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Environmental Protection Agency

Evaluation of health hazards by exposure to

Chlorinated paraffins

and proposal of a health-based quality criterion for ambient air.

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Title:

Evaluation of health hazards by exposure to Chlorinated paraffins and proposal of a health-based quality criterion for ambient air.

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Content

Preface

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to chlorinated paraffins, and a proposal of a health based quality criterion for ambient air. This resulted in 2006 in the present report, which was prepared by Elsa Nielsen and Ole Ladefoged, Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research, i.e. the present Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark.

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

The Danish Working Environment Authority,
The Danish Ministry of Food, Agriculture and Fisheries (The Faculty of Agricultural Sciences),
The Danish Veterinary and Food Administration,
The National Board of Health, Denmark,
The Danish Environmental Protection Agency

The Danish Environmental Protection Agency
Copenhagen, September 2013.

1 General description

Commercial chlorinated paraffins, of which there are more than 200, are very complex mixtures of *n*-alkanes characterised by an average carbon chain length and chlorination degree. They are usually mixtures of different carbon chain lengths and have different degrees of chlorination, although all have a common structure in that no secondary carbon atom carries more than one chlorine atom. The length of the carbon chains is usually between 10 and 30 carbon atoms, and the chlorine content is between 20 and 70% by weight, although the commercial products normally fall within the 40-70% chlorine range. The commercially available chlorinated paraffins are usually subdivided into three types depending on chain length: 1) Short-chain chlorinated paraffins (typically C_{10-13} with an average of C_{12}), 2) medium-chain chlorinated paraffins (typically C_{14-17} with an average of C_{15}), and 3) long-chain chlorinated paraffins (typically C_{18-30} with an average of C_{24}). (WHO 1996, IARC 1990).

In this assessment, the different isomers will be referred to as C_x,y%, i.e., a chlorinated paraffin with a carbon chain length of 12 and a chlorination degree of 60% will be referred to as C_{12},60%.

This assessment is primarily based on the European Union Risk Assessment Reports (EU RAR) for the short-chain chlorinated paraffins (SCCPs) (EU RAR 1999/2004a) and the medium-chain chlorinated paraffins (MCCPs) (EU RAR 2004b/2005) carried out in accordance with Council Regulation (EEC) 793/93 (EEC 1993) on the evaluation and control of the risks of existing substances, as well as on WHO (1996) and IARC (1990).

1.1 Identity

IUPAC Name:	1) Alkanes, C_{10-13}, chloro 2) Alkanes, C_{14-17}, chloro 3) Alkanes, C_{18-30}, chloro
Molecular formula:	1) $C_xH_{(2x-y+2)}Cl_y$, where x=10-13 and y=1-13 2) $C_xH_{(2x-y+2)}Cl_y$, where x=14-17 and y=1-17 3) $C_xH_{(2x-y+2)}Cl_y$, where x=18-30 and y=1-30
Structural formula:	CH_3-$(CH_2)_x$-Cl_y-CH_3 x = number of carbon atoms y = number of chlorine atoms
Molecular weight:	The relative molecular mass depends on the carbon chain length and the degree of chlorination.
CAS-no.:	Around 40 CAS numbers have been used to describe the whole chlorinated paraffin family. For the purpose of the EU risk assessments of SCCPs and MCCPs, the CAS number listed in IUCLID has been taken to represent the commercial product. For LCCPs, the CAS-no. most

similar to those for SCCPs and MCCPs has been selected.

1) 85535-84-8
2) 85535-85-9
3) 85535-86-0

Synonyms:

1) Chlorinated paraffin (C_{10-13})
   Chloroalkanes, C_{10-13}
   Short-chain chlorinated paraffins
   SCCPs

2) Chlorinated paraffin (C_{14-17})
   Chloroalkanes, C_{14-17}
   Medium-chain chlorinated paraffins
   MCCPs

3) Chlorinated paraffin (C_{18-30})
   Chloroalkanes, C_{18-30}
   Long-chain chlorinated paraffins
   LCCPs

General synonyms:
Alkanes, chlorinated
Chlorinated alkanes
Chloroparaffin
Chlorocarbons
Polychlorinated alkanes
Paraffins-chlorinated

1.2 Physical / chemical properties

The physical and chemical properties of chlorinated paraffins are determined by the average carbon chain length and the chlorine content. For SCCPs, the chlorine content for commercial products is typically 49-70% (EU RAR 1999). For MCCPs, the chlorine content in commercial products varies from 40-63%, but the largest tonnages of MCCPs have chlorine contents between 45 and 52% (EU RAR 2005).

The physico-chemical properties for SCCPs and MCCPs given below are the representative values selected as key parameters used for environmental modelling in the EU RARs (EU RAR 1999, EU RAR 2005). For LCCPs, the physico-chemical properties are quoted from WHO (1996) and IARC (1990).

Description:

1) Clear to yellowish liquid.
2) Liquid.
3) Liquid or solid depending on carbon chain length and chlorine content.

Purity:

Impurities present in commercial products are likely to be related to those present in the *n*-paraffin feedstock as e.g., isoparaffins (usually less than 0.1%) and aromatic compounds (usually less than 0.01%).

Additives:	Various stabilisers are often added to commercial products in order to improve the thermal stability or lights stability as e.g., epoxidised vegetable oil (typical concentration < 0.5-1 % by weight).
Pour point:	1) -30.5 to +20 °C (chlorine content 49-70%) 2) -45 to +25 °C 3) -20 to +10 °C (chlorine content 39-48%)
Boiling point:	> 200°C, above which decomposition with release of hydrogen chloride occurs.
Density:	1) 1.2 to 1.6 g/ml (at 25°C) (chlorine content 49-70%) 2) 1.1 to 1.3 g/ml (at 20°C) (chlorine content 41-56%) 3) 1.1 to 1.7 g/ml (at 25°C) (chlorine content 39-70%)
Vapour pressure:	1) 1.6×10^{-4} mmHg (0.021 Pa) (at 40°C) (chlorine content 50%) 2) 1.7×10^{-5} mmHg (0.0023 Pa) (at 40°C) (chlorine content 45%) 1.0×10^{-6} - 2.0×10^{-6} mmHg (0.00013-0.00027 Pa) (at 20°C) (chlorine content 52%) 3) -
Concentration of saturated vapours:	1) 0.21 ppm (at 40°C and 760 mmHg) 2) 0.001-0.002 ppm (at 20°C and 760 mmHg) 3) -
Conversion factors:	-
Vapour density:	-
Flash point:	1) 166 / 202 °C (chlorine content 50 / 56%) 2) > 210 °C (chlorine content > 40%) 3) -
Flammable limits:	-
Auto ignition temp.:	-
Solubility:	Water: Practically insoluble (below 0.5 mg/l at 20°C). Also practically insoluble in lower alcohols, glycerol and glycols. Soluble in chlorinated solvents, aromatic hydrocarbons, ketones, esters, ethers, mineral oil, and some cutting oils.
$\log P_{octanol/water}$:	1) 4.39-6.93 / 5.68-8.69 (chlorine content 49 / 70 %) 2) 5.52-8.21 / 5.47-8.01 (chlorine content 45 / 52 %) 3) 9.29->12.83 / 8.69-12.83 (chlorine content 42 / 48 %)
Henry's constant:	-
pK_a-value:	-

Stability:	Decomposes with liberation of hydrogen chloride above 200 °C
Incompatibilities:	-
Odour threshold, air:	-
Odour threshold, water:	-
Taste threshold, water:	-
References:	1) EU RAR (1999) 2) EU RAR (2005) 3) WHO (1996), IARC (1990)

1.3 Production and use

Chlorinated paraffins are produced by chlorination of normal paraffin fractions (straight-chain hydrocarbons, at least 98% linear, obtained from petroleum refining); small quantities of a stabiliser (e.g. epoxidised vegetable oil) may be added to the product. (EU RAR 1999, EU RAR 2005, WHO 1996, IARC 1990).

According to EU RAR (1999), the main uses of SCCPs (based on 1994 data from Western Europe) were as an extreme pressure additive in metal working fluids (approximately 71% of total use), as a flame retardant in rubber (approximately 10%), and in paints (approximately 9%). Minor uses (approximately 10% in total) included use in leather finishing, sealants and textiles. However, according to EU RAR (2004a), the use of SCCPs (based on 2001 data from the EU) in metal working fluids has reduced markedly compared with the 1994 use and SCCPs are currently used as a flame retardant in textiles and rubber, in paint, and in sealants and adhesives. There has also been a small use in PVC reported during the late 1990s. A further use of SCCPs reported in the late 1990s was in lava lamps; this use is now thought to be very small.

The main uses of MCCPs (based on 1997 data from the EU) are as a secondary plasticiser in PVC (approximately 79% of total use), in metal working fluids (approximately 9%), in paints, adhesives and sealants (approximately 5%), in rubbers and other polymeric materials (approximately 3%), in fat liquors used in leather processing (approximately 1.5%), and in carbonless copy paper (approximately 1%) (EU RAR 2005).

LCCPs are used as flame retardants for rigid plastics (polyesters and polystyrene) and as plasticisers for PVC and plastics (WHO 1996, IARC 1990).

1.4 Environmental occurrence

Chlorinated paraffins are not known to occur naturally (WHO 1996, IARC 1990, EU RAR 2004a,b) and thus, anthropogenic sources contribute solely to the occurrence of chlorinated paraffins in the environment.

For SCCPs, the total emissions in the EU have been estimated to approximately 394 kg/year to air and approximately 1784 tones/year to water. These emissions (particularly to water) were dominated by the use of SCCPs in metal working fluids and in leather fat liquors. (EU RAR 1999).

In EU RAR (2004a), new emission estimates have been carried out showing an increased emission to air (2994-10924 kg/year), and a reduced emission to water (waste water: 40-97 tonnes/year; surface water: 20-46 tonnes/year) when compared to the original risk assessment report. In, addition, a significant emission to urban/industrial soil is now predicted (33-65 tonnes/year).

For MCCPs, the total emissions in the EU have been estimated to be around 171-172 tonnes/year to air, 1263-1309 tonnes/year to waste water treatment plants, 816-885 tonnes/year direct to surface water, and 826-973 tonnes/year to urban/industrial soil (EU RAR 2004b).

No specific data have been located for LCCPs.

In the EU Risk Assessment Reports, environmental concentrations are generally estimated by the use of the European Union System for the Evaluation of Substances (EUSES, a computer modelling program); these estimated concentrations are called Predicted Environmental Concentrations (PECs). The PECs are estimated for three different spatial scales: 1) the local scale i.e., concentrations near a point source of the substance, 2) the regional scale i.e., average concentrations due to all releases in a larger area, and 3) the continental scale defined as the sum of all EU Member States.
Local and regional PECs estimated for SCCPs and MCCPs in atmospheric air, surface water, effluents from waste water treatment plants, sediment, soil, and various food items have been summarised in Table 1.4.

1.4.1 Air

SCCPs are widely found at very low levels, generally in the range 1×10^{-9}-1×10^{-5} mg/m^3, in the atmosphere, including remote Arctic environments. They are also present in household dust. (EU RAR 2004a). For MCCPs, no measured data are available (EU RAR 2004b). No specific data have been located for LCCPs.

1.4.2 Water and sediment

Measured levels of SCCPs in surface water close to industrial activity (UK) were generally below 0.1 µg/l (EU RAR 2004a), and for MCCPs of the order of 0.4 to 4 µg/l with lower levels (of the order of 0.1 to 0.5 µg/l) being found in areas more remote from industry (EU RAR 2004b).
For LCCPs (C_{20-30}), levels found in fresh water remote from industry (UK) ranged from < 0.5 to 2 µg/l, and in fresh water close to industry < 0.5 µg/l (WHO 1996).

SCCPs are widely found in sediment, including in samples taken from remote Arctic regions. The highest levels are generally associated with industrial activities and concentrations of up to 24.2 mg/kg (wet weight) (mixture of SCCPs and MCCPs) have been reported close to a chlorinated paraffin production site in the UK. (EU RAR 2004a).
For MCCPs, levels measured upstream from the sites of discharge were generally in the range of < 0.08 to 5.2 mg/kg (wet weight) with an approximate mean level of 0.7 mg/kg (wet weight). For sediment samples taken downstream from sources of release, the levels were in the range 1 to 25 mg/kg (wet weight). (EU RAR 2004b).
For LCCPs (C_{20-30}), levels < 250 µg/kg were measured at places remote from industrial areas in the UK, with levels up to 3.2 mg/kg at places close to industry (WHO 1996).

1.4.3 Soil

The major source of chlorinated paraffins in agricultural soil is considered to be the application of sewage sludge containing these substances.
Measured levels of SCCPs in agricultural soil receiving sewage sludge containing chlorinated paraffins were generally below 0.088 mg/kg (wet weight) (EU RAR 2004a).
Measured levels of MCCPs found in soil where sewage sludge has been applied were generally below 0.088 mg/kg (wet weight) (EU RAR 2004b).
No specific data have been located for LCCPs.

1.4.4 Foodstuffs

Chlorinated paraffins have been found to be present in various foodstuffs at locations both close to industrial sources and from more remote areas. The levels of SCCPs found in aquatic organisms, including fish are generally up to a few mg/kg, for MCCPs in the range < 0.1 to 5.2 mg/kg (wet weight), and for LCCPs (C_{20-30}) from < 50 to 200 µg/kg (mean: 10-30 µg/kg). (EU RAR 2004a,b, WHO 1996).

Chlorinated paraffins (C_{10-20}) were found in approximately 70% of the foodstuff samples taken with average concentrations of around 300 µg/kg in dairy products, around 150 µg/kg in vegetable oils and derivatives, around 5 µg/kg in fruit and vegetables, and < 50 µg/kg in beverages. C_{20-30} chlorinated paraffins were detected only in a few samples. (Campbell & McConnell 1980 – quoted from EU RAR 1999,2004b).

Levels of chlorinated paraffins (C_{10-24}) in food and fish have also been reported by Greenpeace (1995 – quoted from EU RAR 2005) with mean levels of 271 µg/kg lipid in mackerel, 62 µg/kg lipid in fish oil (herring), 98 µg/kg lipid in margarine containing fish oil, 69 µg/kg lipid in pork, and 74 µg/kg lipid in cow's milk.

Chlorinated paraffins have been measured in cow's milk (one sample) and in single butter samples from various regions of Europe (Denmark, Wales, Normandy, Bavaria, Ireland, and southern and northern Italy). SCCPs were not detected in the cow's milk sample (detection limit < 1.2 µg/kg lipid), but MCCPs were present at a concentration of 63 µg/kg lipid. SCCPs were found in butter samples from Denmark (1.2 µg/kg lipid) and Ireland (2.7 µg/kg lipid), and MCCPs in butter samples from Denmark (11 µg/kg lipid), Wales (8.8 µg/kg lipid) and Ireland (52 µg/kg lipid). (Thomas & Jones 2002 – quoted from EU RAR 2004a,b).

Chlorinated paraffins have also been found to be present in breast milk.
Levels of 45 µg/kg lipid have been reported for chlorinated paraffins (C_{10-24}) in breast milk (Greenpeace 1995 – quoted from EU RAR 2005).
SCCPs (around 60-70% chlorine) were present at a concentration of 11-17 µg/kg lipid (mean: 13 µg/kg lipid) in breast milk from Inuit women living in the northern Canada (Tomy 1998 – quoted from EU RAR 2004a).
In a recent study from the UK (Thomas & Jones 2002 – quoted from EU RAR 2004a,b), SCCPs were found in 5 of 8 breast milk samples from Lancaster (4.6 to 110 µg/kg lipid), and in 7 of 14 samples from London (4.5 to 43 µg/kg lipid). MCCPs were found in one sample from London (61 µg/kg lipid), but were below the detection limit (16-740 µg/kg lipid) in the remaining 21 samples.
In a follow-up study (Thomas et al. 2003 – quoted from EU RAR 2004a,b,2005), SCCPs were detected in all 5 samples from Lancaster and in 16 of 20 samples from London at concentrations ranging from 49 to 820 µg/kg lipid (median: 180 µg/kg

lipid; 95 percentile: 680 µg/kg lipid); there were no significant difference between the concentrations in the samples from Lancaster and those from London. MCCPs were found to be present in all 25 samples analysed at concentrations ranging from 6.2 to 320 µg/kg lipid (median: 21 µg/kg lipid; 95 percentile: 127.5 µg/kg lipid; 97.5 percentile 130.9 µg/kg lipid).

Table 1.4 Local and regional PECs in the environment (from EU RAR 2004a,b)

	SCCPs		MCCPs	
	Local	Regional	Local	Regional
Atmospheric air (mg/m^3)	1.9×10^{-6}-1.1×10^{-5} a) 5.3×10^{-7}-3.6×10^{-6} b)	5.3×10^{-7}-1.3×10^{-6}	3.5×10^{-6}-1.4×10^{-4} a) 4.3×10^{-6}-1.7×10^{-4} b)	3.35×10^{-6}
Surface water (µg/l)	< 0.04-2.7	0.012-0.027	0.10-46.6	0.389-0.745
Effluents from waste water treatment plants (µg/l)	0.15-350		0.88-875	
Sediment (mg/kg ww c))	0.10-11.8	0.09-0.21	1.28-86.9	8.8-16.9
Agricultural soil (mg/kg ww)	0.071-17.2	0.54-1.41	1.52-14.6	53.8-55.8
Natural soil (mg/kg ww)		0.0011-0.0025		1.85-2.01
Industrial soil (mg/kg ww)		1.53-3.04		147-173
Fish (mg/kg)	0.12-9.7	0.092-0.21	0.42-6.4	0.42
Root crops (mg/kg)	0.14-35	1.09-2.87	6.75-379	309
Leaf crops (mg/kg)	0.0005-0.0015	0.0005-0.0011	0.017-0.70	0.02
Meat (mg/kg)	0.0012-0.089	0.0073-0.019	0.13-4.03	1.99
Milk (mg/kg)	0.0004-0.028	0.0023-0.006	0.042-1.27	0.63
Drinking water (µg/l)	0.02-4.9	0.2-0.4	0.11-1.4	4.9

a) Annual average concentration
b) During an emission episode
c) wet weight

1.5 Environmental fate

Chlorinated paraffins adsorb strongly to sediment. In water, they are probably transported adsorbed on suspended particles, and in the atmosphere adsorbed to airborne particulates. (WHO 1996).

For SCCPs, a soil organic carbon - water partition coefficient (K_{OC}) of 199500 l/kg has been measured for a commercial $C_{10\text{-}13}$,55% (EU RAR 1999). For MCCPs, a K_{OC} of 588844 l/kg has been estimated (EU RAR 2004b). These high K_{OC} values indicate that the chlorinated paraffins are expected to be relatively immobile in soil and would not be expected to leach from soil into groundwater. No specific data have been located for LCCPs.

1.5.1 Degradation

The half-lives for chlorinated paraffins in air have been estimated to range from 0.85 to 7.2 days (WHO 1996). For SCCPs, the half-life for atmospheric degradation by reaction with hydroxyl radicals has been estimated to be 7.2 days (EU RAR 1999,2004a), and for MCCPs to be 1-2 days (EU RAR 2004b).

Chlorinated paraffins are not readily biodegradable (EU RAR 1999,2004a,2004b, WHO 1996). SCCPs with a chlorine content of less than 50% may biodegrade slowly in the environment under aerobic conditions, particularly in the presence of

adapted micro-organisms, whereas the degradation appears inhibited at a chlorine content above 58% (EU RAR 1999,2004a, WHO 1996). Similarly, there is evidence that some micro-organisms may be capable of degrading MCCPs in acclimated systems; the potential for biodegradation appears to increase with decreasing chlorine content (EU RAR 2004b).

No specific data have been located for LCCPs.

1.5.2 Bioaccumulation

Chlorinated paraffins are bioaccumulated in aquatic organisms with reported bioconcentration factors (BCF) in the range of 7 to 7155 for fish and 223 to 138000 for mussels. In fish, SCCPs are accumulated to a higher degree than MCCPs and LCCPs. The uptake seems to be more efficient for SCCPs with low chlorine content and the elimination rate is slowest for SCCPs with high chlorine content. The retention in fat-rich tissues appears to increase with increasing degree of chlorination. (WHO 1996).

For SCCPs, a fish BCF of 7816 l/kg has been estimated. Uptake into fish via food has also been shown to occur, with accumulation factors of up to 1-2 being determined on a lipid basis for SCCPs with high chlorine contents. (EU RAR 1999,2004a).

