Health Council of the Netherlands

Hexachlorobenzene

Health-based recommended occupational exposure limit

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies over *Hexachlorobenzene*
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Geachte staatssecretaris,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan hexachloorbenzeen.

Dit advies maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige adieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Het genoemde advies is opgesteld door de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. L.J. Gunning-Schepers,
voorzitter

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Hexachlorobenzene

Health-based recommended occupational exposure limit

Dutch Expert Committee on Occupational Safety
A Committee of the Health Council of the Netherlands

to:

the State Secretary of Social Affairs and Employment

No. 2011/35, The Hague, December 6, 2011

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Samenvatting en advieswaarde

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in lucht waaraan mensen beroepsmatig blootgesteld kunnen worden. Deze advieswaarden vormen vervolgens de basis voor grenswaarden – vast te stellen door de minister – waarmee de gezondheid van werknemers beschermd kan worden.

In dit advies bespreekt de commissie de gevolgen van blootstelling aan hexachloorbenzeen en stelt zij een gezondheidskundige advieswaarde vast. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór mei 2010 zijn verschenen.

Fysische en chemische eigenschappen

Hexachloorbenzeen (CAS nummer 118-74-1) is een kristallijne, witte vaste stof, die slecht water- en goed vetoplosbaar is. Het molaire gewicht is 285 g/mol. In Nederland wordt geen hexachloorbenzeen geproduceerd; het kan echter wel ontstaan als bijproduct of verontreiniging in de productie van andere chloorkoolwaterstoffen.

Monitoring

Er is een groot aantal methoden beschikbaar voor de analyse van hexachloorbenzeen in milieu- en biologische monsters, allen gebaseerd op gaschromatografie met diverse detectiemethoden.

Grenswaarden

Momenteel is de 8-uurs tijdgemiddelde beroepsmatige blootstellingslimiet voor hexachloorbenzeen 0,03 mg/m^3 (0,0024 ppm). In België, Spanje en de USA geldt een 8-uurs limietwaarde van 0,002 mg/m^3 (0,00016 ppm), terwijl in Denemarken en Canada een 8-uurs limietwaarde van 0,025 mg/m^3 (0,002 ppm) geldt. Frankrijk en Polen hanteren een 8-uurs limietwaarde van 0,5 mg/m^3 (0,04 ppm). In Denemarken geldt naast de genoemde 8-uurs limietwaarde een *short term* limietwaarde van 0,05 mg/m^3 (0,004 ppm). Tot dusverre is op Europees niveau geen limietwaarde voor beroepsmatige blootstelling aan hexachloorbenzeen vastgesteld.

Kinetiek en toxisch werkingsmechanisme

Bij de mens wordt de orale absorptie van hexachloorbenzeen op 85% geschat; dit percentage wordt minder naarmate het bloed meer hexachloorbenzeen bevat. Absorptie van hexachloorbenzeen uit het dierlijke maagdarmkanaal varieert van 6% bij toediening in water tot 82% bij toediening in plantaardige oliën. Er zijn geen gegevens beschikbaar over absorptie van hexachloorbenzeen via de ademhaling bij mensen of dieren. Er zijn evenmin geen gegevens beschikbaar over absorptie via de humane huid; in ratten is een absorptiesnelheid via de huid van circa 0,9 microgram/cm^2/uur vastgesteld.

Na orale blootstelling van zoogdieren verdeelt hexachloorbenzeen zich over de weefsels, waarbij het zich snel verspreidt naar het bloed, de lever, het vetweefsel, het beenmerg en de ovaria, in het algemeen bij voorkeur naar weefsels of organen met een hoog vetgehalte. Hexachloorbenzeen hoopt zich op in de weefsels, alsmede in melk, en kan dus worden overgedragen naar de zuigeling. Bij dieren wordt hexachloorbenzeen gemakkelijk via de placenta overgebracht naar de foetus. Er is weinig informatie over de distributie na respiratoire of dermale blootstelling.

In zoogdieren wordt hexachloorbenzeen langzaam gemetaboliseerd. De belangrijkste omzettingsproducten zijn pentachloorfenol, pentachloorthiofenol en pentachloorbenzeen, daarnaast worden lager-gechloreerde benzenen, chloorfenolen, en zwavel-geconjugeerde fenolen en benzenen gevormd.

Inhalatoir of oraal opgenomen hexachloorbenzeen wordt bij mensen grotendeels onveranderd via de feces uitgescheiden. Omzettingsproducten van hexachloorbenzeen worden voornamelijk via de urine uitgescheiden. Ook bij orale blootstelling van dieren is dat het geval. Er is geen informatie beschikbaar over de uitscheiding van hexachloorbenzeen na inhalatieblootstelling van dieren, noch na huidblootstelling van mensen of dieren.

Hexachloorbenzeen veroorzaakt porfyrie, een ziekte waarbij de productie van hemoglobine in de lever wordt verstoord, voornamelijk door het remmen van uroporfyrinogeen decarboxylase, een enzym betrokken bij de productie van het heemmolecuul.

In de lever van ratten zijn tumor-promoverende effecten van hexachloorbenzeen aangetoond bij doses die geen tumoren initieerden. Hexachloorbenzeen veroorzaakt geen directe DNA-schade. Op basis van de beschikbare gegevens beschouwt de commissie hexachloorbenzeen als een promotor – en geen initiator – van levertumoren via een niet-genotoxisch werkingsmechanisme waarbij geen directe interactie met het DNA optreedt.

Effecten

Acute toxiciteit, irritatie en sensibilisatie

Er zijn geen gegevens over de irriterende en sensibiliserende eigenschappen van hexachloorbenzeen, noch gegevens over acute gezondheidseffecten bij mensen.

De acute lethaliteit van oraal ingenomen hexachloorbenzeen bij dieren is relatief laag. De orale LD_{50}* in dieren varieert van 1.700 tot 4.000 mg/kg lichaamsgewicht. De lever is een belangrijk doelorgaan na (sub)acute orale blootstelling. De levereffecten kenmerken zich door verstoring van de heemsynthese (culminerend in porfyrie), inductie van microsomale enzymen, leververgroting en celschade. De laagste gerapporteerde dosis die levereffecten veroorzaakt is 16 mg/kg

* Eenmalige dosis ten gevolge waarvan gemiddeld 50% van de dieren binnen enkele dagen overlijdt.

lichaamsgewicht/dag in een 7-daagse studie in ratten, bij 5 mg/kg lichaamsgewicht/dag werden geen effecten gezien.

Er zijn geen gegevens over acute toxiciteit van hexachloorbenzeen na dermale of respiratoire blootstelling.

Toxiciteit na kortdurende blootstelling

Er zijn geen gegevens over effecten bij mens na kortdurende blootstelling aan hexachloorbenzeen.

Er is slechts beperkte informatie over kortdurende effecten na inhalatoire blootstelling bij dieren. Bij inhalatoir blootgestelde ratten zijn geringe aanwijzingen gevonden dat hexachloorbenzeen het immuunsysteem beïnvloedt.

Er zijn geen dierstudies over de effecten van hexachloorbenzeen na dermale blootstelling.

In kortdurende orale studies met apen, ratten, muizen, en varkens zijn ernstige effecten op de lever waargenomen. Bij apen en ratten veroorzaakte een dosis van 1 mg/kg lichaamsgewicht/dag leverschade, geen effecten werden waargenomen bij een dosis van 0,1 mg/kg lichaamsgewicht/dag. Bij varkens werd bij doses van 0,5 mg/kg lichaamsgewicht/dag en hoger een hypertrofie van levercellen waargenomen, terwijl bij een dosis van 0,05 mg/kg lichaamsgewicht/dag geen nadelige effecten werden gezien. Een hondenstudie toonde effecten op het immuunsysteem aan na één jaar blootstelling aan een dosis van 0,1 mg/kg lichaamsgewicht/dag, Relatief hoge doses van hexachloorbenzeen in kortdurende orale studies lieten ook effecten op de schildklier zien.

Toxiciteit na langdurige blootstelling

De lever is het belangrijkste doelorgaan van hexachloorbenzeentoxiciteit. Gegevens van de mens laten ook effecten op andere organen en orgaansystemen zien, zoals de huid, de botten, de schildklier en het immuunsysteem. Verder ontstonden ten gevolge van hexachloorbenzeenblootstelling huidbeschadigingen door activering van in de huid opgehoopte porfyrines* door zonlicht (fototoxiciteit). In

* Een gevolg van de door hexachloorbenzeen veroorzaakte leverporfyrie.

de vergiftigingsepidemie in Turkije*, veroorzaakt door orale blootstelling aan hexachloorbenzeen, werden blootgestelde kinderen jonger dan 2 jaar het meest getroffen, maar ook kinderen tot 15 jaar oud vertoonden ernstige vergiftigingsverschijnselen. De effecten werden waargenomen bij een geschatte blootstelling van 0,8 – 3,3 mg/kg lichaamsgewicht/dag. Er kon geen dosis zonder nadelige effecten worden geïdentificeerd.

Uit dierstudies zijn alleen gegevens na orale inname beschikbaar. De kritische effecten van hexachloorbenzeen zijn levertoxiciteit, reproductietoxiciteit en kanker. Verscheidene studies in ratten laten zowel porfyrie als andere levereffecten zien, zoals ophoping van witte bloedcellen en verbindweefseling rondom de galgangen, leververgroting en verhoogd levergewicht, enzyminductie en degeneratieve pathologische veranderingen. In een levenslange orale studie met hamsters werd een laagst waargenomen schadelijke dosis van 16 mg/kg lichaamsgewicht/dag geconstateerd, gebaseerd op een vermindering in gewichtstoename. In een 18-maands orale studie met muizen werd een laagst waargenomen schadelijke dosis van 13 mg/kg lichaamsgewicht/dag vastgesteld, gebaseerd op verminderd lichaamsgewicht en hypertrofie van levercellen. Voor ratten werd een laagste effect-dosis van 0,016 mg/kg lichaamsgewicht/dag vastgesteld, gebaseerd op een twee-generatiestudie waarin fibrose van de lever en lymfocytose van de galgangen werden waargenomen.

Schadelijkheid voor het erfelijk materiaal van de cel en carcinogeniteit

In *in vitro* testen met bacteriën en dierlijke cellen, in studies met ratten en muizen, en in een klinische studie bij de mens zijn diverse aspecten van de mogelijke erfelijke schade door blootstelling aan hexachloorbenzeen onderzocht, zoals veranderingen in het DNA en chromosoomafwijkingen. Op grond van deze gegevens concludeert de commissie dat hexachloorbenzeen niet genotoxisch is.

Er zijn slechts weinig humane studies, en veelal zijn ze inconsistent. De oudere studies onder de algemene bevolking hebben in het algemeen geen verband gevonden tussen de hexachloorbenzeengehalten in bloed of weefsels en het vóórkomen van borst- of andere kankers, terwijl in één meer recente studie enig verband met niet-Hodgkin lymfekanker is aangetroffen. Gegevens over mensen die

* In de vijftiger jaren van de 20ste eeuw veroorzaakte de consumptie van brood gemaakt van graan behandeld met hexachloorbenzeen als bestrijdingsmiddel, een epidemie in het zuidoosten van Turkije.

via inademing zijn blootgesteld aan hexachloorbenzeen, verschaffen slechts zwakke aanwijzingen voor een verband tussen deze blootstelling en lever-, schildklier- en hersenkanker. Alles afwegend, is het bewijs vanuit studies in de mens te gering om daaruit te concluderen dat hexachloorbenzeen kanker veroorzaakt bij de mens.

Verscheidene dierstudies hebben aangetoond dat orale blootstelling aan hexachloorbenzeen de incidentie van levertumoren vergroot. Het bewijs voor carcinogeniteit van hexachloorbenzeen is het sterkst in de lever; aangetoond is dat de stof daar goedaardige en kwaadaardige tumoren veroorzaakt. Verder is aangetoond dat hexachloorbenzeen tumoren veroorzaakt in de nier, in de bijnieren, in de bijschildklier, en in de schildklier. Er zijn geen onschadelijke doses voor kankereffecten na orale blootstelling geïdentificeerd; de laagst waargenomen schadelijke dosis voor kankereffecten is 4 mg/kg lichaamsgewicht/dag bij ratten en hamsters en 12 mg/kg lichaamsgewicht/dag bij muizen. Er zijn in de openbare literatuur geen carcinogeniteitsstudies met huid- of ademhalingsblootstelling aangetroffen. Op grond van de dierproefgegevens is de commissie van mening dat, in overeenstemming met de richtlijnen van de Europese Unie, hexachloorbenzeen geclassificeerd moet worden als een stof met veronderstelde kankerverwekkende potentie voor mensen (categorie 1b) via een niet-genotoxisch werkingsmechanisme.

Reproductietoxiciteit

Epidemiologische studies in de mens hebben geen betrouwbaar bewijs voor nadelige effecten van hexachloorbenzeen op de vruchtbaarheid van man en vrouw geleverd.

In dierstudies zijn veelvoudige hormonale effecten en pathologische veranderingen in de voortplantingsorganen aangetoond. In apen is 0,01 mg/kg lichaamsgewicht/dag vastgesteld als no-effect dosis, gebaseerd op afsterving van de epitheelcellen van de ovaria, waargenomen in 90-dagenstudies.

In een vergiftigingsepidemie in Turkije waren blootgestelde kinderen sterker getroffen dan volwassenen. De commissie is echter van mening dat de hogere incidentie van gezondheidseffecten onder kinderen niet het gevolg behoeft te zijn van een specifieke verstoring van het ontwikkelingsproces: de hogere morbiditeit onder kinderen kan veroorzaakt zijn door een relatief hogere blootstelling van jonge kinderen door borstvoeding en bij oudere kinderen door een grotere

inname van eventueel verontreinigd brood. Andere studies naar ontwikkelingseffecten bij mensen zijn beperkt door de kleine omvang van de studies en de lage niveaus van hexachloorbenzeenblootstelling; deze studies suggereren een verhoogd risico op niet-ingedaalde testes en een geremde ontwikkeling van de spierbeheersing in pasgeboren baby's.

Dierstudies hebben bevestigd dat hexachloorbenzeen de ontwikkeling van het zenuwstelsel remt en de levensvatbaarheid en groei van pasgeborenen vermindert. Het vóórkomen van gespleten verhemelte, van het niet-vormen van de nieren en van kleine skeletafwijkingen bij muizen is consistent met een mogelijke aantasting van de ontwikkeling door hexachloorbenzeen. Een studie naar de ontwikkeling van het zenuwstelsel vond bewijs voor hyperactiviteit in pups van ratten. In deze studie kon een laagst waargenomen schadelijke dosis van 2,5 mg/kg lichaamsgewicht/dag worden vastgesteld op grond van minimale ontwikkelingseffecten op het zenuwstelsel; er is geen onschadelijke dosis waargenomen. Bij ratten is een onschadelijke dosis voor ontwikkelingseffecten gevonden van 0,4 mg/kg lichaamsgewicht/dag gebaseerd op verminderde levensvatbaarheid van de pups bij hogere doses; in deze studie werden geen vruchtbaarheidseffecten waargenomen. Ontwikkelingseffecten op het immmuunsysteem zijn gevonden bij ratten blootgesteld *in utero* en gedurende de lactatie: de antilichamenrespons op tetanus toxoïd was verhoogd bij doses (voor de moederdieren) van 0,2 mg/kg lichaamsgewicht/dag (laagste toegepaste dosis) en hoger.

Epidemiologische studies bij mensen laten geen duidelijk verband tussen hexachloorbenzeenblootstelling en ontwikkelingseffecten zien. Echter, een aantal van deze studies heeft methodologische beperkingen, en dierstudies laten wel ontwikkelingseffecten zien. Studies bij mensen lieten eveneens geen duidelijk verband tussen hexachloorbenzeenblootstelling en vruchtbaarheidseffecten zien, maar bij apen werden wel vruchtbaarheidseffecten waargenomen. Daarom moet hexachloorbenzeen, in overeenstemming met de richtlijnen van de Europese Unie, worden geclassificeerd als schadelijk voor de voortplanting (categorie 1b: stoffen waarvan verondersteld wordt dat zij toxisch zijn voor de menselijke voortplanting), zowel voor vruchtbaarheid als ook voor ontwikkeling toxiciteit. Aangezien hexachloorbenzeen wordt overgedragen via de moedermelk, moet het eveneens worden geclassificeerd als schadelijk via de borstvoeding.

Evaluatie en advies

De beschikbare humane gegevens zijn onvoldoende om er een limietwaarde op te kunnen baseren. Derhalve is de commissie uitgegaan van dierexperimentele gegevens en heeft voor hexachloorbenzeen een gezondheidskundige limiet-waarde voor de beroepsbevolking van 0,006 mg/m^3 afgeleid. De basis voor die afleiding is de 90-dagen apenstudie waarbij effecten op de voortplantingsorganen werden waargenomen. De door de Commissie voorgestelde gezondheidskundige advieswaarde wordt uitgedrukt in concentratie gemiddeld over een achturige werkdag.

Executive summary

Scope

At request of the Minister of Social Affairs and Employment, the Dutch Expert Committee on Occupational Exposure Safety (DECOS), a Committee of the Health Council of The Netherlands, proposes health-based recommended occupational exposure limits (HBROEL) for chemical substances in the air at the workplace. These recommendations serve as a basis in setting legally binding occupational exposure limits by the minister.

In this report, the Committee discusses the consequences of occupational exposure to hexachlorobenzene and recommends a health-based occupational exposure limits. The Committee's conclusions are based on scientific papers published prior to May 2010.

Physical and chemical properties

Hexachlorobenzene is a white crystalline solid, which is highly lipophilic and poorly soluble in water. It has a molar weight of 285 g/mol. Hexachlorobenzene is not produced in The Netherlands. However, it can arise as a by-product or contamination in the production of other chlorinated hydrocarbons.

Monitoring

Many analytical methods are available for the determination and quantification of hexachlorobenzene in environmental and biological samples. All are based on gas chromatography (GC), with various detection methods.

Guidelines

Currently, the 8 hr time-weighted average (TWA) occupational exposure limit for hexachlorobenzene in The Netherlands is 0.03 mg/m^3 (0.0024 ppm). Belgium, Spain and the USA have an 8-hrs limit value of 0.002 mg/m^3 (0.00016 ppm), while Denmark and Canada have an 8-hrs limit value of 0.025 mg/m^3 (0.002 ppm). France and Poland have an 8-hrs limit value of 0.5 mg/m^3 (0.04 ppm). In addition to the above mentioned 8-hrs limit value, Denmark has also a "short term" limit value of 0.05 mg/m^3 (0.004 ppm). So far there is no limit value for exposure to hexachlorobenzene at the European level.

Kinetics and mechanism of action

Oral absorption in humans of hexachlorobenzene is estimated at 85%, this percentage decreases with increasing amounts of hexachlorobenzene in the blood. Gastrointestinal absorption of hexachlorobenzene in animals varies from 6% when administered in water to 82% when administered in vegetable oils. No data are available on absorption of inhaled hexachlorobenzene in humans or animals. No data are available on dermal absorption of hexachlorobenzene in humans; in rats a dermal absorption rate of approximately 0.9 µg/cm^2/h was established.

Orally absorbed hexachlorobenzene distributes widely in mammalian tissues, rapidly partitioning to blood, liver, adipose tissue, endocrine organs, bone marrow, and ovarian follicular fluid, preferentially distributing to adipose tissue or organs with high fat content. In animals, it is readily transferred through the placenta to the foetus. Hexachlorobenzene accumulates in mammalian tissues, including milk, and can thus be transferred to the suckling neonate. There is no information on distribution following inhalation or dermal exposure.

Hexachlorobenzene is slowly metabolized in mammals, and the major part of hexachlorobenzene is excreted unchanged in faeces. Major metabolites are pentachlorophenol, pentachlorothiophenol and pentachlorobenzene; other

metabolites include lower-chlorinated benzenes, chlorophenols, and S-conjugated phenols and benzenes.

In humans, inhaled or ingested hexachlorobenzene is mainly excreted unchanged via faeces. Metabolites are excreted mainly in urine; also in orally exposed animals this is the case. No information is available on the excretion of hexachlorobenzene following inhalation exposure in animals or following dermal exposure in humans or animals.

Hexachlorobenzene causes hepatic porphyria mainly by reducing the activity of uroporphyrinogen decarboxylase, an enzym involved in heam biosynthesis.

Tumour-promoting effects of hexachlorobenzene in the liver have been demonstrated in rats at doses that did not initiate tumours. Hexachlorobenzene does not induce direct DNA damage. Therefore, based on the available evidence, the Committee considers hexachlorobenzene to act as a promoter of liver carcinogenesis via a non-genotoxic mechanism of action.

Effects

Acute toxicity, irritation and sensitisation

No human nor animal data have been retrieved on the irritating and sensitising properties of hexachlorobenzene. No information was located regarding acute health effects in humans following exposure to hexachlorobenzene by any route of exposure.

The acute lethality of ingested hexachlorobenzene in animal studies is relatively low. Oral LD_{50} values varies between 1,700 to 4,000 mg/kg bw. The liver is an important target organ for hexachlorobenzene following (sub)acute oral exposure. Hepatic effects include disruption of haem synthesis (culminating in porphyria), induction of microsomal enzymes, hepatomegaly, and cellular damage. The lowest dose reported to induce hepatic effects was 16 mg/kg bw/day in a 7-day study in rats, while no effects were found at 5 mg/kg bw/day.

No acute toxicity studies in animals investigating the dermal and respiratory routes were retrieved.

Short-term toxicity

No information was located regarding short-term health effects in humans following exposure to hexachlorobenzene.

Animal data on inhaled hexachlorobenzene are limited. Observations in rats exposed to hexachlorobenzene aerosol included a slight impairment of pulmonary immune defences.

No toxicity studies investigating the effects of hexachlorobenzene after dermal exposure on animals were located.

In oral toxicity studies, serious effects on the hepatic system were observed in short-term animal studies with monkeys, rats, mice and pigs. In monkeys and rats a dose of 1 mg/kg bw/day (LOAEL) has been reported to induce liver damage, no effects were observed at 0.1 mg/kg bw/day (NOAEL). In pigs, the oral NOAEL was 0.05 mg/kg bw/day, based on hepatocellular hypertrophy observed at doses of 0.5 mg/kg bw/day and higher. A study in dogs showed immunological effects at a dose of 0.1 mg/kg bw/day after 1 year exposure (LOAEL). Relatively high doses of hexachlorobenzene tested in short-term gavage and diet studies also showed adverse thyroid effects.

Long-term toxicity

The liver, specifically the haem biosynthesis pathway, is the major systemic target of hexachlorobenzene toxicity. Human data have also shown effects on other systemic targets, including the skin, bone, thyroid and immune system. Additionally, skin lesions occurred as porphyrins, accumulated in the skin, were activated by sunlight (phototoxicity). In the Turkish epidemic*, caused by oral hexachlorobenzene exposure, exposed children under 2 years of age were the most affected, but also children under 15 showed serious toxicity. The effects were seen at an exposure level of 0.8 - 3.3 mg/kg bw/day; the data did not allow the derivation of a NOAEL.

Only animal toxicity studies employing the oral route are available. The critical effects of hexachlorobenzene are hepatic toxicity, reproductive toxicity, develop-

* In the 1950s, widespread ingestion of bread made from grain that had been treated with hexachlorobenzene as a pesticide caused an epidemic in Southeastern Turkey.

mental toxicity, and carcinogenesis. Several studies in rats and mice have observed hepatic porphyria, as well as other hepatic effects such as fibrosis and peribiliary lymphocytosis, hepatomegaly and increased liver weight, enzyme induction, and degenerative pathological changes. In a lifespan oral study with hamsters, a LOAEL of 16 mg/kg bw/day was observed, based on a marked decrease in weight gain. In a 18-month oral study with mice a LOAEL of 13 mg/kg bw/day was observed, based on decreased body weight and hepatocyte hypertrophy. For rats, a LOAEL of 0.016 mg/kg bw/day was identified in a two-generation study showing peribiliary lymphocytosis and liver fibrosis.

Genotoxicity and carcinogenicity

Hexachlorobenzene tested negative in an *in vitro* chromosomal aberration assay and in three out of four bacterial mutation assays. Ambiguous results were obtained in one *in vitro* bacterial mutation assay and in *in vitro* and *in vivo* DNA-binding assays. Two *in vivo* dominant lethal studies in rats and two *in* vivo Comet assays in, respectively, rats and mice were negative. Also a micronucleus test with human peripheral lymphocytes of hexachlorobenzene-exposed workers was negative. Based on these data, the Committee considers hexachlorobenzene to be a non-genotoxic compound.

Human studies are scarce and often inconsistent. Among the general population, the older case-control studies have generally found no association between hexachlorobenzene levels in blood or tissues and incidence of breast or other cancers, while one new study found some association with non-Hodgkin lymphoma. Data from men exposed to hexachlorobenzene by inhalation provide only weak evidence for an association between hexachlorobenzene exposure and cancer of the liver, thyroid, and brain. By and large, the weight of evidence from human studies is insufficient to conclude that hexachlorobenzene causes cancers in humans.

