels from 27-64 mg/kg b.w./day, tremors in suckling pups from 17-31 mg/kg b.w./day, and, at higher dose levels, decreases in pre-weaning growth rats, and mortality of pups.
(WHO 1991).

2.9.7 Evaluation

HCB is the most thoroughly tested chlorobenzene in intermediate- and long-term experimental studies; very few studies are available for 1,2,3,4-TeCB and PeCB. The available data on chlorobenzenes in general indicate a trend for the toxicity of chlorobenzenes to increase with increased chlorination of the benzene ring for many of the endpoints examined.

Repeated oral exposure to HCB is associated with effects in the liver, kidney, thyroid, lung, heart, haematopoietic system, bone, thymus, spleen, immune and nervous systems, and carcinogenic, reproductive and developmental effects.
For chlorobenzenes other than HCB, effects in the liver, kidney, thyroid, haematopoietic system, and developmental effects have been reported.

For HCB, the liver, and specifically, the haem biosynthesis pathway, is the major target of toxicity in humans, but effects in other targets, including the skin, bone, thyroid, and central nervous system have also been reported; these effects were less common than inhibition of haem biosynthesis in exposed individuals. In 20-30-year follow-ups of exposed individuals, neurological, dermatological, and orthopaedic abnormalities persisted, and there were elevated levels of porphyrins in excreta from some individuals. No specific information of exposure (dose and duration) is available for humans, but a dose level of 50-200 mg/day (0.8-3.3 mg/kg b.w./day for a 60-kg person) for a number of months has been estimated for the effects of HCB to become apparent and thus constitutes a LOAEL.
No human data are available for 1,2,3,4-TeCB or PeCB.

For 1,2,3,4-TeCB, a NOAEL of 3.4 mg/kg b.w./day has been observed for renal and hepatic toxicity in male rats, the most sensitive sex.

For PeCB, effects in the liver and thyroid were observed from 33 mg/kg diet in both rats (corresponding to about 2.0 mg/kg b.w./day) and mice (corresponding to about 6 mg/kg b.w./day) thus constituting a LOAEL.

For HCB, the pathway for the biosynthesis of haem in the liver is a major target, leading to porphyria. Porphyria has been reported in rats following oral exposure at dose levels between 2.5 and 15 mg/kg b.w./day for 8 to 15 weeks and from 4 mg/kg b.w./day for 9-10 months; a NOAEL cannot be determined for porphyria based on these studies. Other effects in the liver include induction of microsomal enzymes, increased liver weight, hepatocellular hypertrophy, and cellular damage; NOAELs for enzyme induction and histopathological changes were between 0.05 and 0.07 mg/kg b.w./day.
Repeated oral exposure to HCB has also caused changes in calcium homeostasis and bone morphometry in rats at dose levels from 0.7 mg/kg b.w./day for 5 and 15 weeks, respectively, but not at 0.07 mg/kg b.w./day.
Oral exposure to HCB affects the immune system, with immunosuppressive effects observed in mice and monkeys, and immunostimulatory effects in rats and dogs. Immunosuppresive effects have been observed in mice exposed to approximately 0.6 mg/kg b.w./day. The developing immune system of the rat seems to be particularly vulnerable to the immunotoxic action, a LOAEL of 0.2 mg/kg b.w./day has been reported.
Other effects observed in experimental animals following repeated oral exposure to HCB include effects in the kidneys, thyroid, lungs, heart, nervous system, and changes in haematological parameters. These effects have generally been observed at higher dose levels than those at which the effects in the liver, bone and the developing immune system have been observed.

The available epidemiological studies taken together do not support an association between HBC exposure and increased cancer incidence, but due to limitations in these studies, no clear conclusion can be drawn. HCB is carcinogenic by oral administration in rats, mice, and hamsters. Liver cell tumours have been observed in all three species following dietary administration, in rats from 4 mg/kg b.w./day for up to 2 years, in mice from 12 mg/kg b.w./day for 120 weeks, and in hamsters from 4 mg/kg b.w./day for lifetime. Individual studies have also reported increased incidences of kidney, thyroid, parathyroid, and adrenal gland tumours. Studies on the mode of action for the hepatocarcinogenicity indicate that HCB is a promoter but not an initiator of liver cancer.
HCB is considered by IARC (2001) as possibly carcinogenic to humans (Group 2B; evidence in humans inadequate, evidence in animals sufficient). US-EPA has considered HCB as a probable human carcinogen (Group B2; evidence in humans inadequate, evidence to animals sufficient) (IRIS 2004b) and PeCB as not classifiable as to human carcinogenicity (Group D; no human data and no animal data available (IRIS 2004a). In the EU, HCB is classified for carcinogenic effects in category 2 (Carc. Cat. 2, R45 – may cause cancer) (MM 2002).
No data are available regarding carcinogenic effects of 1,2,3,4-TeCB and PeCB.
HCB has been tested in several *in vitro* and *in vivo* genotoxicity studies. Most studies have yielded negative results, but weak positive results have been reported as well. PeCB was negative in the available studies (three *in vitro*, 2 *in vivo*); however, positive results have been observed for some of the metabolites. No data are available regarding mutagenic and genotoxic effects of 1,2,3,4-TeCB. Overall, the three chlorobenzenes included in this evaluation is considered as being non-genotoxic substances; however, a genotoxic potential cannot be fully excluded as weak positive results have been obtained in assays with HCB.

Overall, a carcinogenic potential of HCB cannot be excluded for humans in relation to dietary exposure to HCB, but is not considered to be significant at the exposure levels at which the effects in the liver, bone and the immune system have been observed.
The carcinogenic potential of 1,2,3,4-TeCB and PeCB cannot be evaluated as no data are available.

Several miscarriages and stillbirths have been reported among women with previous oral exposure to HCB in the Turkish poisoning incident (estimated intake: 0.8-3.3 mg/kg b.w./day for a number of months); serious developmental toxicity, including a high mortality rate, has been reported in infants of exposed mothers; and persistent symptoms of developmental toxicity has been reported among men and women who had been exposed as children in the poisoning incident.
Studies in experimental animals using oral administration have shown that exposure to HCB is associated with reproductive effects, including ovarian lesions and hormonal and menstrual changes in female rats and monkeys, reduced fertility in rats, reduced mating index in male rats, and testicular effects in male rats. The ovary is the most sensitive target and subchronic studies in monkeys have revealed dose-related degenerative changes in the ovaries at all dose levels tested (from 0.01 mg/kg b.w./day). Overall, a NOAEL cannot be established for reproductive toxicity of HCB; a LOAEL of 0.01 mg/kg b.w./day is considered.
HCB is also a developmental toxicant in experimental animals following oral administration with effects in the developing immune system observed in rats from 0.2 mg/kg b.w./day and in mice from 0.5 mg/kg b.w./day; neurodevelopmental effects in rats at 1.3 mg/kg b.w./day but not at 0.6 mg/kg b.w./day; and reduced neonatal viability and growth, and organ weight changes in rats from 4-5 mg/kg b.w./day. In a 2-generation study in rats, peri-biliary lymphocytosis and fibrosis of the liver were observed in F_1 adult males at the lowest dose level tested (0.016 mg/kg b.w./day); however, these effects were, according to US-EPA (IRIS 2004b), not being considered to be induced by HCB because the effects were observed in a large number of F_1 adult control males as well. Some evidence of teratogenic effects has been reported in studies of rats and mice at rather high exposure levels (> 40 mg/kg b.w./day). Overall, a NOAEL of 0.016 mg/kg b.w./day is considered for developmental toxicity of HCB.

No human data are available regarding reproductive and developmental effects of 1,2,3,4-TeCB and PeCB, and in experimental animals, only limited data are available. Some data indicate that TeCBs and PeCB are embryotoxic and foetotoxic at dose levels that are not toxic for the mother; however, the available data are inconsistent. Overall, a NOAEL of 100 mg/kg b.w./day can be established for developmental effects of 1,2,3,4-TeCB. For PeCB, a NOAEL cannot be established for reproductive or developmental toxicity; a LOAEL of 17 mg/kg b.w./day is considered.

2.9.8 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of HCB are the effects observed in the liver, bone and the immune system, carcinogenicity, and reproductive and developmental effects.
A dose level of 50-200 mg/day (0.8-3.3 mg/kg b.w./day for a 60-kg person) constitutes a LOAEL for effects of HCB observed in humans in the Turkish poisoning incident. In experimental animals, a LOAEL of 2.5 mg/kg b.w./day is considered for porphyria, a NOAEL of 0.05 mg/kg b.w./day for enzyme induction and histopathological changes in the liver, a NOAEL of 0.07 mg/kg b.w./day for changes in calcium homeostasis and bone morphometry, a LOAEL of 0.2 mg/kg

b.w./day for effects in the immune system, a LOAEL of 0.01 mg/kg b.w./day for reproductive toxicity, and a NOAEL of 0.016 mg/kg b.w./day for developmental toxicity. Overall, a LOAEL of 0.01 mg/kg b.w./day is considered for adverse health effects of HCB. This LOAEL is considered to be sensitive and to provide adequate protection against the adverse effects observed in the liver, bone and the immune system in experimental animals, and is taken forward to the risk characterisation.

For 1,2,3,4-TeCB, the critical effects following dietary intake are the effects in the liver and kidney and for PeCB, the effects in the liver and thyroid.
No human data are available for 1,2,3,4-TeCB or PeCB. In experimental animals, a NOAEL of 3.4 mg/kg b.w./day is considered for toxicity in the liver and the kidneys of 1,2,3,4-TeCB, and is taken forward to the risk characterisation. For PeCB, a NOAEL cannot be established based on the available data; a LOAEL of 2.0 mg/kg b.w./day is considered for effects in the liver and thyroid, and is taken forward to the risk characterisation.

A carcinogenic potential of HCB for humans in relation to dietary exposure cannot be excluded, but is not considered to be significant at the exposure levels at which the effects in the liver, bone, and immune system, and reproductive and developmental effects have been observed. Furthermore, a genotoxic potential of HCB cannot be excluded either. The carcinogenic potential of 1,2,3,4-TeCB and PeCB cannot be evaluated as no data are available.

2.9.9 References

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2.10 Hexachlorocyclohexanes

Hexachlorocyclohexane (HCH) consists of eight isomers. Only α-, β-, γ-, and δ-HCH are of commercial significance. The term 'lindane' refers to pesticide products containing >99% γ-HCH. Technical HCH (t-HCH) is a mixture of several isomers and consists of approximately 60-70% α-HCH, 5-12% β-HCH, 10-15% γ-HCH, 6-10% δ-HCH, and 3-4% ε-HCH.

HCH levels in traditional Greenland food items have been presented as sHCH (sum of α-, β- and γ-HCH) (Johansen et al. 2004). Therefore, this evaluation only considers these three isomers of HCH and the term 'HCH' in this evaluation refers to these three isomers unless otherwise stated. Data on t-HCH are included in this evaluation when considered relevant.

γ-HCH is a broad-spectrum insecticide, which has been used world-wide for agricultural (mostly for seed and soil treatment) as well as non-agricultural purposes (wood and timber protection, treatment of animals against ectoparasites). Several countries have restricted the use of γ-HCH and it is not used in Denmark nowadays. β-HCH is the most persistent HCH isomer in the environment. (WHO 1991a,b, WHO 1996, ATSDR 2005).

The general population is exposed to HCH mainly through the diet and HCH is found in dairy products, meat, fish, poultry, garden fruits, oils and fats, leafy and root vegetables, and sugar. The estimated daily human intake has decreased with time probably due to the restriction or banning of the use of HCH. Daily intake of HCH isomers in adult diets in the USA in 1981-1982 has been estimated to be 0.010 µg/kg b.w./day for total HCH, 0.008 µg/kg b.w./day for α-HCH, <0.001 µg/kg b.w./day for β-HCH and δ-HCH, and 0.002 µg/kg b.w./day for γ-HCH. (ATSDR 2005, WHO 1996, WHO 1991a,b).

In Denmark, the estimated average dietary intake of α-HCH for adults (1993-1997) was 0.2 µg/day and the 95 percentile was 0.3 µg/day, for β-HCH 0.3 µg/day and 0.5 µg/day, and for γ-HCH 0.3 µg/day and 0.4 µg/day, respectively (FDIR 2000).

2.10.1 Toxicokinetics

Absorption of HCH following oral exposure has been inferred from humans who have experienced adverse health effects or who had increased serum levels of the various isomers following oral exposure (ATSDR 2005).
In experimental animals, HCH is readily absorbed from the gastrointestinal tract following oral administration. Absorption is enhanced in the presence of lipids and is almost complete when administered in the feed (97.4% for α-HCH, 90.7% for β-HCH, 99.4% for γ-HCH, and 94.9% for t-HCH). (Nielsen 1999, WHO 1996, WHO 1991a,b, ATSDR 2005).

After uptake, HCH is distributed to all organs and tissues in the body within a few hours. For α-HCH, the highest concentrations have been found in liver, kidneys, body fat, brain and muscles, and substantial deposition occurs in fatty tissues; the fat/blood ratio has been reported to be about 400. For β-HCH, the highest concentrations are also found in fat followed by kidney, lungs, liver, muscle, heart, spleen, brain and blood; the fat/blood ratio has been reported to be about 170. For γ-HCH, the highest concentrations are found in fat followed by brain, kidney, muscle, lungs, heart, spleen, liver, and blood; the fat/blood ratio has been reported to be about

150-200. β-HCH accumulates in tissues to a greater degree than γ-HCH except in the brain, where γ-HCH accumulates at a higher concentration. The greater accumulation of β-HCH in tissues is expected since this isomer is metabolised more slowly. In addition, γ-HCH is known to induce the liver mixed-function oxygenase system, and thus self-induced metabolism is an important factor that minimises the accumulation of γ-HCH residues in the tissues. Following 4 to 6 weeks after cessation of exposure, γ-HCH concentrations in all organs, including adipose tissue, are close to the control values. (Nielsen 1999, WHO 1991a,b, ATSDR 2005).

γ-HCH is metabolised mainly in the liver by four enzymatic reactions (dehydrogenation, dehydrochlorination, dechlorination, and hydroxylation) with a large number of metabolites and end-products occurring during the metabolism, including chloro-cycloalkenes, chlorobenzenes, and chlorophenols. The metabolism of α-HCH in rats involves dechlorination and the major metabolite is 2,4,6-trichlorophenol; other identified metabolites include other chlorophenols. Isomeration of α-HCH to γ-HCH has not been reported to occur after repeated doses. β-HCH is metabolised by cis-dehydrochlorination and the major metabolite is 2,4,6-trichlorophenol; β-HCH is metabolised more slowly than γ-HCH. (Nielsen 1999, WHO 1991a,b, ATSDR 2005).

The metabolites are excreted mainly via the urine in the free form or in conjugated form. γ-HCH and α-HCH are readily conjugated with glutathione whereas β-HCH seems to resist conjugation with glutathione. γ-HCH metabolites are also excreted in the form of glucuronides and sulfate conjugates. The elimination of γ-HCH is relatively fast, with a half-time in rats of 3 to 4 days. (Nielsen 1999, WHO 1991a,b, ATSDR 2005).

HCH crosses the placenta of both humans and experimental animals and reaches the foetus and is also excreted in human milk (WHO 1991a,b, ATSDR 2005).

2.10.2 Single dose toxicity

Several cases of fatal poisoning and numerous cases of non-fatal illness caused by γ-HCH have been reported. The most commonly reported effects associated with oral exposure to γ-HCH are neurological effects and symptoms include nausea, restlessness, headache, vomiting, tremor, ataxia, and tonic-clonic convulsions. The toxic or lethal dose appears to vary considerably; 10 to 20 mg/kg b.w. can present a lethal hazard to humans, but higher doses has also been tolerated. Some information indicates that children are more sensitive to γ-HCH than adults. No specific information on α-HCH and β-HCH is available. (Nielsen 1999, WHO 1991a,b, WHO 1996, ATSDR 2005).

In experimental animals, acute toxicity of γ-HCH is moderate with oral LD_{50}-values for mice and rats in the range of 55 to 250 mg/kg b.w. and 90 to 270 mg/kg b.w., respectively. For α-HCH, reported oral LD_{50}-values for mice and rats are between 500 and 4670 mg/kg b.w.; for β-HCH between 1500 and 2000 mg/kg b.w.; and for t-HCH about 2400 mg/kg b.w. (rats). Signs of toxicity are mainly those of central nervous system stimulation. (Nielsen 1999, WHO 1991a,b, WHO 1996, ATSDR 2005).

2.10.3 Repeated dose toxicity

The most commonly reported effects associated with occupational exposure to γ-HCH are neurophysiological and neuropsychological disorders, gastrointestinal disturbances, and increased activity of drug metabolising enzymes in the liver (WHO 1991a,b, WHO 1996, ATSDR 2005). However, information on the duration and exposure concentration is generally not available in these studies and they can therefore not be used in the risk characterisation. No oral studies are available.

γ-HCH is the isomer most thoroughly tested in intermediate- and long-term studies in experimental animals. Following repeated oral administration of HCH, target organs and tissues identified in experimental animals include the liver, kidney, and the immune and nervous systems.

2.10.3.1 Effects on the liver

The liver is a major target organ in rodents and dogs following oral administration of HCH. Effects observed include increased activity of liver enzymes, increased liver weight, liver cell hypertrophy, and histopathological changes. (Nielsen 1999, JMPR 1997, WHO 1991b, WHO 1996, ATSDR 2005, IRIS 2004c).

A number of repeated dose toxicity studies have been carried out in which rats were administered γ-HCH with the feed. In one 2-year study, no effects were observed in the liver at 0.5 mg/kg b.w./day; at dose levels from about 5.0 mg/kg b.w./day, liver cell hypertrophy was observed. In a 90-day study, the NOAEL for liver effects was considered to be 0.3 mg/kg b.w./day with increased enzyme activities and organ weight, and histological changes being observed from about 1.5 mg/kg b.w./day. In a 2-year dietary study with dogs, the liver was slightly enlarged at 2.9 mg/kg b.w./day but without any detected histopathological change; no effects were observed in the liver at 1.6 mg/kg b.w./day. (Nielsen 1999, JMPR 1997, WHO 1991b, WHO 1996, ATSDR 2005, IRIS 2004c).

For α-HCH, a valid 90-day dietary study in rats revealed increased liver weight from 0.5 mg/kg b.w./day, and increased enzyme activities and histological changes from 2.5 mg/kg b.w./day; a NOAEL of 0.1 mg/kg b.w./day was observed for liver effects. In a 2-week dietary study in male rats, α-HCH caused a clear increase in the activity of liver enzymes following administration of 0.25 mg/kg b.w./day (WHO 1991a, ATSDR 2005).

For β-HCH, a valid 90-day dietary study with rats revealed increased enzyme activities from 0.1 mg/kg b.w./day (the lowest dose level in the study) and increased liver weight, centrilobular liver cell hypertrophy, and histological changes from 0.5 mg/kg b.w./day. In short-term studies (2 weeks) on liver enzyme induction in rats, the highest dose level without effect was 10 mg/kg b.w. (WHO 1991a, ATSDR 2005).

For t-HCH, a NOAEL of 0.4 mg/kg b.w./day has been observed in a 360-day study in rats; increased liver weight was observed in males from 2 mg/kg b.w./day and enlargement of hepatocytes, nuclear pyknosis, vacuolation and necrosis from 20 mg/kg b.w./day. In short-term studies with rats, increased liver enzyme activities have been observed from 5 mg/kg b.w./day. (ATSDR 2005).

2.10.3.2 Effects on the kidney

Adverse effects on the kidneys have been observed only in male rats following exposure to γ-HCH and include nephritis, accumulation of protein droplets, renal cell hypertrophy, and necrosis (Nielsen 1999, JMPR 1997, WHO 1991b, WHO 1996, ATSDR 2003). These effects on the kidneys in male rats are associated with a mechanism of toxicity specific for male rats (based on a male rat specific protein α-2μ-globulin, a protein that is not present in humans) and therefore, the effects are not toxicologically relevant for humans. (Nielsen 1999, JMPR 1997).

Exposure to α-HCH has resulted in nephritis in rats fed 72-80 mg/kg b.w./day for an average of about 36 weeks; no effects were observed at 5 mg/kg b.w./day (ATSDR 2005, WHO 1991a).

Increased kidney weight has been observed in female rats exposed to β-HCH at about 0.2 mg/kg b.w./day for 13 weeks; both males and females exhibited renal calcinosis at 22.5 mg/kg b.w./day (ATSDR 2005, WHO 1991a).

t-HCH exposure has resulted in tubular necrosis and glomerular degeneration in male rats from 20 mg/kg b.w./day in the diet for 360 days; the NOAEL was 2 mg/kg b.w./day. (ATSDR 2005).

2.10.3.3 Immunological effects

Studies in experimental animals indicate that HCH may be immunosuppressive, at least during short to intermediate duration exposures (ATSDR 2005, JMPR 1997, WHO 1991a,b).

For γ-HCH, immunomodulating effects (increase in serum albumin/globulin following injection with tetanus toxoid, impaired increase in total IgM and IgG levels in response to immunisation, altered cellular immune function) have been observed in rats from 2.0 mg/kg b.w./day in the diet for 12-22 weeks; no effects were observed at 0.25 mg/kg b.w./day. A biphasic dose-dependent immunological effect on components of cellular and humoral mediated immunity, characterised by initial stimulation (all doses up to weeks 4-8 of exposure) followed by immunosuppression (until termination of study), was reported in mice administered γ-HCH from 0.012 mg/kg b.w./day for 24 weeks; histological alterations in lymphoid organs were noted at 1.2 mg/kg b.w./day. Immunosuppression has also been noted in rabbits administered γ-HCH orally from 1.5 mg/kg b.w./day for 5-6 weeks. (Nielsen 1999, JMPR 1997, ATSDR 2005).

For α-HCH, serum levels of IgG and IgM were decreased in rats following dietary administration from 2.5 mg/kg b.w./day for 13 weeks, but not at 0.5 mg/kg b.w./day (WHO 1991a).

For β-HCH, changes in several immune functions were observed in female mice following dietary administration at 60 mg/kg b.w./day, but not at 20 mg/kg b.w./day (ATSDR 2005, WHO 1991a).

2.10.3.4 Effects on the nervous system

A range of various neurological effects, both central and peripheral, have been observed in rats and mice following oral exposure to HCH (ATSDR 2005, JMPR 1997, WHO 1991a,b).

The neurotoxic effects of γ-HCH have been examined in a number of studies. A recent 13-week study using a neurotoxicity screening battery revealed neurotoxic effects in rats at about 30 mg/kg b.w./day, but not at about 7.5 mg/kg b.w./day. Other studies have shown changes in the electroencephalogram and decrements in a variety of behavioural parameters in rats at 5 mg/kg b.w./day for 40 days, but not at 2.5 mg/kg b.w./day for 22 days. (ATSDR 2005, WHO 1991b).