For MCCPs, a fish BCF of 1087 l/kg has been estimated. The available food uptake studies indicate that the 35% chlorine, 52% chlorine and 69% chlorine products can be taken up by organisms from food with accumulation factors of 1-3 being determined on a lipid basis. The potential for uptake from food appears to reduce with increasing chlorine content. MCCPs are also taken up by organisms from sediment/soil, and a BCF of 5.6 (on a wet weight basis) has been estimated for earthworms and of 0.034 (on a wet weight basis) for plants. (EU RAR 2004b).

No specific data have been located for LCCPs.

1.6 Human exposure

Humans can be exposed to chlorinated paraffins indirectly via the environment, via consumer products and via the working environment.

Chlorinated paraffins have several uses that can result in releases into the environment and have been shown to bioconcentrate in aquatic organisms. Chlorinated paraffins have been detected in low levels in atmospheric air, surface water and sediment, soil, and in some food items and breast milk. For details, see section 1.4.
In the EU Risk Assessment Reports, the estimated total daily human intake of SCCPs from the environment range from 0.00099 to 0.20 mg/kg bw/day based on local PECs, and from 0.0062 to 0.016 mg/kg bw/day based on regional PECs (EU RAR 2004a). The estimated total daily human intake of MCCPs range from 0.0007 to 2.10 mg/kg bw/day based on local PECs, and at 1.71 mg/kg bw/day based on regional PECs (EU RAR 2005). Root crops were predicted to be the major source of human uptake for both SCCPs and for MCCPs. For MCCPs, the daily uptake from breast milk for the first 3 months of infant life has been estimated to 30.5 x 10^{-5} mg/kg b.w./day (EU RAR 2005).
For SCCPs, a total daily human intake of 0.02 mg/kg bw/day has been considered as a reasonable worst case prediction based upon measured data and has been used

for the risk characterisation for man exposed indirectly via the environment (EU RAR 1999).
For MCCPs, a total daily human intake of 2.10 mg/kg bw/day has been used for the risk characterisation for man exposed indirectly via the environment from local exposure, and of 1.71 mg/kg bw/day from regional exposure (EU RAR 2005).

Chlorinated paraffins are not sold directly as consumer products but are found in a number of consumer products, including leather clothing, metal working fluids and textiles, in certain paints, sealants and adhesives, and in plastic and rubber products. The exposure is predominantly via dermal contact and inhalation exposure is only considered to be significant for metal working fluids. Aside from the wearing of leather clothing and the use of metal working fluids, the consumer exposures are considered to be negligible. (EU RAR 1999,2005).
For SCCPs, the total systemic dose has been estimated to 0.03 mg/kg bw/day for use of metal working fluids and to 0.02 mg/kg bw/day for wearing of leather clothing (EU RAR 1999).
For MCCPs, the total systemic dose has been estimated to 0.008 mg/kg bw/day for use of metal working fluids and to 0.00016 mg/kg bw/day for wearing of leather clothing (EU RAR 2005).

The manufacture and use of chlorinated paraffins may give rise to exposure of workers. Skin contact is the predominant occupational route of exposure, but there is also a potential for inhalation exposure in some use areas. (EU RAR 1999,2005).
For SCCPs, the estimated total systemic doses for manufacture and use range from being negligible to 0.6 mg/kg bw/day with the exception of use in formulation at high temperature (9.3 mg/kg bw/day) (EU RAR 1999).
For MCCPs, the estimated total systemic doses for manufacture and use range from 0.013 to 0.72 mg/kg bw/day (EU RAR 2005).

No specific exposure data have been located for LCCPs.

2 Toxicokinetics

2.1 Absorption, distribution and excretion

2.1.1 Inhalation

No data have been located for.

2.1.2 Oral intake

2.1.2.1 SCCPs

F344 rats were treated with 10 or 625 mg/kg bw/day of a C_{10-12},58%, daily in the diet for 13 weeks (Unpublished study 1984 – quoted from EU RAR 1999, WHO 1996). After 13 weeks, all animals received a single oral (gavage) dose of ^{14}C-labelled C_{10-12},58%, same dose level as received daily in the previous weeks. Tissue levels were proportional to the administered dose and were similar, irrespective of dosing regime. The highest initial concentrations of radioactivity were found in the liver, kidney, adipose tissue and ovaries. Approximately 54-66% of the radioactivity was recovered in the faeces in 7 days, 14% in the urine, and less than 1% in exhaled air as carbon dioxide.

Absorption, distribution and excretion have been investigated in a study with C57B1 mice treated (single dose by gavage) with ^{14}C-labelled C_{12},17.5%, 55.9% or 68.5% (Darnerud et al. 1982 – quoted from EU RAR 1999, WHO 1996, IARC 1990). Uptake of radioactivity 24 hours after administration (whole-body autoradiography) was highest in tissues with high metabolic activity and/or high rates of cell proliferation, e.g., intestinal mucosa, bone marrow, brown fat, salivary glands, thymus and liver. The accumulation of radioactivity apparently increased with increasing degree of chlorination. Twelve hours after administration of C_{12}, 55.9% or 67% was recovered with 33% as carbon dioxide in exhaled air, 29% in urine, and 5% in faeces. After administration of C_{12}, 68.5%, 33% was recovered with 8% as carbon dioxide in exhaled air, 4% in urine, and 21% in faeces.

2.1.2.2 MCCPs

F344 rats were treated with 10 or 625 mg/kg bw/day of a C_{14-17}, 52%, daily in the diet for 13 weeks (Unpublished study 1984 – quoted from EU RAR 2005, WHO 1996). After 13 weeks, all animals received a single oral (gavage) dose of ^{14}C-labelled C_{14-17}, 52%, same dose level as received daily in the previous weeks. Tissue levels were greater (up to an order of magnitude) in high-dose animals compared with low-dose animals. The highest initial (first 7 days) concentrations of radioactivity were found in the liver, kidney, and ovaries followed by an increase in adipose tissue levels as the levels in the former tissues declined. Approximately 40-48% of the radioactivity was recovered in the faeces in 7 days in low-dose males, around 53-61% in high-dose males, around 30% in low-dose females, and around 62-74% in high-dose females. Recovery of radioactivity in urine and exhaled air within 7 days amounted to 0.8-3% and 0.1-0.3%, respectively, at both dose levels.

In male Wistar rats administered a diet containing 0.4 or 40 mg/kg of a ^{36}Cl-labelled $C_{14\text{-}17}$, 52% for 8 weeks (40 mg/kg, approximately 3 mg/kg bw/day) or 10 weeks (0.4 mg/kg, approximately 3 mg/kg bw/day), equilibrium levels of radioactivity were reached within 1 week for the liver (7 mg/kg at the high dose) and 7 weeks for adipose tissue (30-40 mg/kg at the high dose). The half-life for elimination of radioactivity from the abdominal fat was estimated as approximately 8 weeks at the low dose; no radioactivity was detected in the liver at 1 week after dosing. No radioactivity was detected in the brain or the adrenals. (Birtley et al. 1980 – quoted from EU RAR 2005, WHO 1996, IARC 1990).

In a very recent study (Unpublished study 2005 – quoted from EU RAR 2005), male F344 rats were given a single dose of an [8-^{14}C]-labelled C_{15} in corn oil at 525 mg/kg bw by gavage. The liver, kidney, fat and skin/fur contained the highest concentrations of radioactivity at 24 hours post-dosing. The elimination half-life was approximately 2-5 days for the liver and kidney, and about 2 weeks for white adipose tissue. Approximately 50% of the administered dose was eliminated in the faeces within the first 24 hours after dosing, and approximately 70% by day 5 post-dosing. Approximately 5% of the dose was eliminated in the urine by day 5 post-dosing.

In female C57B1 mice administered a single dose of 10 mg/kg bw of a ^{14}C-labelled C_{16}, 69% by gavage, whole-body autoradiography revealed that the administered radioactivity on days 1-4 post-dosing was concentrated mainly in the corpora lutea, liver, adrenal cortex and brown and white adipose tissue; a high level of radioactivity was still present in corpora lutea and brown fat 30 days post-dosing. The major route of elimination after administration of a single dose of 1 mg/kg bw was via the faeces with 22% of the radioactivity being eliminated within 8 hours, 57% within 16 hours, and 66% within 4 days. Urinary excretion accounted for 1.2% within 8 hours and for 2.9% within 4 days. (Biessman et al. 1983 – quoted from EU RAR 2005, WHO 1996).

In another study with C57B1 mice (Darnerud & Brandt 1982 – quoted from EU RAR 2005, WHO 1996, IARC 1990), a ^{14}C-labelled C_{16}, 34.1% was administered either by gavage (females) or intravenously (both sexes) at 0.15 mg/kg bw. No difference in the distribution pattern was found between the oral and intravenous administration routes. High levels of radioactivity were observed in brown adipose tissue, liver, kidneys, adrenals, bone marrow, Harderian gland, salivary gland, pancreas, and intestinal mucosa at 24 hours post-administration. At 12 days, following intravenous injection, high levels of radioactivity were seen in the adrenal cortex, adipose tissue, and gall bladder, and after 30 days, prominent levels in the brain and in the liver. When administered by intravenous administration to pregnant mice, uptake of the radioactivity in the foetuses was observed; the distribution pattern was similar to that of adult mice. Twelve hours after gavage administration, 6% of the radioactivity was recovered in the urine, 33% in the expired air and 14% in the faeces with 12% in the urine, 44% in expired air and 4% in the faeces 12 hours after intravenous administration.

The distribution of radioactivity in the brain has been studied in pre-weaning NMRI mice (aged 3, 10 or 20 days old) administered a single dose of 1.1 mg/kg bw of a ^{14}C-labelled C_{16}, 69% by gavage (Eriksson & Darnerud 1985 – quoted from EU RAR 2005, WHO 1996). The radioactivity declined more rapidly in the 3-day-old mice compared to the 10- and 20-day-old mice. The radioactivity was found primarily in the white matter of the cerebellum, in the space between the neocortex and the mesencephalon and thalamus, the corpus callosum, the pons, and the outer part of medulla spinalis. After whole-body autoradiography (single oral dose of 7

mg/kg bw), high levels of radioactivity were found in the liver, intestinal contents, adipose tissue and adrenals.

An interim report of a study (CXR 2005) investigating the bioaccumulation potential of MCCPs in the rat following repeated administration has recently become available for the rapporteur. F344 rats (48/sex) were administered MCCPs, 52% in the diet at a concentration of 3000 mg/kg (equivalent to 200/233 mg/kg b.w./day in males/females) for 14 weeks, time at which steady state level of MCCPs in white adipose tissue was achieved. After 14 weeks exposure, the remaining rats were transferred to control diets and groups of 8 rats were then sacrificed at weeks 15, 16, 18, 22 and 30. In the interim report, only data for the white adipose tissue were provided. The MCCPs content in white adipose tissue increased with time until week 13. The elimination of MCCPs from adipose tissue appeared to be biphasic. Both male and female rats eliminated MCCPs with an initial half-life of approximately 4 weeks, followed by a markedly slower second phase. The concentration of MCCPs in adipose tissue remained fairly constant between weeks 22 and 30, and the elimination phase of the study has been extended in order to accurately evaluate the elimination half-life of MCCPs.

2.1.2.3 LCCPs

After oral administration of a ^{14}C-labelled C_{22-26}, 70% to F344 rats at the end of a 90-day exposure period, a small part of the dose (no further details) was absorbed. The highest level of radioactivity was found in the liver; retention of radioactivity in adipose tissue was also observed. In an identical study, a ^{14}C-labelled C_{20-30},43% gave the highest levels in the liver and ovary. (Serrone et al. 1987 – quoted from WHO 1996).

A ^{14}C-labelled C_{18}, 50-53% was administered by gavage as a single dose of 500 mg/kg to female Sprague-Dawley rats. After 24 hours, 1% of the radioactivity was recovered in the urine, 1.5% in the expired air, and 22% in the faeces. After 96 hours, 1.9% was recovered in the urine, 3.3% in the expired air, 5% in body tissues, and 76% in the faeces. (Yang et al. 1987 – quoted from WHO 1996).

2.1.3 Dermal contact

^{14}C-labelled LCCPs (C_{18}, 50-53% or C_{28}, 47%) were applied to the dorsal skin of Sprague-Dawley rats at a concentration approximately equivalent to 2000 mg/kg bw. Around 0.6-0.7% of the C_{18}-dose was absorbed after 96 hours, and around 0.02% of the C_{28}-dose. Of the absorbed C_{18}-dose, 40% was exhaled as carbon dioxide, 20% was excreted in the urine and 20% in the faeces. (Yang et al. 1987 – quoted from WHO 1996, EU RAR 1999, IARC 1990).

No data have been located for SCCPs and MCCPs.

2.1.4 *In vitro* studies

The absorption of SCCPs (C_{10-13}, 56%) and MCCPs (C_{14-19}, 52%) through human skin has been studied *in vitro* (Scott 1989 – quoted from EU RAR 1999,2005, WHO 1996). There was no absorption of SCCPs following a 7-hour application to the surface of the epidermal membranes using five different receptor media, but after 23 hours a slow but steady rate of absorption was detected; less than 0.01% of

the applied dose was absorbed during the 56-hour application. There was no absorption of MCCPs following a 54-hour application period.

2.1.5 Other routes

In bile-duct cannulated female Sprague-Dawley rats given a ^{14}C-labelled MCCP (C_{16}, 65%) by intravenous injection, around 20-30% of the radioactivity was eliminated via the bile within 3 days with around 10% within 24 hours; less than 0.5% of the radioactivity was found in urine and in faeces (Åhlman et al. 1986 – quoted from EU RAR 2005, WHO 1996, IARC 1990).

In female C57B1 mice administered a single dose of 10 mg/kg bw of a ^{14}C-labelled MCCP (C_{16}) by intravenous injection, the major route of elimination was via the faeces with 2% of the radioactivity being eliminated within 8 hours post-dosing, and 43% within 4 days. Urinary excretion accounted for 3% of the administered dose and exhalation for approximately 1%. (Biessman et al. 1983 – quoted from EU RAR 2005, WHO 1996).

2.2 Metabolism

In bile-duct cannulated female Sprague-Dawley rats given a ^{14}C-labelled MCCP (C_{16}, 65%) by intravenous injection, the metabolites tentatively identified in the bile and in the urine appeared to be conjugates of glutathione; unchanged parent compound accounted for up to 3% of the radioactivity in the bile (Åhlman et al. 1986 – quoted from EU RAR 2005, WHO 1996, IARC 1990).

In C57B1 mice treated by intravenous injection with ^{14}C-labelled SCCPs (C_{12}, 17.4, 55.9 or 68.5%), inducers and inhibitors of cytochrome P450 affected the rate of degradation to carbon dioxide. Pre-treatment with the inhibitors piperonyl butoxide and metyrapone inhibited carbon dioxide production by 84 and 60%, respectively, following administration of C_{12}, 68.5%. With piperonyl butoxide, the decrease was more pronounced with increasing degree of chlorination. The P450 (CYP2B1; CYP2B2) inducer phenobarbital increased the rate of carbon dioxide production from C_{12}, 68.5%, whereas the P448 (CYP1A1; CYP1A2) inducer 3-methylcholanthrene did not affect the degradation rate. These studies suggest a cytochrome P450 dependent metabolism of chlorinated paraffins yielding carbon dioxide, possibly by a de-chlorination reaction followed by β-oxidation and incorporation of the carbon chain into cellular metabolism. (Darnerud 1984 – quoted from WHO 1996, IARC 1990, ER RAR 1999).

2.3 Mode of action

Studies in experimental animals have demonstrated that the liver, kidneys and thyroid are the target organs for the toxicity of chlorinated paraffins. In addition, developmental effects indicative of internal haemorrhaging have been observed in offspring of rats in a range-finding study to a 2-generation study. Mode of actions regarding these effects, with the view to assessing their relevance to humans, are briefly summarised in this section based upon the evaluations performed in the EU RARs of SCCPs (EU RAR 1999) and MCCPs (EU RAR 2005).

2.3.1 Effects in the liver

Repeated dose toxicity studies have revealed a number of effects in the liver, including tumours, of rats and mice exposed to chlorinated paraffins (see sections 4.4 and 4.7).

Studies performed specifically in order to elucidate the mode of actions underlying the observed effects in the liver have revealed that MCCPs are capable of eliciting hepatic enzyme induction and proliferation of smooth endoplasmic reticulum indicative of increased metabolic demand arising from xenobiotic metabolism (EU RAR 2005). The EU RARs of SCCPs (EU RAR 1999) and MCCPs (EU RAR 2005) have concluded that the changes related to xenobiotic metabolism are indicative of physiological adaptation and are not considered to be of toxicological significance.

Hepatic peroxisome proliferation is induced by SCCPs and MCCPs in rats and mice as evidenced by microscopy, morphometric analysis, and enzyme marker activity; peroxisome proliferation was not observed in guinea pigs (EU RAR 1999,2005). The EU RARs of SCCPs (EU RAR 1999) and MCCPs (EU RAR 2005) have concluded that the changes related to peroxisome proliferation in rats and mice are considered to be of limited relevance to human health.

Regarding tumours in the liver, peroxisome proliferation has been suggested to be the plausible mode of action, and the EU Commission Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity has accepted this view at their meeting in June 1997 (EU RAR 1999).

2.3.2 Effects in the kidney

Repeated dose toxicity studies have revealed a number of effects in the kidneys, including tumours, of rats and mice exposed to chlorinated paraffins (see sections 4.4 and 4.7).

Studies performed specifically in order to elucidate the mode of actions underlying the observed effects in the kidney have revealed some deposition of α2µglobulin in proximal convoluted tubules of male rats, but this was considered as being unrelated to the histopathological findings in the kidney (EU RAR 2005). The EU RAR of MCCPs (EU RAR 2005) have concluded that the effects observed in the kidneys are not to be considered as a male rat-specific phenomenon and thus, the effects are considered as being potentially relevant to human health; the NOAEL for repeated dose toxicity has been set based on effects seen in the rat kidney. The EU RAR of SCCPs (EU RAR 1999) has also set the NOAEL for repeated dose toxicity based on effects seen in the rat kidney.

Regarding tumours in the kidney, the EU Commission Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity, at their meeting in June 1997, has considered that no plausible mechanism has been suggested as studies have failed to show significant levels of α2µglobulin in the kidneys of male rats. Other evidence had shown that there was chronic nephropathy, which might be a contributing factor in the tumour development. The Specialised Experts considered that there was still insufficient evidence to conclude a male rat specific event and that the consequences for humans could not be ruled out. (EU RAR 1999).
The IPCS conceptual framework for evaluating a mode of action in SCCPs, and by analogy MCCPs, male rat kidney carcinogenesis has been performed in the EU

RAR for MCCPs (EU RAR 2005). The available evidence suggests that the underlying mechanism would not be relevant to human health. However, uncertainties have still been highlighted and hence, in January 2004, the issue was again referred to the Specialised Experts Group. The group agreed that there were still data gaps leading to uncertainty about the relevance of these tumours to humans. (EU RAR 2005).

2.3.3 Effects in the thyroid

Repeated dose toxicity studies have revealed a number of effects in the thyroid, including tumours, of rats and mice, but not guinea pigs, exposed to chlorinated paraffins (see sections 4.4 and 4.7).

Studies performed specifically in order to elucidate the mode of actions underlying the observed effects in the thyroid indicate that the effects in the thyroid appear to be due to stimulation of the thyroid via negative feed back mechanisms. Initially, the liver enzyme UDPG-transferase activity is increased resulting in increased glucuronidation and excretion of T4 with a resultant decrease in plasma T4 levels. The pituitary responds by releasing more TSH, which in turn leads to increased production of T4 by the thyroid. The increased activity in the thyroid is predicted to result in hypertrophy, hyperplasia and as a consequence, a tendency to develop thyroid tumours. Rodents are particularly susceptible to thyroid changes due to absence of a T4-binding globulin, which is present in humans and has a very high affinity for T4. Other binding proteins are present in rodents, but their binding efficiency is considerably less than T4-binding globulin. In rodents, more free T4 is thus available for metabolism and excretion from the body. (EU RAR 1999,2005). The EU RARs of SCCPs (EU RAR 1999) and MCCPs (EU RAR 2005) have concluded that the effects seen in the thyroid in rats and mice would be of little relevance to human health.

Regarding tumours in the thyroid, hormonal imbalance has been suggested to be the plausible mode of action, and the EU Commission Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity has accepted this view at their meeting in June 1997 (EU RAR 1999).

