Lindane (g-hexachlorocyclohexane)

Health-based recommended occupational exposure limit

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatsecreetaris van Sociale Zaken en Werkgelegenheid

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Mijnheer de Staatssecretaris,

Bij brief van 3 december 1993, nr DGV/MBO/U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid de Gezondheidsraad om gezondheidskundige advieswaarden af te leiden ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. In dat kader bied ik hierbij een advies aan over lindaan. Het is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de Minister van Volksgezondheid, Welzijn en Sport en de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Hoogachtend,

w.g.

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Lindane (γ-hexachlorocyclohexane)

Health-based recommended occupational exposure limit

report of the Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of The Netherlands

to

the Minister and State Secretary of Social Affairs and Employment

No. 2001/07OSH, The Hague, 6 December 2001

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Samenvatting en advieswaarde

1 Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid beveelt de de Gezondheidsraad gezondheidskundige advieswaarden aan voor beroepsmatige blootstelling aan toxische stoffen in de lucht op de werkplek. Deze aanbevelingen worden opgesteld door de Commissie WGD van de Raad, de opvolgster van de Werkgroep van Deskundigen. Zij vormen de eerste stap in een drietrapsprocedure die moet leiden tot wettelijke grenswaarden (MAC-waarden).

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan lindaan (γ-hexachlorocyclohexaan) in de lucht op de werkplek, en beveelt zij een gezondheidskundige advieswaarde aan. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór januari 1997 zijn verschenen.

2 Vóórkomen; fysische en chemische eigenschappen

Lindaan (γ-HCH) is een kristallijn poeder met een licht muffe geur. Het is weinig vluchtig. Het is praktisch onoplosbaar in water, maar oplosbaar in organische oplosmiddelen. Lindaan komt niet van nature voor.

In Nederland is het gebruik van lindaan beperkt tot bepaalde toepassingen in de agri-, horti-, flori- en arboricultuur. Het gebruik van lindaan is niet toegestaan in cosmetische en huishoudelijke produkten. Het voorkomen van lindaan is aangetoond in

geneesmiddelen voor plaatselijke behandeling van kleine huisdieren en in geneesmiddelen voor mensen, die op recept verkrijgbaar zijn.

3 Monitoring

Er zijn diverse gaschromatografische analysetechnieken beschreven voor het bepalen van lindaan in lucht, waarbij gebruik gemaakt wordt van verschillende soorten detectoren.

4 Huidige grenswaarden

De huidige grenswaarde voor lindaan in Nederland, Duitsland, Denemarken en de Verenigde Staten (ACGIH, NIOSH, OSHA) bedraagt 0,5 mg/m^3 (8-h TWA). In het Verenigd Koninkrijk is een grenswaarde van 0,1 mg/m^3 vastgesteld. Daarnaast heeft lindaan in al deze landen een huidnotatie. In Duitsland is een biologische limietwaarde van 0,02 mg lindaan/L totaal bloed en van 0,025 mg/L serum (plasma) vastgesteld. De WHO beveelt een biologische limietwaarde van 0,02 mg/L totaal bloed aan.

5 Kinetiek

De commissie ken geen onderzoek met gegevens over de inhalatoire absorptie van lindaan.

Lindaan kan bij de mens via de huid opgenomen worden, maximaal 9% van een lokaal op de huid aangebrachte dosis wordt binnen een dag geabsorbeerd. Bij verschillende diersoorten, waaronder de rhesus aap, blijkt de huidabsorptie hoger dan bij de mens.

Er zijn weinig gegevens over absorptie in het maagdarmkanaal bij de mens. Bij de rat blijkt lindaan zeer efficiënt opgenomen te worden via het maagdarmkanaal. Na opname via het maagdarmkanaal verdeelt lindaan zich over de verschillende organen en weefsels, waaronder bloed, vetweefsel, hersenen, cerebrospinale vloeistof, moedermelk, spierweefsel van de uterus, testikels en zaadcellen. Bij aan lindaan blootgestelde zwangere vrouwen is de verbinding aangetoond in de foetus, wat wijst op opname via de placenta.

Bloedhalfwaardetijden van 8-10 dagen werden berekend voor mensen die chronisch blootgesteld waren aan lindaan op de werkplek. Bij proefdieren concentreert lindaan zich met name in huid en vetweefsel en in mindere mate in andere organen en weefsels.

Bij de biotransformatie van lindaan spelen de microsomale cytochroom P450 isozymen CYP1A1, CYP1A2, CYP2B2 en glutathionconjugatie, glucuronidering,

sulfatering en mercaptuurzuurvorming een rol. Hierbij ontstaan verschillende vrije en geconjugeerde metabolieten, waaronder chloorphenolen, chloorbenzenen en cycloalkenen. Metabolieten worden voornamelijk via de urine uitgescheiden als vrije en geconjugeerde tetra-, tri- en dichloorphenolisomeren. Geringe hoeveelheden van de metabolieten wordt uitgescheiden met de faeces. Sommige van de metabolieten zijn ook in het bloed aangetoond.

Lindaan wordt relatief snel uitgescheiden met geschatte urinehalfwaardetijden van ongeveer 26 uur bij mensen na een eenmalige intraveneuze injectie en van 1-4 dagen bij proefdieren afhankelijk van diersoort en blootstellingsroute. Er zijn geen aanwijzingen voor stapeling van lindaan bij proefdieren.

Met behulp van biologische monitoring is een verband aangetoond tussen de concentratie van lindaan in bloed en bepaalde gezondheidseffecten. De metabolieten uitgescheiden in de urine worden onvoldoende specifiek geacht voor het schatten van beroepsmatige blootstelling aan lindaan.

6 Effecten

Er zijn geen aanwijzingen dat lindaan huid- of oogirriterend is voor de mens. Er zijn geen gegevens beschikbaar over mogelijk sensibiliserende eigenschappen van lindaan.

Symptomen indicatief voor effecten op het zenuwstelsel zijn in beroepsmatig blootgestelde werkers waargenomen bij gemiddelde serumwaarden groter dan 57 µg lindaan per liter bloed. In het algemeen zijn er geen gegevens over lindaanconcentraties in de lucht en is er ook blootstelling aan andere HCH isomeren (vaak resulterend in hogere serum niveaus).

Rapporten over casussen van fatale en niet-fatale vergiftigingen ten gevolge van accidentele of opzettelijke inneming van lindaan beschrijven verschillende symptomen, waaronder misselijkheid, duizeligheid, rusteloosheid, hoofdpijn, evenwichtsstoornissen, ataxie en tremoren. De beschreven resultaten suggereren, dat hoeveelheden tot 5 mg/kg lichaamsgewicht niet resulteren in acute (neurotoxische) effecten. Onder bepaalde condities zijn doseringen van 10-20 mg/kg lichaamsgewicht letaal, terwijl hogere doseringen getolereerd kunnen worden als tijdig een adequate medische behandeling wordtgegeven. Epileptische aanvallen (’seizures’) en braken zijn waargenomen bij bloedconcentraties van 130 µg/L en verval van dwarsgestreept spierweefsel (rhabdomyolysis), intravasculaire stolling en sterfte bij 1300 µg/L.

Lindaan is niet huid- of oogirriterend of sensibiliserend bij proefdieren.

LC_{50}/LD_{50}-waarden voor verschillende soorten proefdieren, verschillend proefdiergeslacht en verschillende blootstellingsroute zijn van dezelfde orde van grootte. Bij ratten lijkt lindaan echter na dermale blootstelling minder toxisch. De mate van

toxiciteit wordt beïnvloed door het gebruikte vehikel en het eiwitgehalte van het voer. Op basis van de EU-criteria zou lindaan geclassificeerd moeten worden als giftig bij inhalatoire blootstelling en schadelijk bij dermale blootstelling. In het acute toxiciteitsonderzoek lijken de hersenen een belangrijk doelorganen te zijn. Neurotoxische effecten manifesteren zich bij éénmalige orale doses van 10-20 mg/kg lichaamsgewicht als gedragsveranderingen en bij éénmalige orale doses van 30-60 mg/kg lichaamsgewicht als stuiptrekkingen en epileptische aanvallen ('seizures'). Er kon geen NOAEL voor neurotoxische effecten worden vastgesteld, omdat bij een éénmalige orale dosis van 5 mg/kg lichaamsgewicht, de laagst geteste dosering, nog meervoudige en enkelvoudige klonische spiercontracties werden waargenomen.

Herhaalde inhalatoire, dermale of orale blootstelling resulteerde in effecten op de lever en de nieren. De veranderingen in de nier werden alleen gezien in mannelijke ratten en waren karakteristiek voor α2u-globuline-nefropathie, een afwijking die alleen bij mannelijke ratten voorkomt, en daarom toxicologisch gezien niet relevant geacht wordt voor de mens. Er werden geen nadelige effecten gevonden bij muizen blootgesteld aan 0,3 mg/m^3 gedurende drie maanden. Een dermale studie van dertien weken bij de rat resulteerde in een NOAEL van 10 mg/kg lichaamsgewicht per dag. De laagste NOAEL in orale studies van dertien weken bij ratten was 0,75 mg/kg lichaamsgewicht per dag. In subacuut onderzoek werd een verscheidenheid aan neurotoxische effecten waargenomen, waaronder gedragsveranderingen en neuropsychologische en neurochemische effecten. Op basis van deze studies werd een LOAEL van 2.5 mg/kg lichaamsgewicht per dag afgeleid; bij ratten blootgesteld aan deze dosis gedurende 40 dagen werden gedragsveranderingen geconstateerd.

Nadelige effecten op het immuunsysteem werden geïnduceerd bij ratten, muizen en konijnen. De effecten op het immuunsysteem werden gekenmerkt door een disfunctioneren van de cellulaire en humorale immuunresponse bij blootstelling aan T-cel afhankelijke en onafhankelijke antigenen, waarbij ook een afname van de weerstand tegen infecties werd gezien. Bij muizen werd een afname van de immuunfuncties voorafgegaan door een tijdelijke potentiëring van de immunologische response gedurende de eerste vier tot acht weken van de blootstellingsperiode. Naast functionele effecten werden ook histologische veranderingen aangetoond. Bij ratten en konijnen werden effecten waargenomen bij doseringen overeenkomend met 1 mg/kg lichaamsgewicht per dag en hoger, bij ratten werden geen effecten gezien bij een dosis van 0,25 mg/kg lichaamsgewicht per dag. Bij muizen werden zowel immunostimulerende als immunosuppressieve effecten waargenomen bij doseringen van 0,012 mg/kg lichaamsgewicht per dag en hoger.

De mogelijke carcinogeniteit van lindaan is onderzocht bij ratten en muizen, waarbij verschillende blootstellingsroutes (uitgezonderd de inhalatoire route) werden onderzocht. De meeste onderzoeken zijn echter inadequaat van opzet of zijn summier

gerapporteerd. Ondanks deze tekortkomingen gaven de orale studies geen aanwijzing voor een carcinogene werking bij ratten of muizen.

De mogelijk genotoxische activiteit van lindaan werd uitgebreid onderzocht in bacteriën, gisten, schimmels en zoogdiercellen *in vitro*. Hierbij werd naar de meest relevante eindpunten gekeken, waaronder genmutaties, chromosoomafwijkingen en primaire DNA schade. Op een enkele uitzondering na (clastogene effecten in wortelcellen van de ui, enkelstrengschromosoombreuken in bepaalde celcultures) bleek lindaan niet genotoxisch in deze testen. Inconsistente resultaten werden verkregen met *Drosophila*. Bij zoogdieren *in vivo* induceerde lindaan geen genotoxische effecten; de dominant letaaltest en testen met beenmergcellen (onderzochte eindpunten: inductie van chromosoomafwijkingen, SCEs en micronucleï) waren negatief, maar er waren wel aanwijzigingen voor het optreden van primaire DNA-schade (comet assay) in coloncellen na orale blootstelling en in neusslijmvliescellen na inhalatoire blootstelling.

Lindaan was niet teratogeen bij ratten. Bij een maternaal toxische dosering hoger dan 10 mg/kg lichaamsgewicht, werden minimale skeletvariaties waargenomen. Er werden geen veranderingen te zien in een drie-generatie reproductieproef bij doseringen tot 4 mg/kg lichaamsgewicht per dag (de hoogst geteste dosering in betreffend onderzoek). In andere onderzoek werden bij hogere doseringen embryotoxiciteit en effecten op mannelijke en vrouwelijke fertiliteit waargenomen. Op basis van de beschikbare orale gegevens, stelt de commissie de NOAEL voor reproductie toxiciteit op 5 mg/kg lichaamsgewicht per dag. De waarnemingen bij muizen waren inconsistent. In een teratogeniteitsonderzoek werden geen irreversibele teratogene effecten waargenomen, maar bij maternaal toxische doseringen van 60 mg/kg lichaamsgewicht per dag, trad foetotoxiciteit (verminderd foetaal lichaamsgewicht van de foeten) op; de NOAEL was 30 mg/kg lichaamsgewicht per dag. In een ander onderzoek bleken doseringen van 5-10 mg/kg lichaamsgewicht verstrekt gedurende de drachtigheid op respectievelijk dag 1-4, dag 6-12, en dag 14-19 te resulteren in ernstige embryotoxisch en foetotoxische effecten, afhankelijk van de toedieningsperiode bestaande uit respectievelijk volledig pre-implantatieverlies (100%), post-implantatie verlies (100%) of de dood van alle pups; in de publicatie ontbrak informatie over eventuele maternale toxiciteit. Lindaan induceerde geen teratogene effecten bij konijnen na orale inname. Bij 20 mg/kg lichaamsgewicht, een maternaal toxische dosering, werd een marginale toename in skeletvariaties waargenomen, terwijl zulke effecten niet werden gezien bij 10 mg/kg lichaamsgewicht per dag.

7 Evaluatie

Het immunologische afweersysteem blijkt het meest gevoelig voor lindaan blootstelling. Nadelige effecten op het immuunsysteem zijn waargenomen bij ratten, muizen en

konijnen. De effecten op het immuunsysteem worden gekenmerkt door een disfunctioneren van de cellulaire en humorale immuunresponse bij blootstelling aan T-cel-afhankelijke en -onafhankelijke antigenen en een waarneembare afname van de weerstand tegen infecties. Bij muizen wordt een afname van de immuunfuncties voorafgegaan door een tijdelijke potentiëring van de immunologische response gedurende de eerste vier tot acht weken van de blootstellingsperiode. Naast functionele effecten zijn ook histologische veranderingen waargenomen. Bij ratten en konijnen treden deze effecten op bij doseringen die overeenkomen met 1 mg/kg lichaamsgewicht per dag en hoger; bij ratten worden geen effecten gezien bij een dosis van 0,25 mg/kg lichaamsgewicht per dag. Bij muizen zijn zowel immunostimulerende als immunosuppressieve effecten waargenomen na blootstelling aan 0,012 mg/kg lichaamsgewicht per dag en hoger gedurende 24 weken.

Onderzoek naar de effecten na inhalatie ontbreekt. De commissie neemt daarom de zojuist genoemde orale 24-weken studie bij muizen, met de waarde van 0.012 mg/kg lichaamsgewicht per dag, als uitgangspunt voor het afleiden van een advieswaarde (HBR-OEL). Deze waarde is een 'lowest observed adverse effect level' (LOAEL).

Bij het schatten van de HBR-OEL houdt de commissie rekening met intra- and interspecies variatie, verschillen tussen de blootstellingscondities in het experiment en die van de werker, het soort effect dat dient als uitgangspunt voor de risicoschatting, de dosis-effect curve en de betrouwbaarheid van de gegevens.

Voor interspeciesvariatie lijkt volgens de commissie geen correctie noodzakelijk omdat de muis een gevoelig species lijkt te zijn met betrekking tot de gevonden effecten op de immunologische parameters. De commissie acht een factor 2 voldoende hoog voor het verdisconteren van intraspecies verschillen. Compensatie voor de verschillen in blootstellingscondities tussen experiment en werkplek acht zij niet noodzakelijk, omdat de waargenomen veranderingen in immunologische parameters zeer waarschijnlijk niet beïnvloed worden door een verlenging van de blootstellingsduur. Op basis van de dosis-effect relatie waargenomen in het uitgangsonderzoek met muizen, stelt de commissie een factor 10 voor om te corrigeren voor het feit dat wordt uitgegaan van een LOAEL en niet van een 'geen effect waarde (NOAEL)'. Op basis van bovenstaande overwegingen komt de commissie tot een factor 20 voor extrapolatie van een orale LOAEL bij muizen van 0.012 mg/kg lichaamsgewicht per dag naar de werker blootgesteld via inhalatie.

Uitgaande van een ademhalingsvolume van 10 m^3 per acht uur en een lichaamsgewicht van 70 kg voor de werker berekent de commissie een HBR-OEL van 4 $\mu g/m^3$ (= 0.012 mg/kg x 1/20 x 70 kg x 1/10 m^3).

Gezien de relatief hoge dermale absorptie waargenomen in vrijwilligers stelt zij een huidnotatie voor.

8 Gezondheidskundige advieswaarde

De Commissie WGD van de Gezondheidsraad stelt voor lindaan een gezondheidskundige advieswaarde voor van 4 µg/m^3, 8-uur t.g.g.

Executive summary

1 Scope

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands recommends health-based occupational exposure limits for the concentration of toxic substances in air at the workplace. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards. They constitute the first step in a three-step procedure that leads to legally-binding limit values.

In the present report, the committee discusses the consequences of occupational exposure to lindane or γ-hexachlorocyclohexane (further referred to as γ-HCH) and recommends a health-based occupational exposure limit. The committee's conclusions are based on scientific publications prior to January 1997.

2 Occurrence, physical and chemical properties

γ-HCH is a crystalline solid with a slightly musty odour. Its volatility is very low. It is nearly insoluble in water, and soluble in organic solvents.

γ-HCH does not occur naturally. In The Netherlands, it is permitted for specified uses in agri-, horti-, flori-, and arboriculture. Although it is not permitted in cosmetic and household products, it is still present in products for topical treatment of pets and, on medical prescription, of humans.

3 Monitoring

Methods for the determination of γ-HCH in air have been described, and are based on gas chromatography using a variety of detectors.

4 Current limit values

In The Netherlands, Germany, Denmark, and the USA (ACGIH, NIOSH, OSHA), the current occupational exposure limit is 0.5 mg/m^3 (8-h TWA). In the UK, an occupational exposure standard of 0.1 mg/m^3 has been established. In all these countries, a skin notation has been added.

Biological limit values of 0.02 mg γ-HCH/L whole blood and of 0.025 mg/L serum (plasma) have been established in Germany. WHO recommends a value of 0.02 mg/L whole blood.

5 Kinetics

The committee did not find any studies that can supply information on inhalatory absorption of γ-HCH.

γ-HCH is readily absorbed by the human skin *in vivo* although less than in several animal species, including the rhesus monkey. One day after topical application, up to 9% of an administered dose is absorbed.

There is little information on absorption by the gastrointestinal route in humans. In rats, γ-HCH appears to be efficiently absorbed from the intestines.

After uptake, γ-HCH is distributed to various organs and tissues including blood, adipose tissue, the brain, cerebral spinal fluid, mother's milk, uterine muscle, testes, and semen. In exposed pregnant women, γ-HCH can be transferred to the fetus via the placenta. In long-term occupationally exposed workers, elimination half-lives of γ-HCH from the blood were calculated to be 8-10 d. Experimental animal studies show that γ-HCH is preferentially deposited in skin and adipose tissue and, to a lesser extent, to other organs and tissues

The hepatic microsomal P-450 isozymes CYP1A1, CYP1A2, CYP2B2 and conjugation reactions with glutathione, glucuronic acid, sulphate, and mercapturic acid are involved in the biotransformation of γ-HCH. This gives rise to the formation of a variety of free and conjugated metabolites, among which chlorophenols, chlorobenzenes, and cycloalkenes. Metabolites are excreted predominantly in urine in the form of free and conjugated tetra-, tri- and dichlorophenol isomers. Minor amounts of metabolites are excreted with the faeces. Some metabolites were detected in the blood.

Elimination of γ-HCH after exposure occurs relatively rapid with estimated urinary half-lives of approximately 26 h in humans following a single intravenous injection and of 1-4 days in experimental animals depending on the species and the route of dosing. No evidence for bioaccumulation of γ-HCH in animal tissues has been observed.

With respect to biological monitoring, blood levels of γ-HCH are found to be associated with health effects. Metabolites in urine are not considered to be sufficiently specific for assessment of occupational exposure to γ-HCH.

6 Effects

Human data do not show irritating properties of γ-HCH. There was no information on sensitization.

In occupationally exposed workers, symptoms of nervous system effects have been found at average serum levels of γ-HCH of at least 57µg/L. Generally, no air levels were available, and co-exposure to other HCH isomers occurred (frequently resulting in higher serum levels).

Case reports on fatal and non-fatal intoxication following accidental or intentional ingestion describe a variety of signs including nausea, dizziness, restlessness, headache, disturbances of equilibrium, ataxia, and tremors. These reports suggested that exposures somewhat higher than 5 mg/kg bw do not result in acute (neurotoxic) effects. Under some conditions, doses of 10-20 mg/kg bw may be lethal, but higher doses can be tolerated when followed by timely and appropriate medical treatment. Seizures and emesis have been observed at blood levels of 130 µg/L, rhabdomyolysis, disseminated intravascular coagulation, and death at 1300 µg/L.

In experimental animals, γ-HCH did not show skin- or eye-irritating or sensitizing properties.

LC_{50}/LD_{50}-values for different species, sex and exposure routes are of the same order of magnitude. However, in rats γ-HCH seems to be less acutely toxic following dermal exposure. Toxicity is influenced by the vehiculum used and by the protein content of the diet. Based on EC criteria, γ-HCH should be classified as toxic by inhalation and if swallowed, and as harmful in contact with skin.

In the acute animal toxicity, the brain is one of the major targets of γ-HCH. The neurotoxic effects after single oral doses of 10-20 mg/kg bw are apparent as behavioural changes and after single oral doses of 30-60 mg/kg bw as convulsions and seizures. From the neurotoxicity studies, no NOAEL could be established since multiple myoclonic jerks and single clonic seizures were induced by a single oral dose of 5 mg/kg bw, the lowest dose tested.

Repeated inhalatory, dermal and oral exposure caused effects on the liver and the kidneys. The latter effects were seen in the kidneys of male rats only, and were

induced by the male-rat-specific α2u-globulin-mediated mechanism and, therefore, considered to be toxicologically irrelevant to humans. No adverse effects were reported to occur in mice exposed to 0.3 mg/m^3 for three months. From a thirteen-week dermal rat study, a NOAEL of 10 mg/kg bw/d is derived. Thirteen-week oral rat studies indicate a NOAEL of 0.75 mg/kg bw/d. A variety of neurotoxic effects at the neurobehavioural, neuropsychological, and neurochemical level resulted from short-term exposure. From these studies, a LOAEL of 2.5 mg/kg bw per day was derived; in rats exposed for 40 days to this level, alterations in operant conditioning behaviour occurred.

Adverse effects on the immune system were induced in rats, mice and rabbits. Impaired cellular and humoral immune responses against T-dependent and T-independent antigens, including decreased infection resistance, were observed. In mice, impaired immune functions were preceded by a temporary potentiation of immune responses during the first four to eight weeks of exposure. In addition to functional effects, histological changes were demonstrated. In rats and rabbits, effects were seen at doses equivalent to 1 mg/kg bw/day and higher, but in rats, not at a dose of 0.25 mg/kg bw/day. In mice exposed to doses of 0.012 mg/kg bw/day and higher, immunostimulatory and immunosuppressive effects were observed at all dose levels tested.

The potential carcinogenicity of γ-HCH has been studied in rats and mice following several routes of administration (except inhalation), but most studies were inadequately designed or reported. Despite these flaws, the oral studies did not give an indication for carcinogenic activity in rats and mice.

The potential genotoxic activity of γ-HCH has been comprehensively tested *in vitro* in bacteria, yeasts, fungi, and mammalian cells. The most relevant end points were investigated, among which gene mutations, chromosomal aberrations, and primary DNA damage. With a sole exception (clastogenic effects in onion root tip cell systems, DNA single strand breaks in certain cell cultures), γ-HCH appeared not genotoxic in these tests. Inconsistent results were obtained in testing in *Drosophila*. *In vivo*, γ-HCH did not induce genotoxic effects; the dominant lethal and bone marrow assays (end points: induction of chromosome aberrations, SCEs, and micronuclei) were negative, although there were indications for the occurrence of primary DNA damage (comet assay) in colon cells following oral administration and in nasal mucosa cells following inhalation.

In rats, γ-HCH did not induce teratogenic effects. At maternally toxic doses higher than 10 mg/kg bw, minor skeletal variations were observed. No effects were seen in a three-generation study at doses up to 4 mg/kg bw (highest dose level tested in this study). In other studies, at higher dose levels, embryotoxicity and effects on male and female fertility were reported. From the oral data available, the committee concluded that the NOAEL for reproduction toxicity is 5 mg/kg bw per day. In mice, data were not consistent. In a teratogenicity study, there were no irreversible teratogenic effects,

but at maternally toxic doses of 60 mg/kg bw per day, fetotoxicity (decreased fetal bw) is seen; the NOAEL was 30 mg/kg bw per day. In a separate study, doses of 5-10 mg/kg bw given during gestational days 1-4, 6-12, or 4-19 induced severe embryotoxic and fetotoxic effects, depending on the period of administration being complete inhibition of implantation, complete resorption, or death of all pups, respectively; there was no information presented on maternally toxicity. In rabbits, no teratogenic effects found after oral exposure. Doses of 20 mg/kg bw caused maternal toxicity and a marginal increase in skeletal variations, while no such effects were seen at 10 mg/kg bw per day.

7 Hazard assessment

The committee considers the immune system as the most sensitive system with respect to exposure to γ-HCH. Adverse effects were observed in rats, mice and rabbits. Impaired cellular and humoral immune responses against T-dependent and T-independent antigens, including decreased infection resistance were found. In mice, impaired immune functions were preceded by a temporary potentiation of immune responses during the first four to eight weeks of exposure. In addition to functional effects, histological changes were demonstrated. In rats and rabbits, effects were seen after exposure to doses equivalent to 1 mg/kg bw/day and higher, but in rats, not at a dose of 0.25 mg/kg bw/day. In mice exposed for 24 weeks to doses of 0.012 mg/kg bw/day and higher, immunostimulatory and immunosuppressive effects were observed at all dose levels tested.

No studies were available concerning the effects after inhalatory exposure to γ-HCH. Therefore the committee uses the study (in which mice were orally exposed) and the concentration of 0.012 mg/kg bw per day as starting point in deriving a health-based recommended occupational exposure limit (HBR-OEL). This concentrations is the lowest observed adverse effect level (LOAEL)

For the assessment of the HBR-OEL, the committee took the following considerations into account: intra- and interspecies variation, differences between experimental conditions and the exposure pattern of the worker, type of critical effect, dose-response-curve, and the confidence of the data base.