Neurological effects (changes in the electroencephalogram or decrements in a variety of behavioural parameters) have not been observed in rats following oral exposure to α-HCH at dose levels up to about 100 mg/kg b.w./day for 30 days (ATSDR 2005, WHO 1991a).

β-HCH induced a decrease in peripheral nerve conduction velocity in rats fed about 60 mg/kg b.w./day for 30 days, but no changes in the electroencephalogram were observed at dose levels up to about 300 mg/kg b.w./day for 30 days. Ataxia and coma have been observed in rats at 22.5-25 mg/kg b.w./day for 13 weeks, but not at 5 mg/kg b.w./day. (ATSDR 2005, WHO 1991a).

For t-HCH, convulsions, tremors and paralysis were observed in male rats exposed to 0.4 mg/kg b.w./day for 270 days and histological changes in the brain at 20 mg/kg b.w./day; no effects were observed at 0.04 mg/kg b.w./day (ATSDR 2005).

2.10.4 Genotoxicity

No increase in the frequency of chromosome aberrations was observed in humans exposed primarily to γ-HCH by inhalation in a pesticide production factory for at least 6 months (ATSDR 2005, IARC 1987).

γ-HCH has been tested in several *in vitro* and *in vivo* genotoxicity studies. The vast majority of the studies have yielded negative results, but positive results have been reported as well. It gave negative results for the ability to induce gene mutations in bacteria, yeast and in mammalian cells *in vitro*; for unscheduled DNA synthesis in mammalian cells *in vitro*; and for the capacity to induce sex-linked recessive lethal mutations in *Drosophila melanogaster*. Equivocal results have been obtained for chromosomal damage *in vitro* but negative results *in vivo*; negative results were also reported for sister chromatid exchange in mammalian cells *in vivo* and for induction of micronucleus *in vivo*. The results of assays for covalent binding to DNA in the liver of rats and mice *in vivo* following oral administration were also negative. Negative or equivocal results have been observed in the dominant lethal test in rats and mice. (Nielsen 1999, ATSDR 2005, WHO 1991b, IARC 1987). According to WHO (1991b), the few studies, in which positive results were obtained involved invalid study designs or γ-HCH of unknown purity.

α-HCH was negative in yeast for the ability to induce gene mutations and did not cause DNA damage in bacteria. In a test for unscheduled DNA synthesis in rat hepatocytes *in vitro*, α-HCH gave an equivocal result. It has been reported to bind to calf thymus DNA *in vitro* and to mouse liver DNA *in vivo*, and increased the mitotic rate and frequency of polyploidy cells in rat hepatocytes *in vivo*. (ATSDR 2005, WHO 1991a, IARC 1987).

β-HCH was negative in tests with bacteria for the ability to induce gene mutations and DNA damage, but a positive result for chromosome aberrations has been reported in bone marrow cells of rats (ATSDR 2005, WHO 1991a, IARC 1987).

t-HCH induced a dose-dependent increase in chromosomal aberrations and in sister chromatid exchanges in cultured human lymphocytes, but only at high doses. It was also reported to induce dominant lethal mutations but not chromosomal aberrations in bone marrow cells of mice exposed *in vivo*. (ATSDR 2005, IARC 1987).

2.10.5 Carcinogenicity

No data are available regarding the carcinogenicity of the individual isomers of HCH or t-HCH following ingestion by humans (ATSDR 2005).

Attempts to evaluate the carcinogenic potential of γ-HCH have been carried out in a number of studies with mice and rats using dose levels of up to 90 mg/kg b.w./day in mice and up to 80 mg/kg b.w./day in rats. No treatment-related increase in tumour incidences has been observed in rats. Liver tumours have been observed in some studies in mice at relatively high dose levels; however, other studies using similar dose levels did not reveal a carcinogenic potential in mice. (Nielsen 1999, ATSDR 2005, WHO 1991b, IARC 1987).

Dietary α-HCH has been shown to cause increased incidences of liver tumours in five mouse strains at dose levels from about 12.5 mg/kg b.w./day for 24-32 weeks and in one strain of rats at about 20 mg/kg b.w./day for lifetime (IRIS 2004a, ATSDR 2005, WHO 1991a, IARC 1987).

β-HCH induced liver tumours in one dietary study of mice at about 30 mg/kg b.w./day for 110 weeks. No evidence of increased tumour incidence was seen in several small (5-20 animals/group) studies with male and female mice at dietary dose levels of up to about 90 mg/kg b.w./day for 24-32 weeks, or in rats up to about 50 mg/kg b.w./day. (IRIS 2004b, ATSDR 2005, WHO 1991a, IARC 1987).

Increased incidences of liver tumours have been observed in four strains of mice exposed to t-HCH at dietary dose levels from about 20 mg/kg b.w./day for more than 18 months. Dietary administration of t-HCH at levels up to about 25 mg/kg b.w./day has not been shown to produce tumours in rats after 30 months of treatment. (IRIS 2004d, ATSDR 2005).

2.10.6 Toxicity to reproduction

The only available human data are from one study on hormone levels in pesticide workers, in which increases in the levels of serum luteinising hormone were noted following exposure to γ-HCH for 8 years (Nielsen 1999, ATSDR 2005).

γ-HCH has been tested for reproductive effects in a 2- and in a 3-generation study in rats. No reproductive effects were observed at dose levels up to 5-7.5 mg/kg b.w./day. In the 2-generation study, decreased viability and increased incidences of hydronephrosis and hydroureter were observed in the offspring at 7.5 mg/kg b.w./day. In the 3-generation study, morphological changes in the liver indicating enzyme induction occurred in the offspring of the third generation from 2.5 mg/kg b.w./day. The NOAEL for developmental toxicity was 1-1.25 mg/kg b.w./day. γ-HCH has been tested for embryotoxic and teratogenic effects after oral administration in rats, mice, and rabbits. In rats, no embryotoxic or teratogenic effects were observed at dose levels up to 12.5 mg/kg b.w./day during organogenesis; maternal toxicity was noted from 10 mg/kg b.w./day. In mice, no embryotoxic or teratogenic effects were observed at dose levels up to 30 mg/kg

b.w./day during organogenesis; increased pup mortality, decreased pup weight and maternal toxicity was noted at 60 mg/kg b.w./day. In rabbits, no embryotoxic or teratogenic effects were observed at dose levels up to 10 mg/kg b.w./day during organogenesis; increased number of pups with extra ribs was noted at 20 mg/kg b.w./day; maternal effects were observed from 5 mg/kg b.w./day. In a very recent study, developmental and reproductive effects were observed in male offspring of rats that were exposed to 1 mg/kg b.w./day on days 9-14 of lactation. (Nielsen 1999, ATSDR 2005, WHO 1991b, WHO 1996).

For α-HCH, no data are available.

In a 2-generation study in rats, litter size was reduced and there was almost complete infertility following dietary exposure to β-HCH from 0.5 mg/kg b.w./day; no effects were observed at 0.1 mg/kg b.w./day. Histological changes in the ovaries and testes and disruption of spermatogenesis have been observed in rats exposed to dietary levels of 12.5 mg/kg b.w./day for 13 weeks; decreased testis weight was observed at 2.5 mg/kg b.w./day; no effects were observed at 0.1 mg/kg b.w./day. In rats, increased pup mortality was observed at 20 mg/kg b.w./day during gestation and 5 mg/kg b.w./day during gestation and lactation resulted in increased liver weights of pups. (ATSDR 2005, WHO 1991a).

t-HCH has been tested for reproductive effects in a 3-generation study in rats. There were no effects on reproduction in any of the three parental generations or no effects in any of the offspring generations at dose levels up to 32 mg/kg b.w./day; effects were observed in the first parental generation from 16 mg/kg b.w./day, but not in the subsequent parental generations. Testicular degeneration has been reported in male rats exposed to 20 mg/kg b.w./day but not at 2 mg/kg b.w./day in the diet for 360 days, and in mice at 90 mg/kg b.w./day for 3 months. (ATSDR 2005).

2.10.7 Evaluation

γ-HCH is the isomer most thoroughly tested in intermediate- and long-term experimental studies. γ-HCH is the most toxic isomer in acute toxicity studies, but the available data from intermediate- and long-term experimental studies do not indicate noteworthy differences in the general toxicity of the isomers. However, isomer specific differences are indicated for reproductive/developmental and carcinogenic effects.

Repeated oral exposure to HCH is associated with effects in the liver, kidney, and the immune and the nervous systems, carcinogenicity, and reproductive and developmental effects.

No adequate human data are available in order to evaluate the potential of HCH to induce toxic effects in humans.

The liver is a major target organ in rodents and dogs and the effects observed include increased activity of liver enzymes, increased liver weight, liver cell hypertrophy, and histological changes. Based on the available data in experimental animals, the NOAEL for liver effects is considered to be 0.1 mg/kg b.w./day for α-HCH in rats, 0.1 mg/kg b.w./day for β-HCH in rats, 0.3-0.5 mg/kg b.w./day for γ-HCH in rats and 1.6 mg/kg b.w./day in dogs, and 0.4 mg/kg b.w./day for t-HCH in rats. For β-HCH, increased liver enzyme activities were observed in rats from 0.1 mg/kg b.w./day; however, in short-term studies (2 weeks) on liver enzyme induction in rats, the highest dose level without effect was 10 mg/kg b.w. Overall, a

NOAEL of 0.1 mg/kg b.w./day is considered for adverse effects in the liver following dietary administration of HCH; this dose level is a LOEL for increased liver enzyme activities for β-HCH.

Functional effects and histological changes in the immune system are induced by γ-HCH in mice, rats, and rabbits; a NOAEL of 0.25 mg/kg b.w./day has been observed for rats. For mice and rabbits, a NOAEL cannot be established based on the available data; a LOAEL of 0.012 mg/kg b.w./day and of 1.5 mg/kg b.w./day is noted for mice and rabbits, respectively. For α-HCH, a NOAEL of 0.5 mg/kg b.w./day has been observed for immunological effects in rats and for β-HCH, a NOAEL of 20 mg/kg b.w./day in female mice. Overall, a LOAEL of 0.012 mg/kg b.w./day is considered for immunological effects of HCH.

A range of various neurological effects, both central and peripheral, has been observed in rats and mice following oral exposure to HCH. Based on the available data in experimental animals, the NOAEL for neurological effects is considered to be 100 mg/kg b.w./day for α-HCH in rats, 5 mg/kg b.w./day for β-HCH in rats, 2.5 mg/kg b.w./day for γ-HCH in rats, and 0.04 mg/kg b.w./day for t-HCH in rats. Overall, a NOAEL of 0.04 mg/kg b.w./day is considered for neurological effects of HCH.

Other effects observed in experimental animals following repeated oral exposure to HCH include effects in the kidneys. For γ-HCH, the effects in the kidneys are only observed in male rats and associated with a mechanism of toxicity specific for male rats, and are not toxicologically relevant for humans. For α-HCH and β-HCH, effects have been observed in both sexes of rats, but generally at higher exposure levels than those at which the effects in the liver have been observed. For t-HCH, effects have been observed in male rats only, but at higher exposure levels than those at which the effects in the liver have been observed.

t-HCH as well as the specific isomers have produced liver tumours in mice when administered orally; however, negative results have also been observed for β-HCH and γ-HCH. In two studies in rats, an increased incidence of liver tumours was observed with α-HCH whereas no evidence of increased tumour incidence in rats have been observed in dietary studies with β-HCH, γ-HCH and t-HCH.
HCH is considered by IARC (1987) as possible carcinogenic to humans (Group 2B; evidence in humans inadequate, evidence in animals sufficient for t-HCH and for α-HCH but limited for β-HCH and γ-HCH). US-EPA has considered α-HCH as a probable human carcinogen (Group B2; evidence in humans inadequate, evidence to animals sufficient) (IRIS 2004a); β-HCH as a possible human carcinogen (Group 3; evidence in humans inadequate, evidence to animals limited) (IRIS 2004b); and t-HCH as a probable human carcinogen (Group B2; evidence in humans inadequate, evidence to animals sufficient) (IRIS 2004d); γ-HCH has not been assigned a cancer classification by US-EPA. In the EU, HCH except γ-HCH is classified for carcinogenic effects in category 3 (Carc. Cat. 3, R40 – limited evidence of a carcinogenic effect); no classification for carcinogenic effect has been assigned to γ-HCH (MM 2002).
γ-HCH has been tested in several *in vitro* and *in vivo* genotoxicity studies. The vast majority of the studies have yielded negative results, but positive results have been reported as well. α-HCH, β-HCH and t-HCH have given both negative and positive results in the few available studies. γ-HCH appears to have no genotoxic potential *in vivo*, but the data provide no conclusive evidence; the genotoxic potential of the two other isomers and of t-HCH cannot be evaluated based on the available data. Overall, a genotoxic potential of HCH cannot be excluded.

According to WHO (1991a,b), the results of studies on initiation-promotion, on mode of action, and on genotoxicity indicate that the carcinogenic effect of α-HCH, β-HCH and γ-HCH in mice may be due to non-genotoxic mechanisms. Overall, a carcinogenic potential of HCH cannot be excluded for humans in relation to dietary exposure to HCH, but is not considered to be significant at the exposure levels at which other effects in the liver have been observed.

γ-HCH had no reproductive effects in rats (2- and 3-generation studies) at dietary dose levels up to 5-7.5 mg/kg b.w./day; the NOAEL for developmental toxicity was 1-1.25 mg/kg b.w./day. In a very recent study, a LOAEL of 1 mg/kg b.w./day has been noted for developmental and reproductive effects in male offspring of rats exposed on days 9-14 of lactation. Embryo-foetal effects including malformations were only noted in rats, mice and rabbits at dose levels, which also gave rise to maternal effects. β-HCH produced reproductive and developmental effects in rats (2-generation study) from 0.5 mg/kg b.w./day, but no teratogenic effects; the NOAEL was 0.1 mg/kg b.w./day. t-HCH showed no reproductive or developmental effects, including teratogenic effects, in rats (3-generation study) at dose levels up to 32 mg/kg b.w./day. No data are available for α-HCH. Overall, a NOAEL of 0.1 mg/kg b.w./day is considered for reproductive and developmental effects of HCH.

2.10.8 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of HCH are the effects observed in the liver, the immune and nervous systems, carcinogenicity, and reproductive and developmental toxicity.

No human data are available.

In experimental animals, a NOAEL of 0.1 mg/kg b.w./day is considered for adverse effects in the liver following dietary administration of HCH (this dose level is a LOEL for increased liver enzyme activities for β-HCH); a LOAEL of 0.012 mg/kg b.w./day for immunological effects, a NOAEL of 0.04 mg/kg b.w./day for neurological effects, and a NOAEL of 0.1 mg/kg b.w./day for reproductive and developmental effects. Overall, a LOAEL of 0.01 mg/kg b.w./day is considered for adverse health effects in experimental animals. This LOAEL is considered to be sensitive and to provide adequate protection against liver toxicity and probably also carcinogenicity, as well as against neurotoxicity, and reproductive and developmental effects of HCH, and is taken forward to the risk characterisation.

A carcinogenic potential of HCH for humans in relation to dietary exposure cannot be excluded, but is not considered to be significant at the exposure levels at which the effects on the immune system have been observed. Furthermore, a genotoxic potential of HCH cannot be excluded either.

2.10.9 References

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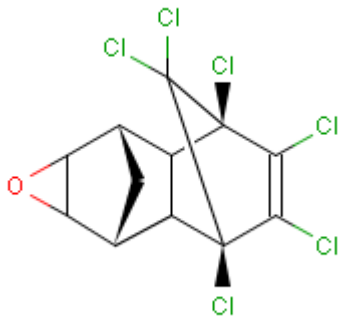
2.11 Dieldrin

Dieldrin is the common name of an insecticide containing 85% HEOD. HEOD is a synthetic organochlorine compound with the IUPAC chemical name 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro- *endo*-1,4- *exo*-5,8-dimethanonapthalene (see the structural formula).



Aldrin, also an organochlorine insecticide, is readily metabolised to dieldrin in animals; dieldrin is the corresponding epoxide to aldrin (WHO 1989, ATSDR 2002). Therefore, data on aldrin are included in this evaluation when relevant.

Dieldrin has been used world-wide in agriculture for the control of many soil pests and in the treatment of seed. Since the early 1970s, the use of dieldrin has been restricted or banned, in a number of countries, especially in agriculture. (WHO 1989, WHO 1996).

The general population is exposed to dieldrin mainly through the diet and dieldrin has been detected in dairy products, meat products, fish, oils and fats, potatoes, and certain other root vegetables. Maximum residue limits in the range of 0.02 to 0.2 mg/kg product have been recommended. Recent studies have shown that the actual concentrations of dieldrin in these food commodities are generally lower probably due to the restriction or banning of the use of dieldrin. The intake in 1980-1982 was estimated to be 0-0.2 µg/kg b.w. per day in several countries. (WHO 1989, WHO 1996).

In Denmark, the estimated average dietary intake of dieldrin for adults (1993-1997) was 0.3 µg/day and the 95 percentile was 0.4 µg/day (FDIR 2000).

2.11.1 Toxicokinetics

In both animals and human beings, aldrin and dieldrin is readily absorbed from the gastrointestinal tract. Animal studies indicate that dieldrin is absorbed rather quickly and that the amount absorbed is proportional to the dose. Aldrin is rapidly converted to dieldrin, mainly in the liver but also in some other tissues. (ATSDR 2002, WHO 1989, WHO 1996).

After absorption, dieldrin is rapidly distributed from the blood to adipose tissue, brain, and liver, and is then redistributed primarily to fat. Dieldrin tends to concentrate in the lipid rich tissues, and particularly in adipose tissue. A steady state between intake, storage, and excretion is reached following repeated dosing.

Significant relationships have been found between the concentrations of dieldrin in the blood and those in other tissues and at equilibrium, the ratio of dieldrin concentrations in the adipose tissue, liver, brain, and blood has been reported to be about 150:15:3:1. (ATSDR 2002, WHO 1989, WHO 1996).

Part of the ingested dieldrin passes unabsorbed through the intestinal tract and is eliminated with the faeces, part is excreted unchanged from the liver into the bile, part is stored in the various organs and tissues particularly in the adipose tissue, and part is metabolised in the liver to more polar metabolites. In mammals, two degradation routes of dieldrin seem to be predominant. One is direct oxidation by cytochrome oxidases, resulting in 9-hydroxy dieldrin, which is the major metabolite. The other is opening of the epoxide ring by epoxide hydrases, resulting in 6,7-*trans*-dihydroxy dihydroaldrin. Both metabolites can undergo glucuronidation. In humans and in most animals, the metabolites are excreted primarily via the bile in the faeces. (ATSDR 2002, WHO 1989, WHO 1996).

Dieldrin is transported via the placenta and reaches the foetus. Accumulation takes place in the same organs and tissues as in the adult, but to a much lower level, and there seems to be an equilibrium between the levels in the mother and in the foetus. Dieldrin is also excreted in human milk. (WHO 1989, ATSDR 2002).

In humans, an estimated half-life for dieldrin elimination has been reported to be 369 days (ATSDR 2002).

2.11.2 Single dose toxicity

Aldrin and dieldrin are highly toxic to human beings. Data from cases of accidental or intentional poisonings indicate that the central nervous system is a major target organ in humans after oral intake. Acute oral exposure has also been reported to cause renal toxicity in humans. The lowest dose with a fatal outcome has been estimated to be 10 mg/kg b.w. Survivors of acute intoxications have recovered completely and irreversible effects have not been reported. (WHO 1989, ATSDR 2002, WHO 1996).

Aldrin and dieldrin is also of high acute toxicity in experimental animals with oral LD_{50}-values in the mouse and rat ranging from 33 to 70 mg/kg b.w. (ATSDR 2002, WHO 1989, WHO 1996).

2.11.3 Repeated dose toxicity

Data from human studies indicate that the nervous system is a major target organ after exposure to aldrin and dieldrin and studies in experimental animals have confirmed this observation. Other targets identified in experimental animals include the immune system, the kidney, and the liver. Intoxication following long-term exposure is characterised by involuntary muscle movements and epileptiform convulsions. In the liver, there is an increased activity of microsomal biotransformation enzymes, particularly of the cytochrome P-450 monooxygenase system. This induction of the microsomal enzymes is reversible and, if it exceeds a certain level, it appears to be linked to cytoplasmic changes and hepatomegaly in the liver of rodents. (ATSDR 2002, WHO 1989).

In a study on male volunteers exposed to dieldrin in capsules at doses up to approximately 3 µg/kg b.w./day for 18 months, no adverse health effects including effects on the nervous system (central as measure by EEG, peripheral nerve

activity, or muscle activity) and the liver (as indicated by normal serum levels of liver enzymes) were observed (ATSDR 2002, WHO 1989, WHO 1996).

Adverse effects from aldrin and dieldrin are related to the level of dieldrin in the blood. In workers exposed for a long time, the level of dieldrin in the blood below which adverse effects did not occur, as indicated by enzyme induction measurements, has been reported to be 105 µg/l corresponding to an oral intake of 17.4 µg/kg b.w./day. (WHO 1989).

The liver is a major target organ in rodents and dogs following oral administration of aldrin or dieldrin. The effects observed include increased relative liver weight, liver cell hypertrophy, cytoplasmic eosinophilia, and an increase in the smooth endoplasmic reticulum, microsomal protein, cytochrome P-450 contents, and/or in microsomal enzyme activity. The histopathological changes in the liver have become known as "Chlorinated Hydrocarbon Insecticide Rodent Liver" (CHIRL). A number of long-term studies have been carried out in which rats of different strains were administered dieldrin with the feed. In one 2-year study, no effects were observed in the liver at 0.005 mg/kg b.w./day of dieldrin. At dose levels from 0.025 mg/kg b.w./day, increased liver weight and histopathological changes were observed. In a 2-year study with dogs receiving dieldrin in olive oil, increased relative liver weight was observed at 0.05 mg/kg b.w./day, but not at 0.005 mg/kg b.w./day. (ATSDR 2002, WHO 1989, WHO 1996).

Adverse effects on the kidneys have been observed following exposure of rats and dogs to aldrin and/or dieldrin. Gross and microscopic examinations did not show adverse effects in the kidneys of rats exposed to 3.25 mg/kg b.w./day of dieldrin for up to 80 weeks or 0.5 mg/kg b.w./day of dieldrin for up to 2 years; or in dogs exposed to 0.05 mg/kg b.w./day dieldrin for up to 2 years. (ATSDR 2002).

Studies in animals, predominantly in mice indicate that aldrin and dieldrin may be immunosuppressive agents, at least during short to intermediate duration exposures. Effects have been observed in mice at dietary dose levels from 0.13 mg/kg b.w./day of dieldrin for 10 to 18 weeks; a no effect level has not been identified. (ATSDR 2002).