2.3.4 Haemorrhaging effect in offspring

Effects indicative of internal haemorrhaging (increased incidences in the occurrence and severity of subcutaneous haematoma, pallor, blood around the orifices, pale liver, kidneys, lungs and spleen, blood in the cranial cavity and brain, haematoma) and reduced pup survival during lactation, but not at birth, have been observed in offspring of rats administered MCCPs ($C_{14\text{-}17}$,52%) in a dietary range-finding study to a 2-generation study (see section 4.5.2). Studies have been performed with the aim of investigating the possible mode of action of internal haemorrhages. Overall, the information from these studies indicates that MCCPs induce a perturbation of the clotting system in lactating neonates of treated mothers. In adult animals, decreased levels of vitamin K and of the clotting factors VII and X were observed, but their prothrombin times were not affected indicating that the functional reserve in these adult animals is sufficient. The foetus *in utero* apparently receives sufficient vitamin K via the placenta, but after birth becomes deficient in vitamin K and related clotting factors when reliant of these factors via the mother's milk. They also receive MCCPs through the milk, which may further reduce their vitamin K levels. This in turn will lead to vitamin K deficiency in the

neonates and consequently haemorrhaging. The effect is considered as being relevant to humans. (EU RAR 2005).

3 Human toxicity

3.1 Single dose toxicity

No data have been located.

3.2 Irritation

3.2.1 Skin irritation

SCCPs (C_{10-13}, 50% or 63%) were applied, under occlusive dressing, to the upper arm of 26 volunteers (Unpublished study 1975 – quoted from EU RAR 1999, WHO 1996) for 24 hours and skin reactions were examined one hour later. A second application was made and reactions assessed after a further 24 hours of contact. Mild erythema and dryness were recorded with average scores at the 25- and 50-hour time points of less than 2 and 1, respectively. These reactions were comparable to those observed in a liquid paraffin control group.

When LCCPs (C_{20-30}, 40-41% or C_{24}, 70%) were applied to the skin of 200 male and female volunteers for a 5-day period, and then re-applied for 2 days beginning 3 weeks after the initial exposure, no local irritation was observed; the dose level was not reported. In a similar study, in which an SCCP (C_{12}, 59%) or LCCPs (C_{24}, 40% or C_{24}, 70%) were applied to the skin of 200 male and female volunteers, no local irritation was observed; the exposure duration and dose level were not reported. (Howard et al. 1975 – quoted from WHO 1996, EU RAR 1999).

No data have been located for MCCPs.

3.2.2 Eye irritation

No data have been located.

3.2.3 Respiratory irritation

No data have been located.

3.3 Sensitisation

When LCCPs (C_{20-30}, 40-41% or C_{24}, 70%) were applied to the skin of 200 male and female volunteers for a 5-day period, and then re-applied for 2 days beginning 3 weeks after the initial exposure, no allergic response was observed; the dose level was not reported. In a similar study, in which an SCCP (C_{12}, 59%) or LCCPs (C_{24}, 40% or C_{24}, 70%) were applied to the skin of 200 male and female volunteers, no allergic response was observed; the exposure duration and dose level were not reported. (Howard et al. 1975 – quoted from WHO 1996, EU RAR 1999).

No positive reactions were obtained among 75 exposed employees patch tested with various constituents of cutting fluid coolants, including chlorinated paraffins (Menter et al. 1975 – quoted from EU RAR 1999, WHO 1996).

No data on skin sensitisation have been located for MCCPs.

No data on respiratory sensitisation have been located.

3.4 Repeated dose toxicity

No data have been located.

3.5 Toxicity to reproduction

No data have been located.

3.6 Mutagenic and genotoxic effects

No data have been located.

3.7 Carcinogenic effects

No data have been located.

4 Animal toxicity

4.1 Single dose toxicity

4.1.1 Inhalation

No signs of toxicity were observed in rats exposed to an SCCP (C_{12}, 59%) at 3300 mg/m^3 for 1 hour (Howard et al. 1975 – quoted from WHO 1996, EU RAR 1999).

No data have been located for MCCPs or LCCPs.

4.1.2 Oral intake

No deaths occurred in F344 rats or B6C3F$_1$ mice treated by gavage with 0.8-13.6 g/kg bw or 1.6-27 g/kg bw, rats and mice respectively, of an SCCP (C_{12}, 60%) or an LCCP (C_{23}, 43%) in corn oil. Animals were inactive and ataxic after dosing. Rats showed diarrhoea for 2-6 days after dosing, while mice had ruffled fur on days 2-6 after treatment. (NTP 1986 – quoted from EU RAR 1999, WHO 1996).

No deaths occurred in rats, which received single oral gavage doses of up to 15 g/kg bw of MCCPs ($C_{14\text{-}17}$, 40-52%), signs of toxicity included urinary incontinence or "oily/moist pelt around the anal-genital region" (Unpublished studies 1978,1986 – quoted from EU RAR 2005).

Several unpublished studies have been summarised by Birtley et al. (1980 – quoted from EU RAR 1999,2005, WHO 1996) in which Wistar rats were given single oral dose of up to 10 g/kg bw of SCCPs ($C_{10\text{-}13}$, 41-50%, 51-60% or 61-70%), MCCPs ($C_{14\text{-}17}$, 51-60%), or LCCPs ($C_{20\text{-}30}$, 41-50%, 51-60% or 61-70%). No deaths were reported except for one rat treated with 13 g/kg bw $C_{10\text{-}13}$, 63%. Signs of toxicity included piloerection, lethargy and urinary incontinence. The toxicity of MCCPs and LCCPs were reported to be lower than that of SCCPs.

The LD_{50}-values have been reported to be > 17.7 or > 50 g/kg bw for oral exposure of rats to LCCPs (C_{24}, 40% or C_{24}, 70%, respectively) and for guinea-pigs given a C_{24}, 70% to be > 25 g/kg bw (Howard et al. 1975 – quoted from WHO 1996).

4.1.3 Dermal contact

No signs of systemic toxicity were observed in rats when 2.8 g/kg bw of an SCCP ($C_{10\text{-}13}$, 52%) was applied under an occlusive dressing for 24 hours; slight erythema and slight desquamation were noted on days 3 and 7, respectively, after application (Unpublished report 1971 – quoted from EU RAR 1999).

The LD_{50}-value for dermal exposure of rabbits to an SCCP (C_{12}, 59%) has been reported to be above 13 g/kg bw (Howard et al. 1975 – quoted from WHO 1996, EU RAR 1999).

No data have been located for MCCPs or LCCPs.

4.2 Irritation

4.2.1 Skin irritation

4.2.1.1 SCCPs

Two studies conducted according to modern standards and well reported are, according to EU RAR (1999), available:
In one study, no signs of irritation was observed following application of 0.5 ml an undiluted C_{10-13}, 59%, under a semi-occlusive dressing, to the shaven skin of three rabbits for four hours and observation for up to 72 hours post-application (Unpublished study 1986 – quoted from EU RAR 1999, WHO 1996).
In the second study, 0.5 ml an undiluted C_{10-13}, 70% was tested as above. One rabbit showed clearly defined erythema (grade 2 on a 0-4 scale score) at 48 and 72 hours. The other two animals showed slightly noticeable erythema (grade 1). Very slight oedema (grade 1) was noted in two animals for up to 24 hours. By day 7, all signs of irritation were completely resolved. (Unpublished study 1983 – quoted from EU RAR 1999, WHO 1996).

According to EU RAR (1999), SCCPs have also been tested in several other unpublished studies, which were not conducted according to modern protocols and were less well, and often only briefly reported. These studies were conducted using rats, and most studies used six 24-hour applications of 0.1 or 0.2 ml to shaven skin, under occlusive dressings. Treatment periods were separated by 24-hour treatment-free periods. The samples were usually undiluted but contained low percentages of epoxy stabilisers and/or various additives. These studies are briefly summarised below:
In one study with a C_{10-13}, 70% containing 1 or 2% of an epoxidised vegetable oil stabiliser with and without additives (0.1% oxalic acid or 0.05% benzotriazole), very mild to mild desquamation (occasional, transient and inconsistent) was only noted following the applications containing additives (Unpublished study 1965 – quoted from EU RAR 1999, WHO 1996).
In another study with a C_{10-13}, 70% containing 0.1 or 2% benzoyl peroxide initiator, no signs of irritation were noted (Unpublished study 1974 – quoted from EU RAR 1999, WHO 1996).
Two studies have tested three C_{10-13}, 63% containing up to 3% epoxy soy oil stabilisers or other unspecified additives. Erythema was usually noted following 2 or 4 application; the severity was not described. Desquamation was also noted following 3 or 4 applications and increased in severity with further treatments. (Unpublished studies 1973,1974 – quoted from EU RAR 1999, WHO 1996).
Five studies have been conducted using C_{10-13}, 48, 50, 52 or 55% containing 0.2 or 2% epoxy stabilisers in most studies. In one study (C_{10-13}, 48 or 55%, 0.2% epoxy octyl stearate), no signs of irritation were observed. In the other studies, there was mild or slight erythema, and mild desquamation was usually noted following the second or third application. In 4/5 studies, the reactions did not worsen following further application, whereas in one study (C_{10-13}, 52%, unspecified additives) slight erythema noted after the second application worsened to severe erythema with slight necrosis after the third application. (Unpublished studies 1967,1968,1969,1971,1974 – quoted from EU RAR 1999, WHO 1996).
Slight desquamation was observed following the second application of a C_{10-13}, 40% containing 1% epoxy soy oil stabiliser, and mild erythema after the third application. This condition persisted throughout the remaining applications and at the end of the study, small scattered ulcers developed. (Unpublished study 1966 – quoted from EU RAR 1999, WHO 1996).

Two studies have tested C_{10-13}, 49 or 60% using single and repeated application to rats. No signs of irritation were noted following a single application of C_{10-13},60%, whereas slight desquamation was noted in 2/6 animals, three to six hours after application of C_{10-13}, 49%. Both SCCPs produced slight erythema and/or slight desquamation with repeated applications. (Unpublished studies 1980,1982 – quoted from EU RAR 1999, WHO 1996).

A C_{10-13}, 61% and a 50% SCCP of unidentified chain length produced mild or moderate skin irritation following a single occlusive application to intact and abraded skin of rabbits. Various degrees of erythema (not further specified) persisted for 72 hours. (Unpublished study 1975 – quoted from EU RAR 1999, WHO 1996).

4.2.1.2 MCCPs

Two studies conducted according to contemporary OECD test guidelines are, according to EU RAR (2005), available (Unpublished studies 1986 – quoted from EU RAR 2005). Undiluted C_{14-17}, 40 or 52% containing 1% epoxy stabiliser was applied (0.5 ml) to the shaved skin of 6 rabbits under occlusive dressing for 4 hours. Mean 24-72 scores for erythema and oedema were 1.5 and 0.6, and 1.3 and 0.3, respectively, for the two MCCPs. Scales were also seen from the 6^{th} to 10^{th} day following exposure. For C_{14-17}, 40%, drying and hardness (at 72 hours) and peeling (days 6-8) were also observed.

No signs of skin irritation were seen following application to the skin of rabbits of an undiluted C_{14-17}, 45% for 24 hours under occlusive dressing, whereas slight erythema was reported in one animal at 24 hours following application of a C_{14-17}, 40%. In rats, slight erythema and/or desquamation were noted after 3-5 applications of 0.1 ml an undiluted C_{14-17}, 40% (6 daily applications, under occlusive dressing), progressing to cracking and thickening of the skin. (Chater 1978 – quoted from EU RAR 2005).

Following a single application of a C_{14-17}, 45% containing 0.2% epoxy stabiliser, slight desquamation was noted in 3/6 rats. Following repeated applications, 1/6 rats developed slight desquamation after the 4^{th} and 5^{th} application. (Moses 1980 – quoted from EU RAR 2005).

Mild irritation was seen in rats treated with 0.1 ml of C_{14-17}, 51-60% under occlusive dressing for up to six 24-hour treatment periods separated by 24-hour treatment-free periods. Some of the MCCPs contained 0.2% epoxidised vegetable oil stabiliser. (Birtley et al. 1980 – quoted from EU RAR 2005, WHO 1996).

4.2.1.3 LCCPs

No signs of irritation were seen in rats treated with 0.1 ml of C_{20-30}, 41-50%, 51-60% or 61-70% for up to six 24-hour treatment periods separated by 24-hour treatment-free periods. In some of the studies, the LCCPs contained epoxy stabilisers. (Birtley et al. 1980 – quoted from WHO 1996).

4.2.2 Eye irritation

4.2.2.1 SCCPs

Three different C_{10-13}, 40-63% containing either 2.5 or 2% of two different additives or 0.7% of an epoxy stabiliser have been tested in two studies (Unpublished studies 1973,1974 – quoted from EU RAR 1999, WHO 1996). Both studies were, according to EU RAR (1999), conducted according to modern protocols with either 0.1 ml or "one drop" of the SCCP being instilled into the conjunctival sac of each of three rabbits. Similar results were reported for all three formulations. Practically no initial pain (2 on a 6 point scale) was noted. Slight irritation (3 on an 8 point scale), evidenced by redness and chemosis (only noted for the SCCP containing epoxy stabiliser) of the conjunctiva with some discharge, lasted for 24 hours.

One drop of a C_{10-13}, 40 or 52% containing unspecified additives or 1% epoxy stabiliser were also tested as above. With the C_{10-13}, 40%, mild congestion was noted at 1 hour with no effects being seen at 24 hours. With the C_{10-13}, 52%, slight immediate irritation was followed by slight redness of the conjunctiva, which lasted for 24 hours. (Unpublished study 1971 – quoted from EU RAR 1999, WHO 1996).

4.2.2.2 MCCPs

Two studies conducted according to contemporary OECD test guidelines are, according to EU RAR (2005), available (Unpublished studies 1986 – quoted from EU RAR 2005). Undiluted C_{14-17}, 40 or 52% containing 1% epoxy stabiliser were instilled (0.1 ml) into the conjunctival sac of one eye of each of 3 rabbits. No iridial or corneal effects were noted. Conjunctival redness (score 1) was noted in all 3 animals at 1 hour with C_{14-17}, 52%, in 1 animal at 24 hours with both MCCPs, and in 1 animal at 48 hours with C_{14-17}, 40%. Discharge was noted in 1-2 animals at 1 hour with both MCCPs, and also at 48 hours with C_{14-17}, 52%.

A C_{14-17}, 40% or 45% containing 0.2% epoxy stabiliser was instilled into the conjunctival sac of each of 3 rabbits. A slight initial pain (2 on a 0-5 point scale) was seen for both MCCPs, and a slight transient conjunctivitis (score 3 out of 8 for conjunctival effects) was seen within 1-2 hours of instillation. No effects were seen at 24, 48 or 72 hours. (Chater 1978 – quoted from EU RAR 2005).

No signs of eye irritation were seen following a single application of 0.1 ml of C_{14-17}, 51-60% into the eyes of groups of 3 rabbits (Birtley et al. 1980 – quoted from EU RAR 2005, WHO 1996).

4.2.2.3 LCCPs

No signs of eye irritation was seen following a single application of 0.1 ml of C_{20-30}, 41-50%, 51-60% or 61-70% into the eyes of rabbits (Birtley et al. 1980 – quoted from WHO 1996).

4.2.3 Respiratory irritation

No data have been located.

4.3 Sensitisation

4.3.1 Skin sensitisation

4.3.1.1 SCCPs

According to EU RAR (1999), three studies are available, which have been well-conducted according to modern protocols and using suitable induction regimes:
In a guinea pig maximization test (GPMT) with a C_{10-13}, 50% and containing 1% stabiliser, 2/20 animals showed marked diffuse redness at 24 hours after challenge (undiluted SCCP) and 1/20 showed slight redness and dryness at 24 hours. When the same animals were challenged (50% SCCP) 1 week later, no skin reactions were observed. No skin reactions were observed in the control group. (Unpublished study 1988 – quoted from EU RAR 1999).
In another GPMT with a C_{10-13}, 56% containing 1% epoxide stabiliser and 1% trisnonylphosphite, 1/20 test animals showed hardly perceptible erythema at 24 hours after challenge (undiluted SCCP), and 1/20 test animals and 1/10 control animals showed clearly defined erythema and slight oedema at 72 hours (Unpublished study 1983 – quoted from EU RAR 1999, WHO 1996).
In a third GPMT with a C_{10-13}, 56% containing 1% of different epoxide stabilisers and 1% trisnonylphosphite, 5/20 test animals showed clearly defined erythema following challenge (undiluted SCCP), and another 2 animals showed slight, hardly perceptible erythema; none of the control animals showed any evidence of a skin reaction. When a second challenge was performed 2 weeks after the first one, 4/20 test animals showed clearly defined erythema and another four showed slight, hardly perceptible erythema and slight oedema. (Unpublished study 1984 – quoted from EU RAR 1999, WHO 1996).

When an undiluted C_{10-13}, 52% was applied to the ears of 6 guinea-pigs on three successive days, slight erythema was noted when challenged four days later with undiluted SCCP applied to the animal flanks; no further details on number of animals were provided. Four control animals also showed slight erythema at challenge. (Unpublished study 1971 – quoted from EU RAR 1999, WHO 1996).

4.3.1.2 MCCPs

In a GPMT with a C_{14-17}, 40% containing 1% stabiliser, a 20% dilution in maize oil was used for intradermal induction. At topical induction with undiluted MCCP, inflammation (intense, sometimes haemorrhagic, purulent) was noted. Following challenge (undiluted MCCP), one test and one control animal showed a reaction at 48 hours (scores of 1 and 3, respectively). No skin reactions were observed following a second challenge (50% MCCP in maize oil). (Murmann 1988 – quoted from EU RAR 2005).

In two other GPMTs conducted with C_{14-17}, 40-45% and containing 0.2% epoxy stabiliser, no skin reactions were observed following intradermal induction (5% MCCP in olive oil), topical induction (20% MCCP), or challenge (20% MCCP) (Chater 1978 – quoted from EU RAR 2005).

4.3.1.3 LCCPs

No data have been located.

4.3.2 Respiratory sensitisation

No data have been located.

4.4 Repeated dose toxicity

Studies with oral administration of chlorinated paraffins to experimental animals have demonstrated that the liver, kidneys and thyroid are the target organs for the toxicity of chlorinated paraffins. The NOAELs and LOAELs stated in this section are quoted from WHO (1996) unless otherwise stated. Modes of actions are addressed in section 2.3 and NOAELs and LOAELs for effects in the target organs will be discussed in section 6.7 taken the mechanistic data into account.

No studies with inhalation of or dermal contact to chlorinated paraffins have been located.

4.4.1 SCCPs

All the available studies on SCCPs included in EU RAR (1999) and/or WHO (1996) are summarised in Table 4.4.1 and supplementary information on the studies is given in the text.

Rat, 14-day dietary study, C_{10-12}, 58% (Unpublished study 1983 – quoted from EU RAR 1999, WHO 1996): SCCP was administered in the diet at concentrations of 0, 900, 2700, 9100 or 27300 mg/kg feed. No deaths occurred and no clinical signs of toxicity were observed. Reduction in body weight and food consumption (high-dose) was approximately 50% at day 14. Liver weights (absolute and relative) were significantly increased from 20% (low-dose) to 240% (high-dose). A dose-related increase in the incidence of hepatocellular hypertrophy was present in all dose groups. A dose-related increase in the liver enzyme activity or microsomal levels was noted for all treatment groups with significance in females from 300 mg/kg bw/day and in males from 1000 mg/kg bw/day. The NOAEL was considered by WHO (1996) as below 100 mg/kg bw/day based on the effects in the liver observed at all dose levels.

Rat, 14-day gavage study, C_{10-12}, 58% (Unpublished study 1981 – quoted from EU RAR 1999, WHO 1996): SCCP was administered in corn oil. No deaths occurred. Clinical signs of toxicity included laboured breathing, decreased motor activity, excessive lachrymation and staining around nose, mouth and ano-genital region. Reduced body weight gain and food consumption observed in high-dose animals was only significant in females. Increased hepatic microsomal enzyme activity was dose-related. The hepatocellular hypertrophy at the two highest dose levels was stated as being mild. The NOAEL was considered by WHO (1996) as 30 mg/kg bw/day based on increased liver weight observed at higher dose levels.

Rat, 16-day gavage study, C_{12}, 60% (NTP 1986 – quoted from EU RAR 1999, WHO 1996). SCCP was administered in corn oil 5 days/week. Three deaths occurred in high-dose animals (1 male, 2 females). Enlarged livers were observed in all dose groups except the females given 469 mg/kg bw/day. Histopathology was not performed. The NOAEL was considered by WHO (1996) as below 469 mg/kg bw/day based on enlarged liver observed at all dose levels.