Several animal studies have demonstrated that oral exposure to hexachlorobenzene increases the incidence of tumour formation. The evidence of carcinogenicity is strongest in the liver: hexachlorobenzene has been shown to induce hyperplasia, metaplasia, benign tumours, and malignant tumours in this organ. Additionally, exposure to hexachlorobenzene has been shown to induce renal metaplasia, adenomas and renal cell carcinomas, lymphosarcomas, adrenal hyperplasia and pheochromocytoma, parathyroid adenomas, and thyroid tumours. No oral NOAELs for cancer effects have been identified. The lowest

oral doses for carcinogenic effects were 4 mg/kg bw/day for hamsters and rats and 12 mg/kg bw/day for mice. No animal carcinogenicity studies addressing the dermal and respiratory routes were retrieved from public literature. On the basis of the animal data the Committee is of the opinion that, in accordance with the guidelines of the European Union, hexachlorobenzene needs to be classified as a substance presumed to have carcinogenic potential for humans (category 1b) by a non-genotoxic mechanism of action.

Reproduction toxicity

In human studies, no reliable evidence of adverse effects of hexachlorobenzene on fertility has been uncovered.

Multiple endocrine effects and pathological changes in reproductive organs have been demonstrated in animal studies. The oral NOAEL for fertility effects in monkeys is 0.01 mg/kg bw/day, based on necrosis of surface epithelium cells of the ovaries in 90-day studies.

With respect to a poisoning epidemic in Turkey, the Committee feels that the higher incidence of health effects among children as compared to adults was not necessarily due to a specific interference with developmental processes. The higher incidence of adverse health effects among children may be linked to a higher body burden in children due to breast feeding (infants) and a higher intake of possibly contaminated bread (older children). Other human studies investigating developmental toxicity have been limited by small study size and low levels of hexachlorobenzene exposure; they have found suggestive evidence of an increased risk of undescended testes and impaired development of locomotor skills in newborn babies.

Animal studies have verified that hexachlorobenzene impaired neurological development and reduced neonatal viability and growth. The occurrence of cleft palate, renal agenesis, and minor skeletal abnormalities in mice are consistent with a possible teratogenic role for hexachlorobenzene. A neurodevelopmental study detected evidence of hyperactivity in rat pups. Based on this study, a LOAEL of 2.5 mg/kg bw/day could be established based on minimal neurodevelopmental effects; a NOAEL could not be established. A NOAEL for developmental effects in rats was found to be 0.4 mg/kg bw/day, based on reduced pup viability in a two-generation study; in this study fertility effects were not observed. Immunodevelopmental effects were seen in rats exposed in utero and

during lactation: the antibody response to tetanus toxoid was increased at doses (for the dams) of 0.2 mg/kg bw/day (lowes dose applied) and higher.

Human epidemiological studies do not show a clear association between hexachlorobenzene exposure and developmental effects. However, a number of these studies have methodological limitations and animal studies do show serious developmental effects. Human studies also did not show a clear association between hexachlorobenzene exposure and fertility effects. However, 90-day monkey studies did reveal adverse fertility effects. Therefore, in accordance with the guidelines of the European Union, hexachlorobenzene should be classified as a category 1b reproductive toxicant (presumed human reproductive toxicant), both for efffects on fertility and developmental toxicity. As hexachlorobenzene can be transferred to breast milk, it should also be classified as hazardous to breastfed babies.

Evaluation and advice

For hexachlorobenzene the Dutch Expert Committee on Occupational Safety (DECOS) derived a health-based recommended occupational exposure limit (HBROEL) of 0.006 mg/m^3. Since human-toxicologial data are insufficient, this recommendation is based on data from animal experiments. The basis for the derivation of the HBROEL is the 90-days study with monkeys, in which effects on the reproductive organs were observed. The recommended HBROEL is expressed as an 8 hr time-weighted average concentration.

Chapter 1

Scope

1.1 Background

In the Netherlands, occupational exposure limits for chemical substances are set using a three-step procedure. In the first step, a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Exposure Safety (DECOS), a committee of the Health Council of the Netherlands, at the request of the Minister of Social Affairs and Employment (Annexes A and B). The purpose of the Committee's evaluation is to set a health-based recommended occupational exposure limit for the atmospheric concentration of the substance, provided the database allows the derivation of such a value.

1.2 Committee and procedure

This document contains the assessment of DECOS, hereafter called the Committee, of the health hazard of hexachlorobenzene. The members of the Committee are listed in Annex B.

In 2011, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex C. The Committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The Committee's recommendations on the health-based occupational exposure limit of hexachlorobenzene have been based on scientific data, which are publicly available. For evaluation of the available data before 2001, the toxicological profile on hexachlorobenzene of the ATSDR (2002)[1] was used as starting document. In some instances also the previous report on Health-based recommended occupational exposure limits for hexachlorobenzene[2], published in 1988, was used as a source. For more detailed bibliographic information on the data retrieved from the ATSDR, the reader is referred to the toxicological profile on hexachlorobenzene of the ATSDR (2002).[1]

In Sections 2.2-7.2, first the ATSDR data, which describe the data available before 2001, are mentioned. Subsequently, the additional data (time period 2002-2010) are described. These data were obtained from the following online databases: Toxline, Medline, and Chemical Abstracts. Several chemical names, including the CAS number, were used:

hexachlorobenzene, snieciotox, sanocide, saatbeizfungizid, phenyl perchloryl, perchlorobenzene, pentachlorophenyl chloride, no bunt, hexachlorbenzol, granox nm, esaclorobenzene, anticarie, amatin, 118-74-1.

The literature from this search was selected based on titles and abstracts. The last search was performed on May 2010.

In addition, the following websites were searched for new analytical methods:

- Nederlands Normalisatie-instituut: http://www2.nen.nl/nen/servlet/dispatcher.Dispatcher?id=HOME
- NIOSH Manual of Analytical Methods (NMAM): http://www.cdc.gov/niosh/nmam/
- OSHA: Index of Sampling and Analytical Methods: http://www.osha.gov/dts/chemicalsampling/data/CH_244700.html
- HSE: Methods for the Determination of Hazardous Substances: http://www.hse.gov.uk/pubns/mdhs/#3253
- Air Monitoring Methods (Analyses of Hazardous Substances in Air): http://www.wiley-vch.de/publish/en/

Chapter 2

Identity, properties and monitoring

2.1 Chemical identity

Chemical name	:	hexachlorobenzene
Synonyms	:	Amatin; Anticarie; Bunt-cure; Bunt-no-more; CEKU C.B.; CO-OP Hexa; Esaclorobenzene (Italian); Granox NM; Hexachlorobenzene; Hexa C.B.; Hexachlorbenzol (German); Hexachlorobenzene; Hexachlorobenzene (ACGIH); Julin's carbon chloride; NO Bunt; NO Bunt 40; NO Bunt 80; NO Bunt liquid; Pentachlorophenyl chloride; Perchlorobenzene; Phenyl perchloryl; RCRA waste number U127; Saatbeizfungizid (German); Sanocid; Sanocide; Smut-Go; Snieciotox
Molecular formula	:	C_6Cl_6
CAS-number	:	118-74-1
EC-number	:	204-273-9 (EINECs-number)
RTECS-number	:	DA2975000

2.2 Physical and chemical properties

Hexachlorobenzene is a white, crystalline solid that is practically insoluble in water. When heated to decomposition, it emits toxic fumes of chlorides (ATSDR, 2002[1]). Information regarding the physical and chemical properties of hexachlorobenzene is presented in Table 1.

Table 1 Physical and chemical properties of hexachlorobenzene.

Property	Information
physical description	crystalline solid
colour	white
molar mass (g/mol)	284.78
melting point (°C)	231 °C
boiling point (°C)	325 °C
density (kg/m^3; 23 °C)	2.044
solubility in water (25 °C)	0.006 mg/L
solubility in organic solvents	slightly soluble in ethanol, soluble in ethyl ether, very soluble in benzene
Log $P_{octanol/water}$	5.73
vapour pressure (at 20 °C)	$1.09x10^{-5}$ mmHg
relative density (air=1)	no data
relative vapour density	no data
conversion factor	1 ppm = 11.8 mg/m^3 1 mg/m^3 = 0.08 ppm
flash point	242 °C
odour threshold (mg/m^3)	no data

2.3 EU Classification and labelling

The classification of hexachlorobenzene based on the Regulation on the classification, labelling and packaging of substances and mixtures (1272/2008/EC[3]; see Annex G) and is represented in Table 2. No concentration limits are specified for hexachlorobenzene.

Table 2 Classification of hexachlorobenzene.

compound	CAS number	classification[a]
hexachlorobenzene	118-74-1	carc. Cat. 2; R45 T; R48/25 N; R50-53

[a] carc. = carcinogenicity, Cat. = category, N = dangerous for the environment, R = risk phrase, T = toxic

Also DECOS classified hexachlorobenzene in its earlier evaluation (DECOS, 1988)[4,5] as carcinogenic, stating that it acts presumably via a non-genotoxic mechanism.

2.4 Validated analytical methods

In this Section the analytical methods which are available for detecting and/or measuring and monitoring hexachlorobenzene in air and in biological samples are described. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify hexachlorobenzene. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis.

2.4.1 Environmental monitoring

ATSDR data*

Atmospheric hexachlorobenzene is usually sampled by pulling a volume of air through an adsorbent trap. A filter may be included in the sampling system in order to determine the amount of hexachlorobenzene in particulate. Filters and polyurethane foam (PUF) adsorbent are Soxhlet extracted; XAD-2 adsorbent is extracted in a Soxhlet apparatus or by solvent desorption. Clean-up on adsorbent columns may be utilized. A variety of analytical methods is used: gas chromatography (GC) coupled with electron capture detection (ECD), capillary GC/ECD, GC coupled with photo-ionisation detection (PID), and capillary GC coupled with mass spectrometry (MS). In Table 3, a summary is presented of methods reported in the literature for detecting hexachlorobenzene in air samples.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

* The references used in the ATSDR (2002)[1] have been maintained in the summary presented here and are listed in Annex E.

Table 3 Analytical methods for determining hexachlorobenzene in air samples

Sample matrix	Preparation method	Analytical method	Limit of detection	Reference
Air	Collection on PUF; Soxhlet extraction; cleanup on alumina	(EPA Method TO-10) GC/ECD	No data	EPA 1988a
Ambient air	2,200 m^3 collected on GFF and XAD-2; Soxhlet extraction; cleanup on layered silica gel; alumina partition	cap. GC/MS	0.18 pg/m^3 (calculated)	Hippelein *et al*. 1993
Ambient air	Collection on XAD-2; solvent desorption	GC/PID	0,826 mg/m^3	Langhorst and Nestrick 1979
Ambient air	Collection on PUF; Soxhlet extraction; concentration	dual column megabore GC/ECD or GC/ECD and GC/MS	5 ng/m^3	EPA 1988b

2.4.2 *Biological monitoring*

ATSDR data

Methods for the determination of organochlorine compounds such as hexachlorobenzene generally consist of the following steps: extraction of the analyte from the sample matrix; clean-up to remove interfering compounds; and analysis (separation and quantitation). The primary method of analysis is gas chromatography (GC) coupled with electron capture detection (ECD) or mass spectrometry (MS). Analytical methods have been developed for the determination of hexachlorobenzene in blood or serum, urine, faeces, adipose tissue, and breast milk. A summary of methods is shown in Table 4 (ATSDR, 2002).[1]

Table 4 Analytical methods for determining hexachlorobenzene in biological samples.

Sample matrix	Sample preparation	Analytical method	Limit of detection	Reference
adipose tissue	extraction, GPC clean-up, florisil fractionation, optional additional clean-up	GC/ECD; confirmation by GC/MS	12 ng/g	EPA 1986
adipose tissue	maceration with sodium sulphate, extraction and back extraction, florisil fractionation	GC/ECD	No data	EPA 1980
adipose tissue	soxhlet extraction, clean-up on florisil	GC/PID	1 ng/g	Alawi *et al*. 1992
adipose tissue	solvent extraction, filtration, florisil, fractionation	cap. GC/ECD; confirmation by NICI	0.12 ng/g	Mes *et al*. 1982
adipose tissue	SFE with alumina (to remove lipids, purification by column chromatography	cap. GC/ECD	10 ng/g (fatty tissue)	Djordjevic *et al*. 1994
breast milk	separation of fat; column clean-up	cap. GC/ECD	0.4 ng/g fat	Abraham *et al*. 1994
breast milk	acid treatment, elute from silica gel, concentrate	GC/ECD	9 ng/g	Stachel *et al*. 1989
blood	solvent (hexane) extraction, concentration	GC/ECD	No data	EPA 1980
blood	solvent extraction, clean up on silica gel, concentration	GC/PID	16 ng/g	Langhorst and Nestrick 1979
blood	homogenization with benzene, filtration, Florisil fractionation	cap. GC/ECD; confirmation by GC/MS	0.2 ng/g	Mes *et al*. 1982
blood	hexane extraction, concentration	GC/ECD; confirmation by GC/MS	0.16 ng/g	Bristol *et al*. 1982
serum	solvent extraction of denatured serum, fractionation on micro-florisil column, acid treatment/silica gel clean-up	GC/ECD	1 ng/g	Burse *et al*. 1990
urine	solvent extraction, clean-up on silica gel, concentration	GC/PID	4.1 ng/g	Langhorst and Nestrick 1979
semen	solvent extraction, clean-up on florisil, concentration	cap. GC/ECD; confirmation by NICI	0.3 ng/mL	Stachel *et al*. 1989
saeces	boiling with solvent, clean-up on alumina	cap. GC/ECD	No data	Abraham *et al*. 1994

cap. = capillary; ECD = electron capture detector; GC = gas chromatography; GPC = gel permeation chromatography; MS = mass spectrometry; NICI = negative ionization chemical ionization; PID = photo ionization detector; SFE = supercritical fluid extraction.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

Chapter 3

Sources

3.1 Natural occurrence

Hexachlorobenzene does not occur naturally.

3.2 Man-made sources

3.2.1 Production

ATSDR data and data from previous Health Council report[2,5]

Processes for direct production of hexachlorobenzene use benzene or hexachlorocyclohexane as raw materials. Hexachlorobenzene can be produced by reacting benzene with excess chlorine in the presence of ferric chloride at 150-200 °C. The reaction products are cooled to 100 °C to allow the hexachlorobenzene to crystallize. Another direct process uses isomers of hexachlorocyclohexane which are refluxed with sulphuryl chloride or chlorosulphonic acid. Reaction temperatures of 130 to 200 °C are used in the presence of a ferric chloride or an aluminium chloride catalyst. In addition, at least one former producer isolated hexachlorobenzene from distillation residues obtained as a by-product in the manufacture of tetrachloroethylene.

In the Netherlands, hexachlorobenzene is not produced in a direct way. Hexachlorobenzene can arise as a by-product or impurity in the manufacture of several chlorinated solvents (*e.g.*, tetrachloroethylene, trichloroethylene, carbon tetrachloride), other chlorinated compounds (*e.g.*, vinyl chloride, trichlorobenzenes, trichlorotoluenes, chlorophenols), and several pesticides, including tetrachloroisophthalonitrile (chlorothalonil), pentachloronitrobenzene (PCNB), 4-amino-3,5,6-trichloropicolinic acid (picloram), pentachlorophenol (pentachlorophenol), dimethyltetrachloroterephthalate (DCPA or Dacthal®), atrazine, propazine, simazine, and mirex. Of the mentioned pesticides, chlorothalonil is the only one currently in use in The Netherlands.[6]

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

3.2.2 Use

ATSDR data and data from previous Health Council report[2]

Hexachlorobenzene has been used as a fungicide on the seeds of onions, sorghum, wheat, and other grains. At present the use of hexachlorobenzene as grain fungicide is prohibited in most Western countries. Hexachlorobenzene was also used in the production of pyrotechnic and ordinance materials for the military, the production of synthetic rubber, as a porosity controller in the manufacture of electrodes, a chemical intermediate in dye manufacturing, a wood preservative and as a feedstock in the production of pentachlorophenol. Hexachlorobenzene is not used in the Netherlands as raw material or semi-manufactured product.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

Chapter 4

Exposure

4.1 General population

ATSDR data and data from previous Health Council report[2]

Hexachlorobenzene is no longer produced (as an end-product) or used as a pesticide. Consequently, the current potential for exposure of the general population appears to be very limited. However, some exposure is possible, as many studies have detected small amounts in food and air samples, particularly in those with high lipid content such as meat, poultry, and fish. Traces of hexachlorobenzene have been found in almost all people tested for hexachlorobenzene or its metabolites. These amounts of hexachlorobenzene are most likely the result of consumption of low levels in food.

Other sources of exposure may include contact with contaminated soil and air, but general population exposure to hexachlorobenzene via inhalation or dermal contact would be much less compared to potential oral exposure.

In survey studies conducted in the USA in the early 1970s, hexachlorobenzene was detected in 14 out of 3,246 soil samples (0.4%), the levels varied from 10 to 440 ng/g. Lake sediments measured in the USA in 1992 contained <1 to 1.5 ng/g (dry weight), in 1986 two out of twelve lake sediment samples in Russia contained 3.5 and 14.6 ng/g.

Air samples taken in a semirural area in the UK in 2000 contained at average 39 pg/m^3 (ranging from <29 to 76 pg/m^3). In 1997 air samples in Croatia ranged from 29-31 pg/m^3.

Surface waters from mountain lakes in Austria, Norway and Spain analysed in 2001 contained hexachlorobenzene in levels from 4 to 8 pg/L. In 1998 the Mediterranean Sea near Alexandria (Egypt) had levels from 12-27 ng/L.

Hexachlorobenzene has a very low solubility in water, so exposure by water is not likely to be significant. Children are expected to be exposed to hexachlorobenzene by the same routes as adults. Additionally, if hexachlorobenzene is present in their mothers, unborn children may be exposed through the placenta, and nursing children may be exposed to hexachlorobenzene present in milk. Human milk samples from the general population found hexachlorobenzene concentrations in the range of 4-640 ng/g fat (see also Section 5.4.2).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

Wang *et al*. (2010)[7] reported hexachlorobenzene levels in the environment in China. In soil they found levels ranging from 0.007-12.7 ng/g, and in lake and river sediments levels ranging from 0.03-16.7 ng/g. Water levels generally varied between 0.5 and 30 ng/L, but in the Tonghui River levels up to 660 ng/L were found, in the Yellow River estuary 1,260 ng/L, and in the Huaihe River 4,700-12,200 ng/L. Sediment levels were between 0.05 and 16 ng/g, but soils and sediments in the neighbourhood of chemical plants were in general quite contaminated: in the Hubei province sediment levels up to 2.3 mg/g have been found. Median concentrations in human milk from inhabitants of various cities were between 10 and 200 ng/g fat, but in the cities of Luqiao and Pingqiao (Zhejiang province) median levels of 38,500 and 48,200 ng/g fat were found, respectively. Liu *et al*. (2009)[8] analysed air samples taken in 37 Chinese cities, and found levels from 16 to 1,750 pg/m^3, with distinctive seasonal and regional variations.

Perelló *et al*. (2009)[9] showed that cooking processes hardly have any influence on the level of hexachlorobenzene in the foodstuffs they investigated (fish, meat, vegetables, and olive oil).

4.2 Working population

ATSDR data

Although hexachlorobenzene is not currently manufactured as a commercial product, some hexachlorobenzene can arise as a by-product or impurity in the manufacture of chlorinated solvents and other chlorinated compounds (Section 3.2.1). Therefore, occupational exposure is possible for workers involved in this type of production or in the formulation/application of plant protection products containing chlorothalonil as an active ingredient. However, because quantitative information on the production of chemicals in which hexachlorobezene is potentially formed as an impurity is lacking, it is not possible to estimate the potential hexachlorobenzene-exposure of current workers in the Dutch chemical industry or elsewhere in the Netherlands.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

Chapter 5

Kinetics

5.1 Absorption

ATSDR data

In humans, inhalation accounts for an unknown amount of exposure. Burns *et al.* (1974) and Currier *et al.* (1980) reported blood levels of hexachlorobenzene in occupationally exposed men and found a relation with working years with pesticides (Burns *et al.* 1974) and working years in plants manufacturing chlorinated solvents, but did not find a relation with hexachlorobenzene levels in the work environment. Burton and Bennet (1987) took representative values of hexachlorobenzene concentrations in human adipose tissue and quantified the relative contributions from oral and respiratory exposure using average hexachlorobenzene concentrations in food, air, soil and water. For this the authors applied a PBPK model in which the oral and respiratory absorptions were assumed to be 80% and 50%, respectively (according to the authors these assumptions have no solid basis). They concluded that respiratory exposure to hexachlorobenzene contributed less than 1% to the total body burden of hexachlorobenzene in adipose tissues.

Based on information from an epidemic resulting from ingestion of hexachlorobenzene-contaminated bread in Turkey (see Section 7.1.4, reproduction toxicity), ingested hexachlorobenzene is moderately absorbed from the gastrointestinal tract (Albro and Thomas 1974; Cam and Nigogosyan 1963; Gocmen

et al. 1989; Peters *et al.* 1982). However, most of the hexachlorobenzene body burden in the US population derives from dietary intake of fatty foods (Burton and Bennett 1987). Schlummer *et al.* (1998) estimated that 85.4% of ingested hexachlorobenzene will be absorbed when the blood contains no hexachlorobenzene, and that this percentage will be reduced by 0.2% for each ng of hexachlorobenzene per g blood lipid.

Data from animal studies indicate that the gastrointestinal absorption of hexachlorobenzene is quite variable, depending upon the solvent vehicle used for administration, ranging from 6% when administered in aqueous solution to 82% when administered with squalene in cottonseed oil (Albro and Thomas 1974), olive oil (Freeman *et al.* 1989; Goldey *et al.* 1990; Knauf and Hobson 1979; Koss and Koransky 1975; Mehendale *et al.* 1975; Sundlof *et al.* 1982; Villeneuve and Hierlihy 1975), or peanut oil (Ingebrigtsen and Nafstad 1983; Ingebrigtsen *et al.* 1981). The lymphatic system has been shown to play an important role in the gastrointestinal uptake of hexachlorobenzene in animals (Iatropoulos *et al.* 1975).

No empirical data are available on the dermal absorption of hexachlorobenzene in humans. The absorption of dermally applied solid ^{14}C-hexachlorobenzene (ca. 625 µg/cm^2) was investigated in the rat under occluded conditions. The absorbed portion* increased from 1% at 6 hours and 2.7% at 24 hours to 9.7% at 72 hours after dosing, and blood concentrations of ^{14}C increased linearly with time. The average dermal absorption constant for hexachlorobenzene was calculated as $1.40 \pm 0.33 \times 10^{-3}$ per hour (absorption rate 0.9 ± 0.2 µg/cm^2/h), while for the first 6 h of exposure it was 1.76×10^{-3} per hour (absorption rate 1.1 µg/cm^2/h) (Koizumi 1991).** [10] The assumption of linearity was justified based on the linearity of cumulative absorption (measured at 6, 24 and 72 h after onset of exposure). The authors defined the dermal absorption constant as the ratio of absorption rate (mg/cm^2/h) and applied dose (mg/cm^2). However, in view of the considerable amount of radioactivity recovered from the skin after 72 h of exposure (>80%) and the very poor solubility of hexachlorobenzene in water the Committee concluded that saturation of uptake was reached. Therefore, the absorption rate would have been at its maximum and independent of the applied dose of solid hexachlorobenzene. This renders the absorption constant meaningless for doses other than the one investigated. Consequently, for risk assessment

* Defined as the total amounts of radioactivity recovered from urine, faeces, liver, carcass, and the remaining skin (except the dose area) and subcutaneous tissues.

** The summary of this study, although also summarized in the ATSDR document, is based on the original article.

purposes dermal absorption should be estimated using the average (maximum) flux calculated by Koizumi (1991)[10]: 0.9 ± 0.2 μg/cm^2/h.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

5.2 Distribution

5.2.1 Distribution through the body

ATSDR data

Information on distribution in people following inhalation exposure to hexachlorobenzene is limited (Ataniyazova *et al.* 2001; Sala *et al.* 2001b) and no information is available on the distribution of hexachlorobenzene in animals after inhalation.

Orally absorbed hexachlorobenzene distributes widely in mammalian tissue, rapidly partitioning to blood, liver, breast milk, adipose tissue, endocrine organs, bone marrow, and ovarian follicular fluid (Ellenhorn and Barceloux 1988; Foster *et al.* 1995[11]; Ingebrigtsen 1986; Ingebrigtsen and Nafstad 1983; Knauf and Hobson 1979; Wickstrom *et al.* 1983), and preferentially distributing to adipose tissue or organs with high fat content (Burton and Bennett 1987; Cohn *et al.* 1978; Koss and Koransky 1975; Lecavalier *et al.* 1994; Mehendale *et al.* 1975; Robinson *et al.* 1990; Teufel *et al.* 1990; Van Raaij *et al.* 1993a; Verschueren 1983). In a survey of the US population, it was found that the concentration of hexachlorobenzene in adipose tissue tended to increase with increasing age, a testimony to the propensity of hexachlorobenzene to bioaccumulate in mammalian tissue (Robinson *et al.* 1990). Animal studies of oral dosing have shown that levels of hexachlorobenzene increase in a dose-dependent manner in all tissues up to doses of 100 mg/kg bw/day (Foster *et al.* 1995[11]; Jarrell *et al.* 1993[12]; Sundlof *et al.* 1982).