In monkeys, impaired learning (difficulty in learning a successive discrimination reversal task) was observed following administration of 0.1 mg/kg b.w./day of dieldrin orally for 55 days, but not at 0.01 mg/kg b.w./day. No effects of operant behaviour were observed in rats following 0.025 mg/kg b.w./day for 60-120 days. Irritability, tremors, and/or convulsions have been observed in rats exposed to dieldrin at dose levels ranging from 0.65 to 3.25 mg/kg b.w./day, but not at 0.05 mg/kg b.w./day, for 1.5-2 years. (ATSDR 2002).

2.11.4 Genotoxicity

In a study of a population occupationally exposed to several pesticides including aldrin, significant increases in sister chromatid exchanges and chromosome aberrations were observed. In another study, no chromosome aberrations were observed in peripheral blood lymphocytes from workers in a dieldrin manufacturing facility. (ATSDR 2002, IARC 1987).

Numerous studies investigating the *in vitro* and *in vivo* genotoxic effects of aldrin and dieldrin have been conducted. The vast majority of the studies have yielded negative results, but positive results have been reported as well. Dieldrin did not induce dominant lethal mutations in mice or chromosomal aberrations in bone

marrow cells of Chinese hamsters treated *in vivo*. It induced unscheduled DNA synthesis in transformed human fribroblasts but not in rat hepatocytes, and did not induce single-strand breaks in Chinese hamster V79 cells. Dieldrin inhibited intercellular communication in human and rodent cell systems. It did not induce sex-linked recessive lethal mutations in *Drosophila*, was not mutagenic to bacteria and did not induce breakage of plasmid DNA. (IARC 1987, ATSDR 2002, IRIS 2004, WHO 1989, WHO 1996).

2.11.5 Carcinogenicity

Epidemiological studies have examined cancer mortality in two cohorts of workers employed in the manufacture of aldrin and dieldrin. In one cohort, nine deaths from cancer occurred among 233 workers with 12 expected. In the other cohort, mortality from all cancers among 1155 workers was lower than expected although there was a slight excess of cancer of oesophagus, rectum, liver, and of the lymphatic and haematopoietic systems based on very small numbers. More recent follow-ups have suggested possible increases in liver and biliary tract cancer, and rectal cancer, but provide no conclusive evidence of carcinogenicity in humans. (IARC 1987, ATSDR 2002, IRIS 2004, WHO 1989, WHO 1996).

Equivocal evidence exists for an association between dieldrin and breast cancer risk from three recent human epidemiological studies (ATSDR 2002).

Dieldrin has been tested by oral administration in mice, rats, hamsters, dogs and monkeys. Several studies conducted in mice of different strains have all revealed benign and/or malign liver cell tumours, females seemed to be less sensitive than males; no other types of tumours were observed in mice. No carcinogenic effect was observed in seven studies with four strains of rats, or in hamsters, which had been given relatively high doses. The studies in dogs and monkeys were inadequate for evaluation. (IARC 1987, ATSDR 2002, IRIS 2004, WHO 1989, WHO 1996).

2.11.6 Toxicity to reproduction

In one study in humans, no association was found between blood levels of dieldrin and premature labour or spontaneous abortions in pregnant women. No other studies on reproductive and developmental effects in humans are available. (ATSDR 2002).

In the reproductive toxicity studies (over one to six generations) carried out with aldrin or dieldrin on mice and rats, the major effect observed in most of the studies was an increased mortality rate in pre-weaning pups. Reproductive performance was only affected at doses causing maternal intoxication. The studies available on dogs are of a too limited nature to draw firm conclusions, apart from the consistent increase in pre-weaning pup mortality. The results of these reproductive studies indicate that dieldrin at levels of 2 mg/kg diet (0.1 mg/kg b.w./day) in the rat and of 3 mg/kg diet (0.4 mg/kg b.w./day) in the mouse are no-effect levels for reproductive effects. No evidence of a teratogenic potential is indicated from developmental toxicity studies on rats, mice, or rabbits using oral doses of up to 6 mg/kg b.w./day; and no gross malformations have been reported in reproductive studies. (WHO 1989, WHO 1996, ATSDR 2002).

2.11.7 Evaluation

Repeated oral exposure to dieldrin is associated with effects in the nervous system, the liver, the kidney, and the immune system, carcinogenicity, and reproductive and developmental effects.

Data from human studies indicate that the nervous system is a major target organ after exposure to dieldrin. Adverse effects from dieldrin are related to the level of dieldrin in the blood. In workers, the level of dieldrin in the blood below which adverse effects did not occur has been reported to be 105 µg/l corresponding to an oral intake of 17.4 µg/kg b.w./day. In male volunteers, no adverse health effects including effects on the nervous system were observed following administration of dieldrin at doses up to approximately 3 µg/kg b.w./day for 18 months.

The liver is a major target organ in rodents and dogs and the effects observed include increased activity of microsomal biotransformation enzymes, increased relative liver weight, and histopathological changes (known as "Chlorinated Hydrocarbon Insecticide Rodent Liver" (CHIRL). In rats and dogs, no effects were observed in the liver at 0.005 mg/kg b.w./day.

Other effects observed in experimental animals following long-term oral exposure to dieldrin include effects in the kidneys, immune system and the central nervous system. These effects have been reported to occur at higher exposure levels than those at which the effects in the liver have been observed.

The available epidemiological studies regarding an association between dieldrin and cancer risk provide no conclusive evidence of carcinogenicity although possible increases in liver, biliary, rectal, breast cancer have been suggested in some of the later studies. Dieldrin has been shown to be carcinogenic in various strains of mice of both sexes with only liver tumours being observed. No carcinogenic effect has been observed in several strains of rats or hamsters. Dieldrin is considered by IARC (1987) as not classifiable as to its carcinogenicity to humans (Group 3; evidence in humans inadequate, evidence to animals limited). US-EPA (IRIS 2004) has considered dieldrin as a probable human carcinogen (Group B2; evidence in humans inadequate, evidence to animals sufficient). In the EU, dieldrin is classified for carcinogenic effects in category 3 (Carc. Cat. 3, R40 – limited evidence of a carcinogenic effect) (MM 2002).
The vast majority of studies investigating the *in vitro* and *in vivo* genotoxic effects of aldrin and dieldrin have yielded negative results, but positive results have been reported as well. Overall, the data provide no conclusive evidence for genotoxic effects, particularly for a direct action on the DNA molecule.
A number of special studies have failed to elucidate the mechanism of the induction of the liver tumours in mice. According to ATSDR (2002), accumulating evidence indicates that aldrin and dieldrin are non-genotoxic tumour promoters acting through species-specific susceptibility of the mouse to induction of oxidative stress and inhibition of gap junctional communication. Overall, a carcinogenic potential cannot be fully excluded in relation to dietary exposure to dieldrin, but is not considered to be significant at the exposure levels at which the effects on the nervous system and liver have been observed.

Several reproductive toxicity studies have reported a decrease in post-natal survival for offspring of mice, rats and dogs exposed to aldrin or dieldrin by the oral route; a NOAEL of 0.1 mg/kg b.w./day can be established for the rat and of 0.4 mg/kg b.w./day for the mouse. No evidence of a teratogenic potential is indicated for rats, mice, or rabbits at oral doses up to 6 mg/kg b.w./day.

2.11.8 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of dieldrin are the effects observed in the nervous system and the liver, and the carcinogenic effect.

No adverse effects in the nervous system and in the liver have been observed in workers with dieldrin blood levels of 105 µg/l corresponding to an intake of 0.02 mg/kg b.w./day or in volunteers following administration of dieldrin at doses up to approximately 0.003 mg/kg b.w./day. The liver is the major target organ in rodents and dogs; a NOAEL of 0.005 mg/kg b.w./day can be established for increased liver weight (rat, dog) and histopathological changes (rat). A NOAEL of 0.005 mg/kg b.w./day is considered to be sensitive and to provide adequate protection against liver toxicity and neurotoxicity and is taken forward to the risk characterisation.

A carcinogenic potential cannot be fully excluded in relation to dietary exposure to dieldrin, but is not considered to be significant at the exposure levels at which the effects in the nervous system and the liver have been observed.

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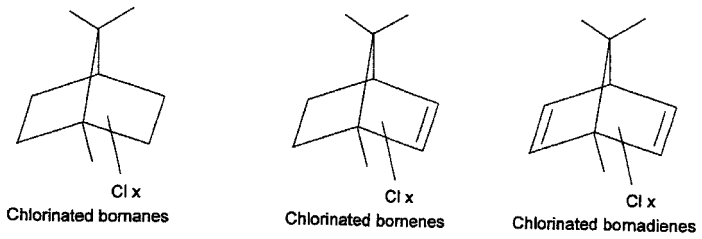
2.12 Toxaphene

Toxaphene is a complex mixture of many structurally related chlorinated bornanes containing 67 to 78 % chlorine resulting from chlorination of camphene. Commercial toxaphene consists on average of a mixture of 76% chlorinated bornanes, 18% chlorinated bornenes, 2% chlorinated bornadienes, 1% other chlorinated hydrocarbons and 3% non-chlorinated hydrocarbons.



Toxaphene has been widely used as an insecticide on crops and to control parasites on livestock since 1949 and was by far the most heavily used pesticide in the world during the years between 1972 and 1984. Due to its toxicity, persistence in nature and its widespread distribution on a global scale far from its application sites, the use of toxaphene was banned or restricted in many countries in the beginning of the eighties. (NCM 1997, IARC 2001).

The general population is exposed to toxaphene mainly through the diet. Main dietary exposures are eating of fatty fish, but milk, including human milk, and other foodstuffs also contribute. A dietary daily intake of toxaphene in USA and Germany of 2-6 ng/kg b.w. has been estimated. (NCM 1997, IARC 2001).

2.12.1 Toxicokinetics

Toxaphene and several of its fractions or individual constituents are absorbed from the gastrointestinal tract of experimental animals as evidenced by the finding of toxaphene residues in fat and various tissues and by the excretion of toxaphene and metabolites in urine and faeces. There are no data on the extent of absorption of toxaphene from the gastrointestinal tract, but it appears to be extensive. (NCM 1997).

In rodents, there is an initial accumulation in the adrenal cortex and the liver, but most other tissues also contain toxaphene. A gradual redistribution takes place to the adipose tissue, the main storage tissue. On cessation of exposure, tissue residues seem to decrease rather rapidly. (NCM 1997).

Most toxaphene components appear to be rapidly metabolised by liver enzymes and probably also by microorganisms in the gastrointestinal tract. The major metabolic processes are reductive dechlorination, reductive dehydrochlorination, and oxidative dechlorination to produce hydroxyl derivatives, acids and ketones. The complex composition of toxaphene and the complex pattern of metabolism, may result in formation of metabolites that are either more or less polar than their parent constituents. There are indications that toxaphene is more extensively metabolised in monkeys than in rodents. (NCM 1997).

Toxaphene components and/or metabolites are able to cross the placenta; however, the levels found in the foetus have consistently been much lower than in the dams. Toxaphene components and/or metabolites are also excreted in human milk. (NCM 1997).

From the experimental studies, the overall biological half-life of commercial toxaphene is in the order of a few days. However, some of the components are more persistent as evidenced by the finding of residues in fat tissue 2 to 3 weeks after administration of a single dose. (NCM 1997).

2.12.2 Single dose toxicity

Accidental poisonings of persons due to toxaphene have been reported. Most fatalities have involved children. Symptoms of poisoning often begin very soon after ingestion and include central nervous system and abdominal symptoms as well as breathing difficulties. The acute lethal dose has been estimated to be 2 to 7 g per person. (NCM 1997).

Toxaphene is of intermediate acute toxicity in experimental animals with oral LD_{50}-values in the mouse and rat ranging from 60 to 300 mg/kg b.w. Acute toxic doses of toxaphene affect the brain and spinal cord and produce central nervous system stimulation, salivation, and generalised epileptiform convulsions, which may lead to respiratory arrest. (NCM 1997).

2.12.3 Repeated dose toxicity

Chronic exposure may possibly cause liver enzyme induction in humans (NCM 1997).

In experimental animals, the liver, kidney and thyroid, and the nervous system and immune system are targets following short-term, intermediate duration, and long-term exposure to toxaphene.

The liver is reported to be the most sensitive organ to toxaphene exposure and effects observed include increased weight, inhibition of hepatobiliary function, and a variety of histopathological changes. A number of studies have demonstrated that toxaphene is a potent inducer of hepatic monooxygenases. Biochemical studies have also shown that toxaphene is able to interfere with the activities of a number of other enzymes, many of which are important for intermediary cell metabolism. The effects observed in the liver are probably partly adaptive in nature due to the hepatic enzyme induction. (NCM 1997).
In a 13-week study of rats, dose-dependent histological changes were seen in the liver from 20 mg/kg in the diet (corresponding to 1.8 / 2.6 mg/kg b.w./day in males / females, respectively), and were to a slight degree also present at 4 mg/kg (corresponding to 0.35 / 0.50 mg/kg b.w./day in males / females, respectively). In a life-time study, the NOAEL for liver toxicity was reported to be 25 mg/kg in the diet (corresponding to approximately 1.25 mg/kg b.w./day). For induction of monooxygenases, the lowest dietary level that produced a significant increase in any enzyme system was 5 mg/kg (corresponding to approximately 0.25 mg/kg b.w./day) for 13 weeks.
In a 13-week study of dogs, mild to moderate dose-dependent histological changes were observed in the liver from 2.0 mg/kg b.w./day; no effects were observed at 0.2 mg/kg b.w./day. In a 2-year study, 20 mg/kg in the diet (about 0.5 mg/kg

b.w./day) was reported to be without effects as judged from gross or histological examinations.

The thyroid is also affected in rats and dogs and morphological changes have been reported. In a 13-week study of rats, histopathological changes were observed from 4 mg/kg in the diet (corresponding to 0.35 / 0.50 mg/kg b.w./day in males / females, respectively), but the incidences were only increased from 20 mg/kg (corresponding to 1.8 / 2.6 mg/kg b.w./day in males / females, respectively). In a 13-week study of dogs, mild to moderate dose-dependent histological changes were observed in the thyroid from 2.0 mg/kg b.w./day; no effects were observed at 0.2 mg/kg b.w./day. (NCM 1997).

Adverse effects on the kidneys have been observed following exposure of rats and dogs to toxaphene. In a 13-week study of rats, renal injuries were seen in the proximal tubules from 20 mg/kg in the diet (corresponding to 1.8 / 2.6 mg/kg b.w./day in males / females, respectively); the NOAEL for kidney toxicity was 4 mg/kg (corresponding to 0.35 / 0.50 mg/kg b.w./day in males / females, respectively). Only very slight effects were observed in the kidneys of dogs at dose levels of up to 5.0 mg/kg b.w./day for 13 weeks. (NCM 1997).

Effects on the nervous system (central nervous system stimulation, convulsive seizures, and functional (EEG, behavioural), biochemical (neurotransmitter) and morphological changes) have been observed in prolonged studies of rats and dogs, but only at high doses (NCM 1997).

In mice, antibody formation was depressed following dietary administration of toxaphene from 100 mg/kg (corresponding to approximate 12.5 mg/kg b.w./day) for 8 weeks; no effects were observed at 10 mg/kg (corresponding to approximate 1.25 mg/kg b.w./day). Cell-mediated immune responses were not affected. (NCM 1997).

2.12.4 Genotoxicity

An increased frequency of chromosomal aberrations has been observed in the lymphocytes of workers exposed to toxaphene (IARC 2001). No significant differences were found between rates of chromosomal aberrations in leukocytes of workers occupationally exposed to toxaphene and of unexposed workers (IRIS 2004).

In mammalian cells *in vivo*, toxaphene did not bind to DNA or produce dominant lethal mutations in mice. *In vitro*, toxaphene was mutagenic to bacteria, but did not induce mutations in mammalian cells. It induced micronuclei in the only assay for this end-point performed in mammalian cells. It also induced sister chromatid exchange and inhibited gap junctional intercellular communication in cultured mammalian cells. (IARC 2001, NCM 1997, IRIS 2004).

2.12.5 Carcinogenicity

One case-control study of non-Hodgkin lymphoma and one of leukaemia not otherwise specified in the same populations showed no significant increase in risk associated with exposure to toxaphene (IARC 2001).

Toxaphene has been tested for carcinogenicity by oral administration in rats and in mice. In rats, it induced thyroid follicular-cell adenomas and carcinomas in both

sexes and pituitary adenomas in females. In mice, it increased the incidence of hepatocellular adenomas and carcinomas combined in both sexes. (IARC 2001, IRIS 2004, NCM 1997).

2.12.6 Toxicity to reproduction

In rats, toxaphene at concentrations of up to 500 mg/kg in the diet (corresponding to 25 mg/kg b.w./day) had no effects on fertility or pup survival. At 500 mg/kg, increased kidney and liver weights, hepatic enzyme induction, slight tubular damage in the kidney, and alterations in the thyroid were observed in the offspring, a dose level at which growth depression was observed in the dams. In mice, exposure for five or sex generations to 25 mg/kg in the diet (corresponding to 3.25 mg/kg b.w./day) resulted in little or no effects on the reproduction. (NCM 1997).

No gross malformations have been reported in teratogenicity studies in rats and mice. Effects such as weight depression, reduced number of ossification centres, and an increased incidence of supernumerary ribs have been observed at dose levels of 25-35 mg/kg b.w./day in rats and of 100-200 mg/kg b.w./day in mice at which maternal toxicity and/or mortality also was observed. At lower dose levels, toxaphene has been shown to affect the behaviour of rat offspring (0.05 mg/kg b.w./day) and the immune responses in mouse offspring (from 1.25 mg/kg b.w./day). (NCM 1997).

Recent reports indicate that toxaphene is able to interfere with several sex hormones and/or their receptors *in vitro* and has been reported to cause oestrogen-like responses in cell systems (NCM 1997).

2.12.7 Evaluation

Repeated oral exposure to toxaphene is associated with effects in the liver, thyroid and kidney, effects in the nervous system and immune system, carcinogenicity, and developmental neurotoxicity.

Human data on the toxicity of toxaphene are very scarse. Chronic exposure may possibly cause liver enzyme induction in humans; however, no specific data are available.

In experimental animals, the liver, kidney and thyroid are major target organs. Toxaphene causes changes in liver morphology associated with enzyme induction, morphological changes in the thyroid, and renal tubular injury. The available long-term studies are of an older date; in these studies, the NOAELs for liver toxicity (only end-point reported) are generally higher than those found in more recent short-term studies (13-week studies) in rats and dogs. Due to deficiencies in design and reporting of the long-term studies, the more recent short-term studies are considered to be more valid and reliable for establishing NOAELs for toxaphene induced toxicity in the target organs. Based on the results reported in a 13-week study of rats, a NOAEL of 0.35 mg/kg b.w./day is considered for histological changes in the liver, thyroid and kidney. Another 13-week study in rats has revealed that the lowest dietary level for enzyme induction is 0.25 mg/kg b.w./day. From a 13-week study of dogs, a NOAEL of 0.2 mg/kg b.w./day is considered for histological changes in the liver and thyroid; only very slight effects were observed in the kidneys at dose levels of up to 5.0 mg/kg b.w./day. Overall, a NOAEL of 0.2 mg/kg b.w./day is considered for effects in the liver, thyroid and kidney.

Other effects observed in experimental animals following prolonged oral exposure to toxaphene include effects in the immune system and in the central nervous system. These effects have been reported to occur at higher exposure levels than those at which the effects in the liver, thyroid and kidney have been observed.

Toxaphene induces hepatocellular adenomas and carcinomas in mice, thyroid follicular-cell adenomas and carcinomas in both sexes of rats, and pituitary adenomas in female rats. However, the available human data did not indicate a significant increase in cancer risk associated with exposure to toxaphene. Toxaphene is considered by IARC (2001) as possibly carcinogenic to humans (Group 2B; evidence in humans inadequate, evidence in animals sufficient). US-EPA (IRIS 2004) has considered toxaphene as a probable human carcinogen (Group B2; no data in humans, evidence to animals sufficient, supported by mutagenicity in *Salmonella*). In the EU, toxaphene is classified for carcinogenic effects in category 3 (Carc. Cat. 3, R40 – limited evidence of a carcinogenic effect) (MM 2002).
The mechanism underlying the carcinogenic effect is at present unclear. Toxaphene has shown both positive and negative results in the available studies investigating the *in vitro* and *in vivo* genotoxic effects; however, based on the current limited data set, it cannot be concluded whether toxaphene is genotoxic *in vivo* or not. The enzyme inducing activities may suggest a promoter effect for the induction of liver carcinogenicity in mice and this is supported by the inhibition of gap junctional intracellular communication in cell culture systems; however, no specific data are available regarding promoter effects of toxaphene.
Overall, a carcinogenic potential of toxaphene cannot be excluded for humans in relation to dietary exposure to toxaphene.

No effects of toxaphene on fertility or teratogenic effects have been reported in studies of experimental animals. Effects such as weight depression, reduced number of ossification centres, and an increased incidence of supernumerary ribs have been observed in offspring of rats and mice at dose levels, which caused maternal toxicity and/or mortality. There are indications of slight neurobehavioural developmental effects in rats at low dose levels (0.05 mg/kg b.w./day). Recent reports indicate that toxaphene is able to interfere with several sex hormones and/or their receptors *in vitro* and has been reported to cause oestrogen-like responses in cell systems.

2.12.8 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of toxaphene are the effects observed in the liver, thyroid and kidney, carcinogenicity, and developmental neurotoxicity.

No human data are available.

In experimental animals, a NOAEL of 0.35 mg/kg b.w./day is considered for histological changes in the liver, thyroid and kidney of rats, and of 0.2 mg/kg b.w./day for histological changes in the liver and thyroid of dogs; only very slight effects were observed in the kidneys of dogs at dose levels up to 5.0 mg/kg b.w./day. Overall, a NOAEL of 0.2 mg/kg b.w./day is considered for effects in the liver, thyroid and kidney. This NOAEL is considered to provide adequate protection against effects in the immune system and the central nervous system, and is taken forward to the risk characterisation.

There are indications of slight neurobehavioural developmental effects in rats at lower dose levels (0.05 mg/kg b.w./day) than the NOAEL derived from the

subchronic studies in rats and dogs. Furthermore, recent reports indicate that toxaphene is able to interfere with several sex hormones and/or their receptors *in vitro* and has been reported to cause oestrogen-like responses in cell systems.

A carcinogenic potential of toxaphene for humans in relation to dietary exposure cannot be excluded. Furthermore, based on the current limited data set, it cannot be concluded whether toxaphene is genotoxic *in vivo* or not.

2.12.9 References

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3 Risk characterisation

3.1 Introduction

3.1.1 Risk characterisation of chemical substances in the EU

Risk characterisation is basically an integration of the findings from the exposure assessment and the effect assessment in order to reach a characterisation of the risk.

