Hexachlorocyclopentadiene

(CAS No: 77-47-4)

Health-based Reassessment of Administrative Occupational Exposure Limits

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

No. 2000/15OSH/081, The Hague, 22 October 2003

Preferred citation:
Health Council of the Netherlands: Committee on Updating of Occupational Exposure Limits. Hexachlorocyclopentadiene; Health-based Reassessment of Administrative Occupational Exposure Limits. The Hague: Health Council of the Netherlands, 2003; 2000/15OSH/081.

all rights reserved

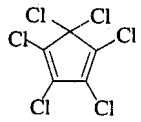
1 Introduction

The present document contains the assessment of the health hazard of hexachlorocyclopentadiene (HEX) by the Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was prepared by MA Maclaine Pont, M.Sc. (Wageningen University and Research Centre, Wageningen, the Netherlands).

The evaluation of he toxicity of HEX has been based on reviews published by the American Conference of Governmental Hygienists (ACG99), Agency for Toxic Substances and Disease Registry (ATSDR) (ATS99), the Advisory Committee on Existing Chemicals of Environmental Relevance of the German Chemical Society (BUA88), the German MAK-committee (Gre01), and the World Health Organization (International Programme on Chemical Safety) (WHO91). Where relevant, the original publications were reviewed and evaluated as will be indicated in the text. In addition, in August 2000, literature was searched in the databases Toxline, Medline, and Chemical Abstracts covering the period of 1989 until August 2000, and using the following key words: hexachlorocyclopentadiene, perchlorocyclopentadiene, and 77-47-4. The final search was carried out in Toxline and Medline in January 2003.

In April 2003, the President of the Health Council released a draft of the document for public review. Comments were received by the following individuals and organisations: A Aalto (Ministry of Social Affairs and Health, Tampere, Finland). These comments were taken into account in deciding on the final version of the document.

2 Identity

name	:	hexachlorocyclopentadiene
synonyms	:	1,2,3,4,5,5'-hexachloro-1,3-cyclopentadiene; perchlorocyclopentadiene; hexachloro-1,3-cyclopentadiene; 1,2,3,3,4,5-hexachloro-1,4-cyclopentadiene; HCCP
molecular formula	:	C_5Cl_6
structural formula	:	
CAS number	:	77-47-4

Data from BUA88, WHO91.

3 Physical and chemical properties

molecular weight	:	272.77
boiling point	:	239°C
melting point	:	-9°C to -11°C
flash point	:	not available
vapour pressure	:	at 25°C: 10 Pa
solubility in water	:	insoluble (at 22°C: 0.1 mg/100 mL)
log $P_{octanol/water}$	:	5.04-5.51 (experimental); 4.30-4.63 (estimated)
conversion factors	:	at 20°C and 101.3 kPa: 1 ppm = 11.4 mg/m^3 1 mg/m^3 = 0.09 ppm

Data from: BUA88, WHO91, http://esc.syrres.com.

HEX is a faint yellow to greenish-yellow, non-flammable liquid with a pungent odour (ACG99, BUA88). HEX is a highly reactive compound that readily undergoes addition and substitution reactions, for example in Diels-Alder reactions (BUA88, WHO91). The compound dissociates in a flame or when in contact with a hot surface, forming toxic and corrosive vapours (phosgene, hydrochloric acid and chlorine gas). In contact with air, the compound forms corrosive fumes that can spread over the floor (Che99). Odour thresholds of

0.002 (0.17 ppb) (WHO91), 0.34 (0.03 ppm) (Amo83), and 1.5-3.3 mg/m^3 (1.4-3 ppm) (Rut86) have been reported.

4 Use

HEX is an intermediate used in the manufacture of organochlorine pesticides (e.g., endosulfan) and of flame-retardants for resins and polymers (ACG99, BUA88).

5 Biotransformation and kinetics

Human data

One human volunteer received a dose of 1.3 mg ^{14}C-labelled HEX following exposure to its vapour (exposure concentration of ^{14}C-HEX and duration of exposure not given). Excretion of radioactivity in the urine started 30 minutes after the end of exposure, and most of the radioactivity was excreted within 5 days. No unchanged HEX was detected in urine. Radioactivity comprised polar metabolites, which could not be identified (Kha81).

Animal data

Groups of female Sprague-Dawley rats were exposed 'nose-only' to vapours of [^{14}C]-HEX (radiochemical purity: >95%) for 30 to 120 min, at air concentrations ranging from 0.11 to 0.56 mg/m^3. The percent retention of HEX increased with the time of exposure, being 77% after 30 min of exposure and 95% after 120 min exposure to the vapour. Retained doses received by the rats ranged from 1 to 40 µg/kg bw, but the retention was not influenced by the quantity received within this range of doses. At a retained dose of 24 µg/kg bw following 1-hour exposure, the maximum concentration of radioactivity in blood was achieved at the end of the exposure period. At 72 hours after the end of exposure, 33.1% of the dose was excreted in the urine, 23.1% in the faeces, and less than 1% in expired air. Most of the urinary radioactivity (29.7 % of the dose) was already excreted within 24 hours. The amount of radioactivity retained in the body was 12.9%, most of it in the trachea, lungs, and kidneys (Law81, Law82). In another inhalation study, 2 groups of Fischer rats were exposed to [^{14}C]-HEX in air (radiochemical purity: >95%; exposure times and air concentrations not given). The average absorbed doses were 1.3 and 1.8 mg/kg bw, respectively. At 6 and 72 hours after the end of exposure, 28.7% and 47.5% of the dose were excreted

in the faeces, 41.0 and 40.0% in the urine, and 1.4% and 1.0% in expired air (as $^{14}CO_2$), respectively. Radioactivity retained in the body amounted to 11.5% of the dose at 72 hours post-exposure, most of it in the kidneys, the lungs, and the blood (ElD83). In either inhalation study, no unchanged HEX or metabolites were found in excreta or tissues; the majority of the radiolabel comprised water-soluble, polar material (ElD83, Law82).

The kinetics of HEX in rats following oral dosing has been reported in several studies. Rats given a single oral (gavage) dose of 5.5 mg/kg bw of [^{14}C]-HEX excreted 35 and 10% of the dose in the urine and the faeces, respectively, within 7 days. At day 7, only 0.5% of the dose was found in the kidneys and the liver (Meh77). In an unpublished study, groups of Sprague-Dawley rats received oral doses ranging from 8.5 to 25.6 mg/kg bw. The concentration of radioactivity in the blood reached a maximum at 4 hours after administration. Within 48 hours, 16 and 70% of the radioactivity were excreted in the urine and faeces, respectively. At 48 hours after dosing, 2.8% of the radioactivity was retained in the organs (liver, kidneys, adipose and muscle tissue, brain, and heart). No unchanged HEX was detected in urine or faeces, and most of the radiolabel comprised polar products (Yu81). In another study, groups of female Sprague-Dawley rats were given single oral doses of 5 to 10 µg/kg bw of [^{14}C]-HEX (radiochemical purity: >95%). The maximum blood level of radioactivity was reached at 2 to 5 hours after dosing. About 24 and 68% of the dose were excreted in the urine and faeces, respectively, within 72 hours after administration. More than 90% of the dose was already excreted within the first 24 hours. Biliary excretion accounted for 18% of the administered dose, indicating that the majority of the dose excreted in faeces comprised unabsorbed material. Bile and faeces contained predominantly polar or unextractable material. At 72 hours after administration, only 0.2% of the dose was retained in the organs. When the rats received a much higher dose (6 mg/kg bw), the amounts of radioactivity excreted in urine or faeces were comparable to those excreted at the low dose (about 15% of the dose in the urine and 64% in the faeces). However, the retention of radioactivity in tissues was appreciably higher (2.8%), most of it in the kidneys (1.9%) (Law82). Similar results were obtained in Fischer rats, dosed with 61 mg/kg bw of [^{14}C]-HEX. Within 72 hours, about 65% of the dose was excreted in faeces, 29% in urine, and 0.9% in expired air, while 2.4% of the dose was retained in the tissues (ElD83). Groups of rats and mice were given [^{14}C]-HEX, either as a single oral (gavage) dose of 2.5 or 25 mg/kg bw, or as daily dietary doses of approximately 0.07 to 1.7 mg/kg bw/day for rats and 0.18 to 4.6 mg/kg bw/day for mice for 30 days. In the repeated study, the animals were returned to a

normal diet for up to 30 days. In the single dose study, in either species or sexes, the majority of radioactivity (73-96% of the dose) was excreted within 2 days, most of it in the faeces. In the repeated dose study, the total excretion of radioactivity ranged from 63 to 79% of the dose, on average about 64% in the faeces and 8.4% in the urine. In all cases, the kidneys, the liver, and the fat contained the highest amounts of radioactivity. Steady state levels of radioactivity in these tissues were reached after 15 days of feeding. In a separate experiment male rats, in which the bile duct was cannulated, received a single oral dose of 25 mg/kg bw of [^{14}C]-HEX. About 16% of the dose was excreted in the bile over a 24-hour period confirming poor absorption of orally administered HEX. No unchanged HEX was detected in either bile or faeces. The radioactivity comprised polar metabolites formed in all probability in the intestine, and most of these were not resorbed (Dor84).