No studies were located regarding distribution of hexachlorobenzene following dermal exposure in humans. Male Fischer 344 rats that received a single dermal dose of approximately 2.5 mg/kg bw ^{14}C-hexachlorobenzene dissolved in tetrachloroethylene applied to a 4 cm^2 clipped area on the back absorbed only 9.7% of the dose; 90.3% of the applied dose remained unabsorbed on the skin after 72 hours. The concentration of hexachlorobenzene in the liver and blood

increased steadily after dermal application. Washing the test area with soap 6 h after hexachlorobenzene application removed 34% of the applied dose and reduced the cumulative amount absorbed after 72 h by 50%. The authors developed a compartment model based on the data collected, for application to a 70-kg worker (Koizumi 1991).[10]

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

5.2.2 Placental transfer

ATSDR data

Hexachlorobenzene body burden is readily transferred from pregnant mother to the foetus through the placenta in animals (Courtney and Andrews 1985; Courtney *et al.* 1979; Cripps 1990; Goldey *et al.* 1990; Nakashima and Ikegami 2000; Nakashima *et al.* 1997, 1999; Villeneuve and Hierlihy 1975; Villeneuve *et al.* 1974).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

5.3 Metabolism

ATSDR data

In mammals, hexachlorobenzene is slowly metabolized to pentachlorophenol by the hepatic cytochrome P-450 system, conjugated with glutathione and further metabolized to yield pentachlorothiophenol, or reductively dechlorinated to form pentachlorobenzene. Other metabolites include lower chlorinated benzenes, chlorophenols, thiophenols, and benzenes (Den Besten *et al*. 1994; Hahn *et al.* 1988, 1989; Koss *et al*. 1979, 1986; Ingebrigtsen *et al.* 1986; Linko *et al.* 1986; Mehendale *et al.* 1975; Mehmood *et al*. 1996; Renner 1988; Schielen *et al*. 1995a; Rozman *et al.* 1977). Pentachlorophenol is subsequently converted to tetrachlorohydroquinone (Mehmood *et al*. 1996, Van Ommen *et al*. 1985). It has

been suggested that epoxide formation also occurs in the biotransformation (Lui *et al.* 1976). The main biotransformation pathways of hexachlorobenzene are depicted in Figure 1.

After oral hexachlorobenzene exposure of mammals, the faeces contain mostly unchanged parent compound, about 1% pentachlorobenzene, and traces of pentachlorophenol. Urinary excretion mostly consists of the metabolites, pentachlorobenzene, 2,4,5-trichlorophenol, *N*-acetyl-*S*(pentachlorophenyl)cysteine, mercaptotetrachlorothioanisole, and tetrachlorobenzene, 2,3,5,6-tetrachlorobenzene-1,4-diol, and unchanged parent compound (Koss *et al.* 1978;

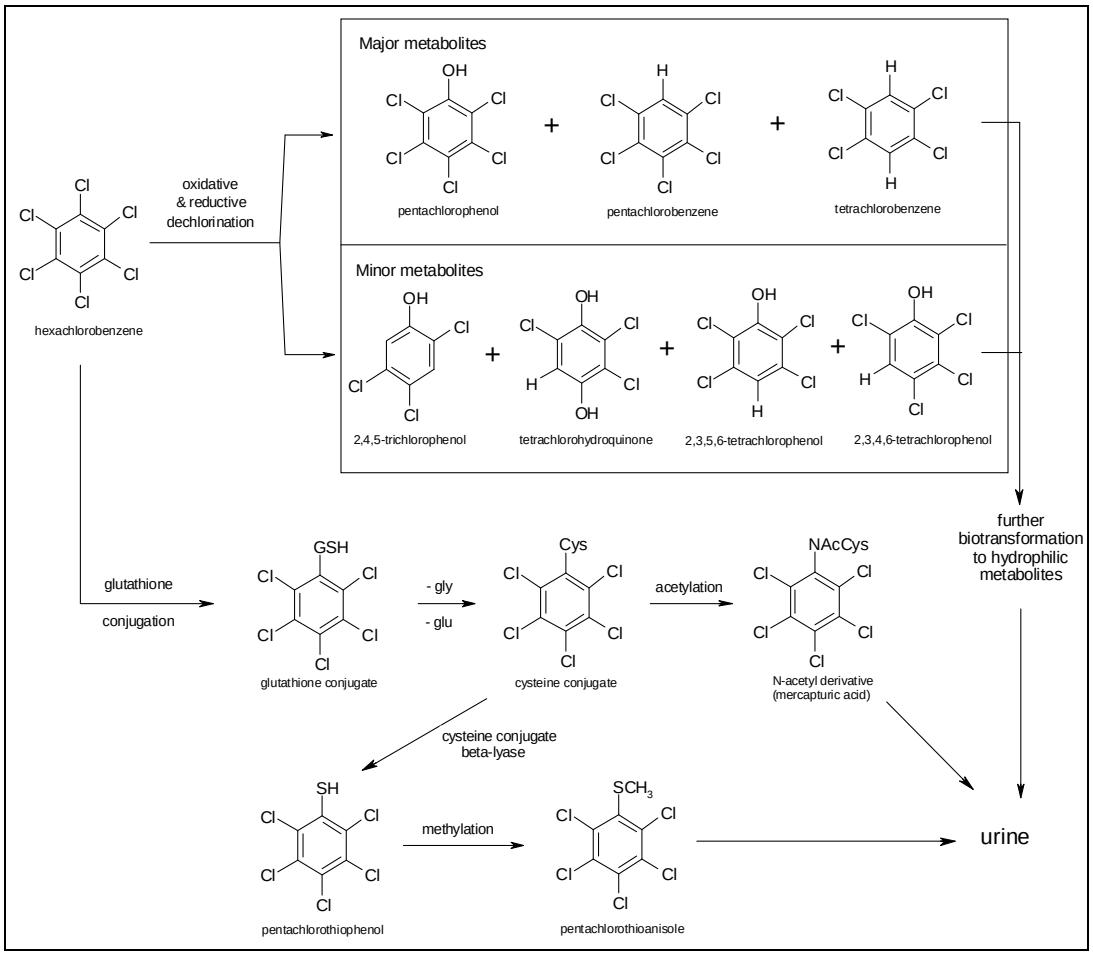

Figure 1 Metabolism of hexachlorobenzene (derived from ATSDR 2002).[1]

Mehendale *et al.* 1975; Rizzardini and Smith 1982; Rozman *et al.* 1978). Pentachlorothiophenol, pentachlorophenol, methylthiopentachlorobenzene, 1,4-bis-(methylthio)-2,3,5,6-tetrachlorobenzene, chlorophenols, S-conjugated phenols and benzenes, and lower chlorinated benzenes have also been identified in the liver following oral exposure in animals (D'Amour and Charbonneau 1992; Engst *et al.* 1976; Ingebrigtsen *et al.* 1981, 1986; Jansson and Bergman 1978; Koss *et al.* 1976, 1979; Lui and Sweeney 1975; Renner 1988; Richter *et al.* 1981; Stewart and Smith 1986; To-Figueras *et al.* 1992; Van Ommen *et al.* 1985, 1989; Yang *et al.* 1978). Sex differences in the metabolism of hexachlorobenzene in the adult animals have been reported: urinary excretion of pentachlorophenol, 2,3,5,6-tetrachlorobenzene-1,4-diol, and pentachlorothiophenol was greater in females than in males in this study (Rizzardini and Smith 1982).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

5.4 Elimination

5.4.1 Elimination from the body

ATSDR data

To-Figueras *et al.* (2000) observed a high correlation between faecal and blood levels of hexachlorobenzene in a group of 25 men and 28 women from Flix (Catalonia region), Spain. This population was highly exposed to airborne hexachlorobenzene from a nearby plant that produced chlorinated solvents. The geometric mean of hexachlorobenzene concentration in blood was 30 µg/L. The estimated faecal excretion of unchanged hexachlorobenzene was 10 µg/day. No unchanged hexachlorobenzene was detected in urine; urinary excretion of metabolites was 5 µg/day.

No information is available on the excretion of hexachlorobenzene following inhalation exposure in animals or following dermal exposure in humans or animals.

In humans, ingested hexachlorobenzene is excreted in the urine mainly as its metabolites pentachlorophenol and pentachlorothiophenol (To-Figueras *et al.* 1992). In animals (rats and rhesus monkeys), the excretion of hexachlorobenzene appears to be quite variable, depending upon the solvent vehicle used (Albro and

Thomas 1974; Rozman *et al.* 1977). Based on decreasing concentrations in the liver, the biological half-life of hexachlorobenzene has been estimated to be 8 days at the start of the elimination phase, 10 weeks after 3 months, and 12 months after 1.5 years (Koss *et al.* 1983), suggesting differential release of hexachlorobenzene from tissue stores, perhaps as a function of lipophilicity. Ingested hexachlorobenzene is excreted predominantly in the faeces, mainly as unchanged parent compound, and to a lesser extent in the urine, as its metabolites (pentachlorophenol, pentachlorothiophenol, pentachlorobenzene) (Mehendale *et al.* 1975). In rhesus monkeys treated with 0.03 mg/kg bw/day in the diet for 15 months, approximately 99% of ingested hexachlorobenzene was excreted in the faeces as the parent compound; 50% of the urinary excretion was pentachlorophenol, 25% was pentachlorobenzene, and 25% was parent compound (Rozman *et al.* 1977). Based on animal studies, the urinary excretion of hexachlorobenzene exhibits sex- and age-specific differences; the excretion of pentachlorothiophenol increases with sexual maturity in female rats and slightly decreases in male rats (To-Figueras *et al.* 1991). Biliary excretion was not an important excretory pathway in rats given a single hexachlorobenzene dose of 10 mg/kg bw by gavage in peanut oil, accounting for <4% of the administered dose (Ingebrigtsen *et al.* 1981).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

5.4.2 *Elimination in human milk*

ATSDR data

Hexachlorobenzene accumulates in the milk and can be transferred to the suckling neonate (Bailey *et al.* 1980; Cripps 1990; Goldey *et al.* 1990; Nakashima and Ikegami 2000; Nakashima *et al.* 1997, 1999). Levels in milk samples from the general population generally varied between 20 (women living in New Zealand) and 640 (women living in Prague, Czech Republic) ng/g fat, equalling approximately 0.6-19.2 ng/g milk (Bates *et al.* 1994; Schoula *et al.* 1996). In a group of 350 German children, blood hexachlorobenzene levels (and levels of other organochlorines) correlated strongly with the length of breast-feeding (Karmaus *et al.* 2001).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

Eggesbø *et al.* (2009)[31] reported levels of 4.4-42.0 ng hexachlorobenzene per g fat (equalling approximately 0.1-1.3 ng/g milk) in human milk in Norway, with a mean level of 12.0 ng/g fat or 0.36 ng/g milk. In human milk in China levels varying between 20 and 800 ng/g fat (equalling approximately 0.6-24 ng/g milk) were found (Wang *et al*. 2010).[7]

5.5 Possibilities for biological monitoring

ATSDR data

Human tissues and body fluids that have been analyzed for hexachlorobenzene to identify and quantify exposure include blood and serum (Ataniyazova *et al.* 2001; Glynn *et al.* 2000; Hagmar *et al.* 2001; Karmaus *et al.* 2001; Rutten *et al.* 1988; Sala *et al.* 1999b, 2001b; Schlummer *et al.* 1998; Siyali 1972; Waliszewski *et al.* 2001; Weiderpass *et al.* 2000; and many others), liver (Dewailly *et al.* 1999; Weistrand and Noren 1998), bone marrow (Bucholski *et al.* 1996; Scheele *et al.* 1995), brain (Dewailly *et al.* 1999), fat (Ansari *et al.* 1986; Dewailly *et al.* 1999; Lordo *et al.* 1996; Robinson *et al.* 1990; Scheele *et al.* 1995; Siyali 1972; Szymczynski and Waliszewski 1981; Teufel *et al.* 1990; Weistrand and Noren 1998), semen (Szymczynski and Waliszewski 1981), the placenta (Poli *et al.* 1999), the umbilical cord (Burse *et al.* 2000; Darvill *et al.* 2000), and breast milk (Ataniyazova *et al.* 2001; Craan and Haines 1998; Czaja *et al.* 1997; Darvill *et al.* 2000; Dewailly *et al.* 2000; Fitzgerald *et al.* 2001; Gladen *et al.* 1999; Gocmen *et al.* 1989; Huang *et al.* 1989; Lunden and Noren 1998; Newsome and Ryan 1999; Scheele *et al.* 1995; Weisenberg 1986; Wickstrom *et al.* 1983; and others).

Reliable methods are also available to measure hexachlorobenzene in faeces (Albro and Thomas 1974; Koss and Koransky 1975; Schlummer *et al.* 1998) and urine. Trace amounts of unchanged hexachlorobenzene have been detected in urine; however, urinary metabolites are more easily detected and quantified (Ingebrigtsen *et al.* 1981, 1986; Koss *et al.* 1976; Lui and Sweeney 1975; Rozman *et al.* 1977; To-Figueras *et al.* 1992; Van Ommen *et al.* 1985; Yang *et al.* 1978) as biomarkers for hexachlorobenzene exposure. Although urinary pentachlorophenol and tissue hexachlorobenzene correlated in 60 patients studied, it

is possible that the urinary pentachlorophenol originated from other chlorinated hydrocarbons such as pentachlorobenzene, alpha-hexachlorocyclohexane, or pentachloronitrobenzene (Burton and Bennett 1987; Currier *et al.* 1980; Koss *et al.* 1986; To-Figueras *et al.* 1992).

Indirect biomarkers of hexachlorobenzene exposure include measurement of gamma-glutamyl transferase in blood, uroporphyrin and δ-aminolevulinic acid in urine, and coproporphyrin in faeces (Koss *et al.* 1986; To-Figueras *et al.* 1992). Because these biomarkers are not specific for hexachlorobenzene, their usefulness in monitoring exposed populations is limited.

Several studies have correlated hexachlorobenzene levels with different end points. In humans, hexachlorobenzene levels are correlated between faeces and serum (To-Figueras *et al.* 2000), maternal and umbilical cord blood levels (Ataniyazova *et al.* 2001; Sala *et al.* 2001a; Waliszewski *et al.* 2000), length of breast-feeding and infant serum levels (Abraham *et al.* 2000), and the presence of other organochlorines in serum (Burse *et al.* 2000; Glynn *et al.* 2000; Hoppin *et al.* 2000; and others).

Sufficient data of air levels of hexachlorobenzene have not been available to determine quantitative biomarkers of inhalation exposure. However, hexachlorobenzene levels have been assayed in people of Flix (Spain), who were exposed to hexachlorobenzene from a nearby plant (see Section 5.4.1; Ballester *et al.* 2000; Grimalt *et al.* 1994; Herrero *et al.* 1999; Sala *et al.* 1999a, 1999b; To-Figueras *et al.* 1997). These studies found higher serum levels in factory workers compared to non-workers, in male workers compared to females (presumably due to increased work-related exposure), in non-workers who lived with factory workers compared to non-workers who did not live with factory workers, and in people living near the factory compared to people living further away.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

5.6 Possibilities for biological effect monitoring

ATSDR data

Although not specific to hexachlorobenzene, hepatic porphyria is the primary biomarker of effect from human exposure to hexachlorobenzene. Disturbance of the haem biosynthesis pathway of the body's porphyrin metabolism in the liver is

the major action of hexachlorobenzene in short- or long-term exposure. Due to this disturbance, abnormal levels of porphyrin precursors are found in exposed individuals. In some cases, porphyria cutanea tarda, displayed as scarring or cutaneous annular erythema (a condition termed pembe yara, or pink sore), is present. Such exposed people also exhibited painless arthritis, osteoporosis, and small distinctive hands (Cripps *et al.* 1984; Peters *et al.* 1982, 1987). Increases in serum γ-glutamyl transferase, uroporphyrin (red-tinged urine), and δ-aminolevulinic acid in the urine, and uroporphyrin and coproporphyrin in the stool are also indicative of the effect of hexachlorobenzene. While low levels of hexachlorobenzene have been found in human tissues and body fluids, such reported low levels have not generally been associated with adverse health effects (Booth and McDowell 1975). Recently, associations have been found between increased hexachlorobenzene levels and decreased interferon-γ (Daniel *et al.* 2001), decreased lymphocyte IL-10* secretion (Belles-Isles *et al.* 2000), ear infections in infants (Dewailly *et al.* 2000), undescended testis (Hosie *et al.* 2000), and locomotor skill impairment in newborns (Sala *et al.* 1999b).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

5.7 Summary

No data are available on the inhalatory absorption of hexachlorobenzene in humans or animals. No data are available on dermal absorption of hexachlorobenzene in humans, while in rats a dermal absorption rate of 0.9 μg/cm^2/h was established.

Oral absorption of hexachlorobenzene in humans via dietary intake with fatty foods is estimated at approximately 85%, the absorption decreases with increasing hexachlorobenzene blood levels. Data from animal studies indicate that the gastrointestinal absorption of hexachlorobenzene is variable, depending upon the solvent vehicle used for administration, ranging from 6% when administered in aqueous solution to 82% when administered in vegetable oils.

Information on distribution in people following inhalation exposure to hexachlorobenzene is limited and no information is available on the distribution of hexachlorobenzene after inhalation in animals. Orally absorbed hexachlo-

* IL = interleukin, cytokine immune system signalling molecule.

robenzene distributes widely in mammalian tissue, rapidly partitioning to blood, liver, adipose tissue, endocrine organs, bone marrow, and ovarian follicular fluid, and preferentially distributing to adipose tissue or organs with high fat content. In animals, hexachlorobenzene body burden is readily transferred from pregnant mother to the foetus through the placenta.

Hexachlorobenzene is slowly metabolized in mammals, and the majority is excreted unchanged (in faeces). Reductive dechlorination of hexachlorobenzene (to pentachlorobenzene) appears to be an important pathway. But also oxidation to pentachlorophenol and conjugation with glutathione yielding pentachlorothiophenol does take place. Other metabolites include lower chlorinated benzenes, chlorophenols, and S-conjugated phenols and benzenes. Pentachlorophenol is subsequently converted to tetrachlorohydroquinone.

No information is available on the excretion of hexachlorobenzene following inhalation exposure in animals or following dermal exposure in humans or animals. In animals, the excretion of hexachlorobenzene appears to be quite variable, depending upon the solvent vehicle used. Based on decreasing concentrations in the liver, the biological half-life of hexachlorobenzene has been estimated to be 8 days at the start of the elimination phase, 10 weeks after 3 months, and 12 months after 1.5 years, suggesting differential release from tissue stores, perhaps as a function of lipophilicity.

Hexachlorobenzene accumulates in mammalian tissues including milk and can be transferred to the suckling neonate.

Chapter 6

Mechanisms of action

The mechanisms of the two important effects of hexachlorobenzene, hepatic porphyria and carcinogenesis, have been extensively investigated and are described below. In searching recent literature, several studies regarding effects of hexachlorobenzene on signalling pathways or gene expression were encountered (Plante *et al.* 2005, 2007; Randi *et al.* 2008). These studies are not summarized here as they do not yet elucidate the mechanisms underlying the hexachlorobenzene-induced carcinogenesis or hepatic porphyria.

6.1 Porphyria

ATSDR data

The main effect of hepatic porphyria is slowed or stopped formation of haem, the oxygen-carrying part of the haemoglobin molecule found in red blood cells and an important chemical in the body. Hepatic porphyria is identified by elevation of haem precursors (porphyrins), in the blood, urine and faeces. Hexachlorobenzene induces porphyria via an increased δ-aminolevulinic acid synthase activity (the enzyme that controls the rate of porphyrin production) and a decreased uroporphyrinogen decarboxylase activity (the enzyme that converts uroporphyrinogen III to coproporphyrinogen III; see Figure 2). Uroporphyrinogen decarboxylase (a cytosolic enzyme) converts uroporphyrinogen III to coproporphyrinogen III by the stepwise decarboxylation of the four acetic acid side chains to leave methyl

residues, but the corresponding porphyrin (uroporphyrin III) cannot be decarboxylated and will not be metabolized further. However, the accumulation of uroporphyrinogen intermediates in the liver is mainly due to a deficiency of uroporphyrinogen decarboxylase. This hypothesis led to the proposal that in certain porphyrias where uroporphyrin accumulates (uroporphyrias), the mechanism responsible may be an accelerated oxidation of uroporphyrinogen, causing an "oxidative escape" of this intermediate from the pathway of haem biosynthesis (Meola and Lim 1993; Dowdele *et al*. 1967; Rajamanickam *et al*. 1972; Smith and De Matteis 1990; Strand *et al*. 1971; Thadani *et al*. 2000[57]).

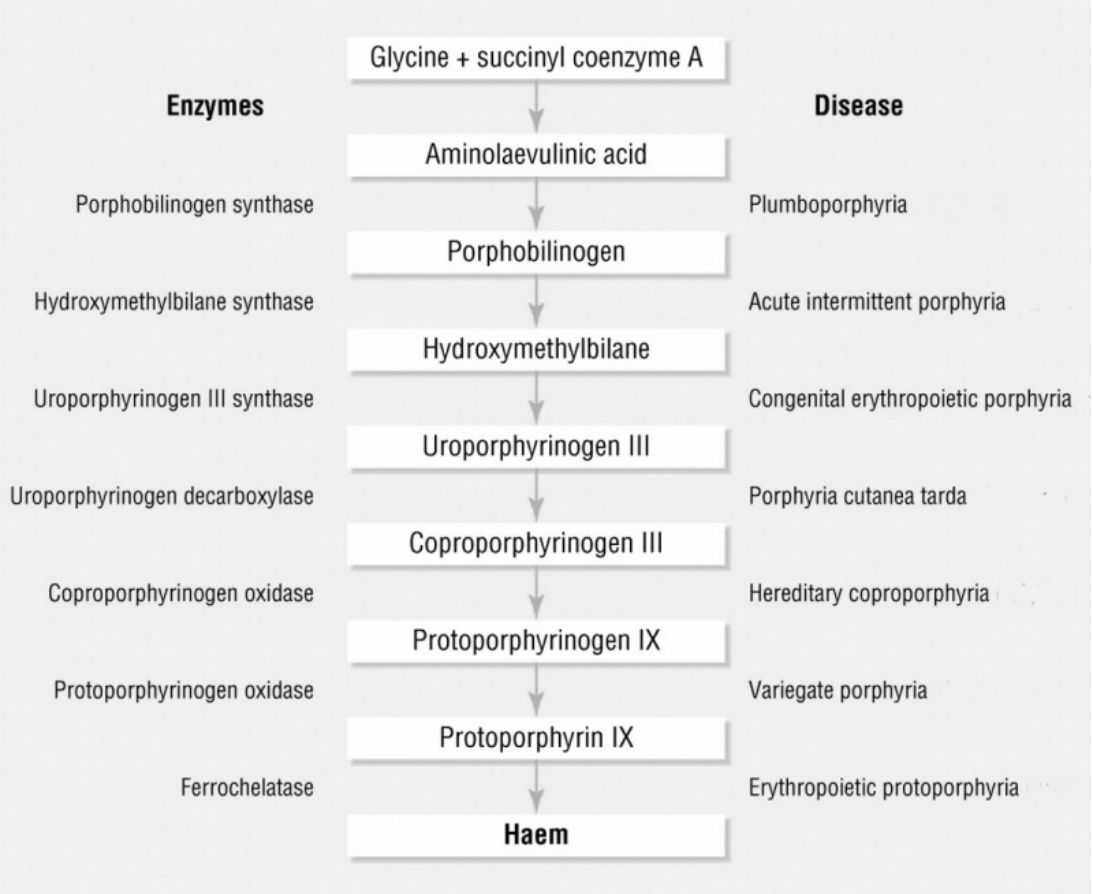

Figure 2 Haem synthesis (taken from Thadani *et al*. 2000[57], with permission).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

Two studies investigated biochemical effects of hexachlorobenzene; one study demonstrated an increase in arachidonic acid (AA) metabolism caused by hexachlorobenzene[13], another showed that it causes a reduction in plasma corticosterone concentration and in the number of hepatic glucocorticoid receptors[14]. Both effects were speculatively linked to the onset or modulation of hepatic porphyria.

6.2 Carcinogenesis

ATSDR data

Several animal studies have demonstrated that oral exposure to hexachlorobenzene increases the incidence of tumour formation. The mode-of-action for the carcinogenicity of hexachlorobenzene has been investigated in several mechanistic studies. In a tumour promotion study, treatment of Sprague-Dawley rats with 10 mg/kg bw/day of hexachlorobenzene for 45 days did not induce hepatic foci. However, foci were induced following liver initiation by partial hepatectomy with and without N-nitrosodiethylamine (Pereira *et al.* 1982). Tsuda *et al.* (1993) found no altered foci in male Fisher 344 rats pre-treated with partial hepatectomy and treated with a single gavage dose of 5,000 mg/kg bw hexachlorobenzene followed by liver tumour promotion with carbon tetrachloride and cholic acid for 12 weeks. The authors suggest that hexachlorobenzene may act as a promoter, but not as an initiator of liver cancer.