Within the European Union, specific programs on risk assessment for new and existing chemical substances are on-going. A directive[14] provides the regulation on the risk assessment for new notified chemical substances[15] and two Council regulations[16] on the risk assessment for existing chemical substances. A Technical Guidance Document[17] (TGD) supporting the risk assessment regulations has been prepared and provides the detailed methodology for the risk assessment process.

Below, the principles of the risk assessment process are introduced based on the guidance provided in the TGD as well as on a recent report[18], which has reviewed the current knowledge and experiences on risk assessment of chemical substances.

The risk assessment process entails a sequence of actions:

1) Assessment of effects, comprising

a) Hazard identification: identification of the adverse effects, which a chemical substance has an inherent capacity to cause, and

b) Dose (concentration) - response (effects) assessment: estimation of the relationship between dose (or level of exposure) to a chemical substance, and the incidence and severity of an effect, where appropriate.

The principles of the effect assessment process are briefly summarised in section 2.1 of this report.

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[14] Commission Directive 93/67/EEC of 20 July 1993, laying down the principles for the assessment of risks to man and the environment of substances notified in accordance with Council Directive 67/548/67.

[15] Substances not on the EU market in the 10 years prior to 18 September 1981 and therefore not appearing in the European INventory on Existing Commercial chemical Substances (EINECS).

[16] Council Regulation 793/93/EEC of 23 March 1993 on the evaluation and control of the risks of existing substances. Commission Regulation (EC) No 1488/94 of 28 June 1994 on risk assessment for existing substances.

[17] Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances. European Chemicals Bureau, European Commission, Joint Research Centre, EUR 20418 EN.

[18] Nielsen E, Østergaard G, Larsen JC and Ladefoged O (2005). Principper for sundhedsmæssig vurdering af kemiske stoffer med henblik på fastsættelse af kvalitetskriterier for luft, jord og drikkevand. Miljøprojekt nr. 974 2005, Miljøstyrelsen, Miljøministeriet. In Danish, with an English summary.

2) Exposure assessment: estimation of the concentrations or doses of the chemical substance to which human populations are or may be exposed.

Exposure to xenobiotics can occur via different sources as e.g., food, drinking water, ambient air, consumer products, and the working environment.

3) Risk characterisation: estimation of the incidence and severity of the adverse effects likely to occur in a human population due to actual or predicted exposure to a chemical substance, and may include “risk estimation”, i.e., the quantification of that likelihood.

3.1.2 Risk characterisation for threshold effects, ‘MOS approach’

According to the TGD, the risk characterisation for a toxicological endpoint with a threshold for the effect (threshold effects) generally relies on the dose (or concentration) below which the effect is unlikely to occur, i.e., the threshold dose (or exposure concentration). Usually the ‘no observed adverse effect level’ (NOAEL), obtained from human data or in experimental studies with animals, serves as a direct measure for the threshold dose and is used as the starting point for the risk characterisation. If a NOAEL has not been identified, the ‘lowest observed adverse effect level’ (LOAEL) might be used as the starting point. Alternatively, a benchmark dose (BMD) can be determined if possible, and is preferred over LOAEL-to-NOAEL extrapolation.

The risk characterisation is carried out by quantitatively comparing the outcome of the effects assessments to the outcome of the exposure assessments. The ratio resulting from this comparison is called the Margin of Safety (MOS).

The risk characterisation for threshold effects as outlined above is called the ‘MOS approach’ or the ‘threshold approach’.

3.1.2.1 Reference MOS

In order to reach a conclusion as to whether a MOS indicates a concern (or risk) for humans to experience adverse health effects following exposure to a specific chemical substance, a so-called ‘reference MOS’ (MOSref) is derived and applied.

The MOSref is an overall assessment factor, as a numerical value, addressing differences between experimental effect assessment data (usually from animal studies) and the real human exposure situation, taking into account variability and uncertainty. The uncertainties are related to the extrapolation of NOAEL (or LOAEL) obtained from studies in experimental animals to the human situation (interspecies variation), to differences in susceptibility among individuals in the human population (intraspecies or inter-individual variation), and to other uncertainties in the establishment of a NOAEL for the critical effect for a specific chemical substance than the interspecies and inter-individual variation.

The MOSref is derived by combining a number of individual assessment factors addressing the interspecies and inter-individual variations as well as several issues related to the available data set for a specific chemical substance. Preferably, the value for each individual assessment factor is based on substance-specific information; however, in practice this is generally not possible as data are often scarce or inadequate and therefore, a default value for the individual assessment factors most often are applied.

In the characterisation of the risk, the MOS is compared with the MOSref.
MOS values clearly above the MOSref, i.e., the ratio 'MOS/MOSref' >> 1, do not lead to concern (or risk) for adverse health effects following exposure to a specific chemical substance. MOS values clearly below the MOSref, i.e., the ratio 'MOS/MOSref' << 1, lead to concern for adverse health effects and risk reduction measures are recommended in order to reduce the exposure to the specific chemical substance. Thus, it is not the value of the MOS in it self but the ratio 'MOS/MOSref', which is crucial for the identification of a concern. This means strictly spoken that the lower the ratio 'MOS/MOSref' is, the higher the concern (or risk) is for adverse health effects following exposure to a specific chemical substance.
MOS values in the range of the MOSref indicate borderline cases and need to be interpreted more carefully by an overall qualitative evaluation on a case-by-case basis.

3.1.2.2 Assessment (or uncertainty) factors

As mentioned above, the MOSref is an overall assessment factor, as a numerical value, addressing the differences between the experimental data and the human situation, taking into account the uncertainties in the extrapolation procedure and in the available data set. When the available data do not allow a derivation of individual substance-specific assessment factors, default values for the individual assessment factors are applied.
Several aspects are involved in the extrapolation of experimental data to the human situation, inter alia:

- Interspecies differences
- Intraspecies (inter-individual differences)
- Differences in duration of exposure
- Uncertainty in route-to-route extrapolation
- Issues related to dose-response, including severity of effect
- Other aspects related to the overall quality of the data set

3.1.2.2.1 Interspecies differences

Data from animal studies are the typical starting points for risk characterisation and thus differences in sensitivity between experimental animals and humans to a specific chemical substance need to be addressed with the default assumption that humans are more sensitive to a specific chemical substance than experimental animals. Interspecies differences result from variation in the sensitivity between different species due to differences in toxicokinetics and toxicodynamics. Some of the toxicokinetic differences can be explained by differences in body size and related differences in basal metabolic rate.

A default value of 10 has traditionally been applied for the assessment factor accounting for the interspecies variation. It has been proposed to divide this default value into a factor of 4 to account for differences in toxicokinetics and a factor of 2.5 to account for differences in toxicodynamics.
Recently, a number of analyses have been performed in order to evaluate the default value of 10 for interspecies variation (metabolic as well as the remaining species-specific differences); these analyses support to continue the application of a default value of 10.

According to the TGD, it is suggested as a default to correct for differences in metabolic rate (allometric scaling) and to apply an additional factor of 2.5 for other interspecies differences. Allometric scaling is based on the assumption that the toxicological effects of a specific chemical substance are driven by metabolic rate of a given individual. Allometric scaling extrapolates doses (for systemic oral and dermal effects) according to an overall assumption that equitoxic doses (when expressed in mg/kg body weight/day) scale with the body weight to the power of 0.75.

3.1.2.2.2 Intraspecies (inter-individual) differences

Humans differ in sensitivity due to biological factors such as age, gender, genetic composition, health status, and nutritional status reflecting both differences in toxicokinetics and in toxicodynamics. This inter-individual variation is greater in humans than in the more inbred experimental animal population. In order to always cover the most sensitive individual in the human population exposed to any chemical substance would require a very high default value for the assessment factor accounting for inter-individual differences; however that is not realistic in practise.

A default value of 10 has traditionally been applied for the assessment factor accounting for the inter-individual variation and has been assumed as being sufficient to protect the larger part of the population, including e.g., children, pregnant women, the elderly, or sick people.
Recently, a number of analyses have been performed in order to evaluate the default value of 10 for inter-individual variation; these analyses support to continue the application of a default value of 10.

According to the TGD, a default value of 10 is suggested for the assessment factor accounting for the inter-individual variation. It is pointed out specifically that there are differences between children and adults in toxicokinetics (especially babies in their first months) and toxicodynamics (especially at different stages of development), which may render children more or less susceptible to the toxic effects of a chemical substance. A higher default value for the inter-individual assessment factor (from 10 to 100) could be applied for children in some situations.

3.1.2.2.3 Other uncertainties in the establishment of a NOAEL

Traditionally, no assessment factor(s) has generally been applied to account for other uncertainties than the interspecies and inter-individual variation in the establishment of a NOAEL for the critical effect for a specific chemical substance, however, this has currently become a more commonly used practise within various regulations.

Other uncertainties include elements such as:

- The quality of the data set, e.g., data on specific toxic endpoints are lacking or inadequate;
- Exposure duration, e.g., no chronic studies on which to establish the NOAEL are available;
- Route-to-route extrapolation, e.g., no studies using the appropriate exposure route are available;
- LOAEL-to-NOAEL extrapolation, e.g., a NOAEL cannot be established for the critical effect;

- Nature and severity of toxicity, e.g., the critical effect is carcinogenicity, toxicity to reproduction, or sensitisation.

Recently, a number of analyses have been performed in order to evaluate or propose default values to account for the various elements of the other uncertainties related to the establishment of a NOAEL for the critical effect for a specific chemical substance. Based on these analyses, a default values for the various elements as well as for an overall factor cannot be suggested.

According to the TGD, default values are suggested for the uncertainties related to 1) exposure duration, 2) route-to-route extrapolation, and 3) issues related to dose-response, including severity of effect.

1) A factor accounting for differences in the experimental exposure duration and the duration of exposure for the human population needs to be considered taking into account that a) in general the experimental NOAEL will decrease with increasing exposure duration and b) other and more serious adverse effects may appear with increasing exposure duration. Default values for the assessment factor accounting for exposure duration are suggested as follows: a value of 2 for sub-chronic (90-day study) to chronic (1.5-2 year study), of 6 for sub-acute (28-day study) to chronic, and of 3 for sub-acute to sub-chronic.

2) A factor accounting for route-specific differences in susceptibility might be relevant in some cases but should be considered on a case-by-case basis. Consequently, a default value of 1 is suggested for the assessment factor accounting for route-to-route extrapolation.

3) For the dose-response relationship, consideration should be given to the uncertainties in the NOAEL as the surrogate for the true ‘no adverse effect level’ (NAEL) as well as to the extrapolation of a LOAEL to the NAEL. The value of an assessment factor should take into account the dose spacing in the experiment, the shape and slope of the dose-response curve, and the extent and severity of the effect observed at the LOAEL. When the starting point for the MOS calculation is a LOAEL, it is suggested to use a default value between 3 (as minimum / majority of cases) and 10 (as maximum / in exceptional cases) for the assessment factor accounting for issues related to dose-response. When the starting point is a NOAEL, the default value is 1; however, a larger value may be applied in specific cases such as e.g., a shallow dose-response curve, a steep dose-response curve for serious/severe effects, poor quality of the study from which the NOAEL is derived, as well as other concerns related to the identified NOAEL.

3.1.2.3 Assessment factors applied by FAO/WHO and US-EPA

As mentioned above, the MOSref is an overall assessment (or uncertainty) factor and can therefore be compared with the uncertainty factors applied in the setting of an acceptable or tolerable daily intake (ADI or TDI) by e.g., FAO/WHO (FECFA/JMPR), or an oral reference dose (RfD) by US-EPA.

The FAO/WHO generally applies a default value of 10 for interspecies and for inter-individual variation, respectively. For some substances, a further uncertainty factor has been applied in order to take into account various limitations in the data set.

US-EPA generally applies five different uncertainty factors: 1) interindividuel variation (UF_H), 2) interspecies variation (UF_A), 3) subchronic-to-chronic (UF_S), 4) LOAEL-to-NOAEL (UF_L), and 5) quality and relevance of the data (UF_D). The default value for the various factors is 10, but a combined value above 3000 is generally not applied.

3.1.3 Risk characterisation for non-threshold effects

Non-threshold effects are effects for which a threshold cannot be identified, e.g., genotoxic effects and carcinogenic effects, which are caused by damage of the genetic material (DNA). For such effects, it is assumed that there is a dose-dependent response at all doses above zero and thus, some risk is considered to exist at any exposure level.

For non-threshold effects, there is currently no clear consensus on an appropriate methodology for the estimation of a no-effect level. A number of approaches based largely on characterisation of dose response analyses and low-dose extrapolation have been adopted for assessment of such effects, which all require administrative/ political judgements of an acceptable health risk.

A number of mathematical models have been developed for extrapolation from responses at the high experimental doses generally used in animal carcinogenicity studies to those of the substantially lower exposure levels encountered in human situations.

According to the TGD, the default approach for non-threshold carcinogens is to assume low-dose linearity, and as a default assumption that even at very low exposure levels residual carcinogenic risks cannot be excluded. However, it is also recognised that linear extrapolation may in some cases result in overestimation of risks at low exposures, but this may be acceptable from a precautionary principle standpoint.

With regard to the quantitative risk characterisation, two approaches presenting risk-based considerations are described in the TGD: 1) The lifetime cancer risk approach, and 2) the MOE (Margin of Exposure) approach.

1) Determination of the lifetime cancer risk is carried out in several distinct steps. An animal dose descriptor is calculated from the experimental data and is converted to a human dose descriptor. Subsequently, the lifetime cancer risk is determined by linear extrapolation to the actual exposure dose. A commentary statement is generated whether an overall evaluation of all data available indicates that the actual risk may be higher or lower than the calculated risk. Finally, the calculated lifetime cancer risk is compared to a lifetime cancer risk of very low concern. Based on this comparison together with the commentary statement, conclusions with respect to the risks associated with the specific exposure situations are made.

2) In the MOE approach, which formally is similar to the MOS approach for threshold effects, the starting point is the animal dose descriptor as well. This dose descriptor is divided by the exposure level and the result is the co-called ‘MOE’. To reach a conclusion, the MOE has to be compared with a standard, the ‘reference MOE’ (MOEref). The MOEref contains the overall information that bridges the gap between the animal dose descriptor chosen, which describes a high risk situation for experimental animals, and a risk situation for human populations

considered to be of very low concern. The MOEref is derived as the product of the relevant assessment factors and a so-called 'risk extrapolation factor'.

According to the TGD, the dose descriptor T25 from animal studies should be used in relation to linear extrapolation. T25 is defined as the chronic dose rate (in mg/kg b.w./day), which will give 25% of the animals tumours at a specific tissue site, after correction for spontaneous incidence, within the standard lifetime of that species.

There are no regulations on acceptable health risk, but there is an administrative practice followed by various authorities. Generally, a lifetime risk between 10^{-6} and 10^{-7} is considered a tolerable level. A lifetime risk of 10^{-6} for developing tumours means that exposure for life time to a specific dose or concentration may result in that one individual of a million of exposed individuals develops one tumour.

The question whether a threshold exists for the effects of carcinogens, which also are genotoxic, has been debated internationally during the last years.
Recently, FAO/WHO (JECFA) has used the MOE approach for the evaluation of dietary intake of three substances, which are both genotoxic and carcinogenic (PAH, acrylamide and diethyl carbamate). The benchmark dose '$BMDL_{10}$' representing the lower bound of a 95% confidence interval on the BMD corresponding to a 10% tumour incidence was used as the starting point. An MOE of 10000 or higher was considered to implicate low concern for human health.
The European Food Safety Authority (EFSA) has also recommended the MOE approach and proposes that an MOE of 10000 or higher, based on the $BMDL_{10}$ would be of low health concern. The MOE of 10000 has been proposed as the product of a factor of 100 for interspecies and inter-individual variation and an additional factor of 100 for the nature of the carcinogenic process and the use of a reference point on the dose-response curve.

3.1.4 Risk characterisation, contaminants selected in this report

In this report, the risk to Greenlanders of experiencing adverse health effects from intake of the eleven selected contaminants from traditional food items is characterised and evaluated by using the principles for the risk characterisation process according to the TGD as outlined above, i.e., the MOS approach.

Thus, the NOAEL (or LOAEL) established for the critical effect(s) of a given contaminant is divided by the mean daily human intake of that specific contaminant from traditional Greenland food items as presented in Johansen et al. (2004a[19],b[20]) in order to calculate the MOS, i.e., N(L)OAEL / mean daily intake of the contaminant.

In the characterisation of the risk, the MOS is compared with the MOSref.
When a MOS value for a specific contaminant is clearly above the MOSref, i.e., the ratio 'MOS/MOSref' >> 1, a concern (or risk) of experiencing adverse health effects among Greenlanders due to long-term intake of a specific contaminant from the traditional Greenland food items is not identified. When a MOS value is clearly below the MOSref, i.e., the ratio 'MOS/MOSref' << 1, a concern for adverse health effects is identified and it is recommended to reduce the intake of the

[19] Johansen P, Muir D, Asmund G and Riget F (2004a). Human exposure to contaminants in the traditional Greenland diet. Sci Total Environ **331**, 189-206.
[20] Johansen P, Asmund G and Riget F (2004b). High human exposure to lead through consumption of birds hunted with lead shot. Environ Pollut **127**, 125-129.

specific contaminant from the traditional Greenland food items as well as from other sources. Thus, it is not the value of the MOS in it self but the ratio 'MOS/MOSref', which is crucial for the identification of a concern. This means strictly spoken that the lower the ratio 'MOS/MOSref' is, the higher the concern (or risk) is for adverse health effects following dietary intake of a specific contaminant. When a MOS value is in the range of the MOSref, a more careful overall evaluation on a case-by-case basis is performed.

The MOSref is, as addressed in section 3.1.2.2, an overall assessment (or uncertainty) factor (AF), as a numerical value, addressing the differences between the experimental data and the human situation, taking into account the uncertainties in the extrapolation procedure and in the available data set.
A default value of 10 is applied for the assessment factor (AF_I) accounting for the interspecies variation, i.e., to account for that humans are assumed to be more susceptible to a given effect of a contaminant than experimental animals.
A default value of 10 is applied for the assessment factor (AF_{II}) accounting for the inter-individual variation, i.e., to account for that individuals in the human population such as children and the unborn child, pregnant women, elderly, or sick people may be more susceptible than the general population.
No default assessment factor (AF_{III}) is applied to account for other uncertainties than the interspecies and inter-individual variation in the establishment of a NOAEL for the critical effect(s) for a specific contaminant, but a value from 1 to 10(100) is applied dependent on the uncertainties in the various elements included in assessment factor (AF_{III}).

The MOSref considered for the selected contaminants has been compared with the overall uncertainty factor applied by e.g., FAO/WHO (JECFA/JMPR) in establishing an acceptable or tolerable daily intake (ADI/TDI), or by US-EPA in establishing an oral reference dose (RfD) when available in order to evaluate whether significant differences can be identified between the risk characterisation of the contaminants as performed in this report (the MOS approach) versus the more traditional approach (ADI/TDI or RfD approach).

For contaminants, which are both genotoxic and carcinogenic, a quantitative risk characterisation is not performed for this endpoint. The reason for this decision is that there currently is no clear consensus on an appropriate methodology for the estimation of a no-effect level although recognising that the MOE approach probably will be the default approach in the future. For these contaminants, it is recommended that the intake of the specific contaminant from the traditional Greenland food items as well as from other sources should be reduced.

3.2 Cadmium

3.2.1 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of cadmium are the effects observed in the kidney and bone, and the carcinogenic effect.

A LOAEL in the range of 0.5 to 3 µg/g creatinine is considered for the kidney and bone effects corresponding to about 10 to 60 µg Cd/day (0.0002 – 0.001 mg Cd/kg b.w./day for a 60 kg adult person). It should be recognised that the clinical significance of the biochemical changes observed at this dose level is subject to an on-going scientific debate. The LOAEL is considered to provide adequate protection against reproductive and developmental effects of cadmium, and is taken forward to the risk characterisation.

A carcinogenic potential of cadmium for humans in relation to dietary exposure cannot be excluded. Furthermore, a genotoxic potential of cadmium cannot be excluded either.

3.2.2 ADI / TDI / PTWI / RfD

FAO/WHO (JECFA) has derived a Provisional Tolerable Weekly Intake (PTWI) of 7 µg/kg b.w. According to JECFA, the kidney and in particular the renal cortex has been identified as the critical organ in relation to chronic exposure to relatively low levels of cadmium. In order that levels of cadmium should not exceed 50 µg/g in the renal cortex, assuming an absorption rate of 5% and a daily excretion of 0.005% of body burden, the total intake should not exceed about 1 µg/kg b.w./day for 50 years leading to a PTWI of 7 µg/kg b.w. (JECFA 1989).
FAO/WHO (JECFA) has recently re-evaluated cadmium and re-affirmed that renal tubular dysfunction is the critical health outcome with regard to cadmium toxicity and that an excess prevalence of renal tubular dysfunction will not be expected to occur if urinary cadmium level remains below 2.5 µg/g creatinine. Furthermore, the PTWI of 7 µg/kg b.w. was maintained. (JECFA 2003).

US-EPA has set an oral Reference Dose (RfD) of 0.0005 mg/kg b.w./day for water and of 0.001 mg/kg b.w./day for food based on a NOAEL of 0.005 mg/kg b.w./day for water and of 0.01 mg/kg b.w./day for food and an uncertainty factor of 10 to account for inter-individual variability. The NOAELs were derived from a concentration of 200 µg Cd/g wet human renal cortex being the highest renal level not associated with significant proteinuria and assuming that 0.01% of the cadmium body burden is eliminated per day, and 2.5% absorption of cadmium from food and 5% from water. (IRIS 2004).

3.2.3 Risk characterisation

The mean human intake of cadmium from traditional Greenland food items has been estimated to be 346 µg/day/person in the spring and 182 µg/day/person in the fall (Johansen et al. 2004).

A LOAEL in the range of 10 to 60 µg Cd/day (0.0002 – 0.001 mg Cd/kg b.w./day for a 60 kg adult person) has been considered for the kidney and bone effects observed in humans.

A MOSref of 3 is derived. AF_I is set to 1 as the LOAEL is based on human data. AF_{II} is set to 1 as the inter-individual variation in sensitivity is considered to be implicitly included in the LOAEL as the LOAEL has been derived from a large set of epidemiological data in the population at risk, including individuals exposed during their childhood, smokers, women with depleted iron stores, and individuals with possible predisposing conditions such as renal diseases or diabetes. AF_{III} is set to 3 to take into account that, based on the available data, a NOAEL cannot be estimated for the kidney and bone effects observed in humans.