When given intravenously, rats given a single dose of 0.73 mg of [^{14}C]-HEX, excreted 21% of the dose via the faeces and 18% via the urine within 48 hours, while some 28% of the radioactivity remained in the tissues (Yu81). In another study, 34% of the dose was excreted in the faeces and 16.8% in the urine within 72 hours after administering 0.59 mg/kg bw. Radioactivity retained in tissues amounted to 39% of the dose (ElD83). Female Sprague-Dawley rats excreted about 31% of the radioactivity in the faeces and 22% in the urine within 72 hours after administration of 10 µg/kg bw. Radioactivity in tissues amounted 31% of the dose, most of it in the kidneys and the lungs (Law82).

In vitro studies

When incubated with [^{14}C]-HEX *in vitro*, HEX bound to components of liver, intestinal contents, and faecal homogenates, as well as to components of whole blood and plasma (ElD83).

In summary, following inhalation or intravenous administration, HEX was completely metabolised and rapidly excreted. In case of inhalation, 95% of the vapour inhaled was retained and approximately equal fractions of this dose were excreted in the faeces and in the urine (ca. 36%) and ca. 1% in expired air. Significant amounts (ca. 12%) were retained in the body (especially trachea, lungs, and kidneys). Orally administered HEX was poorly absorbed, probably due to its reactivity with the contents of the gastrointestinal tract, and considerably more of orally dosed HEX was excreted in the faeces when compared to HEX administered by inhalation or intravenous injection. The polar faecal and urinary metabolites have been isolated but not identified. The failure to identify metabolites represents a major difficulty in assessing the pharmacokinetics and potential mechanisms of action of HEX.

After inhalation exposure, HEX can bind to epithelial lung tissue and extracellular lining in the lung and to bronchiolar Clara cells in rats (Ran82b).

No studies on the kinetics of HEX following dermal application were found, but toxic responses reported after dermal application suggest that HEX is absorbed via the dermal route (WHO91).

6 Effects and mechanism of action

Human data

In March 1977, a municipal sewage treatment plant was illegally contaminated with a large volume of HEX and related chlorinated hydrocarbons, e.g., octachlorocyclopentene (OCCP), for several days. A number of 193 plant employees had worked at the sewage plant for 2 or more days from the beginning of the dumping, which was detected by an objectionable odour, until closure of the plant when analysis showed the wastewater to be contaminated with HEX, and to a lesser extent with OCCP. Although the airborne concentrations of HEX at the time of exposure were not known, concentrations in the primary treatment areas (screen and grit chambers) ranged between 3 and 11 mg/m^3 (0.27-0.97 ppm), 4 days after the plant closed. A questionnaire was sent to each of the 193 plant workers and 145 (75%) responded. Most of the workers were men (85%). They were generally healthy and had an average age of 35 years. The most common symptoms reported were eye irritation (59%), headache (45%), and throat irritation (27%). Furthermore, nausea, skin irritation, cough, chest pain, difficult breathing, nervousness, abdominal cramps, decreased appetite, decreased memory, and increased saliva were reported (19-21 %). Even 6 weeks after the episode, a follow-up questionnaire of 177 plant workers showed residual symptoms, i.e., headache (18%), fatigue (15%), respiratory tract (9%) and eye irritation (9%). Informal follow-up indicated that complaints of persistent symptoms slowly declined over 6 months. Of the 41 workers who received a medical examination 27% had increased serum LDH activity and 27% proteinuria. Three weeks later, these abnormalities had disappeared. Similar complaints were noticed in a group of workers (n=97) who were engaged in the clean-up of the plant. No abnormalities were seen in haematology parameters, urinalyses, or biochemical renal function tests. However, in 18 workers, slight abnormalities were recorded in one or more liver function tests, i.e., serum aspartate aminotransferase (in 11 workers), serum alkaline phosphatase (in 5), serum bilirubin and aspartate aminotransferase (in 1), or serum LDH (in 1); 8 persons had abnormalities on more than one occasion. Seven workers showed an

increased aspartate aminotransferase activity, probably related to exposure to contaminated sewage (Kom80, Mor79).

In a cross-sectional study, hepatic and renal functions were measured of a group of 73 male operators employed in a chemical plant producing chlorinated hydrocarbons for on average 8.2 years (range: 0.5-23 years). Exposure data were given over the period 1980 to 1993 for allyl chloride, HEX, epichlorohydrin, and 1,3-dichloropropene. Air concentrations of allyl chloride and HEX occasionally exceeded the occupational exposure limits of 3 and 0.11 mg/m^3, respectively. Biochemical liver function tests (serum ALAT, ASAT, alkaline phosphatase, LDH, GGT, and total bile acids) were not statistically significantly different compared to a control group. Nor were differences found in biochemical tests for renal tubular damage (urinary alanine aminopeptidase, *N*-acetyl-β-D-glucosaminidase, retinol binding protein, and total protein). However, urinary albumin concentrations were significantly higher in the exposed group, but this (small) increase was not related to exposure. It was concluded that long-term exposure to the above chemicals at air levels below or near the respective MAC-values does not lead to clinically significant effects on kidney and liver (Boo93).

Several epidemiological studies have been reported on workers engaged in the manufacture of HEX or chlorinated 'cyclodiene' pesticides. Only one of these studies dealt with workers who had been engaged in the manufacture of HEX. This unpublished mortality study comprised 341 workers (27 males and 54 females) who had worked in the plant for at least 3 months between 1953 and 1974. The standardised mortality ratio (SMR) for all causes of death was 69. Deaths caused by specific cancers, all cancers, and diseases of the circulatory and digestive systems were fewer compared to the overall USA population. No data were provided on HEX exposures during the above period. The authors noted that the time that had elapsed since the initial exposure (25 years at most) reduced the power of the study to detect cancers that may have a 10-40 year latent period (Bun80). The other reported epidemiological studies dealt with workers who might have been exposed to HEX during the production of 'cyclodiene' pesticides chlordane, heptachlor, and aldrin and dieldrin. However, in none of the reports, information on HEX exposure was given.

In an unpublished study, the mortality was examined of a cohort of 783 workers who had been involved in the manufacture of chlordane for 3 months or more between 1946 and 1979. There were no significant differences in mortality rates between these employees and the overall USA population. The observed value for death from all causes, including heart disease and cancer, was less than the expected value in the overall USA population (Shi80). In another unpublished report, the examination of 1115 workers who had worked for 3

months or more in a heptachlor production facility between 1952 and 1979 likewise showed no differences in mortality compared to the overall USA population (Shi81). A retrospective study using the combined data of 1403 workers of both plants revealed a SMR for all causes of death of 72. Among the various causes of death, the 2 highest SMRs were 134 for lung cancer (not significant) and 183 for cerebrovascular disease (statistically significant). There was one death from liver cancer. The excess mortality due to cerebrovascular disease was not related to the duration of employment (Wan79, Shi86).

Another epidemiological study was conducted on a cohort of 570 workers who had been engaged in the production of aldrin and dieldrin for 1 year or more between 1954 and 1970. The vital status was determined through 1987. There was no statistically significant increase in the SMR of all cancers (103.6). The incidence of primary liver cancer was 1 out of 570 workers (Jon91).

Animal data

Irritation and sensitisation

HEX was extremely irritating to the skin of rabbits following an occluded single application of 430 mg/kg bw. A purplish-black local discolouration and subcutaneous oedema was observed. About 12 days later, the skin was hard, encrusted, and fissured. Single applications of diluted HEX solutions, at concentrations ranging from 0.001% to 90%, to the skin of a monkey caused prompt discolouration of the skin at a concentration of 10% and above, the colour ranging from a very light to a dark tan as the concentration increased. In another monkey, daily 2-hour applications of 0.05 mL of a 10% HEX solution/kg bw for 3 consecutive days produced severe skin irritation and necrosis. Five days after the application, the skin was hard, encrusted, fissured, necrotic, and haemorrhagic. At 13 months after the application, a scar was visible on the injured area, with atrophy and complete absence of hair. In a guinea pig, no effect on the skin was seen following single applications of 0.05 mL of a 0.01% to 1% solution. At concentrations ranging from 40% to 90%, discolouration and necrosis was observed (Tre55).