Additional data to the ATSDR toxicological profile

The potential of hexachlorobenzene to promote the development of carcinogenesis has been confirmed in several additional studies. The promoting role of hexachlorobenzene in tumour promotion model systems has been demonstrated by Ou *et al*. (2003)[15] in a combined experimental and simulation approach of hepatic pre-neoplastic foci development. In this study, hexachlorobenzene was found to increase the volume of glutathione-S-transferase (GST) Pi positive foci induced by N-nitrosodiethylamine. Modelling analysis with various chlorobenzenes did not show a correlation between induction of normal hepatocyte proliferation (which hexachlorobenzene increases), altered GSH/GSSG status, induction of cytochrome P450 2E1 (CYP 2E1) or induction of hepatic porphyria and the stimulation of clonal growth of GST-Pi positive foci. Secondly, Ichihara

et al. (2005)[16] found that hexachlorobenzene causes a weak promotion of hepatocarcinogenesis in a rat medium-term liver bioassay. The development of glutathione S-transferase placental positive foci in the male F344/DuCrj rat liver was evaluated with 11 different dietary doses, ranging from 0.002 to 75 ppm (equivalent to 0.17 µg to 6.5 mg per kg bw per day)*. After initiation of N-nitrosodiethylamine, the numbers and areas of GST-placental positive foci in the 0.002 to 40 ppm group were comparable to the control value. Only at the level of 75 ppm a marginal increase in both parameters was observed, thus suggesting (in this experiment) the existence of a threshold for this endpoint. Thirdly, Randi *et al*. (2006)[17] found no mammary tumour formation when hexachlorobenzene was administered alone in a medium-term bioassay, but after initiation of N-nitroso-N-methylurea (NMU) the latency period, the tumour incidence, the tumour number per rat and the tumour volume were significantly increased. It was also found that the levels of insulin receptor (IR) and insulin-like growth factor-I receptor (IGF-IR) were higher in hexachlorobenzene treated rats than in NMU treated rats and controls. According to the authors these results suggest that the IR and/or IGF-IR signalling pathway may be involved in the mechanism of action of hexachlorobenzene. However, IR and IGF-IR levels were not significantly altered in the group exposed to both NMU and hexachlorobenzene, which casts doubt on the involvement of this pathway in the tumour promoting activity of hexachlorobenzene. Finally, Pontillio *et al.* (2011)[60] concluded in their study that hexachlorobenzene stimulates c-Src/HER1/STAT5b and HER1/ERK1/2 signaling pathways in the MDA-MB-231 human breast cancer cell line. c-Src, HER1 and AhR are involved in hexachlorobenzene-induced increase in cell migration in relation to mammary carcinogenesis and breast cancer.

6.3 Summary

Hexachlorobenzene induces hepatic porphyria characterized by accumulation of uroporphyrinogen in the liver due to decreased uroporphyrinogen decarboxylase activity. It also increases the incidence of tumour formation, particularly in the liver. But in the available literature, no clear link has been established between tumour promotion of hexachlorobenzene and its other hepatotoxic effects. Hexachlorobenzene has shown a lack of genotoxic potential in various mutagenicity assays (summarized in Section 7), indicating it does not induce direct DNA damage.

* Assumptions: body weight 250 g, food consumption 21.7 g/day.

Chapter 7

Effects

7.1 Observations in humans

7.1.1 *Irritation and sensitisation, and local effects to the eyes*

ATSDR data

No information was found regarding irritation and sensitisation following respiratory or dermal exposure to hexachlorobenzene; and also no information was found with respect to local effects on the eyes following ocular exposure to hexachlorobenzene.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

7.1.2 *Acute and short-term exposure*

ATSDR data

No information was located regarding short-term health effects in humans following exposure to hexachlorobenzene by any route.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional studies were located on acute and short-term effects of hexachlorobenzene after inhalation, dermal and oral exposure.

7.1.3 Long-term exposure

Hepatotoxicity

ATSDR data

The main long-term effect following exposure of humans to hexachlorobenzene is hepatic porphyria. Porphyrins can damage the liver by cirrhosis, siderosis (accumulation of iron), focal necrosis and hyperplasia. Hepatic porphyria, diagnosed by the presence of high levels of porphyrins in the blood, faeces, or urine, has been detected following exposures to hexachlorobenzene in workers, in the residents of Flix, Spain (primarily inhalation exposures resulting from a nearby plant [Grimalt *et al.* 1994; Herrero *et al.* 1999; Sala *et al.* 1999b; To-Figueras *et al.* 1997]; see below in the section on reproduction toxicity), and in the Turkish epidemic (oral exposure via contaminated grain; see below in the section on reproduction toxicity). Exposed patients from the Turkish epidemic also exhibited hepatomegaly (Cam and Nigogosyan 1963; Cripps *et al.* 1984; Peters *et al.* 1982, 1987). The exposure at which hepato- and reproductive toxicity was observed in adults was estimated to have been 0.05-0.2 gram per day, corresponding to 0.8-3.3 mg/kg bw/day for a 60 kg adult. A NOAEL could not be derived (Cam and Nigogosyan 1963).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional studies were found regarding hepatic toxicity in humans.

Carcinogenicity

ATSDR data

Among the general population, epidemiology studies have generally found no association between hexachlorobenzene levels in blood or tissues and incidence

of breast or other cancers (pancreas and endometrium cancer, leukemia, Ewing's sarcoma of the bone), but all of these have limitations such as small study sizes, and/or co-exposure to other organochlorines. ATSDR (2002)[1] considered these studies to be inconclusive.

Data from men exposed to hexachlorobenzene by inhalation (occupationally or as a result of nearby air pollution in Flix, Spain) provide weak evidence for an association between hexachlorobenzene exposure and cancer of the liver, thyroid, and brain (Grimalt *et al*. 1994). But the findings were based on small numbers of observed cases, and were not duplicated in a companion analysis of cancer mortality reported in the same paper. ATSDR (2002)[16] considered this study also to be inconclusive.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

Eight additional epidemiological studies were found studying the relationship between hexachlorobenzene exposure and cancer. The cancer sites examined were: breast cancer, non-Hodgkin's lymphoma, prostate and testicular germ cell carcinoma. All units of (internal) exposure were converted to either µg/L (serum) or µg/kg lipid (fat) for reasons of easy comparison.

Breast cancer

One case-control study[18] and two cohort studies[19,20] were found relating hexachlorobenzene exposure to breast cancer. The hypothesis for this relationship is found in the estrogenic or anti-estrogenic effects of organochlorines in experimental studies[19]. One of the studies was positive (positive association between hexachlorobenzene serum levels and breast cancer), one was negative, while the third showed a negative association (inverse relation between breast cancer and hexachlorobenzene levels in adipose tissues). However, the three studies used different analytical techniques, which makes a comparison difficult. In addition, they all suffer from a relatively small number of cases. In Tables D.1.1 and D.1.2 these studies are summarized.

Charlier *et al.* (2003)[18] determined serum levels of hexachlorobenzene as measure of exposure in 159 women with breast cancer and 250 presumably healthy controls. The controls were selected from women visiting the hospital for a routine vaginal cytological examination. Serum samples were collected in 1999 and 2000 after the diagnosis of breast cancer, but before the start of the treatment. In 32% of the cases hexachlorobenzene level in serum was higher than the level

of quantification (level of quantification (LOQ) in serum: 0.05 ng/g). A total of 4% of the controls had a level of hexachlorobenzene higher than the LOQ. The association between the presence of detectable hexachlorobenzene and breast cancer was estimated and an increased risk with an odds ratio (OR) of 9.1 (95% confidence interval: 2.8-29.4) was found. The OR was adjusted for detectable concentrations of DDT (strongly associated with breast cancer[21]) and breast feeding.

Iwasaki *et al.* (2008)[19] studied the relationship between breast cancer and hexachlorobenzene concentration in plasma among Japanese women (cohort of 24226 women with 139 cases). The plasma levels of hexachlorobenzene were measured between 1990 and 1993. The following potential confounders were taken into account: age at menarche, menopausal status, number of births, age at first birth, height, body man index (BMI) and alcohol consumption. No increased risk was found.

Raaschou-Nielsen *et al.* (2005)[20] also studied the relationship between hexachlorobenzene and breast cancer in a cohort study (24,697 women with 409 cases). Hexachlorobenzene concentration was measured in adipose tissue collected between 1993 and 1997. The following confounders were taken into account: previous benign breast tumour, level of education, BMI, alcohol consumption, nulliparity, number of deliveries, age at first birth, duration of hormone replacement therapy use and lifetime duration of lactation. The results showed an inverse relation between the risk of breast cancer and hexachlorobenzene, more pronounced in oestrogen-receptor-negative (ER) breast cancer. Also, there was a significant trend between increasing hexachlorobenzene concentration and decreasing risk in all groups (all breast cancer p=0.002, ER+ breast cancer p=0.08, ER- breast cancer p=0.004). For ER- breast cancer the OR (with 95% confidence interval) in quintile 2 (Q2) was 0.6 (0.2-1.7), Q3: 0.5 (0.2-1.6) and Q4: 0.2 (0.0-0.6)*.

Non-Hodgkin's lymphoma

Three case-control studies were found regarding the relationship between non-Hodgkin's lymphoma and hexachlorobenzene[22-24]. In Table D.1.3 these studies are summarized.

* The distribution of the women over hexachlorobenzene serum levels was divided into five equally sized groups with a different degree of exposure. The separation markers (at 20, 40, 60 and 80% of the distribution) are called quintiles. An interquintile range refers to the group between two quintiles.

Cocco *et al.* (2008)[22] studied the relationship between (subtypes of) non-Hodgkin's lymphoma and hexachlorobenzene concentration in plasma in a multi-centre case-control study, the so-called Epilymph study. The 174 cases and 203 controls were recruited between 1998 and 2004. Controls were either hospital-based or from the general population, depending on the study centre. As high serum lipids imply higher hexachlorobenzene serum levels at the same body burden, the concentration of hexachlorobenzene in serum was adjusted for total serum lipids. Confounders included were age, gender, education and centre. No increased risk was found.

Spinelli *et al.* (2007)[24] conducted a large case-control study (422 cases, 460 controls) to examine the relationship between hexachlorobenzene and non-Hodgkin's lymphoma in subjects recruited among the populations of the Vancouver and Ottawa regions from 2000 until 2004. Spinelli *et al.* excluded cases with >10% weight loss and performing blood sampling before chemotherapy. Controls with weight loss were not excluded. Analyses were adjusted for age, gender, region, education, BMI, ethnicity, farming and family history of non-Hodgkin's lymphoma. Not all possible confounders could be taken into account, for example current BMI, body fat index and lactation in women were not accounted for. In this study, an increased risk of non-Hodgkin's lymphoma was seen, also among some of the subtypes. A significant positive trend for the relationship between hexachlorobenzene plasma concentration and non-Hodgkin's lymphoma was found.

Quintana *et al.* (2004)[23,24] collected human adipose tissue during surgery and post-mortem among a sample of the general US population between 1969 and 1983. It should be noticed that the original study was designed to estimate exposure levels in the general US population. Of these individuals, 175 had a diagnosis of non-Hodgkin's lymphoma, and the 481 controls were selected among subjects with a diagnosis of accidental injury (or death) and subjects with a myocardial infarction. This study did not show an association between hexachlorobenzene exposure and an increased risk of non-Hodgkin's lymphoma. However, it shows several limitations: first the exposure to hexachlorobenzene, measured in adipose tissue, is determined after the onset of the disease, either during disease related surgery or post-mortem. Another limitation is that the cases are likely to be more severe cases of non-Hodgkin's lymphoma, since the participants predominantly had a poor diagnosis or died of the disease. Furthermore, the confounders were limited to age, gender, race, and region.

Prostate cancer

One case-control study was found exploring the relationship between hexachlorobenzene and prostate cancer, this study is summarized in Table D.1.4. In this study by Hardell *et al.* (2006)[25] no relationship was found between hexachlorobenzene and prostate cancer. Tissue samples were sampled between 1997 and 1999. The analyses were adjusted for age at tissue sampling and BMI. This is a limited control of confounding factors, as no other potential confounders were taken into account. Furthermore, the power of this study is limited, since the number of cases (n=58) and controls (n=10) is very low.

Testicular germ cell carcinoma

One case-control study was found exploring the relationship between hexachlorobenzene and testicular germ cell carcinoma (TGCC). This study by Biggs *et al.* (2008)[26] is summarized in Table D.1.5. Cases (246) and controls (630) were recruited between 1999 and 2008 and a blood sample was collected. The ORs were adjusted for age, race, change in BMI, assay run number and serum lipids. Overall, no increased risk was found.

Genotoxicity

ATSDR data

An increased incidence of micronuclei was observed in the peripheral lymphocytes of 41 chemical workers in San Paulo, Brazil, who had been exposed (presumably via the respiratory and dermal routes) to a mixture of chlorinated solvents that included hexachlorobenzene, as well as carbon tetrachloride and perchlorethylene (Da Silva Augusto *et al.* 1997). Due to this mixed exposure, however, this study can not be used to address the genotoxicity of hexachlorobenzene.

No studies were located regarding the genotoxic effects of hexachlorobenzene in humans following oral exposure.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data was found regarding genotoxicity in humans.

Reproduction toxicity (fertility and development)

No studies were located regarding reproductive effects in humans following inhalation or dermal exposure to hexachlorobenzene.

Fertility

ATSDR data

In a study carried out among the people of Xixin, China, no changes in reproductive outcomes were detected following the cessation of agricultural uses of hexachlorobenzene (Huang *et al.* 1989).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

One epidemiological study was found regarding the relationship between hexachlorobenzene exposure and fertility.[27] In Table D.2.1.1 this study is summarized. Pregnant women from the region stricken in the Turkish poisoning epidemic (see below) with a confirmed diagnosis of porphyria cutanea tarda as indirect exposure estimate of hexachlorobenzene were studied with respect to the proportion of male offspring by Jarrell *et al.* (2002).[27] Controls were women living in the same region not exposed to hexachlorobenzene and women living in Ankara. Overall, no effect was found.

Developmental effects

ATSDR data

Between 1955 and 1959 people in Southeastern Turkey were exposed to hexachlorobenzene due to the consumption of bread made with hexachlorobenzene-treated grain. Exposure of adults during this period was estimated at 0.05-0.2 g hexachlorobenzene per day, corresponding to 0.8-3.3 mg/kg bw/day for a 60 kg adult (Cam and Nigogosyan 1963). Epidemiological studies of this population suggest an association between oral hexachlorobenzene exposure and spontaneous abortion, stillbirth and death in early childhood (Cam and Nigogosyan 1963; Gocmen *et al.* 1989; Jarrell *et al.* 1998; Peters *et al*. 1982, 1987). However, the number of cases studied was low (40-60 mothers) and in the first two studies

no control group was included. According to ATSDR (2002)[1], this poisoning epidemic demonstrated hexachlorobenzene to be a developmental toxin via the oral route: children exposed under 2 years of age were the most susceptible (95% mortality, skin lesions). It should be noted, however, that children under 15 were also more susceptible than adults, and exhibited both immediate (10% mortality, skin lesions) and persistent (dermal, neurological, skeletonmuscular, hepatic, and thyroid effects) symptoms.

Developmental effects (spontaneous abortions, low birth weight, and congenital malformations) occurred with a similar prevalence among females who ever worked at the plant near Flix (Spain) that produced chlorinated solvents (n=46-60 for the different end points) and never exposed female residents (n=719-936), despite a 5-fold higher blood hexachlorobenzene levels in the workers (Sala *et al.* 1999b). The small number of women factory workers is a limitation of this study.

Studies of other populations with exposure to multiple organochlorines did not find significant differences in blood hexachlorobenzene levels between controls and cases of spontaneous abortion in Italy (Leoni *et al.* 1986, 1989) or Germany (Gerhard *et al.* 1998).

Other human studies investigating developmental toxicity have been limited by small study size and low levels of hexachlorobenzene exposure; they have found suggestive evidence of an increased risk of undescended testis and impaired development of locomotor skills in newborn babies.

Based on human findings, hexachlorobenzene did not demonstrate clear developmental effects.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

Four additional studies were found regarding the relationship between hexachlorobenzene and developmental effects (see Tables D.2.1.2-D.2.1.4).

The relationship between hexachlorobenzene exposure and erectile dysfunction was examined in a case-control study by Polsky *et al*. (2007).[28] Subjects were recruited between 1997 and 1999. Due to the design of case-control studies selection bias and an influence of the disease on the hexachlorobenzene concentration can not be excluded. Several confounders (smoking, education, cardiovascular disease medication, diabetes, marital status) were tested and found not to influence the estimate. The risk estimate was adjusted for BMI and alcohol intake. No increased risk was found (see Table D.2.1.2).

In 1993 and 1994, Gladen *et al.* (2003)[29] recruited 197 pregnant women from two regions in the Ukraine to study the relation between hexachlorobenzene exposure and birth weight. However, when taking possible confounders into account, the sample size reduced to 162 due to missing data. Hexachlorobenzene exposure of the infants was determined in a breast milk sample on the 4th or 5th day after birth and used as a proxy for exposure to hexachlorobenzene during the pregnancy. The quality of this proxy is unknown. A limitation of this study is that the sample size was restricted to women having the ability to lactate. Overall, no relationship between hexachlorobenzene exposure and birth weight was found (see Table D.2.1.3).

Pierik *et al.* (2007)[30] recruited pregnant women in the USA between 1959 and 1965 to study the risk of cryptorchidism in offspring. Several blood samples of the mothers were collected, every 8 weeks during the pregnancy, at delivery and 6 weeks post partum. The authors studied the relationship between hexachlorobenzene exposure of the mother during pregnancy and cryptorchidism of the offspring in the first year of life in a nested case-control study. A large number of possible confounders were evaluated and found not to influence the outcome. Risk estimates were adjusted for cholesterol and triglycerides. No significantly increased risk of cryptorchidism was found (see Table D.2.1.4).

Eggesbø *et al*. (2009)[31] investigated the levels of hexachlorobenzene in breast milk in relation to birth weight in a Norwegian cohort of 300 randomly selected mothers, to which in a later stage 26 mothers of small for gestational age infants were added. They found slight (but not significant) inverse associations between HCB levels in milk and gestational age, and between HCB levels in milk and birth weight. A large number of possible confounders were evaluated and found not to influence the outcome (see Table D.2.1.3).

Other effects

ATSDR data

Next to hepatic toxicity, human data suggest that hexachlorobenzene also adversely affects the endocrine system; specifically the thyroid is a target organ. In addition there are indications that hexachlorobenzene may cause musculoskeletal effects, neurological toxicity, kidney damage and immunotoxicity in humans. Some adverse health effects of hexachlorobenzene are clearly associated with the hepatic porphyria it induces: high levels of porphyrins in the body have shown to induce renal failure and may activate the immune system. Additionally, skin lesions can occur when porphyrins, accumulated in the skin, are

activated by sunlight to generate reactive oxygen species, causing tissue damage most commonly on areas exposed to sunlight, such as the hands and face (phototoxicity).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

Four epidemiological studies were found on the association of serum concentration of hexachlorobenzene with thyroid function[32-35] (a deficit in thyroid hormone concentration may interfere with neurodevelopment). These studies are summarized in Table D.2.2.

Meeker *et al.* (2007)[35] examined the relationship between hexachlorobenzene exposure and thyroid hormones in 341 men recruited from a fertility clinic between 2000 and 2003. They used multivariate regression analyses to examine the relationship, and found an inverse relationship between total triiodothyronine (triiodothyronine) and serum levels of hexachlorobenzene. In the most elaborate model a regression coefficient of -0.034 was found, indicating a decline of 3.4% in total triiodothyronine per interquintile range (IQR) change in hexachlorobenzene level*.

Chevrier *et al.* (2008)[33] examined the relationship between hexachlorobenzene exposure and thyroid hormones in pregnant women enrolled in 1999 and 2000. A 10-fold increase in hexachlorobenzene concentration was associated with an 8% decrease in free thyroxin and a 51% decrease in total thyroxin (thyroxin).

Bloom *et al.* (2003)[32] also studied the relationship between hexachlorobenzene levels in serum and thyroid hormone levels in sportsmen, who donated blood in 2004. The sample size was small, limiting the power of the study. Hexachlorobenzene was found to be a negative predictor of thyroxin.

The relationship between hexachlorobenzene exposure and thyroid hormones was also studied in two regions in Canada among neonates enrolled between 1993 and 1996 by Dallaire *et al.* (2008)[34]. Exposure to hexachlorobenzene, as determined by the serum concentration in the umbilical cord, was found to be positively associated to free thyroxin levels in both populations, contradicting the results of the studies above.

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* The distribution of the pregnant women over hexachlorobenzene serum levels was divided into five equally sized groups with a different degree of exposure. The separation markers (at 20, 40, 60 and 80% of the distribution) are called quintiles. An interquintile range refers to the group between two quintiles.

7.2 Animal experiments

7.2.1 Irritation and sensitisation, and local effects to the eyes

ATSDR data

No information was found regarding irritation and sensitisation following respiratory or dermal exposure to hexachlorobenzene; and also no information was found with respect to local effects on the eyes following ocular exposure to hexachlorobenzene.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were found.

7.2.2 Acute and short-term toxicity

ATSDR data

No studies were located regarding acute and short-term effects in animals following dermal exposure to hexachlorobenzene. Many studies investigated acute and short-term effects after ingestion of hexachlorobenzene, but only one animal study investigated toxic effects in animals following inhalation. The critical studies will be summarized here.

Acute toxicity

The acute lethality of ingested hexachlorobenzene in animal studies is relatively low. LD_{50} data are limited to a report from the Russian literature of single-dose oral LD_{50} values of 3,500 mg/kg bw for rats, 4,000 mg/kg bw for mice, 2,600 mg/kg bw for rabbits, and 1,700 mg/kg bw for cats (Savitskii 1964, 1965).

After one day exposure increased carboxylated porphyrins were detected in the liver of female Wistar rats administered 50 mg/kg bw (Kennedy and Wigfield, 1990).

Short-term toxicity

Animal data provide weak support for short-term effects of inhaled hexachlorobenzene. Observations in male rats exposed to 4.4 and 33-35 mg/m^3 of hexachlorobenzene aerosol for durations of 1 day (both doses), 4 and 16 days (high dose only) (4 hours per day, 4 days per week) were limited to pulmonary host defence parameters (pulmonary bactericidal activity, macrophage phagocytic activity, alveolar macrophage activity, and B- and T-cell mitogenesis from lung associated lymph nodes and mesenteric lymph nodes), and body weight (Sherwood *et al.* 1989)[36]. No effects were found at 4.4 mg/m^3 (one day exposure only). At an exposure of 33-35 mg/m^3, treatment related effects were only observed after 16 days of exposure: mitogenesis of lung associated lymph node T-cells was doubled (immunostimulation) and mitogenesis of mesenteric T-cells was halved (immunosuppression). Doubling of the mitogenesis of the lung associated lymph node T-cells is an indication that hexachlorobenzene may stimulate the immune system via respiratory exposure. ATSDR (2002)[1] concluded to a slight impairment of host defences. This rather limited study does not allow the Committee to derive a NOAEL or a LOAEL.

No dermal toxicity studies investigating the effects of hexachlorobenzene exposure on animals were located.

Studies with rats, mice, guinea pigs, and monkeys show that the liver is the most important target organ for hexachlorobenzene following short-term oral exposure. Hepatic effects include disruption of haem synthesis (culminating in hepatic porphyria), induction of microsomal enzymes, hepatomegaly, and cellular damage. In a 7-day study with female rats the lowest dose reported to induce hepatic effects (increased activity of δ-aminolevulinic acid synthase, a key enzyme in porphyrin biosynthesis) was 16 mg/kg bw/day, while no effects were found at 5 mg/kg bw/day (Goldstein *et al.* 1978).

In monkeys a hexachlorobenzene dose of 1 mg/kg bw/day for 13 weeks has been reported to induce hepatocellular vacuolation and intrahepatic cholestasis, while no effects were observed at 0.1 mg/kg bw/day (Jarrel *et al*. 1993[12]). In a 5 weeks study in rats similar effect levels were seen: 1 mg/kg bw/day induced effects on the liver, mainly detected as an increased liver weight; no effects were observed at 0.1 mg/kg bw/day (Andrews *et al*. 1988). In specified pathogen free (SPF) pigs which received 0.05, 0.5, 5.0, and 50 mg/kg bw/day of hexachlorobenzene for 13 weeks, serious liver effects were observed. Animals given the highest dose (50 mg/kg bw/day) showed clinical signs associated with hepatic porphyria and died during the experiment. At lower dosages these signs were not observed. An increased excretion of coproporphyrin was found in the 5.0 and 0.5

mg/kg bw/day groups, as well as induction of microsomal liver enzymes accompanied by hepatocellular hypertrophy. Significant increases in by increased liver weight (and kidney and thyroid weight) were observed 5.0 mg/kg bw/day. Based on the hepatocellular hypertrophy observed at doses of 0.5 mg/kg bw/day and higher, the authors derived a NOAEL of 0.05 mg/kg bw/day (Den Tonkelaar *et al*. 1978).[37]

Effects on both bone and muscle have been reported in several animal studies including monkeys. The lowest effects levels were reported in studies conducted by Andrews *et al.* (1989, 1990)[38,39] in Fischer 344 rats, which were dosed 5 days/ week for 5, 10, or 15 weeks with 0, 0.1, 1.0, 10.0, or 25.0 mg hexachlorobenzene/kg bw (summarized in Table 5). They found significant increases in serum 1,25-dihydroxy-vitamin D3, femur density (only measured after 5 weeks of exposure) and cortical area at doses starting at 1 mg/kg bw/day. The levels of parathyroid hormone (PTH) were increased starting at a dose of 10 mg/kg bw/day. From these data ATSDR derived a NOAEL of 0.1 mg/kg bw/day after exposure up to 15 weeks. However, changes in critical parameter values (femur density, cortical area) were less than 10%. Bone strength was increased in a dose related manner starting at a dose of 1 mg/kg bw/day, while bone flexibility was not affected. A decrease of de medullar area could have adverse effects on the synthesis of blood cells and immunocompetent cells. However, these parameters were not investigated by the authors.