Table 4.4.1. Repeated dose toxicity studies on SCCPs

Species / strain	Duration / Dose levels (mg/kg bw/day)	Effects	NOAEL (mg/kg bw/ day)	LOAEL (mg/kg bw/ day)	Reference
F344 rat 5/sex/group C_{10-12},58%	14-day, diet 0, 100, 300, 1000, 3000	3000: ↓ bw/fc, hist spleen/thymus/testes ≥ 1000: hist myocard. ≥ 300: ↑ enz liver ≥ 100: ↑ w liver, hist liver	< 100	100	Unpublished study (1983)
F344 rat 5/sex/group C_{10-12},58%	14-day, gavage 0, 30, 100, 300, 1000, 3000	3000: clinical signs, ↓ bwg/fc, ↓ w thymus/ovary ≥ 1000: hist liver ≥ 300: ↑ enz liver ♀ ≥ 100: ↑ w liver	30	100	Unpublished study (1981)
F344 rat 5/sex/group C_{12},60%	16-day, gavage 469, 938, 1875, 3750, 7500	3750: deaths, diarrhoea, ↓ bwg ≥ 469: enlarged liver	< 469	469	NTP (1986)
F344 rat C_{10-12},58%	13-week, diet 0, 10, 100, 625	625: ↓ bwg ♂, ↑ w thyroid, ↑ enz liver ♂ ≥ 100: ↑ w liver/kidney, hist liver/kidney/thyroid	10	100	Unpublished study (1984)
F244 rat 15/sex/group C_{10-12},58%	13-week, gavage 0, 10, 100, 625	625: ↓ bwg ♂, ↑ w thyroid, ↑ enz liver ♂ ≥ 100: ↑ w liver/kidney, hist liver/kidney/thyroid	10	100	Unpublished study (1984)
F344 rat 10/sex/group C_{12},60%	13-week, gavage 0, 313, 625, 1250, 2500, 5000	≥ 2500: ↓ bwg ♂, hist liver/kidney ≥ 313: ↑ w liver	< 313	313	NTP (1986)
F344 rat 50/sex/group 20/sex/group C_{12},60%	104-week, gavage 0, 312, 625	625: ↓ bwg ♂, ≥ 312: ↓ survival, ↑ w liver/kidney, hist liver/kidney/stomach/ forestomach/parathyroid	< 312	312	NTP (1986)
B6F3C1 mouse 5/sex/group C_{12},60%	16-day, gavage 938, 1875, 3750, 7500, 15000	≥ 1875: deaths ≥ 938: diarrhoea, enlarged liver	< 938	938	NTP (1986)
B6F3C1 mouse 10/sex/group C_{12},60%	13-week, gavage 0, 125, 250, 500, 1000, 2000	2000: ↓ bwg ♂, ≥ 250: hist liver ≥ 125: ↑ w liver	125	250	NTP (1986)
B6F3C1 mouse 50/sex/group C_{12},60%	104-week, gavage 0, 125, 250	250: ↓ survival, hist kidney ♀ ≥ 125: ↓ bwg ♀, hist thyroid	<125	125	NTP (1986)

↓: reduced
↑: increased
♂ / ♀: male / female
fc: food consumption
bw: body weight
bwg: body weight gain
enz: enzyme activity / activities or levels
hist: histopathological changes
w: weight

Rat, 13-week dietary study, C_{10-12}, 58% (Unpublished study 1984 – quoted from EU RAR 1999; WHO 1996). No deaths occurred and no clinical signs of toxicity were observed. Reduced body weight gain (high-dose males) was slight (9%). No changes were observed in haematological parameters. Slight dose-related increases in liver protein content were noted in treated males with corresponding increases in cytochrome P450, particularly in high-dose males; no changes were observed in liver enzyme levels or activities in females. Absolute and relative liver weights were significantly increased in mid- and high-dose group (20 and 140%), kidney weights (10 and 30%), and thyroid weights in high-dose group (32%). Histopathological changes were observed in high-dose animals and in mid-dose males and included hepatocellular hypertrophy, mild nephritis (males only), brown pigmentation in the renal tubules (females only), and thyroid hypertrophy. The NOAEL was considered by WHO (1996) as 10 mg/kg bw/day based on increased liver and kidney weights and hypertrophy in liver and thyroid observed at higher dose levels.

Rat, 13-week gavage study, C_{10-12}, 58% (Unpublished study 1984 – quoted from EU RAR 1999, WHO 1996). No deaths occurred and no clinical signs of toxicity were observed. Reduced body weight gain was stated to be slight. Absolute and relative liver weights were significantly increased in mid- and high-dose group (30 and 110%), kidney weights (20 and 100%), and thyroid weights in high-dose group. Histopathological changes were observed in mid- and high-dose animals and included hepatocellular hypertrophy, thyroid hypertrophy and hyperplasia, and mild nephritis (mid-dose: males only) and brown pigmentation in the renal tubules (high-dose females only). The NOAEL was considered by WHO (1996) as 10 mg/kg bw/day based on increased liver and kidney weights and hypertrophy in liver and thyroid observed at higher dose levels.

Rat, 13-week gavage study, C_{12}, 60% (NTP 1986 – quoted from EU RAR 1999, WHO 1996). SCCP was administered in corn oil 5 days/week. No deaths occurred. Reduced body weight gain was approximately 11-12%. Increased relative liver weight was significant at all dose levels and dose-related, from 25% (low-dose) to 100% (high-dose). Hepatocellular hypertrophy was noted in all high-dose rats, and nephropathy in all high-dose males, in 3/10 high-dose females, and in 8/10 male controls. Only high-dose and control animals were examined histopathologically. There were no changes observed in the thyroid, thymus, heart, or spleen. The NOAEL was considered by WHO (1996) as below 313 mg/kg bw/day based on increased liver weight observed at all dose levels.

Rat, 104-week gavage study, C_{12}, 60% (NTP 1986 – quoted from EU RAR 1999, WHO 1996, IARC 1990). SCCP was administered in corn oil 5 days/week. Additional groups (20/sex/group) were included for concurrent 6- and 12-month studies. Survival in the main study was reduced in low- and high-dose males and in low-dose females (control: 27/34; low-dose: 6/23; high-dose: 3/29 for males/females, respectively). Reduced body weight gain was 12% at the end of the 6- and 12-month studies, and 23% in the main study. Increased liver and kidney weights (absolute and relative) were dose-related and significant at both 6 and 12 months (up to 124% for liver and up to 46% for kidney), but were not different at these two examinations; organ weights were not reported in the main study. Non-neoplastic histopathological changes was observed in the liver (hepatocellular hypertrophy, necrosis, focal cellular changes and gross dilation of the blood vessels), in the kidney (cysts in the cortex (males), nephropathy (incidence and severity in females, severity in males), tubular cell hyperplasia in males), glandular stomach (oedema and erosion in males), forestomach (ulcers, inflammation, epithelial hyperplasia and hyperkeratosis in males), and parathyroid (fibrous osteodystrophy in males). The NOAEL for non-neoplastic lesions is considered as

below 312 mg/kg bw/day based on effects observed at both dose levels. Neoplastic lesions are addressed in section 4.7.1.

Mouse, 16-day gavage study, C_{12}, 60% (NTP 1986 – quoted from EU RAR 1999, WHO 1996). SCCP was administered in corn oil 5 days/week. All animals receiving doses from 3750 mg/kg bw/day died, and 4/5 males and 2/5 females at 1850 mg/kg bw/day. Diarrhoea was observed in all dose groups except low-dose females. Enlarged livers were observed in all treated surviving mice. Histopathology was not performed. The NOAEL is considered as below 938 mg/kg bw/day based on effects observed at all dose levels.

Mouse, 13-week gavage study, C_{12}, 60% (NTP 1986 – quoted from EU RAR 1999, WHO 1996). SCCP was administered in corn oil 5 days/week. No deaths occurred and no clinical signs of toxicity were observed. Reduced body weight gain was 13%. Increased relative liver weights were dose-related (from 17% at low-dose to 160% at high-dose) and were significant from 250 mg/kg bw/day in females and from 500 mg/kg bw/day in males. Hepatocellular hypertrophy was noted from 250 mg/kg bw/day, and focal hepatic necrosis from 500 mg/kg bw/day in males and at 2000 mg/kg bw/day in females. There were no changes observed in the thyroid. The NOAEL was considered by WHO (1996) as 125 mg/kg bw/day based on hepatocellular hypertrophy observed at higher dose levels.

Mouse, 104-week gavage study, C_{12}, 60% (NTP 1986 – quoted from EU RAR 1999, WHO 1996, IARC 1990). SCCP was administered in corn oil 5 days/week. Survival of high-dose females was reduced after week 100 (control: 34/35; low-dose: 30/31; high-dose: 30/25 for males/females, respectively). Body weights were about 10% lower in treated females than in controls. Non-neoplastic histopathological changes was observed in the kidney (nephropathy increased incidence in females) and in the thyroid (follicular cell lesions in all groups ranging from early hyperplasia to multi-layered projections that extended in to the lumen, 10/32%, 12/55%, 24/45% in control, mid- and high-dose males/females, respectively). Non-neoplastic lesions were not noted in the liver. The NOAEL for non-neoplastic lesions is considered as below 125 mg/kg bw/day based on effects observed at both dose levels. Neoplastic lesions are addressed in section 4.7.1.

4.4.2 MCCPs

All the available studies on MCCPs included in EU RAR (2005) and/or WHO (1996) are summarised in Table 4.4.2 and supplementary information on the studies is given in the text.

Rat, 14-day dietary study, $C_{14\text{-}17}$, 52% (Unpublished study 1981 – quoted from EU RAR 2005, WHO 1996): MCCP was administered in the diet at concentrations of 0, 150, 500, 1500, 5000 or 15000 mg/kg feed. No deaths occurred and no clinical signs of toxicity were observed. Food consumption was reduced by up to 31%. Liver weights (absolute and relative) were increased (up to 80%) and ovary weights (absolute and relative) decreased (by 38%). Histopathological changes in the liver included diffuse mild hepatocellular hypertrophy; there were no changes observed in the ovary. The NOAEL was considered by WHO (1996) as 170/180 mg/kg bw/day based on an increased liver weight and hepatocellular hypertrophy at higher dose levels.

Rat, 13-week dietary study, $C_{14\text{-}17}$, 52% (Unpublished study 2005 – quoted from EU RAR 2005): MCCP was administered in the diet at concentrations of 0, 30,

100, 300 or 3000 mg/kg feed. Investigations included clinical observations, body weight

Table 4.4.2. Repeated dose toxicity studies on MCCPs

Species / strain	Duration / Dose levels (mg/kg bw/day)	Effects	NOAEL (mg/kg bw/ day)	LOAEL (mg/kg bw/ day)	Reference
F344 rat 5/sex/group C_{14-17},52%	14-day, diet 0, 18/18, 58/58, 170/180, 550/580, 1540/1290 ♂/♀	1290: ↓ fc ♀, ↓ w ovary ≥ 550: ↑ w liver, hist liver	170/180	550/580	Unpublished study (1981)
F344 rat 10/sex/ treated group 20/sex/control C_{14-17},52%	13-week, diet 0, 2.38/2.51, 9.34/9.70, 23.0/24.6, 222/242 ♂/♀	222: ↓ TG/Chol, ↑ w liver/kidney, ↑ T4 ♀, ↑ enz liver ♂, hist liver ♂ ≥ 23.0: ↓ T3 ♂, ↑ TSH ≥ 9.7: ↑ enz liver ♀	23.0/24.6	222/242	Unpublished study (2005)
Sprague-Dawley rat 10/sex/group C_{14-17},52%	13-week, diet 0, 0.4/0.4, 3.6/4.2, 36/42, 363/419, ♂/♀	363: ↑ w kidney, ↑ enz liver ≥ 36: ↑ w liver, hist liver ≥ 4.2: ↑ Chol ♀, hist kidney ♀, thyroid ♀	4	36/42	Poon et al. (1995)
F344 rat 15/sex/group C_{14-17},52%	13-week, diet 0, 10, 100, 625	625: ↓ bwg/fc, ↑ Chol ♀, ↑ w kidney/thyroid/ adrenal, hist liver ≥ 100: ↑ w liver ≥ 10: hist thyroid /kidney	10	100	Unpublished study (1984)
Wistar rat 24/sex/group C_{14-17},52%	13-week, diet 0, 33/32, 167/160, 333/320, ♂/♀	320: ↑ w kidney ≥ 167: ↓ bwg ♂, ≥ 32: ↑ w liver, hist liver	< 32	32	Birtley et al. (1980)
Beagle dog 4/sex/group C_{14-17},52%	13-week, diet 0, 10, 30, 100	100: ↑ w liver ≥ 30: hist liver	10	30	Birtley et al. (1980)

↓: reduced
↑: increased
♂ / ♀: male / female
fc: food consumption
bw: body weight
bwg: body weight gain
enz: enzyme activity / activities or levels
hist: histopathological changes
w: weight

and food consumption analysis, clinical chemistry, and extensive histopathological examinations of liver, thyroid and kidney. In addition, detailed investigations on parameters related to MCCPs induced liver, thyroid and kidney toxicity (hepatic T4-UDPGA glucuronyl transferase activity, hepatic peroxisome proliferation, free and total plasma T4, T3 and TSH levels, and renal and hepatic α2μglobulin levels) were also performed at the end of the study. No deaths occurred, no clinical signs of toxicity were observed, and there were no adverse effects on body weight, body weight gain or food consumption. Decreases in plasma triglycerides (by 28-39%) and cholesterol (by 14-23%) were small but significant. Liver and kidney weight increases were significant by 13-31% and by 9-13% of the control values, respectively. Plasma free T3 levels were significantly decreased in males at the two highest dose levels (by 26 and 22%) with no effects being observed on total T3 levels, or on free and total T4 levels. Plasma free T4 levels were significantly increased in high-dose females (by 41%) with no effects being observed on total T4 levels, or on free and total T3 levels. Plasma TSH levels were increased in

females at the two highest dose levels (by 20 and 39%) and in high-dose males (by 17%). Hepatic microsomal T4-UDPGA glucuronyl transferase activity was increased in high-dose males (by 82%) and in females at the three highest dose levels (by 30, 30 and 252%). There was no effect on hepatic peroxisome proliferation as determined by palmitoyl CoA oxidation. α2µglobulin levels from kidney or liver homogenates were unaffected by treatment in males, and was not detected in females. Histopathological changes in the liver included minimal centrilobular hepatocellular hypertrophy; no changes were observed in the kidney or thyroid. The NOAEL was considered by EU RAR (2005) as 23.0/24.6 mg/kg bw/day based on decreases in plasma triglycerides and cholesterol and increased kidney weights at 222/242 mg/kg bw/day.

Rat, 13-week dietary study, C_{14-17}, 52% (Poon et al. 1995): MCCP was administered in the diet at concentrations of 0, 5, 50, 500 or 5000 mg/kg feed. Investigations included urinalysis at 4 and 12 weeks, and haematological and blood biochemistry determinations at study termination. Extensive histopathological examinations were also performed at the end of the study. No deaths occurred, no clinical signs of toxicity were observed, and there were no adverse effects on body weight, body weight gain or food consumption. Serum cholesterol was significantly increased (by 7, 18, 18, 28%) in females at the three highest dose levels. Increased relative liver weights were observed in females at the two highest dose levels (by 7 and 47%) and in high-dose males (by 27%), and relative kidney weights in high-dose animals (by 8-11%). Hepatic UDP-glucuronosyl transferase activity was significantly increased in high-dose males and females (by 58 and 70%). Histopathological changes were observed in the liver (minimal to mild anisokaryosis and vesiculation of the nuclei at the two highest dose levels, increase in perivenous homogeneity in high-dose males and in females at the two highest dose levels, and single cell necrosis in high-dose animals), kidney (hyaline-droplet like cytoplasmic inclusions in all treated male dose groups but significant only in high-dose males, dose-related inner medullary tubular dilation of minimal severity in 0/10, 0/10, 1/10, 4/10, 8/10 females at 0, 0.4, 4.2, 42, 419 mg/kg bw/day, respectively), and thyroid (reduced follicle sizes, collapsed angularity, increased height, cytoplasmic vacuolation, nuclear vesiculation – minimal to mild in nature and observed in males from 36 mg/kg bw/day and in females from 4 mg/kg bw/day). According to the authors, female rats appeared to be more sensitive than male rats with effects being observed in the liver from 42 mg/kg bw/day, and thyroid changes and increased serum cholesterol from 4.2 mg/kg bw/day.
The NOAEL was considered by WHO (1996) as 4 mg/kg bw/day.
According to EU RAR (2005) "*Questions have been raised over the validity and reliability of the findings in this study, in particular in relation to the scoring system used for classifying the histopathological findings. It is noted that the effects on female kidney reported in this study starting from the relatively low dose of 4 mg/kg/day have not been seen in other rat 90-day studies even at higher dose levels. It is also noted that although histopathological findings of the thyroid have been described in other rat 90-day studies, only this study have reported them from the relatively low dose of 4 mg/kg/day. It is clearly apparent that this study is unrepresentative of the repeated dose toxicity profile of MCCPs and hence, it should not be used for risk characterisation purposes.*" In the previous version of the draft EU RAR on MCCPs (May 2004), it is stated "*Overall, no adverse effects were seen in female kidneys at exposure levels up to 0.4 mg/kg/day, although the profile of the dose response relationship is shallow and changes at 4 mg/kg/day or more are minor in degree in terms of incidence and severity, but cannot be dismissed as being irrelevant to human health.*"

Rat, 13-week dietary study, C_{14-17}, 52% (Unpublished study 1984 – quoted from EU RAR 2005, WHO 1996): MCCP was administered in the diet to provide dose

levels of 0, 10, 100, 625 mg/kg bw/day. No deaths occurred and no clinical signs of toxicity were observed. The reduction in body weight gain was slight (< 5%) and was observed in both sexes. Serum cholesterol was increased (by 25%) in high-dose females. Increased liver weights were observed in mid- and high-dose animals of both sexes, increased kidney and adrenal weights in high-dose animals of both sexes, and increased thyroid weights in high-dose males. Histopathological changes were noted in the liver (hepatocellular hypertrophy of mild severity), thyroid (mild to moderate hypertrophy and hyperplasia in almost all control and treated males with a trend towards increasing severity with increasing dose), and kidney (chronic nephritis in males 1/15, 3/15, 4/15, 10/15; renal tubular pigmentation in high-dose females). The NOAEL was considered by WHO (1996) as 10 mg/kg bw/day based on increased liver and kidney weights at higher dose levels.

Rat, 13-week dietary study, C_{14-17}, 52% (Birtley et al. 1980 – quoted from EU RAR 2005, WHO 1996): MCCP containing an epoxidised vegetable oil stabiliser was administered in the diet at 0, 500, 2500 or 5000 mg/kg diet. Assuming a mean body weight of 300 g for males and 250 g for females, and a food consumption of 20 g/day for males and 16 g/day for females, the mean intakes were calculated (EU RAR 2005). No deaths occurred and no clinical signs of toxicity were observed. The reduction in body weight gain was 17 and 25%). Increased relative liver weight was observed in males from 2500 mg/kg (by 15 and 22%) and in females from 500 mg/kg (by 11, 21 and 48%). Histopathological changes in the liver included a dose-related proliferation of smooth endoplasmic reticulum (using electron microscopy). The LOAEL was considered by WHO (1996) as 32 mg/kg bw/day based on increased relative liver weights and proliferation of smooth endoplasmic reticulum.

Dog, 13-week dietary study, C_{14-17}, 52% (Birtley et al. 1980 – quoted from EU RAR 2005, WHO 1996): MCCP containing an epoxidised vegetable oil stabiliser was administered in the diet corresponding to 0, 10, 30 or 100 mg/kg bw/day. Histopathological changes in the liver were reported as enlarged hepatocytes and increase in smooth endoplasmic reticulum in these cells in some dogs. No further details are available. The NOAEL was considered by WHO (1996) as 10 mg/kg bw/day based on an increase of hepatic smooth endoplasmic reticulum at higher dose levels.

4.4.3 LCCPs

All the available studies on LCCPs included in WHO (1996) are summarised in Table 4.4.3 and supplementary information on the studies is given in the text.

Rat, 14-day dietary study, C_{22-26}, 70% (IRDC 1982 – quoted from WHO 1996): LCCP was administered in the diet at concentrations of 0, 150, 500, 1500, 5000 or 15000 mg/kg feed.

Rat, 16-day gavage study, C_{22-26}, 43% (NTP 1986 – quoted from WHO 1996). LCCP was administered in corn oil 5 days/week. Histopathology was not performed.