The committee is of the opinion that no compensation is necessary for the interspecies differences because the mouse seems to be a sensitive species for the observed immunological effects. The committee considers a factor of 2 sufficient for intraspecies differences. Although a study of relatively short duration (i.e., 24 weeks) is taken as a starting point, the committee expects that the changes in the immunotoxicity parameters most likely will not be influenced by prolongation of exposure. Therefore, a factor which compensates for differences between experimental conditions and occupational exposure patterns is not deemed necessary. Based on the dose-response

relationship observed in the mouse study, the committee proposes a factor of 10 to compensate for the absence of a NOAEL. In summary, the committee is of the opinion that an overall assessment factor of 20 is sufficient for extrapolation from an oral LOAEL in mice to the worker exposed by inhalation.

Therefore, assuming a respiratory volume of 10 m^3 per eight hours and a body weight of 70 kg for the worker, the committee derives a HBR-OEL of 4 $\mu g/m^3$*.

In view of the dermal absorption found in human volunteers, the committee recommends a skin notation.

8 Recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit for γ-HCH of 4 $\mu g/m^3$, as an eight-hour time-weighted average concentration, as well as a skin notation.

* from: 0.012 mg/kg x 1/20 x 70 kg x 1/10 m^3

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 Standards (DECOS), a committee of the Health Council of the Netherlands, on request of the Minister of Social Affairs and Employment (Annex A). This evaluation should lead to a health-based recommended exposure limit for the concentration of the substance in the air. Such an exposure limit cannot be derived if sufficient data are not available, or if the toxic action cannot be evaluated using a threshold model. In the latter case, an exposure-response relationship is recommended for use in regulatory standard setting.

In the next phase of the three-step procedure the Social and Economic Council advises the Minister on the feasibility of using the health based value as a regulatory Occupational Exposure Limit (OEL) or recommends a different OEL. In the final step of the procedure, the Minister of Social Affairs and Employment sets the official Occupational Exposure Limit.

1.2 Committee and method of work

The present document contains the assessment of DECOS, hereafter called the committee, of the health hazard of γ-HCH. The members of DECOS are listed in

Annex B. The committee consulted two additional experts, dr A Penninks (TNO Nutrition and Food Research Institute) and dr H Loveren (National Institute of Pubic Health and the Environment) with respect to the immunological data.

The first draft was prepared by dr KJ van den Berg, drs K Mahieu, and drs H Stouten, from the TNO Nutrition and Food Research Institute, Zeist, The Netherlands, by contract with the Ministry of Social Affairs and Employment.

In 2000, 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

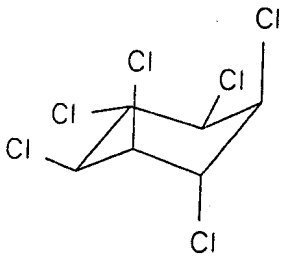
Starting point in searching literature on the health effects of exposure to γ-HCH are, amongst others, the reviews by the US Agency for Toxic Substances and Disease Registry (ATSDR; ATS94), the Dutch National Institute of Public Health and Environmental Protection (Jan88), and the World Health Organization (WHO; WHO91a). Unless otherwise indicated, data were derived from these documents. Data which were considered to be critical were evaluated by reviewing the original publications. In addition, literature has been retrieved from on-line and CD-ROM data bases. The final on-line search was carried out in January 1997 and included Embase 970109/ED, Medline 961219/UP, Toxline 970121/ED, and Chem Abs 1997 vol 126/4 (970121/ED).

Chapter **2**

Identity, properties and monitoring

2.1 Identity

2.1.1 Structure



2.1.2 *Chemical names and synonyms/registry numbers*

name	:	γ-hexachlorocyclohexane
CAS registry number	:	58-89-9
CAS chemical name	:	1α ,2α ,3β ,4α ,5α ,6β -hexachlorocyclohexane
synonyms	:	lindane; cyclohexane, 1,2,3,4,5,6-hexachloro-γ-isomer; γ-1,2,3,4,5,6-hexachlorocyclohexane; 1,2,3,4,5,6-hexachlorocyclohexane, γ-isomer; hexachlorocyclohexane (γ-isomer); γ-benzene hexachloride; benzene hexachloride-γ-isomer; γ-hexachlor; γ-hexachlorane
trade names[a]	:	Asepta Lindaan vloeibaar; Holland Fyto Lindaflow; Lindafor Flo; Luxan Lindaan 21% vloeibaar
EINECS number	:	200-401-2
EC number	:	602-043-00-6
EC labelling[b]	:	R: 23/24/25 - 36/38 - 50/53 S: (1/2-) 13 - 45 - 60 - 61
EC classification[b]	:	T; R 23/24/25; Xi; R 36/38; N; R 50/53
RTECS registry number	:	GV4900000
abbreviation	:	γ-HCH (used in this report); HCCH; γ-BHC

[a] Of products containing lindane (-hexachlorocyclohexane) registrated in The Netherlands (date: October 1997) (CTB97)

[b] Data from CEG93

Theoretically, there are 16 isomers of 1,2,3,4,5,6-hexachlorocyclohexane possible. Chair-chair interconversions reduce this number to eight. The isomers differ in whether the positions of the chlorine atoms are equatorial or axial. γ-HCH has the following conformation, aaaeee (a = axial; e = equatorial).

2.2 Physical and chemical properties*

Molecular formula:	:	$C_6H_6Cl_6$
Molecular weight:	:	290.8
Boiling point (101.3 kPa)	:	288°C (WHO91a); 323°C (ATS94)
Melting point (101.3 kPa)	:	113°C
Density	:	1.9
Vapour pressure (20°C; 101.3 kPa)	:	1.3×10^{-3} Pa (ATS94); 4.3×10^{-4} Pa (WHO91a)
Concentration in saturated vapour/air mixture (20°C; 101.3 kPa)	:	560 mg/m^3
Relative vapour density in saturated vapour/air mixture (air=1; 101.3 kPa)	:	1.0
Flashpoint,closed cup	:	150°F (65°C)
Flammability	:	not flammable
Solubility in water:	:	nearly insoluble 10-17 mg/L at 20°C
Solubility in organic solvents:	:	soluble 6.4 g/100 g ethanol; 20.8 g/100 g ether; 28.9 g/100 g benzeen
Partition coefficient (Log P_{ow})	:	3.2-3.7
Physical form	:	crystalline solid
Odour	:	slightly musty odour
Odour threshold, air	:	no data available
water	:	12 mg/L in water
Conversion factors	:	1 ppm = 12.1 mg/m^3
(20 °C, 101.3 kPa)		1 mg/m^3 = 0.08 ppm

γ-HCH is an insecticide for agricultural and horticultural uses as single active ingredient formulations including liquid formulations (140 or 750 g/L) and emulsifiable concentrate (210 g/L) (CTB97). Furthermore, it is available in formulations for use in human and veterinary medicine.

* data from ATS94, Stu96, WHO91a

2.3 Validated analytical methods

2.3.1 Environmental monitoring

NIOSH method 5502

This method is suitable for measuring γ-HCH and aldrin. γ-HCH is sampled using a glass fiber filter, organic binder-free, held without backup pad in a two-piece polystyrene cassette filter holder connected in series with a midget bubbler (containing iso-octane). The glass filter, the bubbler solution, and the iso-octane used to rinse the bubbler are transferred to one vial. For analysis, a gas chromatograph equipped with a electrolytic conductivity detector (ELCD) is used.

The overall precision is 0.086 at a range of 0.3 to 1.7 mg/m^3. The working range is 0.05 to 1.5 mg/m^3 for a 90-l air sample (Ell84).

Other methods reported are: sampling air on polyurethane foam or Florisil adsorbent tubes and analysing using gas chromatography with electron capture detection (GC/ECD), high-resolution gas chromatography with ECD (HRGC/ECD), or gas chromatography with dual detection by ECD and ELCD. With (some of) these methods, it should be possible to measure concentrations in the pg/m^3 range (see ATS94 and Slo88).

2.3.2 Biological monitoring

DFG has established a biological monitoring method for γ-HCH (Wic96) and published a gas chromatographic method using ECD for the analysis of γ-HCH in serum (Ang91).

Other methods for measuring γ-HCH in serum, semen, adipose tissue, and milk using GC or HRGC combined with ECD and GC/mass spectrometry (MS) are available (see ATS94).

Chapter 3

Sources

3.1 Natural occurrence

γ-HCH is not known to occur naturally (IARC79).

3.2 Man-made sources

3.2.1 Production

γ-HCH is formed by the addition (oxidation) of chlorine (photochlorination) to benzene. This reaction is started by free radical initiators such as visual or ultraviolet light, X-rays, or γ-rays, and produces a number of stereoisomeric compounds with the molecular formula $C_6H_6Cl_6$. This mixture is commonly called technical benzene hexachloride (BHC), and contains approximately 12-18 % (IARC79: 20-22%) of the γ-isomer. Higher contents of the γ-isomer can be obtained when the reaction of benzene and chlorine takes place in a non-reactive solvent of high dielectric constant, e.g., methylene chloride. In these solvents, the content of γ-isomer may increase to 26%. Fortified BHC is the result of selective crystallization to a crystalline solid containing 40 to 48% of the γ-isomer. At least 99% pure γ-HCH can be obtained by further purification, either by the supersaturation process or the fluid classification process (treatment with methanol or acetic acid followed by fractional crystallization). The remaining impurities consist of the other (unspecified) isomers (Col79).

3.2.2 Uses

γ-HCH is or has been used as an insecticide on fruit and vegetable crops (including greenhouse vegetables and tobacco), for seed treatment, in forestry (including Christmas-tree treatment), and for animal treatment. Medically, it is used for topical treatment against head and body lice and scabies (1% preparations) (ATS94).

γ-HCH is used for agricultural and horticultural uses including soil treatment for the culture of maize, beet, certain cereals, flower bulbs, and perennial plants, and in flori- and arboriculture, and treatment of seeds of maize, beet, and certain cereals (CTB94; CTB97). In 1992, it was the seventh mostly used insecticide at an estimated amount of 8850 kg (i.e., 0.1% of the total amount of pesticides and 3.1% of the total amount of insecticides applied in agri- and horticulture). In agriculture, it was the seventh mostly used insecticide (estimated amount: 7949 kg; approx 7% of the total amount of insecticides applied), mainly for the culture of green maize (6456 kg; application rate: 0.4 times/y) and sugar-beet (1381 kg; application rate: 0.8 times/y). As to horticulture, it was the third mostly used insecticide in the culture of flower bulbs (estimated amount: 901 kg; approx 8.5% of the amount applied in this sector; application rate: 0.7 times/y), mainly gladiolus (estimated amount: 489 kg) (CBS94). In addition, it is in use as a human and veterinary medicine. In stockbreeding, it was used as an insecticide for the treatment of pigs only (data of 1992: third mostly used insecticide; 3-5% of total amount) (CBS94).

In the EU, it is not permitted anymore in cosmetics (see also Section 7.1).

Chapter **4**

Exposure

4.1 General population

The general population may be exposed to γ-HCH due to its presence as an insecticide in cosmetics (shampoos) and household products.

In The Netherlands, γ-HCH is not permitted in cosmetic and household products anymore. However, it is still available in products for topical treatment of pets and, on medical prescription, of humans.

4.1.1 Ambient air

(γ-)HCH was not listed among compounds emitted into air due to industrial activities of 700-800 selected companies in The Netherlands (years of registration: 1990, 1992, 1993, 1994) (Ber95, Ber96).

In a review by the Dutch National Institute of Public Health and the Environment (RIVM), data on air levels from before approximately 1985 were summarized. In The Netherlands, annual average air levels were $\leq$ 0.5 ng/m^3. In homes built on dumping grounds, average concentrations were 6 ng/m^3. In homes treated for insect control, γ-HCH was rather persistent; when measured the first weeks to months after treatment, concentrations were 40000-60000 ng/m^3 (with peak levels $\geq$ 100000 ng/m^3), while after one to ten years, levels were 500-2500 ng/m^3. Ambient air levels in other countries were of the similar order of magnitude (Slo88, WHO91a).

According to a recent report, RIVM does not conduct measurements of organochloro-compounds in ambient air. From this report, it can be seen that the wet deposition of γ-HCH in rain water over the period 1971-1993 was at a rather constant level of 20000-40000 ng/m^2 (CCRX95).

From γ-HCH air levels measured in the beginning of the 1980s, it was calculated that the daily average intake of a 70-kg Dutch individual by inhalation will be 7.2 ng (WHO91a).

4.1.2 *Water*

Due to industrial activities in The Netherlands, 0.001 tonnes of (γ-)HCH were emitted into surface water in 1994. Due to agriculture and consumer uses, 1.03 and 0.2 tonnes, respectively, were emitted (Ber96).

The levels of γ-HCH in suspended solids in the fresh national waters of up to 6 µg/kg exceeded the Dutch MILBOWA (i.e., environmental soil and water quality objectives) limit and target values of 2 and 1 µg/kg, respectively (CCRX95).

4.1.3 *Drinking water and food*

In The Netherlands, there was no monitoring program concerning organochloro-compounds in drinking water in 1993 (CCRX95); there were no data presented on previous years (Slo88).

From the statement that the levels of organochloro-pesticides in animal feed in the Netherlands in 1993 were at a very low level without approaching the ADI, it may be concluded that γ-HCH levels are below residue tolerance that vary from 0.1 mg/kg for fishery products to 1 mg/kg for meat and eggs (CCRX95).

Based on a survey on the pesticide content of a series of foods, beverages, and drinking water conducted between 1976 and 1978 in The Netherlands, the mean daily dietary intake of γ-HCH was estimated to be 2 µg/person. In another study in the Netherlands over the period 1984-1985, the median value was estimated to be lower than 1 µg/kg (maximum level: 4 µg/kg) (Slo88).

Diet studies carried out in the UK between 1966 and 1985 showed a decline in estimated dietary daily intake from 6.6 µg/person in 1966 to 0.5 µg/person in 1985. In a US diet study over the period 1964-1980, average daily intakes of 0.05 (1964-1969) and 0.0028 (1980) µg/kg bw were reported (WHO91a).

4.2 Working population

No data on occupational air levels in The Netherlands were presented in the aforementioned RIVM review (Slo88) or were located elsewhere.

During commercial seed treating operations using a dust formulation of γ-HCH and maneb, dermal and respiratory exposure to γ-HCH has been measured using dermal (chest, arms, hands) and respiratory pads. Amounts on the chest and arm pads were below the detection limit (i.e., <0.1mg/25 cm^2 pad). Dermal and respiratory exposure rates were approximately 55-80 and 0.5 mg/h, respectively (Gre83).

Air levels of up to 1800 $\mu g/m^3$ were found in two US plants where γ-HCH and γ-HCH-containing products were processed and stored (Mil68). In other US studies, levels of 2 $\mu g/m^3$ inside a formulation plant and of 1.1 ng/m^3 inside a shorage shed were reported (ATS94). Spraying lawns or preparing emulsions and subsequent spraying conifers resulted in levels of approximately 5 $\mu g/m^3$. During digging up activities in the latter situation, levels were 5-180 and 40 $\mu g/m^3$ seven and eleven days after spraying, respectively (Slo88).

Typically, people are not exposed to the α, β, and δ forms of HCH separately, but to γ-HCH only or to technical-grade HCH, which contains a mixture of the isomers. The most severe exposures to lindane have occurred in workers who make lindane or in other workplaces such as fertilizer manufacturing sites.

Chapter **5**

Kinetics*

5.1 Absorption*

5.1.1 Pulmonary

Humans studies

There are no quantitative human data on pulmonary absorption of γ-HCH.

Animal studies

No quantitative information is available from animal studies on pulmonary absorption of γ-HCH.

5.1.2 Percutaneous

Human studies

Dermal absorption of γ-HCH in humans is indicated in a qualitative way by the occurrence of serious toxic effects in a few cases of accidental dermal exposure to

* Data from ATS94 and WHO91a, unless otherwise indicated

high doses (see 6.1.3). A few studies have been reported with data on dermal absorption of γ-HCH by human skin, both *in vivo* and *in vitro*.

In volunteers and scabies patients, maximum blood levels of γ-HCH were reported within four to six hours following whole body application of γ-HCH from an antiscabies lotion.

After a single application of radio-labelled γ-HCH in acetone to the unprotected forearm skin, at least 9% of the applied dose was absorbed after 24 hours. Although the rate of absorption was faster in the first twelve hours, absorption continued for at least five days, indicating further uptake from a depot (Fel74).

Four volunteers received an application of 120 mg γ-HCH in acetone to a non-occluded area of 75 cm^2 on the forearm (1.6 mg/cm^2). Tape-stripping data indicated that 14.3% of the dose was associated with the stratum corneum after six hours, while 79.3% of the applied dose was recovered in a surface wash (Dic93). Under these experimental conditions, an *in vivo* absorption rate of approximately 40 $\mu g/cm^2/h$ can be determined.

The dermal penetration of γ-HCH has been determined in a number of *in vitro* studies using human skin (Hun91, Nit84, She88). However, the relevance of this type of *in vitro* studies in order to predict dermal penetration *in vivo* in workers is unclear at the time. In addition, data from human volunteer studies are available. Therefore, these *in vitro* studies will not be discussed here.

In conclusion, γ-HCH is absorbed by human skin under *in vivo* conditions. Absorption of γ-HCH in humans is, in first instance, fairly rapid followed by a slower phase. It is estimated that approximately 9% of an applied dose to the forearm under non-occluded conditions may be absorbed in a period of 24 h.

Animals

In Rhesus monkeys, 18% of the applied dose was absorbed by the forearm and excreted in urine, 34% by the forehead and 54% by the palm, in a period of 7 days following topical application of 1.5 µg γ-HCH in acetone per cm^2 of skin. In rats, a similar dose applied to the mid-dorsal region resulted in absorption of 31% of the applied dose.

Dermal absorption of γ-HCH was found to depend on the applied dose. When groups of rats were given a single dermal application of γ-HCH at doses of 0.02, 0.2, and 2 mg γ-HCH/cm^2, a mean fraction of 14% of the applied dose was recovered in urine. Total absorption in 24 hours was 5% of the applied amount at the high dose and 28% at the low dose. Dermal penetration rates for γ-HCH in rats were determined to be 0.2, 2, and 4 $\mu g/cm^2/h$. In a similar study with rabbits, the same doses of γ-HCH

were applied dermally. A mean fraction of 39% of the applied dose was found in urine, which is higher than in rats (14%). In rabbits, total absorption was higher than in rats, with corresponding values of 17 to 56% of the applied dose, at the high and low dose, respectively. Penetration rates of γ-HCH in rabbits were also higher than in rats, giving values of 0.5, 3, and 14 µg γ-HCH/cm^2/h after 24 h at similar doses as applied to rats. Percutaneous absorption of γ-HCH from the tail of rats was studied following a topical application of 6.2 µg. Total urinary recovery was 52% after 20 days, with a half-time of 2.7 d.

In an extensive interspecies comparison on the dermal absorption of γ-HCH *in vivo*, the highest level of absorption was observed in the nude mouse model (75% of applied dose), followed by the nude mouse with grafted pig skin (30%), nude mouse with grafted human skin (25%), hairless dog (12%), and weanling pig (9%). The latter model gave the best correlation with human data, although it is not clear why the nude mouse with grafted human skin gave higher absorption values than human volunteer studies.

Various *in vitro* models have been used to study the uptake of γ-HCH by animal dermal tissue (Cha94, Dic93, Hun91). However, since the relevance of these *in vitro* animal model systems in predicting dermal penetration *in vivo* in workers is questionable, these studies will not be discussed here.

In conclusion, in experimental animals such as monkeys, rabbits, and rats, γ-HCH is readily absorbed by dermal tissues. Of an applied dose, a fraction ranging from 5 to 54% is dermally absorbed in experimental animals. Percutaneous absorption appears to be determined by several variables that include species, type of dermal tissue, and dose. Dermal uptake of γ-HCH is higher in rabbits than in rats, while monkeys and rats may have a comparable percutaneous absorption. Uptake of γ-HCH is higher by skin tissue from the palm of the hand than from the forehead and is lowest from the forearm. γ-HCH is absorbed more efficiently from a low dose than from a high dose, indicating a limited capacity for uptake.

5.1.3 Gastrointestinal

Humans

No human data are available on gastrointestinal uptake of γ-HCH.

Animals

γ-HCH is absorbed rapidly and efficiently from the intestines of rodents. In rats, 29-53% of small aliquots of γ-HCH (0.05 or 0.1 µmol) were absorbed into the blood within the first 30 minutes after injection into the prepared loops of the small intestines. In rats given single oral (feed) doses of 30 to 125 mg of technical-grade HCH/kg, approximately 96% was absorbed within four days. When administered in the feed for fourteen days, the overall degree of absorption of technical-grade HCH and γ-HCH were reported to be approximately 95% and 99%, respectively.

In fasted mice, half of the dose was absorbed from the gastrointestinal tract within fourteen minutes following administration by gavage.

5.2 Distribution*

Humans

The γ-HCH isomer has been detected in blood and adipose tissue of humans exposed either occupationally or via the ambient environment. A group of 45 forestry workers were exposed to γ-HCH by either dipping conifer seedlings in the pesticide solution, transporting or planting activities. Protective clothing was provided to all the workforce. During the summer months, plasma γ-HCH levels reached mean values of 11 µg (40 nmol)/L, while in a few workers peak levels of 21 and 35 µg (75, 123 nmol)/L were determined. When exposure ceased, plasma γ-HCH levels returned below the detection levels within a period of two months. The mean half-life for elimination from the plasma was calculated to be approximately 8 d (Drum88). In workers engaged in the production and formulation of γ-HCH, mean plasma levels ranging from 16-57µg/L were reported (Nig86). In another γ-HCH-producing facility, γ-HCH air levels of 0.004-0.15 mg/m^3 were associated with serum levels in the exposed workers of 5-188 µg/L. The content of γ-HCH in subcutaneous adipose tissue amounted to 11 mg/kg extractable lipids (Bau80). In this group of workers, the half-life for eliminating γ-HCH from the blood was reported to be approximately 10 days (Wic96).

In cases of fatal γ-HCH poisoning following ingestion, γ-HCH levels in blood as high as 1300 µg/L (Aks95) were found, while γ-HCH was also detected in cerebral spinal fluid and brain tissue, indicating that at least high blood levels of γ-HCH is distributed to the CNS.

In pregnant women, γ-HCH accumulates to some extent in fatty tissue and to be transferred to the fetus through the placenta and to neonates through breastmilk.

* Data from ATS94 and WHO91a, unless otherwise indicated

Concentrations of γ-HCH in human milk were approximately 5-7 times greater than those in maternal or umbilical blood. During pregnancy, higher levels were reported in the fetal blood tissue, uterine muscle, placenta, and amniotic fluid than in maternal adipose tissue. Males exposed to γ-HCH accumulate γ-HCH in adipose tissue and to a smaller extent in the testes or semen.

Animals

Distribution of γ-HCH in rats was studied after inhalatory exposure to doses of 0.02, 0.1, 0.5, or 5 mg/m^3 for 90 days. The highest concentrations were found in fatty tissues. The organ/tissue:serum ratios decreased with increasing doses. At the highest dose, brain:blood ratios were approximately 5 times higher than liver:blood ratios. A gender-specific effect was observed, since both brain:blood ratios and liver:blood ratios were on average 2.4 times higher in females than in males.

After single and multiple topical applications to guinea pigs, accumulation of γ-HCH was greater in the brain than in the blood, and was also dependent on the number of applications.

After oral administration of 8 mg radiolabeled γ-HCH/kg bw to rats for ten days, 35% of the radioactivity was deposited in fat. Muscle and kidneys contained 3.5 and 3.7%, respectively, while all other organs that were examined contained less than 1%. Additional measurements of organ radioactivity showed that the half-life value of γ-HCH in most organs was approximately 48 h.

When Wistar rats were exposed to 12.5, 125, and 250 mg γ-HCH/kg diet (≈0.6, 5.5, 11mg/kg bw/d*) for 30 days, concentrations were highest in fat tissue, kidneys, and adrenals, while brain, heart, and liver contained much less γ-HCH.

Oral administration of 1, 10, or 100 mg/kg diet (i.e., ≈ 0.05, 0.5, 4.5 mg/kg bw/d*) for 56 days resulted in fat:blood ratios throughout the study of approximately 150, whereas the liver:blood was approximately 3.5. Highest concentrations were found in adipose tissue. γ-HCH concentrations in the organs reached a maximum after two to three weeks, slowly decreasing thereafter. In a thirteen-week study in which rats were exposed to 0.2, 0.8, 4.0, 20, or 100 mg γ-HCH/kg diet (i.e., ≈ 0.01, 0.04, 0.18, 0.9, 4.5 mg/kg bw/d ; conversion see footnote Table 4-Annex D), the highest concentrations of γ-HCH were measured in fatty tissue. The fat:blood ratios were 7-8 times higher than the liver:blood ratios. In female animals, both fat and liver ratios were approximately 1.7 times higher than in males.

The internal distribution of γ-HCH in the rat brain was studied using quantitative autoradiography and dissection-liquid scintillation counting techniques after administra-

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* conversion see footnote Table 4-Annex D)

tion of an oral dose of 30 mg/kg or i.v. dose of 0.3 mg/kg. The distribution of label in brain regions showed regional heterogenicity, radiolabel concentrations being highest in the white matter, followed by the thalamus, mid-brain, pons and medulla, and lowest in the brain as a whole.

When a single dose of 10 mg γ-HCH/kg bw was administered ip to rats, concentrations of 40 mg/kg in fat, 1.5 mg/kg in brain, 0.7 mg/kg in skeletal muscle, and 0.2 mg/L in blood were found after one day.

In another experiment, daily doses of 20 mg γ-HCH/kg bw were injected ip to rats for three days. The highest concentrations of γ-HCH were found in adipose tissues (≈ 150 mg/kg). Much lower concentrations were found in erythrocytes (≈ 2 mg/kg), liver (≈1.5 mg/kg), brain (≈ 1 mg/kg), blood (≈ 0.3 mg/L), and plasma (≈ 0.3 mg/L) (Jun94).

When rats were injected ip with a mixture of ^{14}C - and ^{36}Cl-γ-HCH in rapeseed oil, the highest concentrations were found in skin (15.7%) and fat (10.7%) one day of after dosing. All other organs, including the central nervous system, contained less than 1%.

The transfer of γ-HCH from mothers to progeny was studied in pregnant rabbits receiving oral doses of 30 mg γ-HCH/kg bw on gestational day 15 through 21. On gestational day 28, the concentration of γ-HCH in fetuses was about 4% of the total quantity found in maternal tissues. Fetal γ-HCH levels were highest in brain (≈6 mg/kg). Lower levels were found in liver (≈1.6 mg/kg), gastric content (≈1.2 mg/kg), and lungs (≈0.3 mg/kg). In five-day-old new-borns, concentrations of γ-HCH in these tissues and organs were lower than in fetuses (Pom94).

Conclusion

γ-HCH is distributed to the blood, brain, cerebral spinal fluid, adipose tissue, testes, and semen in humans after occupational or accidental exposure. In long-term occupationally exposed workers, elimination half-lives of γ-HCH from plasma is about 8-10 d. Pregnant females may transfer γ-HCH to the fetus via the placenta and to the new-born via the milk.