A MOS in the range of 0.03 to 0.17 is calculated for the mean daily human intake of cadmium from traditional Greenland food items in the spring and of 0.05 to 0.33 in the fall. These MOS values are well to far below the MOSref of 3 (about 9-100 times below) implicating a concern of experiencing adverse health effects in the kidney and bone among Greenlanders due to long-term intake of cadmium from the traditional Greenland food items. Furthermore, a carcinogenic and a genotoxic

potential of cadmium for humans in relation to dietary exposure cannot be excluded. Consequently, it is recommended to reduce the intake of cadmium from the traditional Greenland food items as well as from other sources.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied by FAO/WHO (JECFA) and US-EPA in the setting of a PTWI and an RfD, respectively.
No uncertainty factor has been applied in the setting of the PTWI of 7 µg/kg b.w. by JECFA (1989) whereas US-EPA has applied an uncertainty factor of 10 to account for inter-individual variability in the setting of the oral RfD of 1 µg/kg b.w./day for food (IRIS 2004).
The MOSref of 3 is in between the uncertainty factors applied by JECFA (a factor of 1) and by US-EPA. It should be recognised, however, that the JECFA PTWI of 7 µg/kg b.w. (corresponding to a PTDI of 1 µg/kg b.w./day) is equal to the US-EPA oral RfD as well as to the upper value of the LOAEL range used as the starting point for the MOS calculation in the risk characterisation of cadmium. Thus, a significant difference between the risk characterisation of cadmium in this report (the MOS approach) versus the more traditional approach (TDI or RfD approach) is not identified.

3.2.4 References

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3.3 Lead

3.3.1 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of lead are the effects observed in the nervous, haematopoietic and reproductive systems, and carcinogenicity, and lead has particularly significant effects in children.

The most sensitive endpoints appear to be the neurodevelopmental effects and the effects on haem synthesis, effects for which no threshold apparently exists. There is some evidence of an association between lead exposure and cognitive deficits below a PbB level of 10 µg/dl. ALAD, one of the enzymes in the haem synthesis, is inhibited at very low PbB levels (about 5 µg/dl) although adverse effects are not associated with its inhibition at this level. Based on the available data, a PbB level of 10 µg/dl is considered as a LOAEL for effects on the developing nervous system. For children, the relationship between PbB level and lead intake from food has, according to (WHO 1995), been determined to be 0.16 µg/dl per µg Pb/day for a median PbB level of approximately 10 µg/dl and the LOAEL of 10 µg Pb/dl corresponds to 62.5 µg Pb/day (about 6 µg Pb/kg b.w./day for a child weighing 10 kg) based on this relationship. This LOAEL is considered to provide adequate

protection against the other reproductive effects of lead as well as of the haematological effects, and is taken forward to the risk characterisation.

A carcinogenic potential cannot be fully excluded in relation to dietary exposure to lead, but is not considered to be of any great significance at the exposure levels at which the effects in the nervous system have been observed.

3.3.2 ADI / TDI / PTWI

FAO/WHO (JECFA1987) has derived a Provisional Tolerable Weekly Intake (PTWI) of 25 µg/kg b.w. based on the information that a mean daily intake of 3-4 µg/kg b.w. of lead by infants and children was not associated with an increase in blood lead levels.
The risk of dietary exposure of infants and children was assessed by JECFA (JECFA 2000). The most critical effect of lead at low concentrations was considered to be impaired neurobehavioural development indicated by reduced cognitive development and intellectual performance in children. The Committee concluded: *"A number of studies in which various tests of behavioural performance were used have shown an association between blood lead concentration and reduced intelligence quotient (IQ) in children exposed pre- and postnatally. The effects of confounding variables and limits to the precision of analytical and psychometric measurements increase the uncertainty of any estimate of the effect of blood lead concentrations below 10-15 µg/dl. If a threshold does exist, it is unlikely to be detected because of these limitations; nevertheless, there was some evidence of an association between cognitive deficits and PbB below 10 µg/dl"*. The PTWI of 25 µg/kg b.w. was not reconsidered but was retained.

US-EPA has not set an oral Reference Dose (RfD) and has concluded *"…it is still inappropriate to develop reference values for lead."* because of *"…the continued apparent lack of a threshold."* and because *"Lead body burdens vary significantly with age, health status, nutritional state, and maternal body burden during gestation and lactation."* (IRIS 2004).

3.3.3 Risk characterisation

The human intake of lead from a meal of bird meat has been estimated to be 146 µg for murre and 1220 µg for eider (Johansen et al. 2004a).

An intake of 62.5 µg Pb/day from food has been considered as a LOAEL for effects on the developing nervous system in humans. As lead has particularly significant effects in children, the risk characterisation will be performed for young children. Assuming that one meal of either murre or eider is consumed every week, the estimated daily intake is about 21 µg/day for murre and about 174 µg/day for eider.

A MOSref of 3 is derived. AF_I is set to 1 as the LOAEL is based on human data. AF_{II} is set to 1 as the inter-individual variation in sensitivity is considered to be implicitly included in the LOAEL as the LOAEL has been derived from a large set of epidemiological data in the population at risk, i.e., children. AF_{III} is set to 3 to take into account that, based on the available data, a NOAEL cannot be estimated for the effects in the nervous and the haematopoietic systems observed in humans.

A MOS of 3 is calculated for the mean daily human intake of lead from murre and of 0.4 for eider. The MOS value for murre is equal to the MOSref and the MOS

value for eider is below the MOSref of 3 (about 7 times below) implicating a concern of experiencing adverse health effects in the developing nervous system among Greenland children due to long-term intake of lead through the consumption of eider hunted with lead shot. The concern of experiencing adverse health effects from the consumption of murre is low. A carcinogenic potential cannot be fully excluded in relation to dietary exposure to lead, but is not considered to be of any great significance through consumption of birds hunted with lead shot. However, as no threshold apparently exists for the critical effect, it is recommended to reduce the intake of lead from the traditional Greenland food items as well as from other sources.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied by FAO/WHO (JECFA) and US-EPA in the setting of a PTWI and an RfD, respectively.
No uncertainty factor has been used in the setting of the PTWI of 25 µg/kg b.w. by JECFA (1987). US-EPA (IRIS 2004) has not set an RfD because of the apparent lack of a threshold for the critical effects.
The MOSref of 3 is higher than the uncertainty factor (of 1) applied by JECFA. The JECFA PTWI of 25 µg/kg b.w. (corresponding to a PTDI of 3.6 µg/kg b.w./day) is in the same range as the "TDI" of about 2 µg/kg b.w./day, which can be estimated by dividing the LOAEL used as the starting point for the MOS calculation in the risk characterisation of lead in this report by the MOSref (LOAEL / MOSref = 62.5/10 µg/kg b.w./day / 3 = 2.1 µg/kg b.w./day).
Thus, a significant difference between the risk characterisation of lead in this report (the MOS approach) versus the more traditional approach (TDI approach) is not identified. However, it should be noted that the risk characterisation of lead in this report is more in line with that of the US-EPA, i.e., assuming that no threshold exists for the critical effect.

3.3.4 References

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3.4 Mercury

3.4.1 Critical effect(s) and NOAEL / LOAEL

3.4.1.1 Inorganic mercury

The critical effect following dietary intake of inorganic mercury compounds is the effects observed in the kidney.

According to US-EPA (IRIS 2004b), the Brown Norway rat should be used for mercury risk assessment as it is a good surrogate for the study of mercury-induced kidney damage in sensitive humans. The most sensitive adverse effect is the formation of auto-immune glomerulo nephritis, the first step being the production and deposition of IgG antibodies to the glomerular basement membrane. An oral LOAEL of 317 µg Hg/kg b.w./day has been estimated for this effect. Based on the subcutaneous animal LOAEL of 15.8 µg Hg/kg b.w./day, US-EPA (IRIS 2004b) and WHO (1991) has estimated an equivalent oral LOAEL of 226 µg Hg/kg b.w./day (by assuming 100% absorption following subcutaneous injection and 7% absorption following oral administration). A LOAEL of 300 µg Hg/kg b.w./day is taken forward to the risk characterisation.

3.4.1.2 Methylmercury

The critical effect following dietary intake of methylmercury is the effects observed in the developing nervous system.

There is a general agreement that the developing nervous system is the most sensitive target organ for methylmercury toxicity; however, no consensus has been achieved regarding a threshold for neurodevelopmental effects. Based on the evaluations performed by US-EPA (IRIS 2004a), a maternal daily intake of 1.0 µg/kg b.w./day (range, 0.857-1.472 µg/kg b.w./day) is considered as a LOAEL for neurodevelopmental effects (see section 3.4.2 for further details), and is taken forward to the risk characterisation.

3.4.2 ADI / TDI / PTWI

FAO/WHO (JECFA 1972) has derived a Provisional Tolerable Weekly Intake (PTWI) of 5 µg/kg b.w. of total mercury of which no more than 3.3 µg/kg b.w. should be methylmercury compounds (expressed as Hg). The evaluation was *"…based on a high total mercury intake in the diet, due to the consumption of fish containing high levels of methylmercury compounds. Where high total mercury intakes occur for other reasons, (for example due to inorganic mercury as a consequence of local natural conditions) the evaluation does not necessarily apply and the whole situation has to be reconsidered on its merits."* No specific data on toxicological effects of inorganic mercury compounds were included in the evaluation.
The PTWI for methylmercury has been re-evaluated several times since 1972 as new information became available, and up to the evaluation performed in 1999, the PTWI of 3.3 µg/kg b.w. was confirmed.
However, FAO/WHO (JECFA 2004) has derived a PTWI of 1.6 µg/kg b.w. based on two epidemiological studies from the Faroe Islands and the Seychelles that investigated the relationship between maternal exposure to mercury and impaired neurodevelopment in their children. A steady-state intake of methylmercury of 1.5 µg/kg b.w. per day was estimated to represent the exposure that would be expected to have no appreciable adverse effects on children. An uncertainty factor of 2 was applied to allow for inter-individual variability in the hair-to-blood ratio and a factor of $10^{0.5}$ (3.16) to account for inter-individual variability in the rate of elimination. Uncertainty factors for inter-individual variability in toxicodynamic vulnerability or for incompleteness of the database were considered not to be necessary. EFSA (2004) pointed out that the maternal hair level of 14 µg Hg/g was not a NOAEL in the data from the Faroe Islands.

No toxicological data on inorganic mercury compounds are available in these evaluations and no PTWI or TDI has been established for inorganic mercury.

US-EPA has set an oral Reference Dose (RfD) for methylmercury of 0.0001 mg Hg/kg b.w./day based on the studies from the Faroe Islands. The benchmark dose lower limit ($BMDL_{05}$, the lower 95% confidence limit of the BMD_{05}) was calculated and ranged from 46 to 79 ppb in maternal blood for different neuropsychological effects in the offspring at 7 years of age, corresponding to a range of maternal daily intakes of 0.857 to 1.472 µg/kg b.w./day. An uncertainty factor of 10 was used to account for inter-individual variability with a factor of 3 to account for pharmacokinetic variability and uncertainty in estimating an ingested mercury dose from cord-blood mercury concentration and a factor of 3 to account for pharmacodynamic variability and uncertainty. Further BMD analyses were performed for a number of endpoints from all three studies (Faroe Islands, Seychelles, and New Zealand) and led basically to the same outcome. (IRIS 2004a).
US-EPA has set an oral Reference Dose (RfD) for mercuric chloride of 0.0003 mg Hg/kg b.w./day based on the rat subchronic feeding and subcutaneous studies in Brown Norway rats. An uncertainty factor of 1000 was applied with a factor of 10 for LOAEL to NOAEL conversion, 10 for use of subchronic studies, and a combined 10 for both animal to human and sensitive human populations. (IRIS 2004b).

EFSA (2004) noted that the health based guidance values set by FAO/WHO (JECFA) and US-EPA for methylmercury differed by a factor of approximately two, largely because of different interpretations of the main epidemiology studies and because of the different uncertainty factors used. It was beyond the scope of the present EFSA opinion to perform a refinement of the hazard characterisation for methylmercury.

3.4.3 Risk characterisation

The mean human intake of mercury from traditional Greenland food items has been estimated to be 66 µg/day/person in the spring and 42 µg/day in the fall (Johansen et al. 2004).

Two sources, seal muscle and seal liver, dominate the intake (59% of the total intake in the spring and 64% in the fall) with about half coming from muscle and the other half from liver; other sources are scattered among fish, birds and whales (Johansen et al. 2004). In meat from fish and marine animals, mercury is predominantly present in the form of methylmercury whereas in the liver, mercury is predominantly present in the form of inorganic mercury. Inorganic mercury in food is considered as being considerably less toxic than methylmercury (EFSA 2004). Therefore, the risk characterisation will be performed for two scenarios: 1) the intake of total mercury from traditional Greenland food items is assumed as all of the mercury was methylmercury; and 2) the intake of total mercury from traditional Greenland food items is assumed as all of the mercury was inorganic mercury.

3.4.3.1 Scenario 1: total intake as methylmercury

A maternal daily intake of methylmercury of 1.0 µg/kg b.w./day (corresponding to 60 µg/day for a 60 kg adult person) is considered as a LOAEL for neurodevelopmental effects observed in humans.

A MOSref of 3 is derived. AF_I is set to 1 as the LOAEL is based on human data. AF_{II} is set to 3 to take into account the inter-individual variation in sensitivity. AF_{III} is set to 1 because the LOAEL used as the starting point for the risk characterisation in fact is the calculated benchmark dose lower limit ($BMDL_{05}$, the lower 95% confidence limit of the BMD_{05}).

A MOS of 0.91 is calculated for the mean daily human intake of methylmercury in the spring and of 1.4 in the fall. These MOS values are below the MOSref of 3 (about 3 times below in the spring and about 2 times below in the fall) implicating a concern of experiencing adverse health effects in the developing nervous system among Greenlanders due to long-term intake of methylmercury from the traditional Greenland food items. As no threshold has been identified for the critical effect, it is recommended to reduce the intake of methylmercury from the traditional Greenland food items as well as from other sources.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied by FAO/WHO (JECFA) and US-EPA in the setting of a PTWI and an RfD, respectively.
In the setting of the PTWI of 1.6 µg/kg b.w., JECFA (2004) has applied an uncertainty factor of 2 to allow for inter-individual variability in the hair-to-blood ratio and a factor of $10^{0.5}$ (3.16) to account for inter-individual variability in the rate of elimination resulting in a combined uncertainty factor of 6.3.
An uncertainty factor of 10 was applied by US-EPA (IRIS 2004a) in the setting of an RfD of 0.1 µg Hg/kg b.w./day to account for inter-individual variability with a factor of 3 to account for pharmacokinetic variability and uncertainty in estimating an ingested mercury dose from cord-blood mercury concentration and a factor of 3 to account for pharmacodynamic variability and uncertainty.
The MOSref of 3 is about 2 times lower than the uncertainty factor applied by JECFA and about 3 times lower than that applied by US-EPA. The JECFA PTWI of 1.6 µg/kg b.w. (corresponding to a PTDI of 0.23 µg/kg b.w./day) and the US-EPA oral RfD of 0.1 µg/kg b.w./day is of the same order of magnitude as the "TDI" of about 0.3 µg/kg b.w./day, which can be estimated by dividing the LOAEL used as the starting point for the MOS calculation in the risk characterisation of methylmercury in this report by the MOSref (LOAEL / MOSref = 1 µg/kg b.w./day / 3 = 0.33 µg/kg b.w./day).
Thus, a significant difference between the risk characterisation of methylmercury in this report (the MOS approach) versus the more traditional approach (TDI or RfD approach) is not identified.

3.4.3.2 Scenario 2: total intake as inorganic mercury

A LOAEL of 300 µg/kg b.w./day (corresponding to 18000 µg/day for a 60 kg adult person) is considered for effects in the kidney of mercuric mercury in rats and is taken as a LOAEL for inorganic mercury compounds.

A MOSref of 1000 is derived. AF_I is set to 10 (default value) as the LOAEL is based on data from studies in experimental animals. AF_{II} is set to 10 (default value). AF_{III} is set to 10 to account for the scarce toxicological data set from which a NOAEL cannot be established based on subchronic or chronic toxicity studies.

A MOS of 273 is calculated for the mean daily human intake of inorganic mercury in the spring and of 429 in the fall. These MOS values are below the MOSref of 1000 (about 3 times below in the spring and about 2 times below in the fall) implicating a concern of experiencing adverse health effects in the kidney among

Greenlanders due to long-term intake of inorganic mercury from the traditional Greenland food items. Therefore, it is recommended to reduce the intake of inorganic mercury from the traditional Greenland food items as well as from other sources.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied by FAO/WHO (JECFA) and US-EPA in the setting of a PTWI/TDI and an RfD, respectively.
No PTWI or TDI has been set by JECFA.
An uncertainty factor of 1000 was applied by US-EPA (IRIS 2004b) in the setting of an RfD of 0.3 µg Hg/kg b.w./day with a factor of 10 for LOAEL to NOAEL conversion, 10 for use of subchronic studies, and a combined 10 for both animal to human and sensitive human populations.
The MOSref of 1000 is equal to the uncertainty factor applied by US-EPA, and the US-EPA oral RfD of 0.3 µg/kg b.w./day is equal to the "TDI" of 0.3 µg/kg b.w./day, which can be estimated by dividing the LOAEL used as the starting point for the MOS calculation in the risk characterisation of inorganic mercury in this report by the MOSref (LOAEL / MOSref = 300 µg/kg b.w./day / 1000 = 0.3 µg/kg b.w./day).
Thus, a difference between the risk characterisation of inorganic mercury in this report (the MOS approach) versus the more traditional approach (RfD approach) is not identified.

3.4.4 References

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3.5 Selenium

3.5.1 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of selenium are selenosis and the effects in the liver. A NOAEL of about 850 µg Se/day (corresponding to about 14 µg/kg b.w./day for an adult person weighing 60 kg) is considered for the critical effects, and is taken forward to the risk characterisation.

3.5.2 ADI / TDI / PTWI

SCF has derived a tolerable upper intake level (UL) of 300 µg Se/day for adults based on a NOAEL of 850 µg/day for clinical selenosis and an uncertainty factor of 3 to allow for the remaining uncertainties of the studies used in deriving an upper level (SCF 2000).

US-EPA has set an oral Reference Dose (RfD) of 0.005 mg/kg b.w./day based on a NOAEL of 0.015 mg/kg b.w./day for clinical selenosis and an uncertainty factor of 3 to account for sensitive individuals (IRIS 2004).

ATSDR has derived a minimal risk level (MRL) of 0.005 mg/kg b.w./day for oral chronic exposure based on a NOAEL of 0.015 mg/kg b.w./day for nail disease and an uncertainty factor of 3 for human variability (ATSDR 2003).

3.5.3 Risk characterisation

The mean human intake of selenium from traditional Greenland food items has been estimated to be 127 µg/day/person in the spring and 112 µg/day in the fall (Johansen et al. 2004).

A NOAEL of about 850 µg Se/day is considered for the critical effects, which are selenosis and effects on the liver observed in humans.

A MOSref of 10 is derived. AF_I is set to 1 as the NOAEL is based on human data. AF_{II} is set to 10 (default value). AF_{III} is set to 1 as a NOAEL is used as the starting point for the risk characterisation.

A MOS of 6.7 is calculated for the mean daily human intake of selenium in the spring and of 7.6 in the fall. These MOS values are below the MOSref of 10 implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of selenium from the traditional Greenland food items. However, as the calculated MOS values are very close to the MOSref and as the NOAEL used as the starting point for the risk characterisation is a conservative estimate, the concern is very low.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied by SCF and US-EPA in the setting of an upper intake level and an RfD, respectively.
In the setting of a tolerable upper intake level (UL) of 300 µg Se/day for adults, SCF (2000) applied an uncertainty factor of 3 to allow for the remaining uncertainties of the studies used in deriving an upper level.
An uncertainty factor of 3 was applied by US-EPA (IRIS 2004) in the setting of an RfD of 5 µg/kg b.w./day to account for sensitive individuals.

The MOSref of 10 is about 3 times higher than the uncertainty factor applied by SCF and US-EPA. The SCF UL of 300 µg/day (corresponding to 5 µg/kg b.w./day) is equal to the US-EPA oral RfD of 5 µg/kg b.w./day. Both are about 3 times higher than the "TDI" of about 1.5 µg/kg b.w./day, which can be estimated by dividing the NOAEL used as the starting point for the MOS calculation in the risk characterisation of selenium in this report by the MOSref (NOAEL / MOSref = 850/60 µg/kg b.w./day / 10 = 1.4).
Thus, the risk characterisation of selenium in this report (the MOS approach) is a little more conservative than the more traditional approach (TDI or RfD approach); however, a significant difference is not identified. The difference is solely due to the difference in the MOSref and the uncertainty factor applied by SCF and US-EPA.

3.5.4 References

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3.6 PCBs

3.6.1 Critical effect(s) and NOAEL / LOAEL

Effects observed following dietary intake of PCBs include effects in the liver, stomach, thyroid, adrenals, and skin and eyes; in the haematological, immune and nervous systems; carcinogenicity, and reproductive and developmental effects.
The N/LOAELs considered for commercial mixtures are based on the results for Aroclor 1254 in studies with monkeys unless otherwise stated, and for individual congeners included in the sPCB10 based on the results for PCB 105 in studies with rats unless otherwise stated.

For commercial PCB mixtures, a LOAEL of 0.08 mg/kg b.w./day is considered for liver effects based on increased liver weight; 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 b.w./day is a LOEL for effects in the liver. A LOAEL of 0.005 mg/kg b.w./day is considered for dermal, ocular, immunotoxic, and developmental effects.
A NOAEL of 0.08 mg/kg b.w./day is considered for effects in stomach tissue, the thyroid, the adrenal glands, and the haematological system, and a NOAEL of 0.005 mg/kg b.w./day is considered for reproductive effects.

A LOAEL of 0.0075 mg/kg b.w./day is considered for neurotoxic effects following postnatal exposure to a defined PCB mixture analogous to the congener composition found in human milk based on the study in monkeys. This LOAEL is,

according to ATSDR (2000), the lowest dose level tested in intermediate-duration studies of any PCB mixture in any species.

For individual congeners included in the sPCB10, a LOAEL of 0.04 mg/kg b.w./day is considered for effects in the liver and the thyroid, of 0.01 mg/kg b.w./day for neurotoxic effects, and of 4 mg/kg b.w./day for haematological and immunotoxic effects based on the data for PCB 105. A NOAEL of 4 mg/kg b.w./day is considered for effects in stomach tissue, adrenal glands, and skin and eyes, and for reproductive effects based on the data for PCB 105. A NOAEL of 0.001 mg/kg b.w./day is considered for developmental effects of PCB 126 (a congener which is not included in PCB10); no data for developmental effects of PCB 105 have been located.

Overall, a LOAEL of 0.005 mg/kg b.w./day is considered for adverse health effects of commercial PCB mixtures, a LOAEL of 0.0075 mg/kg b.w./day for a defined PCB mixture analogous to the congener composition found in human milk, and a LOAEL of 0.01 mg/kg b.w./day for individual PCB10 congeners. These LOAELs are taken forward to the risk characterisation.