HEX was a skin sensitiser in the Magnusson and Kligman guinea pig maximisation test. All 24 animals used showed positive responses at both 24 and 48 hours after the challenge (Pri82).

Instillation of 0.1 mL of HEX into the eyes of New Zealand white rabbits for 5 minutes or 24 hours resulted in severe eye irritation and in death of all animals within 9 days (IRD72).

Acute toxicity

Results of acute lethal toxicity tests with HEX are summarised in Table 1.

Table 1 Summary of acute lethal toxicity studies in experimental animals.

exposure route	species	strain (sex)	LD_{50} or LC_{50} (duration)	reference
inhalation	rat	Carworth	35 mg/m^3 (3.5 h)	Tre55
	rat	Sprague-Dawley (male) Sprague-Dawley (female)	18 mg/m^3 (4 h) 40 mg/m^3 (4 h)	Ran82a
	rat	albino	41.2 mg/m^3 (4 h)	Jac87
	guinea pig		80 mg/m^3 (3.5 h)	Tre55
dermal	rabbit	(female)	430-610 mg/kg bw[a]	Tre55
	rabbit	(male) (female)	<200 mg/kg bw 340 mg/kg bw	IRD72
oral	rat	Carworth (male)	505 mg/kg bw	Tre55
	rat	Charles River (male, female)	926 mg/kg bw	IDR68
	rat	Sprague-Dawley (male, female)	651 mg/kg bw	Dor79
	rat	Fischer 344 (male) Fischer 344 (female)	425 mg/kg bw 315 mg/kg bw	SRI80
	rat	Sprague-Dawley (male) Sprague-Dawley (female)	1500 mg/kg bw 1300 mg/kg bw	Gar86
	mouse	Charles River (male, female)	679 mg/kg bw	Dea77
	mouse	Unspecified (male, female)	600 mg/kg bw	Dor79
	mouse	$B6C3F_1$ (male, female)	680 mg/kg bw	SRI80
	rabbit	(female)	420-640 mg/kg bw[a]	Tre55

[a] Minimum lethal dose.

The committee remarks that HEX is much more toxic by the inhalation route of exposure than by the dermal or oral routes.

The vapours caused serious irritation of the mucous and respiratory membranes. Signs of intoxication after inhalation exposure included lachrymation, salivation, gasping, and irregular breathing. Pathology revealed diffuse degeneration of the brain, heart, and adrenal glands, and degeneration and

necrosis of the liver and kidney tubules, together with severe pulmonary hyperaemia and oedema, acute bronchitis, and interstitial pneumonitis (Tre55). In another study, lachrymation, salivation, ataxia, loss of weight, and pulmonary abnormalities, characterised by red focal or diffuse consolidation that progressed to generalised haemorrhage and hepatisation, were reported (Ran82a). After oral administration, clinical signs and symptoms were: diarrhoea, lethargy, retarded respiration, decreased limb tone, ataxia, and ptosis (Dea77, Tre55).

Short-term toxicity

- Inhalation toxicity

In an old inhalation study, small groups of rats (n=4), mice (n=5), rabbits (n=6), and guinea pigs (n=2) were exposed to concentrations of 3.9 mg/m^3 (0.34 ppm), 7 hours/day, 5 days/week. Guinea pigs survived 30 exposures. Four rabbits died before the 25th and all rats and mice between the 6th and 20th exposure. When exposed for 30 weeks to 1.7 mg/m^3 (0.15 ppm) all species, except 4 of 5 mice, survived. Pathology revealed mild degenerative changes in the livers and kidneys of all species. The mice were found to have pulmonary oedema and bronchitis, and some of the guinea pigs and rats had developed pneumonia (Tre55).

Groups of Sprague-Dawley rats (n=10/sex/group) were exposed to concentrations of HEX (purity: 97.7%) of 0, 0.25, 1.25, or 5.7 mg/m^3 (0, 0.022, 0.11, 0.5 ppm), 6 hours/day, 5 days/week, for 2 weeks. In the high-exposure group, 9 male and 2 female animals died during the exposure period. In the low- and mid-exposure groups, no mortality or treatment-related clinical signs were observed. Males had a significant dose-related decrease in mean body weight compared to controls. In the animals of the high-exposure group, clinical signs observed were dark red eyes, laboured respiration, and paleness of the extremities. Haematological examination revealed increases in haematocrit, haemoglobin level, and erythrocyte count and decreased lymphocyte counts. In the mid-exposure group, males showed a decrease in haematocrit, but an increase in haemoglobin level. In all male exposure groups, there was a dose-dependent increase of serum total protein levels. Absolute liver weights were reduced in males of the low-exposure group and in females of the low- and mid-exposure group. In animals exposed to 5.7 mg/m^3, absolute lung weights were increased and the absolute weights of kidneys, adrenals, and ovaries were decreased, compared with the controls. Data on relative organ weights were not given. Microscopic examination on rats of the 5.7 mg/m^3 group revealed lung changes mainly confined to the bronchioles, characterised by epithelial erosion, focal areas of hyperplastic cuboidal and columnar epithelium, inflammatory cell

infiltration and/or exudate in the lumen. Minimal atrophy of olfactory epithelium occurred in the nasal passages. There were no significant treatment-related microscopic changes in the mid- and low-exposure groups compared with the controls. These nasal and lung changes in the high-exposure group were consistent with observed impaired respiratory function, confirming the lung as the main target organ, according to Rand et al. In a parallel experiment, groups of rats (n=5/sex/group) were treated similarly, but served as separate recovery groups to observe effects during a 14-day (control, low- and mid-exposure groups) or 21-day (high-exposure group) period without exposure. Rats in the high-exposure group received only 5 exposures, because of the high mortality in the main study (see above). All animals survived during the exposure period, but 3 males in the high-exposure group died within 7 days after the end of exposure. A decrease in mean body weight was observed for both sexes in the high-exposed group. Haematological abnormalities in the high- and mid-exposure group were reversible during the recovery period, except increased haemoglobin levels in the males of both groups. Changes in absolute organ weights were not observed in any of the groups at 14 or 21 days after the end of exposure. Microscopic changes of the lung and the nasal area observed in the high-exposure group recovered 2 to 3 weeks after termination of exposure. According to Rand et al., the NOAEL was 1.25 mg/m^3 (Ran82a).

The same authors conducted a 14-week inhalation study with groups of rats (n=40/sex/group) receiving whole-body exposure to HEX at concentrations 0, 0.11, 0.57, or 2.28 mg/m^3 (0, 0.01, 0.05, 0,2 ppm), 6 hours/day, 5 days/week. No treatment-related mortality was observed, but rats in the high- and mid-exposure groups displayed a transient dark red colouring of the eyes. However, ophthalmoscopic examination did not reveal chemical induced abnormalities. No treatment-related changes were seen in body weight gain, food consumption, or clinical chemical parameters. A small, but occasionally statistically significant increase in haemoglobin level and erythrocyte count was measured in males exposed to 0.11 mg/m^3, females exposed to 0.57 mg/m^3, and males and females exposed to 2.28 mg/m^3. These changes were probably related with impaired respiratory function, according to Rand et al. In males and females of all treatment groups, absolute mean liver weights were statistically significantly reduced by 3 to 15%, relative to controls, and in all treated males absolute kidney weights were reduced by 10 to 11%. Upon macroscopic and microscopic examination, no treatment-related abnormalities were observed. Rand et al. set the NOAEL at 2.28 mg/m^3 (Ran82a). In a further study, dose-dependent abnormalities were found in the Clara cells of the lungs at 0.11 mg/m^3 and above. These abnormalities comprised an increase in the mean number of inclusions in

the apex and base of the Clara cells, the biological significance of which is unclear (Ran82b).