In the Andrews *et al.* studies[38,39] also renal effects such as increased weight of kidney and increased urinary LDH and alkaline phosphatase were observed at doses starting at 10 mg/kg bw/day. In pigs increased kidney weight was found with doses as low as 5 mg/kg bw/day, while no effects were observed at 0.5 mg/kg bw/day (Den Tonkelaar *et al*. 1978).[37] Direct and indirect evidence of renal tissue damage and accumulation of porphyrins in the kidney were observed in rats at higher doses (Smith *et al.* 1985).

Table 5 Relative changes (%) in critical parameters of bone formation in the rat due to 15 weeks hexachlorobenzene exposure (taken from Andrews *et al*., 1989, 1990).[38,39]

hexachlorobenzene (mg/kg bw/d, 5d/w)	PTH I	Ca I	femur density		femur cross sectional areas		femur performance	
			I	II	medulla II	cortex II	strength II	flexibility II
0.1	+19	-44	+0.6	-0.9	**+6**	+1.4	+2.8	+18
1.0	+13	-12	**+3.8**	**+1.5**	-1	**+4.5**	**+6.6**	+2
10.0	**+106**	**-57**	**+4.5**	**+2.8**	**-12**	**+5.1**	**+9.4**	-4
25.0	**+114**	+20	**+5.8**	**+3.6**	**-16**	**+6.9**	**+10.4**	+1.3

I = first study[38]; II = second study[39]; PTH = parathyroid hormone serum level; Ca = calcium serum level.
Percentages in bold denote statistically significant changes ($p<0.05$).

Multiple studies have demonstrated that serum thyroxin levels decrease rapidly in rats following gavage treatment with high doses of hexachlorobenzene (Den Besten *et al.* 1993; Den Tonkelaar *et al.* 1978[37]; Foster *et al.* 1993; Kleiman de Pisarev *et al.* 1989, 1990, 1995; Smith *et al.* 1986; Sopena de Kracoff *et al.* 1994; Van Raaij *et al.* 1993a, 1993b). Effects on serum TSH levels (both increases and decreases) are delayed and appear secondary to decreases in thyroxin. The most sensitive acute study observed statistically significant decreases in thyroxin levels in female rats at doses as low as 50 mg/kg bw/day for 5 days (Foster *et al.* 1993). A time-course in female Wistar rats gavaged with 1,000 mg/kg bw/day of hexachlorobenzene in corn oil found that serum thyroxin levels rapidly decreased, reaching a steady-state after 8 days, approximately 75% below controls. In contrast, serum TSH levels reached a steady state after 30 days, at 80% below controls (Sopena de Kracoff *et al.* 1994). Similarly, experiments in female Wistar rats gavaged with 1,000 mg/kg bw/day for at least 8 weeks observed significantly decreased serum thyroxin and protein-bound iodine and elevated TSH levels and thyroid weight (Kleiman de Pisarev *et al.* 1989, 1990, 1995). The most sensitive subchronic studies were conducted in male Syrian hamsters (Smith *et al.* 1986, 1987), which responded differently than rats to hexachlorobenzene. In hamsters exposed to 10 mg/kg bw/day in feed for 28 weeks (the lowest effective subchronic daily dose identified) or to a higher dose for a shorter time (50 mg/kg bw/day or more for 6 weeks), thyroid gland weights were significantly increased (ca. 2.5-fold), serum triiodothyronine levels were decreased, and sodium iodide uptake was increased (ca. 3-fold), while serum thyroxin levels were unchanged. Thyroid weight increased significantly, and correlated with histopathological observations of large and irregularly shaped follicles in the thyroid. Den Besten *et al.* (1993) observed decreased triiodothyronine and thyroxin levels in female Wistar rats exposed to hexachlorobenzene in the feed at doses up to 19 mg/kg bw/day for 13 weeks and hyperplasia and hypertrophy of the thyroid at 19 mg/kg bw/day (NOAEL: 9.5 mg/kg bw/day), and Den Tonkelaar *et al.* (1978)[37] detected significantly increased thyroid weight in male Landvarken pigs fed 5 mg/kg bw/day of hexachlorobenzene for 12 weeks.

A study in dogs (Beagle) showed immunological effects such as increased severity of nodular hyperplasia of the gastric lymphoid tissue at a dose of 0.1 mg/kg bw/day after 1 year exposure (Gralla *et al.* 1977).

Chiappini *et al.* (2009)[40] studied rat thyroid follicular cells and demonstrated that hexachlorobenzene triggers apoptosis in these cells (see Table 6). Female Wistar rats received hexachlorobenzene by gavage as a suspension in water with 0.5% Tween 20 at doses of 0.1, 1, 10, 100, and 500 mg/kg bw for 5 days/week during 4 weeks. Hexachlorobenzene did not influence thyroid follicular cell proliferation, nor did it have any effect on the serum levels of thyroxine (T_4), triiodothyronine (T_3), thyroid-stimulating hormone (TSH), and relative thyroid weights. Transforming growth factor (TGF-β1) mRNA levels in thyroid glands revealed a significant upregulation at doses of 1 mg/kg bw (LOAEL) and higher. In addition these doses induced apoptosis (evaluated by *in situ* end labelling of fragmented DNA). The authors reported that at 0.1 mg/kg bw no effects were observed (NOAEL), and they concluded that doses of hexachlorobenzene that do not disrupt thyroid economy induce TGF-β1 expression and apoptosis in the thyroid gland.

Two animal studies addressing very specific target organs (inner ear and teeth) after oral exposure of rats were located, see Table 6.

Effects on incisor degeneration were studied in a 13-week toxicity study, in which Sprague-Dawley rats (10/group) were fed 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10 and 25 mg hexachlorobenzene/kg bw, 5 days a week, by oral gavage in corn oil (Long *et al*. 2004[41]). Dose-dependent, region-specific maxillary incisor degeneration was observed at doses of 1.0 mg/kg bw/day and higher. Lesions were restricted to mild incisor degenerations at LOAEL, while a high incidence of bilaterally symmetric, lateral and medial, dentin niches were observed in animals treated with 25 mg/kg bw/day. The authors concluded to a NOAEL of 0.3 mg/kg bw/day.

The effects of hexachlorobenzene on the cochlea were investigated in rats, which were orally exposed by gavage to hexachlorobenzene for four weeks at doses of 0, 0.16, 4 or 6 mg/kg bw/day in olive oil[42]. The effects of hexachlorobenzene were evaluated at various time intervals, by measuring auditory nerve acoustic thresholds and plasma thyroid hormone concentration by radioimmunoassay. Histological evaluation involved surface preparation and scanning electron microscopy observations of cochlear hair cells. At a dose of 0.16 mg/kg bw/day, hexachlorobenzene induced no loss of acoustic sensitivity, whereas at 4 mg/kg bw/day, it induced cochlear sensitivity deficits at the mid-frequencies (2-16 kHz) with complete recovery once treatment was stopped. At a dose of

Table 6 Short-term animal toxicity studies of hexachlorobenzene, 2002 – 2010 (taken from Chiappini *et al.* 2009[40], Hadjab *et al*. 2004[42], and Long *et al.* 2004[41]).

Species and sex / Strain / Number	Exposure duration	Concentration tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	(Critical) effects	Reference
Oral						
Female rats / Wistar / n=4-8	4 weeks	0, 0.1, 1, 10, 100, 500 mg/kg bw, 5 days per week by gavage in water with 0.5% Tween20	0.1	1.0	Induction of TGF-β1 expression and apoptosis in thyroid gland	40
Male rats / SD / n=10	13 weeks	0, 0.03, 0.1, 0.3, 1.0, 3.0, 10 and 25 mg/kg bw, 5 days per week, by gavage in corn oil	0.3	1.0	maxillary incisor degeneration	41
Male rats / SD / n=12	4 weeks	0, 0.16, 4 or 6 mg/kg bw/d by gavage in olive oil	0.16	4.0	cochlear sensitivity deficits at the mid-frequencies (2–16 kHz)	42

16 mg/kg bw/day, permanent threshold shifts were observed at all frequencies tested (from 1 to 32 kHz). Morphological studies showed no cochlear hair cell loss or alteration of stereocilia. Hexachlorobenzene treatment significantly halved circulating thyroxin concentrations at doses of 4.0 and 16 mg/kg bw/day. Thyroidectomy had no effect on cochlear sensitivity in control animals. The authors concluded to a NOAEL of 0.16 mg/kg bw/day.

No further studies were located on acute and short-term effects of hexachlorobenzene after respiratory, dermal and oral exposure.

7.2.3 *Long-term toxicity*

Only toxicity studies employing the oral route were available. The critical effects of hexachlorobenzene are hepatic toxicity, carcinogenicity, reproductive toxicity and developmental toxicity.

Hepatic toxicity

ATSDR data

Several studies in rats and mice have observed hepatic porphyria, but no clear relationship has been established between porphyria and other hepatic effects seen in animals such as peribiliary lymphocytosis and fibrosis, hepatomegaly and increased liver weight, enzyme induction, and degenerative pathological changes.

In a lifespan oral study with hamsters, a LOAEL of 16 mg/kg bw/day was observed, based on a marked decrease in weight gain (Cabral *et al.* 1977).

In a 18-month oral study with mice a LOAEL of 13 mg/kg bw/day was observed, based on decreased body weight and hepatocyte hypertrophy (Smith *et al.* 1989).

A rat study showed mitochondrial swelling and elongation in Sprague-Dawley rat liver after one year oral exposure at a dose of 0.25 mg/kg bw/day, while no effects were observed at 0.05 mg/kg bw/day (Mollenhauer *et al.* 1975).

The toxicological effects of hexachlorobenzene were also examined in a two-generation feeding study in which weanling Sprague-Dawley rats were fed diets containing 0.0 ppm (64 m/64 f), 0.32 ppm (0.016 mg/kg bw/day; 40 m/40 f), 1.6 ppm (0.08 mg/kg bw/day; 40 m/40 f), 8.0 ppm (0.4 mg/kg bw/day; 40 m/40 f) or 40.0 (2.0 mg/kg bw/day; 66 m/66 f) hexachlorobenzene (Arnold *et al*. 1985, 1986[43,44])* (higher doses were considered not appropriate, due to high pup mortality observed in a range-finding study). After 3 months on test, the F_0 rats were bred and 50 pups (F_1) of each sex were randomly selected from every group. At weaning the F_0 animals were killed, and the F_1 animals were fed their parents' diet for the remainder of their lifetimes (130 weeks). There were no treatment-related effects on growth, feed consumption, haematological parameters or survival in either (adult) generation. Increased heart and liver weights were found in the F_0 generation males exposed to 0.4 and 2.0 mg/kg bw/day. Histopathological changes in the F_1 generation included significantly increased incidences of centrilobular basophilic chromogenesis of the liver in both sexes and chronic nephrosis in males. Significant increases of peliosis of the liver in F_1 females, of parathyroid adenomas in F_1 males, of adrenal phaeochromocytomas in both sexes, and of hepatocellular adenomas in females were observed at the highest administered dose. Incidence of peribiliary lymphocytosis of the liver in F_1 males was increased in all dosed males, significanly in the highest and the two lowest doses. This lymphocytosis was accompanied by peribiliary fibrosis, also seen in all dosed males, significanly in the highest and the lowest dose (see Table 7). Although these effects did not show a consistent dose-response effect, and analysis with the dose-response modelling software of the US Environmental Protection Agency showed that none of their dichotomous models was able to produce a good fit, ATSDR (2002)[1] concluded that hexachlorobenzene appears to affect the peribiliary tissue in liver at a (marginal) LOAEL of 0.016 mg/kg bw/day. Since this was the lowest dose administered, a NOAEL can not be derived. The other liver effects observed in the males showed a NOAEL at 0.08 mg/kg

* Dose conversion calculated by ATSDR (2002).[1]

bw/day. Hence the LOAEL for hepatotoxicity in this study is 0.016 mg/kg bw/day, based on peribiliary lymphocytosis and fibrosis of the liver of males. The Committee characterizes this LOAEL as being marginal.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were retrieved from literature.

Carcinogenicity

ATSDR data

Several animal studies have demonstrated that oral exposure to hexachlorobenzene increases the incidence of tumour formation. The evidence of carcinogenicity is strongest in the liver; hexachlorobenzene has been shown to induce hyperplasia (in rats, mice, pigs, dogs, and monkeys), metaplasia (in rats), benign tumours (hepatoma in mice and rats; haemangiohepatoma and bile duct adenoma in rats), and malignant tumours (hepatocarcinoma in rats, mice, and hamsters; bile duct adenocarcinoma in rats). Additionally, exposure to hexachlorobenzene has been shown to induce renal metaplasia, adenomas and renal cell carcinomas (in rats, mice, and hamsters), lymphosarcomas (in rats, mice, and hamsters), adrenal hyperplasia and pheochromocytoma (in rats); parathyroid adenomas (in rats), and haemangioendothelioma and thyroid tumours (in hamsters). Based on the studies reviewed by ATSDR (2002)[1], this organisation concluded that carcinogenic effects in hamsters and rats were observed at 4 mg/kg bw/day (lowest doses tested; Ertürk *et al.* 1986), in mice carcinogenic effects were seen at 12 mg/kg bw/day (lowest dose tested; Cabral *et al.* 1979).

Table 7 Incidences of liver lesions in male F_1 rats in a two-generation study (taken from Arnold *et al.* 1985, 1986).[43,44]

intake level (mg/kg bw/d)[a]		0	0.016	0.08	0.4	2.0
no. of animals		48	48	48	49	49
centrilobular basophilic chromogenesis	- slight	3	4	3	**14**	**16**
	- moderate	0	0	1	3	**21**
peribiliary lymphocytosis		16	**27**	**26**	21	**32**
peribiliary fibrosis		13	**23**	21	21	**23**

[a] Dose conversion calculated by ATSDR (2002).[1]

In bold: statistically significant differences with control group ($p<0.05$).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

Additional studies on the potential of hexachlorobenzene to induce carcinogenicity in animals were found.[16,45] Medium-term assays with rats orally exposed to hexachlorobenzene were performed with and without initiation of tumour induction with the genotoxic substances N-nitrosodiethylamine and N-nitroso-N-methylurea. Both studies revealed that hexachlorobenzene promotes carcinogenesis only when initiated by a genotoxic carcinogen. These studies and the mechanisms underlying the induction of tumours in the liver have been extensively described in Section 6.

Genotoxicity

ATSDR data

Genotoxicity of hexachlorobenzene has been investigated in *in vitro* and *in vivo* experiments. The results are shown in Tables 8 and 9.

In vitro hexachlorobenzene tested negative in reverse mutation assays in *S. typhimurium* (Haworth *et al.* 1983; Siekel *et al.* 1991) and *E. coli* (Siekel *et al.* 1991) with and without metabolic activation, although an assay for reverse mutation in the yeast *Saccharomyces cerevisiae* was positive (Guerzoni *et al.* 1976). One study showed weakly positive results in *S. typhimurium* and weakly positive results for DNA binding capacity in a mammalian system (Gopalaswamy and Nair 1992). Hexachlorobenzene did not produce chromosomal aberrations in human peripheral lymphocytes *in vitro* (Siekel *et al.* 1991).

In vivo studies in rats revealed the lack of significant genotoxic activity in mammals following oral exposures to hexachlorobenzene: negative results were observed in two dominant lethal mutation assays that orally exposed rats at doses ranging from 60 to 221 mg/kg bw (Khera 1974; Simon *et al.* 1979). In an alkaline single-cell gel electrophoresis (Comet) assay no evidence of genotoxicity was observed in mouse liver, lung, kidney, spleen, or bone marrow after oral dosing (Sasaki *et al.* 1997). Studies with rats suggest that the metabolism of hexachlorobenzene to pentachlorobenzene and other more polar metabolites produce cell damage either through a free-radical mechanism or by initial formation of an arene oxide. These reactive intermediates may form covalent bonds with cellular constituents such as protein amino acids or DNA (Lui and Sweeney 1975; Gopalaswamy and Nair 1992; Rajamanickam and Padmanaban 1974; Van

Ommen *et al.* 1985). An oral exposure study to test DNA binding of hexachlorobenzene in Wistar rats provided equivocal evidence that hexachlorobenzene reacts directly with DNA. However, evidence was found that reactive electrophilic metabolites of hexachlorobenzene formed by cytochromes P-450 cause the binding to cellular proteins rather than hexachlorobenzene itself (Gopalaswamy and Nair 1992).

No studies were found regarding genotoxic effects in animals following inhalation or dermal exposure to hexachlorobenzene.

Table 8 Genotoxicity of hexachlorobenzene *in vitro* (taken from ATSDR, 2002).[a]

End point	Species (test system)	Results (with activation)	Results (without activation)	Reference
Gene mutation	S. typhimurium	-	-	Haworth *et al.* 1983
Gene mutation	S. typhimurium	±	-	Gopalaswamy and Nair 1992
Gene mutation	S. typhimurium	-	-	Siekel *et al.* 1991
Gene mutation	E. coli	-	-	Siekel *et al.* 1991
Gene mutation	Saccharomyces cerevisiae		+[b]	Guerzoni *et al.* 1976
DNA repair	E. coli	-	-	Siekel *et al.* 1991
Chromosomal aberration	Human peripheral blood lymphocytes	-	-	Siekel *et al.* 1991
DNA binding	Mammalian system: rat (Wistar)	±	-	Gopalaswamy and Nair 1992

[a] no study details other than those listed in this table were reported by ATSDR (2002).[1]
[b] data on metabolic activation is missing in ATSDR (2002).
- = negative result; ± = weakly positive; + = positive.

Table 9 Genotoxicity of hexachlorobenzene *in vivo* (taken from ATSDR, 2002).[1]

End point	Species (test system)	Exposure route	Results	Reference
Mammalian systems: dominant lethals	Rat	Oral	-	Khera 1974
Mammalian systems: dominant lethals	Rat	Oral	-	Simon *et al*. 1979
Genotoxicity, different organs	Mouse	Oral	-	Sasaki *et al*. 1997
DNA binding	Rat (Wistar)	Oral	±	Gopalaswamy and Nair 1992

- = negative result; ± = weakly positive.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

The lack of genotoxic potential of hexachlorobenzene has been confirmed in the *in vivo* Comet assay (Table 10). Groups of four mice or rats were treated once orally with 2000 mg hexachlorobenzene/kg. The stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow were sampled 3, 8, and 24 h after treatment. Hexachlorobenzene did not induce DNA damage in any mouse or rat organ studied[46].

Reproduction toxicity (fertility and development)

Fertility

ATSDR data

Multiple endocrine effects and pathological changes in reproductive organs have been demonstrated in animal studies. Increased adrenal and liver weights were found in monkeys dosed 10 mg/kg bw/day for 90 days (Jarrel 1993).[12] In rats, higher doses showed a rapid decrease of serum thyroxin levels. In a two-generation rat study (Arnold *et al*. 1985[43,44], discussed in full detail in the paragraph on developmental efects) hexachlorobenzene had no effects on fertility.

A series of reproduction toxicity studies with monkeys (Sims *et al*. 1991[50], Babineau *et al*. 1991[47], Foster *et al*. 1992a[49], 1995[11], Jarrell *et al*. 1993[12], Bourque *et al*. 1995[48]) provide the lowest effect doses for adverse effects on fertility (see Table 11*). Female Cynomolgus monkeys (four animals per dose) were fed doses of up to 10 mg/kg bw/day of hexachlorobenzene in glucose in gelatine capsules for 90 days. The studies focused predominantly on end points relevant to fertility (ovarian function and histopathology). Five studies used

Table 10 Genotoxicity of hexachlorobenzene in animals (*in vivo* data), 2002-2010 (taken from Sekihashi *et al.* 2002).[46]

Test system	Dose / concentration	Endpoint	Result	Reference
male ddY mouse and Wistar rat	2000 mg/kg bw, single dose	comet assay in stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	negative	[46]

* The summaries of these studies, although also summarized by ATSDR[1], are based on the original articles.

doses of 0.1, 1, and 10 mg/kg bw/day of hexachlorobenzene.[11,12,47,49,50] In all treated animals, hexachlorobenzene caused follicular degeneration and induced histopathological changes in the ovarian epithelium (cell stratification, decreased nuclear membrane distinction, increased density and granularity of oocyte nuclei, and increased numbers of aggregated lysosomes, vesicles, and vacuoles).

The severity was dose-dependent. These effects were not associated with changes in oocyte fertility (measured *in vitro*) and also other parameters associated with fertility were either not affected (number of antral (=Graafian) follicles, number of corpora lutea), or only at the highest dose (number of primordial follicles); they were considered minimally adverse. A dose-related decrease in serum progesterone levels during the luteal (but not follicular or periovulatory) phase of the menstrual cycle was observed at doses of 1 mg/kg bw/day and higher. At these doses also a lengthening of the menstrual cycle was seen.

The follow-up study of Bourque *et al.* (1995)[48] extended the observation of ultrastructural effects in the ovaries to a dose of 0.01 mg/kg bw/day. At this dose some condensed ova mitochondria with swollen cristae and some abnormal nuclei were observed in developing follicles. These effects were not severe and only seen electron-microscopally. At higher doses (up to 10 mg/kg bw/day), the mitochondria were progressively more damaged and other changes were noted, such as indentation of nuclear membranes and abnormal accumulation of lipid in the cytoplasm of follicular cells. ATSDR[1] considers the dose of 0.01 mg/kg bw/day to be the LOAEL for degenerative ovarian changes. The Committee, however, is of the opinion that the severity of the effects as observed electron-microscopically at this dose are only marginal, and that these effects do not yet have functional relevance, but represent the onset for degenerative and functional changes at higher doses. Hence the Committee considers 0.01 mg/kg bw/day to be the reproductive NOAEL for fertility effects. The reproductive LOAEL for fertility effects in these studies is based on the degenerative lesions in ovarian follicles and amounts to 0.1 mg/kg bw/day. Hepatotoxicity was seen at dose levels of 1 mg/kg bw/day and higher[11,12,47,49,50]. Hence the NOAEL for non-reproductive toxic effects is 0.1 mg/kg bw/day, based on liver histopathology at the next higher dose (see Table 11).

In female Rhesus monkeys, gavage doses of at least 64 (but not lower doses up to 32) mg/kg bw/day of hexachlorobenzene for 60 days induced degenerative changes of the ovarian follicle, stroma, and germinal epithelium (Iatropoulos *et al.* 1976), and suggestive evidence of unusual steroidogenic activity (depressed serum potassium) was seen in Rhesus monkeys given 128 mg/kg bw/day for at least 60 days (Knauf and Hobson 1979).

Table 11 Overview of results of critical studies in monkeys (Babineau *et al*. 1991[47]; Bourque *et al*. 1995[48]; Foster *et al*. 1992a[49], 1995[11]; Jarrell *et al*. 1993[12]; Sims *et al*. 1991[50]).

Parameter	Dose (mg/kg bw/d)					Reference
	0	0.01	0.1	1.0	10	
antral follicles (n)	58 ± 11	nt	115 ± 26	110 ± 9	59 ± 26	12
primordial follicles (n)	26,348 ± 9,860	nt	19,473 ± 4,504	24,027 ± 3,717	**8,737 ± 3,047**	12
corpora lutea (n)	1.8 ± 2.5	nt	1.5 ± 0.3	1.5 ± 0.7	1.8 ± 0.7	12
% fertilisation	15 ± 8.7	nt	14 ± 4.3	22 ± 8.2	9.7 ± 6.6	12
serum progesterone, luteal phase (ng/mL)	7.9 ± 3.9	nt	8.5 ± 2.0	**1.8 ± 0.6**	**2.5 ± 0.4**	49
serum estradiol, luteal phase (pg/mL)	165 ± 4	nt	180 ± 32	69 ± 10	116 ± 26	49
ovulatory levels of estradiol (AUC, relative units)	6,600 ± 1,500	nt	4,600 ± 1,500	5,000 ± 2,500	**2,500 ± 1,600**	49
change in menstrual cycle length (d)[a]	1.5 ± 0.9	nt	2.5 ± 1.5	**9.1 ± 8.8**	**12 ± 8.5**	11
ultrastructural changes ova mitochondria	none	condensed and swollen cristae	condensed and swollen cristae; granular matrices	large cristae	pertubated morphologic integrity	48
other ultrastructural changes in follicles incl. abnormal nuclei	-	±	+	++	+++	12,48
ultrastructural changes surface epithelium of ovaria	-	nt	+	++	+++	47
% of normal (cuboidal cells) in epithelium of ovaria	78 ± 5	nt	**23 ± 7**	**12 ± 5**	**30 ± 6**	50
liver weight	-	nt	-	-	ic	12
combined adrenal weight	-	nt	-	-	ic	12
liver histopathological effects	-	nt	-	+	++	12

[a] Comparing pre-treatment and treatment period for the individual animals.

Duration of all studies was 90 days; per dose 4 female Cynomolgus monkeys were used.
nt = not tested; ic = significant increase; - = absent; + = present (number indicates relative severity); AUC = area under the curve.
In bold: statistically significant differences with control group (p<0.05).

Rat studies have confirmed the reproductive toxicity of hexachlorobenzene in the ovary. Increased serum progesterone levels and elevated ovarian weights were observed in superovulated female Sprague-Dawley rats orally administered 1 mg/kg bw/day hexachlorobenzene by gavage for 21 days (Foster *et al.* 1992b). Super-ovulated (but not normal cycling) female Sprague-Dawley rats gavaged with 50 mg/kg bw/day of hexachlorobenzene for 5 days exhibited significant elevation of serum levels of progesterone (Foster *et al.* 1993). In a subsequent study with ovariectomized female Sprague-Dawley rats, gavage doses of at least 1 mg/kg bw/day for 30 days significantly decreased circulating corticosterone

and cortisol levels, without affecting levels of circulating aldosterone and progesterone levels or adrenal gland weight (Foster *et al.* 1995)[11]. The investigators concluded that hexachlorobenzene exposure induces alterations in steroidogenesis of cells of the adrenal cortex inner zone.