Rat, 13-week dietary study, C_{22-26}, 70% (IRDC 1984 – quoted from WHO 1996): LCCP was administered in the diet to provide dose levels of 0, 100, 900, 3750 mg/kg bw/day. Histopathological changes in the liver included hepatocellular

Table 4.4.3. Repeated dose toxicity studies on LCCPs

Species / strain	Duration / Dose levels (mg/kg bw/day)	Effects	NOAEL (mg/kg bw/ day)	LOAEL (mg/kg bw/ day)	Reference
F344 rat 5/sex/group C_{22-26},70%	14-day, diet 0, 17.1, 55, 169, 565, 1715	No effects observed	1715	> 1715	IRDC (1982)
F344 rat C_{22-26},43%	14-day, gavage 0, 30, 100, 300, 1000, 3000	No effects observed	3000	> 3000	IRDC (1982)
F344 rat 5/sex/group C_{22-26},43%	16-day, gavage 0, 235, 469, 938, 1875, 3750	No effects observed	3750	> 3750	NTP (1986)
F344 rat C_{22-26},70%	13-week, diet 0, 100, 900, 3750	3750: ↑ w liver, hist liver	900	3750	IRDC (1984)
F344 rat C_{20-30},43%	13-week, gavage 0, 100, 900, 3750	3750: hist kidney ≥ 100: ↑ w liver ♀, hist liver ♀	< 100	100	IRDC (1984)
F344 rat 10/sex/group C_{23},43%	13-week, gavage 0, 235, 469, 938, 1875, 3750	≥ 235: hist liver ♀	< 235	235	NTP (1986)
F344 rat 50/sex/group 20/sex/group C_{23},43%	103-week, gavage 0, 1875, 3750 ♂ 0, 100, 300, 900 ♀	≥ 1875 ♂: ↑ w liver, hist ≥ 100 ♀: ↑ w liver, hist	< 100	100	NTP (1986)
B6C3F1 mouse 5/sex/group C_{22-26},43%	16-day, gavage 0, 469, 938, 1875, 3750, 7500	No effects observed	7500	> 7500	NTP (1986)
B6C3F1 mouse 10/sex/group C_{23},43%	13-week, gavage 0, 469, 938, 1875, 3750, 7500	No effects observed	7500	> 7500	NTP (1986)
B6C3F1 mouse 50/sex/group C_{23},43%	103-week, gavage 0, 2500, 5000	No non-neoplastic effects observed	7500	> 7500	NTP (1986)

↓: reduced
↑: increased
♂ / ♀: male / female
fc: food consumption
bw: body weight
bwg: body weight gain
hist: histopathological changes
w: weight

hypertrophy and cytoplasmic fat vacuolation. The NOAEL was considered by WHO (1996) as 900 mg/kg bw/day based on liver effects at high dose level.

Rat, 13-week gavage study, C_{22-26}, 70% (IRDC 1984 – quoted from WHO 1996): LCCP was administered in corn oil. Histopathological changes in the liver of females included multifocal granulomatous hepatitis characterised by inflammatory changes and necrosis; no effects were observed in males. Histopathological changes in the kidney included mineralisation (females) and nephrosis (males). The

NOAEL was considered by WHO (1996) as < 100 mg/kg bw/day based on liver effects at all dose levels in females.

Rat, 13-week gavage study, C_{23}, 43% (NTP 1986 – quoted from WHO 1996). No clinical signs of toxicity and no effects on body or organ weights were observed. Histopathological changes in the liver of females included a dose-related increased incidence of granulomatous inflammation; no effects were observed in males. The NOAEL was considered by WHO (1996) as < 235 mg/kg bw/day based on liver effects at all dose levels in females.

Rat, 103-week gavage study, C_{23}, 43% (NTP 1986 – quoted from WHO 1996, IARC 1990). LCCP was administered in corn oil 5 days/week. Additional groups (20/sex/group) were included for concurrent 6- and 12-month studies. Survival was not affected, and no clinical signs of toxicity and no effects on body or organ weights were observed. Relative liver weights were dose-related increased in treated males at 12 months and in treated females at 6 and 12 months. Histopathological changes included a diffuse lymphohistiocytic inflammation in the liver and in the pancreatic and mesenteric lymph nodes in both sexes in all exposed groups; splenic congestion was a secondary effect. The NOAEL for non-neoplastic effects is considered as < 100 mg/kg bw/day based on effects observed at all dose levels in females. Neoplastic lesions are addressed in section 4.7.3.

Mouse, 16-day gavage study, $C_{22\text{-}26}$, 43% (NTP 1986 – quoted from WHO 1996). Histopathology was not performed.

Mouse, 103-week gavage study, C_{23}, 43% (NTP 1986 – quoted from WHO 1996, IARC 1990). LCCP was administered in corn oil 5 days/week. Survival was not different between treated and control groups, and no clinical signs of toxicity were observed. However, a *Klebsiella* infection affected the animals after week 65, and 60-70% of the early deaths in each group were attributed to the infection. No significant non-neoplastic lesions were observed. The NOAEL for non-neoplastic effects is considered as 5000 mg/kg bw/day. Neoplastic lesions are addressed in section 4.7.3.

4.4.4 Comparative studies

The effects of representative chlorinated paraffins on liver function and thyroid hormone function has been studied in male rats (Alpk:APFSD, 5/group) and male mice (Alpk:APFCD-1, 5/group) given 0, 10, 50, 100, 250, 500 or 1000 mg/kg bw/day of chlorinated paraffins by gavage in corn oil, once daily for 14 days (Wyatt et al. 1993 – quoted from WHO 1996, EU RAR 1999). The chlorinated paraffins included $C_{10\text{-}13}$, 58%, $C_{10\text{-}13}$, 56% and $C_{14\text{-}17}$, 44%.
All three chlorinated paraffins caused increases in liver weight (significant from 100 mg/kg bw/day) and palmitoyl CoA oxidation, indicative of peroxisome proliferation, (significant from 250 mg/kg bw/day). In general, the rat was more sensitive to the effects than the mouse. The doses required to cause peroxisomal proliferation were, in general, greater than those causing effects on liver weight. The magnitude of the increase in palmitoyl CoA oxidation caused by SCCPs (approximately 10-fold increase as a maximal change) was greater than for MCCPs (approximately 4-fold increase as a maximal change).
The effect on thyroid function was studied in the male rats receiving 1000 mg/kg bw/day. All three chlorinated paraffins caused a reduction in plasma T4 levels (both free and total) and an increase in plasma TSH levels; no effect was observed on plasma T3 levels. All three chlorinated paraffins also caused an increase (2-fold) in the rate of glucuronidation of T4 by hepatic microsomal UDP glucuronosyl

transferase activity, suggesting that the impact on plasma T4 and TSH levels is due to increased clearance of T4 by hepatic metabolism.

The hepatic effects of chlorinated paraffins have been studied in F344 rats, B6C3F1 mice and male Alpk:Dunkin Hartley guinea-pigs, and their effects were compared with a range of known inducers of hepatic enzymes (Elcombe et al. 1994 – quoted from WHO 1996). Groups of 4-5 animals received 1000 to 2000 mg/kg bw/day of the following chlorinated paraffins by gavage in corn oil for 14 days: C_{10-13}, 58%, C_{10-13}, 56%, C_{14-17}, 40%, and C_{20-30}, 43%. The SCCPs and MCCPs, but not the LCCPs, increased relative liver weight (approximately 1.5 times) and elicited hepatocellular hypertrophy, peroxisome proliferation (assessed as increases in peroxisomal volume and palmitoyl CoA oxidase activity) and proliferation of hepatic cell smooth endoplasmic reticulum in both rats and mice. SCCPs and MCCPs also caused induction of cytochrome P450 IVA1 and P450 IIB1/IIB2 in the rat liver, but only P450 IVA1 in the mouse liver. In guinea-pigs administered 1000 mg/kg bw/day of SCCPs and MCCPs had a similar effect on relative liver weight (1.5-fold increase) but had no effect on any of the hepatic ultrastructural or biochemical parameters measured.

4.5 Toxicity to reproduction

No studies with inhalation of or dermal contact to chlorinated paraffins have been located.

4.5.1 SCCPs

No studies specifically investigating effects on fertility have been located.

In a developmental toxicity study, well-conducted according to EU RAR (1999), pregnant Charles River COBS CD rats (25/group) were treated by gavage with 0, 100, 500 or 2000 mg/kg bw/day of a C_{10-13}, 58% in corn oil from day 6 to 19 of gestation (Unpublished study 1982 – quoted from EU RAR 1999, WHO 1996). Eight high-dose dams died. Clinical signs of toxicity (emaciated appearance, excessive salivation and decreased activity) were observed in mid- and high-dose animals. Decreased body weight gain (by 35%) was observed in the high-dose group. Increased incidence (significant) of post-implantation losses (due to both early and late resorptions), decreased number of viable foetuses per dam, and adactyly (absence of digits) and/or shortened digits (in 19 foetuses from 3/15 litters) was noted in the high-dose group. There were no changes observed in developmental parameters in the mid-dose group, and no effects on dams or foetuses in the low-dose group. According to WHO (1996), the NOAEL for teratogenic effects was 500 mg/kg bw/day, which was also a slightly maternally toxic dose.

In another developmental toxicity study, less well-conducted according to EU RAR (1999), pregnant Dutch Belted rabbits (16/group) were treated by gavage with 0, 10, 30 or 100 mg/kg bw/day of a C_{10-13}, 58% in corn oil from day 6 to 27 of gestation (Unpublished study 1982 – quoted from EU RAR 1999, WHO 1996). No maternal deaths occurred and no signs of toxicity were noted in any of the dams. Whole litter resorptions occurred in 2/14 high-dose dams and in 1/15 mid-dose dams; no other effects were noted. According to WHO (1986), the NOAEL was 100 mg/kg bw/day.

4.5.2 MCCPs

In a range-finding study for a 2-generation study (which, according to EU RAR 2005 was not then conducted), Wistar rats (5 males and 10 females per group) were administered a $C_{14\text{-}17}$, 52% at concentrations of 0, 100, 1000, or 6250 mg/kg diet for 28 days prior to mating, during mating and up to post-natal day 21 (females only) (Unpublished study 1985 – quoted from EU RAR 2005, WHO 1996). The average doses received were 0/0, 6/8, 62/74, or 384/463 mg/kg bw/day for males/females, respectively. Five male and 10 female F_1 pups were selected from each group and were retained, on the same diet as their parents from weaning, for up to 70 days of age. No deaths occurred amongst the parental F_0 generation, and there were no abnormalities noted in the histopathological examination (females only). Food consumption was significantly decreased (by 12%) in high-dose females during week 5 only. No treatment-related effects on fertility indices were observed. F_1 survival at birth in treated groups was comparable to that of the control group, whereas a marked and significant decrease in pup survival was noted in the high-dose group during lactation, such that none of the pups survived until weaning. Reduced pup survival (by 12%) was also evident in the mid-dose group, although not significantly different from that in the control group. Decreased activity and swollen and dark or black eye(s) were noted in a few F_1 pups in 1 or 2 mid- and high-dose litters. Haematological analyses revealed decreased erythrocyte counts, haemoglobin concentration and haematocrit among a single litter of high-dose F_1 pups on lactation day 6. Necropsy of pups revealed dose-related, but significant, increases amongst mid- and high-dose F_1 pups in the occurrence and severity of subcutaneous haematoma, pallor, blood around the orifices, pale liver, kidneys, lungs and spleen, and blood in the cranial cavity and brain. Haematoma was noted in all high-dose litters. According to WHO (1996), the pup weights were lower in the low- and mid-dose groups than in the control group on lactation day 21, although not achieving statistical significance in the low-dose group. According to EU RAR (2005), the NOAEL for developmental effects was 8 mg/kg bw/day. According to WHO (1996), the LOAEL was 6/8 mg/kg bw/day for males/females, respectively, in the F_1 generation based on decreased pup weight.

In a developmental toxicity study, pregnant Charles River COBS CD rats (25/group) were treated by gavage with 0, 500, 2000, or 5000 mg/kg bw/day of a $C_{14\text{-}17}$, 52% in corn oil from day 6 to 19 of gestation (Unpublished study 1984 – quoted from EU RAR 2005, WHO 1996). No adverse effects on mortality, body weight gain, or uterine weight of dams were observed. Clinical signs of toxicity (wet and/or matted fur in the ano-genital region with red or yellow staining, increased incidence of soft stool prior to sacrifice) were observed in mid- and high-dose animals. There were no changes observed in developmental parameters, including malformations at dose levels up to 5000 mg/kg bw/day.

In another developmental toxicity study, pregnant Dutch Belted rabbits (16/group) were treated by gavage with 0, 10, 30 or 100 mg/kg bw/day of a $C_{14\text{-}17}$, 52% in corn oil from day 6 to 27 of gestation (Unpublished study 1983 – quoted from EU RAR 2005, WHO 1996). No maternal deaths occurred and no signs of toxicity were noted in any of the dams. Abortions occurred in the control group (1 dam), mid-dose group (2 dams), and in the high-dose group (2 dams). There were no changes observed in developmental parameters, including malformations.

4.5.3 LCCPs

No studies specifically investigating effects on fertility have been located.

In developmental toxicity studies with rats, pregnant Charles River COBS CD rats (25/group) were treated by gavage with 0, 500, 2000 or 5000 mg/kg bw/day of a C_{22-26}, 43% or a C_{22-26}, 70% from day 6 to 19 of gestation (IRDC 1983,1984 – quoted from WHO 1996). No maternal effects and no signs of developmental effects were observed.

In developmental toxicity studies with rabbits, Pregnant Dutch Belted rabbits (16/group) were treated by gavage with 0, 500, 2000 or 5000 mg/kg bw/day of a C_{22-26}, 43% or with 0, 100, 300 or 1000 mg/kg bw/day of a C_{22-26}, 70% from day 6 to 27 of gestation (IRDC 1982,1983 – quoted from WHO 1996).
C_{22-26}, 43%: No maternal effects were noted. In the high-dose group, there was a slight increase in mean implantation loss and a slight decrease in the mean number of viable foetuses; the changes were not statistically significant when compared with the control group. No other developmental effects were noted, and no teratogenic effects.
C_{22-26},70%: No maternal effects and no signs of developmental effects were observed.

4.6 Mutagenic and genotoxic effects

4.6.1 SCCPs

SCCPs have been tested in a number of bacterial studies in the absence or presence of a metabolic activation system, predominantly Aroclor-induced rat liver S9:
A C_{12}, 57% did not produce an increase in revertants in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, or in *Escherichia coli* WP2uvrA, at concentrations up to 5000 µg/plate (Unpublished study 1988 – quoted from EU RAR 1999, WHO 1996). Negative results were also obtained in *Salmonella typhimurium* strains TA 97, TA 98, TA 100 and TA 1535 with a C_{12}, 60%, at concentrations up to 3333 µg/plate (NTP 1986 – quoted from EU RAR 1999); and in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1538 with a C_{10-13}, 50%, at concentrations up to 2500 µg/plate (Birtley et al. 1980 – quoted from EU RAR 1999, WHO 1996).
One study has reported positive findings (around a two-fold increase in revertants) with a C_{10-13}, 50% containing 1% epoxy stabiliser in *Salmonella typhimurium* strains TA 98 (without activation) and TA 100 (with and without activation); negative results were observed in *Salmonella typhimurium* strains TA 1535, TA 1537 and TA 1538, and in *Escherichia coli* WP2uvrA, at concentrations up to 10000 µg/plate (Unpublished study 1986 – quoted from EU RAR 1999, WHO 1996).

According to EU RAR (1999), no standard cytogenetics studies in mammalian cells are available, but a well-conducted gene mutation (HPRT locus) study, performed to modern protocols was available: When tested up to cytotoxic concentrations, a C_{10-13}, 56% did not induce a significant, reproducible increase in the number of mutant colonies in Chinese hamster V79 cells, with or without metabolic activation (Unpublished study 1986 – quoted from EU RAR 1999, WHO 1996).

A C_{12}, 60% has been reported to be mutagenic in L5178Y mouse lymphoma cells at concentrations of 48 and 60 µg/ml in the absence of metabolic activation (Myhr et al. 1990 – quoted from WHO 1996).

In a cell transformation assay using baby hamster kidney cells (BHK21/C13), cells were treated with a C_{10-13}, 50% in the absence of metabolic activation up to toxic concentrations (up to 2500 µg/ml); there was no evidence of an increase in cell transformation frequency (Birtley et al. 1980 – quoted from EU RAR 1999, WHO 1996). In contrast, increases in transformation frequency were observed when cells were treated with a C_{12}, 58% with and without metabolic activation, at both cytotoxic and non-toxic concentrations (Unpublished study 1982 – quoted from EU RAR 1999, WHO 1996).

When a C_{12}, 60% was administrated to male rats (4/group) by gavage at 0, 500, 1000, or 2000 mg/kg bw, no effects on unscheduled DNA synthesis (UDS) in hepatocytes could be detected at 2 or 12 hours after administration (Ashby et al. 1990 – quoted from WHO 1996).

In a rat bone-marrow cell chromosomal aberration study, male F344 rats (8/group) were given 0, 250, 750, or 2500 mg/kg bw/day of a C_{10-12}, 58%, by gavage, daily for five days. Reduced body weight was observed in mid-dose animals, and 7 high-dose animals died. There was no increase in the frequency of chromosomal aberrations, excluding gaps, in bone marrow samples taken at day 6. Cytotoxicity was not assessed, but toxicokinetic data indicate distribution to the bone-marrow to be likely. (Unpublished study 1982 – quoted from EU RAR 1999, WHO 1996).

In a micronucleus test, NMRI mice (5/sex/group) were given single doses of 50 or 5000 mg/kg bw of a C_{10-13}, 58% by gavage. There were no differences from control values either in polychromatic cells with micronuclei or in the ratio of polychromatic erythrocytes to normocytes when examined at 24, 48 and 72 hours after administration (high-dose), or 24 hours after administration (low dose). (Hoechst 1989 – quoted from WHO 1996).

In a germ cell mutagenicity study, dominant lethality was assessed in male rats (15/group) treated with 0, 250, 750, or 2500 mg/kg bw/day of a C_{10-12}, 58%, by gavage, daily for five days. Each male was then mated with 20 untreated females. During treatment, high-dose males showed a slight decrease in body weight, and mid-dose males a slight decrease in body weight gain. There was no evidence of a mutagenic effect on the post-meiotic stage of spermatogenesis at any dose level, as shown by the absence of effect on the mean number of viable embryos during the first four weeks of mating. (Unpublished study 1983 – quoted from EU RAR 1999, WHO 1996).

4.6.2 MCCPs

MCCPs have been tested in a number of bacterial studies in the absence or presence of a metabolic activation system, predominantly Aroclor-induced rat liver S9:
A C_{14-17}, 40% gave negative results in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, at concentrations up to 5000 µg/plate (Wiegand 1989 – quoted from EU RAR 2005). Negative results have also been reported with a C_{14-17}, 42% in *Salmonella typhimurium* strains TA 98, TA 199, TA 1535, TA 1537 and TA 1538, at concentrations up to 5000 µg/plate (Conz & Fumero 1988 – quoted from EU RAR 2005, WHO 1996), and with a C_{14-17}, 45% in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, at concentrations up to 5000 µg/plate (Elliott 1989 – quoted from EU RAR 2005, WHO 1996).
When two C_{14-17}, 52% (with or without 0.2% epoxidised vegetable oil stabiliser) were tested in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA

1538 at concentrations from 4 to 2500 mg/plate, a greater than 2-fold increase in the number of revertants was observed at 4 and 20 mg/plate MCCP without stabiliser; no increases were seen under any other test conditions (Birtley et al. 1980 – quoted from EU RAR 2005, WHO 1996).

There was no evidence of increased cell transformation frequency in baby hamster kidney cells (BHK21/C13) treated with a C_{14-17}, 52% in the absence of metabolic activation at concentrations up to 2500 µg/ml (Birtley et al. 1980 – quoted from WHO 1996).

In a rat bone-marrow cell chromosomal aberration study, male F344 rats (8/group) were given 0, 500, 1500, or 5000 mg/kg bw/day of a C_{14-17}, 52%, by gavage, daily for five days. No deaths occurred and no signs of toxicity were noted. There was no increase in the frequency of chromosomal aberrations (including and excluding gaps) in bone marrow samples taken at day 6. Cytotoxicity was not assessed, but toxicokinetic data indicate distribution to the bone-marrow to be likely. (Unpublished study 1983 – quoted from EU RAR 2005, WHO 1996).