Quantitative studies in experimental animals indicate that γ-HCH is predominantly distributed to fatty tissues such as adipose tissue and skin. γ-HCH can also be found in the brain, where it is mainly located in the white matter, a lipid-rich myelin fraction and kidney. Other tissues and organs such as muscle, lungs, heart, spleen, blood, and also liver are of minor importance in the distribution of γ-HCH.

Animal studies confirm that γ-HCH is distributed to various organs and tissues of fetuses and new-borns.

5.3 Biotransformation*

Overall metabolism

From the recent reviews on biotransformation of γ-HCH, it is clear that a fairly large number of metabolites are formed which can be found in organs and tissues, body fluids, urine and faeces.

There appears to be four major reaction pathways for metabolism of γ-HCH, involving various initial reactions, e.g., (I) *dehydrogenation* to γ-hexachlorocyclohexene, (II) *dehydrochlorination* leading to γ-pentachlorocyclohexene, (III) *dechlorination* to form γ-tetrachlorocyclohexene and (IV) *hydroxylation* leading to the formation of hexachlorocyclohexanol. Most of these metabolic products should be considered as intermediates leading to a further chain of reactions.

Overall, the following major classes of metabolites have been identified:

Cycloalkenes, including γ-1,2,3,4,5,6-hexachlorocyclohex-1-ene (γ-HCCH), β- and γ-1,3,4,5,6-pentachlorocyclohex-1-ene (β -, γ-PCCH), 3,4,5,6- tetrachlorocyclohexene (TCCH), γ-2,3,4,5,6-pentachloro-2-cyclohexene-1-ol (PCCOL), 2,3,4,6- and 2,4,5,6-tetrachloro-2-cyclohexene-1-ol (TCCOL);

Chlorobenzenes, including 1,2,3,4,5,6-hexachlorobenzene (HCB), 1,2,3,4,5-pentachlorobenzene (PCB), 1,2,3,5-, 1,2,4,5- and 1,2,3,4-tetrachlorobenzene TTCB);

Chlorophenols, including pentachlorophenol (PCP), 2,3,4,5-, 2,3,4,6- and 2,3,5,6-tetrachlorophenol (TTCP), 2,3,5-, 2,4,5-, and 2,4,6-trichlorophenol (TCP), and 2,4- and 3,4- dichlorophenol (DCP) and, in addition, *glutathione conjugates* of 2,3- and 2,6-DCP;

glucuronides of PCP, TCCOL, 2,3,4,5-, 2,3,4,6- and 2,3,5,6-TTCP, 2,3,5-, 2,4,5-, and 2,4,6-TCP, and 2,4-DCP;

sulphate conjugates of PCCOL, TCCOL, 2,3,4,5- and 2,3,4,6-TTCP, 2,3,5-, 2,4,5- and 2,4,6-TCP;

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* Data from ATS94 and WHO91a, unless otherwise indicated

mercapturic acid conjugates of 2,3,5- and 2,4,5-TCP, 2,4- and 3,4-DCP and 4-monochlorophenol.

Metabolites in humans

Metabolites of γ-HCH were studied in a group of 21 men who were employed for a mean period of 10.6 years in the production of technical grade HCH (16% α-, 7% β -, and 45% γ-HCH) and had been exposed to mean air levels of 3 µg α-HCH/m^3, 2 µg β-HCH/m^3, and 43 µg γ-HCH/m^3. Some twenty metabolites including mono-, di-, tri-, tetrachlorophenols, and dihydroxychlorobenzenes of unknown configuration were identified in the urine. The main metabolites (accounting for ≈ 58%) were 2,4,6-, 2,3,5-, and 2,4,5-TCP in nearly equal quantities. The formation of metabolites in exposed workers was associated with serum levels of α-, β-, and γ-HCH of 49, 82, and 52 µg/L, respectively.

In another study of workers exposed to technical-grade HCH (an unspecified mixture of α-, β-, γ- and δ-HCH isomers), HCB, PCB, γ- and δ-PCCH, PCP, 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-TTCP, several trichlorophenols, as well as glucuronides of some of these metabolites were detected in the urine. The metabolites PCCHs, TTCPs, HCB, and PCP were also identified in blood .

The extensive metabolism of γ-HCH in humans is supported by data from studies using isolated microsomal enzyme preparations *in vitro*. When human liver microsomes were incubated with γ-HCH, four major metabolites were detected: γ-HCCH, β- and γ-PCCH, and 2,4,6-TCP while smaller amounts of 2,3,4,6-TTCP, and PCB were also found. In another *in vitro* study, it was observed that human liver microsomes converted the γ-HCH metabolite β-PCCH into 1,2,4-TCB, 1,2,3,4-TTCB, 2,4,5-TCP, β-PCCOL and β-PCCH oxide. This oxide is a relatively stable compound and, although it is structurally related to epichlorohydrin and epoxides, it was not found to be mutagenic.

Metabolites in animals

Metabolite formation was studied in rats (Wistar) receiving a single oral dose of 17 mg γ-HCH. In urine samples, the prevalent metabolites were 2,3,5,6-TTCP and 2,4,6-TCP while also 2,3-DCP and 2,4,5-TCP were present, partly in conjugated form. Phenol conjugation declined with time after administration (Bal89).

In another study, rats (Sprague-Dawley) fed 400 mg γ-HCH/kg diet (i.e., ≈ 15 mg/kg bw/d*) for five weeks were found to excrete mainly not-conjugated 2,3,4,6- and 2,3,4,5-TTCP, 2,4,6-, 2,3,5-, and 2,4,5-TCP, and 3,4-DCP in the urine, within 24 hours.

In addition, a study was reported in which γ-HCH was administered ip to rats at daily doses of 40 mg/kg bw to a total of 4 g. Urinary excretion of the metabolites 2,3,5- and 2,4,5-TCP was observed either in free form or as conjugates of sulphuric acid and glucuronic acid.

In brain homogenates prepared from rats sacrificed five hours following a single oral dose of 30 mg γ-HCH/kg, tetra-, penta-, and hexachlorocyclohexenes, and tetra- and pentachlorobenzene were identified in addition to non-metabolized γ-HCH. The major metabolites formed were γ-PCCH (250 ng/g) followed by HCCH (107 ng/g), while minor amounts of PCB (6 ng/g) and HCB (1 ng/g) were present. The level of unmetabolized γ-HCH was 5.1 µg/g, indicating that the concentration of the metabolites was 7% of the parent compound. These results suggest that γ-HCH is not metabolized very extensively in the brain (Art88).

In a study with rat liver microsomal preparations *in vitro*, extensive metabolism of γ-HCH was found resulting in mainly γ-HCCH, but also in significant amounts of 2,4,6-TCP and 2,3,4,6-TTCP. In another *in vitro* study, it was observed that incubation of the γ-HCH metabolites β-PCCH with rat liver microsomes resulted in the same products as with human liver microsomes (see above), i.e., amongst others the β-PCCH oxide.

Glutathione was found to be an important factor for microsomal metabolism of γ-HCH *in vitro*. It enhanced the conversion of γ-HCH to dichlorophenol by a factor 3-4, but conjugates were formed only in the presence of liver cytosol protein as a source of glutathione transferase. The rate of formation of *S*-(dichlorophenyl) glutathione from γ-HCH in rat liver cytosol was dependent on gradual mono-dehydrochlorination. The enzymatic transfer of reduced L-glutathione into pentachlorocyclohexene was not preceded by a second dehydrochlorination.

Enzyme induction

A few studies have been reported indicating that hepatic cytochrome P-450 dependent mono-oxygenases are involved in the metabolism of γ-HCH and that γ-HCH induces these enzymes.

When rats were fed a normal-fat or a high fat diet containing 60 or 93 mg γ-HCH/kg diet (≈ 5.5, 7 mg/kg bw/d), respectively, for four weeks, an increase in

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* conversion see footnote Table 4-Annex D;

hepatic cytochrome P-450 enzyme activity as well as an acceleration of aminopyrine-N-demethylation was found (Alb79).

Another study with rats revealed that γ-HCH was a "mixed type" inducer, leading to increased activities of the cytochrome P-450 isozymes b/e, c, and d (i.e. CYP1A1, CYP2B2, and CYP1A2), after an ip dose of 25 mg/kg γ-HCH for four days.

γ-HCH was administered to DBA/2 and C57BL/6 mouse strains that genetically differ with respect to the induction of microsomal enzymes by xenobiotics, DBA/2 being the unresponsive and C57BL/6 the responsive strain. This lack of microsomal enzyme induction in the DBA/2 strain may explain the higher toxicity observed in the DBA/2 strain (when compared with the C57BL/6 strain) which was associated with higher blood and brain concentrations.

Induction of specific hepatic microsomal enzymes was studied in CF1 and B6C3F1 mice and in Osborne-Mendel rats after feeding a diet containing γ-HCH at doses of 50-360 ppm (rats: ≈ 2-16 mg/kg bw/d*, mice: ≈ 6-40 mg/kg bw/d*), for three days or three months. γ-HCH dose-dependently increased 7-ethoxycoumarin-O-dealkylase, epoxide hydrolase, glutathione-S-transferase, and UDP-glucuronosyltransferase activities in all species and strains, in both sexes, and at both time points (Oes82).

Conclusion

Biotransformation of γ-HCH is extensive, giving rise to a fairly large number of metabolites. Three major classes of metabolites are formed, cycloalkenes, chlorobenzenes, and chlorophenols as well as their glutathione, glucuronic acid, sulphate, and mercapturic acid conjugates. In the urine of occupationally exposed men, trichlorophenols are the major metabolites while a number of other metabolites are present in small amounts. Some of these metabolites are detected in blood as well.

In the rat, urinary excretion of metabolites include di-, tri- and tetraphenols both in free as in conjugated form. Only a very limited amount of metabolites is formed in the brain.

The extensive *in vivo* metabolism of γ-HCH has been confirmed *in vitro*, using human and rat liver microsomal preparations. Various cytochrome P-450 isozymes, including CYP1A1, CYP2B2, CYP1A2, and other hepatic microsomal enzymes such as 7-ethoxycoumarin-O-dealkylase, epoxide hydrolase, glutathione-S-transferase and UDP-glucuronosyltransferase are induced by γ-HCH.

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* conversion see footnote Table 4-Annex D

5.4 Elimination*

Humans

Male workers occupationally exposed to technical grade HCH, excreted nonmetabolized HCH isomers and numerous (≈ 20) metabolites in their urine (see also Section 5.3).

In healthy volunteers, who had received a whole body application of a 0.3% γ-HCH emulsion, nonmetabolized γ-HCH was excreted in the urine and faeces.

After i.v. injection of radiolabelled γ-HCH to human volunteers, approximately 19 and 25% of the administered dose were excreted in urine within one day and five days, respectively. A half-time value of 26 hours was determined on the basis of these findings. In the same study, the cumulative urinary excretion of radioactivity over five days amounted to approximately 5% of the dose only, after dermal application.

Humans excrete γ-HCH and its metabolites in milk and semen as well.

Animals

There are no data on excretion following exposure by inhalation.

Data from dermal, oral, and ip experiments indicate that the major route of elimination is via de urine. Minor amounts are eliminated in the faeces and very little in exhaled air. Most γ-HCH is excreted in a great number of free and conjugated metabolites. In the WHO review, it was stated that the results of rat feeding studies did not indicate that γ-HCH accumulate in significant amounts in the body.

When dermally applied to either the forehead, forearms, or ventral forepaw of monkeys, 18-54% of the dose (% depending on the region) was excreted in the urine with half-lives of approximately 1 d. In rats, urinary excretion amounted to 31% of the dose applied on the back (half-life: ≈3 d) (Moo89b).

One day after oral administration of 8 mg radiolabelled γ-HCH/kg bw/d, for ten days, to rats, approximately 18 and 14% of the radioactivity was recovered from the urine and faeces, respectively. After an additional two days, these figures were approximately 25 and 21% for urine and faeces, respectively. The ratio of free and conjugated metabolites excreted via these routes was estimated to be approximately 1:3.

In mice given a single oral dose of 1.2 mg/mouse, approximately 80% of this dose was reported to be excreted within three days.

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* Data from ATS94 and WHO91a, unless otherwise indicated

Rabbits repeatedly dosed with 3-12 mg radiolabelled γ-HCH/animal (2 times/w, 26 w) excreted 54 and 13% of the label in urine and faeces, respectively.

The transfer of γ-HCH from mothers via milk and placenta to progeny was studied in rabbits. Pregnant rabbits received an oral dose of 30 mg γ-HCH/kg bw. Over one lactation, the mothers excreted via the milk 30% of γ-HCH present in tissues at the 28th day of pregnancy. The total amount of γ-HCH distributed via milk to 5 day-old new-borns was higher than that transferred across the placenta. These results indicate that lactation is an efficient elimination pathway for γ-HCH in mothers. The average concentration in body tissues in 5 day-old pups was already considerably lower than in fetuses and declined further with increasing age. On the basis of tissue concentrations at the ages of 5 d, 10 d, and 20 d, half-life values of γ-HCH for fetal tissues can be determined that are 2.6 d for the total fetal body, 3.4 d for lung, 5 d for brain and gastrointestinal tissue, and 15 d for liver. It was concluded that in pups, despite considerable intake of γ-HCH via milk, no accumulation occurred and that elimination pathways prevailed.

Conclusion

γ-HCH is eliminated from the human body via urine, faeces, mother milk, and semen, in the form of metabolites (free and conjugated) as well as partly in the form of the nonmetabolized parent compound. Excretion of γ-HCH is relatively fast, one half of an administered dose is excreted in urine after 26 h.

In experimental animals, γ-HCH is eliminated similarly; following dermal exposure in the monkey with a half-time value of appoximately 1 d, following oral exposure in the mouse and rat 2-3 days and 3-4 days, respectively. γ-HCH does not accumulate in significant amounts.

In lactating animals, γ-HCH is efficiently excreted since up to 30% of a body dose is transferred to offspring within one lactation. In pups, elimination of γ-HCH prevails despite intake via milk.

5.5 Possibilities for biological monitoring*

Only a few studies are known that have focused on a relationship between occupational exposure levels of γ-HCH in air and blood levels in exposed workers.

Milby *et al* (1968) studied a group of 56 production workers and 21 non-production workers in a γ-HCH production plant. In production workers having little or no skin contact with γ-HCH, mean blood levels of 4.1-4.6 µg/L were observed under exposure

* Data from ATS94 and WHO91a, unless otherwise indicated

levels ranging from 11-1800 of µg/m^3, while in productionworkers having frequent and prolonged skin contact blood levels of 30.6 µg/L were found. In the non-production workers of this plant, blood levels < 1 µg/L were associated with exposure levels of 9-49 µg/m^3. When blood levels of γ-HCH were examined on the basis of years of employment, no increase with the length of exposure was found. The authors concluded that these findings indicate that blood γ-HCH levels are correlated with the intensity of exposure and are a reflection of recent γ-HCH absorption (Mil68).

In another study in which forestry workers were monitored by measuring plasma γ-HCH and urinary trichlorophenols, it was concluded that measurement of γ-HCH offers a valid routine biological monitoring method. There was no statistical relation between the urinary levels of the separate trichlorophenol isomers, but a weak correlation (r=0.46) was found between plasma γ-HCH levels and total urinary trichlorophenol concentrations. This weak relation may reflect the extensive and variable metabolism of γ-HCH, making measurement of urinary trichlorophenols less suitable for biological monitoring (Dru88).

In 21 workers exposed during the production of γ-HCH from the technical grade product, a significant correlation (r=0.74) was found between γ-HCH serum levels and urinary levels of 2,3,4,6-tetrachloro-phenol. The ratio between the concentration of γ-HCH in serum and that of the chlorophenol in urine was 1:6 (Ang83).

In a number of studies, workers occupationally exposed to γ-HCH were investigated concerning health effects versus γ-HCH blood levels. In production workers (n=37) exposed for up to two years, there was clear evidence that increasing estimated individual intensity of exposure resulted in increased γ-HCH levels in whole blood (2-340 µg/L; cf general population: average: 8 µg/L, range: 3-17 µg/L). At blood levels >20 µg/L, there was an increase in the number of workers with clinical symptoms and EEG changes (non-specific, for occurring in 10-20% of the general population). These EEG changes were found in approximately 55 and 90% of the workers examined at blood levels ≥16 and ≥20 µg/L, respectively. Slight clinical symptoms were seen at blood levels >24 µg/L (Cze70). In evaluating these data, it should be noted that about 60% of the workers had previously been exposed to aldrin with severe poisoning in approximately 1% of them.

In another study on production workers (n=60) exposed to α-, β- and γ-HCH, no changes attributable to HCH were found in the EEGs recorded for eight of them (average γ-HCH serum level: 41µg/L, range: 10-72 µg/L). Four of these workers had γ-HCH serum levels >50 µg/L. In addition, the results from electrophysiological examinations of the peripheral nervous system of these 60 workers (average γ-HCH serum level: 25 µg/L) did not differ from those of controls (Bau81).

Non-specific symptoms were reported in 4% of 45 forestry workers at plasma concentrations of 22 and 36 µg/L (Dru88) and in 90% of 19 formulators and 75% of 26

production workers at mean serum levels of 57 and 16 µg/L (Kas86). However, the two latter groups were also exposed to other HCH isomers (with much higher serum levels).

Conclusion

Although measurement of urinary metabolites may offer a non-invasive method for biological monitoring, this method is less suitable because γ-HCH is converted extensively and variably, resulting in metabolites which can be formed from other, structurally related, compounds as well.

Blood levels of γ-HCH in workers could not be correlated with occupational γ-HCH exposure levels. However, blood levels could be related to the absence/presence of nervous system effects, and offer, therefore, a suitable method for monitoring workers (as was concluded by WHO and DFG; see Section 7.2.2).

5.6 Summary

There are no studies known that can supply information on inhalatory absorption of γ-HCH.

γ-HCH is readily absorbed by the human skin *in vivo* although less than in several animal species, including the rhesus monkey. One day after topical application, up to 9% of an administered dose is absorbed.

There is little information on absorption by the gastrointestinal route in humans. In rats, γ-HCH appears to be efficiently absorbed from the intestines.

After uptake, γ-HCH is distributed to various organs and tissues including blood, adipose tissue, the brain, cerebral spinal fluid, mothers milk, uterine muscle, testes and semen. γ-HCH can be transferred to the fetus via the placenta and to neonates via breastmilk. In long-term occupationally exposed workers, elimination half-lives of γ-HCH from the blood were calculated to be 8-10 d. Experimental animal studies show that γ-HCH is preferentially deposited in skin and adipose tissue and, to a lesser extent, to other organs and tissues.

The hepatic microsomal P-450 isozymes CYP1A1, CYP1A2, CYP2B2 and conjugation reactions with glutathione, glucuronic acid, sulphate, and mercapturic acid are involved in the biotransformation of γ-HCH in rats. This gives rise to the formation of a variety of free and conjugated metabolites, among which chlorophenols, chlorobenzenes, and cycloalkenes. Metabolites are excreted predominantly in urine in the form of free and conjugated tetra-, tri- and dichlorophenol isomers. Minor amounts of metabolites are excreted with faeces. Some metabolites were detected in the blood.

Elimination of γ-HCH after exposure occurs relatively rapid with estimated urinary half-lives of approximately 26 h in humans following a single iv injection and of 1-4 days in experimental animals depending on the species and the route of dosing. No evidence for bioaccumulation of γ-HCH in animal tissues has been observed.

With respect to biological monitoring, blood levels of γ-HCH are found to be associated with health effects. Metabolites in urine are not considered to be sufficiently specific for assessment of occupational exposure to γ-HCH.

Chapter 6

Effects

6.1 Observations in man

6.1.1 Irritation and sensitization

A number of cases have been reported with allergic reactions and dermatitis in workers exposed during the manufacture of technical grade HCH. Upon further testing using patch methods, negative results were found with pure HCH isomers, but positive reactions with residual fractions (WHO91a).

Two additional studies in a large number of agricultural workers using patch tests with γ-HCH, did not reveal evidence for a positive reaction (WHO91a).

6.1.2 Toxicity due to acute and short term exposure

There are case reports of acute poisoning following inhalatory exposure to γ-HCH due to household use in vaporizers. However, since exposures were not quantified and other compounds were involved, γ-HCH could not be clearly related to the effects observed, including haematological effects and death (ATS94).

γ-HCH has been dermally used as a scarbicide (ATS94). Clinical reports suggested that exposures somewhat higher than 5 mg/kg bw/d do not usually result in acute neurotoxic symptoms (WHO91a). There were occasional reports of haematological (aplastic anaemia, bone marrow hyperplasia) and neurological (convulsions) effects in adults (ATS94).

Several cases of fatal and non-fatal intoxication ascribed to accidental or intentional ingestion, as well as reports of grossly neglecting safety precautions to using γ-HCH. Clinical signs of intoxication can appear from a few minutes to some hours after intake. In mild cases, these signs include indisposition, nausea, dizziness, restlessness, frontal headaches, and sometimes vomiting. Muscular fasciculation, disturbances of equilibrium, ataxia, and tremor may also occur. Pains in the upper abdomen are frequently coupled with diarrhoea and uncontrolled micturition. Clonic-tonic convulsions of some minutes' duration can occur, with recurrence after several hours or days in response to optical, tactile, or acoustic stimuli. In fatal cases, death may follow several hours to several days after intake. Central respiratory failure or acute circulatory collapse, often after convulsions, is usually the cause of death. Under certain conditions, 10-20 mg/kg bw may be lethal, but higher doses can be tolerated when followed by timely and appropriate medical treatment (WHO91a). In adults, seizures and emesis have been observed in the presence of blood levels of 130 µg/l, lethargy and resting tremor at 250 µg/l, seizures and myonecrosis at 600 µg/l, and seizures, rhabdomyolysis, disseminated intravascular coagulation, and death at 1300 µg/l (Aks95).

6.1.3 Epidemiological studies

A few epidemiological studies have addressed the question of health effects in workers engaged in the manufacture of γ-HCH. Generally, workers were exposed to other isomers of HCH or other pesticides, and exposure levels were not available. No studies were found reporting on the potential carcinogenic effects of γ-HCH in humans (ATS94, WHO91a).

No exposure-related, overt signs of impairment of nervous system functioning (reflexes and sensibility, manual skills by means of a tracking test, motor nerve conduction velocity, and electroencephalography (EEG)) were observed in a group of sixty male workers (24-62 years-old) employed in the production of γ-HCH for a mean period of 7.2 years (range 1-30 y) when compared with non-exposed control groups. Small deviations were found in a few haematological parameters such as higher polynuclear leukocyte count, lower lymphocyte count, higher reticulocyte count, lower prothrombin level and, in addition, lower blood concentrations of creatinine and uric acid and increased serum LH levels. Occupational exposure levels of γ-, α-, and β-HCH were up to 0.15, 1.99, and 0.38 mg/m^3, respectively; average serum levels amounted to 25 (range: 5-188 µg/L), 56 (range; 10-273 µg/L), and 149 µg/L (range: 17-760 µg/L), respectively (Bau81).

In a group of 64 employees of a HCH manufacture plant consisting of handlers and packers, plant operators and supervisors, and maintenance staff, most of the directly and indirectly exposed workers had paraesthesia of the face and extremities, headache,

and giddiness, and some had symptoms of malaise, vomiting, tremors, apprehension confusion, loss of sleep, impaired memory, and loss of libido. The same symptoms were found in the group of maintenance workers but were less severe and occurred in fewer cases. Occupational exposure levels of γ-HCH were not provided in this study. Serum concentrations of γ-HCH in maintenance staff was 22.7 µg/L, in exposed handlers 57 µg/L, but α-, β-, and δ-HCH isomers were also present (total HCH concentrations: staff: 144 µg/L; handlers: 604 µg/L. A control group without occupational exposure to γ-HCH (serum concentration of γ-HCH was 0.7 µg/L) was included (Nig86).

A group of 37 workers, exposed to γ-HCH for a period of two years, was subjected to neurological studies. Occupational exposure levels of γ-HCH were not provided in this study. Mean serum levels of γ-HCH in exposed workers were 2-340 µg/L. Serious EEG disturbances were seen in three and minor symptoms and signs in fourteen workers. The frequency of clinical symptoms and EEG changes was higher among workers with γ-HCH blood levels of 20 µg/L or more (Cze70).

Toxic signs and symptoms including paraesthesia of face and extremities, headache, vomiting, giddiness, apprehension confusion, loss of sleep and tremors were present in 90% of the formulators (n=19) and in 75% of the production workers (n=26) from a HCH manufacturing and formulating plant. In formulators, increased serum enzyme concentrations were observed, including lactate dehydrogenase, γ-glutamyl transpeptidase, and leucine aminopeptidase as well as increased IgM levels. In some 15% of the workers, also cardiac effects were observed in the form of abnormalities in ECG recordings. Occupational exposure levels of γ-HCH were not presented. Mean serum concentrations were 57 and 16 µg/L in formulation and productionworkers, respectively, while appreciable concentrations of other HCH isomers were also present (mean total HCH concentrations: formulators: 604 µg/L; production workers: 413 µg/L) (Kas86).

No increased frequency of stable chromosomal aberrations was found in the lymphocytes of workers engaged in the production of γ-HCH for at least six months. Workplace air or blood levels were not reported (Kir79).

In 50 female, γ-HCH- and pentachlorophenol (PCP)-containing wood preservative-exposed patients attending a hospital endocrine department and admitted with diagnoses including amenorrhea, repeated and habitual abortions, menopausal syndrome and hyperandrogenaemia, significantly differing distributions, were reported for some changes in immune and endocrine parameters in comparison to normal values. The patients had blood γ-HCH levels ranging from 0.01-1.16 µg/l, but much higher PCP levels (ranging from 5.5-133.0 µg/l) (Der95). Because dependent parameters were not analyzed for several different γ-HCH exposure categories and the results may well be influenced by several confounding factors, the results do not allow any conclusions

regarding γ-HCH as a possible causative agent in the altered endocrine and immune parameters.

Other studies have been performed in which the effects of (mostly environmental) exposure to, amongst others, wood preservatives and organochlorine pesticides (to which γ-HCH belongs) on pregnancy outcome (parameters: birthweight/lenght (Kar95); preterm labour (Sha96)) or the nervous system (i.e., multiple chemical sensitivity disorder (Loh96); Parkinson's disease (Sei96)) were investigated. Although exposure-related differences between exposed groups or cases and non-exposed controls have been found, in none of these studies specific chemical compounds could be identified as aetiological agents.

Conclusion

γ-HCH has not been found to be irritating in occupationally exposed workers. No information is available concerning sensitization in workers.

At high doses, exposure to γ-HCH is causing severe toxicity involving the nervous system that can lead to coma and death. Symptoms of nervous system effects have been found in workers after occupational exposure to γ-HCH during manufacturing and formulating at average serum concentrations of at least 57 µg/L, while these workers were also exposed to other HCH isomers (α-, β- and δ-HCH), generally at higher serum levels.

A limited study found no evidence of genotoxicity in production workers.

No studies are known that have reported carcinogenic effects of γ-HCH in humans.

6.2 Animal experiments

6.2.1 Irritation and sensitization

Slight primary eye irritation was observed, when 0.1 g γ-HCH was placed in the conjunctival sac of New Zealand white rabbits (WHO91a).