Additional data to the ATSDR toxicological profile (time period 2002-2011)

Gregoraszczuk *et al*. (2011)[61] studied the direct effect of HCB on ovarian steroid synthesis in pigs. They concluded that HCB is involved in steroid synthesis inhibitory action on CYP17, 17β-HSD and CYP19 enzymes. These enzymes are involved in the synthesis of testosterones, while the CYP19 enzyme is involved in estradiol sythesis.

Developmental effects

ATSDR data

No studies were located regarding developmental effects in animals by inhalation or dermal exposure to hexachlorobenzene.

Animal studies have verified that hexachlorobenzene impaired neurological development and reduced neonatal viability and growth. Although an increased risk of undescended testis has not been observed in animals, the occurrence of cleft palate, renal agenesis (*i.e.*, no kidney development), and minor skeletal abnormalities in mice are consistent with a possible teratogenic role for hexachlorobenzene. These were observed in pregmant CD-1 mice gavaged with 100 mg hexachlorobenzene per kg bw per day during gestation days (GD) 7-16 (Courtney *et al*. 1976). At this dose maternal relative liver weights were increased significantly (0.080 versus 0.061 for controls). Developmental toxicity experiments in pregnant Wistar rats administered 40 mg/kg bw/day during GD 6-21 showed increased incidences of sternal defects and 14th rib formation (Khera 1974). At this dose no maternal toxicity was seen (maternal toxicity became apparent at the next higher dose: 80 mg/kg bw/day).

Evidence of hyperactivity in rat pups was detected in a study evaluating neurodevelopmental end points (Goldey and Taylor 1992[51]; Taylor and Goldey 1990)*. Virgin female Sprague-Dawley rats were gavaged for 4 days with 2.5 or

* The ATSDR summary (ATSDR 2002)[1] of this study was added upon based on the original 1992 article. The 1990 reference could not be located, but probably refers to a poster abstract of the same study as reported in the 1992 article.

25 mg/kg bw/day of hexachlorobenzene 2 weeks prior to mating. Compared to controls, pups from both treatment groups reoriented themselves significantly more quickly in a negative geotaxis test, required less time in an olfactory discrimination test (postnatal days 6, 8, and 10), and demonstrated increased exploratory activity in a motor activity test (postnatal days 15-20). Pups of which the mothers were exposed to 25 mg/kg bw/day, exhibited decreased acoustic startle response (ASR) on postnatal day 23 and increased ASR on postnatal day 90. ATSDR (2002)[1] concluded to a LOAEL of 2.5 mg/kg bw/day, based on minimal neurodevelopmental effects. A NOAEL could not be established. No maternal toxicity was observed, but body weight was the only parameter measured. Therefore no conclusions can be drawn from this study with respect to maternal toxicity.

Pups of pregnant BALB/c mice fed doses as low as 0.5 mg/kg/day on GD 1-18 exhibited a marked, significant decrease in delayed type hypersensitivity (DTH) response when tested on postnatal day 45 (Barnett *et al*. 1987). However, a dose-response relationship was not observed. Analyses of collected spleen cells found that 5 (but not 0.5) mg/kg bw/day decreased mixed lymphocyte response and B cell numbers; neither dose affected spleen blastogenesis induced by T- or B-cell mitogens. No overt signs of toxicity in either the treted females or their offspring were noted.

Developmental effects on the immune system were also reported by Vos *et al*. (1979). They exposed pregnant rats to HCB at concentrations of 0, 50 or 150 mg/kg diet (equivalent with 0, 2.5 or 7.5 mg/kg bw/day) and continued the exposure through lactation until weaning when the pups were 5 weeks of age. The authors observed elevated IgM and IgG serum levels as well as an increase in the number of blood basophilic and eosinophilic granulocytes in the blood of the pups from dams exposed at the highest dose. Testing of immune function showed a decrease in resistance to *Trichinella spiralis* and to *Listeria monocytogenes* infection for pups in the highest dose group. In a follow-up study (Vos *et al*., 1983), pups were exposed to HCB during pre- and postnatal development through maternal exposure at doses of 0, 4, 20 or 100 mg/kg diet (equivalent with 0, 0.2, 1 or 5 mg/kg bw/day). Pups in the highest dose group had elevated serum IgM levels and increased numbers of basophilic peripheral granulocytes, while animals in the 1 and 5 mg/kg bw/day dose group had elevated popliteal lymph node weights. Primary and secondary IgM and IgG antibody responses to tetanus toxoid were increased in animals from the 0.2 and 1 mg/kg bw/day groups.

In a two-generation study with Sprague-Dawley rats the animals were dietary administered 0.0, 0.016, 0.08, 0.4 or 2.0 mg/kg bw/day hexachlorobenzene

(Arnold *et al*. 1985[43,44]; this study is described in more detail in the preceding paragraph on hepatotoxicity). After 3 months on test, the F_0 rats were bred and 50 pups (F_1) of each sex were randomly selected from every group. At weaning the F_0 animals were killed, and the F_1 animals were fed their parents' diet for the remainder of their lifetimes (130 weeks). Hexachlorobenzene significantly reduced pup viability of the 2.0 mg/kg bw/day parental group, indicating a treatment related developmental effect. For developmental effects a NOAEL of 0.4 mg/kg bw/day is derived, based on reduced pup viability at the next higher level. Fertility effects were not observed in these studies.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional studies on developmental effects in animals were found.

Other effects (neuro- and immunotoxicity)

ATSDR data

Serious neurological effects (tremors, convulsions) were observed in mice at doses of 24 mg/kg bw/day and higher in a 120 week study (Cabral *et al.* 1979)*.

The effects of oral exposure to hexachlorobenzene to the immune system of animals appear to be species- and strain-dependent (Michielsen *et al*. 1997), with immunosuppression observed in mice, monkeys (Iatropoulos *et al*. 1976) and bears (Bernhoft *et al*. 2000), and at least a partial stimulation of the immune system in rats and dogs (Gralla *et al*. 1977). Additionally, a number of animal studies have observed inflammation and immune cell infiltration in tissues such as the liver (Arnold *et al*. 1985[44]; Ertürk *et al*. 1986), respiratory tract (Goldstein *et al*. 1978; Kimbrough and Linder 1974; Kitchin *et al*. 1982; Michielsen *et al*. 1997, 1999, 2001; Vos *et al*. 1979, 1983), and skin (Koss *et al*. 1978; Michielsen *et al*. 1997, 2000; Schielen *et al*. 1993, 1995b; Torinuki *et al*. 1981) following oral exposure to hexachlorobenzene. The most sensitive effects in mice were seen at dietary doses as low as 0.6 mg/kg bw/day; 3, 6 and 18 weeks of exposure significantly reduced spleen cell cytocidal activity against mKSA tumor cells by 13 and 20%, respectively, compared to the control groups, and 6 and 18 weeks of exposure significantly decreased the mean survival time following challenge with injected ascites L1210 tumor cells (Loose *et al*. 1981)[52]**. Because the

* The conclusion of ATSDR (2002)[1] was adapted based on the evaluation of the original paper.

mode-of-action is unclear, it is not known if these immune effects are secondary following toxicity to target organs or if they are involved in the etiology of disease in these organs. Mode-of-action studies in rats have suggested that the immune effects of hexachlorobenzene may be secondary to the accumulation of porphyrins produced by the liver in the spleen or other organs of immunological importance (Kennedy and Wigfield 1990; Kuiper-Goodman *et al*. 1977) or by the metabolic products of hexachlorobenzene (Schielen *et al*. 1995a).

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data were located.

7.3 Summary

Irritation and sensitisation

No human nor animal data have been retrieved on the irritating and sensitising properties of hexachlorobenzene.

Acute toxicity

No information was located regarding acute health effects in humans following exposure to hexachlorobenzene by any route of exposure.

The acute lethality of ingested hexachlorobenzene in animal studies is relatively low: single-dose oral LD_{50} values for several animal species varied between 1,700 and 4,000 mg/kg bw.

Studies with rat, mouse and guinea pig show that the liver is an important target organ for hexachlorobenzene following acute oral exposure. Hepatic effects include disruption of haem synthesis (culminating in porphyria), induction of microsomal enzymes, hepatomegaly, and cellular damage.

No acute toxicity studies in animals investigating the dermal and respiratory routes were retrieved.

** Summary based on original study.

Short-term toxicity

No information was located regarding short-term health effects in humans following exposure to hexachlorobenzene by any route of exposure.

Mechanistic studies have demonstrated hexachlorobenzene causes hepatic porphyria, mainly by reducing the activity of uroporphyrinogen decarboxylase.

Animal data concerning the short-term effects are limited. Observations in male rats exposed to 4.4 and 33-35 mg/m^3 of hexachlorobenzene aerosol for durations up to 4 weeks showed some immunostimulating effect on T-cells.

No dermal toxicity studies investigating the effects of hexachlorobenzene exposure on animals were located.

In oral toxicity studies, serious effects on the hepatic system were observed in short-term animal studies with monkeys, rats, mice and pigs. In monkeys a hexachlorobenzene dose of 1 mg/kg bw/day for 13 weeks induced hepatocellular vacuolation and intrahepatic cholestasis, while no effects were observed at 0.1 mg/kg bw/day. In rats the same NOAEL and LOAEL were established; 1 mg/kg bw/day induced effects on the liver, mainly detected as an increased liver weight; no effects were observed at 0.1 mg/kg bw/day (duration 5 weeks). In pigs which received 0.05, 0.5, 5.0, and 50 mg/kg bw/day of hexachlorobenzene during 13 weeks, serious liver effects were observed. Based on the hepatocellular hypertrophy observed at doses of 0.5 mg/kg bw/day and higher, the oral NOAEL for pigs was 0.05 mg/kg bw/day.

At higher doses, effects on both bone and muscle have been reported in studies with monkeys, rabbits and rats. Renal tissue damage and accumulation of porphyrins in the kidney were observed in rats and increased kidney weight in pigs.

In special studies, in rats also effects on hearing (cochlear sensitivity deficits), teeth (incisor degeneration) and effects on the thyroid gland (decrease of serum thyroxin concentrations, and apoptosis of the thyroid) were seen. A study in dogs showed immunological effects such as increased severity of nodular hyperplasia of the gastric lymphoid tissue at a dose of 0.1 mg/kg bw/day after 1 year exposure (LOAEL). In mice doses as low as 0.6 mg/kg bw/day administered for 18 weeks significantly reduced spleen cell cytocidal activity and decreased the mean survival time of mice following challenge with injected ascites tumor cells.

A study with human peripheral lymphocytes did not demonstrate a clear association between micronucleus formation and hexachlorobenzene exposure. Hexachlorobenzene tested negative in an *in vitro* chromosomal aberration assay and in three out of four bacterial mutation assays. Ambiguous results were obtained in one *in vitro* bacterial mutation assay and in *in vitro* and *in vivo* DNA-binding assays. Two *in vivo* dominant lethal studies in rats and two *in vivo* Comet assays in rats and mice were negative. Based on these data, hexachlorobenzene is considered to be a non-genotoxic compound.

Among the general population, older case-control studies have generally found no association between hexachlorobenzene levels in blood or tissues and incidence of breast or other cancers. Data from men exposed to hexachlorobenzene by inhalation provide weak evidence for an association between hexachlorobenzene exposure and cancer of the liver, thyroid, and brain. However, all of these studies have limitations (such as small study size and co-exposure to other organochlorines).

More recently, 8 epidemiological studies on the relationship between hexachlorobenzene exposure and cancer were reported.

Of the three studies on breast cancer, one showed a positive correlation. The second study was negative, while the third showed an inverse association. But the studies used different analytical techniques, suffered from small numbers of cases, and only a few confounders were taken into account. Due to the lack of reliable data a conclusion can not be drawn.

For non-Hodgkin lymphoma three studies (all case-control) are available, two of which were negative, one was positive. All studies are of limited quality, and thus, also due to the lack of cohort studies, a conclusion can not be drawn.

Prostate and testicular tumours were studied in case-control studies. Both were negative.

Several animal studies have demonstrated that oral exposure to hexachlorobenzene increases the incidence of tumour formation. The evidence of carcinogenicity is strongest in the liver: hexachlorobenzene has been shown to induce hyperplasia (in rats, mice, pigs, dogs, and monkeys), metaplasia (in rats), benign tumours (hepatoma in mice and rats; haemangiohepatoma and bile duct adenoma in rats), and malignant tumours (hepatocarcinoma in rats, mice, and hamsters; bile duct adenocarcinoma in rats). Additionally, exposure to hexachlorobenzene has been shown to induce renal metaplasia, adenomas and renal cell carcinomas (in rats, mice, and hamsters), lymphosarcomas (in rats, mice, and hamsters), adrenal hyperplasia and pheochromocytoma (in rats), parathyroid adenomas (in

rats), and haemangioendothelioma and thyroid tumours (in hamsters). No oral NOAELs for cancer effects have been identified. The lowest oral doses at which carcinogenic effects were seen were 4 mg/kg bw/day for hamsters and rats and 12 mg/kg bw/day for mice.

Tumour-promoting effects of hexachlorobenzene in the liver have been demonstrated in rats at doses that did not initiate tumours. Hexachlorobenzene has shown a lack of genotoxic potential in various mutagenicity assays, indicating the substance does not induce direct DNA damage. Therefore, based on the available evidence, hexachlorobenzene appears to act as a promoter of liver carcinogenesis via a non-genotoxic mechanism of action.

Reproduction toxicity – fertility

In the older human studies, no reliable evidence of adverse effects of hexachlorobenzene on fertility has been demonstrated. A recent epidemiological study investigated the relation between hexachlorobenzene exposure and the proportion of male births; overall no effect was found. The study had obvious limitations since it did not quantify the exposure.

Multiple endocrine effects and pathological changes in reproductive organs have been demonstrated in animal studies. Different repeated dose toxicity studies were performed in female monkeys demonstrating adverse effects on the reproductive organs after approximately 90 days exposure to doses as low as 0.01 mg/kg bw/day. Overall, the oral NOAEL for fertility effects in monkeys is 0.01 mg/kg bw/day, based on necrosis of ovary surface epithelium cells and denuding of ovary in 90-day studies.

Reproduction toxicity – development

In a poisoning epidemic in Turkey, caused by oral hexachlorobenzene exposure due to the consumption of contaminated grain over a period of four years, children exposed under 2 years of age were the most affected. According to ATSDR these effects are attributable to developmental toxicity properties of hexachlorobenzene. But children under 15 were also more affected than adults, and exhibited both immediate and persistent symptoms. The studies did indicate fertility effects: the exposure to hexachlorobenzene was associated with an increased number of abortions, stillbirths and deaths in early childhood. Other human studies investigating developmental toxicity have been limited by small study size and low levels of hexachlorobenzene exposure. Recent epidemiological studies investigated the relation between hexachlorobenzene exposure and birth weight

and gestational age, the risk of erectile dysfunction, and the risk of cryptorchidism in offspring. None of the studies found an increased risk.

Animal studies have verified that hexachlorobenzene impaired neurological and immunological development, and reduced neonatal viability and growth. The occurrence of cleft palate, renal agenesis, and minor skeletal abnormalities in mice are consistent with a possible teratogenic role for hexachlorobenzene. A developmental study with rats evaluated neurodevelopmental end points and detected evidence of hyperactivity in the pups with a LOAEL of 2.5 mg/kg bw/day for minimal neurodevelopmental effects. A two-generation study with rats demonstrated a NOAEL for developmental effects of 0.4 mg/kg bw/day, based on reduced pup viability. Developmental effects on the immune system were observed in rat pups pre- and postnatally exposed to hexachlorobenzene, with a LOAEL of 0.2 mg/kg bw/day for increased IgM and IgG antibody responses to tetanus toxoid.

Long-term toxicity

The main long-term effect following exposure of humans to hexachlorobenzene is hepatic porphyria. Porphyria, diagnosed by the presence of high levels of porphyrins in the blood, faeces, or urine, has been detected following exposures to hexachlorobenzene in workers and residents of Flix, Spain after primarily inhalation exposures resulting from a nearby organochlorine factory, and in the Turkish epidemic due to consumption of contaminated grain.

In addition to hepatic toxicity, hexachlorobenzene has been shown to cause musculoskeletal effects, neurological toxicity, kidney damage and immunotoxicity in humans, which may be secondary effects of hepatic porphyria. Human data also suggest that hexachlorobenzene adversely affects the endocrine system; specifically, the thyroid is a target organ. Additionally, skin lesions occurred as porphyrins, accumulated in the skin, were activated by sunlight to generate reactive oxygen species, causing tissue damage most commonly on areas exposed to sunlight, such as the hands and face (phototoxicity).

Based on the studies of the Turkish epidemic, an oral long-term lowest intake level leading to adverse effects of 0.05 g/day could be derived, corresponding to an intake 0.8 mg/kg bw/day for a 60 kg adult. An oral NOAEL could not be identified.

In oral animal studies the critical effects for hexachlorobenzene are hepatic toxicity, reproductive toxicity, developmental toxicity, and carcinogenesis. Several studies in rats and mice have observed hepatic porphyria, as well as other hepatic effects such as peribiliary lymphocytosis and fibrosis, hepatomegaly and

increased liver weight, enzyme induction, and degenerative pathological changes. In a 18-month oral study with mice a LOAEL of 13 mg/kg bw/day was observed, based on decreased body weight and hepatocyte hypertrophy. In a lifespan oral study with hamsters, a LOAEL of 16 mg/kg bw/day was observed, based on a marked decrease in weight gain. For rats, a marginal LOAEL of 0.016 mg/kg bw/day was derived based on a two-generation study, with peribiliary lymphocytosis and liver fibrosis in F_1 males as the most sensitive effect. In this study mitochondrial swelling, increased liver weight, heart effects and renal damage were observed at dose levels of 0.4 mg/kg bw/day and higher, with a NOAEL of 0.08 mg/kg bw/day.

The most critical studies described above are listed in Table 12.

Table 12 Critical toxicity studies with hexachlorobenzene, per study type sorted in order of increasing N(L)OAEL.

Study	Route	Duration (wk)	Species	Reference	Effect level (mg/kg bw/day)	
					Type: value	Critical effect
subacute and subchronic studies						
subchronic repeated dose	oral	13	monkey	Babineau *et al*. 1991; Bourque *et al*. 1995; Foster *et al*. 1992a, 1995; Jarrell 1993; Sims *et al*. 1991	NOAEL: 0.01	necrosis of ovary surface epithelium cells, and denuding of ovary
subchronic repeated dose	oral	13	pig	Den Tonkelaar *et al*. 1978	NOAEL: 0.05	hepatocellular hypertrophy
subchronic repeated dose	oral	52	dog	Gralla *et al*. 1977	LOAEL: 0.1	increased severity of nodular hyperplasia of gastric lymphoid tissue
subacute repeated dose	oral	4	rat	Hadjab *et al*. 2004	NOAEL: 0.16	cochlear sensitivity defects
subchronic repeated dose	oral	13	rat	Long *et al*. 2004	NOAEL: 0.3	maxillary incisor degeneration
subchronic repeated dose	oral	18	mouse	Loose *et al*. 1981	LOAEL: 0.6	increased susceptibility to tumour challenge, decreased TK activity *in vitro*
reproduction toxicity studies (fertility and development)						
2-generation	oral	104	rat	Arnold *et al*. 1985, 1986	LOAEL: 0.016	peribiliary lymphocytosis and fibrosis in F1 males
developmental	*in utero* and oral	gestation and lactation	rat	Vos *et al*. 1979, 1983	LOAEL: 0.2	increased IgM & IgG responses to tetanus toxoid
human epidemiological studies						
epidemiology (cohort)	oral	ca. 210	human	Cam and Nigogosyan 1963; Cripps *et al*. 1984; Peters *et al*. 1982, 1987	LOAEL: 0.8	hepatic porphyria, hepatomegaly, musculoskeletal effects, neurological toxicity, kidney damage, phototoxicity, immunotoxicity
carcinogenicity studies in animals						
carcinogenicity	oral	life	hamster	Cabral *et al*. 1977	LOAEL: 4	hepatoma, liver haemangioendothelioma, thyroid alveolar adenoma
carcinogenicity	oral	104	rat	Ertürk *et al*. 1986	LOAEL: 4	hepatoma, bile-duct adenoma, hepatocarcinoma, renal adenoma
carcinogenicity	oral	120	mouse	Cabral *et al*. 1979	LOAEL: 12	liver tumours

Chapter 8

Existing guidelines, standards and evaluations

8.1 General population

In the Ninth Report on Carcinogens, the US National Toxicology Program classified hexachlorobenzene as *reasonably anticipated to be a human carcinogen*. The US Environmental Protection Agency classified hexachlorobenzene as a *probable human carcinogen*, Group B2, on the basis that oral administration of hexachlorobenzene has been shown to induce tumours in the liver, thyroid, and kidney in three rodent species. IARC has classified hexachlorobenzene as *possibly carcinogenic to humans* (Group 2B), based on inadequate evidence in humans and sufficient evidence in experimental animals for carcinogenicity.

8.2 Working population

Currently, the 8 hr time-weighted average (TWA) occupational exposure limit for hexachlorobenzene in the Netherlands is 0.03 mg/m^3. So far there is no limit value for exposure to hexachlorobenzene at the European level. A number of EU member countries have set a limit for hexachlorobenzene. Furthermore, the American Conference of Industrial Hygienists (ACGIH) has set a limit/recommended standard for exposure to hexachlorobenzene (see Table 13).

In 1988 DECOS classified hexachlorobenzene as carcinogenic, stating that it acts presumably via a non-genotoxic mechanism.[4,5]

Table 13 Existing Occupational Exposure Limits (OELs) for hexachlorobenzene.

country / organisation	level (mg/m^3)	time-relation	source
The Netherlands	0.03	TWA – value (8 hr)	SER[4,5]
Belgium	0.002	Limit value - 8 hr	GESTIS[4]
Canada	0.025	Limit value - 8 hr	GESTIS[4]
Denmark	0.025	Limit value - 8 hr	GESTIS[4]
	0.05	Limit value - short term	
France	0.5	Limit value - 8 hr	GESTIS[4]
Germany	no value established	-	BGIA[4,53]
Poland	0.5	Limit value - 8 hr	GESTIS[4]
Spain	0.002	Limit value - 8 hr	INSHT[59]
United States	0.002	TWA – value (8 hr)	ACGIH[54]

TWA: time-weighted average.

Chapter 9

Hazard assessment

9.1 Assessment of the health risk

Kinetics

No data are available on dermal absorption of hexachlorobenzene in humans. In rats a maximum dermal absorption rate of 0.9 µg/cm^2/h was established.

Oral absorption of hexachlorobenzene in humans via dietary intake of fatty foods is estimated at 85%, this percentage decreases with increasing amounts of hexachlorobenzene in blood. Animal studies indicate that the absorption is variable, ranging from 6% when administered in water up to 82% when administered in vegetable oils. Based on these data, the Committee assumes for risk assessment purposes that after oral exposure 80% hexachlorobenzene is absorbed.

No data are available on human or animal absorption of inhaled hexachlorobenzene. Hence for risk assessment purposes the Committee assumes respiratory absorption to be equal to oral absorption (default approach due to lack of data).

Information on distribution of hexachlorobenzene following inhalation exposure is very limited. Orally absorbed hexachlorobenzene distributes widely in mammalian tissues, preferentially to adipose tissue or organs with high fat content including milk. It can thus be transferred to the suckling neonate. In animals hexachlorobenzene is readily transferred through the placenta from pregnant mother to the foetus.

In humans, ingested or inhaled hexachlorobenzene is predominantly excreted as parent compound in faeces. A lower amount is excreted in the urine, as its metabolites pentachlorophenol, pentachlorothiophenol, and pentachlorobenzene. Based on oral animal studies, the biological half-life of hexachlorobenzene has been estimated to be 8 days at the start of the elimination phase, 10 weeks after 3 months, and 12 months after 1.5 years.

Irritation and sensitisation

No human nor animal data have been retrieved on the irritating and sensitising properties of hexachlorobenzene.

Acute toxicity

No information was located regarding acute health effects in humans following exposure to hexachlorobenzene. The acute lethality of ingested hexachlorobenzene in animals is relatively low: the LD_{50} for rodents is 3,500-4,000 mg/kg bw.

No acute toxicity studies in animals investigating the dermal and respiratory routes were retrieved.

Short-term toxicity

No information was located regarding short-term health effects in humans following exposure to hexachlorobenzene by any route of exposure.

No toxicity studies investigating the effects of hexachlorobenzene after dermal exposure on animals were located.

Observations in male rats exposed to 4.4 and 33–35 mg/m^3 of hexachlorobenzene aerosol for durations ranging from 4 hours to 4 weeks (4 hours/day, 4 days/week) showed an immunostimulating effect on T-cells. In this study only pulmonary host defences and body weight were investigated. It was not possible to derive a LOAEL or NOAEL from this study.

In oral toxicity studies, serious effects on the liver were observed in subchronic studies with monkeys, rats, mice and pigs. Pigs were the most sensitive animals: based on the hepatocellular hypertrophy observed at doses of 0.5 mg/kg bw/day and higher, an oral NOAEL of 0.05 mg/kg bw/day was derived.