In a bone marrow micronucleus test, mice (5/sex) were given a single dose of 5000 mg/kg bw of a C_{14-17}, 42% by gavage. No increase in the frequency of micronuclei occurred when examined at 18, 43 and 66 hours post-application. (Conz & Fumero 1988 – quoted from EU RAR 2005, WHO 1996).
Similarly, there were no increases in micronucleus formation in a second bone marrow micronucleus test in mice (5/sex) given a single dose of 0, 3125, or 5000 mg/kg bw of a C_{14-17}, 45% by gavage when examined at 24, 48 and 72 hours after administration (high-dose), or 24 hours after administration (low dose) (Elliott 1989 – quoted from EU RAR 2005, WHO 1996).

4.6.3 LCCPs

Negative results have been reported in bacterial studies in the absence or presence of a metabolic activation system, predominantly Aroclor-induced rat liver S9, with a C_{20-30}, 42% in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1538, at concentrations up to 2500 µg/plate (Birtley et al. 1980 – quoted from WHO 1996), and with a C_{23}, 43% in *Salmonella typhimurium* strains TA 97, TA 98, TA 100 and TA 1535, at concentrations up to 10000 µg/plate (NTP 1986 – quoted from WHO 1996).

A C_{23}, 43% induced chromosome aberrations in Chinese hamster ovary cells *in vitro* in the absence of metabolic activation at a concentration of 5000 µg/ml, but not at lower concentrations, and not at concentrations up to 5000 µg/ml in the presence of metabolic activation. Sister chromatid exchange was induced in Chinese hamster ovary cells *in vitro* with and without metabolic activation at all concentrations (5, 500, 1700 and 5000 µg/ml). (Anderson et al. 1990 – quoted from WHO 1996).

There was no evidence of increased cell transformation frequency in baby hamster kidney cells (BHK21/C13) treated with a C_{20-30}, 42% at concentrations up to 2500 µg/ml (Birtley et al. 1980 – quoted from WHO 1996). In contrast, increases in transformation frequency were observed when cells were treated with a C_{20-28}, 70% or a C_{22-28}, 70% with and without metabolic activation, at both cytotoxic and non-toxic concentrations (Unpublished studies 1982 – quoted from WHO 1996).

When a C_{22-26}, 43% or a C_{20-30}, 70% was administered by gavage to F344 rats (8/group) at doses of 0, 500, 1500, or 5000 mg/kg bw/day daily for five days, no

increased frequency of chromosome aberrations in bone marrow cells was observed. Body weight gain was decreased in the high-dose C_{20-30}, 70% group, whereas no signs of toxicity were observed with C_{22-26}, 43%. (IRDC 1983 – quoted from WHO 1996).

4.7 Carcinogenic effects

Studies with oral administration of chlorinated paraffins to experimental animals have demonstrated toxicologically significant, dose-related increases in the incidence of several tumour types. Probable underlying modes of actions involved are addressed in section 2.3 and the relevance to humans of the carcinogenic effects observed in experimental animals will be discussed in section 6.7 taken the mechanistic data into account.

No studies with inhalation of or dermal contact to chlorinated paraffins have been located.

4.7.1 SCCPs

Rat, 104-week gavage study, C_{12}, 60% (NTP 1986 – quoted from EU RAR 1999, WHO 1996, IARC 1990). SCCP was administered by gavage in corn oil 5 days/week at doses of 0, 312 or 625 mg/kg bw/day to F344 rats (50/sex/group). Survival was reduced in low- and high-dose males and in low-dose females (control: 27/34; low-dose: 6/23; high-dose: 3/29 for males/females, respectively). Significantly increased incidences were observed for tumours in the liver, kidney and thyroid, see Table 4.7.1.1. Treated males also showed slightly increased incidences of squamous cell papillomas in the forestomach (control: 0/50; low-dose: 0/50; high-dose: 3/49), pancreatic acinar cell carcinomas (control: 0/50; low-dose: 0/50; high-dose: 2/49), and pancreatic acinar adenomas (control: 11/50; low-dose: 22/50; high-dose: 15/49). Non-neoplastic lesions are addressed in section 4.4.1.

Table 4.7.1.1 Incidence of tumours in rats administered SCCPs

	Males			Females		
Dose (mg/kg bw/day)	Control	312	625	Control	312	625
Hepatocellular neoplastic nodules	0/50	10/50[a]	16/48[a]	0/50	4/50	7/50[a]
Hepatocellular carcinomas	0/50	3/50[a]	2/48	0/50	1/50	1/50
Hepatocellular neoplastic nodules and carcinomas	0/50	13/50[a]	16/48[a]	0/50	5/50[a]	7/50[a]
Thyroid follicular cell adenomas and carcinomas	3/50	3/50	3/50	0/50	6/50[a]	6/50[a]
Mononuclear cell leukaemia	7/50	12/50[b]	14/50[b]	11/50	22/50[b]	16/50
Renal tubular-cell adenomas or adenocarcinomas	0/50	9/50[b]	3/49	0/50	0/50	0/50

a: Incidental tumour test for trend, $p < 0.05$, increase relative to control (WHO 1996)
b: Life table analysis, $p < 0.05$, increase relative to control (WHO 1996)

Mouse, 104-week gavage study, C_{12}, 60% (NTP 1986 – quoted from EU RAR 1999, WHO 1996, IARC 1990). SCCP was administered by gavage in corn oil 5 days/week at doses of 0, 125 or 250 mg/kg bw/day to B6C3F1 mice (50/sex/group). Survival of high-dose females was reduced after week 100

(control: 34/35; low-dose: 30/31; high-dose: 30/25 for males/females, respectively). Significantly increased incidences were observed for tumours in the liver and thyroid, see Table 4.7.1.2. The increased incidence of Harderian gland carcinomas in females was significant, but not dose-related; no effects were seen in males. The incidences of alveolar/bronchiolar carcinomas were increased significantly in high-dose males, and the trend with dose was also significant; an increase in adenomas was not observed and there were no increases in lung tumour incidence in females. Non-neoplastic lesions are addressed in section 4.7.1.

Table 4.7.1.1 Incidence of tumours in mice administered SCCPs

	Males			Females		
Dose (mg/kg bw/day)	Control	125	250	Control	125	250
Hepatocellular adenomas	11/50	20/50[a]	29/50[a]	0/50	18/50[a]	22/50[a]
Hepatocellular carcinomas	11/50	15/50	17/50	3/50	4/50	9/50[b]
Hepatocellular adenomas and carcinomas	20/50	34/50[a]	38/50[a]	3/50	22/50[a]	28/50[a]
Thyroid follicular cell adenomas and carcinomas	3/49	4/50	3/49	8/50	12/49[a]	15/49[a]
Harderian gland carcinomas	-	-	-	1/50	6/50	2/50
Alveolar/bronchiolar carcinomas	0/50	3/50[a]	6/50[a]	-	-	-

a: Incidental tumour test for trend, $p < 0.05$, increase relative to control (WHO 1996)
b: Life table analysis, $p < 0.05$, increase relative to control (WHO 1996)

The EU Commission Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity, at their meeting in June 1997, considered the NTP cancer studies to be of poor quality and that no significance should be attributed to the slight excess of tumours seen in the lung, pancreas, stomach, leukaemia or Harderian gland. They agree that of the tumours observed, only those in the liver, thyroid and kidney should be considered significant. Mechanisms for two of these had been suggested: peroxisome proliferation for the liver tumours, and hormonal imbalance for the thyroid; these mechanisms were accepted by the Specialised Experts. Regarding kidney tumours, the Specialised Experts considered that no plausible mechanism has been suggested as studies have failed to show significant levels of α2μglobulin in the kidneys of male rats. Other evidence had shown that there was chronic nephropathy, which might be a contributing factor in the tumour development. The Specialised Experts considered that there was still insufficient evidence to conclude a male rat specific event and that the consequences for humans could not be ruled out.

4.7.2 MCCPs

No data have been located.

4.7.3 LCCPs

Rat, 103-week gavage study, C_{23}, 43% (NTP 1986 – quoted from WHO 1996, IARC 1990). LCCP was administered by gavage in corn oil 5 days/week at doses of 0, 1875 or 3750 mg/kg bw/day to male F344 rats and of 0, 100, 300 or 900 mg/kg bw/day to female F344 rats (50/sex/group). Survival was not affected. In female rats, an increased incidence of adrenal gland medullary phaeochromocytomas was observed (1/50, 4/50, 6/50, 7/50); the increase was significant at the highest dose level, and there was also a significant positive trend.

An increased incidence of endometrial stromal polyps in the uterus was also observed (9/50, 17/50, 10/50, 10/50); the increase was significant at the lowest dose level only, and the increase was not dose-related. In males, acinar cell tumours of the pancreas occurred with a negative trend. The incidence of benign hepatocellular neoplasia was not increased in treated rats. Non-neoplastic lesions are addressed in section 4.7.3.

Mouse, 103-week gavage study, C_{23}, 43% (NTP 1986 – quoted from WHO 1996, IARC 1990). LCCP was administered by gavage in corn oil 5 days/week at doses of 0, 2500 or 5000 mg/kg bw/day to B6C3F1 mice. Survival was not significantly different between treated and control groups. However, a *Klebsiella* infection affected the animals after week 65, and 60-70% of the early deaths in each group were attributed to the infection. The incidence of malignant lymphomas was significantly increased in high-dose males and occurred with a positive trend (6/50, 12/50, 16/50). The incidences of hepatocellular carcinomas in females occurred with a positive trend, but the increase was not significant (1/50, 1/49, 6/50), and the incidences of adenomas and carcinomas of the liver (combined) were marginally increased in females (4/50, 3/49, 10/50). Thyroid follicular cell carcinomas in males occurred with a positive trend (0/49, 0/48, 3/49); however, the incidence of follicular cell adenomas or carcinomas (combined) was not significantly greater than that of the controls (1/49, 3/48, 5/49). Non-neoplastic lesions are addressed in section 4.7.3.

5 Regulations

5.1 Ambient air

Denmark (C-value): -

WHO: -

US-EPA:

5.2 Drinking water

Denmark: -

WHO: -

US-EPA:

5.3 Soil

Denmark: -

The Netherlands: -

5.4 Occupational Exposure Limits

Denmark: -

ACGIH: -

Germany: -

5.5 Classification

SCCPs are classified for carcinogenic effects (C;R40 – possible risks of irreversible effects) and for environmental effects (N;R50/53 – very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment) (MM 2002).

MCCPs and LCCPs are currently not classified.

For MCCPs, the following classification has been proposed: R64 – may cause harm to breast-fed babies, R66 – repeated exposure may cause skin dryness or cracking (EU RAR 2005).

5.6 IARC

SCCPs: Chlorinated paraffins of average carbon-chain length C_{12} and average degree of chlorination approximately 60% are possibly carcinogenic to humans (Group 2B). There is sufficient evidence for the carcinogenicity of a commercial chlorinated paraffin product of average carbon-chain length C_{12} and average degree of chlorination 60% in experimental animals; no data were available from studies in humans on the carcinogenicity of chlorinated paraffins. (IARC 1990).

LCCPs: There is limited evidence for the carcinogenicity of a commercial chlorinated paraffin product of average carbon-chain length C_{23} and average degree of chlorination 43% in experimental animals; no data were available from studies in humans on the carcinogenicity of chlorinated paraffins. (IARC 1990).

5.7 US-EPA

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6 Summary and evaluation

6.1 Description

Commercial chlorinated paraffins are very complex mixtures of *n*-alkanes characterised by an average carbon chain length and chlorination degree. The commercially available chlorinated paraffins are usually subdivided into three types depending on chain length: 1) Short-chain chlorinated paraffins (SCCPs, C_{10-13}), 2) medium-chain chlorinated paraffins (MCCPs, C_{14-17}), and 3) long-chain chlorinated paraffins (LCCPs, C_{18-30}).

Chlorinated paraffins are liquids (SCCPs, MCCPs, most LCCPs) or solids depending on carbon chain length and chlorine content. They have very low vapour pressures, are practically insoluble in water, and have high octanol-water partition coefficients

6.2 Environment

Chlorinated paraffins are not known to occur naturally but are released into the environment due to manufacture and uses, They have been detected at low levels in atmospheric air (SCCPs: 1 x 10^{-9}-1 x 10^{-5} mg/m^3), surface water close to point sources (SCCPs: < 0.1 µg/l; MCCPs: 0.4-4 µg/l; LCCPs: < 0.5-2 µg/l) and sediment close to point sources (SCCPs: up to 24.2 mg/kg wet weight; MCCPs: 1-25 mg/kg wet weight; LCCPs: up to 3.2 mg/kg wet weight), agricultural soil receiving sewage sludge containing chlorinated paraffins (generally below 0.088 mg/kg wet weight), and in some food items (up to 300 µg/kg), and in breast milk (median concentration up to 180 µg/kg lipid).

In the EU Risk Assessment Reports, environmental concentrations (Predicted Environmental Concentrations - PECs) are generally estimated by the use of EUSES for three different spatial scales: local, regional and continental. Regional PECs, representing average concentrations due to all releases in a larger area as e.g., DK, have been estimated for SCCPs and MCCPs in atmospheric air, surface water, sediment, soil, and various food items (fish, root crops, leaf crops, meat, milk, and drinking water), see Table 6.2.1.

Table 6.2.1 Regional PECs in the environment

	SCCPs	MCCPs
Atmospheric air	5.3 x 10^{-7}-1.3 x 10^{-6} mg/m^3	3.35 x 10^{-6} mg/m^3
Surface water	0.012-0.027 µg/l	0.389-0.745 µg/l
Sediment	0.09-0.21 mg/kg wet weight	8.8-16.9 mg/kg wet weight
Agricultural soil	0.54-1.41 mg/kg wet weight	53.8-55.8 mg/kg wet weight
Natural soil	0.0011-0.0025 mg/kg wet weight	1.85-2.01 mg/kg wet weight
Industrial soil	1.53-3.04 mg/kg wet weight	147-173 mg/kg wet weight
Fish	0.092-0.21 mg/kg	0.42 mg/kg
Root crops	1.09-2.87 mg/kg	309 mg/kg
Leaf crops	0.0005-0.0011 mg/kg	0.02 mg/kg
Meat	0.0073-0.019 mg/kg	1.99 mg/kg
Milk	0.0023-0.006 mg/kg	0.63 mg/kg
Drinking water	0.2-0.4 µg/l	4.9 µg/l

Chlorinated paraffins adsorb strongly to particulate matter and are expected to be relatively immobile in soil and would not be expected to leach from soil into groundwater. The half-lives for atmospheric degradation by reaction with hydroxyl radicals in air have been estimated to range from 0.85 to 7.2 days. Chlorinated paraffins are not readily biodegradable.
Chlorinated paraffins are bioaccumulated in aquatic organisms. In fish, SCCPs are accumulated to a higher degree than MCCPs and LCCPs with a BCF of 7816 l/kg and of 1087 l/kg for SCCPs and MCCPs, respectively. Uptake into fish via food has also been shown to occur, with accumulation factors for SCCPs of up to 1-2 on a lipid basis, and for MCCPs of 1-3.

6.3 Human exposure

The general population can be exposed to chlorinated paraffins indirectly via the environment and via consumer products.

In the EU Risk Assessment Reports, the total daily human intakes of SCCPs and MCCPs from the environment have been estimated based on local PECs and on regional PECs, see Table 6.3.1. Root crops were predicted to be the major source of human uptake for both SCCPs and for MCCPs. For SCCPs, a total daily human intake of 0.02 mg/kg bw/day has been considered as a reasonable worst case prediction based upon measured data and has been used for the risk characterisation for man exposed indirectly via the environment. For MCCPs, a total daily human intake of 2.10 mg/kg bw/day has been used for the risk characterisation for man exposed indirectly via the environment from local exposure, and of 1.71 mg/kg bw/day from regional exposure.

For MCCPs, the daily uptake from breast milk for the first 3 months of infant life has been estimated to 30.5 x 10^{-5} mg/kg b.w./day (EU RAR 2005).

According to the EU Risk Assessment Reports, chlorinated paraffins are found in a number of consumer products, including leather clothing, metal working fluids and textiles, in certain paints, sealants and adhesives, and in plastic and rubber products. Aside from the wearing of leather clothing and the use of metal working fluids, the consumer exposures are considered to be negligible. The total systemic dose for use of metal working fluids has been estimated to 0.03 mg/kg bw/day for SCCPs and to 0.008 mg/kg bw/day for MCCPs, and for wearing of leather clothing to 0.02 mg/kg bw/day for SCCPs and to 0.00016 mg/kg bw/day for MCCPs.

Table 6.3.1 Estimated total daily human intake based on local and regional PECs

	SCCPs	MCCPs
Local PECs	0.00099 to 0.20 mg/kg bw/day	0.0007 to 2.10 mg/kg bw/day
Regional PECs	0.0062 to 0.016 mg/kg bw/day	1.71 mg/kg bw/day

6.4 Toxicokinetics

Chlorinated paraffins are absorbed following oral administration to rats and mice and studies in mice indicate that the absorption of SCCPs and MCCPs may be as high as up 60-70%.

One study with LCCPs using dermal application to rats indicated a very low degree (< 1%) of dermal absorption. *In vitro* studies with SCCPs and MCCPs showed less than 0.01% absorption through human epidermal skin for SCCPs and no absorption for MCCPs.

Following absorption, chlorinated paraffins were initially distributed to tissues of high metabolic activity and/or high rate of cell proliferation (e.g., intestinal mucosa, bone marrow, thymus, and liver) with subsequent re-distribution to fatty tissue. An elimination half-life of approximately 2-5 days was estimated for the liver and kidney, and about 2 weeks for white adipose tissue following a single gavage dose to rats. Following repeated dietary administration to rats, the half-life for elimination from the abdominal fat was estimated as approximately 8 weeks. Distribution to the brain has been demonstrated in pre-weaning mice administered a single gavage dose. Chlorinated paraffins are transferred to the developing foetus *in utero* and also to the offspring via maternal milk.

One study in mice indicated that metabolism of chlorinated paraffins involves cytochrome P450, possibly by a de-chlorination reaction followed by β-oxidation and incorporation of the carbon chain into cellular metabolism yielding carbon dioxide. Another study in rats indicated conjugation with glutathione.

Excretion of chlorinated paraffins and/or their metabolites occurs via the faeces and via exhaled air, and to a lower extent in the urine. Studies with intravenous injection and one study in bile-duct cannulated rats have shown that chlorinated paraffins and their metabolites are excreted via the bile.

6.5 Human toxicity

No data have been located regarding single dose toxicity, eye irritation, respiratory sensitisation, repeated dose toxicity, toxicity to reproduction, genotoxic effects, or carcinogenic effects.

Mild erythema and dryness have been reported in volunteers following skin application of SCCPs (C_{10-13}, 50% or 63%) under occlusive dressing. No local irritation or allergic reactions were reported when an SCCP (C_{12}, 59%) or LCCPs (C_{20-30}, 40-41%, C_{24}, 40% or C_{24}, 70%) were applied to the skin of volunteers. No positive reactions were observed among exposed employees patch tested with various constituents of cutting fluid coolants, including chlorinated paraffins.

6.6 Animal toxicity

6.6.1 Single dose toxicity

No signs of toxicity were observed in rats exposed to an SCCP (C_{12}, 59%) by inhalation at 3300 mg/m^3 for 1 hour.

No deaths have been observed in rats or mice following single oral doses of SCCPs or LCCPs up to around 13 or 27 g/kg bw (rats or mice, respectively); in rats at doses of up to 15 g/kg bw MCCPs; in rats at doses of up to 17.7 or 50 g/kg bw LCCPs (C_{24}, 40% or C_{24}, 70%, respectively), or in guinea-pigs at doses of up to 25 g/kg bw LCCPs. Signs of toxicity observed included piloerection, ataxia, lethargy, urinary incontinence, and diarrhoea (one study with rats).

No signs of systemic toxicity were observed in rats dermally applied 2.8 g/kg bw of an SCCP; slight erythema and slight desquamation were noted on days 3 and 7, respectively. The LD_{50}-value for dermal exposure of rabbits to an SCCP has been reported to be above 13 g/kg bw.

6.6.2 Irritation

Slight skin irritation has been observed in rats and rabbits following a single application of either SCCPs or MCCPs. In rats, signs of skin irritation, as well as desquamation, were also observed following repeated applications of SCCPs or MCCPs, whereas no signs of irritation were observed following repeated applications of LCCPs.

Slight eye irritation has been observed in rabbits following instillation of either SCCPs or MCCPs into the conjunctival sac, whereas no signs of irritation were observed following instillation of LCCPs.

6.6.3 Sensitisation

Erythema has been noted in few animals following challenge with SCCPs or MCCPs in Guinea-Pig Maximisation Tests. In an ear-flank test, slight erythema was observed following challenge with SCCPs.