Application of 0.5 g of γ-HCH to the intact skin of New Zealand white rabbits did not cause irritation (study performed in compliance with the guidelines of the OECD and EPA). γ-HCH had no sensitization potential in guinea pigs (n=10/sex) when tested in a Magnusson-Kligman maximization test (according to OECD guidelines). Similar testing with other formulations (liquid, powder) containing approximately 20-79% γ-HCH showed negative results as well (WHO91a).

In conclusion, γ-HCH did not show skin- or eye-irritating or sensitizing properties in experimental animals.

6.2.2 *Toxicity due to acute exposure*

The acute lethal effects of γ-HCH have been investigated in numerous studies in various species (and strains) following several exposure routes (see Annex D: Table 1).

Generally, the LC_{50}/LD_{50}-values are of the same order of magnitude when comparing species, sex, and exposure route, although in rats γ-HCH seems to be less acutely toxic following dermal exposure. The differences in the results of the oral experiments are due to the vehicle used, viz, oily solutions were more toxic than suspensions in water: when administered in oily solutions or as an emulsifiable concentrate, the LD_{50} was approximately 90 mg/kg, when wettable powders, granules, aqueous suspensions, etc, 170 mg/kg or higher. Furthermore, low protein levels in the diet enhanced toxicity.

In LC_{50}-tests, signs of neurotoxicity including curved body posture, paddling movements, and spasms were observed at (not specified) toxic concentrations (WHO91a).

No effects were observed in young adult male rabbits following a single topical application of 60 mg γ-HCH/kg bw on the shaved skin. When the skin was shaved, depilated, and stripped, this dose induced excitability in 2/4 rabbits after about 24 hours. Weanling rabbits, exposed to similar concentration (blood concentrations after 24 h were 0.7-2.5 µg/ml), were more susceptible, since severe anorexia and convulsions, and, in some cases, death occurred (ATS94, WHO91a).

Neurotoxicity

There are numerous reports on the neurotoxic effects of γ-HCH following single oral, ip, or iv administration (for summary: see Annex D, Tables 2a-c). No inhalation studies were found.

From these tables, it can be seen that single oral doses of 30-60 mg/kg bw caused convulsions and seizures in rats. At levels of 10-20 mg/kg bw, behavioural changes were reported.

In one study with male Long-Evans rats given single oral doses of 0, 5, 10, and 20 mg/kg bw, a dose-dependent increase in the number of animals displaying convulsions was observed. A dose of 5 mg/kg bw caused both multiple myoclonic jerks and single clonic seizures. The ED_{50} (i.e., the dose producing multiple myoclonic jerks in 50% of the animals) was 12-14 mg/kg bw. In electrically kindled animals, γ-HCH decreased the ED_{50} by more than 60% (Gil95b).

When given iv to rats, 1.5 and 3.1 mg/kg bw were the doses required to induce convulsions and generalized seizures, respectively. Brain levels at which convulsions occurred were approximately 6 µg/g wet brain weight, a concentration that was achieved following a single ip dose of 60 mg/kg bw as well (Por88).

In some other experiments, relationships between neurotoxic responses and γ-HCH levels in brain and blood were determined (Llo89, Tus87, Zis95). Threshold concentrations may be approximately 5 µg/g brain and 1.5 mg/L blood (Tus87).

In addition to overt acute neurotoxic effects such as convulsions/seizures and more subtle manifestations in the form of altered behaviour, a number of acute alterations at the neurochemical level have been observed in animals following exposure to γ-HCH.

In the brain of rats orally given single doses of 0, 5, 10, 15, 20, 30 and 40 mg/kg bw, a rapid transient increase in *c-fos* expression was induced persisting for three hours and reaching a maximum after one hour. The proto-oncogene *c-fos* is thought to participate in the control of genetic events that lead to the establishment of prolonged functional changes in neurons. The lowest dose of 5 mg/kg bw caused an increase in *c-fos* mRNA that was further increased in a dose-dependent manner. The *c-fos* gene was expressed in the absence of overt convulsions but levels of mRNA were attenuated by seizures. Immunohistochemical studies showed a dose-response effect on appearance of the Fos protein in brain at doses of 5, 10, 20, and 30 mg/kg bw (Ven92b). The *c-fos* gene is considered to be an indicator for "neuronal stress", but the relation of the above mentioned findings with neurological dysfunction is not known at the moment.

Alterations in levels of biogenic amines in rat brain were found after a single oral dose of 20 mg γ-HCH/kg bw, including changes in noradrenaline and serotonin levels. In another study, effects on regional levels were reported showing decreased levels of noradrenaline in cerebral cortex and hippocampus, of serotonin (5HT) and dopamine in the cerebral cortex, and increased levels of the dopamine metabolite DOPAC in the hypothalamus (Llo91).

From toxicokinetic data, it is known that γ-HCH has a relatively short half-time in the brain of experimental animals of approximately one to two days and does not accumulate in tissues (see Section 5.4).

Nevertheless, γ-HCH may produce marked CNS effects that outlast their presence in brain. For instance, inhibition of Na^+/K^+-ATPase activity was observed in synaptosomes prepared from the brain of mice that were pre-treated with γ-HCH eighteen hours earlier, although neither γ-HCH nor metabolites could be detected. After a single dose of γ-HCH, limbic evoked potentials were still evident after a period of two weeks. In addition, a dose of γ-HCH below that required to produce seizures was found to potentiate the olfactory-evoked hippocampal potential for a period of about twelve days (Woo95).

Conclusion

Based on EC criteria, γ-HCH should be classified as toxic by inhalation and if swallowed, and as harmful in contact with skin.

The brain is one of the major targets in the acute animal toxicity, γ-HCH inducing neurobehavioural, neurophysiological, and neurochemical effects. These effects are apparent as behavioural changes at single oral doses of 10-20 mg/kg bw and as convulsions and seizures following single oral doses of 30-60 mg/kg bw. At a single oral dose of 5 mg/kg bw, multiple myoclonic jerks and single clonic seizures were induced.

6.2.3 Toxicity due to repeated exposure

General toxicity studies: inhalation

Two unpublished reports and one Russian paper were available on the effects following short-term exposure to γ-HCH by inhalation. They were reviewed by JMPR (FAO89), RIVM (Jan88), and WHO (WHO91a).

In the first study, rats (Wistar Han/Boe SPF; N=12/sex/group) were exposed (whole body) to aerosol (average particle size: 0.92 µm) concentrations of 0, 0.02, 0.12, 0.6, or 4.5 mg/m^3, 6 h/d, for three months. No adverse effects were seen following exposure to concentrations up to 0.6 mg/m^3. In the highest concentration group (4.5 mg/m^3) slight diarrhoea and ruffled fur were seen temporarily in the male animals only. Significant findings included increased hepatic cytochrome P-450 levels in the animals of the high dose group at the end of the exposure period, increased relative kidney weights in the male animals of the high dose group, and histological changes in the kidneys (cloudy swelling of the tubular epithelium) of the male animals of the two highest dose groups. These effects were transient, as they were not found in an additional recovery group exposed to 4.8 mg/m^3 and kept for another exposure-free period of six weeks. In the RIVM review, it was noted that the input concentrations were markedly higher than the concentrations measured at the exits probably because of deposition of the compound from the aerosol. This implies a possibility of oral uptake by the test animals (Jan88). Furthermore, it raises questions concerning the actual concentrations that were inhaled by these animals.

In the second experiment, mice (CD-1; n=45/sex/group) were exposed by whole body inhalation to aerosol (geometric mean particle diameter ~3 µm) concentrations of 0, 0.3, 1.0, or 10 mg/m^3, 6 h/d, 5 d/w, for fourteen weeks. Subgroups were sacrificed after seven, fourteen and twenty weeks (the latter being a recovery group with an additional exposure-free period of six weeks). Because of an unexpectedly high mortality rate in the female animals of the highest dose group in the first week, the

concentration was lowered to 5 mg/m^3. From autopsy on the mice that died during the study, no cause of death could be ascertained. WHO states that concentrations of 1 and 5 mg/m^3 were highly toxic to female mice, but does not present details; the no effect level was concluded to be 0.3 mg/m^3. JMPR points to the possibility of oral uptake due to deposition of test material from the aerosols (FAO89, WHO91a).

In the Russian study, mice were exposed to nominal concentrations of 1 mg/m^3, 6 h/d, for 2.5 months. During the exposure period, leukocytosis and leukopenia were observed, as well as the appearance of toxic granulocytes and vacuoles in the nuclei and cytoplasm of some leucocytes, and a reduced mitotic rate. The relationship between the different cell types in the bone marrow was undisturbed (WHO91a).

Furthermore, in its documentation on the occupational exposure limit for γ-HCH, ACGIH refers to two additional inhalation studies carried out around 1950 in which several species were intermittently (7 h/d, 5 d/w) exposed for about one year and rats continuously (24 h/d) for 655 days to 0.7 and 0.19 mg/m^3, respectively. There was no evidence for histological changes at the lower level, while minimal pathology was seen at 0.7 mg/m^3. No details were presented (ACG91).

General toxicity studies: dermal

There is one unpublished report on the effects of γ-HCH following dermal exposure. In this study, doses of 0, 10, 60, or 400 mg/kg bw were applied (vehiculum: carboxymethyl cellulose) under occlusion (for 6 h) on the clipped area of the skin of rats (Crl:(WI)BR; n=49/sex/group), 5 d/w, for thirteen weeks. Subgroups were sacrificed six (13/sex), thirteen (23/sex) and twenty weeks (recovery group, 13/sex) after starting the experiment. Concerning the treated female animals, JMPR mentions a total of 18 unscheduled deaths and an unusually large number of replacements during the initial phase of the study. At the two higher dose levels, increased relative liver weights, and a reversible (for not found in the recovery group) centrilobular hepatocellular hypertrophy were found in both sexes. In addition, an apparent dose-dependent increase in the incidence of focal necrosis was seen in the liver of some male animals of the recovery group (twenty weeks) only (incidences: 1/10, 2/10, 3/9 vs 0/10 (control)). At the two higher dose levels, there were effects on the kidneys of the male animals: increased relative weight, hyaline droplet formation, tubular degeneration with necrosis, basophilic tubules, and granular casts. Some of these lesions persisted after the six-week exposure-free period (tubular degeneration with necrosis; granular casts). Although there was some evidence of increased intensity of hyaline droplet formation at the lowest dose tested (i.e., 10 mg/kg bw/d), this was considered to be so slight that 10 mg/kg bw/d could be (close to) the NOAEL (FAO89, WHO91a).

General toxicity studies: oral

Data from unpublished reports on subchronic oral general toxicity studies were presented in the reviews by JMPR (FAO89), RIVM (Jan88), and WHO (WHO91a).

Rats (KFM-Han (outbred) SPF; male and female; n=15/sex/group) were given doses of 0, 0.2, 0.8, 4, 20, or 100 mg/kg diet (i.e. male: 0, ~0.02, 0.06, 0.29, 1.55, 7.25; female : 0, ~0.02, 0.06, 0.33, 1.67, 7.90 mg/kg bw/d*), for three months. Groups of animals were sacrificed after five, twelve, and eighteen (6-w exposure-free recovery group) weeks. At 100 mg/kg, decreased bw gain of approximately 8-15% was seen. At 20 and 100 mg/kg, there were a reversible induction of cytochrome P450, reversible dose-related increases in absolute and relative liver and kidney weights, reversible centrilobular hepatocellular hypertrophy (dose-related with respect to intensity and severity), partially reversible renal tubular changes (mainly in males; hyaline droplet induction, tubular degeneration and distension, interstitial nephritis with basophilic proximal tubuli). The dose of 4 mg/kg diet (i.e., ~0.3 mg/kg bw/d) was considered to be a no-effect level.

In a separate experiment, rats (RIV:TOX (C-S); male and female; n=10/sex/group) were exposed to doses of 0, 2, 10, 50, or 250 mg/kg diet (0, ~0.15, 0.75, 3.8, 19 mg/kg bw/d*), for thirteen weeks. At 250 mg/kg, clinical signs of toxicity including increased mortality, decreased body weight gain, aggressive behaviour (especially in females), and bleedy nose were seen. At gross and histological examinations, increased absolute and relative liver (in males and females), kidney (in males), adrenal (in females), thymus (in females), and ovary (in females) weights, centrilobular hepatocellular changes, vacuolization of thyroid (in males), and mild hyperkeratosis of oesophagus (in males and females) were observed. Furthermore, there were changes in some haematological parameters (in females; decreases in number of erythrocytes, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin), and increases in activities of some liver enzymes. At 50 mg/kg, absolute and relative ovary (in females), liver (in males), and kidney (in males) weights were increased. At 10, 50, and 250 mg/kg, there was a dose-dependent increase in incidence of hyaline droplets in proximal tubuli of kidneys of the male animals. The dose of 10 mg/kg diet (i.e., ~0.75 mg/kg bw/d) was considered to be the no-effect level.

The toxicity of repeated oral dosing of γ-HCH has been examined in dogs and pigs as well. Hardly any effects were seen in male and female Beagle dogs (n=4/sex/group) fed diets containing 0, 25, 50, or 100 mg/kg diet (reported to be equivalent to 0, ~0.8, 1.6, 2.9 mg/kg bw/d) for 104 weeks or to 200 mg/kg diet (equivalent to ~11 mg/kg bw/d initially and gradually decreasing to ~7 mg/kg bw/d during the final 3 w). At autopsy,

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* conversion based on diet analyses and food intakes as presented by FAO89

livers were dark, friable, and slightly enlarged, without histological changes, at 100 and 200 mg/kg diet. In these dose groups, serum alkaline phosphatase levels were elevated (Riv78). No effects were seen in pigs (n=5/group) at doses of up to 80 mg/kg diet for nine months (WHO91a).

In a number of experiments with young male Wistar rats fed diets containing 0 (n=5) or 800 mg/kg diet (i.e, ~110 mg/kg bw/d*) (n=8) for two weeks, effects on the kidney (hypertrophy and degeneration of tubular epithelia; glucosuria; increased excretion of creatinine and urea), liver (amongst others: increased weight; hypertrophy; increased fat and DNA content; decreased DNA per unit tissue; increased activity of serum aminotransferases, glucose-6-phosphate dehydrogenase, aldolase; decreased activity of glucose-6-phosphatase; decreases in certain ATPasses), and testes (see Section 6.2.5) were reported (Sri88, WHO91a).

In female weanling rats, 20 mg/kg bw induced increases in body weight after ten weeks of treatment, while at 40 mg/kg bw, seven out of twelve rats died (see also Section 6.2.6) (Cha88).

Since the kidney effects (such as formation of hyaline droplets, enlargement of lysosomes in the proximal tubules, necrosis of proximal tubular epithelial cells) which are reported to be induced by γ-HCH point to a male rat-specific α2u-globulin-mediated mechanism, experiments have been carried out in which γ-HCH and compounds known to induce α2u-globulin-mediated lesions were orally (gavage) administered to male and female F344 rats and to male NBR rats, a strain that does not synthesize the hepatic form of 2u-globulin. The studies demonstrated that the presence of this globulin was causal to the development of the nephropathy following exposure to γ-HCH (Die90, Die91). Since only male rats synthesize 2u-globulin, this kind of rat nephropathy induced by γ-HCH is considered to be toxicologically irrelevant.

Neurotoxicity studies

Data on studies concerning the neurotoxic effects following repeated exposure to γ-HCH are summarized in Table 3 (see Annex D). No inhalation studies were located.

Neurobehavioural effects were studied after feeding γ-HCH at daily doses of 2.5, 5, 10 or 50 mg/kg bw to Wistar rats for 40 days. Increased responses to conditioning in a Skinner box, displayed as a markedly higher rate of lever presses, were seen with 2.5 mg/kg bw. In the maze test, no effect was observed with 2.5 mg/kg bw per day, but at 5 mg/kg bw per day running time was altered accompanied by an increased error rate (Des74).

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* assumption: bw: ~70 g, food intake 10 g/d

Functional, neurological, electro-encephalographic and neurobehavioral effects were studied in female rats that received daily oral doses of γ-HCH at 2.5 or 5 mg/kg bw per day for three months. At a dose of 5.0 mg/kg bw per day, alterations in EEG recordings and in neurobehavioral tests were observed, whereas no alterations were apparent at a dose level of 2.5 mg/kg bw per day (Des83).

The proconvulsant properties of γ-HCH were studied in Sprague Dawley rats receiving daily oral doses of 1, 3, or 10 mg/kg bw for four days. In naive rats, γ-HCH increased the probability of epileptic response to amygdaloid stimulation at a dose of 10 mg/kg bw/day, while a dose of 3 mg/kg/day bw was without effect. In amygdaloid kindled animals, a dose of 3 mg/kg bw/day increased the rate of acquisition of the fully kindled state, e.g., the animals required fewer stimulations, fewer discharges, and fewer seconds of after discharge to kindle. A dose of 1 mg/kg bw/day did not lead to an enhancement of amygdaloid kindling (Joy82). In another related study an oral dose of 5 mg γ-HCH/kg bw/day for five days caused also an increased rate of acquisition of kindling seizures after stimulation of a different brain region, the hippocampus. Lower doses were not studied in this paradigm (Joy83).

A dosing regimen of intermittent exposure to subconvulsant doses of γ-HCH was studied to assess behavioural seizure development. Groups of male Long-Evans rats were administered oral doses of 10 mg/kg bw for 30 days or three times a week for ten weeks. Enhanced behavioural responses to γ-HCH, as myoclonic jerks and clonic seizures, emerged over the course of dosing and persisted for two to four weeks after the last dose. The incidence of generalized convulsions was increased from 0 to 15% at the final day of dosing. EEG recordings also showed alterations in animals that did not show signs of overt behavioural seizures. The results indicate that repeated intermittent exposure to 10 mg γ-HCH/kg bw leads to gradual development of electrographic and behavioural signs of seizures in rats (Gil95a).

The effect of γ-HCH on pineal gland metabolism of serotonin was studied in rats. Animals received a daily dose of 2.75 mg/kg bw/d by gastric intubation for a period of six days. γ-HCH augmented the nocturnal rise in pineal N-acetyltransferase activity, while pineal levels of serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) were reduced. All effects could be blocked by propanolol pretreatment, suggesting involvement of β-adrenergic receptors. Levels of pineal 5-hydroxytrypthophane and melatonin were unaffected, as was the activity of the melatonin forming enzyme (hydroxyindole-*O*-methyltransferase, HIOMT). These results indicate slight changes in pineal serotonin metabolism. However, the effect on circadian rhythms is unknown (Att91).

Myelin deficits in some brain regions were observed by immunohistochemical studies of new-born male and female rats orally dosed with γ-HCH at doses of 5, 10,

15, or 20 mg/kg bw, administered from postnatal day 8 to 10. A dose-dependent effect was found, starting with a dose of 5 mg/kg bw/day. It appears that γ-HCH affects the myelination process, but the relevance of these findings for exposure of adult workers is not clear (Ser90).

Immunotoxicity studies

Humoral immune responses to *Salmonella typhi* and *Salmonella paratyphi* A and B antigens were suppressed in weanling male and female Charles Foster albino rats orally (by gavage) given either 6.25 or 25 mg γ-HCH/kg bw/day for 35 days and intramuscular injections of typhoid-paratyphoid vaccine on experimental days 7 and 14. Control animals received the vehicle only. Antibody titers determined in sera obtained from blood samples collected at weekly intervals from day 14 until 35 indicated slightly lower specific antibody titers already upon primary dosing (d 14 sera) and significantly suppressed responses following booster injection (d 21-35 sera) (Dew80).

When young male Wistar albino rats were fed 0, 5, 20, or 30 mg γ-HCH/kg diet (equivalent to 0, 0.25, 1, or 1.5 mg/kg bw/day) for eight, twelve, eighteen, or 22 weeks, an increase in serum albumin/globulin ratios in tetanus toxoid injected animals (tetanus toxoid in freunds's complete adjuvant administered subcutaneously 20 d before sacrifice) due to a decrease in globulin levels was observed at week 18-22 in the 30 mg/kg diet group and at week 22 also in the 20 mg/kg diet group. These differences correlated with an impaired increase in total IgM and IgG levels in response to the immunization. In addition, a significant decrease in tetanus toxoid-specific antibody titers was observed at week 12-22 in the 20 and 30 mg/kg diet groups. The cellular immune function was also altered following exposure to 20 mg/kg during twelve to 22 weeks and already from week 8 onwards at 30 mg /kg diet, as apparent from a decreased leukocyte and peritoneal macrophage migration inhibition. No immunomodulating effects were noted at 5 mg/kg diet. There were no signs of general toxicity nor changes in body weights, food intake, thymus or spleen weights in any of the treated groups (Sah93).

In adult BALB/c mice fed 0 or 150 mg γ-HCH/kg diet (i.e., 0, 22.5 mg/kg bw/d), from one month prior to the initiation of immune function tests until termination of the study, no effect on the primary IgM response to sheep red blood cells (SRBC) following a single intraperitoneal immunization was observed. Following five consecutive daily intragastric SRBC doses, specific IgA, IgG1, IgG2a, IgG3 and IgM levels were not affected either, but specific IgG2b levels were significantly increased. In addition, the infection resistance to an oral *Giardia muris* infection in control mice and γ-HCH-exposed (same dose for 10 w prior to the initiation of the immune function test) BALB/c mice was assessed by quantification of the number of trophozoites in the small intestine on day 28 after occulation and by anti-*Giardia* IgM, IgA, and IgG antibody

determinations. In the γ-HCH-exposed mice, an increased duration of giardasis (3-59 x 10^4 trophozoites/animal in comparison to < 1 x 10^4 in controls) was demonstrated. In addition, the γ-HCH-treated mice more frequently developed systemic anti-*Giardia* antibodies (And83).

Male albino Hissar mice exposed to 30 or 50 mg γ-HCH/kg diet (i.e., 4.5, or 7.5 mg/kg bw/day) for six to twelve weeks, showed depressed humoral immunity as demonstrated by the determination of the primary and secondary direct splenic plaque forming cell (PFC) response following immunization with sheep red blood cells (SRBC). Upon exposure for three weeks, a reduction was only observed on the secondary PFC response in 50 mg/kg diet exposed mice. The primary antibody response to SRBC as determined by haemagglutination tests were only affected in mice exposed to 50 mg/kg diet for twelve weeks. The secondary haemagglutinating antibody titers were decreased from three weeks of exposure onwards in 50 mg/kg diet exposed mice and after twelve weeks of 30 mg/kg diet exposure. No effects on PFC responses or anti-SRBC haemagglutinating antibody titers were noted at a dose level of 10 mg/kg diet (i.e., 1.5 mg/kg bw/day) (Ban96).

The immune status of young female Swiss albino mice was investigated after oral exposure to doses of 0, 0.012, 0.12, and 1.2 mg γ-HCH/kg bw/day, for up to 24 weeks. Immune functions were assessed in separate groups of animals at monthly intervals. In addition, immunohistology was performed at week 4, 12 and 24. Both cellular and humoral immune functions to T-dependent and T-independent antigens were stimulated dose-dependently, at all dose levels up to week 4 to 8 of exposure, followed by suppression until termination of the study. The *in vitro Staphylococcus aureus* bactericidal activity of lipopolysaccharide-activated peritoneal macrophages was not affected. In addition to the modulated immune functions, histological alterations in lymphoid organs were noted. The immunohistological changes (initially an increase in lymphoid follicular activity followed by depletion of cellular populations in thymus, lymph nodes and spleen) were in line with the observed biphasic functional modulation of the immune system (Mee92).

In male rabbits given oral (capsule) doses of 0, 1.5, 3, 6, and 12 mg/kg bw, 5 d/w, for five to six weeks and weekly iv injections of a *Salmonella typhi* "Ty-3" vaccine, a dose-dependent decrease in *S. typhi* "O"-specific agglutinating antibody titers was observed at all dose levels. The titers in the test groups were lower already from week 1 onwards. Although decreases were reported to be statistically significant, the results of statistical analyses were not presented (Dés78).

Conclusions

γ-HCH induces adverse effects in the liver and the kidneys irrespective of the route of exposure. The kidney effects are mediated by a male-rat-specific mechanism and have, therefore, no toxicological relevance for man. From limited information from inhalation studies, a NOAEL for intermittent exposure of approximately 0.6 mg/m^3 can be established for rats and of 0.3 mg/m^3 for mice. From thirteen-week dermal and oral studies in rats, NOAELs of 10 and 0.75 mg/kg bw/d, respectively, can be derived.

A variety of neurotoxic effects at the neurobehavioural, neurophysiological and neurochemical levels are apparent as a consequence of repeated oral exposure of experimental animals to γ-HCH. From these studies, a LOAEL of 2.5 mg/kg bw/d can be derived. In rats exposed for 40 days to this level, alterations in operant conditioning behaviour occurred.

Adverse effects (functional effects; histological changes) on the immune system were induced in rats, mice, and rabbits. In rats and rabbits, effects were seen at oral doses equivalent to 1 mg/kg bw/day and higher, but in rats not at a dose of 0.25 mg/kg bw/day. In mice orally exposed to doses of 0.012 mg/kg bw/day and higher, immunostimulatory and immunosuppressive effects were observed at all dose levels tested.

6.2.4 Carcinogenicity studies

Besides administration in the diet, other routes (viz, dermal application, sc implantation, ip injection) have been used to study the potential carcinogenicity of γ-HCH. Since the latter studies were inadequately designed or reported, they are not discussed here (for review: see IARC79).

The feeding studies are summarized in Table 4 (see Annex D). From this table, it can be seen that γ-HCH treatment did not induce an increase in tumour incidence, when tested at doses up to approximately 70 mg/kg bw/d. However, it should be noted that all of these studies showed inadequacies, viz, small test groups, short duration, early mortality, or low doses.

Studies using mice suffer from similar flaws. When considering the studies by Herbst *et al*, Weisse and Herbst (Her75, Wei77), NCI (IARC79, Ves83), Thorpe and Walter (Tho73), i.e., the studies most adequately designed (with respect to among others duration) and reported, it can be concluded that administration in the diet of doses up to approximately 20 mg/kg bw/d did not induce tumours in male and female mice. Administration of approximately 50 mg/kg bw/d only increased the incidence of adenomas and hyperplastic nodules in the liver of male mice. In these studies, γ-HCH did not cause an increase in the incidence of malignant tumours.

Initiating and promoting activities of γ-HCH have been studied as well. No initiating activity (parameter: the number of phenotypically altered, γ-glutamyltransferase(GGT)-positive foci) was found in partially hepatectomized female rats (Wistar; n=8) given a single oral dose of 30 mg γ-HCH/kg bw (by gavage under light ether anaesthesia), after a two-week recovery period followed by daily dietary doses of 50 mg phenobarbital/kg bw, for fifteen weeks. When given a single oral (gavage) dose of 250 mg N-nitrosomorpholine/kg bw, after a recovery period of eight weeks followed by daily dietary doses of 0.1-30 mg γ-HCH/kg bw, for four, fifteen, or twenty weeks, tumour-promoting activity was concluded from the increase in the number and size of altered foci at doses of ≥ 2-3 mg/kg bw/d; no changes were noted at doses of 0.5 mg/kg bw/d (Sch87). Janssen *et al* cited a similar experiment in which initiation with a single oral dose of diethylnitrosamine (0.3 mmol/kg bw ≈ 31 mg/kg bw) followed by the administration of a diet containing 76 mg γ-HCH/kg feed (i.e., ≈ 3.4 mg/kg bw/d) for 72 days to rats (Sprague-Dawley; ♂♀; n: not reported) induced a significant increase in GGT-positive foci, being greater in female than in male animals (Jan88). Although γ-HCH may act as a promotor, as can be concluded from the induction of preneoplastic changes, i.e., it causes the outgrowth of foci and increased their areas, it was not determined, whether these events actually led to tumour formation.