A micronucleus test with human peripheral lymphocytes did not demonstrate an association between micronucleus formation and hexachlorobenzene exposure. Hexachlorobenzene tested negative in an *in vitro* chromosomal aberration assay and in three out of four bacterial mutation assays. Ambiguous results were obtained in one *in vitro* bacterial mutation assay and in *in vitro* and *in vivo* DNA-binding assays. Two *in vivo* dominant lethal studies in rats and two *in vivo* Comet assays in rats and mice were negative. Based on these data, the Committee considers hexachlorobenzene to be non-genotoxic.

Among the general population, older case-control studies have generally found no association between hexachlorobenzene levels in blood or tissues and incidence of breast or other cancers. Data from men exposed to hexachlorobenzene by inhalation provide inconclusive evidence for an association between hexachlorobenzene exposure and cancer of the liver, thyroid, and brain. Eight recent epidemiological studies studying the relationship between hexachlorobenzene exposure and cancer were found. The cancers examined were breast cancer, non-Hodgkin's lymphoma, prostate and testicular germ cell carcinoma. None of these studies established a clear relationship between hexachlorobenzene exposure and cancer.

Several animal studies have demonstrated that oral exposure to hexachlorobenzene increases the incidence of tumour formation. The evidence of carcinogenicity is strongest in the liver: hexachlorobenzene has been shown to induce hyperplasia (in rats, mice, pigs, dogs, and monkeys), metaplasia (in rats), benign tumours (hepatoma in mice and rats, haemangiohepatoma and bile duct adenoma in rats), and malignant tumours (hepatocarcinoma in rats, mice, and hamsters, bile duct adenocarcinoma in rats). Additionally, exposure to hexachlorobenzene has been shown to induce renal metaplasia, adenomas and renal cell carcinomas (in rats, mice, and hamsters), lymphosarcomas (in rats, mice, and hamsters), adrenal hyperplasia and pheochromocytoma (in rats), parathyroid adenomas (in rats), and haemangioendothelioma and thyroid tumours (in hamsters). No oral NOAELs for cancer effects have been identified, the lowest oral doses at which carcinogenic effects were seen were 4 mg/kg bw/day for hamsters and rats, and 12 mg/kg bw/day for mice.

Mechanistic studies have demonstrated hexachlorobenzene causes hepatic porphyria, mainly by reducing the activity of uroporphyrinogen decarboxylase. Tumour-promoting effects of hexachlorobenzene in the liver have been demonstrated in rats at doses that did not initiate tumours. Hexachlorobenzene has shown a lack of genotoxic potential in various mutagenicity assays, indicating

the substance does not induce direct DNA damage. Based on the available evidence, the Committee concludes that hexachlorobenzene acts as a promoter of liver carcinogenesis via an non-genotoxic mechanism of action. In accordance with the guidelines of the European Union, the Committee recommends classification as a substance presumed to have carcinogenic (non-genotoxic) potential for humans (category 1B; see Annex H).

Reproduction toxicity

In the older human studies, no reliable evidence of adverse effects of hexachlorobenzene on fertility has been uncovered. Recent epidemiological studies on the relationship between hexachlorobenzene exposure and various fertility and developmental outcomes investigated the proportion of male births, birth weight and gestational age, the risk of erectile dysfunction, and the risk of cryptorchidism in offspring. None of the studies found an increased risk.

Based on the serious toxic effects in children under 2 years of age during the Turkish epidemic in the 1950s, ATSDR concluded hexachlorobenzene to show developmental toxic effects. However, the described symptoms were also observed in adults and do not appear to be the results of interference with specific developmental processes. The high susceptibility of infants is probably more linked to their relatively high level of exposure via breast feeding, as hexachlorobenzene accumulates in breast milk, than to specific developmental effects. Analysis of human milk from exposed women and unexposed controls in this epidemic showed hexachlorobenzene concentrations of 0.51 and 0.07 mg/kg, respectively (Cripps *et al*. 1984; Gocmen *et al*. 1989; Peters *et al*. 1982, 1987). Also the relatively high incidence of symptoms among children under 15 may be related to higher levels of exposure rather than a higher susceptibility, as they have a higher energy consumption per kg body weight than adults and are therefore likely to have consumed relatively higher quantities of contaminated bread resulting in a higher body burden. In addition differences in metabolism between adults and children cannot be excluded as a cause of the observed differences in sensibility.

In 90-day monkey studies, adverse effects were observed on the female reproductive organs (necrosis of ovary surface epithelium cells, denuding of ovary), starting at a dose of 0.01 mg/kg bw/day. Since the releance of the observed effects (which were marginal and only seen microscopally) is doubtful, the Committee decided to characterize this dose as the NOAEL for fertility effects. Functional effects (changes in hormone levels and menstrual cycle length) were seen at higher dosages (0.1 mg/kg bw/day and higher).

Animal studies have verified that hexachlorobenzene impaired neurological development and reduced neonatal viability and growth. The occurrence of cleft palate, renal agenesis, and minor skeletal abnormalities in mice are consistent with a possible teratogenic role for hexachlorobenzene. A neurodevelopmental study with rats showed evidence of hyperactivity. Based on this study, a LOAEL of 2.5 mg/kg bw/day could be established for minimal neurodevelopmental effects; the study did not allow the derivation of a NOAEL. In a two-generation rat study the NOAEL for developmental effects was 0.4 mg/kg bw/day, based on reduced pup viability; fertility effects were not observed. Developmental effects on the immune system were observed in rat pups pre- and postnatally exposed to hexachlorobenzene, with a LOAEL of 0.2 mg/kg bw/day for increased IgM and IgG antibody responses to tetanus toxoid.

Overall, human epidemiological studies do not show a clear association between hexachlorobenzene exposure and fertility or developmental effects. However, a number of these studies have methodological limitations and animal studies do show fertility and developmental effects. Therefore, in accordance with the guidelines of the European Union (see Annex G), the Committee recommends to classify hexachlorobenzene as a category 1B reproductive toxicant for both effects on fertility and developmental toxicity (see Annex I). Since hexachlorobenzene can be transferred to breast milk, it should also be classified as *hazardous to breastfed babies* (see Annex I).

Long-term toxicity

The main long-term effect following exposure of humans to hexachlorobenzene is hepatic porphyria. This has been observed in workers and residents of Flix, Spain after primarily inhalation exposures resulting from a nearby plant that produced chlorinated solvents, and in a Turkish epidemic due to oral exposure to contaminated grain over a period of four years. In this latter epidemic, the ingested dose of hexachlorobenzene was estimated to be in the range of 0.05-0.2 g/day, equivalent to 0.8-3.3 mg/kg bw/day for an average person weighing 60 kg.

Next to hepatic toxicity, human data also indicate that hexachlorobenzene adversely affects the endocrine system; specifically the thyroid is a target organ. The relationship between hexachlorobenzene exposure and thyroid hormones has been evaluated in four different cross-sectional human studies. Although all studies did find a relationship between hexachlorobenzene level in serum and a (small) decrease in various thyroid hormones, the physiological significance is unknown. In animal studies, relatively high doses of hexachlorobenzene tested in short-term gavage and diet studies also showed a decrease of serum thyroxin lev-

els. The results of these animal studies suggest that other, clearly biologically significant effects (*e.g.*, hepatic) will occur at lower doses of hexachlorobenzene than the effects on serum thyroxin. Therefore, these effects are not relevant for the derivation of the HBROEL.

Only animal toxicity studies employing the oral route were available. The critical effects for hexachlorobenzene are hepatic toxicity, reproductive toxicity, developmental toxicity, and carcinogenesis. Several studies in rats and mice have observed hepatic porphyria, as well as other hepatic effects such as hepatomegaly and increased liver weight, enzyme induction, and degenerative pathological changes. Rats were the most sensitive species: a LOAEL of 0.016 mg/kg bw/day was observed based on marginal effects: peribiliary lymphocytosis and liver fibrosis in F_1 males of a two-generation study; a NOAEL could not be derived.

9.2 Recommendation of the health-based occupational exposure limit

Only one set of epidemiological studies was identified in which effects could be linked to external exposure levels. This set of studies reports and analyses the effects of a 4 year exposure to hexachlorobenzene via contaminated grain used in bread in the Southeastern part of Turkey. A major disadvantage of these studies is the fact that only one high level of hexachlorobenzene exposure could be taken into account, which does not allow extrapolation to low no-effect levels.

In the absence of appropriate human data, the HBROEL should ideally be derived from a chronic inhalatory animal toxicity study. However, in the public toxicity database described here, only one inhalatory study is available, and this study is considered not suitable for the derivation of a NOAEL: it is a short-term inhalation study (up to 4 weeks, 4 h/day, 4 days/wk) which only investigated body weight and pulmonary host defence parameters, and was limited to two dosages.

Many oral studies, both short- and long-term, are available. Some of the short-term studies investigated special parameters like the ultrastructure of ovaries, dental development and hearing performance. In Table 12 the critical studies (that is, the studies showing the lowest NOAELs) for each combination of exposure route, species used, and duration of exposure are listed. The available data are considered sufficient for the derivation of a health-based recommended occupational exposure limit (HBROEL) for hexachlorobenzene.

In general, hexachlorobenzene causes systemic (*e.g.*, liver, thyroid, skin, and bone), developmental, endocrine, neurological, and immunological toxicity. The Committee considers the 13 weeks reproductive monkey studies of Babineau *et al*. 1991[47]; Bourque *et al*. 1995[48]; Foster *et al*. 1992a[49], 1995[11]; Jarrell 1993[12];

and Sims *et al*. 1991[50] as the pivotal studies for deriving the HBROEL. Despite the disadvantage of being subchronic (13 weeks), these are scrupulously conducted studies which repeatedly showed similar adverse effects (histopathological as well as functional), all pointing to effects on the reproductive system (see Table 11). The NOAEL in these studies as derived by the Committee was 0.01 mg/kg bw/day, with a LOAEL of 0.1 mg/kg bw/day. A BMD analysis of the data as reported in these studies was not possible (only 4 animals per dose were used, and the number of dosages was too limited).

Taking the NOAEL of 0.01 mg/kg bw/day from the 13 weeks monkey studies as the starting point, the following uncertainty factors (UFs) were applied[55]:

- 2 for extrapolation from subchronic to chronic exposure (a UF of 2 is used taking into account the long biological half-life of hexachlorobenzene leading to bioaccumulation and thus the possibility of adverse effects occurring also after prolonged exposure at a lower dose)
- 2 for interspecies extrapolation from monkey to human
- 3 for intraspecies extrapolation (differences in susceptibility between individual healthy human workers).

This results in a safe oral intake level for humans of $0.01 / (2x2x3) = 0.83x10^{-3}$ mg/kg bw/day.

For conversion of the oral to to the inhalatory exposure route, inhalatory absorption is assumed to be equal to oral absorption. Further, it has to be adjusted for body weight (70 kg) and breathing volume during a 8 h working day (10 m^3)*, which leads to a value of $0.83x10^{-3}$ x (70/10) = $5.8x10^{-3}$ mg/m^3, rounded up to 0.006 mg/m^3.

The Committee thus recommends a HBROEL of 0.006 mg/m^3.

This HBROEL is supported by the two-generation rat study by Arnold *et al.* (1985[44], 1986[43]) which showed a LOAEL of 0.016 mg/kg bw/day based on marginal effects (a NOAEL could not be deived). Taking this LOAEL, the following UFs were applied:

- 2 to extrapolate from the LOAEL to a NOAEL (a UF of 2 is used considering the marginal character of the LOAEL)

* A breathing volume of 10 m^3 for a worker during a 8 h working day is the default assumption of the Committee.[58]

- 3 for rat to human extrapolation
- 3 for intraspecies extrapolation.

This results in a safe oral intake level for humans of 0.016 / (2x3x3) = 0.89×10^{-3} mg/kg bw/day. To convert this oral value into a respiratory value, it has to be adjusted for body weight (70 kg) and breathing volume during a 8 h working day (10 m^3). This would result a limit value of 0.89×10^{-3} x (70/10) = 6.2×10^{-3} mg/m^3 (rounded up to 0.006 mg/ m^3), which is similar to the HBROEL as derived above from the 13-weeks monkey studies.

In view of the hepatotoxic effects of hexachlorobenzene, which probably is the cause of hepatic tumors at high dose (oral) exposure, the Committee checked whether the above HBROEL would also protect against hepatotoxicity. The lowest dose at which hepatotoxicity was observed in animal studies was 0.05 mg/kg bw/day (NOAEL in pigs; Den Tonkelaar *et al*. 1978[37]) as compared to the dose of 0.008 mg/kg bw/day (0.016 mg/kg bw/day divided by 2) in the rat used for the calculation above. The lowest dose at which carcinogenicity was observed in animal studies was 4 mg/kg bw/day (LOAELs; Cabral *et al*. 1977; Ertürk *et al*. 1986). The recommended HBROEL thus also protects against hepatotoxicity and hepatocarcinogenicity. The lowest dose at which toxicity in humans was observed (liver and other tissues and organs) was 0.8 mg/kg bw/day. Finally, the Committee discussed the possible toxic effects of metabolites of hexachlorobenzene. The limited data on the biotransformation of hexachlorobenzene do not suggest essential differences between humans and experimental animals. Hence the Committee considered that the potential toxicity of metabolites is adequately covered by the various studies with experimental animals in which the toxicity of these metabolites – if any – has been included in the overall toxic effects observed.

The overall NOAEL for (sub)acute effects in animals is approximately 5 mg/kg bw, which is over two orders of magnitude higher than the lowest NOAELs observed in the relevant (acute/subacute) animal studies. Thus it is not considered necessary to derive an acute (15 minutes TWA) HBROEL.

9.3 Skin notation

In general, a skin notation is used to indicate that the contribution to the adverse systemic effects of a compound by exposure via the skin is considerable as compared to the contribution via the respiratory route of exposure. To determine whether or not a skin notation is applicable, the critical absorption value (CAV, that is the rate of absorption above which dermal exposure is considered to be an

important contributor to total exposure) has been calculated according to the method described by ECETOC[56]:

(10[m^3] x OEL[mg/m^3] x f x 0.1) / 2000 [cm^2]

in which 10 m^3 is the human inhalation volume per 8 h working day, f is the absorption factor for inhalation (here assumed to be 1), 0.1 denotes the 10% criterion, 2000 cm^2 is the surface area of the hands and forearms, and OEL is the Occupational Exposure Limit, in this case the HBROEL. Thus the CAV will be:

(10 [m^3] x 0.006 [mg/m^3] x 1 x 0.1) / 2000 [cm^2] = 3 $ng/cm^2/h$

The maximum rate of dermal absorption of hexachlorobenzene is 0.9 ± 0.2 $\mu g/cm^2/h$ (see Section 5.1), which is approximately 300 times higher than the CAV. Therefore the Committee recommends a skin notation for hexachlorobenzene.

9.4 Groups at extra risk

ATSDR data

Although infants appear to be especially sensitive to the effects of hexachlorobenzene (ATSDR, 2002)[1], they are not relevant in the risk assessment for workers, and are thus not discussed here any further.

Hexachlorobenzene has been shown to elevate porphyrin levels in humans following inhalation exposure (Herrero *et al*. 1999; Sala *et al*. 1999b; Selden *et al*. 1999) and to cause porphyria cutanea tarda (a specific disease resulting from elevated porphyrin levels) following oral exposure (Cam and Nigogosyan 1963; Cripps *et al*. 1984; Dogramaci 1964; Gocmen *et al*. 1989; Peters *et al*. 1982, 1987). Studies unrelated to hexachlorobenzene-exposure have associated the diagnosis of porphyria cutanea tarda with infections of HIV and hepatitis C virus (Drobacheff *et al*. 1998; Egger *et al*. 2002; Meola and Lim 1993). It is not known if, or the degree to which, these diseases contribute to or exacerbate one another; however, HIV and hepatitis C-infected individuals may have increased susceptibility to porphyria cutanea tarda following hexachlorobenzene exposure.

Additional data to the ATSDR toxicological profile (time period 2002-2010)

No additional data on groups at extra risk were found.

9.5 Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Safety recommends a health-based occupational exposure limit for hexachlorobenzene of 0.006 mg/m^3, as an 8-hours time-weighted average concentration, and a skin notation. There is no need for an acute (15 minutes time-weighted average concentration) health-based occupational exposure limit.

Chapter 10

Recommendation for research

Since information on respiratory absorption of hexachlorobenzene in not available in the public literature, the Committee recommends kinetic experiments with animals to estimate this absorption.

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A	Request for advice
B	The Committee
C	Comments on the public review draft
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F	Carcinogenic classification of substances by the Committee
G	Regulation (EC) No 1272/2008
H	Evaluation of the Subcommittee on the classification of carcinogenic substances
I	Evaluation of the Subcommittee on the classification of reprotoxic substances

Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, an ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in Annex B.

Annex **B**

The Committee

- G.J. Mulder, *chairman*
  Emeritus Professor of Toxicology, Leiden University, Leiden
- R.B. Beems
  Toxicologic Pathologist, formerly employed at the National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  Toxicologist; Shell International BV, The Hague
- B.P.F.D. Hendrikx, *advisor*
  Social and Economic Council, The Hague
- D.J.J. Heederik
  Professor of Risk Assessment in Occupational Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R. Houba
  Occupational Hygienist, Netherlands Expertise Centre for Occupational Respiratory Disorders, Utrecht
- H. van Loveren
  Professor of Immunotoxicology, Maastricht University, Maastricht, and National Institute for Public Health and the Environment, Bilthoven
- T.M. Pal
  Occupational Physician, Netherlands Centre for Occupational Diseases, University of Amsterdam, Amsterdam

- A.H. Piersma
  Professor of Reproductive Toxicology, Utrecht University, Utrecht, and National Institute for Public Health and the Environment, Bilthoven
- H.P.J. te Riele
  Professor of Molecular Biology, VU University Amsterdam, Amsterdam
- I.M.C.M. Rietjens
  Professor of Toxicology, Wageningen University and Research Centre, Wageningen
- H. Roelfzema, *observer*
  Ministry of Health, Welfare and Sport, The Hague
- G.M.H. Swaen
  Epidemiologist; Dow Benelux NV, Terneuzen
- R.C.H. Vermeulen
  epidemiologist, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R.A. Woutersen
  Toxicologic Pathologist, TNO Quality of Life, Zeist, and Professor of Translational Toxicology, Wageningen University and Research Centre, Wageningen
- P.B. Wulp
  Occupational Physician, Labour Inspectorate, Groningen
- A.J. Baars, *scientific secretary*
  Health Council of the Netherlands, The Hague
- A.S.A.M van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment.

An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2011 for public review. The following organisations and persons have commented on the draft document:

- V. Gálvez Perez, Ministerio de Trabajo e Immigración, Madrid, Spain.
- K.E. Dragon, National Institute for Occupational Safety and Health, Pittsburgh (PA), USA.

Annex **D**

Human data

(if not further specified, the data concern the period 2002-2010)

Human studies with regard to carcinogenic effects after exposure to hexachlorobenzene

- Table D.1.1 Overview of case-control studies on breast cancer
- Table D.1.2 Overview of cohort studies on breast cancer
- Table D.1.3 Overview of case-control studies on non-Hodgkin's lymphoma
- Table D.1.4 Overview of case-control studies on prostate cancer
- Table D.1.5 Overview of case-control studies on testicular germ cell cancer

Human studies with regard to non-carcinogenic effects after long-term exposure to hexachlorobenzene

- Table D.2.1 Overview of studies on reproductive toxicity effects
- Table D.2.2 Overview of studies on thyroid effects

Abbreviations used in Tables:

CI = confidence interval
CLL = Chronic lymphocytic lymphoma
DLBCL = diffuse large B-cell lymphoma
HxCB = hexachlorobenzene
LOD = limit of detection

LOQ = limit of quantitation
N = number
OR = odds ratio
Q = quintile
RR = relative risk
SLL = small lymphocytic lymphoma
T = tertile

D.1 Human studies with regard to carcinogenic effects after exposure to hexachlorobenzene

Table D.1.1 Overview of studies regarding exposure to hexachlorobenzene and breast cancer.

Reference	Design	Country	Gender	Type of control	N control	N cases	Exposure assessment	Mean exposure to HxCB among controls	Contrast	OR (95% CI)
[18]	case-control	Belgium	female	hospital	250	159	serum level	0.09 µg/L	HxCB higher or lower than LOQ	9.14 (2.84-29.41)

Table D.1.2 Overview of studies regarding exposure to hexachlorobenzene and breast cancer.

Reference	Design	Country	Gender	Follow up (years)	N cohort	N cases	Exposure assessment	Contrast	Subgroup	RR (95% CI)
[19]	nested case-control	Japan	female	10.7	24,226	139	plasma	Q4 (0.72 µg/L) vs Q1 (0.081 µg/L)	all	0.82 (0.42-1.60)
									premenopausal	0.48 (0.14-1.63)
									postmenopausal	1.09 (0.33-3.54)
[20]	cohort	Denmark	female	4.8	24,697	409	adipose tissue	Q4 (91-704 µg/kg lipids) vs Q1 (8-58 µg/kg lipids)	all, postmenopausal	0.5 (0.3-0.9)
									ER+, postmenopausal	0.6 (0.4-1.1)
									ER-, postmenopausal	0.2 (0.0-0.6)

Table D.1.3 Overview of studies regarding exposure to hexachlorobenzene and non-Hodgkin's lymphoma.

Reference	Design	Country	Cancer site	Gender	Type of control	N control	N cases	Exposure assessment	contrast	OR (95% CI)
[22]	case-control	France, Germany, Spain	Non-Hodgkin's lymphoma	both	general population and hospital-based	203	174	plasma level	Q4 (≥ 556.71 µg/L) vs Q1 (≤ 117.93 µg/L)	1.1 (0.5-2.3)
			DLBCL	both	general population and hospital-based	203	44	plasma level	Q4 (≥ 556.71 µg/L) vs Q1 (≤ 117.93 µg/L)	1.1 (0.4-3.7)
			CLL/SLL	both	general population and hospital-based	203	55	plasma level	Q4 (≥ 556.71 µg/L) vs Q1 (≤ 117.93 µg/L)	0.9 (0.3-2.6)
[24]	case-control	Canada	Non-Hodgkin's lymphoma	both	general population	460	422	plasma level	Q4 (> 22.78-1,050 µg/L) vs Q1 (≤ 11.45 µg/L)	1.94 (1.25-3.03)
			Follicular non-Hodgkin's lymphoma	both	general population	460	141	plasma level	Q4 vs Q1	2.4 (1.2-4.6)
			Diffuse large cell non-Hodgkin's lymphoma	both	general population	460	67	plasma level	Q4 vs Q1	2.5 (1.0-6.0)
			T-cell non-Hodgkin's lymphoma	both	general population	460	47	plasma level	Q4 vs Q1	1.4 (0.5-4.0)
			Other B-cell non-Hodgkin's lymphoma	both	general population	460	167	plasma level	Q4 vs Q1	1.6 (0.9-2.9)
[23]	case-control	USA	Non-Hodgkin's lymphoma	both	hospital	481	175	adipose tissue	triiodothyronine (40 µg/kg lipid) vs T1 (0.00 µg/kg lipid (<LOD))	1.29 (0.58-2.83)

Table D.1.4 Overview of studies regarding exposure to hexachlorobenzene and prostate cancer.

Reference	Design	Country	Gender	Type of control	N control	N cases	Exposure assessment	Mean exposure to HxCB among controls	Contrast	Subgroup	OR (95% CI)
[25]	case-control	Sweden	men	hospital (benign hyperplasia)	20	58	adipose tissue	64 µg/kg lipid	HxCB higher or lower than median concentration among controls (28 µg/kg lipid)	all	2.39 (0.81-7.09)
										PSA ≤ 16.5 ng/ml	1.14 (0.35-3.72)
										PSA > 16.5 ng/ml	9.84 (1.99 - 48.5)
										PSA < 4 *	0.74 (0.15-3.54)
										PSA 4-10 *	1.14 (0.17-7.61)
										PSA >10 *	5.21 (1.46-18.6)

* prostate cancer patients were divided in three groups based on their clinical relevance of PSA (prostate specific antigen).

Table D.1.5 Overview of studies regarding exposure to hexachlorobenzene and testicular germ cell carcinoma.

Reference	Design	Country	Gender	Type of control	N control	N cases	Exposure assessment	Median exposure to HxCB among controls	Contrast	OR (95% CI)
[26]	case-control	USA	men	population	630	246	serum level	0,186 µg/L *	T3 vs T1	0.85 (0.37-1.96)
									per 10 pg/g	1.05 (0.95-1.15)

* median concentration for cases and controls together.

D.2 Human studies with regard to non-carcinogenic effects after long-term exposure to hexachlorobenzene

Tables D.2.1 Overview of studies regarding exposure to hexachlorobenzene and reproductive effects

Table D.2.1.1 Proportion of male offspring.

Reference	Country	Gender	N controls	N cases	Exposure assessment	Contrast	Group	% male offspring
[27]	Turkey	women and male offspring	42 (in south east Turkey), 42 (in Ankara)	42 in south east Turkey	confirmed diagnosis of porphyria cutanea tarda	exposed versus non-exposed, not quantified	exposed	50.17%
							control group 1	53.12%
							control group 2	54.22% (not significant)

Table D.2.1.2 Erectile dysfunction.

Reference	Design	Country	Gender	Type of control	N control	N cases	Exposure assessment	Mean exposure to HxCB among controls	Contrast	Subgroup	OR (95% CI)
[28]	case-control	Canada	men	hospital	234	101	plasma level	19.28 µg/kg lipid	T3 (>23.0 µg/kg lipid) vs T1 (<16.0 µg/kg lipid)	all	0.91 (0.48-1.69)

Table D.2.1.3 Birth weight and gestational age.