6.6.4 Repeated dose toxicity

Studies with oral administration of chlorinated paraffins to experimental animals have demonstrated that the liver, kidneys and thyroid are the target organs for the toxicity of chlorinated paraffins. The available studies on SCCPs are summarised in Table 4.4.1 (section 4.4.1), on MCCPs in Table 4.4.2 (section 4.4.2), and on LCCPs in Table 4.4.3 (section 4.4.3).

Effects in the liver have been observed in 14-day, 13-week and 2-year studies and include increased absolute and relative weights, enzyme induction, and histopathological changes (centrilobular hepatocellular hypertrophy, necrosis, focal cellular changes, gross dilation of the blood vessels, proliferation of smooth endoplasmic reticulum, and peroxisome proliferation).
For SCCPs, effects in the liver have been observed at dose levels from 100 and 125 mg/kg bw/day in rats and mice, respectively; alterations in enzyme activities and/or levels were noted in rats at dose levels from 300 mg/kg bw/day (14-day gavage; 13-week gavage, dietary). The NOAEL for liver effects (increased weight, histopathology) in rats was 30 mg/kg bw/day (increased weight only) in a 14-day study (gavage) and 10 mg/kg bw/day in two 13-week studies (dietary, gavage – Unpublished studies 1984); increased weight and histopathology was observed at the lowest dose level (312 mg/kg bw/day) in the 2-year rat study (gavage). The NOAEL for liver effects (increased weight, histopathology) in mice was 125 mg/kg bw/day in a 13-week study (gavage); effects in the liver were not observed in the 2-year study (gavage) at 125 or 250 mg/kg bw/day.
For MCCPs, effects in the liver have been observed at dose levels from around 30 mg/kg bw/day in rats and dogs. The NOAEL for liver effects in rats (increased weight, histopathology) was 4 mg/kg bw/day in a 13-week dietary study (Poon et al. 1995) and 23.0 mg/kg bw/day in another, very recent, 13-week dietary study (Unpublished study 2005), and in dogs (histopathology) 10 mg/kg bw/day in a 13-week dietary study.

For LCCPs, effects in the liver have been observed at dose levels from 100 and 1875 mg/kg bw/day in female and male rats, respectively. The NOAEL for liver effects in male rats (increased weight, histopathology) was 900 mg/kg bw/day in a 13-week gavage study (IRCD 1984); no NOAEL can be established for liver effects in female rats as 100 mg/kg bw/day was the lowest dose level used in the 13-week (IRCD 1984) and 2-year studies (NTP 1986). No effects have been reported in studies (13-week and 2-year gavage) with mice.

Effects observed in the kidneys include increased absolute and relative weights, and histopathological changes (males: some deposition of α-2μglobulin in proximal convoluted tubules, nephritis, cysts in the cortex, nephropathy, and tubular cell hyperplasia; females: brown pigmentation, inner medullary tubular dilation, and nephropathy).
For SCCPs, effects in the kidneys have been observed at dose levels from 100 and 250 mg/kg bw/day in rats and mice, respectively. The NOAEL for kidney effects (increased weight, histopathology) in rats was 10 mg/kg bw/day in two 13-week studies (dietary, gavage – Unpublished studies 1984); increased weight and histopathology was observed at the lowest dose level (312 mg/kg bw/day) in the 2-year rat study (gavage). The NOAEL for kidney effects (histopathology) in mice was 125 mg/kg bw/day in a 2-year study (gavage); effects in the kidney were not reported in a 13-week study (gavage) at dose levels from 125 mg/kg bw/day.
For MCCPs, effects in the kidneys have been observed at dose levels from 4 mg/kg bw/day in rats; the NOAEL (histopathology) was 0.4 mg/kg bw/day in a 13-week dietary study (Poon et al. 1995), whereas no changes were observed in another, very recent, 13-week dietary study (Unpublished study 2005) at dose levels up to 222/242 mg/kg bw/day (highest dose level in the study). Effects in the kidney were not reported in a 13-week study (dietary) in dogs.
For LCCPs, effects in the kidney have been observed in a 13-week gavage study (IRCD 1984) at a dose level of 3750 mg/kg bw/day; the NOAEL (histopathology) was 900 mg/kg bw/day. No effects have been reported in the 2-year gavage study with rats (NTP 1986) or in 13-week and 2-year gavage studies with mice.

Effects observed in the thyroid include increased absolute and relative weights, alterations in T3, T4 and TSH levels, and histopathological changes (follicular cell hypertrophy, hyperplasia).
For SCCPs, effects in the thyroid have been observed at dose levels from 100 and 125 mg/kg bw/day in rats and mice, respectively. The NOAEL for thyroid effects (histopathology) in rats was 10 mg/kg bw/day in two 13-week studies (dietary, gavage – Unpublished studies 1984); effects in the thyroid were not reported in the 2-year rat study (gavage). The LOAEL for thyroid effects (histopathology) in mice was 125 mg/kg bw/day(lowest dose level in the study) in a 2-year study (gavage); effects in the thyroid were not observed in the 13-week study (gavage) at dose levels from 125 mg/kg bw/day.
For MCCPs, effects in the thyroid have been observed at dose levels from 4 mg/kg bw/day in rats; the NOAEL (histopathology) was 0.4 mg/kg bw/day in a 13-week dietary study (Poon et al. 1995). Decreased T3 (males) and increased TSH (females) has been reported in a very recent 13-week dietary study (Unpublished study 2005) in rats at 23 mg/kg bw/day, and increased T4 (females) and TSH (both sexes) at 222/242 mg/kg bw/day, whereas no histopathological changes or weight changes were observed at dose levels of up to 222/242 mg/kg bw/day (highest dose level in the study). Effects in the thyroid were not reported in a 13-week study (dietary) in dogs.
For LCCPs, no effects have been reported in studies (13-week and 2-year – IRCD 1984, NTP 1986) with rats and mice.

6.6.5 Toxicity to reproduction

In a range-finding study to a 2-generation study, MCCPs administered in the diet (0, 6/8, 62/74, 384/463 mg/kg bw/day for males/females, respectively) to rats had no effects on the parental generation, including fertility, up to the highest dose levels. Effects (reduced pup survival during lactation but not at birth, and subcutaneous haematoma, pallor, blood around the orifices, pale liver, kidneys, lungs and spleen, and blood in the cranial cavity and brain) were observed in the developing offspring at maternal dose levels of 74 and 463 mg/kg bw/day; at 463 mg/kg bw/day, all pups died before weaning and haematoma was noted in all of these litters.

SCCPs produced developmental effects (increased incidence of post-implantation loss, decreased number of viable foetuses per dam, and adactyly and/or shortened digits) in rats following administration by gavage of 2000 mg/kg bw/day (highest dose level in the study) on gestation days 6-19; maternal toxicity (deaths, clinical signs of toxicity, decreased body weight gain (by 35%)) was observed at the same dose level; no effects (except mild clinical signs of toxicity) was observed at 500 mg/kg bw/day (mid-dose). The only effect observed in rabbits treated by gavage at dose levels up to 100 mg/kg bw/day (highest dose level in the study) on gestation days 6-27, was a few instances of whole litter resorptions.

In a developmental toxicity study with MCCPs, clinical signs of toxicity were the only effects observed in rats treated by gavage on gestation days 6-19 at dose levels of 2000 and 5000 mg/kg bw/day; there were no changes observed in developmental parameters, including malformations, at dose levels up to 5000 mg/kg bw/day, and no maternal effects at 500 mg/kg bw/day. The only effect observed in rabbits treated by gavage at dose levels up to 100 mg/kg bw/day (highest dose level in the study) on gestation days 6-27, was a few instances of abortions, including in the control group.

In developmental toxicity studies with LCCPs, no maternal effects and no signs of developmental effects were observed in rats treated by gavage with $C_{22\text{-}26}$, 43% or $C_{22\text{-}26}$, 70% on gestation days 6-19 at dose levels up to 5000 mg/kg bw/day (highest dose level in the studies), or in rabbits treated with $C_{22\text{-}26}$, 70% on gestation days 6-27 at dose levels up to 1000 mg/kg bw/day (highest dose level in the study). In rabbits treated with $C_{22\text{-}26}$, 43%, an increase in mean implantation loss and a decrease in the mean number of viable foetuses was observed at 5000 mg/kg bw/day (highest dose level in the study), but not at the lower dose levels (500, 2000 mg/kg bw/day); the alterations were slight and not statistically significantly different from the incidences in the control group.

6.6.6 Mutagenic and genotoxic effects

Chlorinated paraffins showed generally negative results in bacterial studies with a few exceptions. In mammalian cells *in vitro*, SCCPs were reported to be mutagenic in mouse lymphoma cells in the absence of metabolic activation, whereas no effect was observed in Chinese hamster V79 cells. LCCPs have induced chromosome aberrations and sister chromatid exchange in Chinese hamster ovary cells *in vitro*. Chlorinated paraffins have yielded both positive and negative results in cell transformation assays.

Chlorinated paraffins did not induce chromosomal aberrations in rat bone marrow cells following oral administration by gavage for five days. SCCPs and MCCPs did not increase the frequency of micronuclei in bone marrow cells obtained from mice

given a single oral dose. No effects on unscheduled DNA synthesis were noted in hepatocytes of male rats given a single oral dose of SCCPs. No evidence of a mutagenic potential was observed in a germ cell mutagenicity study with rats administered SCCPs by oral gavage for five days.

6.6.7 Carcinogenic effects

Studies with oral administration of chlorinated paraffins to experimental animals have demonstrated significant and dose-related increases in the incidence of several tumour types.
SCCPs: In F344 rats administered 312 or 625 mg/kg bw/day (gavage) for 104 weeks, significantly increased incidences were observed for tumours in the liver (neoplastic nodules and carcinomas, both sexes), kidney (adenomas or carcinomas, males only), thyroid (follicular cell adenomas and carcinomas, females only), and for mononuclear cell leukaemia (both sexes). Survival was significantly reduced in low- and high-dose males and in low-dose females.
In B6C3F1 mice administered 125 or 250 mg/kg bw/day (gavage) for 104 weeks, significantly increased incidences were observed for tumours in the liver (adenomas and carcinomas, both sexes), thyroid (follicular cell adenomas and carcinomas, females only), Harderian gland (carcinomas, females only, not dose-related) and lungs (alveolar/bronchiolar carcinomas, males only).
MCCPs: No studies have been located.
LCCPs: In F344 rats administered up to 3750 or 900 mg/kg bw/day (males/females, respectively, gavage) for 104 weeks, increased incidences were observed in females for adrenal gland medullary phaeochromocytomas (significant at high-dose only) and for endometrial stromal polyps in the uterus (significant at low-dose only, not dose-related). In B6C3F1 mice administered 2500 or 5000 mg/kg bw/day (gavage) for 104 weeks, a significantly increased incidence was observed for malignant lymphomas (high-dose males).

6.7 Evaluation

Chlorinated paraffins are very complex mixtures of *n*-alkanes characterised by an average carbon chain length and chlorination degree. The commercially available chlorinated paraffins are usually subdivided into three types depending on chain length: SCCPs (C_{10-13}), MCCPs (C_{14-17}), and LCCPs (C_{18-30}). The available toxicological studies have been performed with a range of combinations of chain length and degree of chlorination.
Generally, the vapour pressures of chlorinated paraffins are very low and decrease with increasing chain length (see section 1.2) as well as with increasing degree of chlorination. Based on the available toxicological data, no firm conclusions can be drawn with respect to possible differences in toxicity as a result of different chain length and degree of chlorination. However, for many toxicological endpoints, data indicate a similarity, at least in qualitative terms, in the toxicological profile of the tested chlorinated paraffins, and some data indicate a trend of decreased toxicity with increasing chain length for endpoints such as acute toxicity, skin and eye irritation, repeated dose toxicity, toxicity to reproduction, and carcinogenicity. Overall, it seems reasonable to consider that a ‘read-across’ of toxicological data between the tested chlorinated paraffins is valid for the purpose of setting a health based quality criterion in air for chlorinated paraffins.

In general, there is limited information on the toxicokinetics of chlorinated paraffins in experimental animals, and no data for humans.

Chlorinated paraffins are absorbed following oral administration as indicated by excretion of radioactivity in the urine and in the exhaled air following oral administration of radioactively labelled chlorinated paraffins to rats and mice. Absorption is possibly greater for chlorinated paraffins with lower degrees of chlorination and may be as high as up 60-70% for SCCPs and MCCPs. The EU Risk Assessments Reports (EU RAR 1999,2005) have assumed 100% absorption for SCCPs and MCCPs by the oral route of exposure.
Dermal absorption is very low (< 1%) in rats and *in vitro* studies have shown less than 0.01% absorption through human epidermal skin for SCCPs and no absorption for MCCPs. The EU Risk Assessments Reports (EU RAR 1999,2005) have assumed 1% absorption for SCCPs and MCCPs by the dermal route of exposure.
There is no information available on absorption following inhalation of chlorinated paraffins; in the absence of specific information, the EU Risk Assessments Reports (EU RAR 1999,2005) have assumed 100% absorption for SCCPs and MCCPs by the inhalation route of exposure.
Chlorinated paraffins are initially distributed to tissues of high metabolic activity and/or high rate of cell proliferation with subsequent re-distribution to fatty tissue; the elimination half-life from the abdominal fat has been estimated as around 8 weeks. Distribution to the brain has been demonstrated in pre-weaning mice. Chlorinated paraffins are transferred to the developing foetus *in utero* and also to the offspring via maternal milk.
No specific data are available on metabolism but there are some indications of a cytochrome P450 dependent reaction, possibly by de-chlorination followed by β-oxidation and incorporation of the carbon chain into cellular metabolism yielding carbon dioxide. Conjugation with glutathione has also been indicated.
Excretion of chlorinated paraffins and/or their metabolites occurs via the faeces, via exhaled air, and to a lower extent in the urine. Excretion via the bile has also been demonstrated indicating that much of the faecal excretion following oral dosing represented excretion of material previously absorbed rather than material passing through the gastrointestinal tract.

There are no data available on the effects of acute exposure to chlorinated paraffins in humans.
Limited information available from animal studies indicates that SCCPs are of very low acute toxicity following inhalation or dermal contact, with no signs of toxicity observed in rats following 1-hour inhalation exposure at 3300 mg/m^3, and no signs of systemic toxicity following a dermal dose of 2.8 g/kg bw. No data have been located for inhalation exposure or dermal contact to MCCPs or LCCPs.
Chlorinated paraffins (SCCPs, MCCPs, LCCPs) are of low acute oral toxicity with no deaths reported at dose levels below 13 g/kg bw; signs of toxicity observed included piloerection, ataxia, lethargy, urinary incontinence, and diarrhoea (one study with rats). The acute oral toxicity of MCCPs and LCCPs is apparently lower than that of SCCPs as experimental animals tolerated higher oral single doses of MCCPs and LCCPs compared with SCCPs.

Human data on skin irritation are limited to one study having reported mild erythema and dryness following skin application of SCCPs and another study having reported no local irritation following skin application of SCCPs or LCCPs. Several animal studies are available on the skin irritating potential of particularly SCCPs but also of MCCPs, and some studies are available on LCCPs. Only a few studies have been conducted according to modern standards. Slight skin irritation has been noted in rats and rabbits following a single application of SCCPs or MCCPs. Following repeated applications of SCCPs or MCCPs to rats, signs of skin irritation were more pronounced than following a single application; no signs of irritation were observed following repeated applications of LCCPs. In many of the studies, the chlorinated paraffins contained additives and/or stabilisers, and it

cannot be concluded whether the irritating response was due to those substances rather than the chlorinated paraffins themselves. In the repeated studies, desquamation was also observed indicating a defatting potential of chlorinated paraffins.
No human data are available on eye irritation. Some animal studies are available on the eye irritating potential of chlorinated paraffins. Only a few studies have been conducted according to modern standards. Slight eye irritation has been noted in rabbits following instillation of SCCPs or MCCPs into the conjunctival sac, whereas no signs of irritation were observed following instillation of LCCPs.
No data have been located regarding respiratory irritation.

Human data on skin sensitisation are limited to one study having reported no allergic reactions following skin application of SCCPs or LCCPs; similarly, no positive reactions were observed among exposed employees patch tested with various constituents of cutting fluid coolants, including chlorinated paraffins. Erythema has been noted in few animals following challenge with SCCPs or MCCPs in Guinea-Pig Maximisation Tests, and in an ear-flank test with SCCPs; no data have been located for LCCPs. Based on the available data it is concluded that chlorinated paraffins do not have a skin sensitising potential.
No data have been located regarding respiratory sensitisation.

No human data on effects following repeated exposure to chlorinated paraffins have been located.
For experimental animals, no studies regarding effects following repeated inhalation exposure or dermal contact have been located. In skin irritation studies (see section 4.2.1), no systemic toxicity was reported in rats treated on alternate days with up to six, 24-hour applications of 0.1-0.2 ml of chlorinated paraffins under occlusive dressings.
Studies with oral administration (dietary, gavage) of chlorinated paraffins to experimental animals have demonstrated that the liver, kidneys and thyroid are the target organs for the toxicity of chlorinated paraffins. The available studies on SCCPs are summarised in Table 4.4.1 (section 4.4.1), on MCCPs in Table 4.4.2 (section 4.4.2), and on LCCPs in Table 4.4.3 (section 4.4.3), and N/LOAELs are summarised in section 6.6.4.
The critical studies for SCCPs are two 13-week studies (dietary, gavage – Unpublished studies 1984), for MCCPs two 13-week dietary studies (Poon et al. 1995, Unpublished study 2005), and for LCCPs two 13-week studies (dietary, gavage – IRCD 1984) and a 2-year gavage study (NTP 1996). These studies are summarised in Table 6.7.

The NOAEL for MCCPs (C_{14-17}, 52%) in the study by Poon et al. (1995) was considered by WHO (1996) as 4 mg/kg bw/day. According to the MCCP EU RAR (2005), questions have been raised over the validity and reliability of the findings in the Poon et al. (1995) study (see section 4.4.2), and the EU RAR concludes that this study should not be used for risk characterisation purposes; however, in the previous version of the draft EU RAR on MCCPs (May 2004), a NOAEL of 0.4 mg/kg bw/day was identified for repeated dose toxicity based upon the effects seen in the female rat kidney and thyroid in the Poon et al. (1995) study. In a very recent 13-week study (Unpublished study 2005) with rats administered a C_{14-17}, 52% in the diet at dose levels up to 222/242 mg/kg bw/day, no histopathological changes were observed in the kidney or thyroid; the NOAEL was considered by EU RAR (2005) as 23.0/24.6 mg/kg bw/day based on decreases in plasma triglycerides and cholesterol and increased kidney weights at 222/242 mg/kg bw/day. This very recent study seems very well performed and consequently more reliance will be put on this study than on the Poon et al. (1995) study in terms of setting NOAELs for effects in the target organs.