In a 90-day inhalation study, groups of F344 rats (n=10/sex/group) were exposed to concentrations of HEX of 0, 0.46, 1.7, 4.6, 11.4, or 22.8 mg/m^3 (0, 0.04, 0.15, 0.4, 1, 2 ppm), 6 hours/day, 5 days/week. All rats in the 2 highest exposure groups died within the first 4 weeks. Clinical signs were eye irritation and respiratory distress in all rats at 11.4 mg/m^3 and above, listlessness in all rats at 4.6 mg/m^3 and above, and posterior paresis in all rats at 1.7 mg/m^3. At 4.6 mg/m^3, male animals showed decreased body weights and increased absolute and relative lung weights, compared to controls. No abnormalities in haematology, clinical chemistry, or urinalysis parameters were observed in male or female rats of any exposure group. Macroscopic and microscopic examination revealed inflammation and necrosis of the nose, larynx, trachea, and lungs, as well as metaplasia of the nasal epithelium in males and females exposed to 4.6 mg/m^3 and above. No compound-related changes were observed in any organ of male and female animals exposed to 0.46 mg/m^3 (NTP94).

Groups of Wistar rats (n=27/sex/group) were exposed to concentrations of HEX of 0, 0.57, 1.14, or 5.7 mg/m^3 (0, 0.05, 0.1, 0.5 ppm), 6 hours/day, 5 days/week, for 30 weeks. At the end of the exposure period, the animals, 9 males and 9 females of each group, were retained for a 14-week recovery period. In the high-exposure group, 3 males and 2 females died during the exposure period and 1 male during the recovery period. Most animals of the high-exposure group were sneezing and lethargic at some time during the exposure period, but such signs were absent in the low- and mid-exposure groups. Significant decreased body weights compared to control animals were observed in males at the high-exposure level from week 7 to the end of exposure and in females of the high- and mid-exposure groups at the end of the recovery period. Haematological examination of samples collected at the end of week 30 revealed a statistically significant increase in haematocrit, haemoglobin level, and erythrocyte and neutrophil count, and a significantly lower lymphocyte count in male rats of the high-exposure group, compared to controls. No abnormalities were observed in clinical chemical and urinalysis parameters. At the end of the exposure period, absolute and relative kidney weights were significantly increased in females at the high exposure. The changes in absolute heart weights (significantly decreased in high-exposure males), testes weights (significantly increased at the mid-exposure level), and relative kidney weights (significantly increased in high- and low-exposure males) were judged as ‘probably not of biological significance’. Microscopic examination of tissues of high-exposure animals collected at the end of week 30 revealed pulmonary degenerative changes

ranging from epithelial hyperplasia, oedema, and sloughing of the bronchiolar epithelium of males and females to epithelial ulceration and necrosis in the males. Such changes were absent in the mid- and low-exposure groups and in the recovery groups. Mild degenerative changes were seen in the liver and kidney of a few high-exposure rats after 30 weeks. The NOAEL was 1.14 mg/m^3 (Cla82).

In a 90-day inhalation study, groups of B6C3F$_1$ mice (n=10/sex/group) were exposed to HEX concentrations of 0, 0.46, 1.7, 4.6, 11.4, or 22.8 mg/m^3 (0, 0.04, 0.15, 0.4, 1, 2 ppm), 6 hours/day, 5 days/week. All mice in the 2 high-exposure groups died during the first week of exposure. Of the animals exposed to 4.6 mg/m^3, 5 males and 2 females died. Final body weights of males exposed to 1.7 or 4.6 mg/m^3 were significantly lower compared to control animals. Treatment-related posterior paresis and listlessness were observed at 4.6 mg/m^3 and above. At these levels, microscopic examination revealed necrosis or inflammation of the nose, larynx, or lung. Squamous metaplasia of the larynx or trachea was observed in males at 1.7 mg/m^3 and above and in females at 4.5 mg/m^3 and above. The NOAEL was 0.46 mg/m^3 (NTP94).

Cynomolgus monkeys (n=6/sex/group) were exposed to HEX concentrations of 0, 0.11, 0.57, or 2.28 mg/m^3 (0, 0.01, 0.05, 0.2 ppm), 6 hours/day, 5 days/week, for 14 weeks. There were no mortalities or adverse clinical signs. No changes in body weight gain and food consumption, pulmonary function, haematological, clinical chemical, or urinalyses were found between animals in any of the exposed groups, compared with the controls. Furthermore, no abnormalities were found upon ophthalmoscopic, macroscopic, or microscopic examinations. The NOAEL was 2.28 mg/m^3, the highest level tested (Ran82a).

- Oral toxicity

Groups of F344 rats (n=10/sex/group) were given HEX (purity: 94-97%) by gavage at doses of 0, 10, 19, 38, 75, or 150 mg/kg bw, 5 days/week, for 13 weeks. A significant increase in mortality was observed at the top dose (7 males and 5 females). Clinical signs of toxicity were reduced spontaneous activity and ruffled fur in both sexes at 38 mg/kg bw/day and above. Body weights were significantly reduced compared with controls in males and females, receiving 38 mg/kg bw/day and more, and 75 mg/kg bw/day and more, respectively. In female rats, relative liver weights were significantly increased at 38 mg/kg bw/day and above, and relative kidney weights at 75 mg/kg bw and above. Macroscopic and microscopic examination revealed treatment-related lesions in the forestomach, including proliferative and inflammatory changes of the epithelia at 38 mg/kg bw/day and above in males, and at 19 mg/kg bw/day and above in females. Toxic

nephrosis, characterised by proximal tubular dilation, was observed at 38 mg/kg bw/day in both males and females. The NOAEL was 10 mg/kg bw/day (Abd84).

Groups of $B6C3F_1$ mice (n=10/sex/group) received oral (gavage) doses of HEX (purity: 94-97%) of 0, 19, 38, 75, 150, or 300 mg/kg bw/day, 5 days/week, for 13 weeks. At 300 mg/kg bw/day, all male mice and 3 female mice died. Clinical signs of toxicity were ruffled fur and slight inactivity at 300 and 150 mg/kg bw/day. Body weights were significantly reduced at 300 and 150 mg/kg bw/day in females, and at 150 mg/kg bw/day in males. In females, relative lung weight was significantly increased at 300 mg/kg bw/day, and relative liver and kidney weights were significantly increased in both sexes at 19 mg/kg bw . Macroscopic and microscopic examination revealed treatment-related lesions in the forestomach, including epithelial hyperplasia, and focal inflammation at 38 mg/kg bw/day and above (both sexes), and ulceration at 300 mg/kg bw/day (males only). Toxic nephrosis, characterised by lesions in the terminal portion of the proximal convoluted tubule in the inner cortex was observed in females only at 75 mg/kg bw/day and above. The NOAEL and LOAEL were 19 mg/kg bw/day in males and females, respectively (Abd84).

A summary of short-term studies is shown in Table 2.

Table 2 Summary of short-term toxicity studies in experimental animals.

exposure route	species (strain)	dose level	exposure duration	critical effect	NOAEL[a]	reference
inhalation	rat (Sprague-Dawley)	0, 0.25, 1.25, 5.7 mg/m^3	2 weeks	nasal and lung injury; haematological effects	1.25 mg/m^3	Ran82
	rat (F344)	0, 0.46, 1.7, 4.6, 11.4, 22.8 mg/m^3	13 weeks	posterior paresis	0.46 mg/m^3	NTP94
	rat (Sprague-Dawley)	0, 0.11, 0.57, 2.28 mg/m^3	14 weeks	none	2.28 mg/m^3	Ran82
	rat (Carworth)	1.7 mg/m^3	30 weeks	liver and kidney injury; pneumonia	LOAEL: 1.7 mg/m^3	Tre55
	rat (Wistar)	0. 0.57, 1.13, 5.7 mg/m^3	30 weeks	lung, liver, and renal injury; haematological effects; reduced body weights	1.13 mg/m^3	Cla82
	mouse ($B6C3F_1$)	0, 0.46, 1.7, 4.6, 11.4, 22.8 mg/m^3	13 weeks	effects on respiratory tract; reduced body weights	0.46 mg/m^3	NTP94

	mouse	1.7 mg/m^3	30 weeks	mortality	LOAEL: 1.7 mg/m^3	Tre55
	rabbit	1.7 mg/m^3	30 weeks	liver and kidney injury	LOAEL: 1.7 mg/m^3	Tre55
	monkey (cynomolgus)	0, 0.11, 0.57, 2.28 mg/m^3	14 weeks	none	2.28 mg/m^3	Ran82
oral	rat (F344)	0, 10, 19, 38, 75, 150 mg/kg bw	13 weeks	forestomach lesions	10 mg/kg bw	Abd84
	mouse ($B6C3F_1$)	0, 19, 38, 75, 150, 300 mg/kg bw	13 weeks	forestomach lesions	19 mg/kg bw (males); LOAEL: 19 mg/kg bw (females)	Abd84

Long-term toxicity and carcinogenicity

In a 2-year inhalation study, groups of F344/N rats (n=60/sex/group) were exposed to HEX concentrations of 0, 0.11, 0.57, or 2.28 mg/m^3 (0, 0.01, 0.05, 0.2 ppm), 6 hours/day, 5 days/week. Survival rates and mean body weights of exposed rats were similar to those of the controls. No treatment-related clinical findings were observed in male or female rats during the 2-year study. No differences in urinalysis parameters were found at the 15-month interim evaluation, which could be attributed to exposure to HEX. Macroscopic and microscopic examination revealed that no treatment-related increased incidences of neoplasms occurred in any of the exposure groups. Toxicity was limited to the respiratory tract and included a statistically significantly increased incidence of pigmentation of the respiratory epithelium of the nose (all male and female groups), the trachea (high-exposure males only), and the bronchioles and bronchi of the lung (all male and female groups). At 0.11 mg/m^3, significant nasal effects were found in both males and females and significant lung effects in females.