A carcinogenic potential of PCBs for humans in relation to dietary exposure cannot be excluded, but is not considered to be significant at dose levels not producing liver toxicity, i.e., at the LOAEL of 0.005 mg/kg b.w./day considered for adverse health effects of commercial PCB mixtures.

3.6.2 ADI / TDI / PTWI

US-EPA has set an oral Reference Dose (RfD) for Aroclor 1016 of 0.07 µg/kg b.w./day based on a NOAEL of 0.007 mg/kg b.w./day for reduced birth weights in a monkey reproductive study. 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. (IRIS 2004a).

US-EPA has not set an oral Reference Dose (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 (IRIS 2004b).

US-EPA has set an oral Reference Dose (RfD) for Aroclor 1254 of 0.02 µg/kg b.w./day based on a LOAEL of 0.005 mg/kg b.w./day for ocular, dermal and immunological effects in monkeys. 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 subchronic exposure to a chronic RfD. (IRIS 2004c).

3.6.3 Risk characterisation

PCB levels in traditional Greenland food items have been presented as the sum of 10 congeners (sPCB10). These 10 congeners are CB 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180. This group represents most of the predominant congeners in fish and marine mammals. The mean human intake of sPCB10 has been estimated to be 23 µg/day/person both in the spring and in the fall. (Johansen et al. 2004).

The toxicological evaluation considers commercial and defined PCB mixtures, and the individual congeners included in the sPCB10. A LOAEL of 5 µg/kg b.w./day is considered for adverse health effects of commercial PCB mixtures, a LOAEL of 7.5 µg/kg b.w./day for a defined PCB mixture analogous to the congener composition found in human milk, and a LOAEL of 10 µg/kg b.w./day for the individual congeners included in the sPCB10. Commercial PCB mixtures, particularly Aroclor 1254, are the most thoroughly tested PCBs, whereas the data for the sPCB10 as well as for environmental PCB mixtures and defined mixtures are limited. The mechanisms of toxicity are not completely understood, but there is evidence to suggest that PCB congeners differ qualitatively and quantitatively in biological activities and that multiple mechanisms are involved in responses to PCB mixtures. Because different PCB congeners may produce effects by different mechanisms and humans are exposed to complex mixtures of interacting PCBs with differing biological activities, it seems reasonable to use the LOAEL of 5 µg/kg b.w./day (corresponding to 300 µg/day for a 60 kg adult person) established for commercial PCB mixtures as the starting point for the risk characterisation of PCBs.

A MOSref of 1000 is derived. AF_I is set to 10 (default value) as the LOAEL is based on data from studies in experimental animals. AF_{II} is set to 10 (default value). AF_{III} is set to 10 to account for incompleteness of the database, i.e., a NOAEL cannot be established for adverse health effects of PCBs in general, and limited data are available on the toxicity of the environmental PCB mixtures.

A MOS of 13 is calculated for the mean daily human intake of sPCB10 in the spring and in the fall. This MOS value is far below the MOSref of 1000 (about 75 times below) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of PCBs from the traditional Greenland food items. Consequently, it is recommended to reduce the intake of PCBs from the traditional Greenland food items as well as from other sources.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied by US-EPA in the setting of an RfD. An uncertainty factor of 300 was applied by US-EPA (IRIS 2004c) in the setting of an oral RfD for Aroclor 1254 of 0.02 µg/kg b.w./day.
The MOSref of 1000 is about 3 times higher than the uncertainty factor applied by US-EPA. The US-EPA RfD of 0.02 µg/kg b.w./day is 4 times higher than the "TDI" of 0.005 µg/kg b.w./day, which can be estimated by dividing the LOAEL used as the starting point for the MOS calculation in the risk characterisation of PCBs in this report by the MOSref (LOAEL / MOSref = 5 µg/kg b.w./day / 1000 = 0.005 µg/kg b.w./day).
Thus, the risk characterisation of PCBs in this report (the MOS approach) is a little more conservative than the more traditional approach (RfD approach); however, a significant difference is not identified. It should be noted that the US-EPA evaluation has been performed for one of the commercial PCB mixtures, whereas this evaluation considers the PCBs relevant for human exposure from the traditional Greenland food items, for which the available toxicological data are limited.

3.6.4 References

ATSDR (2000). Toxicological Profile for Polychlorinated Biphenyls (Update). U.S. Department of Health & Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

IRIS (2004a). Aroclor 1016. In: Integrated Risk Information System. Database quest, last revised: 11/01/1996. US-EPA.

IRIS (2004b). Aroclor 1248. In: Integrated Risk Information System. Database quest, last revised: 11/01/1996. US-EPA.

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3.7 DDT

3.7.1 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of DDT and its metabolites are the effects observed in the nervous system, the liver, and the reproductive system.

A NOAEL of 0.05 mg/kg b.w./day has been identified for minimal histological effects in the liver of rats at the next dose level (0.25 mg/kg b.w./day). This NOAEL is considered to be sensitive and to provide adequate protection against liver toxicity and carcinogenicity, neurotoxicity, and reproductive and developmental effects of DDT and DDE, and is taken forward to the risk characterisation.

3.7.2 ADI / TDI / PTWI

JMPR has established a Provisional Tolerable Daily Intake (PTDI) of 10 µg/kg b.w. based on a NOAEL of 1 mg/kg b.w./day for developmental toxicity in rats and a safety factor of 100 (JMPR 2000).

US-EPA has set an oral Reference Dose (RfD) of 0.5 µg/kg b.w./day based on a NOEL of 1 ppm in the diet (equivalent to 0.05 mg/kg b.w./day assuming a food consumption of 5% b.w./day) for hepatocellular alterations in the liver of rats when commercial DDT was administered for 15-27 weeks. A factor of 10 each was applied for the uncertainty of interspecies conversion and to protect sensitive human subpopulations. (IRIS 2004).

3.7.3 Risk characterisation

The mean human intake of sDDT (sum of *p,p'*-DDE, -DDD, -DDT + *o,p'*-DDE, -DDD, -DDT) has been estimated to be 32 µg/day/person in the spring and 25 µg/day in the fall (Johansen et al. 2004).

This toxicological evaluation considers *p,p´*-DDT and technical DDT as well as the two isomeric forms of the metabolite DDE: *o,p*-DDE and *p,p*-DDE. A NO(A)EL (sum of DDT and DDE) of 50 µg/kg b.w./day (corresponding to 3000 µg/day for a 60 kg adult person) is considered for minimal histological effects in the liver of rats. DDE is the most persistent of the two metabolites and also more persistent than DDT, and DDE is usually found at higher levels than DDT (and DDD) in the

tissues. Therefore, it is considered appropriate to compare this NO(A)EL with the intake estimates although the intake estimates also include the metabolite DDD.

A MOSref of 150 is derived. AF_I is set to 15 as the NO(A)EL to account for differences between humans and experimental animals in the accumulation of DDT and DDE. AF_{II} is set to 10 (default value). AF_{III} is set to 1 as a NO(A)EL is used as the starting point for the risk characterisation.

A MOS of 94 is calculated for the mean daily human intake of sDDT in the spring and of 120 in the fall. These MOS values are below the MOSref of 150 (about 1.6 times below in the spring and about 1.3 times below in the fall) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of DDT from the traditional Greenland food items. However, as the calculated MOS values are close to the MOSref and as the NO(A)EL is a conservative estimate for minimal histological effects in the liver, the concern is low.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied by WHO/FAO (JMPR) and US-EPA in the setting of a PTDI and an RfD, respectively.
In the setting of the PTDI of 10 µg/kg b.w., JMPR (2000) has applied an uncertainty factor 100.
An uncertainty factor of 100 was applied by US-EPA (IRIS 2004) in the setting of an oral RfD of 0.5 µg/kg b.w./day.
The MOSref of 150 is 1.5 times higher than the uncertainty factor applied by JMPR and by US-EPA. The JMPR PTDI of 10 µg/kg b.w./day is 20 times higher than the US-EPA oral RfD of 0.5 µg/kg b.w./day due to the different NO(A)ELs used in their respective evaluations. The US-EPA oral RfD is of the same order of magnitude as the “TDI” of 0.3 µg/kg b.w./day, which can be estimated by dividing the NO(A)EL used as the starting point for the MOS calculation in the risk characterisation of DDT in this report by the MOSref (NO(A)EL / MOSref = 50 µg/kg b.w./day / 150 = 0.33 µg/kg b.w./day).
Thus, a significant difference between the risk characterisation of DDT in this report (the MOS approach) versus the more traditional approach (TDI or RfD approach) is not identified.

3.7.4 References

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3.8 Chlordane

3.8.1 Critical effect(s) and NOAEL / LOAEL

3.8.1.1 Chlordane

The critical effects following dietary intake of chlordane are the effects observed in the liver, the carcinogenic effect, and the developmental effects.
The liver is the major target organ in rats and mice; a NOAEL of 1 mg/kg in the diet (corresponding to 0.055 mg/kg b.w./day for rats; 0.15 mg/kg b.w./day for mice) can be established based on histopathological alterations in the liver of female rats and mice of both sexes at higher dietary dose levels. Developmental effects in form of subtle behavioural effects (LOAEL 1 mg/kg b.w./day) and suppressed immune function (NOAEL of 0.16 mg/kg b.w./day) have been observed in the offspring of mice following exposure *in utero*. A NOAEL of 0.055 mg/kg b.w./day for effects in the liver is considered to be sensitive and to provide adequate protection against neurotoxicity and developmental effects as well, and is taken forward to the risk characterisation.

A carcinogenic potential of chlordane for humans in relation to dietary exposure cannot be excluded, but is not considered to be significant at the exposure levels at which other effects in the liver have been observed. Furthermore, a genotoxic potential of chlordane cannot be excluded either.

3.8.1.2 Heptachlor

The critical effects following dietary intake of heptachlor and heptachlor epoxide are the effects observed in the liver, the carcinogenic effect, and reproductive and developmental effects.
The liver is the major target organ in rats, mice and dogs; a NOAEL of 1 mg/kg in the diet for liver toxicity of heptachlor and heptachlor epoxide (corresponding to 0.025 mg/kg b.w./day) can be established based on histopathological alterations in the liver of dogs in the 2-year study with heptachlor epoxide. For reproductive and developmental effects, a NOAEL of 0.025 mg/kg b.w./day can be established based on the 2-generation study in dogs with heptachlor epoxide. A NOAEL of 0.025 mg/kg b.w./day is considered to be sensitive, and is taken forward to the risk characterisation.

A carcinogenic potential of heptachlor and heptachlor epoxide for humans in relation to dietary exposure cannot be excluded, but is not considered to be significant at the exposure levels at which other effects in the liver have been observed. Furthermore, a genotoxic potential of heptachlor and heptachlor epoxide cannot be excluded either.

3.8.1.3 Nonachlor

No data have been located regarding adverse health effects of nonachlor following repeated oral administration.

3.8.2 ADI / TDI / PTWI

JMPR (1986) has established an Acceptable Daily Intake (ADI) for chlordane of 0.5 µg/kg b.w. No information is provided regarding the basis for the ADI, but it is probably established based on a NOAEL of 1 mg/kg diet (0.05 mg/kg b.w./day) in a study of rats.
For heptachlor, JMPR (1991) has established an Acceptable Daily Intake (ADI) of 0.1 µg/kg b.w. based on a NOAEL of 1 mg/kg diet (0.025 mg/kg b.w./day) in a reproduction study in dogs and a 2-year study in dogs and a safety factor of 200. Furthermore it was stated *"Many studies available for evaluation were conducted*

more than 20 years ago and have severe deficiencies. Because of these deficiencies, its carcinogenicity in mice and its ability to bio-accumulate, the Meeting recommended that heptachlor should not be used directly on food crops and its use in the production of food communities should be phased out. Because of its environmental persistence it is found as a contaminant in food commodities. The Meeting therefore maintained an ADI, basing it on the NOAELs derived from studies in dogs. However, recognising the inadequacy of the data base, the Meeting increased the safety factor to 200-fold. This ADI will provide a guideline for assessing the significance of dietary exposure to heptachlor residues."

US-EPA has set an oral Reference Dose (RfD) for chlordane of 0.5 µg/kg b.w./day based on a NOAEL of 1 mg/kg in the diet (equivalent to 0.15 mg/kg b.w./day) for liver toxicity in a 104-week study in mice. A factor of 10 each was employed for consideration of interspecies extrapolation and for intraspecies variation, and a factor of 3 for lack of any reproductive studies. (IRIS 2004a).
For heptachlor, US-EPA has set an RfD of 0.5 µg/kg b.w./day based on a NOAEL of 3 mg/kg in the diet (equivalent to 0.15 mg/kg b.w./day) for liver toxicity in a 2-year study in rats. A factor of 10 each was used to account for inter- and intraspecies differences, and a factor of 3 was considered appropriate because of the lack of chronic toxicity data in a second species. (IRIS 2004b).
For heptachlor epoxide, US-EPA has set an RfD of 0.013 µg/kg b.w./day based on a LOAEL of 0.5 mg/kg in the diet (equivalent to 0.0125 mg/kg b.w./day) for liver toxicity in a 60-week study in dogs. A factor of 10 each was used to account for inter- and intraspecies differences, and for the fact that a NOEL was not established. (IRIS 2004c).

3.8.3 Risk characterisation

Chlordane levels in traditional Greenland food items have been presented as sCHL (sum of heptachlor, heptachlor epoxide, oxychlordane, cis- and trans-chlordane, and cis- and trans-nonachlor). The mean human intake of sCHL has been estimated to be 18 µg/day/person in the spring and 15 µg/day in the fall. (Johansen et al. 2004).

The toxicological evaluation considers the seven compounds representing sCHL. No data have been located regarding adverse health effects of oxychlordane and nonachlor following repeated oral administration. Analytical grade chlordane used in the experimental studies consists of 72% *cis* and 23% *trans* isomers; no data have been located regarding the toxicity of the individual isomers and therefore, specific evaluations of the isomers of chlordane cannot be performed. Technical chlordane is a mixture of more than 140 related substances and consists generally of 60-85% of *cis*- and *trans*-chlordane; other major constituents are chlordene, heptachlor, and *cis*- and *trans*-nonachlor. Technical grade heptachlor used in the experimental studies consists of 73% heptachlor, 18% *trans*-chlordane, 2% *cis*-chlordane. Overall, specific evaluations have not been performed for analytical grades of chlordane / heptachlor versus the technical grades.
A NOAEL of 1 mg/kg in the diet is considered for liver toxicity of chlordane (corresponding to 0.055 mg/kg b.w./day) based on the 30-month study of rats with technical chlordane. A NOAEL of 1 mg/kg in the diet is considered for liver toxicity and for reproductive and developmental effects of heptachlor and heptachlor epoxide (corresponding to 0.025 mg/kg b.w./day) based on the 2-year study in dogs with heptachlor epoxide. Oxychlordane and heptachlor epoxide are the metabolites of chlordane and heptachlor, respectively, that are considered of primary toxicological significance. Based on the dietary NOAEL of 1 mg/kg diet for both technical chlordane and for heptachlor epoxide, it is considered that the

toxicological potency of chlordane and heptachlor, and consequently of oxychlordane and heptachlor epoxide, is quite similar. Overall, a NOAEL of 25 µg/kg b.w./day (corresponding to 1500 µg/day for a 60 kg adult person) is considered as a starting point for the risk characterisation of chlordane (based on the 2-year study in dogs with heptachlor epoxide). No data are available on the concentration of the individual compounds represented in the sum of sCHL in traditional Greenland food items. However, as the NOAEL to be used as the starting point for the risk characterisation of chlordane is considered to be sensitive, it is considered appropriate to compare the NOAEL with the intake estimates.

A MOSref of 1000 is derived. AF_I is set to 10 (default value) as the NOAEL is based on data from studies in experimental animals. AF_{II} is set to 10 (default value). AF_{III} is set to 10 to account for incompleteness of the database, i.e., most of the available long-term studies were conducted more than 30 years ago and have methodological deficiencies, limited data are available regarding reproductive and developmental effects, and a carcinogenic and genotoxic potential of chlordane cannot be excluded either.

A MOS of 83 is calculated for the mean daily human intake of sCHL in the spring and of 100 in the fall. These MOS values are well below the MOSref of 1000 (about 12 times below in the spring and about 10 times below in the fall) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of chlordane from the traditional Greenland food items. Consequently, it is recommended to reduce the intake of chlordane from the traditional Greenland food items as well as from other sources.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied by FAO/WHO (JMPR) and US-EPA in the setting of an ADI and an RfD, respectively.
In the setting of the ADI for chlordane of 0.5 µg/kg b.w., JMPR (1986) has probably applied a safety factor of 100 and in setting of the ADI for heptachlor of 0.1 µg/kg b.w.; JMPR (1991) has applied a safety factor of 200.
An uncertainty factor of 300 was applied by US-EPA (IRIS 2004a) in the setting of an oral RfD for chlordane of 0.5 µg/kg b.w./day with a factor of 10 each to account for interspecies extrapolation and for intraspecies variation, and a factor of 3 for lack of any reproductive studies. Similarly, an uncertainty factor of 300 was applied by US-EPA (IRIS 2004b) in the setting of an oral RfD for heptachlor of 0.5 µg/kg b.w./day with a factor of 10 each to account for inter- and intraspecies differences, and a factor of 3 was considered appropriate because of the lack of chronic toxicity data in a second species. For heptachlor epoxide, US-EPA (IRIS 2004c) applied an uncertainty factor of 1000 in the setting of an oral RfD of 0.013 µg/kg b.w./day with a factor of 10 each to account for inter- and intraspecies differences, and for the fact that a NOEL was not established.

The MOSref of 1000 is 10 times higher than the safety factor used by JMPR for chlordane and 5 times higher than the safety factor used for heptachlor. The MOSref is equal to the uncertainty factor used by US-EPA for heptachlor epoxide, but 3 times higher than the uncertainty factor used for chlordane and heptachlor.
The JMPR ADI for chlordane of 0.5 µg/kg b.w./day is equal to the US-EPA oral RfD. Both are 20 times higher than the “TDI” of 0.025 µg/kg b.w./day, which can be estimated by dividing the NOAEL used as the starting point for the MOS calculation in the risk characterisation of CHL in this report by the MOSref (NOAEL / MOSref = 25 µg/kg b.w./day / 1000 = 0.025 µg/kg b.w./day).
The JMPR ADI for heptachlor of 0.1 µg/kg b.w./day is 5 times lower than the US-EPA oral RfD. The JMPR ADI is 4 times higher than the “TDI” of 0.025 µg/kg

b.w./day for CHL in this evaluation, and the US-EPA oral RfD is 20 times higher. The US-EPA oral RfD of 0.013 µg/kg b.w./day for heptachlor epoxide is 2 times lower than the "TDI" of 0.025 µg/kg b.w./day for CHL in this evaluation.

Thus, the risk characterisation of CHL in this report (the MOS approach) is conservative compared to the more traditional approach (TDI or RfD approach); however, it should be noted that the JMPR and US-EPA evaluations consider these two compounds separately whereas this evaluation considers all the compounds represented in sCHL together.
It should also be noted that the "TDI" for CHL in this evaluation is not conservative compared to the US-EPA evaluation for heptachlor epoxide, which probably is reflected by the fact that the "TDI" for CHL in this evaluation is based on a NOAEL for heptachlor epoxide in dogs, the same species used in the US-EPA evaluation, and that the MOSref in this evaluation is equal to the uncertainty factor used by US-EPA.

3.8.4 References

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IRIS (2004b). Heptachlor. In: Integrated Risk Information System. Database quest, last revised: 03/01/1991 (cancer 07/01/1993). US-EPA.

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3.9 Chlorobenzenes

3.9.1 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of HCB are the effects observed in the liver, bone and the immune system, carcinogenicity, and reproductive and developmental effects.
A dose level of 50-200 mg/day (0.8-3.3 mg/kg b.w./day for a 60-kg person) constitutes a LOAEL for effects of HCB observed in humans in the Turkish poisoning incident. In experimental animals, a LOAEL of 2.5 mg/kg b.w./day is considered for porphyria, a NOAEL of 0.05 mg/kg b.w./day for enzyme induction and histopathological changes in the liver, a NOAEL of 0.07 mg/kg b.w./day for changes in calcium homeostasis and bone morphometry, a LOAEL of 0.2 mg/kg b.w./day for effects in the immune system, a LOAEL of 0.01 mg/kg b.w./day for reproductive toxicity, and a NOAEL of 0.016 mg/kg b.w./day for developmental toxicity. Overall, a LOAEL of 0.01 mg/kg b.w./day is considered for adverse health effects of HCB. This LOAEL is considered to be sensitive and to provide adequate protection against the adverse effects observed in the liver, bone and the

immune system in experimental animals, and is taken forward to the risk characterisation.

For 1,2,3,4-TeCB, the critical effects following dietary intake are the effects in the liver and kidney and for PeCB, the effects in the liver and thyroid.
No human data are available for 1,2,3,4-TeCB or PeCB. In experimental animals, a NOAEL of 3.4 mg/kg b.w./day is considered for toxicity in the liver and the kidneys of 1,2,3,4-TeCB, and is taken forward to the risk characterisation. For PeCB, a NOAEL cannot be established based on the available data; a LOAEL of 2.0 mg/kg b.w./day is considered for effects in the liver and thyroid, and is taken forward to the risk characterisation.

A carcinogenic potential of HCB for humans in relation to dietary exposure cannot be excluded, but is not considered to be significant at the exposure levels at which the effects in the liver, bone, and immune system, and reproductive and developmental effects have been observed. Furthermore, a genotoxic potential of HCB cannot be excluded either. The carcinogenic potential of 1,2,3,4-TeCB and PeCB cannot be evaluated as no data are available.

3.9.2 ADI / TDI / PTWI

JMPR (1970) concluded that no Acceptable Daily Intake (ADI) could be established for HCB for the following reasons: 1) HCB is a highly toxic compound, 2) there was insufficient information on metabolism, 3) effects on the bone marrow gave rise to serious concern, 4) no long-term studies or studies on reproduction were available, and 5) there were little information on the effects in mammalian species other than the rat. JMPR agreed to establish a Tentative Negligible Daily Intake of 0.6 µg/kg b.w./day based on a NOAEL of 1.25 mg/kg b.w./day for toxicity in a 13-week study in rats and using an extremely high safety factor (not further specified). Furthermore, it was stressed that the use of HCB was highly undesirable.

US-EPA has set an oral Reference Dose (RfD) for HCB of 0.8 µg/kg b.w./day based on a NOAEL of 1.6 ppm in the diet (equivalent to 0.08 mg/kg b.w./day) for developmental toxicity in a 2-generation study in rats. A factor of 10 each was applied to account for interspecies variation and to protect sensitive human subpopulations. (IRIS 2004b).