A number of studies on the mode of action concerning tumorigenicity have been performed (for review: see WHO91a). In mice with a dominant mutation at a specific locus (agouti; A^{vy}) which increases susceptibility to strain-specific spontaneous and chemically induced neoplasms, γ-HCH acted as a tumour promoter via cellular proliferation. Furthermore, the influence of repeated oral exposure on specific activities of some drug-metabolizing enzymes have been investigated in mice and rats. Although striking differences were found between species or strains, the influence of these differences on tumour formation was not clear. In addition, there was preferential binding to protein but not to DNA. Finally, in separate experiments the ability of γ-HCH to inhibit metabolic or intercellular (gap junctional) communication has been demonstrated.

Conclusion

γ-HCH did not show carcinogenic activity in oral studies using rats and mice, but it should be noted that most of these studies had some flaws. γ-HCH can promote the development of preneoplastic liver cell nodules.

6.2.5 *Genotoxicity*

γ-HCH has been tested in numerous *in vitro* and *in vivo* test systems for its mutagenic/genotoxic properties as was reviewed by ATSDR (ATS93), the Dutch National Institute of Public Health and Environmental Protection (Jan88), JMPR (FAO89), and WHO (WHO91a) (for summary: see Annex D, Tables 5a-c, 6a-c).

In vitro tests

γ-HCH was negative in numerous studies using a variety of prokaryotic cell systems (frame-shift mutations, gene mutations, or DNA damage in bacteria), except for one study in which, contrary to other studies, a weakly positive result was reported in the presence of a metabolic activating system in *S typhimurium* strain TA98 found (Gop92). However, since positive and vehicle control groups were not included, this study cannot be evaluated adequately.

From host-mediated assays, one weakly positive result (end point: frame-shift mutations) and two negative results (end point: gene mutations) were reported.

In non-mammalian eukaryotic systems, mostly negative results were obtained, but γ-HCH induced clastogenic effects in onion root tip cell systems.

Testing in *Drosophila melanogaster* (endpoints: dominant lethal or sex-linked recessive lethal mutations) gave both positive and negative results.

In mammalian cell systems, γ-HCH was negative, when tested for gene mutations, chromosome aberrations, UDS, and SCEs. The compound was, however, capable of inducing DNA damage (single strand breaks) in some of the tests applied (for example, in human and rat nasal mucosa cells).

In vivo tests

Dominant lethal assays (rat, mouse) and tests in bone marrow for chromosome aberrations (rat, hamster), SCEs (mouse, hamster), and micronuclei (rat, mouse, hamster) were negative.

Assays for DNA damage (single strand breaks) showed possibly positive results in nasal mucosa cells from rats exposed to 0.3 or 3 mg/m^3 (vapour + aerosol) for one hour, as well as in colon cells of rats following a single oral (gavage) dose of 60 mg/kg bw. Assays in other cells (rat: liver cells, gastric cells, peripheral lymphocytes; mouse: liver cells) were considered to be negative.

Conclusion

There are no indications that γ-HCH is mutagenic/genotoxic compound, but it may have some DNA-damaging potential.

6.2.6 *Reproduction toxicity*

Fertility and general reproductive performance

No studies following exposure by inhalation were located.

The reproduction toxicity of γ-HCH has been tested in a three-generation study in which rats (CD; ♂: n=10/group/generation; ♀: n=20/group/generation) were given daily doses of 0, 25, 50, or 100 mg/kg diet (i.e., ≈ 1, 2, 4 mg/kg bw/d) for a 60-day premating period, during mating (two 19-d periods) and gestation. Pups of the first litter were examined for external and internal abnormalities, while the pups of the second litter were exposed according to the aforementioned protocol. Treatment did neither induce effects on reproductive performance parameters, nor major malformations or minor variations. Only slight effects on the livers of the pups of the second litter of the third generation (increased relative liver weights; enlarged hepatocytes) were found, but these were considered of doubtful importance in view of the lack of effects on the growth and reproductive performance in the preceding generations and litters (Pal78b).

WHO refers to a poorly reported Russian study in which oral administration of 10 mg γ-HCH/kg bw/d for 138 days reduced fertilization rate, while a daily dose of 5 mg/kg bw for 90 days did not (WHO91a).

Injection of γ-HCH to female rats (CDF-F344) on the morning of pro-oestrus (route: ip; n=3-8; doses: 0, 25, 33, 50, 75 mg/kg bw) or on the afternoon of di-oestrus (route: ip, po; n=6-18; doses: 0, 10, 25, 33, 50 mg/kg bw) affected the sexual behaviour (dose-dependent reduction of sexual receptivity), and, in the latter case also, the oestrus cycle (prolongation) (Uph87, Uph89).

When orally given 20 mg/kg bw/d for 30 days to ovariectomized rats (albino; n=4-6/group), no histological or weight changes in the uterus, cervix, and vagina were seen, when compared with those in ovariectomized controls. Uterus weights of ovariectomized rats treated with oestradiol diproprionate and γ-HCH were (not significantly) lower than those of rats treated with oestradiol alone. There was a small increase in glycogen content of the uterus, cervix, and vagina (Rai80). In weanling female rats (Fischer 344; n=6-12/group) orally dosed with 0, 5, 10, 20, or 40 mg/kg bw/d for fifteen weeks, γ-HCH treatment induced delayed vaginal opening, disrupted oestrus cycling, reduced pituitary and uterus weights, and increased food consumption (during pro-oestrus), body weight gain, and obesity (Lee index). After 90 days of treatment,

most of the animals in all exposure groups had regular ovarian cycle. At 40 mg/kg bw/day, seven out of twelve animals died. Most of the aforementioned effects occurred at doses of ≥ 20 mg/kg bw/day. Apart from a marginal decrease in the mean number of pro-oestrus days, no effects were seen at 5 mg/kg bw/day (Cha88, Coo89). From these and additional experiments, it was concluded that these effects on reproductive processes were not mediated through oestrogenic activity (Cha88, Coo89, Law94; see also Tie96).

In rabbits orally (gavage) treated with 0.8 mg γ-HCH/kg bw/day, three times a week for twelve to fourteen weeks, the mean ovulation numbers (not specified; combined data obtained at day 1, 6, and 11 post — artificial — insemination after twelve weeks of exposure) was significantly reduced; remarkably, no such effect was seen, when γ-HCH was given in combination with DDT (positive as well when given alone) and PCB (Lin94). Due to limited reporting and the wide variation in results of the controls of the various groups, the significance of this result is unclear.

One single oral dose of 30 mg/kg bw or five doses of 6 mg/kg bw to adult rats (Bor: spf, TNO; n=10/group) reduced the number of spermatids in both groups and the number of sperms in the single dose group when counted two weeks after treatment. Histological examination carried out in the single dose group only showed a pronounced ballooning of Sertoli cells accompanied by fragmentation or complete loss of organelles (Dal96).

Feeding young male rats (Wistar; n=9, controls: n=6) 800 mg γ-HCH/kg diet for two weeks caused tubular atrophy, spermatogenic arrest, and oedematous interstitial space. Furthermore, an increase in testicular protein content and a decrease in testicular DNA content were observed (Sri88).

Daily ip injections of 8 mg/kg bw for ten days to rats (albino; n=10/group) caused complete degeneration of the seminiferous lumens and severely affected spermatocytes and spermatids, while no such effects were seen at a dose of 4 mg/kg bw/day. A statistically significant dose-related decrease in absolute (low dose: not significant) and relative testicular weights was seen as well (Roy87), but this may be a reflection of the accompanying decrease in body weight. Following intratesticular injections of 10 mg/kg bw/d for ten days, hypertrophic and atrophic changes were seen (ATS94).

Developmental effects

No studies following exposure by inhalation were located.

When rats (CFY; n=20/group) were orally (gavage) given doses of 0, 5, 10, or 20 mg/kg bw/d on gestational days (gd) 6-15, (some) maternal toxicity was noted in the mid and high dose groups (decreased food intake and body weight gain). Two animals

of the high dose group died (cause of death not ascertained). There was no effect on litter parameters and there were no major malformations. The incidence of skeletal variations were comparable for all groups, except for the incidence of extra (14th) ribs. This was dose-relatedly increased, being statistically significant ($p<0.05$) in the high dose group (but being just within the range recorded for the control group) (Pal78a).

When given dietary levels of 0, 200, or 400 mg/kg feed (≈ 0, 14, 28 mg/kg /d*) to pregnant rats (Wistar; n=6/group) throughout gestation, no effects on perinatal development (parameters: pregnancy rate; pup bw; pup survival rate) were observed. Dietary doses of 250 mg/kg feed (≈17.5 g/kg bw/d*) throughout gestation and a 28-day lactation period or during the lactation period alone did not affect pup survival rate or pup body weight at postnatal day 28. At necropsy, pups of both treatment groups had increased relative liver weights, while decreased kidney weights were seen in the pups from mothers exposed throughout the gestation and lactation period (Sri91).

Oral (gavage) exposure to a formulation containing 50% γ-HCH (remaining 50% not specified) at levels of 6.25, 12.5, or 25 mg/kg bw/d on gd 6-15 did not induce any treatment-related effect on developmental and maternal toxicity parameters in rats (Wistar; n=20/group) (Khe79).

An increased incidence in fetal resorptions and decreased fetal weights were reported, when rats (n=10/group) were orally (gavage) given 50 or 100 mg/kg bw on gd 9 or 40 mg/kg bw/d on gd 6, 8 and 10 (Koë87).

WHO referred to an oral study using mice (NMRI-EMD (SPF); n=25/group) given doses of 0, 12, 30, or 60 mg/kg bw/d on gd 6-15 or gd 11-12. In the high dose group, increased mortality and decreased body weight gain were observed in the maternal animals as well as increased mortality and decreased body weights in the fetuses. There were no other treatment-related effects including the number of implantations per dam, percentages of early and late resorptions, the number of runts, or the malformation rate (WHO91a).

In a separate experiment, oral (gavage) treatment of mice (Swiss; n=6/group) during gd 1-4 or 6-12 with a total dose of approximately 43 mg/kg bw (i.e., 1/2 LD_{50}) induced complete inhibition of implantation or a complete resorption of implanted fetuses, respectively. The results of simultaneous experiments in which γ-HCH treatment during early pregnancy was combined with progesterone and oestrogen (alone or in combination) suggested that inadequate steroid hormone levels caused by γ-HCH may have played a role. When given during gd 14-19, total doses of approximately 22 and 43 mg/kw bw caused the death of all pups within five days (low dose) or twelve hours (high dose) of parturition. No data on maternal toxicity were presented (Sir89).

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* assuming a bw of 250 g and a food intake of 17.5 g

When given single oral doses of 0, 30 and 45 mg/kg bw on gd 12 to C57BL/6J mice (n=5-7/group), significant decreases in fetal weight, fetal thymic weight and placental weight were reported. At the high dose level, an increase in percentage of fetuses/dam dead or resorbed was found. Similar treatment of DBA/2J mice induced significant reductions in fetal and placental weight and in the mean of implantation number/dam at the high dose level only. In both strains, treatment with both doses caused maternal mortality of 14 to 25%, but no changes in maternal bodyweight. Furthermore, it was stated that doses lower than 30 mg/kg bw neither produced maternal toxicity nor significant fetal effects (Has96).

In rabbits (New Zealand white; n=13/group), orally (gavage) dosed with 0, 5, 10, or 20 mg/kg bw/d on gd 6-18, slight tachypnoea, lethargy, decreased food intake, and decreased body weight gain were seen in all treatment groups during the exposure period. There was no evidence of any teratogenic effect, but some changes considered to be of doubtful importance were seen. Among these differences the incidence of extra ribs which was significantly lower in the low dose group, and significantly higher in the high dose group (but only just greater than the control group range in the latter case) (Pal78a). In a separate experiment using rabbits (n=9/group), a single oral (gavage) dose of 40 or 60 mg/kg bw on gd 9 caused an increase in the incidence of fetal resorptions. In the high dose group, prematurely opened eyes was seen in approximately 21% of the fetuses (no control values given) (Koë87).

In hamsters (n=9/group), a single oral (gavage) dose of 20 or 40 mg/kg bw on gd 8 caused an increased incidence in fetal resorptions (Koë87).

Sc injection of 0, 5, 15, or 30 mg/kg bw/d on gd 6-15 to rats (Sprague-Dawley; n=20/group) induced maternal toxicity in the mid (decreased mean bw gain) and high (decreased mean bw gain; nervous system effects; mortality) dose groups. There were no indications of developmental effects in any of the treatment groups (FAO89, WHO91a).

Similar experiments in rabbits (New Zealand white; n=15/group; doses: 0, 5, 15 mg/kg bw/d on gd 6-18, 30 mg/kg bw/d on gd 10-18, 45 mg/kg bw/d on gd 6-9) did not show teratogenic effects. In the two high dose groups (i.e, at 30 and 45 mg/kg bw), maternal toxicity (body weight loss; nervous system effects; at 45 mg/kg bw: 14/15 rabbits died) and embryotoxicity (increased number of resorptions) were observed (FAO89, WHO91a).

No effects (number of implantations or living embryos per dam; percentage of absorptions or resorptions; malformations) were seen in mice (NMRI; n=25/group) following sc injections of 6mg/kg bw/d on gd 11-13. When given on gd 6-15, the only effect observed was a slight increase in the frequency of runts (FAO89, WHO91a).

Conclusion

In rats, γ-HCH did not induce teratogenic effects. At maternally toxic doses of ≥ 10 mg/kg bw/day, minor skeletal variations were observed. No effects were seen in a three-generation reproduction study at doses up to 4 mg/kg bw/day (highest dose level tested). At higher dose levels, embryotoxicity and effects on male and female fertility were reported. From the oral data available, the overall NOAEL for reproduction toxicity is concluded to be 5 mg/kg bw/day.

In mice, data were not consistent. In a teratogenicity study, there were no teratogenic effects, but at maternally toxic doses of 60 mg/kg bw, fetotoxicity (decreased fetal bw) was seen; NOAEL: 30 mg/kg bw/day. In another study, total doses of 22 and 43 mg/kg bw (i.e., ≈ 5 and 10 mg/kg bw/d) given during gd 1-4, gd 6-12, or gd 14-19 induced severe embryo/fetotoxic effects (complete inhibition of implantation, complete resorption, death of all pups).

In rabbits, oral studies did not show teratogenic effects. Doses of 20 mg/kg bw/day caused maternal toxicity and a marginal increase in skeletal variations, while no such effects were seen at 10 mg/kg bw/day.

6.3 Other studies

The exact mechanism(s) of the neurotoxic action of γ-HCH is not completely known. A number of neurochemical studies have been carried out in order to provide some insight in this issue (see Annex D, Table 7). The most widely accepted mechanism of action by γ-HCH involves interference with one of the GABA receptor subtype complexes (i.e., $GABA_A$) (WHO91a, Woo95). GABA (γ-aminobutyric acid) is an inhibitory neurotransmitter, which mediates the entry of the Cl^- necessary for inhibitory neuronal function. γ-HCH has been found to bind at the $GABA_A$ receptor complex to the same site as the model compound picrotoxin, a potent convulsant and GABA antagonist, does. Thus, γ-HCH is thought to block the $GABA_A$ receptor for interaction with GABA, thereby inhibiting the entry of Cl^- and subsequently the neuron regulating the secretion of excitatory neurotransmitters, from which a continuous excitation of postsynaptic neurons results. The concentrations of γ-HCH necessary to mediate these effects at a neurochemical level *in vitro*, are reached in the brain *in vivo* under conditions when convulsions take place.

At a concentration of 100 µM, γ-HCH inhibited the bicuculline-insensitive $GABA_C$ receptor (a newly discovered GABA receptor subtype) when studied on retinal bipolar cells prepared from adult rats and cultured *in vitro*. γ-HCH inhibited this receptor more strongly than a very potent convulsive compound such as picrotoxin (Fei94). Since this

receptor is thought to play a role in the visual circuitry, this test result implies that γ-HCH may affect visual signal transduction *in vivo*.

In *in vitro* studies concerning the immunotoxicity, suppressing effects of γ-HCH on human peripheral blood mononuclear cell responses to mitogenic stimulation (Rou79) and effects on biochemical murine macrophage functions (For90, Mea84) were demonstrated.

γ-HCH has been found to impair steroidogenesis since pregnenolone production *in vitro* in Y1 adrenocortical cells was dose-dependently inhibited at a concentration of 10 µM (Zis96).

6.4 Summary

6.4.1 Human data

There are no indications for irritating effects of γ-HCH in occupationally exposed workers; there was no information on sensitization.

In occupationally exposed workers, symptoms of nervous system effects have been found at average serum levels of γ-HCH of at least 57 µg/L. Generally, no air levels were available, and co-exposure to other HCH isomers occurred (frequently resulting in higher serum levels).

Case reports of fatal and non-fatal intoxication following accidental or intentional ingestion describe a variety of signs including nausea, dizziness, restlessness, headache, disturbances of equilibrium, ataxia and tremors. These reports suggested that exposures somewhat higher than 5 mg/kg bw do not result in acute (neurotoxic) effects. Under some conditions, doses of 10-20 mg/kg bw may be lethal, but higher doses can be tolerated when followed by timely and appropriate medical treatment. Seizures and emesis have been observed at blood levels of 130 µg/L, rhabdomyolysis, disseminated intravascular coagulation, and death at 1300 µg/L.

6.4.2 Animal data

γ-HCH did not show skin- or eye-irritating or sensitizing properties in experimental animals.

LC_{50} /LD_{50}-values are of the same order of magnitude when comparing species, sex, and exposure route, although in rats γ-HCH seems to be less acutely toxic following dermal exposure. Toxicity is influenced by the vehiculum used and by the protein content of the diet. Based on EC criteria, γ-HCH is classified as toxic by inhalation and if swallowed, and as harmful in contact with skin.

The brain is one of the major targets in the acute animal toxicity of γ-HCH. The neurotoxic effects are apparent as behavioural changes at single oral doses of 10-20 mg/kg bw and as convulsions and seizures following single oral doses of 30-60 mg/kg bw. From the neurotoxicity studies, no NOAEL could be established, since multiple myoclonic jerks and single clonic seizures were induced by a single oral dose of 5 mg/kg bw, the lowest dose tested.

Repeated inhalation, dermal, and oral exposures caused effects on the liver and the kidneys. The latter effects were seen in the kidneys of male rats only, and were induced by the male-rat-specific α2u-globulin-mediated mechanism and, therefore, considered to be toxicologically irrelevant to humans. Limited information from unpublished repeated and continuous inhalation studies suggests that long-term intermittent exposure to approximately 0.6 mg/m^3 does not cause adverse effects in rats. No adverse effects were reported to occur in mice exposed to 0.3 mg/m^3 for three months. From a thirteen-week dermal rat study, a NOAEL of 10 mg/kg bw/d is derived. Thirteen-week oral rat studies indicate a NOAEL of 0.75 mg/kg bw/d. A variety of neurotoxic effects at the neurobehavioural, neuropsychological, and neurochemical level resulted from short-term exposure. From these studies, a LOAEL of 2.5 mg/kg bw/d can be derived. In rats exposed for 40 days to this level, alterations in operant conditioning behaviour occurred.

Adverse effects on the immune system were induced in rats, mice, and rabbits. Impaired cellular and humoral immune responses against T-dependent and T-independent antigens, including decreased infection resistance, were observed. In mice, impaired immune functions were preceded by a temporary potentiation of immune responses during the first four to eight weeks of exposure. In addition to functional effects, histopathological changes were demonstrated. In rats and rabbits, effects were seen at doses equivalent to 1 mg/kg bw/day and higher, but in rats, not at a dose of 0.25 mg/kg bw/day. In mice exposed to doses of 0.012 mg/kg bw/day and higher, immunostimulatory and immunosuppressive effects were observed at all dose levels tested.

The potential carcinogenicity of γ-HCH has been studied in rats and mice following several routes of administration (except inhalation), but most studies were inadequately designed or reported. Although having some flaws too, the oral studies did not show evidence for carcinogenic activity in rats and mice.

The potential genotoxic activity of γ-HCH has been comprehensively tested *in vitro* in bacteria, yeasts, fungi, and mammalian cells. The most relevant end points were investigated, among which gene mutations, chromosomal aberrations, and primary DNA damage. With a sole exception (clastogenic effects in onion root tip cell systems, DNA single strand breaks in certain cell cultures), γ-HCH appeared not genotoxic in these tests. Inconsistent results were obtained in testing in *Drosophila*. *In vivo*, γ-HCH did

not induce genotoxic effects, i.e., the dominant lethal and bone marrow assays (end points: induction of chromosome aberrations, SCEs, and micronuclei) were negative, although there were indications for the occurrence of primary DNA damage (comet assay) in colon cells following oral administration and in nasal mucosa cells following inhalation.

In rats, γ-HCH did not induce teratogenic effects. At maternally toxic doses of ≥ 10 mg/kg bw/day, minor skeletal variations were observed. No effects were seen in a three-generation study at doses up to 4 mg/kg bw /day (highest dose level tested). At higher dose levels, embryotoxicity and effects on male and female fertility were reported. From the oral data available, the overall NOAEL for reproduction toxicity is concluded to be 5 mg/kg bw/day. In mice, data were not consistent. In a teratogenicity study, there were no irreversible teratogenic effects, but at maternally toxic doses of 60 mg/kg bw/day, fetotoxicity (decreased fetal bw) was seen; NOAEL: 30 mg/kg bw/day. In a separate study, doses of 5-10 mg/kg bw given during gd 1-4, gd 6-12, or gd 14-19 induced severe embryo/fetotoxic effects (complete inhibition of implantation, complete resorption, death of all pups). In rabbits, oral studies did not show teratogenic effects. Doses of 20 mg/kg bw/day caused maternal toxicity and a marginal increase in skeletal variations, while no such effects were seen at 10 mg/kg bw/day.

Chapter **7**

Existing guidelines, standards, and evaluations

7.1 General population

The National Institute of Public Health and Environmental Protection (RIVM) of The Netherlands has evaluated the risks of exposure to hexachlorocyclohexanes including γ-HCH to man. From oral animal toxicity data (two thirteen-week rat studies showing liver and kidney effects at doses ≥ 0.3 mg/kg bw/d, but not at 0.15 mg/kg bw/d), an acceptable daily intake (ADI) of 1 µg/kg bw has been proposed. It was concluded that oral exposure levels at that time did not present a risk for the population. Based on limited data from an inhalation study (semi-chronic rat study showing liver and kidney effects at a concentrations ≥ 0.5 mg/m^3, but not at 0.1 mg/m^3) a no-effect level of 100 µg/m^3 was derived. Since large-scale annual average outdoor concentrations in The Netherlands were a factor of 10^6 lower, the risk of inhalatory exposure was considered to be negligible. However, a certain risk from indoor uses leading to persistent levels in the tens of µg/m^3 range could not be excluded (Slo88). The conclusions of this report were adopted by the Health Council of the Netherlands (GR88).

The health risk due to exposure to γ-HCH has been evaluated at the request of the Netherlands Board for the Authorization of Pesticides (College voor de Toelating van Bestrijdingsmiddelen) within the frame work of the registration of pesticides in The Netherlands. It was concluded that γ-HCH is moderately toxic to toxic following oral or dermal exposure, and not irritating to the eyes or skin. Subchronic exposure (oral, dermal, inhalation) caused death and effects on the liver and the kidneys. Similar effects were seen following chronic exposure. It did not affect reproduction toxicology

parameters in rats; it did not induce irreversible teratogenic effects. γ-HCH was not genotoxic. From the NOAEL derived from oral (sub)chronic toxicity studies, an ADI of 5 µg/kg bw was proposed (CTB94).

Based on levels causing no toxicological effect in a thirteen-week oral rat study and a two-year oral dog study (0.75 and 1.6 mg/kg bw/d, resp) an acceptable daily intake (ADI) of 8 µg/kg bw has been estimated by JMPR (FAO89).

Maximum residue limits (MRLs) have been recommended for some 35 commodities, ranging from 0.01 mg/kg in milk to 3 mg/kg on strawberries; a limit of 0.5 mg/kg was recommended for most fruit and vegetables (Cod93). In the EC, MRLs are 0.2 mg/kg for all feed, except fat (2 mg/kg) (WHO91b).

For drinking water, WHO recommends a guideline of 2 µg/L. This level was based on a TDI of 5 µg/kg bw derived from a thirteen-week oral rat study (demonstrating liver and kidney toxicity) and on the assumption that only 1% of the TDI is allocated to drinking-water (WHO93).

EC legislation prohibits the placing on the market, and the use, of hexachlorocyclohexane containing less than 99% of the γ-isomer, and also the placing on the market of cosmetics containing γ-HCH (WHO91b).

In the Germany, γ-HCH may not be handled by adolescents or by pregnant and nursing women (WHO91b).

In its evaluation of the carcinogenicity of γ-HCH, IARC concluded that the evidence was inadequate for humans and limited for experimental animals. IARC stated that γ-HCH produced liver tumours in mice and, in one study, a few thyroid tumours in rats, but many studies were inadequate, including one on skin application in mice. In summarizing genotoxicity data, it was mentioned that γ-HCH did not induce dominant lethal mutations and bone marrow chromosomal aberrations *in vivo* in mice. *In vitro*, it did neither induce UDS in human cells, nor micronuclei, chromosomal aberrations, or UDS in cultured rodent cells. It caused DNA strand breaks, and inhibited intercellular communication in Chinese hamster V79 cells. It was not mutagenic in bacteria, but it caused gene conversion in yeast. γ-HCH did not induce sex-linked recessive lethal mutations in *Drosophila* (IARC79, IARC87).

The aforementioned document of RIVM underlays a subsequent advice of the Committee on the Evaluation of the Carcinogenicity of Chemical Substances of the Health Council of The Netherlands (GR). This Committee concluded from the data presented in the RIVM document that neither mutagenicity nor macromolecular binding could be established. The tumours found in experimental animals were not considered to be the result of a genotoxic mechanism, but were ascribed to toxic effects of γ-HCH on the liver. It was noted that there will be no risk of tumours at levels that do not induce toxic effects (GR88).

7.2 Working population

7.2.1 Occupational exposure limits

Current occupational exposure limit for γ-HCH in The Netherlands and some other countries are presented in Table 8.

From inhalation studies carried out around 1950, ACGIH concluded that the lowest-observed-adverse-effect level is somewhere between 0.19 and 0.7 mg/m^3. Accordingly, an occupational exposure limit of 0.5 mg/m^3 is recommended to prevent central nervous system effects. It was stated that this limit is under review at the time because of the data (not specified) reported in recent reviews of γ-HCH. A skin notation was considered to be appropriate, because skin absorption in humans has been associated with seizures and with measurable quantities of γ-HCH in blood. No STEL was recommended until additional toxicological data and industrial hygiene experience become available to provide a better base for quantifying on a toxicological basis what the STEL should be (ACG91).