Table 6.7 Critical studies in relation to setting NOAELs in the target organs liver, kidney and thyroid

Species / strain	Duration / Dose levels (mg/kg bw/day)	Effects	NOAEL (mg/kg bw/ day)	LOAEL (mg/kg bw/ day)	Reference
SCCP:					
F344 rat C_{10-12},58%	13-week, diet 0, 10, 100, 625	625: ↓ bwg ♂, ↑ w thyroid, ↑ enz liver ♂ ≥ 100: ↑ w liver/kidney, hist liver/kidney/thyroid	10: liver, kidney, thyroid	100: liver, kidney, thyroid	Unpublished study (1984)
F344 rat 15/sex/group C_{10-12},58%	13-week, gavage 0, 10, 100, 625	625: ↓ bwg ♂, ↑ w thyroid, ↑ enz liver ♂ ≥ 100: ↑ w liver/kidney, hist liver/kidney/thyroid	10: liver, kidney, thyroid	100: liver, kidney, thyroid	Unpublished study (1984)
MCCP:					
F344 rat 10/sex/ treated group 20/sex/control C_{14-17},52%	13-week, diet 0, 2.38/2.51, 9.34/9.70, 23.0/24.6, 222/242 ♂/♀	222: ↓ TG/Chol, ↑ w liver/kidney, ↑ T4 ♀, ↑ enz liver ♂, hist liver ♂ ≥ 23.0: ↓ T3 ♂, ↑ TSH ≥ 9.7: ↑ enz liver ♀	23.0/24.6: liver, kidney 9.3/9.7: thyroid	222/242: liver, kidney 23.0/24.6: thyroid	Unpublished study (2005)
Sprague-Dawley rat 10/sex/group C_{14-17},52%	13-week, diet 0, 0.4/0.4, 3.6/4.2, 36/42, 363/419, ♂/♀	363: ↑ w kidney, ↑ enz liver ≥ 36: ↑ w liver, hist liver ≥ 4.2: ↑ Chol ♀, hist kidney ♀, thyroid ♀	3.6/4.2: liver 0.4: kidney, thyroid	36/42: liver 3.6/4.2: kidney, thyroid	Poon et al. (1995)
LCCP:					
F344 rat C_{22-26},70%	13-week, diet 0, 100, 900, 3750	3750: ↑ w liver, hist liver	900: liver	3750: liver	IRDC (1984)
F344 rat C_{20-30},43%	13-week, gavage 0, 100, 900, 3750	3750: hist kidney ≥ 100: ↑ w liver ♀, hist liver ♀	< 100: liver 900: kidney	100: liver 3750: kidney	IRDC (1984)
F344 rat 50/sex/group 20/sex/group C_{23},43%	103-week, gavage 0, 1875, 3750 ♂ 0, 100, 300, 900 ♀	≥ 1875 ♂: ↑ w liver, hist ≥ 100 ♀: ↑ w liver, hist	< 100: liver	100: liver	NTP (1986)

↓: reduced
↑: increased
♂ / ♀: male / female
bwg: body weight gain
hist: histopathological changes
w: weight

Effects in the liver include increased weights, enzyme induction, and histopathological changes (centrilobular hepatocellular hypertrophy, necrosis, focal cellular changes, gross dilation of the blood vessels, proliferation of smooth endoplasmic reticulum, peroxisome proliferation, and hepatocellular adenomas and carcinomas).
Studies performed specifically in order to elucidate the mode of actions underlying the observed effects in the liver have revealed that hepatic peroxisome proliferation is induced by SCCPs and MCCPs in rats and mice as evidenced by microscopy, morphometric analysis, and enzyme marker activity; peroxisome proliferation was not observed in guinea pigs. For SCCPs, a NOEL for peroxisome proliferation has been set at 100 mg/kg bw/day (14-day studies in rats and mice). For MCCPs, hepatic peroxisome proliferation (determined by palmitoyl CoA oxidation) was not

noted at dose levels up to 222/242 mg/kg bw/day in a very recent 13-week dietary study in rats (Unpublished study 2005).
Rats and mice are particularly sensitive to peroxisome proliferation, whereas guinea pigs are relatively insensitive to the effect. Humans are also relatively insensitive to the induction of hepatic peroxisome proliferation and it is now generally accepted that the changes related to peroxisome proliferation in rats and mice are not relevant to human health.
In addition, MCCPs are capable of eliciting hepatic enzyme induction and proliferation of smooth endoplasmic reticulum indicative of increased metabolic demand arising from xenobiotic metabolism. According to the EU RARs for SCCPs and MCCPs (EU RAR 1999,2005), the changes related to xenobiotic metabolism are indicative of physiological adaptation and are not considered to be of toxicological significance. However, for SCCPs, alterations in enzyme activities and/or levels were observed in rats at higher dose-levels (625 mg/kg bw/day in 13-week studies, gavage/dietary) than those resulting in increased liver weight and histopathological changes (from 100 mg/kg bw/day in the 13-week studies, gavage/ dietary). Therefore, the relevance to humans of the effects observed in the liver of experimental animals cannot be fully excluded.
The NOAEL for liver effects is considered to be 10 mg/kg bw/day for SCCPs, 23.0/24.6 mg/kg bw/day for MCCPs, and < 100 mg/kg bw/day for LCCPs. Overall, it seems reasonable to consider that a 'read-across' of toxicological data between SCCPs, MCCPs and LCCPs is valid.
In conclusion, a NOAEL of 10 mg/kg bw/day is considered for liver effects of chlorinated paraffins based on the 13-week studies with SCCPs (Unpublished studies 1984); however, it should be noted that the LOAEL in these studies was 100 mg/kg bw/day and therefore, the NOAEL of 23.0/24.6 mg/kg bw/day for MCCPs in the very recent 13-week dietary study (Unpublished study 2005) probably might be more relevant.

Effects in the kidneys include increased weights and histopathological changes (male rats: hyaline-droplet like cytoplasmic inclusions, nephritis, cysts in the cortex, nephropathy, and tubular cell hyperplasia; female rats: brown pigmentation, inner medullary tubular dilation, and nephropathy; female mice: nephropathy). Studies performed specifically in order to elucidate the mode of actions underlying the observed effects in the kidney have revealed some deposition of α2µglobulin in proximal convoluted tubules of male rats, but this was considered as being unrelated to the histopathological findings. Furthermore, nephropathy has also been observed in the kidneys of female rats and mice, and inner medullary tubular dilation in one 13-week dietary study with rats. Thus, the effects observed in the kidneys are not considered as being a male rat-specific phenomenon and is consequently considered as being relevant to human health.
The NOAEL for kidney effects is considered to be 10 mg/kg bw/day for SCCPs, 23.0/24.6 mg/kg bw/day for MCCPs, and 900 mg/kg bw/day for LCCPs. Overall, it seems reasonable to consider that a 'read-across' of toxicological data between SCCPs, MCCPs and LCCPs is valid.
In conclusion, a NOAEL of 10 mg/kg bw/day is considered for kidney effects of chlorinated paraffins based on the 13-week studies with SCCPs (Unpublished studies 1984); however, it should be noted that the LOAEL in these studies was 100 mg/kg bw/day and therefore, the NOAEL of 23.0/24.6 mg/kg bw/day for MCCPs in the very recent 13-week dietary study (Unpublished study 2005) probably might be more relevant.

Effects in the thyroid include increased weights, alterations in T3, T4 and/or TSH levels, and histopathological changes (follicular cell hypertrophy, hyperplasia, and adenomas/carcinomas) in rats and mice, but not guinea pigs.

Studies performed specifically in order to elucidate the mode of actions underlying the observed effects in the thyroid indicate that the effects in the thyroid appear to be due to stimulation of the thyroid via negative feedback mechanisms. According to the EU RARs for SCCPs and MCCPs (EU RAR 1999,2005), the effects seen in the thyroid in rats and mice would be of little relevance to human health as rodents are particularly susceptible to thyroid changes due to absence of a T4-binding globulin, which is present in humans and has a very high affinity for T4. It is generally accepted that humans seem to be less sensitive than rodents concerning thyroid disturbances even though the basic hypothalamic-pituitary-thyroid axis functions in a similar way in animals and humans. The relevance for humans to a great extent is a question of doses, that is whether the amount of thyroid disturbing substances we are exposed to are sufficient to cause effects. Therefore, the relevance to humans of the effects observed in the thyroid of experimental animals cannot be excluded.
The NOAEL for thyroid effects is considered to be 10 mg/kg bw/day for SCCPs, and 9.3/9.7 mg/kg bw/day for MCCPs; no thyroid effects have been reported for LCCPs. Overall, it seems reasonable to consider that a 'read-across' of toxicological data between SCCPs, MCCPs and LCCPs is valid.
In conclusion, a NOAEL of around 10 mg/kg bw/day is considered for thyroid effects of chlorinated paraffins based on the 13-week studies with SCCPs (Unpublished studies 1984) and the very recent 13-week dietary study with MCCPs (Unpublished study 2005).

No human data on reproductive and developmental effects have been located.
Data on reproductive effects of chlorinated paraffins are limited to a range-finding study in rats with an MCCP in which no effects on the parental generation, including fertility, were noted at dietary dose levels up to 384/463 mg/kg bw/day (highest dose level in the study) for males/females, respectively. Significant effects (reduced pup survival during lactation, but not at birth, and effects indicative of internal haemorrhaging) were observed in the developing offspring during lactation at maternal dose levels of 74 and 463 mg/kg bw/day, and at 463 mg/kg bw/day, all pups died before weaning; no such effects were noted in the offspring at 8 mg/kg bw/day (the lowest dose level in the study). According to WHO (1996), the pup weights were lower at 8 and 74 mg/kg bw/day than in the control group on lactation day 21, although not achieving statistical significance at 8 mg/kg bw/day. The NOAEL for parental toxicity and fertility is considered to be ≥ 463/384 mg/kg bw/day for males/females, respectively; and for developmental effects to be 8 mg/kg bw/day. Follow-up studies performed with the aim of investigating the possible mode of action of internal haemorrhages indicate that MCCPs induce a perturbation of the clotting system in lactating neonates of treated mothers. The neonates also receive MCCPs through the maternal milk, which may further reduce their vitamin K levels. This in turn will lead to vitamin K deficiency in the neonates and consequently haemorrhaging.
No studies specifically investigating effects on fertility of SCCPs or LCCPs have been located. In a 14-day gavage study with SCCPs, decreased relative and absolute ovary weights was observed in female F344 rats given 3000 mg/kg bw/day; no effect was observed at lower dose levels; no changes were seen in the reproductive organs in rats and mice treated for 13 weeks with up to 5000 and 2000 mg/kg bw/day, respectively. Likewise, no effects on reproductive organs of rats and mice have been reported following repeated administration of LCCPs up to 3750 and 7500 mg/kg bw/day, respectively.

In conventional developmental toxicity studies in rats and rabbits with chlorinated paraffins, adverse effects in offspring were observed for SCCPs only, and only at maternally toxic doses in rats (2000 mg/kg bw/day, highest dose level in the study); the NOAEL for maternal and developmental effects of SCCPs in rats is considered

to be 500 mg/kg bw/day, and in rabbits ≥ 100 mg/kg bw/day (highest dose level in the study). For MCCPs, the NOAEL for maternal effects in rats is considered to be 500 mg/kg bw/day, and for developmental effects ≥ 5000 mg/kg bw/day (highest dose level in the study); in rabbits, the NOAEL for maternal and developmental effects is considered to be ≥ 100 mg/kg bw/day (highest dose level in the study). For LCCPs, the NOAEL for maternal and developmental effects in rats and rabbits is considered to be ≥ 5000 mg/kg bw/day (highest dose level in the studies).
In conclusion, a NOAEL for developmental toxicity of chlorinated paraffins is considered to be 8 mg/kg bw/day based on the effects observed at higher dose levels in the range-finding study with an MCCP. The NOAEL for fertility is considered to be ≥ 384/463 mg/kg bw/day for males/females, respectively, as no effects were noted at the highest dose level in the range-finding study.

There are no data available on genotoxic effects of chlorinated paraffins in humans. Chlorinated paraffins do not appear to induce mutations in bacteria. In mammalian cells *in vitro*, SCCPs were reported to be mutagenic in mouse lymphoma cells, but not in Chinese hamster V79 cells, and LCCPs induced chromosome aberrations and sister chromatid exchange in Chinese hamster ovary cells in one study; no data have been located for MCCPs. Chlorinated paraffins have yielded both positive and negative results in cell transformation assays *in vitro*. Neither mutagenic nor clastogenic effects have been reported in several *in vivo* studies using oral administration of chlorinated paraffins, including rat bone marrow cell chromosomal studies (SCCPs, MCCPs, LCCPs), bone marrow micronucleus tests in mice (SCCPs and MCCPs), unscheduled DNA synthesis in rat hepatocytes (SCCPs), and a germ cell mutagenicity study in rats (SCCPs).
In conclusion, the available data indicate that chlorinated paraffins do not possess genotoxic activity *in vivo*.

No human data on carcinogenic effects have been located.
Studies with oral administration of chlorinated paraffins to experimental animals have demonstrated significant and dose-related increases in the incidence of several tumour types. For SCCPs, only the tumours in the liver (rats, mice), kidney (male rats), and thyroid (female rats and mice) should be considered of toxicological significance. For LCCPs, increased incidences were observed in female rats for adrenal gland medullary phaeochromocytomas (significant at high-dose only) and for endometrial stromal polyps in the uterus (significant at low-dose only, not dose-related); in mice, a significantly increased incidence was observed for malignant lymphomas (high-dose males). No data are available for MCCPs.
Regarding tumours in the liver and the thyroid, peroxisome proliferation and hormonal imbalance have been suggested to be the plausible mode of actions, respectively, and the EU Specialised Experts Group has accepted this view.
Regarding tumours in the kidney, the EU Specialised Experts Group has recently (January 2005) agreed that there were still data gaps leading to uncertainty about the relevance of these tumours to humans.

6.7.1 Critical effect and NOAEL

The critical effects following exposure to chlorinated paraffins are considered to be the effects, including tumours, observed in the liver, kidneys and thyroid of rats and mice, as well as the effects observed in developing offspring of rats.

Based on the available toxicological data, no firm conclusions can be drawn with respect to possible differences in the toxicity of chlorinated paraffins as a result of different chain length and degree of chlorination. However, for many toxicological endpoints, data indicate a similarity, at least in qualitative terms, in the

toxicological profile of the tested chlorinated paraffins, and some data indicate a trend of decreased toxicity with increasing chain length for many endpoints including the critical effects. Overall, it seems reasonable to consider that a 'read-across' of toxicological data between the tested chlorinated paraffins is valid for the purpose of setting a health based quality criterion in air for chlorinated paraffins.

Effects in the liver include increased weights, enzyme induction, and histopathological changes. Changes related to peroxisome proliferation are not considered as being relevant to humans. In contrast, the relevance to humans of the changes related to xenobiotic metabolism observed in the liver of experimental animals cannot be fully excluded. A NOAEL of 10 mg/kg bw/day is considered for liver effects of chlorinated paraffins based on the 13-week studies with SCCPs (Unpublished studies 1984). As the LOAEL in the studies with SCCPs was 100 mg/kg bw/day, the NOAEL of 23.0/24.6 mg/kg bw/day for MCCPs in the very recent 13-week dietary study (Unpublished study 2005) probably might be more relevant.

Effects in the kidneys include increased weights and histopathological changes. The effects observed in the kidneys are not considered as being a male rat-specific phenomenon and consequently as being relevant to human health. A NOAEL of 10 mg/kg bw/day is considered for kidney effects of chlorinated paraffins based on the 13-week studies with SCCPs (Unpublished studies 1984). As the LOAEL in the studies with SCCPs was 100 mg/kg bw/day, the NOAEL of 23.0/24.6 mg/kg bw/day for MCCPs in a very recent 13-week dietary study (Unpublished study 2005) probably might be more relevant.

Effects in the thyroid include increased weights, alterations in T3, T4 and/or TSH levels, and histopathological changes in rats and mice, but not guinea pigs. Humans seem to be less sensitive than rodents concerning thyroid disturbances even though the basic hypothalamic-pituitary-thyroid axis functions in a similar way in animals and humans, and the relevance for humans is thus to a great extent a question of doses. Therefore, the relevance to humans of the effects observed in the thyroid of experimental animals cannot be excluded. A NOAEL of around 10 mg/kg bw/day is considered for thyroid effects of chlorinated paraffins based on the 13-week studies with SCCPs (Unpublished studies 1984) and the very recent 13-week dietary study with MCCPs (Unpublished study 2005).

In conventional developmental toxicity studies in rats and rabbits with chlorinated paraffins, adverse effects in offspring were observed for SCCPs only, and only at maternally toxic doses in rats. However, in a range-finding study with an MCCP, significant effects (reduced pup survival during lactation, but not at birth, and effects indicative of internal haemorrhaging) were observed in the developing offspring during lactation at maternal dose levels of 74 and 463 mg/kg bw/day. Internal haemorrhage is probably a result of perturbation of the clotting system due to a vitamin K deficiency in the neonates, which is considered as being of relevance to human health. A NOAEL of 8 mg/kg bw/day is considered for developmental toxicity.

Tumours have been observed in the liver (rats, mice), kidney (male rats), and thyroid (female rats and mice). The available data indicate that chlorinated paraffins do not possess genotoxic activity. Plausible mode of actions have been suggested and accepted for tumours in the liver (peroxisome proliferation) and the thyroid (hormonal imbalance), and these tumours are considered as being of no or limited relevance to humans. Regarding tumours in the kidney, there are still data gaps leading to uncertainty about the relevance of these tumours to humans. As the

chlorinated paraffins are not genotoxic, it is considered that there would be no risk of kidney tumour development associated with exposures lower than those required to induce chronic toxicity in this target organ. A NOAEL of 10 mg/kg bw/day is considered for kidney tumours of chlorinated paraffins.

For the purpose of setting a health based quality criterion in air for chlorinated paraffins, an overall NOAEL of 10 mg/kg bw/day is considered for effects in the liver, kidney and thyroid as well as for the effects observed in developing offspring.

7 TDI and quality criterion in ambient air

7.1 TDI

The TDI is calculated based on an overall NOAEL of 10 mg/kg bw/day for effects in the liver, kidney and thyroid as well as for the effects observed in developing offspring.

$$TDI = \frac{NOAEL}{UF_I * UF_{II} * UF_{III}} = \frac{10 \text{ mg/kg b.w./day}}{10 * 10 * 1} = 0.1 \text{ mg/kg b.w./day}$$

The uncertainty factor UF_I accounting for interspecies variability is set to 10 assuming that humans are more sensitive than animals.
The UF_{II} accounting for intraspecies variability is set to 10 reflecting the range in biological sensitivity within the human population.
The UF_{III} is set to 1 as

1) a NOAEL has been established for the effects in the target organs,
2) the effects in the liver, kidney and thyroid are generally of a mild severity at the LOAEL,
3) the NOAEL for the effects in the liver and kidney is probably higher than 10 mg/kg bw/day as indicated by the NOAEL (23.0/24.6 mg/kg bw/day) for effects in the liver and kidney in the very recent study on MCCPs (Unpublished study 2005),
4) humans seem to be less sensitive than rodents concerning thyroid disturbances implicating that the NOAEL of around 10 mg/kg bw/day observed for alterations in hormone levels in rats in the very recent study on MCCPs (Unpublished study 2005) probably is a very conservative estimate in relation to humans,
5) the relevance of the effects in the liver, kidney and thyroid to human health can been questioned although not to be fully excluded, and
6) there is about one order of magnitude between the NOAEL (8 mg/kg bw/day) and the LOAEL (74 mg/kg bw/day) for the effects observed in developing offspring.

7.2 Allocation

Chlorinated paraffins have several uses that can result in releases into the environment. Chlorinated paraffins have been detected in low levels in atmospheric air, surface water and sediment, soil, and in some food items and breast milk.

In the EU Risk Assessment Reports, the total daily human intakes of SCCPs and MCCPs from the environment have been estimated based on local PECs and on regional PECs, see Table 6.3.1. Root crops were predicted to be the major source of human uptake for both SCCPs and for MCCPs. For SCCPs, a total daily human intake of 0.02 mg/kg bw/day has been considered as a reasonable worst case prediction based upon measured data and has been used for the risk

characterisation for man exposed indirectly via the environment. For MCCPs, a total daily human intake of 2.10 mg/kg bw/day has been used for the risk characterisation for man exposed indirectly via the environment from local exposure, and of 1.71 mg/kg bw/day from regional exposure.

For MCCPs, the daily uptake from breast milk for the first 3 months of infant life has been estimated to 30.5 x 10^{-5} mg/kg bw/day (EU RAR 2005).

According to the EU Risk Assessment Reports, chlorinated paraffins are found in a number of consumer products, including leather clothing, metal working fluids and textiles, in certain paints, sealants and adhesives, and in plastic and rubber products. Aside from the wearing of leather clothing and the use of metal working fluids, the consumer exposures are considered to be negligible. The total systemic dose for use of metal working fluids has been estimated to 0.03 mg/kg bw/day for SCCPs and to 0.008 mg/kg bw/day for MCCPs, and for wearing of leather clothing to 0.02 mg/kg bw/day for SCCPs and to 0.00016 mg/kg bw/day for MCCPs.

Based on the available data, the general population is considered to be exposed to chlorinated paraffins predominantly from foodstuffs and from consumer products containing chlorinated paraffins. Therefore, only 10% of the TDI is allocated to exposure from ambient air. It should be noted that the data on the potential exposure of infants via breast milk are not sufficient in order to evaluate whether chlorinated paraffins from breast milk is an important source to the daily intake.

7.3 Quality criterion in ambient air

The quality criterion in air QC_{air} is calculated based on the TDI of 0.1 mg/kg b.w. per day, assuming 100% absorption following inhalation and oral exposure in experimental animals as well as in humans, and a daily inhalation of 0.5 m^3/kg bw/day of air for children of 1-5 years of age:

$$QC_{air} = \frac{TDI * f}{inhalation_{air}} = \frac{0.1 \text{ mg/kg bw/day} * 0.1}{0.5 \text{ m}^3\text{/kg bw/day}}$$

$$= 0.02 \text{ mg/m}^3$$

A quality criterion of 0.02 mg/m^3 has been calculated.

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Evaluation of health hazards by exposure to Chlorinated paraffins and proposal of a health-based quality criterion for ambient air

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to chlorinated paraffins. This resulted in 2006 in the present report which includes a health-based quality criterion for the substances in ambient air

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