US-EPA has set an oral Reference Dose (RfD) for PeCB of 0.8 µg/kg b.w./day based on a LOAEL of 125 mg/kg in the diet (equivalent to 8.3 mg/kg b.w./day according to the author) for liver and kidney toxicity in a subchronic oral study in rats (including weanlings). The composite uncertainty factor of 10000 represents a factor of 10 each to account for interspecies variation, to protect sensitive human subpopulations, to extrapolate a subchronic effect level to its chronic counterpart, and to drop the LOAEL into the expected range of a NOAEL. (IRIS 2004a).

3.9.3 Risk characterisation

Chlorobenzene levels in traditional Greenland food items have been presented as sCBz (sum of 1,2,3,4-tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene). The mean human intake of sCBz has been estimated to be 4 µg/day/person both in the spring and in the fall. (Johansen et al. 2004).

The toxicological evaluation considers the three chlorobenzenes constituting the sCBz. A LOAEL of 10 µg/kg b.w./day (corresponding to 600 µg/day for a 60 kg adult person) is considered for adverse health effects of HCB based on reproductive toxicity in monkeys, a NOAEL of 3.4 mg/kg b.w./day (corresponding to 204 mg/day) for renal and hepatic toxicity of 1,2,3,4-TeCB, and a LOAEL of 2.0 mg/kg b.w./day (corresponding to 120 mg/day) for effects on the liver and thyroid of PeCB.
The available data on chlorobenzenes in general indicate a trend for the toxicity to increase with increased chlorination of the benzene ring. In addition, HCB is the most thoroughly tested chlorobenzene. Therefore, the LOAEL for HBC is considered as a LOAEL for chlorobenzenes in the risk characterisation. As this LOAEL is considered to be sensitive and to provide adequate protection against adverse health effects of HCB observed in the liver, bone, and the immune system as well as of the observed adverse health effects of 1,2,3,4-TeCB and PeCB, it is considered appropriate to compare the LOAEL with the intake estimates.

A MOSref of 1000 is derived. AF_I is set to 10 (default value) as the LOAEL is based on data from studies in experimental animals. AF_{II} is set to 10 (default value). AF_{III} is set to 10 to account for incompleteness of the database, i.e., a NOAEL cannot be established for reproductive toxicity of HCB and for adverse health effects of PeCB, no or only limited data are available for a number of toxicological endpoints for 1,2,3,4-TeCB and PeCB, and a carcinogenic and genotoxic potential of HCB cannot be excluded either.

A MOS of 150 is calculated for the mean daily human intake of sCBz in the spring and in the fall. This MOS value is well below the MOSref of 1000 (about 6.6 times below) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of chlorobenzenes from the traditional Greenland food items. However, as the LOAEL is considered to be a conservative estimate, the concern is relatively low.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied by FAO/WHO (JMPR) and US-EPA in the setting of a TDI and an RfD, respectively.
JMPR has not set an ADI or TDI for HCB, but has established a Tentative Negligible Daily Intake of 0.6 µg/kg b.w./day by applying an extremely high safety factor (JMPR 1970). The JMPR evaluation was performed in 1969 at a time when very limited data were available for HCB. Therefore, it is not relevant to compare the safety factor used by JMPR with the MOSref considered in this evaluation.
An uncertainty factor of 100 was applied by US-EPA (IRIS 2004b) in the setting of an oral RfD for HCB of 0.8 µg/kg b.w./day with a factor of 10 each to account for interspecies variation, and to protect sensitive human subpopulations.
The MOSref of 1000 is 10 times higher than the uncertainty factor used by US-EPA. The US-EPA RfD of 0.8 µg/kg b.w./day is 80 times higher than the “TDI” of 0.01 µg/kg b.w./day, which can be estimated by dividing the LOAEL used as the starting point for the MOS calculation in the risk characterisation of CBz in this report by the MOSref (LOAEL / MOSref = 10 µg/kg b.w./day / 1000 = 0.01 µg/kg b.w./day).
Thus, the risk characterisation of CBz in this report (the MOS approach) is relatively more conservative than the more traditional approach (RfD approach); however, it should be noted that the US-EPA evaluation of HCB was performed in 1991 and the data on reproductive toxicity used for establishment of the LOAEL for HCB in this evaluation have become available since then. Furthermore, this evaluation also considers two other chlorobenzenes for which the available toxicological data are limited or not available for a number of toxicological endpoints.

3.9.4 References

IRIS (2004a). Pentachlorobenzene. In: Integrated Risk Information System. Database quest, last revised: 03/01/1988 (cancer 02/01/1995). US-EPA.

IRIS (2004b). Hexachlorobenzene. In: Integrated Risk Information System. Database quest, last revised: 04/01/1991 (cancer 11/01/1996). US-EPA.

JMPR (1970). Hexachlorobenzene. FAO/PL:1969/M/17/1. http//www.inchem.org/documents/jmpr/

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3.10 Hexachlorocyclohexanes

3.10.1 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of HCH are the effects observed in the liver, the immune and nervous systems, carcinogenicity, and reproductive and developmental toxicity.

No human data are available.

In experimental animals, a NOAEL of 0.1 mg/kg b.w./day is considered for adverse effects in the liver following dietary administration of HCH (this dose level is a LOEL for increased liver enzyme activities for β-HCH); a LOAEL of 0.012 mg/kg b.w./day for immunological effects, a NOAEL of 0.04 mg/kg b.w./day for neurological effects, and a NOAEL of 0.1 mg/kg b.w./day for reproductive and developmental effects. Overall, a LOAEL of 0.01 mg/kg b.w./day is considered for adverse health effects in experimental animals. This LOAEL is considered to be sensitive and to provide adequate protection against liver toxicity and probably also carcinogenicity, as well as against neurotoxicity, and reproductive and developmental effects of HCH, and is taken forward to the risk characterisation.

A carcinogenic potential of HCH for humans in relation to dietary exposure cannot be excluded, but is not considered to be significant at the exposure levels at which the effects on the immune system have been observed. Furthermore, a genotoxic potential of HCH cannot be excluded either.

3.10.2 ADI / TDI / PTWI

JMPR has established a temporary Acceptable Daily Intake (ADI) for γ-HCH of 1 µg/kg b.w. based on a NOAEL of 0.5 mg/kg b.w./day for toxicity and carcinogenicity in a 2-year study in rats and a safety factor of 500. This ADI provides a 10-fold margin of safety over the LOAEL of 0.012 mg/kg b.w./day in a study of immunotoxicity in mice. (JMPR 1997).
JMPR concluded in 1973 that there is insufficient information to estimate a no-effect level for t-HCH and therefore, an ADI could not be recommended (JMPR 1973).

US-EPA has set an oral Reference Dose (RfD) for γ-HCH of 0.3 µg/kg b.w./day based on a NOAEL of 4 mg/kg in the diet (equivalent to 0.33 mg/kg b.w./day based on measured food intake) for liver and kidney toxicity in a 90-day study in rats. A factor of 10 each was employed for use of a subchronic versus a lifetime assay, to account for interspecies variation, and to protect sensitive human subpopulations. (IRIS 2004c).
No RfDs have been set for the other HCH isomers or for t-HCH.

3.10.3 Risk characterisation

HCH levels in traditional Greenland food items have been presented as sHCH (sum of α-, β- and γ-HCH). The mean human intake of sHCH has been estimated to be 4 µg/day/person in the spring and 3 µg/day in the fall. (Johansen et al. 2004).

The toxicological evaluation considers the three individual HCH isomers as well as t-HCH. A LOAEL of 10 µg/kg b.w./day (corresponding to 600 µg/day for a 60 kg adult person) is considered for adverse health effects in experimental animals based on immunological effects observed in mice exposed to γ-HCH. β-HCH is the most persistent isomer in the environment and accumulates to a greater degree in tissues and organs than the other isomers. γ-HCH is the isomer most thoroughly tested in intermediate- and long-term experimental studies. γ-HCH is the most toxic isomer in acute toxicity studies, but the available data from intermediate- and long-term experimental studies do not indicate noteworthy differences in the general toxicity of the isomers. However, isomer specific differences are indicated for reproductive/ developmental and carcinogenic effects. No data are available on the concentration of the three individual HCH isomers in traditional Greenland food items. However, as the LOAEL for immunological effects of γ-HCH in mice is considered to be sensitive and to provide adequate protection against liver toxicity and probably also carcinogenicity, neurotoxicity, and reproductive and developmental effects of the three individual HCH isomers as well as of t-HCH, it is considered appropriate to compare the LOAEL with the intake estimates.

A MOSref of 1000 is derived. AF_I is set to 10 (default value) as the LOAEL is based on data from studies in experimental animals. AF_{II} is set to 10 (default value). AF_{III} is set to 10 to account for incompleteness of the database, i.e., a NOAEL cannot be established, the LOAEL is based on a subchronic study in stead of a lifetime study, and a carcinogenic and genotoxic potential of HCH cannot be excluded either.

A MOS of 150 is calculated for the mean daily human intake of sHCH in the spring and of 200 in the fall. These MOS values are below the MOSref of 1000 (about 6.6 times below in the spring and about 5 times below in the fall) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of HCH from the traditional Greenland food items. However, as the LOAEL is considered to be a conservative estimate and to provide adequate protection against liver toxicity and probably also carcinogenicity, neurotoxicity, and reproductive and developmental effects of the three individual HCH isomers as well as of t-HCH, the concern is relatively low.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied by FAO/WHO (JMPR) and US-EPA in the setting of an ADI and an RfD, respectively.
In the setting of the temporary ADI for γ-HCH of 1 µg/kg b.w., JMPR (1997) has applied a safety factor of 500 in order to provide a 10-fold margin of safety over the LOAEL of 0.012 mg/kg b.w./day for immunotoxicity in mice.

An uncertainty factor of 1000 was applied by US-EPA (IRIS 2004c) in the setting of an oral RfD for γ-HCH of 0.3 µg/kg b.w./day with a factor of 10 each to account for use of a subchronic versus a lifetime assay, to account for interspecies variation, and to protect sensitive human subpopulations.
The MOSref of 1000 is 2 times higher than the safety factor applied by JMPR and equal to the uncertainty factor applied by US-EPA. The JMPR ADI for γ-HCH of 1 µg/kg b.w./day is 3 times higher than the US-EPA oral RfD for γ-HCH of 0.3 µg/kg b.w./day due to slightly different NOAELs and uncertainty factors applied in their respective evaluations (0.5 / 0.33 mg/kg b.w./day). The JMPR ADI is 100 times higher and the US-EPA oral RfD 30 times higher than the "TDI" of 0.01 µg/kg b.w./day, which can be estimated by dividing the LOAEL used as the starting point for the MOS calculation in the risk characterisation of HCH in this report by the MOSref (LOAEL / MOSref = 12 µg/kg b.w./day / 1000 = 0.012 µg/kg b.w./day).
Thus, the risk characterisation of HCH in this report (the MOS approach) is relatively more conservative than the more traditional approach (TDI or RfD approach); however, it should be noted that the JMPR and US-EPA evaluations only consider γ-HCH, the isomer most thoroughly tested in experimental studies, whereas this evaluation also considers the other isomers as well as t-HCH.

3.10.4 References

IRIS (2004c). Gamma-hexachlorocyclohexane (gamma-HCH). In: Integrated Risk Information System. Database quest, last revised: 03/01/1988 (cancer 10/01/1993). US-EPA.

JMPR (1997). Lindane. Pesticide Residues in Food : 1997. http//www.inchem.org/documents/jmpr/

JMPR (1973). BHC (technical). WHO Pesticide Residues Series 3. http//www.inchem.org/documents/jmpr/

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3.11 Dieldrin

3.11.1 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of dieldrin are the effects observed in the nervous system and the liver, and the carcinogenic effect.

No adverse effects in the nervous system and in the liver have been observed in workers with dieldrin blood levels of 105 µg/l corresponding to an intake of 0.02 mg/kg b.w./day or in volunteers following administration of dieldrin at doses up to approximately 0.003 mg/kg b.w./day. The liver is the major target organ in rodents and dogs; a NOAEL of 0.005 mg/kg b.w./day can be established for increased liver weight (rat, dog) and histopathological changes (rat). A NOAEL of 0.005 mg/kg b.w./day is considered to be sensitive and to provide adequate protection against liver toxicity and neurotoxicity and is taken forward to the risk characterisation.

A carcinogenic potential cannot be fully excluded in relation to dietary exposure to dieldrin, but is not considered to be significant at the exposure levels at which the effects in the nervous system and the liver have been observed.

3.11.2 ADI / TDI / PTWI / RfD

JMPR has in 1966 established an Acceptable Daily Intake (ADI) of 0.1 µg/kg b.w. (combined total for aldrin and dieldrin) based on a NOAEL of 0.025 mg/kg b.w./day for toxic effects in rats and dogs, and an uncertainty factor of 250 for concern about carcinogenicity observed in mice (JMPR 1977).

US-EPA has in 1990 set an oral Reference Dose (RfD) of 0.05 µg/kg b.w./day based on a NOAEL of 0.1 ppm in the diet (equivalent to 0.005 mg/kg b.w./day) for effects in the liver of rats. A factor of 10 each was applied for the uncertainty in the extrapolation of dose levels from laboratory animals to humans and uncertainty in the threshold for sensitive humans. (IRIS 2004).

3.11.3 Risk characterisation

The mean human intake of dieldrin from traditional Greenland food items has been estimated to be 8 µg/day/person in the spring and 7 µg/day in the fall (Johansen et al. 2004).

A NOAEL of 5 µg/kg b.w./day (corresponding to 300 µg/day for a 60 kg adult person) is considered for liver toxicity and neurotoxicity in experimental animals.

A MOSref of 100 is derived. AF_I is set to 10 (default value) as the LOAEL is based on data from studies in experimental animals. AF_{II} is set to 10 (default value). AF_{III} is set to 1 as a NOAEL is used as the starting point for the risk characterisation.

A MOS of 38 is calculated for the mean daily human intake of dieldrin in the spring and of 43 in the fall. These MOS values are below the MOSref of 100 (about 2.6 times below in the spring and about 2.3 times below in the fall) implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of dieldrin from the traditional Greenland food items. However, as the calculated MOS values are at the same order of magnitude as the MOSref and as the NOAEL is considered to be a conservative estimate for minimal effects in the liver, the concern is relatively low.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied by FAO/WHO (JMPR) and US-EPA in the setting of an ADI and an RfD, respectively.
In the setting of the ADI of 0.1 µg/kg b.w. (combined total for aldrin and dieldrin), JMPR (1977) has in 1966 applied an uncertainty factor of 250 for concern about carcinogenicity observed in mice. The MOSref of 100 is 2.5 times lower than the uncertainty factor applied by JMPR. It should be recognised that the available data indicate that aldrin and dieldrin are non-genotoxic tumour promoters acting through species-specific susceptibility of the mouse. Therefore, protection against liver toxicity implicitly also protects against liver tumours and thus, a lower uncertainty factor than that used by JMPR in 1966 is justified.
An uncertainty factor of 100 was applied by US-EPA (IRIS 2004) in the setting of an oral RfD of 0.05 µg/kg b.w./day with a factor of 10 each to account for the uncertainty of interspecies conversion and to protect sensitive human subpopulations. The MOSref of 100 is equal to the uncertainty factor applied by US-EPA as is the NOAEL identified for toxic effects.

Thus, a significant difference between the risk characterisation of dieldrin in this report (the MOS approach) versus the more traditional approach (RfD approach) is not identified.

3.11.4 References

IRIS (2004). Dieldrin. In: Integrated Risk Information System. Database quest, last revised: 09/01/1990 (cancer 07/01/1993). US-EPA.

JMPR (1977). Pesticide Residues in Food 1977 : Aldrin/dieldrin. http//www.inchem.org/documents/jmpr/

Johansen P, Muir D, Asmund G and Riget F (2004). Human exposure to contaminants in the traditional Greenland diet. Sci Total Environ **331**, 189-206.

3.12 Toxaphene

3.12.1 Critical effect(s) and NOAEL / LOAEL

The critical effects following dietary intake of toxaphene are the effects observed in the liver, thyroid and kidney, carcinogenicity, and developmental neurotoxicity.

No human data are available.

In experimental animals, a NOAEL of 0.35 mg/kg b.w./day is considered for histological changes in the liver, thyroid and kidney of rats, and of 0.2 mg/kg b.w./day for histological changes in the liver and thyroid of dogs; only very slight effects were observed in the kidneys of dogs at dose levels up to 5.0 mg/kg b.w./day. Overall, a NOAEL of 0.2 mg/kg b.w./day is considered for effects in the liver, thyroid and kidney. This NOAEL is considered to provide adequate protection against effects in the immune system and the central nervous system, and is taken forward to the risk characterisation.

There are indications of slight neurobehavioural developmental effects in rats at lower dose levels (0.05 mg/kg b.w./day) than the NOAEL derived from the subchronic studies in rats and dogs. Furthermore, recent reports indicate that toxaphene is able to interfere with several sex hormones and/or their receptors *in vitro* and has been reported to cause oestrogen-like responses in cell systems.

A carcinogenic potential of toxaphene for humans in relation to dietary exposure cannot be excluded. Furthermore, based on the current limited database, it cannot be concluded whether toxaphene is genotoxic *in vivo* or not.

3.12.2 ADI / TDI / PTWI

JMPR has in 1968 evaluated toxaphene and concluded *"Although adequate work has been done on the original compound of known composition, the substance at present in agricultural use does not necessarily conform to the specifications of the original material tested biologically. Before an evaluation can be made, the identity of the compounds presently in use must be established, and future toxicological work must be related to them."* (JMPR 1968).
In 1973, JMPR reaffirmed that *"an ADI could not be established for material whose composition may vary with the method of manufacture."* (JMPR 1973).

US-EPA has in 1991 performed a carcinogenicity assessment of toxaphene; no oral Reference Dose (RfD) has been established (IRIS 2004).

The Nordic Committee of Senior Officers for Food Issues under the Nordic Council of Ministers has in 1996 funded a report regarding risk assessment of toxaphene exposure. It was concluded *"Given the uncertainties in the understanding of the underlying mechanisms of toxaphene carcinogenicity and in the determination of NOAELs for non-neoplastic effects in long-term studies, no definitive health effect-based tolerable intake can be established for toxaphene at the present time."* (NCM 1997).

3.12.3 Risk characterisation

Toxaphene levels in traditional Greenland food items have been presented as "total" toxaphene quantified with a technical toxaphene standard and 22 chlorobornane congeners. The mean human intake of toxaphene has been estimated to be 30 µg/day/person in the spring and 31 µg/day in the fall. (Johansen et al. 2004).

There are major difficulties in assessing the hazards of toxaphene and the risks related to toxaphene exposure due to the fact that toxaphene is a complex mixture containing a large number of individual compounds. Furthermore, toxaphene residues in biological samples show a different profile from that of technical toxaphene presumably due to metabolic transformation and degradation of some components. Humans may therefore be exposed to different components than those, which have been tested in experimental systems. Also the toxicological data set of toxaphene is rather scarce and most of the information is from older studies using poorly characterised test materials and lack of compliance with modern test guidelines. (NCM 1997).
A NOAEL of 0.2 mg/kg b.w./day (corresponding to 12000 µg/day for a 60 kg adult person) is considered for effects on the liver, thyroid and kidney. There are, however, indications of slight neurobehavioural developmental effects in rats at considerably lower dose levels (0.05 mg/kg b.w./day) than the NOAEL derived from the subchronic studies in rats and dogs. Furthermore, recent reports indicate that toxaphene is able to interfere with several sex hormones and/or their receptors *in vitro*.

A MOSref of 10000 is derived. AF_I is set to 10 (default value) as the NOAEL is based on data from studies in experimental animals. AF_{II} is set to 10 (default value). AF_{III} is set to 100 to account for the incompleteness of the data set, i.e., neurobehavioural developmental effects indicated at lower dose levels than the NOAEL for effects on the liver, thyroid and kidney; possible differences of technical toxaphene standard used for exposure analyses and the toxaphene used in the experimental studies; and the scarce toxicological data set from which no valid NOAEL can be established from chronic toxicity studies.

A MOS of 400 is calculated for the mean daily human intake of toxaphene from traditional Greenland food items in the spring and of 387 in the fall. These MOS values are far below the MOSref of 10000 (about 25-26 times below) implicating a significant concern of experiencing adverse health effects in the target organs and tissues among Greenlanders due to long-term intake of toxaphene from the traditional Greenland food items. Furthermore, a carcinogenic potential of toxaphene for humans in relation to dietary exposure cannot be excluded. In addition, based on the current limited database, it cannot be concluded whether

toxaphene has a genotoxic potential *in vivo* or not. This implicates that a NOAEL cannot be derived for the carcinogenic effects for the time being. Consequently, it is recommended to reduce the intake of toxaphene from the traditional Greenland food items as well as from other sources.

The MOSref, as an overall assessment (or uncertainty) factor, can be compared with the overall uncertainty factor applied in the setting of a TDI and an RfD, respectively.
According to JMPR (1973) *"an ADI could not be established for material whose composition may vary with the method of manufacture."*
US-EPA has not established an oral Reference Dose (RfD) (IRIS 2004).
According to The Nordic Committee of Senior Officers for Food Issues under the Nordic Council of Ministers *"No definitive tolerable daily intake of toxaphene can be recommended at the present time"* (NCM 1997).
These conclusions are in concordance with the toxicological evaluation in this report.

3.12.4 References

IRIS (2004). Toxaphene. In: Integrated Risk Information System. Database quest, last revised: 01/01/1991. US-EPA.

JMPR (1973). Camphechlor (Toxaphene). Pesticide Residues Series 3. http//www.inchem.org/documents/jmpr/

JMPR (1968). Toxaphene. FAO/PL:1968/M/9/1. http//www.inchem.org/documents/jmpr/

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NCM (1997). Nordic risk assessment of toxaphene exposure. TemaNord 1997:540. Nordic Council of Ministers.

4 Discussion and conclusions

A risk assessment in order to evaluate the risk to Greenlanders of experiencing adverse health effects from intake of contaminants from traditional food items has been performed for eleven contaminants, including lead, cadmium, mercury, selenium, PCBs, DDT, chlordane, HCHs, chlorobenzenes, dieldrin and toxaphene.

The risk has been characterised and evaluated by using the MOS approach as outlined in section 3.1, i.e., by comparing the NOAEL (or LOAEL) established for the critical effect(s) of a given contaminant to the mean daily human intake of that specific contaminant from traditional Greenland food items. The ratio resulting from this comparison is called Margin of Safety (MOS); i.e., MOS = N(L)OAEL / mean daily human intake of the contaminant.
In the characterisation of the risk, the MOS has been compared with a reference MOS (MOSref). When the MOS value was clearly below the MOSref, a concern for adverse health has been identified and it has been recommended to reduce the intake of the specific contaminant from the traditional Greenland food items as well as from other sources. When the MOS value was in the range of the MOSref, a more careful overall evaluation on a case-by-case basis has been performed.