Reference	Country	Gender	Population	N	Exposure assessment	Median exposure to HxCB	Outcome	Contrast	Results
[29]	Ukraine	female	pregnant women	162	breast milk (at day 4 or 5 after birth)	168 µg/kg milk fat	birth weight (gram)	T3 versus T1	3548 ± 66 versus 3408 ± 62 (not significant)
							relative birth weight (%)[a]	T3 versus T1	103.3 ± 1.7 versus 100.1 ± 1.6 (not significant)
[31]	Norway	female	pregnant women	300 + 26 [b]	breast milk (daily for 8 days at age 0-2 months)	11.5 µg/kg milk fat	gestational age	association with HCB conc. in milk	-0.8 (CI -4.4 to 2.9)
							birth weight	association with HCB conc. In milk	-90 (CI -275 to 8)

[a] defined as the ratio of the observed weight to the mean weight for that gestational age.
[b] 300 mothers were randomly selected from the "Norwegian Human Milk Study", later 26 mothers of small for gestational age infants were included.

Table D.2.1.4 Cryptorchidism in male offspring.

Reference	Design	Country	Gender	Follow up (years)	N cohort	N cases	Exposure assessment	Contrast	Subgroup	RR (95% CI)
[30]	nested case-control	USA	pregnant women	1 year	42.000	216	serum level, repeated every 8 weeks	Q5 (≥ 0.59 µg/L) vs Q1 (< 0.11 µg/L)	all	1.06 (0.57-1.98)

Table D.2.2 Overview of cross-sectional studies regarding exposure to hexachlorobenzene and thyroid function.

Reference	Country	Gender	Population	N	Exposure assessment	Mean exposure to HxCB	Outcome	Regression coefficient of hormone level
35	USA	men	20-54 years	341	serum level	15.6 ng/g lipid 0.075 ng/g serum	free thyroxin (ng/dL)[a] TSH (µgIU/mL)[a] total triiodothyronine (ng/mL)[a] total triiodothyronine (ng/mL)[b]	0.021 (-0.018, 0.060) 0.95 (0.87, 1.04) -0.018 (-0.046, 0.010) -0.034 (-0.062, -0.005)
33	USA	women	pregnant	334	serum level	65.8 ng/g lipids	free thyroxin (thyroxin) (ng/dL)[c] total thyroxin (thyroxin) (µg/dL)[c] TSH (mIU/L)[c]	-0.08 (-0.15, -0.01) -0.51 (-0.97, -0.04) 0.02 (-0.05, 0.10)
32	USA	men	sportsmen	66	serum level	0.223 ng/g	total thyroxin full model (µg/dl)[d] total thyroxin reduced model (µg/dl)[d]	-0.086 (-0.245, 0.073) -0.113 (-0.227, 0.000)
34	Canada	neonates	Nunavik region	410	serum level umbilical cord	58.4 µg/kg lipid 0.14 µg/L	TSH (mIU/L)[e] free thyroxin (pmol/l)[e] triiodothyronine (nmol/l)[e] TBG (nmol/l)[e]	-0.07 (p=0.20) 0.12 (p=0.04) 0.11 (p=0.09) 0.03 (p=0.68)
34	Canada	neonates	Lower North Shore St. Lawrence river	260	serum level umbilical cord	21.1 µg/kg lipid 0.05 µg/L	TSH (mIU/L)[e] free thyroxin (pmol/l)[e] triiodothyronine (nmol/l)[e] TBG (nmol/l)[e]	0.02 (p=0.79) 0.19 (p < 0.01) 0.00 (p=0.96) -0.05 (p=0.43)
32	USA	men	sportsmen	66	serum level	0.223 ng/g	total thyroxin (µg/dl)	-0.192 (p=0.123)

[a] coefficient represents the change in hormone level for an IQR (interquintile range) change in hexachlorobenzene concentration, adjusted for serum lipids, age, BMI, current smoking, time of blood draw.

[b] coefficient represents the change in hormone level for an IQR change in hexachlorobenzene concentration, adjusted for serum lipids, age, BMI, current smoking, time of blood draw and p,p-DDE concentration (DDT metabolite).

[c] coefficient represents change in hormone level per 10-fold increase in exposure, adjusted for age, pre-pregnancy BMI.

[d] the full model included all covariates: time of sample, age, BMI, smoking, serum lipids, polychlorinated biphenyl, hexachlorobenzene. The reduced model was based on the statistically significant predictors using a step-wise procedure.

[e] multiple correlation coefficient adjusted for parity, smoking, serum lipids, newborn birth weight, newborn gestational age, maternal age, ethnicity.

TBG = thyroxin-binding globulin, TSH = thyroid stimulating hormone, IU = international units

Annex **E**

ATSDR references

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Annex **F**

Carcinogenic classification of substances by the Committee

The Committee expresses its conclusions in the form of standard phrases:

Category	Judgement of the Committee (GR_{GHS})	Comparable with EU Category	
		67/584/EEC before 12/16/2008	EC No 1272/2008 as from 12/16/2008
1A	The compound is known to be carcinogenic to man. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, the mechanism of action is not known.	1	1A
1B	The compound is presumed to be carcinogenic to man. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, the mechanism of action is not known.	2	1B
2	The compound is suspected to be carcinogenic to man.	3	2
(3)	The available data are insufficient to evaluate the carcinogenic properties of the compound.	Not applicable	Not applicable
(4)	The compound is probably not carcinogenic to man.	Not applicable	Not applicable

Source: Health Council of the Netherlands. Guidline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands, 2010; publication no. A10/07E.

Annex **G**

Regulation (EC) No 1272/2008

of the European Parliament and of the Council on classification, labelling, and packaging of substances and mixtures

3.6 Carcinogenicity

3.6.1 Definition

Carcinogen means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

3.6.2 Classification criteria for substances

See Table on the next page.

3.6.2.1 For the purpose of classification for carcinogenicity, substances are allocated to one of two categories based on strength of evidence and additional considerations (weight of evidence). In certain instances, route-specific classification may be warranted, if it can be conclusively proved that no other route of exposure exhibits the hazard.

3.6.2.2 Specific considerations for classification of substances as carcinogens.

3.6.2.2.1 Classification as a carcinogen is made on the basis of evidence from reliable and acceptable studies and is intended to be used for substances which have an intrinsic property to cause can-

Table 3.6.1 Hazard categories for carcinogens.

Categories	Criteria
Category 1:	Known or presumed human carcinogens. A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
Category 1A:	Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or
Category 1B:	Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.
Category 2:	Suspected human carcinogens. The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

(1) Note: See 3.6.2.2.4.

cer. The evaluations shall be based on all existing data, peer-reviewed published studies and additional acceptable data.

3.6.2.2.2 Classification of a substance as a carcinogen is a process that involves two interrelated determinations: evaluations of strength of evidence and consideration of all other relevant information to place substances with human cancer potential into hazard categories.

3.6.2.2.3 Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms ‘sufficient’ and ‘limited’ have been used here as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows:

(a) Carcinogenicity in humans

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

- sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;
- limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

(b) Carcinogenicity in experimental animals

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals. The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

- sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;
- limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, *e.g.* (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

3.6.2.2.4 Additional considerations (as part of the weight of evidence approach (see 1.1.1)). Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here.

3.6.2.2.5 The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner.

3.6.2.2.6 Some important factors which may be taken into consideration, when assessing the overall level of concern are:

a tumour type and background incidence;
b multi-site responses;
c progression of lesions to malignancy;
d reduced tumour latency;
e whether responses are in single or both sexes;
f whether responses are in a single species or several species;
g structural similarity to a substance(s) for which there is good evidence of carcinogenicity;
h routes of exposure;
i comparison of absorption, distribution, metabolism and excretion between test animals and humans;
j the possibility of a confounding effect of excessive toxicity at test doses;
k mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.

Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity in vivo may indicate that a substance has a potential for carcinogenic effects.

3.6.2.2.7 A substance that has not been tested for carcinogenicity may in certain instances be classified in Category 1A, Category 1B or Category 2 based on tumour data from a structural analogue together with substantial support from consideration of other important factors such as formation of common significant metabolites, *e.g.* for benzidine congener dyes.

3.6.2.2.8 The classification shall take into consideration whether or not the substance is absorbed by a given route(s); or whether there are only local tumours at the site of administration for the tested route(s), and adequate testing by other major route(s) show lack of carcinogenicity.

3.6.2.2.9 It is important that whatever is known of the physico-chemical, toxicokinetic and toxicodynamic properties of the substances, as well as any available relevant information on chemical analogues, i.e. structure activity relationship, is taken into consideration when undertaking classification.

3.6.4 Hazard communication

3.6.4.1 Classification for carcinogenicity:

Category 1A or Category 1B:

Hazard statement H350: May cause cancer *<state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard>*.

Category 2:

Hazard statement H351: Suspected of causing cancer *<state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard>*.

Annex **H**

Evaluation of the Subcommittee on the classification of carcinogenic substances

H.1 Scope

On request of the Dutch Expert Committee on Occupational Safety of the Health Council, the Subcommittee on the Classification of Carcinogenic Substances evaluates the carcinogenic properties of hexachlorobenzene.

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification with reference to the appropriate EU-directive (see annexes F and G). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question.

The members of the Subcommittee on classifying carcinogenic substances are listed at the end of this annex. This evaluation is based on the data summarized in the first part of the present report of DECOS.

H.2 Carcinogenicity of hexachlorobenzene

The International Agency for Research on Cancer (IARC) classified hexachlorobenzene previously as ‘possibly carcinogenic to humans’ (group 2B), based on inadequate evidence in humans and sufficient evidence in experimental animals for carcinogenicity (IARC 2001).

There are three human studies on breast cancer (Charlier *et al*. 2003, Raaschou-Nielsen *et al*. 2005, Iwasaki *et al*. 2008). The first one shows a positive correlation, but the numbers are small and only a few confounders were taken into account. The two other studies are negative. Due to the lack of reliable data a conclusion cannot be drawn.

For non-Hodgkin lymphoma three studies (all case-control) are available, two of which (Cocco *et al*. 2008, Quintana *et al*. 2004) are negative, one (Spinelli *et al*. 2007) is positive. All studies are of limited quality, and thus, also due to the lack of cohort studies, a conclusion cannot be drawn.

Prostate (Hardell *et al*. 2006) and testicular tumours (Biggs *et al*. 2008) were studied in case-control studies. Both were negative.

The Subcommittee resumes that most studies are negative and only some are positive, but generally the numbers are small and the data are not convincing. In many studies mixed exposure to other organochlorine compounds is possible. Regarding the human epidemiological studies the Subcommittee concludes that the human data are insufficient to allow a conclusion as to an association between exposure to hexachlorobenzene and cancer.

Regarding the animal studies, the subcommittee considers that in rats, mice, hamsters, dogs, pigs and monkeys liver hyperplasia and benign and malignant tumours in the liver have been observed. In some species also renal cancers have been seen. Tumour promoting effects have been observed in rats at doses that did not initiate tumours (Pereira *et al*. 1982, Tsuda *et al*. 1993). Also in tumour promotion model systems clear indications for tumour promoting acticity was found (Ou *et al*. 2003, Ichihara *et al*. 2005, Randi *et al*. 2006). Regarding the animal studies the subcommittee concludes that there is sufficient evidence for the carcinogenicity of hexachlorobenzene.

Various mutagenicity and genotoxicity tests show predominantly negative results (reviewed in ATSDR 2002; an *in vivo* Comet assay with mice and rats has been reported negative (Sekihashi *et al*. 2002)). The Subcommittee states that hexachlorobenzene appears to cause cancer via a non-genotoxic mechanism, such as hepatotoxicity. It may act as a promotor of liver carcinogenesis.

H.3 Recommendation for classification

Based on the available data, the Subcommittee recommends classifying hexachlorobenzene in category 1B * (*the compound should be regarded as*

* According to the new classification system of the Health Council, which is based on regulation 1272/2008 of the European Union. This regulation came into force on 20 January 2009.

carcinogenic to humans). Since hexachlorobenzene appears to be a non-genotoxic compound, the subcommittee advises to apply the threshold-approach to derive a HBROEL.

H.4 References

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H.5 The Subcommittee

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Meeting date: 3 December 2010

Annex I

Evaluation of the Subcommittee on the classification of reprotoxic substances

I.1 Scope

On request of the Dutch Expert Committee on Occupational Safety of the Health Council, the Subcommittee on the Classification of Reprotoxic Substances evaluates the reprotoxic properties of hexachlorobenzene.

In the Netherlands a special policy is in force with respect to occupational use and exposure to reprotoxic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the reprotoxic properties of substances, and to propose a classification with reference to the appropriate EU-directive (see Annex G).

The members of the Subcommittee on classifying reprotoxic substances are listed at the end of this annex. This evaluation is based on the data summarized in the first part of the present report of DECOS.

I.2 Human studies, general

The Subcommittee is of the opinion that the available human studies of Cam and Nigogosyan (1963), Gocmen *et al.* (1989), Jarrell *et al.* (1998, 2002), and Peters *et al*. (1982, 1987) are of limited value. These studies deal with the epidemic in Turkey in the mid-1950s and were reported many years later. Although they clearly indicate very serious toxic effects, the data do not allow a conclusion as to the reproduction toxic potential of hexachlorobenzene.

I.3 Human studies, fertility

In a study carried out in China, no changes in reproductive outcomes were detected following the cessation of agricultural uses of hexachlorobenzene (Huang *et al.* 1989).

I.4 Human studies, developmental effects

Developmental effects occurred with a similar prevalence among women who ever worked at a chemical plant in Spain and never exposed female residents, despite a 5-fold higher blood hexachlorobenzene levels in the workers (Sala *et al.* 1999). The small number of female factory workers is a limitation of this study.

Studies of other populations with exposure to multiple organochlorines did not show significant differences in blood hexachlorobenzene levels between controls and cases of spontaneous abortion in Italy (Leoni *et al.* 1986, 1989) or Germany (Gerhard *et al.* 1998).

Hosie *et al*. (2000) studied the association between organochlorine compounds in adipose tissues of pregnant mothers and the risk for undescended testes in the offspring. Although such an association indeed was found, the results are inconclusive because not only hexachlorobenzene but also heptachlorepoxide was associated with undescended testes. Moreover, the study size was very limited.

The association between hexachlorobenzene exposure and erectile dysfunction was examined in a case-control study by Polsky *et al*. (2007); no increased risk was found.

Gladen *et al.* (2003) studied the relation between hexachlorobenzene exposure during pregnancy and birth weight. Hexachlorobenzene levels in breast milk were used as a proxy for exposure. Overall, no association between hexachlorobenzene exposure and birth weight was found.

Pierik *et al.* (2007) studied the association between hexachlorobenzene exposure of the mother during pregnancy and cryptorchidism in offspring in the first year of life in a nested case-control study. A large number of possible confounders were evaluated and found not to influence the outcome. No increased risk of cryptorchidism was found.

Eggesbø *et al*. (2009) investigated the levels of hexachlorobenzene in breast milk in relation to birth weight. They found statistically not significant inverse associations between levels in milk and gestational age, and between levels in

milk and birth weight. A large number of possible confounders were evaluated and found not to influence the outcome.

I.5 Animal studies, fertility

Multiple endocrine effects and pathological changes in reproductive organs have been demonstrated in animal studies. Increased adrenal and liver weights were found in monkeys dosed 10 mg/kg bw/day for 90 days (Jarrel *et al*. 1993). In rats, higher doses showed a rapid decrease of serum thyroxin levels. In a two-generation rat study, however, hexachlorobenzene had no effects on fertility (Arnold *et al*. 1985, 1986). Studies with female monkeys demonstrated effects on reproductive organs after 90 days exposure to doses as low as 0.1 mg/kg bw/day (Babineau *et al.* 1991, Bourque *et al.* 1995, Foster *et al.* 1992a, 1995, Jarrell *et al*. 1993, Sims *et al*. 1991). The studies focused predominantly on end points relevant to fertility (ovarian function and histopathology). In all treated animals, hexachlorobenzene caused follicular degeneration and induced histopathological changes in the ovarian epithelium (cell stratification, decreased nuclear membrane distinction, increased density and granularity of oocyte nuclei, and increased numbers of aggregated lysosomes, vesicles, and vacuoles). The severity was dose-dependent. Hepatotoxicity was seen at dose levels of 1 mg/kg bw per day and higher. At 0.01 mg/kg bw/day mitochondria in developing follicles were condensed and deformed. At higher doses (up to 10 mg/kg bw/day), the mitochondria were progressively more damaged and other changes were noted, such as indentation of nuclear membranes and abnormal accumulation of lipid in the cytoplasm of follicular cells. Follicular degeneration was apparent from doses of 0.1 mg/kg bw/day upwards. Dose levels of 0.01 and 0.1 mg/kg bw/day only induced ultrastructural effects, while functional effects (hormonal and menstrual cycle length) start to appear from doses of 1 mg/kg bw/day upward. The subcommittee agrees to base the LOAEL for fertility effects in these studies on the degenerative lesions in ovarian follicles observed at 0.1 mg/kg bw/day. The effects observed at 0.01 mg/kg bw/day were marginally and only seen electron-microscopally. At this dose level, the adversity of the effects is doubtful in the apparent absence of functional effects. Therefore, the subcommittee considers 0.01 mg/kg bw/day to represent the NOAEL for these ovarian effects (Jarrell *et al*. 1993, Bourque *et al*. 1995).

Rat studies have confirmed the reproductive toxicity of hexachlorobenzene in the ovary. Increased serum progesterone levels and elevated ovarian weights were observed in super-ovulating female Sprague-Dawley rats orally administered 1 mg/kg bw/day hexachlorobenzene by gavage for 21 days (Foster

et al. 1992b). Super-ovulating (but not normal cycling) female Sprague-Dawley rats gavaged with 50 mg/kg bw/day of hexachlorobenzene for 5 days exhibited significant elevation of serum levels of progesterone (Foster *et al.* 1993). In a subsequent study with ovariectomized female Sprague-Dawley rats, gavage doses of at least 1 mg/kg bw/day for 30 days significantly decreased circulating corticosterone and cortisol levels, without affecting levels of circulating aldosterone and progesterone levels or adrenal gland weight (Foster *et al.* 1995).

I.6 Animal studies, developmental effects

Animal studies have verified that hexachlorobenzene impaired neurological development and reduced neonatal viability and growth. The occurrence of cleft palate and minor skeletal abnormalities in mice are consistent with possible developmental effects of hexachlorobenzene. These were observed in pregnant CD-1 mice gavaged with 100 mg hexachlorobenzene per kg bw per day during gestation days 7-16 (Courtney *et al*. 1976). At this dose maternal relative liver weights were increased significantly (0.080 versus 0.061 for controls). Developmental toxicity experiments in pregnant Wistar rats administered 40 mg/kg bw/day during gestation days 6-21 showed increased incidences of sternal defects and 14th rib formation (Khera 1974). At this dose no maternal toxicity was seen (maternal toxicity became apparent at the next higher dose: 80 mg/kg bw/day).

Evidence of hyperactivity in rat pups was detected in a study evaluating neurodevelopmental end points (Goldey and Taylor 1992). Virgin female Sprague-Dawley rats were gavaged for 4 days with 2.5 or 25 mg/kg bw/day of hexachlorobenzene 2 weeks prior to mating. Compared to controls, pups from both treatment groups reoriented themselves significantly more quickly in a negative geotaxis test, required less time in an olfactory discrimination test (postnatal days 6, 8, and 10), and demonstrated increased exploratory activity in a motor activity test (postnatal days 15-20). Pups of which the mothers were exposed to 25 mg/kg bw/day, exhibited decreased acoustic startle response (ASR) on postnatal day 23 and increased ASR on postnatal day 90. ATSDR (2002) concluded a LOAEL of 2.5 mg/kg bw/day, based on minimal neurodevelopmental effects. A NOAEL could not be established. No maternal toxicity was observed, but body weight was the only parameter measured. Therefore, no conclusions can be drawn from this study with respect to maternal toxicity.

Pups of pregnant BALB/c mice fed doses as low as 0.5 mg/kg/day on gestation days 1–18 exhibited a marked, significant decrease in delayed type

hypersensitivity (DTH) response when tested on postnatal day 45 (Barnett *et al*. 1987). However, a dose-response relationship was not observed. Analyses of collected spleen cells revealed that 5 (but not 0.5) mg/kg bw/day decreased mixed lymphocyte response and B cell numbers; neither dose affected spleen blastogenesis induced by T- or B-cell mitogens. No overt signs of toxicity in either the treated females or their offspring were noted.

Developmental effects on the immune system were also reported by Vos *et al*. (1979). They exposed pregnant rats to HCB at concentrations of 0, 50 or 150 mg/kg diet (equivalent with 0, 2.5 or 7.5 mg/kg bw/day) and continued the exposure through lactation until weaning when the pups were 5 weeks of age. The authors observed elevated IgM and IgG serum levels as well as an increase in the number of blood basophilic and eosinophilic granulocytes in the blood of the pups from dams exposed at the highest dose. Testing of immune function showed a decrease in resistance to *Trichinella spiralis* and to *Listeria monocytogenes* infection for pups in the highest dose group. In a follow-up study (Vos *et al*., 1983), pups were exposed to HCB during pre- and postnatal development through maternal exposure at doses of 0, 4, 20 or 100 mg/kg diet (equivalent with 0, 0.2, 1 or 5 mg/kg bw/day). Pups in the highest dose group had elevated serum IgM levels and increased numbers of basophilic peripheral granulocytes, while animals in the 1 and 5 mg/kg bw/day dose group had elevated popliteal lymph node weights. Primary and secondary IgM and IgG antibody responses to tetanus toxoid were increased in animals from the 0.2 and 1 mg/kg bw/day groups.

In a two-generation feeding study Sprague-Dawley rats were administered hexachlorobenzene in doses of 0.016, 0.08, 0.4, and 2 mg/kg bw/day (Arnold *et al*. 1985, 1986). Hexachlorobenzene significantly reduced pup viability of the 2.0 mg/kg bw/day parental group, indicating a treatment related developmental effect. There were no treatment-related effects on growth, food consumption, haematological parameters or survival in either (adult) generation. For developmental effects the subcommittee derives a NOAEL of 0.4 mg/kg bw/day (in accordance with ATSDR 2002), based on reduced pup viability at the next higher dose level. Fertility effects were not observed in these studies.

I.7 Lactation

In order to protect up to 6-month-old breastfed children from the effects of hexachlorobenzene through intake of breast milk, the Subcommittee uses the following default values:

- Body weight infant: 4.5 kg

- Intake human breast milk per infant per day: 900 mL
- An infant is as sensitive to the substance as an adult.

These assumptions are used for the calculation of a tolerable level of hexachlorobenzene in human breast milk. The values are conservative figures estimated from growth curves for the Netherlands (Fredriks *et al*. 2000) and by the WHO (2006), and breast milk intake (Butte *et al*. 2002). Of note, however, are the indications from animal studies that children might be more sensitive to the effects of hexachlorobenzene than adults (ATSDR 2002).

The following health-based limit values have been recommended:
- ATSDR (2002), MRL for intermediate duration exposure (exposure 15 days to 1 year): 0.1 µg/kg bw/day
- ATSDR (2002), MRL for chronic exposure (1 year or longer): 0.005 µg/kg bw/day
- US-EPA (1991), RfD (1991): 0.8 µg/kg bw/day.

The MRL (intermediate) of ATSDR is the most appropriate for suckling infants (it covers the right exposure period, it is a rather recent limit value, and the value is conservatively estimated). This corresponds to:
- a tolerable intake of hexachlorobenzene of at most 0.45 µg/infant/day
- a tolerable concentration of hexachlorobenzene in breast milk of at most 0.5 µg/L.

In 2009, the concentration of hexachlorobenzene was measured in breast milk in a randomly selected cohort of 300 Norwegian mothers (Eggesbø *et al*. 2009). The lowest and highest level found were 0.13 and 1.3 µg/L, respectively, while the median and mean levels were 0.35 and 0.36 µg/L, respectively. In a recent study in China levels varied between 0.6 and 24 µg/L (Wang *et al*. 2010). Earlier studies reported mean levels of 0.6 µg/L in New Zealand (Bates *et al*. 1994) and 19.2 µg/L in the Czech Republic (Schoula *et al*. 1996). Thus a substantial part of the breast milk samples exceeds the tolerable concentration of hexachlorobenzene.

I.8 Conclusion

Indications for the reproduction toxic potential of hexachlorobenzene from human studies are not convincing. However, after weighing the evidence from animal studies, the subcommittee concludes that hexachlorobenzene does affect

both fertility and development in experimental animals. Consequently, the subcommittee proposes to classify hexachlorobenzene as a 'presumed human reproductive toxicant' and recommends classifying the substance in category 1B *. Furthermore, the Subcommittee recommends classifying hexachlorobenzene as a substance that 'may cause harm to breastfed babies'.

In addition the Subcommittee advises to characterize the dose of 0.01 mg/kg bw/day in the 90-days study with monkeys (Jarrell *et al.* 1993, Bourque *et al.* 1995) as the overall NOAEL for reproductive toxicity, with 0.1 mg/kg bw/day as the LOAEL, based on effects on the ovaries.

Proposed classification for effects on fertility

Category 1B; H360F.

Proposed classification for developmental toxicity

Category 1B; H360d.

Proposed labeling for effects during lactation

H362.

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* According to the new classification system of the Health Council, which is based on regulation 1272/2008 of the European Union. This regulation came into force on 20 January 2009.

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