Details are shown in Table 3. Exposure to HEX also caused a significant increase in the incidence of squamous metaplasia of the laryngeal epithelium in females at 0.11 and 2.28 mg/m^3, but not at 0.57 mg/m^3, compared to controls (Table 3). The severity of squamous metaplasia was minimal in all exposed and control females. The apparent change diagnosed as squamous metaplasia consisted of stratified squamous epithelium several cell layers thick and was believed to be located in areas usually lined by columnar epithelium. Due to individual variation in determining where the transition from squamous to columnar epithelium occurs

as well as difficulties in obtaining consistent sections, the relevance of the higher incidences of squamous metaplasia in the 0.11 and 0.57 mg /m^3 groups is uncertain. In conclusion, there was no evidence of carcinogenic activity of HEX in male or female F344/N rats. A NOAEL for toxic effects, however, could not be established (NTP94).

Table 3 Summary of respiratory tract effects[a] in F344 rats and B6C3F_1 mice after inhalation exposure to HEX, 6 hours/day, 5 days/week, for 2 years (NTP94).

	male rats (n=48-50)				female rats (n=48-50)			
	0	0.11	0.57	2.28[b]	0	0.11	0.57	2.28[b]
pigmentation:								
nose	1	46**	48**	48**	0	34**	47**	48**
trachea	0	0	0	5*	0	0	0	1
lung (bronchioles)	0	0	0	49**	0	25**	42**	50**
lung (peribronchiolar	0	0	2	1**	3	1	4	27**
metaplasia:								
larynx	1	2	6	4	9	20*	15	24**
		male mice (n=50)				female mice (n=50)		
mucosal pigmenation:								
nose	0	45**	50**	44**	0	40**	48**	41**
trachea	0	29**	48**	48**	0	6*	43**	42**
lung	0	2	42**	45**	0	0	27**	44**
suppurative pigmentation:								
nose	0	0	1	36**	4	0	3	40**

[a] Incidences expressed as number of animals.
[b] Concentrations in mg/m^3.
* $p<0.05$; ** $p>0.01$.

In a similar study, groups of B6C3F_1 mice were exposed to HEX concentrations of 0, 0.11, 0.57, or 2.28 mg/m^3 (0, 0.01, 0.05, 0.2 ppm), 6 hours/day, 5 days/week, for 2 years. The 2-year survival rate of high-exposure females was marginally lower than that of the controls due to a higher incidence of ovarian inflammation. Mean body weights of high-exposure males (weeks 62 to 103) and females (throughout the study) were lower than those of the controls. No treatment-related clinical findings were observed in males or females in any of the exposure groups. There were no treatment-related differences in urinalysis parameters at the 15-month interim evaluation, compared to the controls. The

site of toxicity of HEX exposure was the respiratory tract. Macroscopic and microscopic examination revealed a statistically significant increased incidence of treatment-related pigmentation of the respiratory epithelium of the nose (all male and female groups), trachea (all male and female groups), and lung (mid- and high-exposure males and females), and suppurative inflammation of the nose (high-exposure males and females) (see Table 3). No statistically significant increased incidences of neoplasms were found in any of the treatment groups, compared to the control group. A NOAEL for toxic effects, however, could not be established (NTP94).

In a separate so-called 'stop-exposure' study, groups of male mice (n=50/group) were exposed to HEX concentrations of 2.28 mg/m^3 (0.2 ppm), for 33 or 66 weeks, or to 5.7 mg/m^3 (0.5 ppm), for 26 or 42 weeks, and then examined at 2 years after the beginning of exposure. Two-year survival rates and mean body weights of 'stop-exposure' groups were similar to that of the controls. No treatment-related clinical abnormalities were observed. Macroscopic and microscopic examination revealed that non-neoplastic respiratory tract lesions were similar to those observed in the above 2-year exposure study. Treatment-related pigmentation and inflammation of the respiratory epithelium were persistent, as indicated by their presence in many mice after recovery periods of 62 to 78 weeks. The incidence and severity of the lesions were related to exposure concentration and duration. This suggests that the pigment could be a reaction product between the chemical and an intracellular component of the respiratory tissues that has a very slow turnover rate, according to the authors. There appears to be a critical burden, below which suppurative inflammation of the trachea and lung does not occur. The critical burden, expressed as a composite unit (concentration x weeks), was estimated at 226-237 mg/m^3 x weeks (NTP94).

Mutagenicity and genotoxicity

- In vitro tests
  - Gene mutation assays. HEX (purity: 98%) did not induce reverse gene mutations in several strains of *S. typhimurium* (TA98, TA100, TA1535, and TA1537) at concentrations up to 3.3 μg/plate without metabolic activation, or up to 100 μg/plate after metabolic activation by a rat liver microsomal S9 preparation (Haw83). In an unpublished study, application of HEX (purity: 98.9%) at concentrations up to 10 μg/mL in the absence, or up to 500 μg/mL in the presence of S9 fraction did not increase the reverse gene mutation frequency in agar layer cultures of

S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538, or of *E. coli* strains WP2 or WP2 *uvrA* (Bro83). In *E. coli* strain K12, HEX did not induce reverse mutations at 2.7 mmol/L in the presence or absence of S9 (Gog78). In 2 studies, without details of the concentrations tested, negative results were reported using *S. typhimurium* strains TA1535 or TA1538 (Gre77) or *S. typhimurium* strain TA100 (WHO91), both with and without metabolic activation. However, in one study, positive results were found in *S. typhimurium* strains TA98 and TA100 and in *E.coli* strain PQ37 with and without metabolic activation. No details were given of the concentrations tested (Raa93). HEX, dissolved in DMSO, did not induce gene mutations in the thymidine kinase ($TK^{+/-}$) assay in cultured L5178Y mouse lymphoma cells at concentrations of 0.002 to 0.04 µL/L without metabolic activation, and 0.4 to 1.25 µL/L with metabolic activation (Mat78). HEX at a concentration of 273 µg/L did not increase the gene mutation frequency in the HGPRT forward mutation assay carried out in rat liver epithelium cells (Wil80). No increased sex-linked recessive lethal mutations were found in *Drosophila*, either by feeding Canton-S wild-type males HEX doses of 40 mg/kg feed for 3 days, and then mated, or by injection of males with HEX dissolved in 10% alcohol at concentrations of 2000 or 3000 mg/L (Zim85). In a later study, the negative results were confirmed when feeding males doses of 10 mg/kg feed for 3 days or by injection of males with a solution of 900 mg HEX/L (Mas92).

- Cytogenicity assays. HEX did induce sister chromatid exchanges (SCE) and chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells with and without S9 (NTP94). In an unpublished study, no statistically significant increase in the frequency of chromosomal aberrations in rat liver (RL_4) cell cultures exposed to HEX (purity: 98.9%) at concentrations up to 0.2 µg/mL was found (Bro83).
- Other assays. HEX in a concentration of 2.73 mg/L produced no increase in the DNA-repair rate in rat hepatocyte primary cultures (Wil80). Negative results were also obtained in an additional unscheduled DNA synthesis (UDS) assay (Bra83). In another unpublished study, HEX (purity: 98.9%) did not induce mitotic gene conversion in *S. cerevisiae* JD1 at concentrations up to 10 µg/mL, either in the presence or absence of rat liver S9 (Bro83). However, HEX was positive in the *B. subtilis* rec-assay at concentrations in the range of 20-30 µg/L in the absence, and 300-500 µg/L in the presence of S9 (Mat89).