HSE concluded that the human data available did not provide an adequate basis for a limit value. Further, γ-HCH was not considered to be genotoxic or carcinogenic. Of the animal studies, the mouse 90-day inhalation study was thought to be the most suitable from which to derive an occupational exposure limit. In this study, no effects were observed at 0.3 mg/m^3. There were two deaths in the 1.0 mg/m^3 group, but the relevance of this finding was unclear due to the lack of pathological changes and one death in the control group. Taking this uncertainty into account and allowing for interspecies extrapolation, a limit of 0.1 mg/m^3 (8-h TWA) was proposed (HSE96b).

In 1981, a Study Group of the WHO has evaluated the toxicity of γ-HCH and proposed a tentative health-based occupational exposure limit of 0.3 mg/m^3 as a time-weighted daily average over a 40-hour working week and the addition of a skin notation to denote the possibility of skin absorption. This limit had been based on the limited data from inhalation studies as presented by ACGIH (see above) (WHO82).

No occupational toxicological risk assessment has been made available to the Netherlands Board for the Authorization of Pesticides (College voor de Toelating van Bestrijdingsmiddelen; see also Section 7.1) within the framework of legislation of pesticides in The Netherlands (CTB94).

Table 8 Occupational exposure standards in various countries.

country -organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	lit ref[b]	year of adoption[c]
	ppm	mg/m^3					
The Netherlands							
-Ministry		0.5	8 h	administrative	S	SZW96	unknown
-DECOS		-		force			
Germany							
-AGS							
-DFG MAC-kom.		0,5	8 h	MAK	S	DFG97	unknown
		5	30 min[d]				
Great-Britain							
-HSE		0,1	8 h	OES	S	HSE96a	1995 (Wil95)
Sweden		-					
Denmark		0,5	8 h		S	Arb92	unknown
USA							
-ACGIH		0,5	8 h		S, A3[e] S	ACG97	1996
-OSHA		0,5	8 h	PEL	S	ACG91	unknown
-NIOSH		0,5	8 h	REL		ACG91	unknown
European Union		-					
-SCOEL							

[a] S = skin notation; which mean that skin absorption may contribute considerably to body burden
sens = substance can cause sensitisation

[b] reference to the most recent official publication of occupational exposure limits

[c] year that this limit was officially adopted or established

[d] limited to maximal one time per shift

[e] classified as an A3 carcinogen, i.e., an animal carcinogen

7.2.2 *Biological limit values*

The aforementioned WHO Study Group also recommended a health-based biological limit for γ-HCH. The determination of γ-HCH in whole blood should be used as the primary basis for the individual monitoring of γ-HCH exposure. This level should not exceed 0.02 mg/L, which should be regarded as a individual maximum. The frequency of determination should be adapted to the exposure pattern. Pre-exposure levels should be known. This level has been based on results from studies on occupational exposed workers showing that EEG changes were present in workers with blood levels exceeding 0.02 mg/L (WHO82).

DFG has established the following biological limit values for γ-HCH: blood: 20 µg γ-HCH/L, determined at the end of a shift after several working days; plasma/serum: 25 µg/L determined at the end of a shift after several working days. This was based on similar considerations as those of the WHO Study Group (see above) and supported by data from additional studies on occupational exposed groups (DFG95, Wic96).

HSE has proposed a "biological monitoring benchmark value" of 5 µg/L (17 nmol/L) in whole blood being equivalent to approximately 35 nmol/L (~10 µg/l) in plasma (HSE96b).

Chapter **8**

Hazard assessment

8.1 Assessment of health hazard

The committee is of the opinion that no human data were sufficient for deriving an occupational exposure limit for γ-HCH. There are no indications for irritating effects of γ-HCH in occupationally exposed workers; there is no information on sensitization as well.

In experimental animals, γ-HCH did not show skin- or eye-irritating or sensitizing properties.

The committee did not consider γ-HCH to be a genotoxic carcinogen. Apart from clastogenic effects in onion root tip cell systems and the induction of DNA single strand breaks in certain cells following *in vitro* or *in vivo* exposure, negative results were reported in a wide variety of *in vitro* and *in vivo* genotoxicity tests, and oral studies (inhalatory exposure route was not tested) did not show evidence for carcinogenic activity in rats and mice, although these studies had some flaws.

γ-HCH did not induce irreversible teratogenic effects in rats, mice or rabbits. Embryotoxic and effects on male and female fertility were reported at relatively high doses. No (such) effects were seen in a three-generation feed study in rats at doses up to 4 mg/kg bw/d (the highest dose level tested). In mice, severe embryo/fetotoxic effects were induced when doses of 5-10 mg/kg bw/d were given during definite periods of pregnancy.

Repeated inhalatory, dermal, and oral exposure caused effects on the liver and the kidneys. The kidney effects are of no toxicological relevance for humans, since they

were male rat-specific. Limited information from unpublished repeated and continuous inhalation studies suggests that long-term intermittent exposure to approximately 0.6 mg/m^3 does not cause effects in rats. No effects were reported to occur in mice exposed to 0.3 mg/m^3 for three months. From a thirteen-week dermal rat study, a NOAEL of 10 mg/kg bw/d is derived. Thirteen-week oral rat studies indicate a NOAEL of 0.75 mg/kg bw/d. A variety of neurotoxic effects at the neurobehavioural, neuropsychological, and neurochemical level result from short-term exposure. From these studies, a LOAEL of 2.5 mg/kg bw/d can be derived. In rats exposed for 40 days to this level, alterations in operant conditioning behaviour occurred.

Adverse effects on the immune system were induced in rats, mice and rabbits. Impaired cellular and humoral immune responses against T-dependent and T-independent antigens, including decreased infection resistance, were observed. In mice, impaired immune functions were preceded by a temporary potentiation of immune responses during the first four to eight weeks of exposure. In addition to functional effects, histopathological changes were demonstrated. In rats and rabbits, effects were seen at doses equivalent to 1 mg/kg bw/day and higher, but in rats, not at a dose of 0.25 mg/kg bw/day. In mice exposed to doses of 0.012 mg/kg bw/day and higher, immuno-stimulatory and immuno-suppressive effects were observed at all dose levels tested.

The committee is of the opinion that the effects on the immune system are the most sensitive effects. Because these effects have not been investigated in the inhalatory studies, the committee takes the LOAEL of 0.012 mg/kg bw/d, observed in mice orally exposed for 24 weeks, as a starting point in deriving a health-based occupational exposure limit.

For the assessment of an HBR-OEL, the committee takes the following considerations into account: intra- and interspecies variation, differences between experimental conditions and the exposure pattern of the worker, type of critical effect, dose-response-curve, and the confidence of the data base.

The committee is of the opinion that a compensation for interspecies differences is not necessary because the mouse seems to be a sensitive species for the observed immunological effects. A factor of 2 is considered sufficient for intraspecies differences. Although a study of relatively short duration (i.e., 24 weeks) is taken as a starting point, the committee expects that the changes in the immunotoxicity parameters most likely will not be influenced by prolongation of exposure. Therefore, the committee is of the opinion that a factor which compensates for differences between experimental conditions and occupational exposure patterns is not necessary.

As to the dose-response-curves in the mouse study, the doses administered differed by a factor of 10, and a reasonable dose-response relationship was observed for most

parameters, but changes in some parameters at the lowest dose were comparable with those at higher doses. Therefore, the committee recommends a factor of 10 to compensate for the absence of a NOAEL.

In summary, the committee considers an overall assessment factor of 20 to be sufficient for extrapolation from an oral LOAEL in mice to the worker exposed by inhalation.

Therefore, assuming a respiratory volume of 10 m^3 per eight hours and a body weight of 70 kg for the worker, the committee derives a HBR-OEL of 4 $\mu g/m^3$*.

In view of the dermal absorption found in human volunteers (see Section 5.1.2), the committee recommends a skin notation.

8.2 Groups at extra risk

The available data do not indicate groups at extra risk

8.3 Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational standards recommends a health-based occupational exposure limit for γ-HCH of 4 $\mu g/m^3$ as an eight-hour TWA, as well as a skin notation.

* from: 0.012 mg/kg x 1/20 x 70 kg x 1/10 m^3

Chapter 9

Recommendations for research

A subchronic toxicity inhalation study in which immunotoxicity parameters are investigated as well is recommended.

References

ACG91 American Conference of Governmental Industrial Hygienists (ACGIH). Lindane. In: Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Cincinnati OH, USA: ACGIH, 1991: 858-61.

ACG97 American Conference of Govermental Industrial Hygienists (ACGIH). 1997 TLVs® and BEIs®. Threshold Limit Values for chemical substances and physical agents. Biological Exposure Indices. Cincinnati OH, USA: ACGIH, 1997: 28.

Agr95 Agrawal D, Subramoniam A, Afaq F. Influence of hexachlorocyclohexane on phosphoinositides in rat erythrocyte membranes and brain. Toxicology 1995; 95: 135-40.

Aks95 Aks SE, Krantz A, Hryhorczul DO, *et al.* Acute accidental lindane ingestion in toddlers. Ann Emerg Med 1995; 26: 647-51.

Alb79 Albrecht R, Dooh-Priso E, Faudemay F, *et al.* Induction par le lindane des monoxygénases microsomales du foie chez le rat: effets d'un régime hyperlipidique. Food Cosmet Toxicol 1979; 17: 463-71.

And83 André F, Gillon J, André C, *et al.* Pesticide-containing diets augment anti-sheep red blood cell nonreaginic antibody responses in mice but may prolong murine infection with *Giardia muris*. Environ Res 1983; 32:145-50.

Ang83 Angerer J, Maaß R, Heinrich R. Occupational exposure to hexachlorocyclohexane.VI. Metabolism of -hexachlorocyclohexane in man. Int Arch Occup Environ Health 1983; 52: 59-67

Ang91 Angerer J. Hexachlorcyclohexan-Isomere (HCH). In: Analytische Methoden zur Prüfung gesundheitsschädlicher Arbeitsstoffe. 1st-10th ed. Weinberg, Germany: VCH Verlagsgesellschaft mbH, 1991; Analysen in biologischem Material; Vol 2.

Arb92 Arbejdstilsynet. Grænseværdier for stoffer och materialer. Copenhagen, Denmark, 1992; 23 (At-anvisning Nr 3.1.0.2).

Ari94 Arisi ACM, Simizu K, Kogake M, *et al.* Brain and liver peroxidation levels following acute and short-term lindane administration in the rat. Toxicol Lett 1994; 1: 61-8.

Art88 Artigas F, Martinez E, Camon L, *et al.* Synthesis and utilization of neurotransmitters: actions of subconvulsant doses of hexachlorocyclohexane isomers on brain monoamines. Toxicology 1988; 49: 49-55.

ATS94 Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for alpha-, beta-, gamma- and delta-hexachlororcyclohexane (Update). Atlanta GA, USA: ATSDR, 1994; rep no TP-93/09.

Att91 Attia AM, Richardson BA, Rodriguez C, *et al.* Lindane may enhance nocturnal pineal *N*-acetyltransferase activity via -adrenergic receptors. Brain Res 1991; 554: 253-6.

Bal89 Balikovà M, Kohli ek J, Rybka K. Chlorinated phenols as metabolites of lindane. Evaluation of the degree of conjugation in rat urine. J Anal Toxicol 1989; 13: 27-30.

Ban96 Banerjee BD, Koner BC, Ray A, *et al.* Influence of subchronic exposure to lindane on humoral immunity in mice. Indian J Exp Biol 1996; 34: 1109-13.

Bar95a Barrón S, Tusell JM, Serratosa J. Effect of hexachlorocyclohexane isomers on calmodulin mRNA expression in the central nervous system. Mol Brain Res 1995; 30: 279-86.

Bar95b Barrón S, Tusell JM, Solà, *et al.* Convulsant agents activate c-*fos* induction in both a calmodulin-dependent and calmodulin-independent manner. J Neurochem 1995; 65: 1731-9.

Bar95c Barrón S, Serratosa J, Tusell JM. Regulation of c-*fos* expression by convulsants and hexachlorocyclohexane isomers in primary cultures of cortical neurons. J Neurochem 1995; 64: 1708-14.

Bau80 Baumann K, Angerer J, Heinrich R, *et al.* Occupational exposure to hexachlorocyclohexane. I. Body burden of HCH-isomers. Int Arch Occup Environ Health 1980; 47: 119-27.

Bau81 Baumann K, Behling K, Brassow HL, *et al.* Occupational exposure to hexachlorocyclohexane. III. Neurophysiological findings and neuromuscular function in chronically exposed workers. Int Arch Occup Environ Health 1981; 48: 165-72.

Ber95 Berdowskii JJM, Jonker WJ, Verhoeve P. Emissies in Nederland. Bedrijfsgroepen en regio's 1993 en ramingen 1994. The Hague, The Netherlands: Hoofdinspectie Milieuhygiene/IPC680, Afd Emissieregistratie en Informatiemanagment, 1995; Publikatiereeks Emissieregistratie Nr 27.

Ber96 Berdowskii JJM, van der Auweraert RJK, Blom JC, *et al.* Emissies in Nederland. Trends, thema's en doelgroepen 1994 en ramingen 1995. The Hague, The Netherlands: Hoofdinspectie Milieuhygiene/IPC680, Afd Emissieregistratie en Informatiemanagment, 1996; Publikatiereeks Emissieregistratie Nr 32.

CBS94 Centraal Bureau voor de Statistiek (CBS): Divisie Landbouw, Nijverheid en Milieu, Sector Milieu. Gewasbescherming in de land- en tuinbouw, 1992. Chemische, mechanische en biologische bestrijding. The Hague, The Netherlands: Sdu Servicecentrum Uitgeverijen, 1994: Sdu/CBS-publicaties.

CEG93 Commissie van de Europese Gemeenschappen (CEG). In: Bijlage bij Richtlijn 93/72/EEG van de Commissie van 1 september tot negentiende aanpassing aan de vooruitgang van de techniek van Richtlijn 67/543/EEG van de Raad betreffende de aanpassing van de wettelijke en bestuursrechtelijke bepalingen inzake de indeling, de verpakking en het kenmerken van gevaarlijke stoffen (vervolg). Maastricht, The Netherlands: Ellis Publications, 1993: 596 (Publicatieblad van de Europese Gemeenschappen L258A, deel I).

CCRX95 Co-ordinating Committee for Monitoring the Environment (CCRX).Bestrijdingsmiddelen. In: Monitoring the environment in The Netherlands, 1993. Bilthoven, The Netherlands: National Institute of Public Health and Environmental Protection, Reporting and Sales Department, 1995: 101-6.

Cha88 Chadwick RW, Cooper RL, Chang J, *et al.* Possible antiestrogenic activity of lindane in female rats. J Biochem Toxicol 1988; 3: 147-58.

Cha94 Chang S.K. Williams PL, Dauterman WC, *et al.* Percutaneous absorption, dermatopharmacokinetics and related bio-transformation studies of carbaryl, lindane, malathion, and parathion in isolated perfused porcine skin. Toxicology 1994; 91: 269-80.

Coc93 Coccini T, Candura SM, Manzo L, *et al.* Interaction of the neurotoxic pesticides ivermectin and lindane with the enteric $GABA_A$ receptor-ionophore complex in the guinea-pig. Eur J Pharmacol 1993; 248: 1-6.

Cod93 Codex Alimentarius Commission. (48) Lindane. Codex Alimentarius. Pesticides residues in food. 2nd ed. Rome, Italy: FAO/WHO, 1993: 46.

Col79 Colson JG. Benzene hexachloride. In: Mark HF, Othmer DF, Overberger, CG, *et al*, eds. Castor oil to chlorosulfuric acid. 3rd ed. New York, USA: John Wiley & Sons, 1979: 808-18 (Kirk-Othmers encyclopedia of chemical technology; Vol 5).

Coo89 Cooper RL, Chadwick RW, Rehnberg GL, *et al.* Effect of lindane on hormonal control of reproductive function in the femal rat. Toxicol Appl Pharmacol 1989; 99: 384-94.

CTB94 College voor de Toelating Bestrijdingsmiddelen (CTB). Verslag G22; agendapunt c-22.3.15 lindaan. Wageningen, The Netherlands: CTB, 1994 (April 22).

CTB97 College voor de Toelating van Bestrijdingsmiddelen (CTB). Register van toegelaten bestrijdingsmiddelen in de landbouw (bijgewerkt t/m oktober 1997). Wageningen, The Netherlands: CTB, 1997.

Cze70 Czeglédi-Janko G, Avar P. Occupational exposure to lindane; clinical and laboratory findings. Br J Ind Med 1970; 27: 283-6.

Dal96 Dalsenter PR, Faqi AS, Webb J. Reproductive toxicity and tissue concentrations of lindane in adult male rats. Hum Experiment Toxicol 1996; 15: 406-10.

Der95 Derner M, Gerhard I, Monga B, *et al.* Immunological, endocrine, and chemical changes in women with hormonal disorders due to exposure to pentachlorophenol and lindane. Zbl Arbeitsmed 1995; 45: 312-28.

Des74 Dési I. Neurotoxicological effect of small quantities of lindane. Int Arch Arbeitsmed 1974; 33: 153-62.

Des78 Dési L, Varga L, Farkas I. Studies on the immunosuppressive effect of the organochlorine and organophosphoric pesticides in subacute experiments. J Hyg Epidemiol Microbiol Immunol 1978; 22: 115-22.

Des83 Dési I. Neurotoxicological evaluation of pesticides in animal experiments. Neurobehav Toxicol Teratol 1983; 5: 503-15.

Dew80 Dewan A, Gupta SK, Jani JP, *et al.* Effect of lindane on antibody response to typhoid vaccine in weanling rats. J Environ Sci Health 1980; B15: 395-402.

DFG97 Deutsche Forschungsgemeinschaft (DFG): Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe. MAK- und BAT-Werte-Liste 1997. Maximale Arbeitsplatzkonzentrationen und Biologische Arbeitsstofftoleranzwerte. Weinheim, Germany: VCH Verlagsgesellschaft mbH, 1997; 69, 162 (Mitteilung 33).

Die90 Dietrich DR, Swenberg JA. Lindane induces nephropathy and renal accumulation of 2u-globulin in male but not in female Fischer 344 rats or male NBR rats. Toxicol Lett 1990: 179-81.

Die91 Dietrich DR, Swenberg JA. NCI-Black-Reiter (NBR) male rats fail to develop renal disease following exposure to agents that induce -2u-globulin ($_{2u}$) nephropathy. Fundam Appl Toxicol 1991; 16: 749-62.

Dru88 Drummond L, Gillanders EM, Wilson HK. Plasma -hexachlorocyclohexane concentrations in forestry workers exposed to lindane. Br J Ind Med 1988; 45: 493-7.

Ell84 Eller PM, ed. Method 1300. NIOSH Manual of analytical methods. 3rd ed. Cincinnati OH, USA: DHHS/NIOSH, 1984; DHHS (NIOSH) publ no 84-100; Vol 1.

Eps72 Epstein SS, Arnold E, Andrea J, *et al.* Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol Appl Pharmacol 1972; 23: 288-325.

FAO89 Food and Agriculture Organization/World Health Organization (FAO/WHO). Lindane. In: Pestides residues in food - 1989. Report of the Joint Meeting of the FAO Panel of Experts on Pesticides in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Italy: FAO/WHO, 1989; 141-55 (FAO Plant Production and Protection Paper 100/2).

Fei94 Feigenspan A, Bormann J. Differential pharmacology of $GABA_A$ and $GABA_C$ receptors on rat retinal bipolar cells. Eur J Pharmacol 1994; 288: 97-104.

Fel74 Feldmann RJ, Maibach HI. Percutaneous penetration of some pesticides and herbicides in man. Toxicol Appl Pharmacol 1974; 28: 126-32.

Fer95 Ferguson CA, Audesirk G. Non-$GABA_A$ -mediated effects of lindane on neurite development and intracellular free calcium ion concentration in cultured rat hippocampal neurons. Toxicol in Vitro 1995; 9: 95-106.

Fis87 Fishman BE, Gianutsos G. Differential effects of gamma-hexachlorocyclohexane (lindane) on pharmacologically-induced seizures. Arch Toxicol 1987; 59: 397-401.

Fis88 Fishman BE, Gianutsos G. CNS biochemical and pharmacological effects of the isomers of hexachlorocyclohexane (lindane) in the mouse. Toxicol Appl Pharmacol 1988; 93: 146-53.

For90 Forgue MF, Pinelli E, Beraud M, *et al.* Chemiluminescence response and arachidonic acid metabolism of macrophages induced by -hexachlorocyclohexane (lindane). Food Addit Contam 1990; 7 (suppl 1): S97-9.

Gil95a Gilbert ME. Repeated exposure to lindane leads to behavioral sensitization and facilitate electrical kindling. Neurotoxicol Teratol 1995; 17: 131-41.

Gil95b Gilbert ME, Mack CM. Seizure tresholds in kindled animals are reduced by the pesticides lindane and endosulfan. Neurotoxicol Teratol 1995; 17: 143-50.

Gop92 Gopalaswamy UV, Nair CKK. DNA binding and mutagenicity of lindane and its metabolites . Bull Environ Contam Toxicol 1992; 49: 300-5.

Gri89 Griffith JA, Woolley DE. “Central” and “peripheral” benzodiazepines and kinetics of lindane-induced toxicity. Pharmacol Biochem Behav 1989; 32: 367-76.

GR88 Gezondheidsraad (GR). Hexachlorocyclohexanen. Toetsing van een ontwerp-basis-document. The Hague, The Netherlands: Health Council, 1988.

Gre83 Grey WE, Marthre DE, Rogers SJ. Potential exposure of commercial seed-treating applicators to the pesticides carboxin-thiram and lindane. Bull Environ Contam Toxicol 1983; 31: 244-50.

Han73 Hanada M., Yutani C, Miyaji T. Induction of hepatoma in mice by benzene-hexachloride. Gann 1973; 64: 511-3.

Has93 Hassoun E, Bagchi M, Bagchi D, *et al.* Comparative studies on lipid peroxidation and DNA -single strand breaks induced by lindane, DDT, chlordane and endrin in rats. Comp Biochem Physiol 1993; 104C: 427-31.

Has96 Hassoun EA, Stohs SJ. Comparative teratological studies on TCCD, endrin and lindane in C57BL/6J and DBA/2J mice. Comp Biochem Physiol 1996; 113C: 393-8.

Haw89 Hawkinson JE, Shull LR, Joy RM. Effects of lindane on calcium fluxes in synaptosomes. Neurotoxicology 1989; 10: 29-40.

Her75 Herbst M, Weisse I and Koellmer H.A contribution to the question of the possible hepatocarcinogenic effects of lindane. Toxicology 1975; 4: 91-6.

Hoy93 del Hoyo N, Pulido JA, Pérez-Albarsanz MA. Characterization of phosphoinositide hydrolysis products induced by hexachlorocyclohexane isomers in rat brain cortex. Biosci Rep 1993; 13: 119-26.

HSE96a Health and Safety Executive (HSE). Occupational exposure limits 1996. Sudbury (Suffolk), England: HSE Books, 1996: 21 (Guidance note: EH40/96).

HSE96b Health and Safety Executive (HSE). Lindane (Gamma-HCH). In: EH64 summary criteria for occupational exposure limits. Sudbury (Suffolk), England: HSE Books, 1996: D78.

IARC79 International Agency for Research on Cancer (IARC). Hexachlorocyclohexane (technical HCH and lindane). In: Some halogenated hydrocarbons. Lyon, France: IARC, 1979: 195-239 (IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans; Vol 20).

IARC87 International Agency for Research on Cancer (IARC). Hexachlorocyclohexanes (Group 2B). In: Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1 to 42. Lyon, France: IARC, 1987: 220-2 (IARC monographs on the evaluation of carcinogenic risks to humans; Suppl 7).

Ish77 Ishidate M, Odashima S. Chromosome tests with 134 compounds on Chinese hamster cells in vitro-a screening for chemical carcinogens. Mutat Res 1977; 48: 337- 54.

Ito73 Ito N, Nagasaki, H, Arai M, *et al.* Histologic and ultrastructural studies on the hepatocarcinogenicity of benzene hexachloride in mice. J Natl Cancer Inst 1973; 51: 817-26.

Ito75 Ito N, Nagasaki H, Aoe H, *et al.* Development of hepatocellular carcinomas in rats treated with benzeze hexachloride. J Natl Cancer Inst 1975; 54: 801-5.

Ive84 Iverson F, Ryan JJ, Lizotte R, *et al.* In vivo and in vitro binding of - and -hexachlorocyclohexane to mouse liver macromolecules. Toxicol Lett 1984; 20: 331-5.

Joy82 Joy RM, Stark LG, Albertson TE. Proconvulsant effects of lindane: enhancement of amygdaloid kindling in the rat. Neurobehav Toxicol Teratol 1982; 4: 347-54.

Joy83 Joy RM, Stark LG, Albertson TE. Proconvulsant actions of lindane compared at two different sites in the rat-Amygdala and hippocampus. Neurobehav Toxicol Teratol 1983; 5: 465-8.

Joy88 Joy RM, Burns VW. Exposure to lindane and two other hexachlorocyclohexane isomers increases free intracellular calcium levels in neurohybridoma cells. Neurotoxicology 1988; 9: 673-44.

Joy95 Joy RM, Walby WF, Stark LG, *et al.* Lindane blocks non-$GABA_A$ mediated inhibitions and modulates pyramidal cell excitability in the rat hippocampal slice. Neurotoxicology 1995; 16: 217-28.

Jan88 Janssen PJCM, Van Koten-Vermeulen JEM, Krajnc EI, *et al*. Integrated criteria document hexachlorocyclohexanes (republication of rep no 758473004, July 1987). Effects. Bilthoven, The Netherlands: National Institute of Public Health and Environmental Protection, 1988; appendix to rep no 758473011.

Jun94 Junqueira VBC, Bainy ACD, Arisi ACM, *et al.* Acute lindane intoxication: a study on lindane tissue concentration and oxidative stress- related parameters in liver and erythrocytes. J Biochem Toxicol 1994; 1: 9-15.

Kar95 Karmaus W, Wolf N. Reduced birthweight and lenght in the offspring of females exposed to PCDFs, PCP, and lindane. Environ Health Perspect 1995; 103: 1120-5.

Kas86 Kashyap SK. Health surveillance and biological monitoring of pesticide formulators in India. Toxicol Lett 1986; 33: 107-14.

Kas93 Kassner JT, Maher TJ, Hull KM, *et al.* Cholestyramine as an adsorbent in acute lindane poisoning: a murine model. Ann Emerg Med 1993; 9: 1392-7.

Khe79 Khera KS, Whalen C, Trivett G, *et al.* Teratogenicity studies on pesticidal formulations of dimethoate, diuron and lindane in rats. Bull Environ Contam Toxicol 1979; 22: 522-9.

Kir79 Kiraly J, Szentesi I, Ruzicska M, *et al.* Chromosome studies in workers producing organophosphate insecticides. Arch Environ Contam Toxicol 1979; 8: 309-19.

Koë87 Koëter HBWM, Dreef-van der Meulen HC, Zielhuis RL, *et al*. Schadelijke effecten van lindaan op voortplanting en nageslacht, anders dan via de werking op het erfelijk materiaal. Een literatuurstudie. Zeist, The Netherlands: TNO Nutrition and Food Research Institute, 1987.