For contaminants, which are both genotoxic and carcinogenic, a quantitative risk characterisation has not been performed for this endpoint. The reason for this decision is that there currently is no clear consensus on an appropriate methodology for the estimation of a no-effect level. For these contaminants, it has been recommended that the intake of the specific contaminant from the traditional Greenland food items as well as from other sources should be reduced.

The outcome of the risk characterisations as performed for the eleven contaminants evaluated in this report are summarised in Table 1 (see last page in this section).

For cadmium, PCBs, chlordane, and toxaphene, the calculated MOS values were well to far below (10-100 times below) the MOSref implicating a high concern of experiencing adverse health effects among Greenlanders due to long-term intake of these contaminants from the traditional Greenland food items. Consequently, it is recommended to reduce the intake of these contaminants from the traditional Greenland food items as well as from other sources.
As concluded by Johansen et al. (2004a)[21], the mean intakes of cadmium, chlordanes and toxaphene significantly exceeded the respective guideline values chosen for comparison by a factor of between 2.5 and 6; the mean intake of PCBs exceeded the guideline value chosen for comparison by about 30%. Although the risk characterisations performed for these contaminants in this report have arrived at higher exceedings (10-100 times) of the calculated MOS in relation to the MOSref, the conclusions point in the same direction, i.e., that the exposure to these contaminants from the traditional Greenland food items should be reduced.

For lead and mercury, the calculated MOS values were below (2-7 times below) the MOSref implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of these contaminants from the traditional Greenland food items. Furthermore, no threshold has been identified for the effects

[21] Johansen P, Muir D, Asmund G and Riget F (2004a). Human exposure to contaminants in the traditional Greenland diet. Sci Total Environ **331**, 189-206.

of lead and methylmercury on the developing nervous system. Consequently, it is recommended to reduce the intake of these contaminants from the traditional Greenland food items as well as from other sources.
As concluded by Johansen et al. (2004a), the mean intake of mercury exceeded the guideline value chosen for comparison by about 55%. This conclusion is in accordance with the risk characterisations performed for mercury in this report, i.e., that the exposure to mercury from the traditional Greenland food items should be reduced.
Johansen et al. (2004b)[22] concluded that, in some cases, the lead intake by Greenland bird eaters will largely exceed the guideline value chosen for comparison. This conclusion is also in accordance with the risk characterisation performed for lead in this report, i.e., that the exposure to lead from the traditional Greenland food items should be reduced.

For chlorobenzenes, hexachlorocyclohexanes (HCH), and dieldrin, the calculated MOS values were below (2-7 times below) the MOSref implicating a concern of experiencing adverse health effects among Greenlanders due to long-term intake of these contaminants from the traditional Greenland food items. However, as the NOAEL for dieldrin and the LOAELs for chlorobenzenes and HCHs used as starting points for the risk characterisation of these contaminants are considered to be conservative estimates, the exposure to these contaminants from the traditional Greenland food items is considered to be of relatively low concern.
As concluded by Johansen et al. (2004a), the mean intakes of HCH and chlorobenzenes were well below the respective guideline values chosen for comparison and it seems unlikely that the guideline values for these contaminants normally are exceeded in the Greenland population. Although the risk characterisations performed for these contaminants in this report have shown exceedings (2-7 times) of the calculated MOS in relation to the MOSref, the conclusions point in the same direction, i.e., that the exposure to these contaminants from the traditional Greenland food items is considered to be of relatively low concern.
For dieldrin, Johansen et al. (2004a) concluded that the mean daily intake exceeded the guideline value chosen for comparison by up to approximately 33%. The risk characterisation performed for dieldrin in this report, although showing an exceeding (2-3 times) of the calculated MOS in relation to the MOSref, concludes that the exposure to dieldrin from the traditional Greenland food items is considered to be of relatively low concern.

For selenium and DDT, the calculated MOS values were a little below (1.3-1.6 times below) the MOSref implicating a risk of experiencing adverse health effects among Greenlanders due to long-term intake of these contaminants from the traditional Greenland food items. However, as the calculated MOS values are very close to the MOSref and as the NOAELs for selenium and DDT used as the starting points for the risk characterisation are considered to be conservative estimates, the exposure to these contaminants from the traditional Greenland food items is considered to be of low concern.
As concluded by Johansen et al. (2004a), the mean intake of DDT was well below the guideline value chosen for comparison and it seems unlikely that the guideline value for DDT normally is exceeded in the Greenland population. This conclusion is in accordance with the risk characterisation performed for DDT in this report, although showing an exceeding (1.3-1.6 times) of the calculated MOS in relation to the MOSref, i.e., the exposure to DDT from the traditional Greenland food items is considered to be of low concern.

[22] Johansen P, Asmund G and Riget F (2004b). High human exposure to lead through consumption of birds hunted with lead shot. Environ Pollut **127**, 125-129.

The MOSref is an overall assessment (or uncertainty) factor (AF), as a numerical value, addressing the differences between the experimental data and the human situation, taking into account the uncertainties in the extrapolation procedure and in the available data set (see section 3.1.2.2).
In order to evaluate whether significant differences could be identified between the risk characterisation of the contaminants as performed in this report (the MOS approach) versus the more traditional approach (ADI/TDI or RfD approach), the MOSref derived for the selected contaminants has been compared with the overall uncertainty factor applied by e.g., FAO/WHO (JECFA/JMPR) in establishing an acceptable or tolerable daily intake (ADI/TDI), or by US-EPA in establishing an oral reference dose (RfD) when available for a given contaminant.

No significant difference has been identified between the risk characterisations of cadmium, lead, mercury, DDT, dieldrin as performed in this report (the MOS approach) versus the more traditional approach (TDI or RfD approach).

The risk characterisations of selenium, PCBs as performed in this report (the MOS approach) are a little more conservative than the more traditional approach (TDI or RfD approach); however, a significant difference has not been identified.
For PCBs, it should be noted that the US-EPA evaluation has been performed for one of the commercial PCB mixtures, whereas the risk characterisation performed in this report includes the PCBs relevant for human exposure from the traditional Greenland food items, for which the available toxicological data are limited.

The risk characterisations of chlordane, chlorobenzenes, hexachlorocyclohexanes (HCH) as performed in this report (the MOS approach) are conservative compared to the more traditional approach (TDI or RfD approach).
For chlordane, it should be noted that the FAO/WHO (JMPR) and US-EPA evaluations consider chlordane and heptachlor separately whereas the risk characterisation in this report considers all the compounds represented in sCHL together. It should also be noted that the “TDI” for chlordane in this evaluation is not conservative compared to the US-EPA evaluation for heptachlor epoxide, which probably is reflected by the fact that the “TDI” for chlordane in this evaluation is based on a NOAEL for heptachlor epoxide in dogs, the same species used in the US-EPA evaluation, and that the MOSref in this evaluation is equal to the uncertainty factor used by US-EPA.
For the chlorobenzenes, it should be noted that the US-EPA evaluation of hexachlorobenzene was performed in 1991 and the data on reproductive toxicity used for establishment of the LOAEL for hexachlorobenzene in this evaluation have become available since then. Furthermore, this evaluation also considers two other chlorobenzenes (1,2,3,4-tetrachlorobenzene, pentachlorobenzene) for which the available toxicological data are limited or not available for a number of toxicological endpoints.
For HCH, it should be noted that the FAO/WHO (JMPR) and US-EPA evaluations only consider γ-HCH, the isomer most thoroughly tested in experimental studies, whereas this evaluation also considers the other isomers as well as technical HCH.

No comparison could be performed for toxaphene as no guidance values have been established. According to The Nordic Committee of Senior Officers for Food Issues under the Nordic Council of Ministers, the most recent evaluation of toxaphene *“No definitive tolerable daily intake of toxaphene can be recommended at the present time”*. This conclusion is formally in concordance with the toxicological evaluation of toxaphene in this report.

According to the current practices, risk assessments of exposures to chemical substances and the subsequent regulatory measures, e.g., establishment of tolerable daily intakes, are generally based upon data from studies on the individual substances. This is also the approach used in the risk characterisations of the eleven contaminants evaluated in this report.
However, humans are simultaneously exposed to a large number of chemical substances that potentially possess a number of similar or different toxic effects, as is also the case for Greenlanders via consumption of traditional food items.

A recently published report[23] has summarised and evaluated the currently available scientific literature in the field of risk assessments of toxicological effects of exposures to chemical mixtures. Below is a very brief summary of the main results considered relevant for the discussion of potential adverse effects of combined exposure to the eleven contaminants evaluated in this report from consumption of the traditional Greenland food items.
Ideally, the prediction of the toxicological properties of a chemical mixture requires detailed information on the composition of the mixture and the mode of action of each of the individual compound. In order to perform a risk assessment, proper exposure data are also needed. Most often such detailed information is not available as is also the case for the eleven contaminants evaluated in this report.
One of the main points to consider is whether there will be no interaction or interaction in the form of either synergism or antagonism. These three basic principles of combined actions of chemical mixtures as e.g., contaminants in the food items, are purely theoretical and one often has to deal with two or all three concepts at the same time when food items consist of more than two compounds and when the toxicity targets are more complex.

Guidelines from national and international organisations have suggested the use of simple 'dose addition' or 'response addition' models for the assessment of chemically mixtures without taking into account the mode of action of the individual chemical substances. Research programmes have been initiated in order to test the hypothesis that exposure to chemical substances at (low) non-toxic doses of the individual substance as a rule would be of no health concern.
The currently available data indicate that combined exposure to a mixture of chemical substances that have either different target organs and/or different target sites within the same organ (i.e., differ in the mode of action) is not associated with a greater hazard than exposure to the individual substances, provided that the exposure levels are at or below the individual NOAELs. At exposure levels higher than the individual NOAELs, both synergistic and antagonistic effects may be observed, dependent on the substances.
The use of the 'dose addition' approach to the risk assessment of chemical mixtures is only scientifically justifiable when all the substances in the mixture act by the same mode of action, and thus differ only in their potencies. The same mode of action might exist if two substances 1) cause the same critical effect, 2) act on the same molecular target at the same target tissue, and 3) act by the same mechanism of action and may share a common toxic intermediate. It should be realised that with the exception of a few groups of chemical substances (e.g., some dioxins and PCBs), information on their mode of action is scarce.

A number of the contaminants evaluated in this report act on the same target organ. Eight contaminants induce toxic effects in the liver, three in the kidneys, two in the

[23] Larsen JC (ed.) (2003). Combined actions and interactions of chemicals in mixture. The toxicological effects of exposure to mixtures of industrial and environmental chemicals. FødevareRapport 2003:12. Published by the Danish Environmental Protection Agency and the Danish Veterinary and Food Administration.

bones, four in the immune system, seven in the nervous system, six in the reproductive system, and eight are carcinogenic. It is beyond the scope of this report to evaluate the potential adverse effects of combined exposure to these contaminants in the traditional Greenland food items; however, the available data indicate that some of the contaminants possibly act by the same mode of action. Thus, a concern for additive effects of some of the individual contaminants is indicated and this should also be taken into account in a consideration of risk reduction to be recommended for the contaminants in the traditional food items.

For some of the contaminants evaluated in this report (cadmium, lead, mercury, PCBs, chlordane, and toxaphene), the risk characterisation indicates a significant concern of experiencing adverse health effects among Greenlanders due to long-term intake of these contaminants from the traditional Greenland food items and risk reduction is recommended, i.e., to reduce the intake of these contaminants from the traditional Greenland food items as well as from other sources. However, for some of the contaminants, the concern is considered as being relatively low (chlorobenzenes, HCH and dieldrin) or low (DDT) and no risk reduction has been recommended for these contaminants. However, the concern for additive effects of some of the individual contaminants via consumption of the traditional food items supports that the intake of these contaminants from the traditional Greenland food items should probably also be reduced.

4.1 Conclusions

The risk characterisations of cadmium, lead, mercury, PCBs, chlordane, and toxaphene, as performed in this report indicate a concern of experiencing adverse health effects among Greenlanders due to long-term intake of these contaminants from the traditional Greenland food items. Risk reduction is therefore recommended, i.e., the intake of these contaminants from the traditional Greenland food items as well as from other sources should be reduced.
The risk characterisations, as performed in this report indicate a relatively low concern of adverse health effects of chlorobenzenes, HCH and dieldrin, and a low concern for selenium and DDT. No risk reduction has been recommended for these contaminants based on the risk characterisations. However, as a concern for additive effects of some of the individual contaminants is indicated, the intake of chlorobenzenes, HCH, dieldrin, and DDT from the traditional Greenland food items as well as from other sources should probably also be reduced.

Table 1 Summary of the risk characterisations performed for the eleven contaminants evaluated in this report

Contaminant	Intake[a] µg/d/person (spring/autumn)	NOAEL / LOAEL[b] µg/d/person	MOS[c] (spring/autumn)	MOSref[d]	MOSref / MOS[e] (spring/autumn)	Conclusion[f]
Lead	174[g]	L: 62.5	0.4	3	7	Concern
Cadmium	346 / 182	L: 10 - 60	0.03-0.17 / 0.05-0.33	3	18-100 / 9-60	Concern
Mercury	66 / 42[h]	L: 60 (org.) L: 18000 (inorg.)	0.91 / 1.4 (org.) 273 / 429 (inorg.)	3 1000	3 / 2 (org.) 3 / 2 (inorg.)	Concern Concern
Selenium	127 / 112	N: 850	6.7 / 7.6	10	1.5 / 1.3	Low concern
PCBs	23 / 23[i]	L: 300	13 / 13	1000	75	Concern
DDT	32 / 25[j]	N: 3000	94 / 120	150	1.6 / 1.3	Low concern
Chlordane / heptachlor	18 / 15[k]	N: 1500	83 / 100	1000	12 / 10	Concern
Chlorobenzenes	4 / 4[l]	L: 600	150 / 150	1000	7 / 7	Relatively low concern
Hexachloroccyclohexanes	4 / 3[m]	L: 600	150 / 200	1000	7 / 5	Relatively low concern
Dieldrin	8 / 7	N: 300	38 / 43	100	3 / 2	Low concern
Toxaphene	30 / 31	N: 12000	400 / 387	10000	25 / 26	Concern

a) The mean daily human intake of a specific contaminant from the traditional Greenland food items (from Johansen et al. 2004a[24],b[25]).
b) NOAEL or LOAEL used as the starting point for the risk characterisation of the individual contaminants.
c) MOS is the ratio between the NOAEL (or LOAEL) and the mean daily human intake (N(L)OAEL / intake).
d) Reference value to be compared with the MOS in order to evaluate whether there is a concern for experiencing adverse health effects among Greenlanders following intake of the specific contaminant from the traditional Greenland food items. The MOSref is an 'overall' assessment (or uncertainty) factor.
e) Ratio between the MOSref and the calculated MOS.
f) A concern is identified if the ratio between MOSref and the calculated MOS is above 1, i.e., the calculated MOS is lower than the MOSref.
g) The human intake of lead from a meal of bird meat has been estimated to be 146 µg for murre and 1220 µg for eider. Assuming that 1 meal of either murre or eider is consumed every week, the estimated daily intake is about 21 µg/day for murre and about 174 µg/day for eider.
h) Total mercury. The risk characterisation has been performed for two scenarios: 1) the intake of total mercury from traditional Greenland food items assumed conservatively as all of the mercury was methylmercury; and 2) the intake of total mercury from traditional Greenland food items assumed as all of the mercury was inorganic mercury.
i) Sum of 10 congeners (CB 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180), which represents most of the predominant congeners in fish and marine mammals.
j) Sum of *p,p'*-DDE, -DDD,-DDT + *o,p'*-DDE,-DDD,-DDT.
k) Sum of heptachlor, heptachlor epoxide, oxychlordane, cis- and trans-chlordane, and cis- and trans-nonachlor.
l) Sum of 1,2,3,4-tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene.
m) Sum of α-, β- and γ-HCH.

[24] Johansen P, Muir D, Asmund G and Riget F (2004a). Human exposure to contaminants in the traditional Greenland diet. Sci Total Environ **331**, 189-206.
[25] Johansen P, Asmund G and Riget F (2004b). High human exposure to lead through consumption of birds hunted with lead shot. Environ Pollut **127**, 125-129.

Terminology

Adverse effect:
Change in morphology, physiology, growth, development or life span of an organism, which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences.

Allometric scaling:
Extrapolates doses (for systemic oral and dermal effects) according to an overall assumption that equitoxic doses (when expressed in mg/kg body weight/day) scale with the body weight to the power of 0.75. Allometric scaling is based on the assumption that the toxicological effects of a specific chemical substance are driven by metabolic rate of a given individual.

Assessment factor:
A product of several single factors by which the NOAEL or LOAEL of the critical effect is divided to derive a tolerable intake. These factors account for adequacy of the pivotal study, interspecies extrapolation, inter-individual variability in humans, adequacy of the overall database, and nature of toxicity.
The term ‘Uncertainty factor (UF)’ is equivalent to the assessment factor.

Benchmark dose - BMD:
The lower confidence limit of the dose calculated to be associated with a given incidence, e.g., 5 or 10%, of effect estimated from all toxicity data on that effect within that study.

Critical effect(s):
The adverse effect(s) judged to be most appropriate for determining the tolerable intake.

Default value (or factor):
A pragmatic, fixed or standard value used in the absence of relevant data. Is often used in relation to uncertainty (or assessment) factors.

Effect assessment:
Consists of a hazard identification and a dose-response / dose-effect assessment (hazard characterisation), is also known as hazard assessment.

Hazard assessment:
Consists of a hazard identification and a dose-response / dose-effect assessment (hazard characterisation), is also known as effect assessment.

Hazard characterisation:
Estimation of the relationship between the dose (or exposure concentration), and the incidence and severity of an effect.

Hazard identification:
Identification of the potential toxic effects, which a given substance has an inherent capacity to cause.

Intraspecies variation:
Differences in sensitivity among individuals of a specific species and are due to biological factors such as age, gender, genetic composition, health status, and nutritional status reflecting both differences in toxicokinetics and in toxicodynamics. This inter-individual variation is greater in humans than in the more inbred experimental animal population.

Inter-individual variation:
Differences in sensitivity among individuals of a specific species to a specific chemical substance. The differences are due to biological factors such as age, gender, genetic composition, health status, and nutritional status reflecting both differences in toxicokinetics and in toxicodynamics. This inter-individual variation is greater in humans than in the more inbred experimental animal population.

Interspecies variation:
Differences in sensitivity between various species to a specific chemical substance.

JECFA:
The FAO/WHO Joint Expert Committee on Food Additives.

JMPR:
The FAO/WHO Joint Meeting on Pesticide Residues.

Margin of Exposure (MOE):
The ratio resulting from a comparison of the outcome of the effects assessment for non-threshold effects (e.g., T25, BMD) to the outcome of the exposure assessment. Is formally similar to the MOS for threshold effects.

Margin of Safety:
The ratio resulting from a comparison of the outcome of the effects assessment for threshold effects (e.g., N/LOAEL, BMD) to the outcome of the exposure assessment.

LOAEL – Lowest Observed Adverse Effect Level:
The lowest concentration or amount of a substance, found by experiment or observation, which causes an adverse alteration of morphology, functional capacity, growth, development or life span of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.

NOAEL – No Observed Adverse Effect Level:
The greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development or life span of the target organism under defined conditions of exposure. Alterations of morphology, functional capacity, growth, development or life span of the target may be detected, which are judged not to be adverse.

NOEL – No Observed Effect Level:
The greatest concentration or amount of a substance, found by experiment or observation, which causes no alterations of morphology, functional capacity, growth, development or life span of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Provisional Tolerable Weekly Intake (PTWI):
An estimate of the weekly intake of a substance which can occur over a lifetime without appreciable health risk.

Reference dose (RfD):
Term used by US-EPA for an acceptable or tolerable daily intake.

Reference MOE (MOEref):
An overall assessment factor, as a numerical value, addressing differences between experimental effect assessment data (usually from animal studies) and the real human exposure situation, taking into account variability and uncertainty, as well as a so-called 'risk extrapolation factor'. Contains the overall information that bridges the gap between the animal dose descriptor chosen, which describes a high risk situation for experimental animals, and a risk situation for human populations considered to be of very low concern. See also 'Margin of Exposure'.

Reference MOS (MOSref):
An overall assessment factor, as a numerical value, addressing differences between experimental effect assessment data (usually from animal studies) and the real human exposure situation, taking into account variability and uncertainty.

Risk characterisation:
Basically an integration of the findings from the exposure assessment and the effect assessment in order to reach a characterisation of the risk.

Technical Guidance Document (TGD):
Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances. European Chemicals Bureau, European Commission, Joint Research Centre, EUR 20418 EN.

Threshold:
The exposure concentration or dose below which no effects are observed, see also NOEL / NOAEL.

Tolerable Daily Intake (TDI):
An estimate of the daily intake of a substance, which can occur over a lifetime without appreciable health risk.
The TDI is similar in definition and intent to terms such as Reference Dose (RfD) and Acceptable Daily Intake (ADI).

Toxicodynamics:
The process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects.

Toxicokinetics:
The process of the uptake of potentially toxic substances by the body, the biotransformation they undergo, the distribution of the substances and their metabolites in the tissues, and the elimination of the substances and their metabolites from the body. Both amounts and the concentrations of the substances and their metabolites are studied. The term has essentially the same meaning as pharmacokinetics, but the latter term should be restricted to the study of pharmaceutical substances.

Uncertainty factor (UF):
A product of several single factors by which the NOAEL or LOAEL of the critical effect is divided to derive a tolerable intake. These factors account for adequacy of the pivotal study, interspecies extrapolation, inter-individual variability in humans, adequacy of the overall data base, and nature of toxicity.
The term 'Assessment factor (AF)' is equivalent to the uncertainty factor.

US-EPA:
The Environmental Protection Agency in the United States of America.

Risk assessment of contaminant intake from traditional Greenland food items

People in Greenland have generally a higher intake of contaminants from their diet than people in the more westernised countries because some traditional food items such as fish, seabirds, seals and whales contain high levels of some heavy metals as well as of some persistent organic pollutants.

This report evaluates the risk to Greenlanders of experiencing adverse health effects from intake of contaminants from traditional food items, including lead, cadmium, mercury, selenium, PCB, DDT, chlordane, HCH, chlorobenzenes, dieldrin and toxaphene.

A concern of experiencing adverse health effects is indicated due to long-term intake of cadmium, lead, mercury, PCBs, chlordane, and toxaphene from the traditional Greenland food items, and risk reduction is recommended for these contaminants , i.e., the intake from the traditional Greenland food items as well as from other sources should be reduced. A relatively low concern is indicated for chlorobenzenes, HCH and dieldrin, and a low concern for selenium and DDT.

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