- *In vivo* tests

In an unpublished study, no dominant lethal mutations were induced when male CD-1 mice (n=10) were given oral (gavage) doses of HEX of 0.1, 0.3, or 1.0 mg/kg bw/day for 5 days, and then mated with female animals throughout spermatogenesis (7 weeks) (WHO91).

No increase in the frequency of micronucleated erythrocytes was observed in male or female $B6C3F_1$ mice, exposed by inhalation to HEX concentrations ranging from 0.11 to 2.28 mg/m^3 (0.01-0.2 ppm), 6 hours/day, 5 days/week, for 13 weeks (NTP94).

- Other tests

In an unpublished study, no malignant cell transformations were observed in cultured BALB/3T3 cells after incubation with HEX at concentrations of 0, 10, 20, 39, 78, or 156 µg/L (WHO91)

In summary, in most *in vitro* tests in microorganisms or mammalian cells, HEX has no mutagenic or genotoxic activity. The induction of SCEs and chromosome aberrations in CHO cells *in vitro* could neither be confirmed in a rat liver cell culture nor in an *in vivo* micronucleus assay in mice. The committee concluded that HEX is not a mutagenic hazard.

Reproduction toxicity

The committee did not find inhalation reproduction toxicity studies on HEX. Following dosing by gavage, HEX was evaluated for developmental toxicity in rats, mice, and rabbits.

In pregnant CD-1 rats (numbers not presented) given HEX (purity: 98.2%) in corn oil at doses of 0, 3, 10, and 30 mg/kg bw/day on gestational days 6 to 15, no significant differences were observed in maternal survival, mean maternal body weight gain, mean number of implantations, corpora lutea, or live fetuses, mean fetal body weights, or male/female sex ratios comparing dosed and control groups. No increased incidences of external, soft tissue, or skeletal abnormalities were found in fetuses in any of the exposed groups compared to the controls. The NOAEL for both maternal and developmental toxicity was 30 mg/kg bw/day, the highest dose tested (IRD78).

Groups of pregnant CF-1 mice (numbers not given) received HEX (purity: 98%) at daily oral doses of 0, 5, 25, or 75 mg/kg bw on gestational days 6-15, and were sacrificed on gestational day 18. No signs of maternal toxicity were observed in any of the groups. Fertility, average numbers of implantations, live fetuses per litter, number of resorptions per litter, fetal body weight, or fetal

crown-rump length of the treated mice were not significantly different compared to the control group. There was no increase in the incidence of fetal malformations examined in 249 to 374 fetuses per group when considered collectively or by individual type. The NOAEL for maternal and developmental toxicity was 75 mg/kg bw/day (Mur80). In another study, 16 pregnant CD-1 mice received HEX doses of 0 or 45 mg/kg bw/day on gestational days 8-12. Neither significant effects on maternal body weights nor any changes in either the number of live offspring or the average fetal weight on the 1st and 3d day after birth were found in the exposed animals compared to controls (Che82). In another study, using the same dosing regimen, the observation period of offspring of CD-1 mice was extended to 250 days. No treatment-related effects were observed on survival rate, and no macroscopic and microscopic abnormalities were found in the liver, testes, seminal vesicles, or right kidney of male animals compared to controls. The females did not show impairment of reproductive functions. The NOAEL for both maternal and developmental toxicity was 45 mg/kg bw/day, the highest level tested (Gra84).

Groups of pregnant New Zealand white rabbits (numbers not given) received daily oral doses of HEX (purity: 98.2%) of 0, 5, 25, or 75 mg/kg bw on gestational days 6-18, and were sacrificed on gestational day 29. At the high dose, mortality, diarrhoea, and body weight loss was observed. Fertility, average numbers of implantations, live fetuses per litter, number of resorptions per litter, fetal body weight, or fetal crown-rump length of the treated mice were not significantly different compared to controls. There was an increase in the number of fetuses with one minor skeletal variation, i.e., a 13th rib, which was significantly higher at the high dose compared to controls (58/171 or 34%, 33/95 or 35%, 33/78 or 42%, and 44/77 or 57%, at 0, 5, 25, and 75 mg/kg bw/day, respectively) (Mur80). The committee concludes that, in this study, the NOAEL for both maternal and developmental toxicity was 25 mg/kg bw (Mur80).

7 Existing guidelines

The current administrative occupational exposure limit (MAC) for HEX in the Netherlands is 0.11 mg/m^3 (0.01 ppm), 8-hour TWA.

Existing occupational exposure limits for HEX in some European countries and in the USA are summarised in the annex.

8 Assessment of health hazard

For workers, engaged in the manufacture or use of HEX, the main routes of exposure to the chemical are through inhalation of its vapour or by direct skin contact with the liquid.

In rats, following 2-hour inhalation exposure to air concentrations ranging from 0.11 to 0.57 mg/m^3 (0.01-0.05 ppm), 95% of the inhaled vapour is retained in the body. No data is available of the percentage of dermal uptake. HEX is very reactive, and, following absorption into the body, the compound or its metabolites may bind to blood, tissues, and the contents of the gastrointestinal tract. The kinetics of HEX have been studied in experimental species following the inhalation, oral, or intravenous routes of exposure. In rats, following a 2-hour exposure up to 0.57 mg/m^3 (0.05 ppm), 95% of the amount inhaled was retained and completely metabolised. Approximately equal amounts of the dose were excreted in the urine and the faeces (ca. 36% each) within 72 hours after exposure. At 72 hours, ca. 12% was retained in the body, principally the trachea, lungs, and kidneys. A small amount (ca. 1%) was excreted as CO_2. Following oral administration, absorption was poor, probably due to its reactivity with the contents of the gastrointestinal tract. Most of the dose is excreted in the faeces (63-80%) and only 15-35% in the urine, within 72 hours. The percentage of the dose retained in the body at 72 hours after application varied from 0.2% to 2.8%, principally in the kidneys. Studies on the metabolism of HEX have been unsuccessful; the polar faecal and urinary metabolites have been isolated but not identified.

Case studies in humans showed that acute exposure to HEX, due to an illegal contamination of a municipal sewage plant with a large volume of HEX and related chlorinated hydrocarbons, resulted in a high incidence of eye, throat, and skin irritation, headaches, nausea, respiratory distress, and nervousness in plant workers and clean-up workers. These complaints persisted for 3 to 6 weeks. Air concentrations, measured 4 days after the plant closed, ranged from 3 to 11 mg/m^3 (0.27-0.97 ppm). In some of the clean-up workers, a slight increase in serum aspartate aminotransferase activity was found, probably related with exposure. In another study, it was demonstrated that long-term exposure (average ca. 8 years) to HEX and other chlorinated hydrocarbons, at air concentrations near or below the occupational exposure limits, did not produce abnormal biochemical liver and kidney function tests in the workforce. Epidemiological

studies on workers involved in the production of HEX or in the use of HEX as an important precursor of chemical of organochlorine pesticide manufacture, such as chlordane, heptachlor, aldrin, or dieldrin, did not give indications for an increased mortality rate (all cases, cancer, or diseases of the circulatory system), compared to the overall USA population. However, information specific to HEX exposure, either qualitative or quantitative, was not available in any of these studies. In addition, study populations were relatively small, and observation times (25 years at most) relatively short. According to the committee, these data, therefore, contribute little to the health hazard assessment of HEX.

In experimental animal studies, the compound was very irritating to the eyes and the skin. In one study, HEX was found to be a skin sensitiser in guinea pigs. HEX is much more toxic when inhaled than when ingested or following dermal contact. Based on the results of acute lethal toxicity studies in animals, the committee considers the compound as very toxic after inhalation exposure, toxic after skin contact, and harmful if swallowed.