Kum95 Kumar D, Khan PK, Sinha SP. Cytogenetic toxicity and no-effect limit dose of pesticides. Food Chem Toxicol 1995; 33: 309-14.

Lah91 Lahiri P, Sircar S. Suppression of adrenocortical function in female mice by lindane (gamma-HCH). Toxicology 1991; 66: 75-9.

Law94 Laws SC, Carey SA, Hart DW, *et al.* Lindane does not alter the estrogen receptor or the estrogen-dependent induction of progesterone receptors in sexually immature or ovariectomized adult rats. Toxicology 1994; 92: 127-142.

Lin94 Lindenau A, Fischer B, Seiler P, *et al.* Effects of persistent chlorinated hydrocarbons on reproductive tissues in female rabbits. Hum Reprod 1994; 9: 772-80.

Llo89 Llorens J, Tusell JM, Suñol C, *et al.* Effects of lindane on spontaneous behavior of rats analyzed by multivariate statistics. Neurotoxicol Teratol 1989; 11: 145-51.

Llo90 Llorens J, Tusell JM, Suñol C, *et al.* On the effects of lindane on plus-maze model of anxiety. Neurotoxicol Teratol 1990; 12: 643-7.

Llo91 Llorens J, Suñol C, Tusell JM, *et al.* Evidence for acute tolerance to the behavioral effects of lindane: concomitant changes in regional monoamine status. Neurotoxicolgy 1991; 12: 697-706.

Loh96 Lohmann K, Pröhl A, Schwarz E. Vielfache Chemikalienunverträchlichkeit (Multiple Chemical Sensitivity Disorder) bei Patienten mit neurotoxischen Gesundheitsstörungen. Gesundheitswesen 1996; 58: 322-31.

Mat88 Matsumoto K, Eldefrawi ME, Eldeframi AT. Action of polychlorocycloalkane insecticides on binding of [^{35}S]*t*-butylbicyclophosphorothionate to *torpedo* electric organ membranes and stereospecificity of the binding site. Toxicol Appl Pharmacol 1988; 95: 220-9.

Mea84 Meade CJ, Harvey J, Boot JR, *et al.* -Hexachlorocyclohexane stimulation of macrophage phospholipid hydrolysis and leukotriene production. Biochem Pharmacol 1984; 33: 289-93.

Mee92 Meera P, Rao PR, Shanker R, *et al.* Immunomedulatory effects of -HCH (lindane) in mice. Immunopharmacol Immunotoxicol 1992; 1-2: 261-82.

Mil68 Milby TH, Samuels AJ, Ottoboni F. Human exposure to lindane; blood levels as a function of exposure. J Occup Med 1968; 10: 584-7.

Miy95 Miyagawa M, Takasawa H, Sugiyama A, *et al.* The in vivo-in vitro replicative DNA synthesis (RDS) test with hepatocytes prepared from male B6C3F1 mice as an early prediction assay for putative nongenotxic (Ames-negative) mouse hepatocarcinogens. Mutat Res 1995; 343: 157-83.

Moo89 Moody RP, Ritter L. Dermal absorption of the insecticide lindane (1 , 2 , 3 , 4 , 5 , 6 -hexachlorocyclohexane) in rats and rhesus monkeys: effect of anatomical site. J Toxicol Environ Health 1989; 28: 161-9.

Nag95a Nagata K, Narahashi T. Multple actions of dieldrin and lindane on the $GABA_A$ receptor-chloride channel comple of rat dorsal root ganglion neurons. Pestic Sci 1995; 44: 1-7.

Nag95b Nagata K, Narahashi T. Differential effects of hexachlorocyclohexane isomers on the $GABA_A$ receptor-chloride channel complex in rat dorsal root ganglion neurons. Brain Res 1995; 704: 85-91.

Nar92 Narahashi T, Frey JM, Ginsburg KS, *et al.* Sodium and $GABA_A$-activated channels as the targets of pyrethroids and cyclodienes. Toxicol Lett 1992; 64/65: 429-36.

Nig86 Nigam SK, Karnik, AB, Majumder SK, *et al.* Serum hexachlorocyclohexane residues in workers engaged at a HCH manufacturing plant. Int Arch Occup Environ Health 1986; 57: 315-20.

Nit84 Nitsche K, Lange M, Bauer E, *et al.* Quantitative distribution of locally applied lindane in human skin and subcutaneous fat in vitro. Dependence of penetration on the applied concentration, skin, state, duration of action and nature and time of washing. Dermatosen 1984; 32: 161-5.

Oes82 Oesch F, Friedberg T, Herbst M, *et al.* effects of lindane treatment on drug metabolizing enzymes and liver weight of CFI mice in which it evoked hepatomas and in non-susceptible rodents. Chem-Biol Interact 1982; 40: 1-14.

Ort95 Ortiz Martinez AO, Martinez-Conde E. The neurotoxic effects of lindane at acute and subchronic dosages. Ecotoxicol Environ Saf 1995; 30: 101-5.

Pal78a Palmer AK, Cozen DD, Spicer EJF, *et al.* Effects of lindane upon reproductive function in a 3-generation study in rats. Toxicology 1978; 10: 45-54.

Pal78b Palmer AK Bottomley AM, Worden AN, *et al.* Effect of lindane on pregnancy in the rabbit and rat. Toxicology 1978; 9: 239-47.

Pom94a Pomés A, Frandsen A, Suñol S, *et al.* Lindane cytotoxicity in cultured neocortical neurons is ameliorated by $GABA_A$ and flunitrazepam. J Neurosci Res 1994; 39: 663-8.

Pom94b Pomés A, Rodríguez-Farré E, Suñol S. Effects of organochlorine pesticides on $^{36}Cl^-$ flux in primary neuronal cultures. Neurotoxicology 1994; 15: 745-50.

Pom94c Pompa G, Fadini L, Di Lauro F, *et al.* Transfer of lindane and pentachlorobenzene from mother to newborn rabbits. Pharmacol Toxicol 1994; 74: 28-34.

Poo93 Pool-Zobel BL, Guigas C, Klein R, *et al.* Assessment of genotoxic effects by lindane. Food Chem Toxicol 1993; 31: 271-83.

Poo94 Pool-Zobel BL, Lotzmann N, Knoll M, *et al.* Detection of genotoxic effects in human gastric and nasal mucosa cells isolated from biopsy samples. Environ Mol Mutagen 1994; 24: 23-45.

Por88 Portig J, Schnorr C. The potency of gamma-1,2,3,4,5,6-hexachlorocyclohexane (lindane). Toxicology 1988; 52: 309-21.

Pul90 Pulido JA, del Hoyo N, Pérez-Albarsanz MA. Effects of lindane on the glucose metabolism in rat brain cortex cells. Life Sci 1990; 47: 1099-1107.

Rai80 Raizada RB, Misra P, Saxena I, *et al.* Weak estrogenic activity of lindane in rats. J Toxicol Environ Health 1980; 6: 483-92.

Rei95 Reinhardt CA, Schein CH. Glutamine synthetase activity as a marker of toxicity in cultures of embryonic chick brain and retina cells. Toxicol In Vitro 1995; 9: 369-74.

Riv78 Rivett KF, Chesterman H, Kellett DN, *et al.* Effects of feeding lindane to dogs for periods up to 2 years. Toxicology 1978; 9: 273-89.

Riv91 Rivera S, Sanfeliu C, Suñol C, *et al.* Regional effects on the cerebral concentration of noradrenaline, serotonine and dopamine in suckling rats after a single dose of lindane. Toxicology 1991; 69: 43-54.

Ros96 Rosa R, Rodríguez-Farré E, Sanfeliu C. Cytotoxicity of hexachlorocyclohexane isomers and cyclodienes in primary cultures of cerebellar granule cells. J Pharmacol Exp Ther 1996; 278: 163-9.

Rou79 Roux F, Treich I, Brun C, *et al.* Effect of lindane on human lymphocyte responses to phytohemagglutinin. Biochem Pharmacol 1979; 28: 2419-26.

Roy87 Roy Chowdhury A, Venkatakrishna-Bhatt H, Gautam AK. Testicular changes of rats under lindane treatment. Bull Environ Contam Toxicol 1987; 38: 154-6.

Sag83 Sagelsdorff P, Lutz WK, Schlatter C. The relevance of covalent binding to mouse liver DNA to the carcinogenic action of hexachlorocyclohexane isomers. Carcinogenesis 1983; 4: 1267-73.

Sah93 Saha S, Banerjee BD. Effect of sub-chronic lindane exposure on humoral and cell-mediated immune responses in Albino rats. Bull Environ Contam Toxicol 1993; 51: 795-802.

San96 Sanfeliu C, Rosa R, Suñol C, *et al.* Stimulation of phosphoinositide hydrolysis by - and -hexachlorocyclo-hexane in primary cultures of cerebellar granule cells: interaction with glutamate and carbachol receptor-mediated phosphoinositide response and effects of specific pharmacological agents. Pestic Biochem Physiol 1996; 55: 64-76.

Sch87 Schröter C, Parzefall W, Schröter H, *et al.* Dose-respons studies on the effects of -, -, and -hexachlorocyclohexane on putative preneoplastic foci, mono-oxigenases, and growth in rat liver. Cancer Res 1987; 47: 80-8.

Sei96 Seidler A, Hellenbrand W, Robra BP, *et al.* Possible environmental, occupational, and other etiologic factors for Parkinson's disease: a case-control study in Germany. Neurology 1996; 46: 1275-84.

Ser90 Serrano MT, Vendrell M, Rivera S, *et al.*. Effect of lindane on the myelination process in the rat. Neurotoxicol Teratol 1990; 12: 577-83.

Sha96 Sharma M, Bhatnagar P. Organochlorine pesticides and preterm labour in human beings. Curr Sci 1996; 628-31.

She88 Shehata-Karam H, Monteiro-Riviere NA, Guthrie FE. In vitro penetration of pesticides through human newborn foreskin. Toxicol Lett 1988; 40: 233-9.

Sin83 Sina JF, Bean CL, Dysart GR, *et al.* Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat Res 1983; 113: 357-91.

Sir89 Sircar S, Lahiri P. Lindane (-HCH) causes reproductive failure and fetotoxicity in mice. Toxicology 1989; 59: 171-7.

Slo88 Slooff W; Matthijsen AJCM, eds. Integrated Criteria Document Hexachloro-cyclohexanes. Bilthoven, The Netherlands: National Institute of Public Health and Environmental Protection, 1988; rep no 758473011.

Sri88 Srinivasan K, Ramesh HP, Radhakrishnamurty R. Changes induced by hexachlorocyclohexane isomers in rat liver and testis. Bull Environ Contam Toxicol 1988; 41: 531-9.

Sri91 Srinivasan K, Mahadevappa KL, Radhakrishnamurty R. Effect of maternal dietary hexachlorocyclohexane exposure on pup survival and growth in albino rats. J Environ Sci Health 1991; B26: 339-49.

Stu96 Stuurgroep Chemiekaarten, eds. Lindaan. In: Chemiekaarten: gegevens voor het veilig werken met chemicaliën. 12th ed. Alphen a/d Rijn, The Netherlands: Samson HD Tjeenk Willink bv, 1996: 695.

Suñ94 Suñol C, Pomes A, Rodriguez-Farre E. Primary neural cultures to study the $GABA_A$ receptor as a target for neurotoxic agents: different effects of stimulant/depressant compounds. Biomed Health Res 1994; 7: 223-34.

SZW96 Ministerie van Sociale Zaken en Werkgelegenheid (SZW): Arbeidsinspectie. De nationale MAC-lijst 1996. The Hague, The Netherlands: Sdu Servicecentrum Uitgeverijen, 1996: 36 (pub no P145).

Tho73 Thorpe E, Walker AIT. The toxicology of dieldrin (HEOD*). II. Comparative long-term oral toxicity studies in mice with dieldrin, DDT, phenobarbitone , -BHC and -BHC. Food Cosmet Toxicol 1973; 11: 433-42.

Tho90 Thompson RG, Menking DE, Valdes JJ. Comparison of lindane, bicyclophosphate and picrotoxin binding to the putative chloride channel sites in rat brain and *torpedo* electric organ. Neurotoxicol Teratol 1990; 12: 57-63.

Tie96 Tieman U, Schneider F, Tuchscherer A. Effects of organochlorine pesticides on DNA synthesis of cultured oviductal and uterine cells and on estrogen receptor of uterine tissue from heifers. Arch Toxicol 1996; 70: 490-6.

Til87 Tilson HA, Shaw S, McLamb RL. The effect of lindane, DDT, and chlordecone on avoidance responding and seizure activity. Toxicol Appl Pharmacol 1987; 88: 57-65.

Tus87 Tusell JM, Suñol C, Gelpí E, *et al.* Relationship between lindane concentration in blood and brain and convulsant response in rats after oral or intraperitoneal administration. Arch Toxicol 1987; 60: 432-37.

Uph87 Uphouse L. Decreased rodent sexual receptivity after lindane. Toxicol Lett 1987; 39: 7-14.

Uph89 Uphouse L, Williams J. Diestrous treatment with lindane disrupts the female rat reproductive cycle. Toxicol Lett 1989; 48: 21-8.

Ven91 Vendrell M, Zawia NH, Serratosa J, *et al. c-fos* and ornithine decarboxylase gene expression in brain as early markers of neurotoxocity. Brain Res 1991; 544: 291-6.

Ven92 Vendrell M, Tusell JM, Serratosa. c-*fos* Expression as a model for studying the action of hexachlorocyclohexane isomers in the CNS. J Neurochem 1992; 58: 862-9.

Ves83 Vesselinovitch SD, Carlborg FW. Lindane bioassay studies and human cancer risk assessment. Toxicol Pathol 1983; 11: 12-22.

Wei77 Weisse I, Herbst M. Carcinogenicity study of lindane in the mouse. Toxicology 1977; 7: 233-38.

WHO82 World Health Organization (WHO). Lindane. In: Recommended health-based limits in occupational exposure to pesticides. Geneva, Switzerland: WHO, 1982: 57-85 (Techn Rep Ser 677).

WHO91a World Health Organization (WHO): International Programme on Chemical Safety (IPCS). Lindane. Geneva, Switzerland: WHO, 1991; Environ Health Crit 124.

WHO91b World Health Organization (WHO): International Programme on Chemical Safety (IPCS). Lindane (Gamma-HCH). Geneva, Switzerland: WHO, 1991; Health and Safety Guide No. 24.

WHO93 World Health Organization (WHO). In: Guidelines for drinking-water quality. 2nd ed. Geneva, Switzerland: WHO, 1993: 85 (Recommendations; Vol 1).

Wic96 Wichmann N, Eistel B. -1,2,3,4,5,6-Hexachlorcyclohexan. In: Greim H, ed. Biologische Arbeitsstoff-Toleranz-Werte (BAT-Werte) und Expositionsäquivalente für krebserzeugende Arbeitssoffe (EKA). Arbeitsmedizinisch-toxikologische Begründungen. 1st-8th ed. Weinheim, Germany: VCH Verlagsgesellschaft mbH, 1996; Vol 1.

Wil95 Williams S. Changes in EH 40/95. Toxic Substances Bulletin 1995; issue no 27; April 1995.

Woo95 Woolley DE. Organochlorine insecticides: neurotoxicity and mechanisms of action. In: Chang LW, Dyer RS, *et al*. Neurological disease and therapy. Handbook of neurotoxicology. New York, USA: Marcel Dekker, 1995: 475-510.

Zis95 Zisterer DM , Suñol C, Moynagh PN, *et al*. PK 11195 reduces the brain availability of lindane in rats and the convulsions induced by this neurotoxic agent. Life Sc 1995; 57: 2359-64.

Zis96 Zisterer DM, Moynagh PN, Williams DC. Hexachlorocyclohexanes inhibit steroidogenesis in Y1 cells. Absence of correlation with binding to the peripheral-type benzodiazepine binding site. Biochem Pharmacol 1996; 51: 1303-8.

A Request for advice

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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, a '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

- GJ Mulder, *chairman*
  professor of toxicology; Leiden University, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- PJ Borm
  toxicologist; Heinrich Heine Universität Düsseldorf (Germany)
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- VJ Feron,
  professor of toxicology; TNO Nutrition and Food Research, Zeist
- DJJ Heederik
  epidemiologist; Wageningen University, Wageningen
- LCMP Hontelez, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- G de Jong
  occupational physician; Shell International Petroleum Maatschappij, The Hague
- TM Pal
  occupational physician; Netherlands Centre for Occupational Diseases Amsterdam
- IM Rietjens
  professor in Toxicology; Wageningen University, Wageningen.

- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague
- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- HG Verschuuren
  toxicologist; DOW Europe, Horgen (Switzerland)
- F de Wit
  occupational physician; Labour Inspectorate, Arnhem
- JL Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague

The first draft of the present advisory report was prepared by KJ van den Berg, K Mahieu and H Stouten, from the TNO Nutrition and Food Research Institute, Zeist, the Netherlands by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: T van der Klugt.
Lay-out: J van Kan.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2000 for public review. The following organisations and persons have commented on the draft document:

- A Aalto
  Occupational Safety and Health Division, Tampere, Finland
- RD Zumwalde,
  National Institute of Occupational Safety and Health, Cincinnati, USA.

Annex **D**

Experimental data

Table 1 Acute lethal toxicity data of g-HCH (from ATS94, WHO91a, unless otherwise noted).

species	route	dose/concentration	effect	ref
rat	inhalation	273, 603 mg/m^3/4h[a]	no deaths	
rat	inhalation	1600 mg/m^3/4h[b]	LC_{50}	
rat	oral	88-270 mg/kg	LD_{50}	
rat	oral	60 mg/kg	1/5 dead	
rat	ip	69 mg/kg	LD_{50}	
rat	dermal	900-1000 mg/kg[c]	LD_{50}	
mouse	oral	55-250 mg/kg	LD_{50}	Lah91, Kas93, WHO91a
mouse	ip	97, 101 mg/kg	LD_{50}	Kas93, WHO91a
mouse	im	152 mg/kg	LD_{50}	
rabbit	oral	90-200 mg/kg	LD_{50}	
rabbit	dermal	200-300 mg/kg	lethal	
guinea pig	oral	100 mg/kg	LD_{50}	
dog	oral	30 mg/kg	no deaths	
dog	oral	40 mg/kg	lethal	
dog	oral	50 mg/kg	4/7 dead	
dog	oral	100 mg/kg	3/3 dead	

[a] Observation time: 14 d; aerosol: average particle size: 0.4 µm

[b] Observation time: 22 d; aerosol: 50% or more of particles had diameter < 7µm (concentration is equivalent to a dose of ≈ 300 mg/kg, assuming a respiratory volume of 0.24 l/min, a bw of 300 g and a retention of 1)

[c] For females and males, resp; compound was not removed during observation time (= 14 d)

Table 2a Neurotoxic effects following single exposure to γ-HCH: convulsions and seizures.

species (sex)	exposure	NOAEL (mg/kg bw)	LOAEL (mg/kg bw)	relevant effect	ref
rat ♂♀	i.v., infusion		1.5	convulsions	Por88
rat ♂	oral		5	seizure behaviour	Gil95b
rat ♂	oral		60	seizures	Tus87
rat ♂	ip	20	40	increased excitability granule cells of hippocampus	WHO91a
rat ?	oral		60	seizures	ATS94
rat ♂♀	oral		30	convulsions	Bar95a
rat ♂	oral		60	convulsions	Ort95
rat ♂	oral		60	convulsions	Zis95
mice ♂	ip	40	80	enhancement of induced seizure activity	WHO91a

Table 2b Neurotoxic effects following single exposure to γ-HCH: neurobehavioural effects.

species (sex)	exposure	NOAEL (mg/kg bw)	LOAEL (mg/kg bw)	relevant effect	ref
rat ♂	oral	(1.8 [a])	10 1.8	increased motor activity	Llo89
rats ♂	oral		15	altered avoidance responses	Til87
rat ♂	oral		20	altered behaviour in plus-maze	Llo90
rat ♂	oral		20	altered behaviour in plus-maze	Llo91
rat ♂♀	oral		30	hypothermia reduced food intake reduced body weight	WHO91a
rat ♂♀	oral		40	hypothermia, anorexia	Gri89
mice ♂	ip	80		locomotor activity	WHO91a

[a] Extrapolated no effect level

Table 2c Neurotoxic effects following single exposure to γ-HCH: neurochemical effects.

species (sex)	exposure	NOAEL (mg/kg bw)	LOAEL (mg/kg bw)	relevant effect	ref
rat ♂	oral		5	*c-fos* proto-oncogene expression in brain	Ven92
rat ♂	oral		20	regional alterations in brain monoamine levels	Llo91
rat ?	oral		20	altered brain norepinephrine and serotonin levels	Riv91
rat ♂	oral		30	*c-fos* proto-oncogene expression in brain ornithine decarboxylase gene expression in brain	Ven91
rat ♂	oral		30	decreased brain calmodulin II gene expression	Bar95a
rat ♂	oral	30	40	altered brain dopamine and serotonine levels	Art88
rat ♂	oral		60	*c-fos* proto-oncogene expression	Bar95c
rat ♂♀	oral		60	decreased dopamine levels	Ort95
rat ♂	ip	60		brain lipid peroxidation	Ari94
			60	brain GSH levels	
mice ♂	ip		80	increased c-GMP accumulation in cerebellum	WHO91a
rat ♂	oral		150	altered brain dopamine and GABA levels	WHO91a
rat ♂	oral	30	150	altered regional cerebral glucose uptake	WHO91a

Table 3 Neurotoxic effects following repeated exposure to g-HCH.

species (sex)	exposure (duration)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	relevant effect	ref
rat ♀	oral (40 d) 2.5, 5, 10, 50 mg/kg bw	2.5	2.5 5	altered operant conditioning behaviour (Skinner box) altered maze behaviour	Des74
rat ♂	oral (4 d); 1, 3, 10 mg/kg bw	1 3	3 10	increased kindling acquisition seizures	Joy82
rat ♂	oral (5d); 5 mg/kg bw		5	Increased kindling acquisition seizures.	Joy83
rat ♀	oral (3 mo); 2.5, 5 mg/kg bw	2.5	5	EEG patterns neurobehaviour: locomotor activity, learning	Des83
rat ?	oral (3 d)		5	decreased myelin	Ser90
rat ♂	oral (6 d)		3	increased pineal N-acetyl-transferase decreased serotonin and metabolite levels	Att91
rat ♂	ip (7 d)		10	altered regional brain glucose uptake	WHO91a
rat ♂	oral (12 d)	5	12	convulsions	WHO91a
rat ♂	oral (30 d)	12.3	25.4	decreased nerve conduction velocity	WHO91a
rat ♂	diet (90 d)	1 g/kg diet (50 mg/kg bw/d)		brain lipid peroxidation	Ari94
rat ♂♀	oral (7d)		10	increased motor activity increased neurmotor reflexes	ATS94
rat ♀	oral (3 mo)		5	decreased level of phosphoinositide-derived second messengers in fore brain	Agr95
rat ♂♀	oral (10x, 30 d)	6	6	decreased brain dopamine levels convulsions	Ort95
rat ♂	oral (intermittent, repeated 30d - 10w)		10	behavioural seizure development EEG alterations	Gil95a

Table 4 Carcinogenicity studies with g-HCH: oral (diet) experiments (data from ATS94, IARC79, Slo88, WHO91a, unless otherwise noted).

authors (ref)	species/strain	exposure	findings	remarks
Fitzhugh *et al*, 1950	rat (Wistar; ♂♀ ; n=10/ sex/dose; controls: n=40)	0, 5, 10, 50, 100, 400, 800, 1600 mg/kg diet in corn oil (≈ 0.2-72 mg/kg bw/d), until death/sacrifice; or 0, 10, 100, 800 mg/kg diet, powdered (≈ 0.5-36 mg/kg bw/d) until death/sacrifice	no treatment-related increase in tumour incidence general toxicity: at doses ≥ 400 mg/kg diet (except 800 mg/kg diet powdered γ-HCH group), dose-dependently shortened lifespan of 20-40% ; at doses ≥ 100 mg/kg, increased relative liver weight, hepatocellular hypertrophy, fatty degeneration, necrosis, and granular degeneration and calcification in kidneys of male rats (these findings being more pronounced in the higher dose groups); at doses 800 mg/kg nervous system symptoms and convulsions	only few animals microscopically examined, with detailed sectioning in even less animals; protocol did not include haematology, blood chemistry, urinalysis mean lifetime: experimental groups (including those receiving other isomers and technical HCH): 33-70 w; controls: 58 w 50 mg/kg diet concluded to be the NOAEL (WHO91a)
Truhaut, 1954	rat (strain not reported; ♂♀ ; n=10/sex/ dose)	0, 25, 50, 100 mg/kg diet (≈ 11, 22.5, 45, mg/kg bw/d), until death/sacrifice	no formation of tumours found at doses ≥ 50 mg/kg diet, histological changes of the liver (hypertrophy, fatty degeneration)	no details given
Ito *et al* (Ito75)	rat (Wistar; ♂; n=18-24/ group)	0, 500 mg/kg diet (≈ 20 mg/kg bw/d), for 24 or 48 w	no tumours found high mortality (survivors); some increase in liver weight.	duration of study is short; size of experimental and control groups is small; only one dose tested
NCI, 1977	rat (Osborne-Mendel; ♂♀; n=50/sex/dose, controls: n= 10/sex, for statistical evaluation combined with 45 untreated animals/sex	♂:0, 320, 640 mg/kg diet, for 38 w, then 160, 320 mg/kg, for 42 w (TWA: 236, 472 mg/kg; i.e., ≈ 9.4, 18.9 mg/kg bw/d); ♀:0, 320, 640 mg/kg, for 2 w, 160, 320 mg/kg, for 49 w, then 80, 160 mg/kg, for 29 w (TWA: 135, 270 mg/kg; i.e., ≈ 6.8, 13.5 mg/kg bw/d)	no treatment-related increase in tumour incidence	time of sacrifice: 108-110 w doses lowered because of high mortality poor survival rates in all experimental groups (♂ : 50%, 48% vs 60%; ♀ : 60%, 60% vs 40%) IARC79 noted the small number of contemporary controls and the lack of adequate evidence that the dose given to male rats corresponded to the maximum tolerated dose
Goto *et al*, 1972	mice (ICR-JCL;♂; n=20/group)	0, 300, 600 mg/kg diet (≈ 36, 72 mg/kg bw/d), for 26 w	high dose: liver nodules or benign liver tumours in 5/10 animals; increased liver weight	IARC noted that this study was in-adequately reported
Hanada *et al* (Han73)	mice (dd; ♂♀ ; n=10-11/ sex/group; con-trols: 21♂, 20 ♀)	0, 100 , 600 mg/kg diet (♂: ≈ 12, 36, 72; ♀: ≈ 13, 39, 78 mg/kg bw/d), for 32 w	tumour incidence: hepatomas: ♂: 0/9, 3/4 vs 0/10; ♀: 0/7, 1/3 vs 0/8; most animals of two higher dose groups had atopical proliferations in the liver.	time of sacrifice: w 37-38 duration of the study is short; size of experimental groups is small
Ito *et al* (Ito73)	mice (dd; ♂; n=20/group)	0, 100, 250, 500 mg/kg diet (≈ 12, 30, 60 mg/kg bw/d), for 24 w	no treatment-related increase in tumour incidence general toxicity: no effect on final bw; increased liver weight in high dose group.	time of sacrifice: 24 w duration of experiment is short

Table 4 Continued.

authors (ref)	species/strain	exposure	findings	remarks
Herbst *et al* (Her75); Weisse/ Herbst (Wei77)	mice (Chbi: NMRI(SPF); ♂♀ ; n=50/ sex/group; controls: n= 100/sex)	0, 12.5, 25, 50 mg/kg diet (♂: ≈ 2.1, 4.1, 8.2; ♀: ≈2.0, 3.9, 7.8 mg/kg bw/d), for 80 w	no treatment-related increase in tumour incidence general toxicity: no treatment-related effect on mortality, body weight, feed consumption, development	time of sacrifice: 80 w doses administered are low and did obviously not induce any kind of toxicity
NCI, 1977 (see also Ves83)	mice (B6C3F1 hybrid; ♂♀ ; n=50/sex/ group (controls: n=10/sex, for statistical evaluation combined with 40 untreated animals/sex)	0, 80, 160 mg/kg diet (♂ : ≈9.6, 19.2, ♀ : ≈ 10.4, 20.8 mg/kg bw/d), for 80 w	hepatocellular carcinomas: ♂: 19/49 (p=0.001), 9/46 vs 5/49; ♀: 2/47, 3/46 vs 2/47; neoplastic hepatic nodules in absence of carcinoma: ♂; 0/49, 1/46 vs 3/49; ♀ : 2/47, 0/47 vs 1/47 reevaluation by Vesselenovitch/Carlborg (Ves83): hepatocellular carcinomas + adenomas: ♂: 16/50 (9/50 + 7/50), 7/46 (2/46 + 5/46) vs 2/10 (matched controls; 1/10 + 1/10), ♀: 1/49 (0/49 + 1/49), 3/48 (1/48 + 2/48) vs 0/10	time of sacrifice: 90-91 w IARC79 noted the relatively low dose and the small number of controls; in view of LD_{50} of 65 mg/kg for B6C3F1 mice dosing was considered to be adequate (Ves83)
Thorpe/Walker (Tho73)	mice (CF1; ♂♀; n=30/ sex/group; controls: 45/ sex	0, 400 mg/kg diet (♂: ≈48, ♀ : ≈52 mg/kg bw/d), for 110 w	benign and malignant liver tumours: ♂: 93% (27/29) vs 24% (11/45), ♀: 69% (20/29) vs 23% (10/44) reevaluation by Vesselenovitch/ Carlborg (Ves83): liver lesions: ♂: carcinomas/ adenomas/hyperplastic nodules: 3/28 (p<0.16), 11/28 (p<0.003), 12/28 (p<0.005) vs 1/45, 0/45, 1/45, ♀: 4/28 (p<0.08), 2/28, 8/28 (p<0.11) vs 1/44, 1/44, 6/44 general toxicity: significant mortality (♂: 10%, ♀+20%) in first 3 mo; no signs in survivors; enlarged livers	

To calculate oral doses in mg/kg bw/d from mg/kg diet, the following default values (or their means) are used throughout this report (unless other data are presented or available in the original papers/reports):
rat ♂: bw: 500 g, daily food intake: 20 g; ♀: bw: 350 g, daily food intake: 17.5 g; mouse:♂ : bw: 30 g, daily food intake: 3.6 g, ♀ : 25 g, daily food intake: 3.25 g).