Effects of HEX exposure in short- or long-term studies in rats and mice included: lung injury and increased erythrocyte, haematocrit, and haemoglobin level in a 2-week inhalation study in rats (NOAEL: 1.25 mg/m^3 (0.11 ppm)), posterior paresis in a 13-week inhalation study in rats (NOAEL: 0.46 mg/m^3 (0.04 ppm)), lung, liver, and kidney injury, and increased erythrocyte and haemoglobin level in a 30-week inhalation study in rats (NOAEL: 1.14 mg/m^3 (0.1 ppm)), pigmentation of respiratory epithelium of the nose, the bronchioles, and the bronchi, and squamous metaplasia of laryngeal epithelium in a 2-year inhalation rat study (LOAEL: 0.11 mg/m^3 (0.01 ppm)), squamous metaplasia of larynx and trachea in a 13-week inhalation study in mice (NOAEL: 0.46 mg/m^3 (0.04 ppm)), pigmentation of respiratory epithelium of the nose and the trachea in a 2-year inhalation mouse study (LOAEL: 0.11 mg/m^3 (0.01 ppm)), forestomach lesions in rats and mice in a 13-week oral study (NOAEL: 10 mg/kg bw/day for the rat and 19 mg/kg bw/day for the male mouse; LOAEL: 19 mg/kg bw/day for the female mouse). No adverse effects were found in a 14-week inhalation study in monkeys (NOAEL: 2.28 mg/m^3 (0.2 ppm)). The brown pigment observed in the mucosa and submucosa of the respiratory tract of rats and mice in the 2-year inhalation study was not reported when rats and mice were exposed to other irritant chemicals, like methyl isocyanate, glutaraldehyde, or formaldehyde. The NTP suggested that the pigmentation might have been the result of a direct reaction between the chemical or one of its metabolites and the respiratory tissues (NTP94).

HEX did not induce gene mutations in most tests, using bacteria or cultured mammalian cells. However, tests for cytogenicity in HEX-treated cultured mammalian cells were conflicting. *In vivo*, HEX did not increase the frequency of micronuclei in erythrocytes or of dominant lethal mutations in mice. The committee concluded that HEX is not a mutagenic hazard *in vivo*.

The 2-year inhalation studies in rats and mice did not show treatment-related increased incidences of neoplastic lesions in either species.

In developmental toxicity studies, oral dosing of HEX did not induce maternal or developmental toxicity in rats and mice at 30 and 75 mg/kg bw, the highest dose levels tested. In rabbits, there was an increase in the incidence of the number of fetuses with a 13th rib at a maternally toxic dose of 75 mg/kg bw, while neither maternal nor developmetal toxicity was seen at 25 mg/kg bw.

From the above-discussed data, the committee takes the well-performed 2-year rat inhalation study (NTP94) as a starting point in deriving a health-based recommended occupational exposure limit (HBROEL). In this study, the committee considered squamous metaplasia of the laryngeal epithelium to be the critical effect. A NOAEL could not be established since at 0.11 mg/m^3 (0.01 ppm), the lowest level tested, these laryngeal lesions as well as pigmentation of the respiratory epithelium of the nose, the bronchioles, and the bronchi were demonstrated. For extrapolation to a HBROEL, the committee establishes an overall assessment factor of 12 covering the absence of a NOAEL, intra- and interspecies variation, and the type of critical effect. Thus, applying this factor of 12 and the preferred-value approach, the committee recommends a health-based occupational limit of 0.01 mg/m^3 (0.0009 ppm) for hexachlorocyclopentadiene.

The committee recommends a health-based occupational exposure limit for hexacyclopentadiene of 0.01 mg/m^3 (0.0009 ppm), as an 8-hour time-weighted average (TWA). A skin notation is not deemed necessary because of the local character of the critical effect.

References

Abd84 Abdo KM, Montgomery CA, Kluwe WM, et al. Toxicity of hexachlorocyclopentadiene: subchronic (13-week) administration by gavage to F344rats and B6C3F1 mice. J Appl Toxicol 1984; 4: 75-81.

ACG99 American Conference of Governmental Industrial Hygienists (ACGIH). Hexachlorocyclopentadiene. In: TLVs® and other occupational exposure values - 1999. [CD-ROM]. Cincinnati OH, USA: ACGIH®, Inc, 1999.

ACG03a American Conference of Governmental Industrial Hygienists (ACGIH). Guide to occupational exposure values - 2003. Cincinnati OH, USA: ACGIH®, Inc, 2003: 65.

ACG03b American Conference of Governmental Industrial Hygienists (ACGIH). 2003 TLVs® and BEIs® based on the documentation of the Threshold Limit Values for chemical substances and physical agents & Biological Exposure Indices. Cincinnati OH, USA: ACGIH®, Inc, 2003: 34.

Amo83 Amoore JE, Hautala E. Odor as an aid to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J Appl Toxicol 1983; 3: 272-90.

Arb02 Arbejdstilsynet. Grænseværdier for stoffer og materialer. Copenhagen, Denmark: Arbejdstilsynet, 2002: 27 (At-vejledning C.0.1).

ATS99 Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for hexachlorocyclopentadiene (HCCPD). Atlanta GA, USA: US Department of Health and Human Services, Public Health Service, 1999; http://www.atsdr.cdc.gov/toxprofiles/tp112.html.

Boo93 Boogaard PJ, Rocchi PSJ, van Sittert NJ. Effects of exposure to low concentrations of chlorinated hydrocarbons on the kidneys and livers of industrial workers. Br J Ind Med 1993; 50: 331-9.

Bra83 Brat SV. The hepatocyte primary culture/DNA repair assay on compound hexachlorocyclopentadiene using rat hepatocytes in culture. Valhalla NY, USA: American Health Foundation, Naylor Dana Institute for Disease Prevention, 1983; unpublished report, cited in WHO91

Bro83 Brooks TM, Hodson-Walker G, Wiggins DE. Genotoxicity studies with hexachlorocyclopentadiene (Hex). London, England: Shell Research Limited, Sittingbourne Research Centre, 1983.

BUA88 German Chemical Society - Advisory committee on existing chemicals of environmental relevance (Gesellschaft Deutscher Chemiker- Beratergremium für umweltrelevante Altstoffe) (BUA). Hexachlorocyclopentadiene., Stuttgart, Germany: S. Hirzel Wissenschaftliche Verlagsgesellschaft, 1988; BUA report 25.

Bun80 Buncher CR, Moomaw C, Sirkoski E. Mortality study of Montague Plant-Hooker Chemical. Cincinnati OH, USA: University of Cincinnati Medical Center, Division of Epidemiology and Biostatistics, 1980; unpublished report, cited in BUA 88 and WHO91.

Che99 Chemiekaarten: Gegevens voor het veilig werken met chemicaliën/[ed by] Samenwerkingsverband-Chemiekaarten (TNO Arbeid, VNCI). 15th ed 2000. The Hague, the Netherlands: Ten Hagen & Stam, 1999: 630.

Cla82 Clark DG , Pilcher A, Blair D, et al. Thirty week chronic inhalation study of hexachlorocyclopentadiene (HEX) in rats. London, England: Shell Research Limited, Sittingbourne Research Centre, 1982.

Dea77 Dean WP. Acute oral toxicity (LD_{50}). Study in albino mice. Mattawan MI, USA: International Research and Development Corporation, 1977 (available from the National Technical Information Service, Springfield VA, USA; order no: NTIS/OTS0512867).

Dor79 Dorough HW. The accumulation, distribution, and dissipation of hexachlorocyclopentadiene (C56) in tissues of rats and mice; unpublished report, cited in WHO91.

Dor84 Dorough HW, Raneiri TA. Distribution and elimination of hexachlorocyclopentadiene in rats and mice. Drug Chem Toxicol 1984; 7: 73-89.

DFG02 Deutsche Forschungsgemeinschaft (DFG): Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. List of MAK and BAT values 2002. Maximum concentrations and biological tolerance values at the workplace. Weinheim, FRG: Wiley-VCH, 2002: 65 (rep no 38).

EC03 European Commission: Directorate General of Employment and Social Affairs. Occupational exposure limits (OELs). http://europe.eu.int/comm/employment_social/h&s/areas/oels_en.htm.

ElD83 El Dareer SM, Noker PE, Tillery KF, et al. Investigations on the basis for the differential toxicity of hexachlorocycopentadiene administered to rats by various routes. J Toxicol Environ Health 1983; 12: 203-11.

Gar86 Gardner JR. Acute oral toxicity to rats of hexachlorocyclopentadiene. Huntingdon (Cambridgeshire), England: Huntingdon Research Centre Ltd, 1986 (available from the National Technical Information Service, Springfield VA, USA; order no: NTIS/OTS0513386).

Gre01 Greim H, ed. Hexachlorcyclopentadien. In: Gesundheitsschädliche Arbeitsstoffe. Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten (Maximale Arbeitsplatz-Konzentrationen). 33rd ed. Weinheim, FRG: Wiley-VCH, 2001.

HSE02 Health and Safety Executive (HSE). EH40/2002. Occupational exposure limits 2002. Sudbury (Suffolk), England: HSE Books, 2002.

IRD68 International Research and Development Corporation. Hexachlorocyclopentadiene and octachlorocyclopentene:Acute oral toxicity LD_{50} in male albino rats.Mattawan MI, USA: International Research and Development Corporation, 1968; unpublished report, cited in WHO91.

IRD72 International Research and Development Corporation. Acute toxicity studies in rats and rabbits. Mattawan MI, USA: International Research and Development Corporation, 1972; unpublished report cited in BUA88 and WHO91.

IRD78 International Research and Development Corporation. Hexachlorocyclopentadiene: teratology study in rats. Mattawan MI, USA: International Research and Development Corporation, 1978; unpublished report, cited in BUA88 and WHO91.

Jac87 Jackson GC, Hardy CJ. Acute inhalation toxicity study. Huntingdon (Cambridgeshire), England: Huntingdon Research Centre Ltd, 1987; unpublished report, cited in Gre01.

Jon91 De Jong G. Long-term health effects of aldrin and dieldrin. A study of exposure, health effects and mortality of workers engaged in the manufacture and formulation of the insecticides aldrin and dieldrin. Toxicol Lett 1991; suppl.

Kha81 Khan MAQ, Sudershan P, Feroz M, *et al*. Biotransformations of cyclodienes and their photoisomers and hexachlorocyclopentadiene in mammals and fish. In: Khan MAQ, Stanton RH, eds. Toxicology of halogenated hydrocarbons: health and ecological effects. Oxford, England: Pergamon Press, 1981: 271-88 (cited from BUA88, Gre01, and WHO91).

Kom80 Kominsky JR, Wisseman CL III, Morse DL. Hexachlorocyclopentadiene contamination of a municipal wastewater treatment plant. Am Ind Hyg Assoc J 1980; 41: 552-6.

Law81 Lawrence LJ, Dorough HW. Retention and fate of inhaled hexachlorocyclopentadiene in the rat. Bull Environ Contam Toxicol 1981; 26: 663-8.

Law82 Lawrence LJ, Dorough HW. Fate of inhaled hexachlorocyclopentadiene in albino rats and comparison to the oral and iv routes of administration. Fundam Appl Toxicol 1982; 2: 235-40.

Mas92 Mason JM, Valencia R, Zimmering S. Chemical mutagenesis testing in Drosophila: VIII. Reexamination of equivocal results. Environ Mol Mutagen 1992; 19: 227-34.

Mat78 Matheson DW. Mutagenicity evaluation of hexachlorocyclopentadiene in the mouse lymphoma forward mutation assay. Final report. Kensington MD, USA: Litton Bionetics, Inc, 1977; LBI proj no 20839 (available from the National Technical Information Service, Springfield VA, USA; order no: NTIS/OTS0512869).

Mat89 Matsui S, Yamamoto R, Yamada Y. The Bacillus subtilis/microsome rec-assay for the detection of DNA damaging substances which may occur in chlorinated and ozonated waters. Water Sci Technol 1989; 21: 875-87.

Meh77 Mehendale HM. Chemical reactivity-absorption, retention, metabolism and elimination of hexachlorocyclopentadiene. Environ Health Perspect 1977; 21: 275-8.

Mor79 Morse DL, Kominsky JR, Wisseman CL III, et al. Occupational exposure to hexachlorocyclopentadiene. How safe is sewage? JAMA 1979; 241: 2177-9.

NTP94 National Toxicology Program (NTP). Toxicology and carcinogenesis studies of hexachlorocyclopentadiene (CAS no. 77-47-4) in F344/N rats and B6C3F$_1$ mice (inhalation studies). Research Triangle Park NC, USA: National Toxicology Program, 1994; NTP Techn Report 437.

Pri82 Price JB. Toxicology of insecticide intermediates: The skin sensitizing potential of hexachlorocyclopentadiene (hex"). London, England: Shell Research Limited, Sittingbourne Research Centre, 1982.

Raa93 Raabe F, Janz S, Wolff G, et al. Genotoxicity assessment of waste products of aluminum plasma etching with the SOS chromotest. Mutat Res 1993; 300: 99-109.

Ran82a Rand GM, Nees PO. Calo CJ, et al. Effects of inhalation exposure to hexachlorocyclopentadiene on rats and monkeys. J Toxicol Environ Health 1982; 9: 743-60.

Ran82b Rand GM, Nees PO, Calo CJ, et al. The Clara cell: An electron microscopy examination of the terminal bronchioles of rats and monkeys following inhalation of hexachlorocyclopentadiene. J Toxicol Environ Health 1982; 10: 59-72

Rut86 Ruth JH. Odor thresholds and irritation levels of several chemical substances: a review. Am Ind Hyg Assoc J 1986; 47: A142-A151.

Shi80 Shindell & Associates. Report of epidemiological study of employees of Velsicol Chemical Corporation plant, Marshall, Illinois, January 1946-December 1979. Milwaukee WI, USA: Shindell & Associates, 1980; unpublished report, cited in BUA88 and WHO91.

Shi81 Shindell & Associates. Report of epidemiological study of employees of Velsicol Chemical Corporation plant, Memphis, Tennessee, January 1952-December 1979. Milwaukee WI, USA: Shindell & Associates, 1981; unpublished report, cited in BUA88 and WHO91.

Shi86 Shindell S, Ulrich S. Mortality of workers employed in the manufacture of chlordane. An update. J Occup Med 1986; 28: 497-501.

SRI80 Southern Research Institute. Acute toxicity report on hexachlorocyclopentadiene (C53607) inFischer-344 rats and B6C3F_1 mice. Birmingham AL, USA: Southern Research Institute, 1980; unpublished report, cited in WHO91.

Swe00 Swedish National Board of Occupational Safety and Health. Occupational exposure limit values and measures against air contaminants. Solna, Sweden: National Board of Occupational Safety and Health, 2000; Ordinance AFS 2000:3.

SZW03 Ministerie van Sociale Zaken en Werkgelegenheid (SZW). Nationale MAC-lijst 2003. The Hague, the Netherlands: Sdu, Servicecentrum Uitgevers, 2003: 30.

Tre55 Treon JF, Cleveland FP, Cappel J. The toxicity of hexachlorocyclopentadiene. Arch Ind Health 1955; 11: 459-72.

TRG00 TRGS 900. Grenzwerte in der Luft am Arbeitsplatz; Technische Regeln für Gefahrstoffe. BArbBl 2000; 2.

Wan79 Wang HH, MacMahon B. Mortality of workers employed in the manufacture of chlordane and heptachlor. J Occup Med 1979; 21: 745-8.

WHO91 World Health Organization/International Programme on Chemical Safety (WHO/IPCS). Hexachlorocyclopentadiene. Geneva, Switzerland: World Health Organization, 1991; Environmental Health Criteria 120.

Wil80 Williams GM. Classification of genotoxic and epigenetic hepatocarcinogenes using liver culture assays. Ann N Y Acad Sci 1980; 349: 273-82.

Yu81 Yu CC, Atallah YH. Pharmacokinetics and metabolism of hexachlorocyclopentadiene in rats. Chicago IL, USA: Velsicol Chemical Corporation, 1981; unpublished report, cited in BUA88 and WHO91.

Zim85 Zimmering S, Mason M, Valencia R, et al. Chemical mutagenicity testing in Drosophila. II. Results of 20 coded compounds tested for the National Toxicology Program. Environ Mutagen 1985; 7: 87-100.

Annex

Occupational exposure limits for hexachlorocyclopentadiene in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	0.01	0.11	8 h	administrative		SZW03
Germany						
- AGS	-	-				TRG00
- DFG MAK-Kommission	-[c]	-				DFG02
Great Britain						
- HSE	-	-				HSE02
Sweden	-	-				Swe00
Denmark	0.01	0.1	8 h			Arb02
USA						
- ACGIH	0.01	-	8 h	TLV	A4[d]	ACG03b
- OSHA	-	-				ACG03a
- NIOSH	0.01	0.1	10 h	REL		ACG03a
European Union						
- SCOEL	-	-				EC03

[a] S = skin notation, which means 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] Listed among compounds for which studies of the effects in man or experimental animals have yielded insufficient information for the establishment of MAK values.

[d] Classified in carcinogenicity category A4, i.e., not classifiable as a human carcinogen: agents which cause concern that they could be carcinogenic for humans but which cannot be assessed conclusively because of a lack of data. *In vitro* or animal studies do not provide indications of carcinogenicity which are sufficient to classify the agent into one of the other categories.