Table 5a In vitro genotoxicity of γ-HCH: prokaryotic and non-mammalian eukaryotic test systems (data from ATS93, Jan88, FAO89, WHO91a, unless otherwise stated).

species	strain	end points	result[a]	test conditions	ref
prokaryotic systems (bacteria)					
Salmonella typhimurium	various strains: TA98, TA100, TA1535, TA1537, TA1538, TA1950, TA1978, G46, C3076, D3052	frame-shift mutation, base-pair substitution	-/-	up to 5000 µg/plate or concentration gradient (various separately performed tests)	
	TA98	frame-shift mutation	(see remark)	50, 100 µg/plate *remark: although Gopalaswamy/Nair reported a weakly positive result, this study cannot be evaluated since a positive and a vehicle control group were not included*	Gop92
Escherichia coli	various strains: WP2 *try*$^-$, WP2,WP2 *uvr* A, WP2 *hcr*	gene mutation DNA damage	-/-	up to 5000 µg/plate or concentration gradient	
Bacillus subtilis	H17 *rec*$^+$, M45 *rec*$^-$	DNA damage	-/nt	0.02 ml of solution of 1 mg/ml in DMSO	
host-mediated assay					
Salmonella typhimurium	TA1535	frame-shift mutation	+ (weakly)	host: mouse (NMRI; ♂); route: ip; doses: 0.5, 5, 50 mg/kg bw (frequency not reported)	
Salmonella typhimurium	G46	gene mutation	-	host: mouse (NMRI); route: sc; dose: 25 mg/kg bw (frequency not reported)	
Serratia marescens	a 21. leu$^-$	gene mutation	-	host: mouse (NMRI); route: sc; dose: 25 mg/kg bw (frequency not reported)	
non-mammalian eukaryotic systems (yeast, algae, plant cells)					
Saccharomyces cerevisiae		gene mutation	-/-	not reported	
Nostoc muscorum		gene mutation	-/nt	not reported	
Allium cepa		mitotic disturbances	+/nt	not reported	
			+/nt	not reported	
		chromosome aberrations	+/nt	0.4-4.0 mg/l *remark: technical grade formulation of γ-HCH-20% has been tested; purity not reported*	Kum95
		mitotic index			Kum95
		chromosome clastogenicity	+/nt	4-14 mg/l *remark: see above*	

[a] Results are from experiments without and with metabolic activation, respectively; nt = not tested

Table 5b Drosophila melanogaster assays with γ-HCH (data from Jan88; WHO91a, unless otherwise stated).

result	end point	test conditions	ref
-	sex-linked recessive lethal mutations	0.001% (aqueous solution) injected into abdomen	
+	sex-linked recessive lethal mutations	0, 1-10 µg/l; administration via food-medium; technical grade formulation of γ-HCH-20% has been tested; purity: not reported; parameter: frequency of sex-chromosome-linked recessive lethal mutations in the M_1 generation of γ-HCH-fed males	Kum95
-	dominant lethal mutations	20 mg/kg food-medium; 6-24-h aged males and females (n=25/sex) were transferred to γ-HCH-containing food-medium, and their progeny were raised on this food; males and females (n=5sex) of F_1 generation were raised on normal food-medium and were allowed to mate with each other and layed eggs. From this generation of flies, three successive mutation-generations were raised on normal medium, and numbers of larvae hatched from eggs laid on each of the first 10 days after enclosion were recorded. Result: significantly decreased percentage of larvae hatched from the total number of eggs laid (cumulated over the entire period) in the 2nd and 3rd generation. Considered to be suggestive of being mutagenic by WHO and to be negative by RIVM (Jan88).	
+	dominant lethal mutations	0, 10-30 µg/l; administration via food-medium; technical grade formulation of γ-HCH-20% has been tested; purity: not reported; parameter: cumulative frequency of larvae hatched from the eggs laid on d 4-6 (post-enclosion) by untreated females mated with males raised on γ-HCH-containing food-medium	Kum95

Table 5c In vitro genotoxicity of γ-HCH: mammalian cell test systems (data from ATS93, Jan88, FAO89, WHO91a, unless otherwise stated).

species	cell line	end points	result[a]	test conditions	ref
Chinese hamster	V79	gene mutation	-/-	0-250 µg/ml; 0-500 µg/ml (both aerobic and anaerobic); plate assay 102 µg/ml	
	V79	gene mutation	-/nt		
	ovary	gene mutation	-/-	0-87 mg/ml (in DMSO)	Poo93
human	peripheral lymphocytes	chromosome aberrations	(see remark)	0-10 µg/ml *remark: chromosome breakage at toxic doses only; WHO considered this study to be inadequate; protocol did not comply with international standards*	
Chinese hamster	fibroblasts	chromosome aberrations	(see remark)	0-2.1 mg/ml (in ethanol) *remark: only percentage of cells with chromosomal aberrations presented; based on percentage found, i.e., 5%, result was considered to be suspicious by Ishidate/Odashima; in view of the cut-off point between "negative" and "suspicious"(4.9%) and the fact that gaps were probably included (which does not comply with OECD guidelines) this study can concluded to be negative*	Ish77
human	fibroblasts SV-40	UDS	-/-	0.29, 290 µg/ml (in aceton)	
rat	primary hepatocytes	UDS	-/nt	29 µg/ml (in DMSO); plate assay	
mouse (B6C3F;♂)	hepatocytes	replicative DNA synthesis	-/nt	not reported	Miy95
Chinese hamster	ovary cells	SCE	-/-	0-87mg/ml (in DMSO)	Poo93
human	peripheral lymphocytes	DNA damage (single strand breaks (SSB))	-/nt	0, 290 µg/tube (in DMSO); comet assay (microgelelectrophoresis); parameters: medium distance of DNA migration in µm, calculated value of medium migration distances in test minus control (T-C) (positive response defined as increase in proportion of damaged cells (migration distance >40 µm) or as a T-C value> 10 µm)	Poo93
human	primary gastric mucosa cells	DNA damage (SSB)	-/nt	0, 145, 290 µg/tube (in DMSO); comet assay (see above)	Poo94
human	primary nasal mucosa cells	DNA damage (SSB)	+/nt	0, 72, 145 µg/tube (in DMSO); comet assay (see above)	Poo94
rat	primary hepatocytes	DNA damage (SSB)	-/nr	0-290 µg/tube (in DMSO); alkaline elution assay; parameter: % DNA retained on filter in the control group minus %DNA retained in treated groups at non-toxic doses (cut-off point for negative/positive effect: 20%)	Poo93

Table 5c Continued.

species	cell line	end points	result[a]	test conditions	ref
rat	primary hepatocytes	DNA damage (SSB)	+/nr	0, 8.7, 87 µg/ml; alkaline elution assay; parameter: elution rate: increase 3.1- to 5.0-fold control and >7.1-fold control, resp (cut-off point for negative/positive result: 3-fold control value)	Sin83
rat	primary gastric mucosa cells	DNA damage (SSB)	+/nt (see remark)	0-290 µg/tube (in DMSO); comet assay (see above); *remark: results were rather variable*	Poo93; Poo94
rat	primary nasal mucosa cells	DNA damage (SSB)	+/nt	0-290 µg/tube; 0-145 µg/ml (in DMSO); comet assay (see above)	Poo93, Poo94

[a] Results are from experiments without and with metabolic activation, respectively; nt = not tested; nr=not relevant

Table 6a In vivo genotoxicity of γ-HCH: mammalian germ cell assays (data from ATS93, Jan88, FAO89, WHO91a, unless otherwise stated).

species	strain	result	test conditions	ref
dominant lethal assay				
rat ♂	Chbb=THOM	-	route: oral; doses: repeated: 0, 1.5, 7.0, 15 mg/kg/bw/d, for 8 w (continuously during whole mating period) vehicle: olive oil	
rat ♂	Wistar	see remark	route: not reported; doses: 0, 1.5, 7, 15 mg/kg bw/d; dose regimen not reported vehicle: olive oil *remark: WHO considered this study to be indequate; protocol did not comply with international standards*	
mouse ♂	ICR/Ha Swiss	-	route: oral; doses: repeated: 0, 15 mg/kg bw/d, 5 x no details presented; γ-HCH listed among agents producing early fetal deaths and/or preimplantation losses beyond control limits but with differences not significant by analysis of variance	Eps72
mouse ♂	ICR/Ha Swiss	-	route: ip; doses: single : 0, 15, 75, 200, 1000 mg/kg bw no details presented	Eps72
mouse ♂	NMRI-EMD	-	route: ip; doses: single: 0, 12.5, 25, 50 mg/kg bw	

Table 6b In vivo genotoxicity of γ-HCH: mammalian somatic cell assays (data from ATS93, Jan88, FAO89, WHO91a, unless otherwise stated).

species	strain	result	test conditions, comments	ref
chromosome aberrations in bone marrow				
rat	not reported	-	route: oral; doses: repeated: 1.5, 7.0, 15 mg/kg bw/d for 12 w sex not reported; no details presented in review	
mouse	Swiss albino	see remark	route: oral (gavage); doses: repeated: 0, 0.2-2.4 mg/kg bw/d for 7 d; n=15/group; 20 metaphases/animal/dose were scored for cytological abnormalities, i.e., chromosome/chromatid breaks and gaps with or without the accompanying acentric fragments; stat sign ($p<0.01$) increases in frequency of chromosomal abnormalities at doses of 1.4 mg/kg bw/d and higher *remark: technical grade formulation of γ-HCH-20% has been tested; purity not reported; test can not be evaluated since only number and frequency of metaphases with abnormalities were reported.; type and number of aberrations should be given and gaps should not be included in the total aberration frequency (OECD guidelines)*	Kum95
hamster	Chinese	-	route: oral; doses: repeated: 0, 0.125, 1.25, 12.5 mg/kg bw/d for 5 d sex not reported; no details presented in review at the highest dose level there was an increase in chromosomal gaps	
sister chromatid exchange in bone marrow				
mouse ♂♀	CF1	-	route: oral; doses: single: ♂ : 0, 2, 10, 50 mg/kg bw, ♀ : 1.6, 8, 40 mg/kg bw no details presented in reviews	
mouse ♂♀	CF1	-	route: ip; doses: single: 0, 1.3, 6.4, 32.1 mg/kg bw no details presented in review	
hamster	Chinese	-	route: oral (gavage); doses: single: 0, 120 mg/kg bw (i.e., ≈ 1/3 LD_{50}); sex not reported; n=6/dose/time point; sacrifice time: 24, 30 h; 50 metaphases/animal/time point were scored; positive control group included	Poo93
micronuclei in bone marrow				
rat	Sprague-Dawley	-	route: oral (gavage); doses: single: 0, 15, 30, 60 mg/kg bw (i.e., high dose=0.8 x LD_{50}); sex not reported; n=6/dose/time point; sacrifice time: 24, 30, 36, 48 h; 1000 polychromatic erythrocytes/animal were scored for micronuclei (1000 normochromatic erythrocytes for control purposes)	Poo93
mouse ♂	CBA	-	route: not reported; doses: 0, 75 mg/kg bw; no details presented in review	
mouse	NMRI	-	route: oral (gavage); doses: single: 0, 35, 50, 70 mg/kg bw (i.e., high dose=0.8 x LD_{50}); see further above, the rat experiment	Poo93

Table 6c γ-HCH: assays for primary DNA damage in somatic cells *in vivo* (data from ATS93, Jan88, FAO89, WHO91a, unless otherwise stated).

species	strain	result	test conditions	ref
rat	Sprague-Dawley	+ (see remark)	route: inhalation; concentrations: 0.3, 3 mg/m^3 for 1 h (vapour and aerosol particels); n=6/group; sacrifice time: not indicated; parameter: DNA single strand breaks (SSB) in nasal mucosa cells: comet assay (see Table 5c) *remark: no data on viability of cells given; a great decrease may lead to a positive result*	Poo93
rat	Sprague-Dawley	-	route: oral (gavage); doses: single: 0, 30, 60 mg/kg bw; n=3/group; sacrifice time: 1 h; parameter: DNA SSB in liver cells; comet assay (see Table 5c)	Poo93
rat	Sprague-Dawley	-	route: oral (gavage); doses: single: 60 mg/kg bw; sacrifice time: 1 h; parameters: DNA SSB in gastric cells and peripheral lymphocytes; comet assay (see Table 5c)	Poo93
rat	Sprague-Dawley	+ (see remark)	route: oral (gavage); doses: single: 60 mg/kg bw; sacrifice time: 16 h; parameter: DNA SSB in colon cells; comet assay (see Table 5c) *remark: γ-HCH treatment strongly affected food consumption inducing reduced weight gain; it is unclear to which extent this unwanted fasting may have contributed to the DNA-damaging effects*	Poo93
rat ♀	Sprague-Dawley	- (see remark)	route: oral; doses: single: 0, 30 mg/kg bw (in corn oil); n=4/group/time point; sacrifice time: 0, 6, 12, 24 h; parameter: DNA SSB in liver cells: elution rate constant calculated from the slope of the plot of % DNA remaining on the filter vs volume of elution fraction (alkaline elution assay); result: increase in elution rate constant of 2.2-fold (stat sign), 1.8-fold (stat sign), 1.3-fold control at t=6h, t=12h, t=24h, resp *remark: applying the criteria of Sina et al (see above, in vitro tests: Sin83), this result is considered to be negative*	Has93
mouse ♂	NMRI, CF1, C3B6F1	(-)[a]	route: oral (gavage); doses: single: 0, 8.7-23 mg/kg bw; n=2/group; sacrifice time: 10 h; parameter: covalent binding to liver DNA very low level of binding: covalent binding index is factor of 10^5-10^6 below that of strong DNA-binding carcinogens	Sag83
mouse ♂	HPB	(-)[a]	route: ip; doses: single: 25 mg/kg bw (in corn oil); n=10 group; sacrifice time: 24 h; an additional phenobarbital-pretreated group included; parameter: covalent binding to liver DNA low level of binding when compared to that of known hepatocarcinogens	Ive84

[a] Weakly positive result

Table 7 Studies on the mechanisms of the neurotoxic action of γ-HCH.

effect	species	tissue	effective Dose	ref
inhibition of GABA receptor binding	rat	brain membranes	IC_{50}=0.15-0.25 µM	Llo90
	rat	brain membranes	IC_{50}=0.46 µM	Tho90
	mouse	cortical neuron cells	IC_{50}=0.19 µM	Pom94a
	mouse	brain membranes	IC_{50}= 4.6 µM	Fis87
	torpedo	electric organ membranes	IC_{50}=0.45 µM	Tho90
	torpedo	electric organ membranes	K_i= 0.40 µM	Mat88
inhibition of GABA-induced Cl^- current	rat	hippocampal neurons	IC_{50}=50 µM	Nar92
	rat	dorsal root ganglia cells	1 µM	Nag95a
	rat	dorsal root ganglia cells	EC_{50}=0.24 µM	Nag95b
inhibition of GABA-mediated Cl^- uptake	rat	brain synaptosomes neuronal membranes		WH091a
	mouse	brain membranes	300 µM	Fis88
	mouse	cortical neuron cells	50 µM	Pom94b
	mouse	cortical neuron cells	10 µM	Suñ94
loss of $GABA_A$-mediated recurrent collateral inhibition	rat	hippocampal slice	25 µM	Joy95
GABA-dependent inhibition of cytotoxicity	mouse	cortical neuron cells	250 µM	Pom94a
inhibition of GABA-mediated contraction	guinea pig	intestinal segments	10 µM	Coc93
increased uptake of Ca^{2+}	rat	brain synaptosomes	0.10 µM	Haw89
	rat	neuroblastoma cells		WHO91a
	rat	neurohybridoma cells	10-400 µM	Joy88
	rat	neuromuscular junction		WHO91a
	rat	hippocampal neurons	50 µM	Fer95
mitochondrial damage	frog	skeletal muscle		WHO91a
inhibition of Na^+/K^+-ATPase	frog	skeletal muscle		WHO91a
inhibition of Ca^{2+}/Mg^{2+} ATPase	bovine	brain		WHO91a
inhibition of glutamine synthetase	chicken	embryonic brain cells	100 µM	Rei95
decreased cell protein	chicken	embryonic brain cells	1000 µM	Rei95
inhibition neurite development	rat	hippocampal neurons	25 µM	Fer95
increased *c-fos* gene expression	rat	cortical brain cells	5 µM	Bar95b
increased phosphoinositide hydrolysis	rat	brain cortex slices	100 µM	Hoy93
increased phosphoinositide hydrolysis	rat	cerebellar granule cells	EC_{50}=100 µM	San96
inhibition glucose uptake	rat	brain cortex cells	300 µM	Pul90
cytotoxicity	rat	cerebellar granule cells	50-200 µM	Ros96

Annex **E**

Abbreviations

bp	boiling point
EC_{50}	concentration at which a described effect is found in 50% of the exposed animals or at which the effect is decreased up to 50% of the control value
HBR-OEL	health based recommended occupational exposure limit
h	hour
IC_{50}	concentration at wAbbreviationshich inhibition of a certain function is found up to 50% of the control value
LC_{50}	lethal concentration for 50% of the exposed animals
LC_{lo}	lowest lethal concentration
LD_{50}	lethal dose for 50% of the exposed animals
LD_{lo}	lowest lethal dose
LOAEL	lowest observed adverse effect level
MAC	maximaal aanvaarde concentratie (maximal accepted concentration)
MAEL	minimal adverse effect level
MAK	Maximale Arbeitsplatz Konzentration
MOAEL	minimal observed adverse effect level
MTD	maximum tolerated dose
NAEL	no adverse effect level
NEL	no effect level
NOAEL	no observed adverse effect level
OEL	occupational exposure limit
PEL	permissible exposure limit
ppb	parts per billion $(v/v)10^{-9}$
ppm	parts per million $(v/v)10^{-6}$
RD_{50}	concentration at which a 50% decrease of respiratory rate is observed
REL	recommended exposure limit

STEL	short term exposure limit
tgg	tijd gewogen gemiddelde
TLV	threshold limit value
TWA	time weighted average
V_{max}	maximal reaction velocity of an enzyme

Organisations

ACGIH	American Conference of Governmental Industrial Hygienists
CEC	Commission of the European Communities
DECOS	Dutch Expert Committee on Occupational Standards
DFG	Deutsche Forschungsgemeinschaft
EPA	Environmental Protection Agency (USA)
FDA	Food and Drug Administration (USA)
HSE	Health and Safety Executive (UK)
IARC	International Agency for Research on Cancer (WHO)
INRS	Institut National de Recherche et de Sécurité (France)
NIOSH	National Institute for Occupational Safety and Health (USA)
NTP	National Toxicology Programme (USA)
OECD	Organisation for Economic Cooperation and Development
OSHA	Occupational Safety and Health Association (USA)
RTECS	Registry of Toxic Effects of Chemical Substances
SER	Social and Economic Council (Sociaal-Economische Raad NL)
WATCH	Working Group on the Assessment of Toxic Chemicals (UK)
WHO	World Health Organisation

Toxicological terms

bid	*bis in diem* (twice per day)
bw	body weight
CARA	chronic non-specific respiratory diseases
CHD	coronary heart disease
CNS	central nervous system
ECG	electrocardiogram
EEG	electro encephalogram
FCA	Freunds Complete Adjuvans
FEV	forced expiratory volume
FSH	follicle stimulating hormone
GD	gestation day(s)
GPMT	guinea pig maximisation test
GSH	glutathione
HLiA	hamster liver activated
IHD	ischaemic heart disease
im	intramuscular
ip	intraperitoneal
ipl	intrapleural
it	intratracheal
iv	intravenous
LH	lutheinising hormone
MAC	minimal alveolar concentration

MFO	mixed function oxidase
NA	not activated
PNS	peripheral nervous system
po	*per os* (= oral)
RBC	red blood cells
RLiA	rat liver activated
SCE	sister chromatid exchange
sc	subcutaneous
UDS	unscheduled DNA-synthesis

Statistical terms

GM	geometric mean
OR	Odds Ratio
RR	relative risk
SD	standard deviation
SEM	standard error of mean
SMR	standard mortality ratio

Analytical methods

AAS	atomic absorption spectroscopy
BEEL	biological equivalent exposure limit
BEI	biological exposure index
BEM	biological effect monitoring
BM	biological monitoring
ECD	electron capture detector
EM	environmental monitoring
FID	flame ionisation detector
GC	gas chromatography
GLC	gas liquid chromatography
GSC	gas solid chromatography
HPLC	high performance liquid chromatography
IR	infrared
MS	mass spectrometry
NMR	nuclear magnetic resonance
PAS	personal air sampling
TLC	thin layer chromatography
UV	ultraviolet

Annex **F**

DECOS-documents

DECOS has produced documents on the following substances.

To be ordered from the Health Council of the Netherlands:

Acetone cyanohydrin	1995/05WGD
p-Aramid fibres	1997/07WGD
Azathioprine	1999/04OSH
Aziridine (ethyl imine)	2000/13OSH
1,2,3-Benzotriazole	2000/14OSH
Bisphenol A and its diglycidylether	1996/02WGD
Bromoethane	1998/10WGD
1,2-and t-Butanol	1994/10
β-Butyrolactone	1999/05OSH
Cadmium and inorganic cadmium compounds	1995/04WGD
Calculating cancer risk	1995/06WGD
Carbadox	1999/06OSH
Carbon disulphide	1994/08
Chlorine dioxide	1995/07WGD
p-Chloroaniline	1998/09WGD
4-Chloro-o-toluidine	1998/08WGD
Chromium and its inorganic compounds	1998/01WGD
Cresols	1998/15WGD
Copper sulphate	1999/01OSH
1996-1997 WGD-rapporten/1996-1997 DECOS reports	1999/01WGD
1,2-Dibromoethane	1999/07OSH
1,2-Dichloroethane	1997/01WGD
Diethylsulphate	1999/08/OSH

Diglycidyl resorcinol ether	1999/09OSH
Diphenylamine	1997/05WGD
Endotoxins	1998/03WGD
Epichlorohydrin (1-Chloro-2,3-epoxypropane)	2000/10OSH
1,2-Epoxybutane	1998/11WGD
1,2-Ethanediamine	1996/03WGD
Ethyleneglycol ethers	1996/01WGD
Ethylene thiourea	1999/03OSH
Formamide and dimethylformamide	1995/08WGD
Hydrazinoethanol, phenylhydrazine, isoniazid, maleic hydrazide	1997/03WGD
Isopropyl acetate	1997/04WGD
Man made mineral fibers	1995/02WGD
2-Meathylaziridine (propylene imine)	1999/10OSH
Methyl Methacrylate	1994/09
Methacrylates. Ethyl methacrylate, n-butyl methacrylate and isobutyl methacrylate	1994/11
Methyl-t-butylether	1994/23
Methyl chloride	1995/01WGD
4,4'-Methylene bis (2-Chloroaniline)	2000/09OSH
4,4'-Methylene dianiline	2000/11OSH
Metronidazole	1999/11OSH
2-Nitropropane	1999/13OSH
N-Nitrosodimethylamine (NDMA)	1999/12OSH
2-Nitrotoluene	1998/12WGD
Pentaerythritol	1997/06WGD
Phenol	1996/04WGD
o-Phenylenediamine	1998/06WGD
Piperidine	1997/08WGD
Procarbazine hydrochloride	1999/14OSH
1- and 2-Propanol	1994/24
Propylene oxide	1997/02WGD
Ronidazole	1998/05WGD
Styrene	1998/07WGD
Quartz	1998/02WGD
1,1,1-Thrichloroethane	1995/03WGD
1,2,3-Trichloropropane	1994/25
1,2,3-Trichloropropane	1998/14WGD
Urethane (ethyl carbamate)	200012OSH
Vinylbromide	1999/15OSH
Wood dust	1998/13WGD